B AND T CELL TUMORS: BIOLOGICAL AND CLINICAL ASPECTS

Ellen Vitetta, Organizer

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Origin and Classification of Tumors

0001 STEM CELL ORIGIN AND CLONAL DEVELOPMENT OF HUMAN HEMATOLOGIC NEOPLASMS, Philip J. Fialkow, Dept. of Medicine, University of Washington, Seattle, WA 98195

The number of cells from which human tumors develop can be determined in patients heterozygous for glucose-6-phosphate dehydrogenase (G6PD). Since the G6PD locus is on the X chromosome, only one G6PD gene is active in each somatic cell. Therefore, women heterozygous for the usual B gene ($\underline{Gd}^B$) and $\underline{Gd}^A$ have two cell populations--one synthesizing B-type G6PD and the other, A-type enzyme. Tumors with a single-cell (clonal) origin exhibit only one type of G6PD, but those arising from many cells may show both B and A enzymes.

Both B and A types of G6PD were found in normal tissues from 23 women heterozygous for G6PD who had chronic myelocytic leukemia (CML), but only a single enzyme was detected in granulocytes, red cells and platelets (13 type B and 10 type A), indicating that this disorder involves multi-potent marrow stem cells and that it develops clonally. Fewer patients with other myeloproliferative disorders such as polycythemia vera, essential thrombocythemia and agnogenic myeloid metaplasia have been studied, but the results are similar to those found in CML. Unlike the blood cells, cultured marrow fibroblasts from patients with agnogenic myeloid metaplasia had double-enzyme phenotypes, suggesting that the marrow fibrosis from patients with agnogenic myeloid metaplasia had double-enzyme phenotypes, suggesting that the marrow fibrosiswas secondary despite the fact that it was the pre-dominant clinical manifestation in that disease. Similar findings were obtained in CML. In contrast to marrow fibroblasts, preliminary data suggest that some endothelial-like marrow microenvironmental cells were transplantable and arose from the hematologic pluripotent stem cell involved by CML. Acute myelogenous leukemia is also a clonal disorder, but it is heterogeneous with respect to stem cell involvement. In some patients, the disease involves multipotent stem cells similar to CML, but in other patients the disorder involves cells with differentiative expression restricted to the granulocytic and not the erythrocytic pathway. Detailed studies with G6PD in CML indicate that some B-lymphoid and perhaps T-lymphoid cells arise from the stem cell involved by the leukemia. These data provide firm evidence for the existence in man of a stem cell pluripotent for both the myelogeneous and the lymphoid series and that it is this stem cell which is involved in CML. Immunoglobin (Ig) diversity was found in B-lymphoid cell populations that arose from the CML clone. Other investigators have reported monoclonal Ig synthesis by B-lymphoid cells in the blastic phase of CML. These data suggest that Ig restriction is fixed in malignant CML lymphoblasts, but not in the more primitive pluripotent stem cell involved by the disease.

0002 MORPHOLOGY, CYTOCHEMISTRY, AND IMMUNOHISTOLOGY OF T-CELL LYMPHOMAS, K. Lennert, H. Stein, and A. Feller, Institute of Pathology, University of Kiel, Germany

T-cell lymphomas, including chronic lymphocytic leukemia of T type (T-CLL) were investigated with subtle morphologic techniques (on paraffin sections and imprints) and cytochemical methods (acid phosphatase, acid nonspecific esterase, DAPIV). The T-cell derivation of all tumors was demonstrated by immunologic techniques applied to cell suspensions or frozen sections. Frozen sections were analyzed with a large number of monoclonal antibodies characteristic, or even specific for various lymphoid cell populations (OKT11, OKT4, OKT8) and B-cell populations (IgM, IgD, kappa, lambda). In addition, macrophages and dendritic reticulum cells were visualized with OKM and R4/23, respectively. Two types of T-CLL were found: one was positive for OKT4, acid nonspecific esterase, and DAPIV, and the other was positive for OKT8 and negative for DAPIV. A majority of the T-cell lymphomas in the present series were classified as T-zone lymphoma on the basis of their morphology. These cases expressed OKT11 and OKT4 and/or OKT8. They also exhibited a characteristic feature, namely, adjunctive nonneoplastic ("bitypic") B-cell areas containing B-cell area-specific dendritic reticulum cells. Cases of T-cell lymphoma of the lymphoblastic type showed a phenotype similar to that of cortical thymocytes (OKT11+, OKT4++, OKT8++, OKT6+). A number of other types of T-cell lymphoma were also studied and defined by morphologic, cytochemical, and immunologic methods.

0003 THE RELATIVE CLINICAL VALUE OF THE VARIOUS CLASSIFICATIONS OF HUMAN NON-HODGKIN'S LYMPHOMAS, Saul A. Rosenberg, Departments of Medicine and Radiology, Stanford University, Stanford, CA 94305

The non-Hodgkin's lymphomas are a diverse group of neoplasms which include some of the most well tolerated and indolent human tumors to some of the most rapidly progressive and fatal. Their clinical features, sites of disease, response to therapy and curability are considerably different among the various types. The clinical variables of the non-Hodgkin's lymphomas have become appreciated as improved histologic classifications have been utilized over the past two decades. The original Rappaport classification has undergone considerable modification since its description in 1956. Newer classifications have been proposed which are scientifically more valid than the modified Rappaport system. Two classifications, the Kiel and Lukes-Collins systems are based on morphologic expressions of more current concepts of lymphoid function.

A recently completed international study, correlating clinical characteristics, response to therapy and survival of 1175 patients with each of six major pathologic classifications will be described. All six classifications are of comparable value, though several identify clinical entities not appreciated by others. Ten major subtypes will be described, each of which can be related to the subtypes of the six classifications.

The Non-Hodgkin's Lymphoma Pathologic Classification Project. National Cancer Institute sponsored study of classifications of non-Hodgkin's lymphomas: summary and description of a working formulation for clinical usage. Cancer (in press), April 1982.

T and B Cell Clones

0004 MURINE T-CELL CLONES USED TO DISSECT THE IMMUNE RESPONSE
C. G. Fathman, A.J. Infante, M. Shigeta and M. Kimoto

Recent studies in our laboratory and elsewhere have shown that it is possible to isolate and propagate murine T cell clones reactive with soluble antigen (1,2). We have used such antigen reactive T-cell clones to address a variety of questions concerning immunoregulation. Using such clones it has been possible to show that murine Ia antigens are responsible for restriction of antigen recognition and that such Ia antigens are formed by free combinatorial association of alpha and beta chains encoded within the murine I region (2). Using monoclonal anti-Ia antibodies and a mutant mouse (bm.12) it has been possible for us to show that there must exist more than one restriction site for each Ia molecular complex. Not only have we been able to show that there exists more than one restriction site on each Ia molecule but also that the "hybrid" molecules are fully functional as restricting determinants for antigen recognition by F_1 T-cell clones. Using such T-cell clones as a source of antigen-specific help for the differentiation of plasma cells to B cells it has been possible to show that a single murine T-cell clone can interact in at least two distinct pathways. One of these results in an $Lyb5^-$B cell response which requires histocompatibility at the macrophage and T-cell, but not at the T-cell and B-cell level. The second is through the $Lyb5^+$B cell response which requires MHC identity for all three interacting cell types (3). Although such help is antigen-specific and I-region restricted, it does not show any isotype selectivity (4). Additionally, studies using such murine T-cell clones have allowed us to suggest that there is sharing of idiotypy between antibodies and helper T cell clones (4).

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0005 ANALYSIS OF LYMPHOKINE PRODUCTION BY T CELL CLONES, Peter H.Krammer, Institute of Immunology and Genetics, German Cancer Research Center, Im Neuenheimer Feld 280, D-6900 Heidelberg, Fed.Rep.Germany

T cell clones from limiting dilution microcultures and from long term cultures were used to determine T cell activity. Mitogen activated T cells plated in limiting dilution microcultures were expanded in the presence of irradiated peritoneal exudate filler cells and medium supplemented with T cell growth factors. After a growth period of 7 to 12 days the progeny of one or very few precursor cells reached a clone size suitable for functional assays. The cells were either tested directly, e.g. for cytolytic activity, or induced by T cell mitogens to secrete lymphokines into the supernatant. Depending on the sensitivity of the lymphokine assay supernatants could simultaneously be tested for various lymphokines. A correlation analysis of direct cellular activity and lymphokine release could be made.

The data obtained with "T cell clones" in limiting dilution microcultures were supported by data with T cell lines and clones growing either in the presence of antigens and medium supplemented with T cell growth factors or in medium supplemented with T cell growth factors alone and exhibiting either no apparent specificity, specific alloreactivity, or specific cytolytic activity.

The data from both assay systems complemented each other and showed that: 1) T lymphocytes are the producer cells of various lymphokines, 2) lymphokines can be released from T cells by mitogen and antigen-specific signals, 3) the progeny of a particular precursor T cell is capable of releasing several lymphokines simultaneously, and 4) various lymphokines produced by the progeny of one particular precursor T cell are not identical.

These data show that complex cellular interactions lead to lymphokine release and that the lymphokines may belong to a large family of molecules. Since antigen-specific signals are involved in triggering the progeny of one T cell precursor to release various lymphokines acting on a variety of target cells, stringent regulatory systems must exist controlling lymphokine activity in vivo and in vitro.

0006 CO-SEGREGATION OF SEVERAL CHARACTERISTICS OF CYTOLYTIC T-CELLS IN HYBRIDS WITH THYMOMAS. M. Nabholz, A. Conzelmann, M. Cianfriglia and W. Haas, Swiss Institute for Experimental Cancer Research, CH-1066 Epalinges, Switzerland.

Cloned murine cytolytic T lymphocyte (CTL) lines depend on T-cell growth factor (TCGF) for survival and proliferation. Crosses between CTL-lines and AKR-thymomas yield cytolytic hybrids if these are selected in the presence of TCGF (1, 2). Active hybrids display the specificity of the parental CTL (2) and resemble CTL-lines in that they are TCGF-dependent and sensitive to growth inhibition by *Vicia Villosa* lectin. They have a differentiated morphology and adhere to tissue culture plastic. Hybrids selected in the absence of TCGF resemble the parental thymoma cells : they are round, non-adherent cells without detectable cytolytic activity and resistant to *Vicia Villosa* (3).

Circumstantial evidence suggests that the difference between the two types of hybrids is genetic and that TCGF-dependence behaves like a dominant trait in thymoma x CTL hybrids. Selection for TCGF independence from cloned growth factor dependent hybrids yields variants which resemble the hybrids originally isolated in the absence of TCGF. The data are consistent with the hypothesis that TCGF-independent CTL x thymoma hybrids have lost a CTL-chromosome carrying one or several genes the expression of which (a) imposes TCGF-dependence on the cells, (b) controls the activity of a glycosyltransferase required for rendering cells sensitive to *Vicia Villosa* lectin, and (c) influences the cytolytic activity of the cells in a quantitative fashion.

These results illustrate the usefulness of cloned T-cell lines as tools for a somatic cell genetic approach to the analysis of the molecular bases of their function.

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0007 HUMAN IMMUNOREGULATORY T CELL SUBSETS: THEIR DIFFERENTIATION AND FUNCTION, Ellis L. Reinherz, Chikao Morimoto, Stefan Meuer and Stuart F. Schlossman, Division of Tumor Immunology, Sidney Farber Cancer Institute, Harvard Medical School, Boston, MA 02115.

Prior studies utilizing heteroantisera and monoclonal antibodies have indicated that two major functionally distinct subpopulations of T lymphocytes exist in man. These subsets arise from a common thymocyte progenitor through an orderly series of differentiation steps. The T4+ subset induces other lymphoid and nonlymphoid cells to fulfill their genetic programs whereas the T5/T8+ subset of T lymphocytes does not. Rather, the T5/T8+ subpopulation contains cells with cytotoxic and suppressor functions. Differentiation of T5/T8+ cytotoxic effectors from pre-cytotoxic T5/T8+ lymphocytes requires induction by T4+ T cells. Similarly, an interaction between a subpopulation of T4+ T cells and T5/T8+ T lymphocytes is required to suppress B cell immunoglobulin production. Within the T4+ population itself, considerable heterogeneity is evident since both autoantibodies and monoclonal antibodies can distinguish between one subpopulation of T4+ lymphocytes which induces suppressor effector function and another which induces B cell immunoglobulin production. Abnormalities in individual regulatory T lymphocyte subsets result in aberrations of immune homeostasis that initiate clinical disorders of autoimmunity as well as immunodeficiency.

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Differentiation of T and B Cell Tumors

0008 DIFFERENTIATION OF LEUKEMIC CELLS IN CHRONIC LYMPHOCYTIC LEUKEMIA, Shu Man Fu, The Rockefeller University, New York, NY 10021

Monoclonal proliferation of sIg^+ B cells is a major feature of chronic lymphocytic leukemia. The leukemic cells were thought to be arrested at an early stage of B cell maturation. Recently, it has been demonstrated in a considerable number of cases that these leukemic cells could be induced to differentiate further in vitro. In certain cases in which a monoclonal serum Ig band was associated with the lymphocytosis, a large number of the leukemic cells were induced to mature to plasma cells and to secrete a significant amount of the monoclonal Ig in vitro by the provision of allogeneic helper T cells or pokeweed mitogen. the leukemic origen of the maturing cells was ascertained with anti-idiotypic antibodies to the serum monoclonal Ig band (1). In cases without a demonstratable serum Ig band, other investigators have shown that polyclonal B cell activators induced a considerably smaller number of the leukemic cells to mature to plaque forming cells (2) and that the tumor promotor, phorbol ester induced increased Ig synthesis without evidence for the newly synthesized Ig being secreted (3). These studies demonstrate the varied responsiveness of leukemic cells to different stimuli and suggest the heterogeneous origin of these cells. This heterogeneity has also been shown by the reactivities of the leukemic cells to a panel of monoclonal antobodies to human B cells.

Despite the responsiveness to certain regulatory processes, the leukemic cells in chronic lymphocytic leukemia have been shown to have certain defects such as their inability to respond to anti-Ig antibody stimulation, their defectiveness as stimulator cells in the mixed lymphocyte reaction and chromosome abnormalities. In addition, T cell dysfunctions have been demonstrated in these patients. These abnormalities undoubtedly play significant roles in the pathogenesis of the disease.

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0009 BIOCHEMICAL AND MOLECULAR STUDIES ON ACTIVATION AND REGULATION OF IMMUNOGLOBULIN SYNTHESIS IN HUMAN MONOCLONAL B CELLS, Tadamitsu Kishimoto, Department of Medicine, Osaka University Medical School, Osaka 553 Japan.

Human leukemic B cells (B-CLL) or an established B cell line, which were capable of differentiation into IgG producing cells by T cell-derived signals, were employed for the molecular studies of B cell activation (1,2). Stimulation of B-CLL cells with anti-idiotypic antibody or anti-μ antibody and T cell-derived helper factors induced proliferation of B-CLL cells as well as production of IgM and IgG with the same idiotype. Neither anti-immunoglobulin nor T cell factors alone induced any proliferation or Ig-production in B-CLL cells. T cell factors were fracionated into proliferation factor(s) (IL-2) and differentiation factor(s) (TRF) by gel filtration and chromatofocusing. Anti-Ig and IL-2 induced proliferation of B-CLL cells but no Ig-production. Three signals, i.e., anti-Ig, IL-2 and TRF, were required for IgM and IgG production in B-CLL cells. These results indicate that anti-Ig activates B cells into the stage responsive to IL-2, which induces proliferation of activated B cells. After IL-2-induced proliferation, TRF induces final differentiation of B cells into Ig-producing cells.

In order to study biochemical mechanisms of the transmission of the signals provided by T cells, we have established a B lymphoblastoid cell line, which differentiates into IgG producing cells in the presence of TRF. Absorption experiment demonstrated that cells expressed acceptors for TRF but did not have any acceptors for IL-2. The binding of TRF to its acceptors induced IgG production without any requirement of cell division. Thus, those cells may represent the final differentiation stage of B cells which can directly differentiate into Ig-producing cells under the influence of the differentiation factor(s). Involvement of stimulus activatable serine esterase in the transmission of TRF-mediated signals was shown by using several organophosphorus inhibitors. Red cell-mediated microinjection showed that the limited proteolysis of the precursor proteins in cytoplasm with TRF-activated serine esterase generated the cytoplasmic factor(s) responsible for the transmission of membrane-mediated signals into nuclei. Thus, injection of the cytoplasm obtained from TRF-stimulated cells induced IgG production in non-stimulated cells. DFP but not actinomycin D inhibited the generation of the cytoplasmic factor(s). Incubation of the membrane fraction from TRF-stimulated cells with cytoplasm from non-stimulated cells could generate the cytoplasmic factor(s) capable of inducing IgG production in non-stimulated cells. Characterization of the cytoplasmic factor(s) will provide useful information on the molecular mechanisms of B cell activation.

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Regulation of Tumor Growth

0010 CELLULAR INTERACTIONS IN TUMOR IMMUNITY AGAINST SYNGENEIC TUMOR IN THE MOUSE, Shigeyoshi Fujimoto, Department of Immunology, Kochi Medical School, Kochi, Japan

It has been demonstrated that suppressor T cells against a growing tumor are generated in thymus, spleen and other lymphoid tissues of tumor bearing hosts. The suppressor T cell induced in A/J mice bearing a transplantable methylcholanthrene-induced sarcoma, S1509a can specifically inhibit the rejection of the tumor secondarily implanted in syngeneic mice that have been previously immunized with unreplicable homologous tumor cells, upon transfer to the immune syngeneic mice. Moreover, the suppressor T cell (Ts) can also specifically inhibit the effect of cytotoxic T cell against S1509a in vitro, which is a major effector cell for the rejection of the homologous tumor in the immune mice. The cytotoxic T cell (Tc) against S1509a in A/J mice could be induced in vitro by restimulating syngeneic immune spleen cells to S1509a with mitomycin C-treated homologous tumor cells. The cytotoxic activity of effector cells was specific for S1509a and was inhibited by anti-H-2^a alloantisera. The effector cells were found to be Thy-1 and Lyt-2 positive cells. On the other hand, the suppressor T cell can be predominantly activated by a soluble tumor antigen(s)(STA) extracted from S1509a tumor cells in vivo although STA is incapable of activating Tc against the tumor cells. Ts has Thy-1 and I-J molecules on their cell surface. The killing activity of Tc is completely inhibited by the live cold target homologous tumor cells but not by STA, whereas Ts activity inhibited by STA in ^{51}Cr release assay. The results indicate that cytotoxic and suppressor T cells are activated under different conditions and that the antigenic determinants recognizable by these two cell types are not the same.

To elucidate more precise mechanism of cellular interactions on suppression of the effector function against the syngeneic tumor by Ts, killing mechanism by Tc has been investigated. It was found that the killing activity of Tc to S1509a disappeared when phagocytic cells were removed by carbonyl iron from Tc source and it was recovered by adding back either normal peritoneal exudate cells or T cell depleted normal spleen cells to Tc source. This evidence was further confirmed by using the cytotoxic T cell clone against S1509a established by cultivating with T cell growth factor (TCGF). The established cytotoxic T cell clone only showed specific killing to S1509a target cells in the presence of small number of accessory cells such as normal peritoneal exudate cells or T cell depleted normal spleen cells. This

result suggests that accessory cells are essentially required for the activation of Tc to show cytotoxic killing to the target cells even at effector phase.

The T cell activating ability of accessory cells obtained from tumor bearing hosts, however, are remarkably impaired. It will be discussed that Ts may act on accessory cells to inhibit their activating ability resulting in the inability to reject the tumor in tumor-bearing hosts.

0011 Regulation of the Immune Response to Cell Surface Antigens, Mark Irwin Greene, Department of Pathology, Harvard Medical School, Boston, Mass

The cellular and molecular basis of immune responses to cell surface antigens have been studied. In the immune response to the S1509a tumor syngeneic to A/J mice, Lyt 1+2- immune T cells respond to unique structures on the S1509a cell presented by I-A$^+$ Antigen Presenting Cells in vivo and in vitro. The regulation of Lyt 1+ T cells from eradicating syngeneic tumors is imposed by interacting subsets of suppressor T cells (Ts). One subset of Ts cells (Ts1) is first activated by tumor antigen, whereas the second set of Ts (Ts2) are stimulated by soluble suppressor molecules of MW 30-68K elaborated by the Ts1 subset. To further evaluate the role of interacting elements, we have extended these studies to the azobenzenearsonate (ABA) system in which it is now clear that there are at least three Ts subsets active in regulating the generation of in vivo and in vitro ABA specific immunity. ABA coupled cells induce Ts1 cells. Ts1 cells elaborate a suppressor molecule (TsF1) which in certain strains A/J($H\text{-}2^a Igh\text{-}1^e$) and and C.AL-20($H\text{-}2^d Igh\text{-}1^d$) can be shown to express products of genes linked to the Igh locus of the XIIth chromosome as well as gene products of the I-J subregion of the XVIIth chromosome. These molecules induce a second set of Ts cells (Ts2) which can be shown to express complementary receptors to TsF1. Ts2 cells trigger the third subset (Ts3) which ultimately suppress Lyt 1+ cells in vivo. Two T cell hybridomas, one representing a Ts1 clone and the other a Ts3 clone have been produced and the molecular characteristics and mode of action have been studied. Moreover we have also evaluated how defined monoclonal anti I-J and anti I-A antibodies can influence the immune response to cell surface antigens in vivo. Finally, to gain insight into the nature of putative tumor antigens we have analyzed the relationship between tumor antigens and transforming genes in a variety of tumor systems. The general relationship of H-2 and Igh-1 linked genes and genes relevant to tumor immunity will be discussed.

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0012 REGULATION OF MYELOMA CELLS BY IMMUNOREGULATORY T CELLS, Richard G. Lynch, Gary L. Milburn and Richard G. Hoover, Department of Pathology, University of Iowa College of Medicine, Iowa City, Iowa 52242

Hapten-specific plasmacytomas of BALB/c mice have been employed to visualize and analyze two distinct populations of immunoregulatory suppressor T cells:

1) One suppressor T cell is an idiotype-specific, $Ly_t\ 1^-2^+$ cell that mediates a rapid, total and reversible inhibition of immunoglobulin biosynthesis. The target cell of this suppressor T cell is the actual antibody-secreting cell.

2) The second suppressor T cell is a short-lived, $Ly_t\ 1^-2^+$ cell with a cell surface Fc receptor. The specificity of the Fc receptor expressed is dictated by the isotype of the immunoglobulin used to induce its expression.

Immunoregulatory interactions in myeloma appear to be exaggerated but otherwise normal activities. They provide a powerful model system for the analysis of immunoregulatory mechanisms. Supported by CA-23217, CA-29208 and CA-00897.

0013 REGULATION OF GROWTH AND IMMUNOGLOBULIN SYNTHESIS IN A MURINE B CELL LEUKEMIA (BCL_1). S. Strober, D. Lafrenz, S. Koretz.

The BCL_1 cell line is derived from a spontaneous B cell leukemia of BALB/c mice (1). The *in vivo* passaged line expresses IgM and IgD on the cell surface, and can be stimulated *in vitro* with lipopolysaccharide (LPS) to proliferate rapidly and secrete IgM (2). An *in vitro* tumor line which also secretes IgM after culture with LPS was established by Gronowicz *et al* (2). We cloned the latter line by limiting dilution techniques, and examined changes in surface, cytoplasmic and secreted IgM after exposure to LPS. Within 24 hours after incubation with LPS there was a considerable reduction in the quantity of surface IgM which reached a minimum at 72 hours. IgM plaque forming cells (PFC) appeared between 24-48 hours, and reached a maximum at 72 hours. PFC's were never more than 10% of the input tumor cells in the reverse hemolytic plaque assay. Experiments with synchronized cells showed no change in the doubling time after the addition of LPS, and no relationship between the kinetics of the development of PFC's and cell cycle. Inhibition of cell proliferation and DNA synthesis with hydroxyurea did not reduce the ability of the tumor cells to secrete IgM in the presence of LPS. Staining of the cytoplasm for IgM showed that large quantities of IgM were present before exposure to LPS, and that little change was observed after exposure. Although the quantity of surface IgM decreased and the quantity of secreted IgM increased after LPS stimulation, the relative levels of mRNA coding for membrane and secreted IgM did not change appreciably. The experimental results suggest that there is post-transcriptional control of the quantity of membrane IgM expressed by BCL_1 cells, and that the secretion of IgM by a small fraction of cells after stimulation with LPS is due to the release into the supernatant of pre-existing pools of cytoplasmic IgM

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0014 LYMPHOMA CELL SURFACE RECEPTORS: THEIR ROLE IN GROWTH AND METASTASIS, I. L. Weissman, M. McGrath, E. Butcher, M. Gallatin and G. Tamura, Department of Pathology, Stanford University, Stanford, CA. 94305

Lymphocytes are mobile cells which possess multiple classes of receptors necessary for their central roles in immune recognition and response. Both B and T lymphocytes possess surface receptors which determine their homing capacity by causing them to bind to postcapillary high endothelial venule (HEV) cells lining lymphoid organs; the bound lymphocytes subsequently migrate through these venules into the parenchyma of these lymphoid organs (e.g., peripheral lymph nodes, spleen, Peyer's patches). While most lymphocytes in all species studied possess genetically restricted receptors which allow them to home either to peripheral lymph nodes or to Peyer's patches, an organ preference does exist. Peyer's patch HEV regularly select ~70% B cells and ~30% T cells from the circulation, while peripheral lymph nodes select ~70% T cells and ~30% B cells. Neoplastic lymphoid clones may also possess receptors specific for Peyer's patch HEV or peripheral node HEV, and these receptors could be involved in organ-preferential metastases. We have raised monoclonal antibodies to some of these lymphomas, and selected for those antibodies which block peripheral lymph node organ-specific HEV recognition by the malignant clones. These monoclonal antibodies also detect those normal mature lymphocytes which possess lymphoid-homing capacity; and these antibodies selectively block lymphocyte binding to HEV in peripheral lymph nodes, but not to HEV in Peyer's patches.

We have also studied another class of cell-surface receptors on retrovirus-induced leukemias and lymphomas. We have proposed that both B and T lymphomas express antigen-specific receptors for retroviral envelope antigens, and that filling of such receptors imparts a mitogenic signal to these cells. According to this hypothesis, such cells may produce their own mitogenic retrovirus, or be in association with cells which produce their mitogenic retrovirus, and in consequence undergo uncontrolled proliferation (neoplasia). We shall present evidence that each T lymphoma indeed expresses retrovirus-specific receptors involved in growth and malignancy of these cells; that B lymphomas express surface immunoglobulin receptors specific for a retrovirus produced either by them or by other cells in their microenvironment; and that an idiotypic determinant on at least one of these B cell lymphoma immunoglobulins is expressed also on two T cell lymphomas at or near their retrovirus receptors.

Tumor Therapy

0015 THE KILLING OF LYMPHOMA CELLS BY UNIVALENT DERIVATIVES OF TUMOR-SPECIFIC ANTIBODY, G.T. Stevenson, M.J. Glennie and J. Gordon, Tenovus Research Laboratory, General Hospital, Southampton SO9 4XY, England.

The idiotypic determinants on cell surface immunoglobulin (Ig) of neoplastic B lymphocytes represent an immunotherapeutic target well characterized at the molecular level. We have used xenogeneic antibody directed against these determinants (anti-Id) to treat both guinea pig and human B lymphocytic leukemias (1,2). However antigenic modulation offers the cells an avenue of escape from complement and effector cells recruited by the antibody. Modulation of surface Ig is rapid, dependent on bivalency of the antibody, and related to redistribution (aggregation and endocytosis) of the antigen-antibody complexes (3). To avoid it we are investigating univalent antibody derivatives which retain their Fc zones and hence their abilities to recruit the adjunctive killing agents. (a) Fab/c derivatives of rabbit anti-Id are prepared by limited papain digestion of antibody IgG. (b) FabFc derivatives of sheep anti-Id are prepared by coupling Fab'γ (from peptic digestion of antibody IgG) with Fc (from papain digestion of normal IgG), via a disulfide bond derived from a hinge region half-cystine in each fragment.

Both fragments have been characterized in vitro against both human and guinea pig target cells. They are seen by indirect immunofluorescence to bind uniformly to the target cell surfaces, with no hint of redistribution after incubation of viable cells plus derivative at 37° for 30 min. They are able to mediate both complement and K-cell killing of the cells, with no evidence of escape via modulation when preincubation occurs at 37° for 30 min without the killing agent present. In an immunotherapeutic trial in guinea pig L_2C leukemia Fab/c has proved vastly more effective than its parent antibody.

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Tumor Viruses

0016 Genetic control of sensitivity to Moloney leukemia virus in mice : demonstration and role of specific T helper lymphocytes. Patrice DEBRE, Brigitte BOYER, Elizabeth GOMARD and Jean Paul LEVY - Hôpital COCHIN et Hôpital PITIE-SALPETRIERE PARIS FRANCE.

The N.B tropic Moloney virus (M-Mulv) is an exogenous virus highly leukemogenic for sensitive mouse strains where it is actively replicated M-Mulv is also the helper agent associated to the defective sarcoma virus which induces a regressing tumor in immunocompetent mice. In these two experimental models virus an virus induced-tumors stimulate both cellular and humoral responses against viral antigenic specificities. Two types of helper T cells could be demonstrated : a) helper T cell under Ir gene control cooperating with B cell to induce anti M-Mulv antibody response b) helper T cell cooperating with H-2 restricted cytolytic T lymphocytes in cell mediated anti-tumor response. Both helper function have similarities : helper T cells express lyt 1.2, thy 1.2 antigens at their surface, are specifically induced by whole virus and appeared radioresistant. However we were able to produce helper T cell lines in long terme cultures with inactivated virus or tumor cells which cooperate with B cell for antibody production but not T cell for cytolytic response;

0017 H-2 Linked Control of Xenotropic Murine Leukemia Virus (X-MuLV) R. A. Yetter, J. W. Hartley, J. H. Stimpfling, H. C. Morse III

Several copies of the genetic information for X-MuLV exist in the cellular DNA of all mice. However, most strains do not express this information as high levels of infectious virus and virus coded cell surface antigen (Xen CSA) F/st mice spontaneously produce high levels of both infectious X-MuLV and Xen CSA. Previous studies from this lab showed that, in crosses with C57/L, NFS, and AKR, the high virus phenotype was controlled by a single recessive gene. This gene assorted independently of the site of the X-MuLV inducibility locus and independently of loci on chromosomes 4, 5, 7, 9 and 14. In the current study, 53 mice in an (AKRxF)xF backcross showed absolute concordance between high X-MuLV production and the H-2 $^{P/P}$ haplotype. Further, B10.F and B10.P both show high levels of Xen CSA and infectious X-MuLV. Results obtained with recombinant H-2 congenic mice indicate that the locus controlling X-MuLV expression in F/ST mice may be in the C region of the H-2 complex.

0018 PHENOTYPIC HETEROGENEITY OF LYMPHOMAS OF INBRED MICE INFECTED WITH NON-TRANSFORMING ONCORNAVIRUS, Jan Cerny and Dale D. Isaak*, University of Texas Medical Branch, Galveston, Texas 77550 and *Kirksville College of Osteopathic Medicine, Kirksville, MO 63501

Mice infected with exogenous, non-transforming murine leukemia viruses (Moloney, MuLV-M or Friend, MulV-F) develop lymphomas/leukemias following a long (several months) latent period occupied by a vigorous virus replication in various target cells. Most of the tumors are considered to be monoclonal; however, we find that a given MuLV strain may induce different tumors in individual inbred mice (BALB/c). The analysis has been facilitated by comparing tumors of euthymic and nude mice. Infection of nude mice with MuLV-F leads to splenic lymphoblastic leukemia (either null cell or B cell), whereas euthymic animals develop either T cell lymphoma (2/3) or a splenic, non-T leukemia (1/3). The T cell neoplasia is distinguished by a unique, MuLV-associated surface antigen. MuLV-M induces T lymphomas in euthymic mice and an array of diseases in nudes (granulocytic leukemias, reticular neoplasias and lymphomas). There is a specific increase of endogenous, xenotropic virus in the presumed target tissue.

Taken together with our studies on lymphocyte permissiveness to MuLV infection, the above observations indicate that (1) MuLV may replicate in various lymphocytes and non-lymphoid cells; (2) there are non-permissive subsets of cells within each class of target cells that appear to represent a particular differentiation stage; (3) neoplastic transformation may occur in almost any permissive cell, but it is an extremely rare event; and (4) there may be unique, target cell-specific antigens generated during the MuLV-driven transformation.

0019 HUMAN T-CELL LYMPHOMA VIRUS (HTLV) IS PRESENT IN T- BUT NOT B-LYMPHOCYTES OF PATIENT OF ORIGIN, M.S. Reitz, M. Robert-Guroff, V.S. Kalyanaraman, F.W. Ruscetti, M.G. Sarngadharan, L. Ceccherini Nelli and R.C. Gallo, Laboratory of Tumor Cell Biology, National Cancer Institute, Bethesda, MD 20205

HTLV, a retrovirus which is not related to any previously described animal retroviruses, has been isolated from T-cell lines from two patients with T-cell malignancies and identified in several other similar cell lines from patients with various types of leukemia and lymphoma involving mature T-cells. We have analyzed three T-cell lines and a B-cell line established from one of the patients of origin for HTLV. Viral protein and RNA, were detectable in all three of the T-cell lines. Proviral DNA was analyzed in one of the T-cell lines and was present at 3-4 copies per haploid genome. In contrast, viral protein and RNA were not detectable in the B-cell line, and proviral DNA was either absent or present at substantially less than one copy per haploid genome. The data indicate that the patient acquired HTLV by infection after fertilization. HTLV has so far been infectious for T-cell lines from some relatives of HTLV-positive patients but not B-cells, suggesting B-cells are not permissive for viral growth, and has not been infectious for normal T-cells obtained from random normal donors.

0020 GREAT SIMILARITIES BETWEEN EPSTEIN-BARR VIRUS ISOLATES FROM DIFFERENT DISEASES AND DIFFERENT GEOGRAPHICAL AREAS, G.W.Bornkamm, G.Bühler, and U.Zimber, Institut für Virologie, 7800 Freiburg, West Germany

Epstein-Barr virus (EBV), the etiological agent of infectious mononucleosis (IM), is associated with two human malignancies, Burkitt's lymphoma (BL) and nasopharyngeal carcinoma (NPC). By blot analysis of the EBV genome of 30 different cell lines we have asked the question, whether different virus strains may be accounted for the different manifestation of the infection. 11 cell lines were derived from BL, including 6 african, 3 european, and 2 new guinean cases, 6 lines from NPCs, and 3 from IM. 10 cell lines were established from the peripheral blood of patients with other diseases. The results are summarized as follows: (i) most isolates exhibit only minor differences in their HindIII fragments; variabilities in (ii) HindIII-A and (iii) HindIII-D2 are due to variable numbers of large and small internal repeats, respectively; (iv) the sequences deleted in B95-8(EBV) are present in all other isolates including 883L(EBV), from which B95-8 was derived, (v) the non-transforming P3HR-1 strain has deleted 6.5 kb including half of the rightmost large internal repeat, (vi) BL lines have a HindIII I1 fragment of 3.0 kb, whereas the size of this fragment in other isolates varies between 3.0 and 3.5 kb. The small differences observed argue against the hypothesis, that disease-specific EBV subtypes exist.

0021 SECRETION OF HUMAN IMMUNOGLOBULINS BY MOUSE MYELOMA X DAUDI SOMATIC CELL HYBRIDS, Jan Erikson and Carlo M. Croce, The Wistar Institute of Anatomy and Biology, Philadelphia, Pa. 19104.

Somatic cell hybrids between non-producer mouse myeloma cells and Burkitt lymphoma-derived Daudi cells, that do not secrete human immunoglobulin chains, secrete human IgM. The P33 protein is also secreted by the hybrids as part of the IgM molecule. Since P33 and κ chains cosegregate in all primary hybrid clones and subclones, we conclude that they are coded for by the same human chromosome 2. The fact that both P33 and κ^1 chains are immunoprecipitated by specific anti-human κ chain antisera, and that both P33 and κ^1 bands disappear and a single band immunoprecipitable by an anti-human κ chain serum appears in tunicamycin-treated hybrid cells, suggest that they are different glycosylated forms of the same polypeptide.

0022 CHARACTERIZATION OF C-TYPE VIRUSES ISOLATED FROM VIRUS-INDUCED LYMPHOMAS IN H-2 CONGENIC C57BL MICE, C.J.M. Melief, M. Zijlstra, H. Schoenmakers, R.E.Y. de Goede, A.H. Schinkel and W.H. Hesselink, Central Lab. Blood Transf. Service, Amsterdam, The Netherlands

In previous studies we have shown an important role of the H-2 complex in a) the humoral response against MuLV, b) resistance against virus-induced leukemogenesis and c) type of lymphomas induced after milk-transmission of a naturally occurring B-ecotropic 810.A virus (Melief et al., J. Nat. Cancer Inst. 64, 1179-1189, 1980). We now report the occurrence of different host-range classes of MuLV in the different types of virus-induced lymphomas. From almost all lymphomas a B-ecotropic XC^+ virus could be cloned. From one tumor an XC^- ecotropic virus was cloned. XC^- dual-tropic viruses were isolated from 11 out of 17 virus-induced lymphomas of different T and B cell phenotypes. Characterization of the virus isolates with monoclonal anti-MuLV antibodies (O'Donnell and Nowinski, Virol. 107: 81-88, 1980 and Portis et al. in press) and tryptic peptide mapping of the gp70 molecule indicate that especially the dual-tropic MCF viruses constitute a heterogeneous group. Some dual-tropic MCF viruses expressed epitopes highly characteristic of the AKR MCF 247 isolate and related viruses. Some eco- and dual-tropic isolates were oncogenic after injection in newborn C57BL and BALB/c mice: they induced generalized lymphomas of various histologic types and cell surface phenotypes after a latent period of 7-12 months. Implications of these findings for the mechanism of viral oncogenesis in this model will be discussed.

Cytogenetics

0023 IMPORTANCE OF THE SPECIFIC TRANSLOCATIONS OBSERVED IN BURKITT TYPE LYMPHOMA, Gilbert M. Lenoir, International Agency for Research on Cancer, 69372 Lyon cedex 2.

Cytogenetic investigations have indicated that Burkitt type lymphoma (BL), a B cell type lymphoma, is characterized by non random chromosomal changes (1). In most of the cases, a translocation between chromosomes 8 and 14 is observed (2) but variant translocations, such as t(2;8) and t(8;22), have also been reported (3). Our ongoing studies performed on BL cases from both endemic and non endemic areas indicate that these cytogenetic changes can be considered as a characteristic feature of this cancer and, as a consequence, may have a diagnostic value. We found that these translocations are independent (i) of Epstein-Barr virus association, (ii) of the geographic origin of the patient, (iii) of the clinical presentation of the disease since they are also detected in Burkitt type leukemias.

Human immunoglobulin (Ig) genes have been recently assigned to the three chromosomes implicated with chromosome 8 in BL translocations: heavy chains to 14, lambda and kappa light chains to 22 and 2 respectively. Our studies on Ig expression in BL cells suggest that the precise localisation of those genes may correspond to the breakpoints observed in BL, i.e., 14q32, 22q11 and 2p12, and that DNA sequences involved in the malignant transformation of these cells may be located on the long arm of chromosome 8 (on 8q24).

(1) Manolov, G. and Manolova, Y. Nature, 237, 33-34 (1972).
(2) Zech, L., Haglund, V., Nilsson, K. and Klein, G. Int. J. Cancer, 17, 47-56 (1976).
(3) Bernheim, A., Berger, R. and Lenoir, G. Cancer Genet. Cytogenet., 3, 307-316 (1981).

Myeloma

0024 IN VITRO PRODUCTION OF MONOCLONAL RHEUMATOID FACTOR (R.F.) BY EPSTEIN-BARR VIRUS (EBV) IMMORTALIZED CELL LINE, Michael Steinitz, Hematology, Hadassah University Hospital il 91 120 Jerusalem, Israel.

Immortalization of human B lymphocytes with Epstein-Barr virus is used to establish antibody producing cell lines (Steinitz, M. et al., Nature, 269, 420, 1977). The initial lymphocytes are collected from immune donors and it is essential to perform a selection of the specific antibody producing cells prior to the viral infection. Lines producing antibodies against various antigens were established by this approach (Steinitz, M. and Klein, E., Immunology Today, 2, 38, 1981). Lymphocytes from patients with autoimmune diseases are suitable for initiation of lines producing monoclonal autoimmune antibodies. Selected lymphocytes from a patient with Rheumatoid arthritis were EBV infected and subsequently twice reselected (=enriched for rheumatoid factor producing cells) and cloned. The resulting line was characterized. It continuously secretes a monoclonal R.F. (19S,μ,λ) (Steinitz, M. et al., Nature, 287, 443, 1980) now for more than two years. This autoimmune antibody, which specifically binds to IgG-immune complexes was purified and characterized. It is now applied as a universal reagent to detect immune complexes. Because of the high efficiency of EBV, even very small populations of selected B cells can be immortalized into antibody producing cell lines. B cells from infiltrated organs and cells producing anti allergens are suitable targets.

0025 THE "MONOCLONAL" LYMPHOCYTES ACCOMPANYING MURINE AND HUMAN MYELOMA ARE FC RECEPTOR-BEARING T CELLS WITH ADSORBED MYELOMA PROTEIN, Richard G. Hoover & Richard G. Lynch Univ. of Iowa College of Medicine, Dept. of Pathology, Iowa City, Iowa 52242.

Myeloma in mouse and man is accompanied by the development of large numbers of circulating T lymphocytes with Fc receptors (FcR) specific for the isotype of the myeloma protein. Thus Tα cells are increased in IgA myeloma, Tμ cells in IgM myeloma, Tγ cells in IgG myeloma and Tε cells in IgE myeloma. Adsorption of myeloma protein to these FcR^+ T cells accounts for the so-called "monoclonal" lymphocytes found in myeloma. FcR^+ T lymphocytes are induced directly by the myeloma protein in a process that requires DNA and protein synthesis and is independent of the presence of a tumor per se. These findings demonstrate that immunoglobulin can directly induce an expansion in a subset of immunoregulatory T cells and provides a mechanism for the expression of idiotypes on T cells. (Supported by N.I.H. CA 06831 and CA 32277).

0026 HYBRID RESISTANCE TO MURINE PLASMACYTOMAS, Mary Clare Walker and Julia M. Phillips-Quagliata, New York University School of Medicine, New York, NY 10016

Patterns of genetic control of hybrid resistance to the BALB/c plasmacytomas MPC-11 and LPC-1 have been compared. Hybrids between BALB/c and A, A.BY, BALB.B, BALB.G, BALB.K, DBA/2 and SJL are as susceptible as BALB/c mice to both tumors. Hybrids between BALB/c and AKR, DBA/1, C57BL/6, C57BL/Ks, C57BL/10 (B10) and nine B10 congenic resistant strains are resistant to moderate doses of both tumors. Heterozygosity within the H-2 complex is neither necessary nor sufficient for resistance to either tumor. Analysis of the resistance to each tumor by (BALB/c x B10)F_1 x BALB/c backcross mice indicates that a single, dominant, autosomal gene, not linked to H-2, controls resistance to MPC-11 whereas two dominant autosomal genes control resistance to LPC-1. One of these genes is sex-limited and only expressed in males; neither is linked to H-2. Hybrids between BALB/c and DBA/1 and B10.D2 which survive exposure to a moderate dose of viable LPC-1 cells are protected on challenge with a high dose of viable MPC-11 cells, indicating that the two plasmacytomas share a transplantation antigen. Both tumors express G_{IX} determinants. However, studies of hybrids between BALB/c and G_{IX}+ and G_{IX}- congenic strain pairs indicate that the expression of G_{IX} or another trait controlled by the Gv-1 gene significantly reduces resistance to MPC-11 but not to LPC-1. Cytotoxic T lymphocytes are generated to MPC-11 in vitro, but there is incomplete correlation with in vivo susceptibility. Supported by ACS grant IM-213B.

0027 PRODUCTION OF MONOCLONAL (m) AND POLYCLONAL (p) IMMUNOGLOBULINS (Ig) BY PERIPHERAL BLOOD MONONUCLEAR CELLS (PBM) IN HUMAN MULTIPLE MYELOMA (MM), Göran Holm, Håkan Mellstedt, Dietrich Peest and Dagny Pettersson, Department of Clinical Immunology, Huddinge Hospital, S-141 86 HUDDINGE, Sweden.

PBM were cultivated in micro wells using Eagle MEM medium with 10 % FBS for 7 days. Using an enzyme linked immunosorbent assay with anti-class and anti-idiotypic (id) antisera it was possible to quantitate separately mIg and pIg of the same class in cell supernatants and in cells. Freshly isolated myeloma PBM contained large amounts of mIg while pIg was low. An early phase of rapid release of mIg was insensitive to inhibition by pyromycin. A later phase of slow release was partly suppressed. It is suggested that the latter required active protein synthesis. mIg production was not enhanced by PWM. In contrast, the cellular pIg was low at start of culture and afer 7 days without PWM. After activation by PWM patients' PBM produced lower amounts of pIg than cells from healthy adults. The production of pIg normalized during remission when no mIg was found. The results support with the notion that the majority of MM monoclonal blood B-cells are late in the differentiation towards plasma cells.

0028 ELUTRIATION AND FLOW CYTOMETRY OF MOPC-315 PLASMACYTOMA, Fred Valeriote, Dean Coulter and Alex Nakeff, Section of Cancer Biology, Department of Radiology, Washington Univesity School of Medicine, St. Louis, MO 63110

In order to study many of the important biological characteristics of tumor cells relating to differentiation and growth control, it is necessary first to obtain viable sub-populations. Two techniques by which this can be done are elutriation and flow cytometry. we have used both techniques to study MOPC-315 plasmacytoma cells. We have separated MOPC-315 cells from normal spleen cells by elutriated and analyzed the latter by flow cytometry. It was necessary to optimize the flow cytometric analysis of the myeloma cells with the supravital DNA Stain Hoechst 33342 since it was differentially cytotoxic for the MOPC-315 cells as compared to normal marrow hematopoietic stem cells. Quantitation of the fraction of SG_2M phase cells labelled by different concentrations of Hoechst 33342 as a function of staining time revealed that a 30 min exposure at 37°C with 5 M dye was optimal to maintain MOPC-315 clonogenicity. By optimizing and combining elutriation with flow cytometry, we have defined 4 fractions of the myeloma population in terms of cell size, DNA content, morphology and cell clonogenicity.

0029 HUMAN MYELOMA CELL LINE SECRETING TWO FORMS OF δ HEAVY CHAINS. Carol J. Thiele and J. Frederic Mushinski, Lab. of Cell Biology, NCI, NIH, Bethesda, MD. 20205

ODA is a human IgD secreting cell line adapted from the subcutaneous tumor of a 61 yr. male with an IgD myeloma.(N.Ishihara et al. Proc. Japan. Cancer Assoc.(1977) 423) We have analyzed the ^{35}S- Methionine labelled ODA cell culture supernatant proteins by immunoprecipitation. An IgD (δ,λ) protein was precipitated from the culture supernatants and it contained a δ heavy chain that appeared as a broad 70-74k dalton band after electrophoreisis on 15% SDS-PAG. Further analysis on 2D-NEPHGE gels resolved 2 immunoprecipitated δ heavy chains: an approx. 68k dalton protein with a pI 4.5-5.5 and a larger protein (approx. 71k daltons) with a pI 5.5-6.5. The more basic nature of the 71k band argued against it being a more heavily glycosylated form of the 68k protein and suggested that 2 forms of δ heavy chains were secreted by ODA cells. In order to determine the size of the unglycosylated proteins, we have studied the secreted products of tunicamycin treated cells. From these immunoprecipitates we have only been able to visualize one 58k dalton band and a remnant of the original band (which may be due to incomplete inhibition of N-glycosylation) on 15% SDS-PAG. The inablility to detect more than one unglycosylated protein band may be due to the limitations of the assay system and the decreased secretion of proteins by tunicamycin treated cells. Currently we are studying the tunicamycin treated δ heavy chains by 2D gel electrophoreisis and the cell free translation products of mRNA isolated from this tumor line.

C.J. Thiele is a postdoctoral fellow of The Cancer Research Institute, Inc. New York, N.Y.

0030 PLASMA CELL ASSOCIATED MONOCLONAL ANTIBODY. Kenneth C. Anderson, Russell Hardy, and Lee M. Nadler. Sidney Farber Cancer Institute, Harvard Medical School, Boston, Mass. 02215

Two plasma cell associated monoclonal antibodies(PC-1 and PC-2) were produced by somatic cell hybridization against tumor cells from a patient with IgGκ plasma cell leukemia. By indirect immunoflourescence, they were reactive with plasma cells from patients with plasma cell leukemia(3) and multiple myeloma(3). In contrast, they were unreactive with B lymphocytes from peripheral blood(3), tonsil(3), lymph node (2), spleen(3), and normal bone marrow(2). Moreover, they were unreactive with T lymphocytes from peripheral blood(3) or tonsils(4) and peripheral blood monocytes(6). Weak cross reactivity has been defined in granulocytes(10) and myeloid leukemia(2). B(SB, Laz 388, and Laz 168), T(CEM and HSB), null cell leukemia(Laz 221), CML in blast crisis(Nalm-1), Burkitt's(Daudi, Ramos, and Raji), and promyelocytic leukemia (HL-60) cell lines were unreactive. CLL(4), ALL(3), AML(5), null cell leukemia(3), DHL(5), and NPDL(9) blood cells were unreactive. Additional studies demonstrated that these antibodies were distinct from T10, conventional immunoglobulin, complement receptor, Fc receptor, and Ia-like antigens. This selective reactivity with plasma cells suggests potential utility in the diagnosis and therapy of plasma cell dyscrasias.

0031 PURIFICATION OF A SOLUBLE IMMUNOREGULATORY SUBSTANCE RELEASED BY PLASMACYTOMA CELLS. S.E. Ullrich & S. Zolla-Pazner, New York VA Med. Ctr. and NYU Med. Ctr., NY,NY 10016

Mice bearing plasmacytomas are severely depressed in their ability to produce antibody in response to primary antigenic stimulation. Previous studies have demonstrated that a cascade of events involving both host macrophages and tumor-derived immunoregulatory factors is responsible for the observed suppression. In this phenomenon, the malignant plasma cells release a soluble immunoregulatory substance (PC-factor) which interacts with normal macrophages, causing the release of another factor which suppresses antigen-stimulated antibody production by B lymphocytes. Experiments were performed to purify and partially characterize the PC-factor. The source of PC-factor for these experiments was the spent culture fluid from an *in vitro* subclone of the MPC-11 plasmacytoma, designated 456,31. The purification procedure consisted of ammonium sulfate fractionation followed by Sephacryl 200 gel filtration chromatography, DEAE-cellulose ion exchange chromatography and Protein A-Sepharose affinity chromatography. Upon further purification using high pressure liquid chromatography, the biologically active material eluted as a homogeneous peak. The biologic activity of PC-factor is retained after heating at 56° for 30 min. and after repeated cycles of freezing and thawing but is destroyed by treatment with protease. Thus, malignant plasma cells mediate immunosuppression through the release of a non-immunoglobulin protein which migrates with a molecular weight of approximately 55,000 daltons. Further analysis of the biochemical characteristics of PC-factor should enhance our understanding of the modulation of host immune responsiveness by tumors. (Supported in part by NIH grants CA 15585, CA 16247 and AM 01431.

Stem Cell and Hematopoietic Tumors

0032 REGULATION OF H-2 ANTIGENS ON TERATOCARCINOMA CELLS GROWING IN RESISTANT MICE, S. Ostrand-Rosenberg, Univ. of Maryland Balt. Co., Balt., MD 21228. Previous studies have demonstrated that resistance to the 402AX testicular teratocarcinoma is under genetic control in the mouse (Ostrand-Rosenberg et al. 1980. Immunogenet.10:607). When passaged in genetically susceptible hosts, the nullipotent 402AX cells do not express H-2 antigens. However, when passaged in genetically resistant mice, the tumor cells become positive for H-2 antigens in the absence of other indications of differentiation (Ostrand-Rosenberg and Cohan 1981. J. Immunol.126: 2190). These studies suggested that H-2 antigen expression on teratocarcinoma cells is mandatory for an effective host cell-mediated immune response against this tumor. The present studies determine which host cell population are regulating H-2 antigens on the tumor cells in vivo. Reconstitution studies demonstrate that resistant bone marrow alone is insufficient to confer complete resistance to the tumor and does not transfer the ability to induce H-2 antigen expression on tumor cells. Two lines of evidence suggest that H-2 antigen expression is mediated by host lymphoid cells: 1) sublethal irradiation of genetically resistant hosts inhibits H-2 antigen expression on in vivo passaged teratocarcinoma cells and 2) immunological priming can overcome the loss of H-2 induction that is associated with aging in genetically resistant hosts. Genetically susceptible mice can be reconstituted for tumor rejection and H-2 antigen expression on teratocarcinoma cells by reconstitution with 1) genetically resistant bone marrow, plus 2) lymphoid cells from tumor primed resistant hosts. These results: 1) imply the necessity for H-2 antigens on tumor cells for effective cell-mediated immunity to this tumor, and 2) indicate that host lymphoid cells regulate H-2 antigen expression on in vivo passaged tumor cells. (Supported by USPHS CA24910).

0033 POSSIBLE HISTOGENETIC BASIS FOR THE DIFFERENTIAL RESPONSE OF LYMPHOMAS TO ALKYLATING AGENTS (AA). J.E. Byfield and P. Calabro-Jones, Division of Radiation Oncology, University of California San Diego 92103

Lymphomas (L) differ in their sensitivity to AA. Greatest responsiveness is usually seen in B cell, high-proliferative fraction types. We have shown[1] that the uptake and toxicity of AA is dependent on their water solubility. Water-soluble AA are analogues of normal compounds and their uptake is mediated by natural membrane carriers while lipid-soluble agents freely penetrate all cells and their toxicity is much less dependent of proliferation per se. As part of these studies we noted that PHA-stimulable circulating T cells were insensitive to active Cyclophosphamide (C, phosphoramide mustard) and to chlorozotocin. These results indicate that some mature T-cells have membrane properties similar to toti-potent marrow stem cells which have also been shown (by others) to be very resistant to C. The resistance is not seen with either normal or malignant B cells. The overall perspective one obtains from these studies is that (for unknown reasons) marrow stem cells can exclude certain AA and that this property is carried on in cellular differentiation into (some) T-cells. There appears therefore to be a histogenetic basis for differential sensitivity seen in L to AA that varies depending on lymphocyte ontogeny. This in turn suggests that it may be possible to develop chemotherapeutic regimens that exploit membrane-related ontogenetic differences.

1. Byfield, J.E. & Calabro-Jones, P. Nature (in press).

0034 IN VIVO TREATMENT OF ADULT MICE WITH A MONOCLONAL ANTIBODY SIMULATES B CELL ONTOGENY. (P.M. Rosoff & V.L. Sato, Biological Laboratories, Harvard University, Cambridge, MA.
We attempted to create an experimental system to study adult B cell development by in vivo elimination of immature B cells using a monoclonal antibody, 5H1, which has activity against pre-B cells but not the CFU-S. Balb/c mice were given once daily intraperitoneal injections of 5H1 for 7 days. They were sacrificed at 48 hour intervals starting 24 hours after the last injection and continuing to 264 hours (11 days). Bone marrow cells were isolated and labelled with ^{35}S-methionine either immediately upon removal or after 24 hours in culture. Labelled cells were lysed, pre-cleared, and immunoprecipitated with heterosera against murine whole Ig and μ and κ chains. Immunoprecipitates were analyzed by SDS-PAGE. 24 hours after the last injection with 5H1 (=day #1), we observed the complete elimination of cells synthesizing Ig components from bone marrow. By day #2, synthesis of cytoplasmic μ without associated light chain could be detected. By 72 hours light chain synthesis was noted (always in association with μ), and by day #5 μ and γ heavy chains and κ light chain could be seen in the regenerating bone marrow population. Controls treated with PBS or normal rat Ig showed little change in Ig production. Control BM has 40% 5H1+ cells: day #1 BM from 5H1-treated animals showed only 24% 5H1+ cells. By 72 hours, this figure had increased to 64% coincident with the resumption of Ig synthesis. The 5H1+ population at day #1 was shown to be responsible for the regenerating Ig+ cells. This pattern closely parallels that seen in fetal liver B cell ontogeny. This technique may represent a very useful tool for analysing B cell development in both fetal and adult life. It may also serve as a means of approaching the cell that serves as the antecedant of the pre-B cell.

0035 EXPRESSION OF MULTIPLE LIGHT AND HEAVY CHAINS mRNA'S IN CLONED MURINE B-LYMPHOMAS, Reuven Laskov, Rivka Ishay-Michaeli, David Givol, Jin Kim and Michael Wallach, Hebrew University-Hadassah Medical School, Jerusalem, Israel.
Expression of Ig genes in 5 B-lymphomas was studied at the polypeptides and mRNA's levels. The presence of light and heavy mRNA's in these cells was determined by Northern blot analyses of the total poly(A)mRNA, using appropriate P^{32}-labeled recombinant plasmid probes. Two lymphomas expressing λ chains contained in addition to the λ-mRNA, a normal size k-mRNA. On the other hand, two k-producing lymphomas did not contain λ-mRNA. One of the lymphomas expressed both k and λ chains and the corresponding mRNA's. It thus appears that the expression of light chains is orderly programmed so that k gene expression is followed by activation of the λ gene. Similar analysis done on the μ- and γ-mRNA revealed that 3 lymphomas which expressed membrane μ chains also contained γ-mRNA. One lymphoma expressing γ chains also produced μ-mRNA. Only one lymphoma which expressed membrane μ chains did not contain γ-mRNA. Three myelomas did not contain more than one heavy chain mRNA. Biosynthetic studies showed that 2 tumors which expressed membrane μ and contained μ- and γ-mRNA, also synthesized γ chains. Two additional tumors containing μ- and γ-mRNA expressed either γ or μ chains only. These results indicate that production of heavy chain mRNA is not always accompanied by synthesis of the intact chain. It will be of interest to find out whether μ- and γ-mRNA are transcribed from a single chromosome or from the two allelic chromosomes.

0036 ABELSON VIRUS TRANSFORMED CELLS AS A MODEL SYSTEM FOR THE STUDY OF B-LYMPHOID CELL DIFFERENTIATION, Frederick W. Alt, Susanna Lewis, Naomi Rosenberg, and David Baltimore, Massachusetts Institute of Technology, Cambridge, Mass. 02139. Infection of murine bone marrow or fetal liver with Abelson Murine Leukemia Virus (A-MuLV)transforms a small fraction of the cells into clonal, continuous cell lines. Various properties of these lines have indicated that they were generated from cells of the B-lymphoid lineage, the majority being related to the most immature cell known in this pathway- the pre-B cell. Surveys of the structure and expression of immunoglobulin genes in large numbers of such lines have proven extremely useful for defining the pre-B differentiation stage at a molecular level. In addition, these studies have also defined several sub-classes of A-MuLV transformants which exhibit a number of unique and useful immunodifferentiative properties including heavy and light chain gene rearrangement and heavy chain class switching during growth in culture. Studies of lines which undergo the recombination processes associated with the formation of complete light or heavy chain variable regions (e.g. V-J joining) during growth in culture have provided new insight into the mechanics and regulation of these processes. Analyses of lines which switch from λ to κ during growth in culture have suggested that the μ to γ class switch can occur, at least in part, by an RNA processing mechanism. Finally, analysis of variants of certain A-MuLV transformants which lose constitutive chain synthesis while acquiring LPS inducible synthesis of that chain offers the possibility of studying the regulation of heavy chain gene transcription. This variation is associated with the expressed allele and correlated with deletions in the J_H-C_μ intron.

0037 DIFFERENTIATION-LINKED EXPRESSION OF SURFACE ANTIGENS BY THE MULTIPOTENTIAL K-562 CELLS, Albert T. Ichiki, Bismarck B. Lozzio, Carmen B. Lozzio, Elena G. Bamberger. University of Tennessee Center for the Health Sciences/Knoxville, Knoxville, TN 37920. K-562 cells differentiate along the erythrocytic, granulocytic, and monocytic series, and as suggested from recent studies, may also differentiate along the megakaryocytic series. Hence, K-562 cells are considered to be multipotential leukemic cells. These cells express both specific leukemia associated antigens (LAA) and Fc receptors. We initiated a study employing monoclonal antibody preparations to determine the presence of other surface membrane antigens on the K-562 cells. When evaluated with the Cytofluorograf for the binding of fluorescein isothiocyanate-conjugated $F(ab')_2$ fragment of goat anti-mouse IgG, K-562 cells from 6 to 7 day-old cultures did not bind anti-HLA-A,B,C, anti-HLA-DR, OKT 10 [reactive with thymocytes, bone marrow (BM), terminal transferase positive cells, BM precursor B cells, and myeloblasts], OKT 11 (reactive with large thymic blasts), or anti-human monocyte antibody. On the other hand, over 90% of the cells bound the five monoclonal anti-K-562 antibody preparations (gift from Dr. N. Young, NIH) of differing specificities. The 24-hour incubation of K-562 cells with thymosin fraction 5 led to changes in the expression of some surface antigens, but no changes were seen at 48 hours. The number of HLA-A,B,C expressing cells was significantly increased while the number of OKT 10 binding cells was only noticeably increased. Moreover, the thymosin-treated cells expressed a higher density of LAA. The fact that the incubation of the K-562 cells with thymosin fraction 5 resulted in the expression of HLA-A,B,C suggests that these multipotential leukemic cells could be induced to differentiate along the lymphocytic series.

0038 T-CELL SUBSETS IN MIXED HEMOPOIETIC COLONIES AND COLONIES EXPANDED IN LIQUID SUSPENSION CULTURE, Bing Lim and Hans A. Messner, Ontario Cancer Institute, Institute of Medical Sciences.

E-rosette forming OKT-3 positive cells with lymphoid morphology can routinely be observed in some mixed colonies grown from human pluripotent progenitors in culture. The frequency of these T-cells is enhanced in the presence of medium conditioned by PHA stimulated T-cells (PHA-TCM). The T-cell component of these mixed colonies can be significantly expanded when transferred into liquid suspension cultures stimulated by PHA-TCM.

We have analyzed subsets of T-cells within primary mixed colonies and within liquid suspension cultures derived from mixed colonies using monoclonal antibodies (OKT-3, 4 and 8). If E-rosette depleted bone marrow cells are plated for mixed colonies, one can usually observe OKT-4 positive cells and only occasionally colonies may contain a sub-component of OKT-8 positive cells. The same finding is observed after expansion in liquid suspension culture. The presence of autologous T-cells in the primary cultures promotes growth of OKT-8 positive cells in primary mixed colonies and in cells subsequently grown in liquid suspension.

In conclusion, OKT-4 positive cells can be observed in mixed colonies derived from E-rosette negative bone marrow cell suspensions in the presence of PHA-TCM. The development of OKT-8 positive lymphocytes in the system appears to be dependent upon the addition of other T-cell subsets, thus indicating a T-T interaction.

0039 EFFECT OF BLOCKING DNA SYNTHESIS ON EXPRESSION OF T-CELL SURFACE ANTIGENS BY BONE MARROW CELLS AND T-CELL TUMOR LINES IN VITRO. Joan Abbott, Huguette Duteau, Theresa Lee and Mary Kirch, Sloan-Kettering Institute, New York, NY 10021.
T-cell surface antigens (Thy1.2, Lyt1.1, Lyt2.2) are not detected on fresh bone marrow cells from 5-8 day mice as assayed by antibody-mediated cytotoxicity. Cells were grown in culture, induced at 24 hour intervals for 3 hours with LPS and assayed for antigen. Thy1.2 and Lyt1.1 can be induced only after 48 hours and Lyt2.2 not until 72-96 hours. If DNA synthesis is blocked from 0-24 hours by hydroxyurea, Thy1.2 and Lyt1.1 can be prematurely induced by LPS but Lyt2.2 is not. Clonal cell lines of the murine T-cell tumor EL4 are variable with respect to Lyt2.2 expression. Blocking DNA synthesis with cytosine arabinoside for 24 hours induces a 4 fold increase in Lyt2.2 positive cells in one low expressing cell line whereas it has no effect on an Lyt2.2 negative cell line. Both fluorescence and cytotoxicity assays were used to detect presence of antigen. These studies imply that slowing of the cell cycle may play a role in regulating the expression of some T-cell surface antigens.

0040 CONSTITUTIVE PRODUCTION OF A UNIQUE LYMPHOKINE (IL-3) BY THE THY 1.2 POSITIVE WEHI-3 CELL LINE, John C. Lee, Andrew J. Hapel, and James N. Ihle, Biological Carcinogenesis Program, NCI-Frederick Cancer Research Facility, Frederick, MD 21701
It has been recently demonstrated that conditioned media from Con A-stimulated splenocyte cultures contain a novel lymphokine termed IL-3. IL-3 is characterized by its ability to induce 20 alpha hydroxysteroid dehydrogenase (20αSDH) in spleen cells from young nu/nu mice. 20αSDH is a specific enzyme marker of early mature T lymphocytes. IL-3 has also been shown to be biochemically and functionally distinct from all presently known lymphokines, including IL-1, IL-2 and immune interferon. In search of a convenient source for biochemical and in vivo studies of this lymphokine, a number of established murine cell lines were screened for the constitutive and induced production of IL-3. It was found that WEHI-3 cells, originally designated as a myelomonocyte cell line, produced high levels of IL-3 constitutively. Added mitogen and/or phorbol myristic acetate did not enhance the production of IL-3. Production of IL-3 varied among various sublines of WEHI-3. IL-3 purified from WEHI-3-conditioned media has biochemical and biological characteristics identical to those obtained from Con A-conditioned media. WEHI-3 conditioned media, however, generally contained 100-fold higher levels of IL-3 than conditioned media from Con A-stimulated splenic lymphocytes. Furthermore, we demonstrate that the properties of Thy 1.2 expression and constitutive IL-3 production clearly distinguish WEHI-3 from other myelomonocytic or macrophage-like cell lines and propose that the WEHI-3 cell line may in fact be of the lymphocytic or mixed lineage. (Research supported by the National Cancer Institute under contract no. NO1-CO-75380 with Litton Bionetics, Inc.)

0041 MONOCLONAL ANTIBODIES TO HUMAN MYELOID DIFFERENTIATION ANTIGENS, P. Mannoni, A. Janowska-Wieczorek, R. Turner, S. Shiuji, A. de Boer, J.-M. Turc. Departments of Pathology and Medicine, University of Alberta and Blood Transfusion Service, Edmonton, Alberta, Canada.
A series of monoclonal antibodies (MCA) to myeloid antigens was produced by fusion of 315-43 mouse myeloma cells with splenocytes from BALB-c mice immunized with human normal granulocytes, myeloid leukemia cells or myeloid cell lines. MCA reactivity was tested by ELISA, IMMUNOFLUORESCENT and CYTOTOXIC assays as well as by fluorescent activated cell sorting analysis. Tested cells were either normal cell subsets isolated from the bone marrow and blood of normal donors, or leukemic cells from patients with AML, AMML, CML or ALL, as well as leukemic and tumor cell lines (K.562, HL.60, KG.1, lymphoid and carcinoma cell lines). Three distinct groups of MCA were identified: Group A recognizes antigens expressed only on the mature forms of myeloid lineage. Group B recognizes antigens expressed on both the progenitor and mature myeloid forms. Group C recognizes normal as well as leukemic cells from different lineages. The expression of antigens identified by the three MCA-groups varies with the different types of acute myeloid leukemia cells and myeloid cell lines. Complement dependent MCAs of the Group B had the ability to specifically inhibit the CFU-GM growth with feeder layers or in conditioned media, while they did not inhibit BFU-E or CFU-E growth. These findings suggest that a new approach to the classification of myeloid leukemias is possible in relation to different stages of cell differentiation.

0042 ABNORMAL DIFFERENTIATION OF MALIGNANT B-CELLS IN NON-HODGKIN'S LYMPHOMA
Carlos A. Izaguirre, John Habeshaw, ICRF Department of Medical Oncology, St. Bartholomew's Hospital, London EC1A 7BE, U.K.

Blast cells from patients with B cell malignancies and common ALL form colonies in culture (Brit. J. Cancer 42:430,1980; Blood 57:823,1981). Colonies derived from blast cells from patients with common ALL contained single cells reacting with both T- and B-cell markers (Proc.AACR 22:615a,1981). In this work we report a case of Non-Hodgkin's lymphoma of the immunoblastic type who also had double marked cells. Direct and indirect immunofluorescence was used to detect a panel of T- and B-cell markers. Double marked single cells were identified using antibodies conjugated to different fluorochromes (FITC and Rhodamine). The patient had a leukaemic relapse, blood mononuclear cells were phenotyped: E-rosette< 1%, OKT3=78%, OKT4=8%, OKT8=0%, OKT6=0%, kappachain=97%, lambdachain=1.7%.

On cultivation B-cell colonies were obtained (150 colonies per 10^4 cells plated). Cells in the colonies were kappa +, lambda -, OKT3+. Two colour immunofluorescence revealed that 15% of cytoplasmic kappa chain positive cells also reacted with OKT3.

OKT3 and Ig are relatively late differentiation markers of the T- and B-cell lineage respectively. The presence of both markers in the same cell indicates that differentiation is abnormal in some B-cell malignancies.

(Supported by The Imperial Cancer Research Fund, London, England)

0043 B LYMPHOCYTE CARCINOGENESIS IN PEYER'S PATCHES, Marvin A. Cuchens and Kenneth L. Bost. Dept. Micro., Univ. Miss. Med. Center, Jackson, Miss. 39216

Spontaneously arising B cell leukemias in experimental animals are rare. Furthermore the experimental protocols used to induce lymphoid malignancies seemingly have little efficacy in the induction of malignancies of B cell origins. Therefore a different approach was used in the study reported here. The basic procedure was to localize a chemical carcinogen in the Peyer's patches of rats which were previously treated with anti-IgD to induce B cell proliferation in vivo. Thus far 9 of 15 3-methylcholanthrene (MCA) and 4 of 14 7,12-dimethylbenzanthracene (DMBA) treated rats have developed lymphoid malignancies which have been characterized, based on morphology, growth kinetics, pathogenesis and cell surface markers, as: 1) B cell chronic lymphocytic leukemias which coexpress membrane IgM and IgD (3 MCA treated rats), 2) Immature B cell leukemias which preferentially express membrane and/or cytoplasmic IgM (3 MCA treated rats), 3) B cell nodular lymphomas with immunoblastic characteristics (3 MCA and 1 DMBA treated rats) and 4) T cell thymomas (3 DMBA treated rats). Studies have also been done to propagate the B cell malignancies in vivo and in vitro. These studies indicate that the treatment of Peyer's patches with chemical carcinogens may provide a selective means of inducing B lymphocytic malignancies. Supported by grants from DHEW (7 R23 CA28046) and ACS (Cancer Research Center Grant, Univ. Miss. Med. Ctr.).

0044 DEFECT OF Il-2 (TCGF) PRODUCTION IN LYMPHOBLASTIC LEUKEMIA (ALL) DEMONSTRATED WITH MITOGENIC MONOCLONAL ANTIBODIES, Salvatore Venuta, Stuart P. Feldman, Cheng Y. Wang, Karl Welte, Malcolm A.S. Moore, Paul Harris, and Roland Mertelsmann, Memorial Sloan-Kettering Cancer Center, New York, NY 10021

Il-2 is a powerful growth factor regulating the proliferation of normal T cells. We have previously shown that ALL cells produce large amounts of Il-2 after PHA stimulation. Pan T2 is a monoclonal antibody recognizing an antigen on 90 % of peripheral blood T cells and thymocytes. A second monoclonal with similar specificity, OKT3, is known to be mitogenic for peripheral blood lymphocytes (PBL). We have studied the effect of these T cell specific reagents on Il-2 production by normal and ALL cells. Pan T2 was found to be strongly mitogenic for normal PBL and proliferation induced by Pan T2 and OKT3 was preceded by release of Il-2. The data obtained so far suggest two determinants on the surface of normal T cells which regulate Il-2 production. Cultures of ALL cells show a strikingly different response. While OKT3 induces Il-2 release by fresh ALL cells as well as by the ALL line Jurkat, Pan T2 is unable to induce Il-2 production under these conditions. However, Pan T2 induction of Il-2 by ALL cells can be restored by co-cultivation with irradiated Daudi cells. Since normal T cells do not require Daudi to produce Il-2 in response to Pan T2 and since Daudi do not produce Il-2 under any conditions, we conclude that the cell producing Il-2 in ALL is a Leukemic cell with an altered mechanism of Il-2 production at the level of the Pan T2 receptor. Since in vitro studies show that ALL cells require Il-2 for proliferation and ALL cells produce Il-2 through an altered mechanism, we hypothesize that autostimulation is essential for leukemogenesis.

0045 IMMUNE DISORDERS ASSOCIATED WITH RADIATION-INDUCED MYELOPROLIFERATIVE DISEASE IN THE DOG, Katherine A. Stitzel and Moshe Shifrine, Laboratory for Energy-Related Health Research, University of California, Davis, CA 95616

We have identified six cases of myeloproliferative disease (MPD) in beagle dogs chronically exposed to whole body gamma irradiation from 21 days of gestation throughout their life. The disease has occurred from 600 to 1000 days of age in dogs exposed at two dose levels, 0.037 and 0.10 Gy/day. The cases were characterized histologically by proliferation of bone marrow elements in the spleen, lymph nodes, liver, kidney, meninges and elsewhere. The cell types vary from highly undifferentiated to monomyelocytic. In one case a frank leukemia was seen, in the other cases abnormal cells were present in the blood in small numbers. Of great interest was the development of immune dysfunction in these animals at approximately the same time as the MPS. All animals had some spherocytosis. Three of the six cases developed a positive reaction in both the direct and indirect Coomb's test. Two other cases were positive in the direct Coomb's test only. One other animal, which has not yet presented with MPD, has developed agglutinating antibodies to unrelated red cells. Immune dysfunction in dogs with MPS has not been previously described. Whether the abnormal immune system allows the malignant cell population to expand or whether immune dysfunction is a consequence of disease is unknown. Manifestation of immunological disorder concomitant with radiation-induced MPD suggests that an interrelationship exists between the two and that immunological disorders may be associated with the development of hematological as well as lymphoid malignancies.

0046 MONOCLONAL ANTIBODIES (MoAb) FOR USE IN ACUTE LYMPHOBLASTIC LEUKEMIA (ALL)/LYMPHOMA J. Kersey, J. Jansen, R. Ash, E. Zanjani, M. Nesbit, D. Hurd, T. LeBien. University of Minnesota, Minneapolis, MN 55455

Our laboratory has produced MoAb against surface antigens of human pre B ALL cells. These MoAb have been tested on a large panel of acute leukemia/lymphoma cells and cell lines. The antigens have been studied immuno-chemically using SDS/PAGE. One of the MoAb, BA-3, binds to gp 100/common ALL antigen present on 80% of ALLs and some lymphomas. A second MoAb, BA-2 binds to p24 (24,000 k) found on 75% of ALL's and other hemopoietic and nonhemopoietic malignancies. BA-1 binds to a determinant present on 80% of cases of ALL and >90% of B cell lymphoma. Our recent studies indicate that neither BA-1 or BA-2 bind to CFU-GM, CFU-E, BFU-E or CFU-GEMM. Thus, these antibodies used alone or as cocktails, should prove useful for serotherapy or marrow cleanup for autologous marrow transplantation in ALL and lymphoma.

0047 CELL SURFACE PHENOTYPING AND DIFFERENTIATION OF FOUR MOUSE HEMOPOIETIC TUMOR CELL LINES OBTAINED AFTER TRANSFORMATION BY RAUSCHER MURINE LEUKEMIA VIRUS, W. van Ewijk[1], R. Delwel[1], E. Rhijnsburger[2] and N.J. de Both[2], [1]Dept. Cell Biology and Genetics, [2]Dept. Pathology from the Erasmus University of Rotterdam, P.O.Box 1738, Rotterdam, The Netherlands.

Inoculation of susceptible mice with R-MuLV complex causes a rapidly developing erythroblastosis due to Spleen Focus Forming Virus (SFFV), whereas injection of cloned lymphatic Leukemia helper Virus (LLV) induces a slowly progressing lymphatic and occasionally a Myeloid Leukemia. Syngeneic transplantation of leukemic blasts results in tumor formation of which permanent erythroid, myeloid and lymphatic cell lines could be established. We have recently isolated four tumor cell lines, RLD, RLB, RED, and RMB. RLD, and RMB show lymphoid, characteristics, whereas RED and RMB show erythroid and myeloid characteristics, respectively. In the present study we further characterize these cell lines based on the presence of cell surface markers, identified by a panel of monoclonal antibodies, and the fluorescence activated cell sorter (FACS). Furthermore, we have stimulated the in vitro differentiation of the RLD cell line with a number of potential inducers, including dimethylsulfoxide (DMSO). Under the present experimental conditions RLD cells show reduced proliferation and express a more mature cell surface phenotype.

Functional Properties of B Cell Tumors

0048 ACTIVATION OF MURINE B CELL LYMPHOMAS Lewis L. Lanier & Noel L. Warner, Univ. New Mexico School of Medicine, Dept. Pathology, Albuquerque, NM 87131 and Becton-Dickinson Monoclonal Laboratories, Mountain View, CA 94043

Cloned, transformed cell lines provide powerful tools to examine the phenotype and function of subsets of lymphocytes. Such lines may be particularly useful in examining the changes accompanying differentiation. Using an extensive panel of murine B lymphoma cell lines, we have examined the phenotypic, proliferative, and functional changes accompanying cellular activation by a variety of inducing agents. We have examined differences in surface density of a variety of differentiation antigens using quantitative flow cytometry, cell proliferation, and cell cycle profile. Lipopolysaccharide (LPS) was found to induce a 4-fold increase in surface IgM density on the NBL B lymphoma cell line, In parallel with sIgM, LPS induced expression of the ThB antigen, but did not significantly alter expression of other markers such as Ly 5, Lym 7.2, Ly 9.1, or E2. Changes in surface Ig isotype or Ig secretion were not observed. In contrast, LPS activation of the L10A/2J B cell line resulted in a 2-3 fold increase in surface density of I-A and I-E/C antigens, but not H-2K or H-2D gene products, or several other differentiation antigens (ThB, Ly 5, Lym 7.2, E2, etc.). LPS activation of L10A/2J and NBL did not significantly alter cell proliferation or cell cycle profile. Furthermore, anti-Ig serum did not inhibit activation. Further studies are underway to examine the influence of certain soluble T cell factors, alone or in combination with B cell mitogens, on these cloned B lymphoma cell lines.

0049 Induction of IgG-production in B lymphoblastoid cell lines and identification of T cell replacing factor (TRF)-like factor(s).

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IgG-PFC was induced in Epstein-Barr virus-transformed B lymphoblastoid cell lines(LCL) by the addition of allogeneic T cells. T cells involved in the induction of IgG-PFC were shown to belong to Leu 3a-positive T cell subset. Furthermore, partially purified soluble factors obtained from the culture supernatant of PPD-stimulated pleural T cells obtained from patients with tuberculous pleurisy or PWM-stimulated tonsillar mononuclear cells was shown to induce IgG-PFC in LCL across the MHC-barrier. The induction of IgG-PFC was observed only in surface IgG-positive LCL cell populations and was not accompanied by the increase of the number of LCL cells. The factors with such a TRF-like activity were found in two fractions corresponding to the molecular weight range of 18,000 to 25,000 (22K) and 28,000 to 38,000 (36K) by gel filtration. Isoelectric focusing of these fractions revealed that TRF-like activity of both 22K and 36K fractions distributed in the pI range of 5.0 to 6.0 and both fractions were found to be devoid of TCGF activity. These results appear to indicate that the factors act on the B cells in terminal stages to trigger final differentiation to immunoglobulin-producing cells.

0050 CONCOMITANT INDUCTION OF THE CELL SURFACE EXPRESSION OF Ia DETERMINANTS AND ACCESSORY CELL FUNCTION BY MURINE MACROPHAGE TUMOR CELL LINES, Edwin B. Walker, Lewis L. Lanier, and Noel L. Warner, University of New Mexico, Albuquerque, N.M. 87131

This study demonstrates that an uncharacterized soluble factor produced in Con A induced rat spleen cell suspensions, Ia inducing factor (IaIF), has the capacity to induce the increased expression of cell surface H-2K and H-2D molecules, and the expression of I region gene products on murine monocyte-macrophage lineage tumors such as WEHI-3 and P388.D1 which are not Ia positive in the absence of the factor. Detailed kinetic analysis of the induced cell surface expression of Ia determinants using monoclonal antibodies and flow cytometry shows that the I-A cistron product is expressed first followed by the appearance of I-E/C subregion products. In parallel with induction of seriologically defined Ia specificities, Ia-induced WEHI-3 and P388.D1 macrophage tumors are capable of providing accessory cell function in stimulating IL-2 production by T-T hybridomas which are activated in an MHC-restricted, antigen-dependent fashion. Uninduced, Ia-negative macrophage tumor cells do not trigger a comparable response in this assay system.

0051 FUNCTIONAL REGULATION OF HUMAN B CELL LINES WITH COWAN I, ANTI-u, PMA AND T CELL FACTOR, OSAMU SAIKI, PETER RALPH, SLOAN-KETTERING CANCER INSTITUTE, RYE, NY 10580
Biological effects of staphylococcal bacteria strain Cowan I (Cowan I), $F(ab')_2$ fraction of goat anti human IgM (anti-u), Phorbol Myristic Acetate (PMA) and partially purified T cell factor on human B cell lines were tested using thymidine incorporation and protein A-red blood cell plaque formation for immunoglobulin secretion. Cowan I, anti-u and PMA decreased thymidine uptake in most B cell lines tested, while anti-u and Cowan I stimulated DNA synthesis in normal human peripheral B cells independently of T cells. Anti-u inhibition was completly abrogated by intact IgM, but Cowan I and PMA inhibition were not. However, cell lines formed subsets with respect to their responses. Anti-u but not Cowan I or PMA decreased thymidine uptake more than 80% in BJAB. Anti-u inhibited immunoglobulin secretion in DAUDI, U698 and 1788 but not other IgM or IgG secreting cell lines. Cowan I, PMA or T cell factor increased immunoglobulin secretion in IgM secreting cell lines up to 10-fold. In CAH, PMA increased IgM-PFC but T cell factor did not. In contrast T cell factor increased the IgM-PFC in DAUDI but PMA had little effect. No inductions from non secreting to secreting cells, and no switch of Ig class synthesis was found in the cell lines tested by these stimulants separately or together. These findings suggest B cell lines are modulated in growth and Ig secretion by anti-u, Cowan I and PMA, receptor for these agents on the cell surface and their signals to B cells are quite different and may depend on the subclass of B cells. The cell lines provide models for biochemical analysis of human B lymphocyte regulation and subset categories.

0052 REGULATION OF IMMUNOGLOBULIN SECRETION IN SUBSETS OF HUMAN B CELL LINES, Peter Ralph and David H. Maurer, Sloan-Kettering Institute, Rye, New York 10580
The degree of differentiation of B cell lines was assayed by Ig secretion measured by protein A-plaque forming cells (PFC). Tumor promoter phorbol myristic acetate (PMA) increased PFC 3 to 20-fold in a number of IgM and IgG lines, requiring several days of incubation for optimal effect. PMA concentrations stimulating PFC were different for each line, but correlated with growth inhibition of the line, suggestive of a terminal differentiation process. Some B lines were insensitive to PMA stimulation of PFC despite growth inhibition, especially secretion-negative lines, suggesting that they are less mature or belong to a subset not responding to this nonspecific activator (J. Clin. Invest. 68:1093,1981). Normal blood and spleen B cells require T cells for PMA-induction of Ig secretion. Therefore T-mitogen induced lymphokine (LK) was tested on cell lines. Helper factors from PWM or PHA-stimulated spleen or blood cells augmented PFC 3 to 10-fold in certain lines without growth inhibition. In IgM (BM) and IgG (ARH-77) lines, the effects of PMA and LK on PFC were approximately additive. No induction of a new Ig class was seen. Binding to ARH-77 of (3)H-phorbol dibutrate which also stimulates Ig secretion, was inhibited by PMA but not by LK, suggesting a different binding site for the two types of modulators. Induction of IgM secretion and of IgG production in certain IgM lines was only found during coculture with normal T cells (cf. Nature 271:756). Therefore the lines appear to belong to B cell subsets: a) requiring direct T cell signals for induction of Ig secretion or switching, b) partially activated to secrete at a low level but requiring direct T cell contact for further secretion, c) partially activated and sensitive to stimulation by one or more soluble agents.

0053 FUNCTIONAL AND PHENOTYPIC PROPERTIES OF IN VITRO DIFFERENTIATING CHRONIC LYMPHOCYTIC LEUKEMIA (CLL) CELLS. Thomas H.Tötterman,Antero Danersund,Kenneth Nilsson,Magnus Gidlund[1] and Ganesha Yogeeswaaran[2] Wallenberg Lab.,Dept Immunology[1],University of Uppsala,Sweden and Dept Microbiology[2],Boston University,Mass.,USA.
We have recently found that the phorbol diester TPA is capable of inducing phenotypic and functional changes in several CLL clones strongly suggesting differentiation(Nature 288:176,1980). TPA induced a lymphoblastoid-plasmacytoid morphology and the synthesis of cytoplasmic clonal heavy and light Ig chains and Ia antigens.This was paralleled by decrease in surface Ig and specific changes in surface glycolipid/protein patterns as well as B cell marker(Fc,C_3,mouse RBC receptors) profile.TPA,especially in combination with antibody to mu heavy Ig chains,induced an Ig secretion in CLL cells corresponding to that of EBV-positive lymphoblastoid cell line cells.Triggering of Ig secretion was dependent of assisting autologous/allogeneous T cells and monocytes.CLL cells induced to differentiate by TPA were highly sensitive to lysis by allogeneous natural killer(NK) cells compared to non-induced cells,and this was correlated to changes in surface glycolipid patterns.Clinical studies demonstrated that the magnitude of TPA-induced differentiation of CLL cells -as revealed by cytoplasmic Ig levels and increased NK cell sensitivity- was strongly correlated with the clinical activity (signs of progressive disease,tumor cell mass) of the leukemia.

0054 MODULATION OF Ia AND H2 ANTIGENS ON BALB/c B LYMPHOID TUMOR CELLS BY INTERFERON, K. Jin Kim, E. De Maeyer, William L. Leiserson and Alain Paraf, NIAID, NIH, Bethesda, MD 20205

It has been shown previously, by flow microfluorometry analysis, that Ia antigens on BALB/c lymphoid tumor cell line, X16C 8.5, were modulated by T soluble factor(s) present in the culture fluid of spleen cells activated with Concanavalin A (CAS). CAS also affects the expression of H-2 antigens on B cell lines in addition to the Ia antigens but not sIg, Fc receptors or gp70 antigens. Using purified T soluble factors, it was found that interferon (IF) but not IL-1, IL-2 or TRF, appear to be responsible for such antigenic modulation. The degree of response of several B lymphoid tumor cells to CAS was variable depending upon the cell line, suggesting the possibility of the presence of B cell subsets depending upon the responsiveness to interferon. Thus, the phenomenon of antigenic modulation of B cells by interferon could be important to understand the immunoregulatory role of interferons.

We are studying the kinetics of the effects of IF on the expression of Ia and H-2 ntigens on B lymphoid cells. Further, we are investigating the relationship between cylostatic effects and the antigen modulation of IF on tumor cells.

0055 HYBRIDOMAS BETWEEN T CELLS AND B CELLS, M. J. Potash, J. Zeuthen, and G. Köhler, Basel Institute for Immunology, Basel Switzerland

Hybridomas were constucted between normal mouse spleen cells, and a pre-B cell line, a T helper cell line and a Ig secreting hybridoma, and between B and T cell tumour lines at different developmental stages. Resulting hybrids were assayed for B and T cell characters including Ig synthesis, LyT markers, Ø, and factor secretion. A hierarchy of expression of B and T cell phenotype can be constucted. B cell character or T cell character is mutually exclusive.

0056 BIOCHEMICAL CHARACTERIZATION OF A FACTOR WHICH INDUCES CELL SURFACE EXPRESSION OF Ia DETERMINANTS, Vernon C. Maino, Edwin B. Walker, Edward K. Wakeland, Lewis L. Lanier and Noel L. Warner, Univ. of Florida, Gainesville, FL 32610, and Univ. of New Mexico, Albuquerque, NM 87131

Preliminary characterization of a soluble factor generated in ConA-induced rat spleen cell cultures, Ia inducing factor (IaIF), has been initiated. Crude supernatant preparations were fractionated by preparative isoelectric focusing and P-100 gel filtration and resulting fractions were analyzed by flow cytometry with monoclonal antibodies for the capacity to induce the increased expression of cell surface H-2K and H-2D molecules, I region gene products, and Fc receptors on a murine macrophage tumor, WEHI-3. Preliminary studies indicated that IaIF has a pI value of 3.5-3.9 which was separable from a fraction which induced Fc receptor expression but was not separated from H-2 induction activity. IaIF activity was recovered in the void volume of gel filtration column suggesting a molecular weight above 80,000 daltons. Partially purified preparations of T cell growth factor (IL-2) from spleen cell supernatants and from a number of IL-2 producing T cell tumor lines were not active in this response indicating that IaIF is not related to IL-2. Preliminary studies have also been initiated to examine the biosynthesis and structure of Ia antigens induced by IaIF.

0057 WEHI 231 AS A TUMOR MODEL FOR TOLERANCE INDUCTION IN IMMATURE B LYMPHOCYTES, Anthony L. DeFranco, Mark M. Davis, and William E. Paul, LI, NIH, Bethesda, MD 20205

WEHI 231 is a B cell lymphoma which has been shown to die in the presence of anti-μ antibodies (Boyd, A.W., and Schrader, J.W., J. Immunol. 126 2466, 1981). Using a short term proliferation assay, we have demonstrated that very low concentrations of anti-μ (1 ug/ml) inhibit completely the DNA synthesis of this cell line within 24 hours. This inhibition is followed by cell death, as determined by uptake of trypan blue, and change in light scatter. Complete inhibition of proliferation was not observed in 5 other B cell lymphomas which were examined. We have found that LPS protects WEHI 231 from this inhibition for short periods of time (several days). We postulate that WEHI 231 may be a B cell lymphoma of the neonatal or immature differentiation state. B cells at this stage of development are believed to be uniquely sensitive to tolerance induction ("clonal abortion"). LPS may act to make these cells differentiate (although unstably) to a more mature state which is no longer sensitive to tolerance induction via the antigen receptor. To examine this idea, we have generated a series of B cell-specific cDNA clones from mRNAs expressed in this cell line. Preliminary results suggest that those mRNAs preferentially expressed in WEHI 231 (as opposed to other B cell lymphomas) are changing their level of expression in cells treated with LPS, an observation which supports our model.

0058 GROWTH AND HOMING OF A PANEL OF AKR T AND B CELL LYMPHOMAS WITH DIFFERENT CELL SURFACE PHENOTYPES, Bonnie J. Mathieson, Marion M. Zatz and Michael Mage, National Institute of Allergy and Infectious Diseases, and the National Cancer Institute, Bethesda, MD 20205, and George Washington University Medical Center, Washington, DC 20037

A panel of 7 different, spontaneous AKR T- and B- cell lymphomas has been isolated and characterized in vivo. These tumors have been analyzed for cell surface expression of the following cell surface markers; Lytl, Lyt2, Ly9, Ia, PNA, 2C2/14-8-18, and sIg expression. Each of the tumors has different biological characteristics of organ growth or intra-organ localization. In particular, one of the T cell tumors grows and replaces the thymus with serial i.v. transfer. The other T-cell lymphomas primarily have peripheral patterns of lymphoid growth. The 2 B-cell lymphomas grow in similar follicular patterns preferentially in the spleen but they differ in their pattern of growth in other organs.

The homing patterns of these tumors have been compared to those of fractionated normal thymocytes and subsets of peripheral T cells. The homing patterns of normal cells can be predicted by their Lyt expression and the homing patterns of the tumor cells may also reflect in part the Lyt phenotype. The patterns of the T-cell lymphomas appear to reflect the homing of specific subsets of both mature and immature T cells.

We have concluded that the characteristics of these B-cell tumors suggest the susceptibility of a particular subset of B cells for lymphoma induction in older AKR mice whereas the T-cell lymphomas have a varied spectrum of phenotype and indicate the probable susceptibility of a common precursor.

0059 THE BCL$_1$ B CELL LINE: A MODEL TO STUDY Fc FRAGMENTS INDUCED PROLIFERATION AND POLYCLONAL ANTIBODY PRODUCTION, Edward L. Morgan, Marilyn L. Thoman and William O. Weigle, Scripps Clinic & Research Foundation, 10666 N.Torrey Pines Rd., La Jolla,CA 92037.

The murine B cell leukemia (BCL$_1$) was induced to proliferate and secrete antibody upon stimulation with Fc fragments derived from papain digestion (Fc) and plasmin digestion (pFc) of human IgG$_1$. BCL$_1$ served as a unique model for studying B cell activation by these probes in that the culture systems employed were free of serum and 2-mercaptoethanol. Fc fragment-induced BCL$_1$ proliferation was found to be dependent upon the presence of macrophages. The addition of increasing numbers of irradiated splenic adherent cells from Balb/c mice resulted in a pronounced proliferate response. Optimal Fc induced BCL$_1$ proliferation occurred when 1×10^5 BCL$_1$ cells were supplemented with 2×10^5 macrophages. In contrast, pFc fragments were capable of inducing BCL$_1$ proliferation in the absence of any other cell population. In this regard, pFc fragment activation resembles the mitogenic Fc subfragment which is derived from macrophage enzymatic digestion of Fc fragments. Fc subfragments also induce B cell proliferation in the absence of macrophages. Fc fragment induced immunoglobulin synthesis requires the presence of both macrophages and T cells. Supplementation of BCL$_1$ cultures with either macrophages or T cells did not result in Ig synthesis, but when added together a significant response was observed.

The results obtained on Fc activation of the BCL$_1$ line are identical to data obtained using conventional murine or human lymphocytes indicating that the BCL$_1$ is an ideal system to study lymphocyte activation.

0060 ANTI-LYB-3 ANTISERUM PROMOTES MOPC-315 SECRETORY DIFFERENTIATION, James W. Rohrer, Richard K. Gershon, John D. Kemp, and Brigitte Huber, Univ. of S. Ala. Coll. of Med., Mobile, AL 36688

During in vivo growth in diffusion chambers(DC), MOPC-315,a TNP-specific IgA (M315)-secreting BALB/c myeloma, differentiates from non-secretory lymphocytic cells(LC) to M315-secreting plasmacytes(PC). 315 clone growth and differentiation are enhanced when DC containing 315 cells and TNP-SRBC are implanted i.p. in SRBC-immune mice. Adoptive transfer experiments show that 315 cell growth and differentiation enhancement are induced by distinct Ly $1^+,2^-$ SRBC-immune Th cell subsets. The clone growth helper(Th_g) is SRBC-specific,does not bind M315, is Qa-1^-, and needs a TNP-SRBC bridge to effect help. The differentiation helper(Th_d) sees both SRBC and M315 idiotopes independently, is Qa-1^+, and requires SRBC near the target B cell to deliver help. Each Th cell is independent of the other. Anti-Lyb-3 antiserum injected after DC implantation can replace the Th_d cells if both TNP-SRBC and Th_g cells are present. Also, anti-Lyb-3 antiserum enhances 315 cell differentiation in M315-immune mice even though Ts cells are present that block 315 PC M315 secretion and inhibit M315-induced Th_d cells. This Th_d cell-replacing activity is removed by 315 cell absorption. This suggests that: 1)the Lyb-3 determinant is on the B cell receptor for Th_d helper signals, and 2)MOPC-315 is an Lyb-3^+ B cell neoplasm. (Supported by NIH research grant CA-28708).

NK Cells

0061 DIFFERENT EFFECTORS AND EFFECTOR-MECHANISMS MEDIATE THE NATURAL KILLER CELL LYSIS OF LYMPHOID AND FIBROBLAST TARGETS. F. Scott Brauer, Paul Q. Patek, John Leslie Collins and Melvin Cohn. Salk Institute, P.O. Box 85800, San Diego, CA 92138

We have found that NK-sensitive lymphoid cells as well as all NK-sensitive and NK-resistant fibroblasts tested (normal and transformed) share NK-recognition determinants. Lymphoid target cell sensitivity or resistance to NK-lysis is determined by the presence or absence of NK-recognition determinants, respectively. In contrast, sensitivity or resistance of fibroblast targets to NK-lysis is determined by the level of a protein synthesis dependent counterlytic mechanism operating in the target cells. This counterlytic mechanism is absent from lymphoid cells. In addition, fibroblast lysis does not require protein synthesis while lymphoid cell lysis does. We have also shown that a cloned NK-like cell line is able to lyse fibroblast targets and not lymphoid targets.

These findings support the concepts that: 1) the NK-effectors that mediate lysis of lymphoid targets are different from those that mediate lysis of fibroblast targets, 2) the two populations of effectors have overlapping recognition specificities, and 3) the lytic signals delivered by the two populations are different or the lytic signals are the same and the responses of lymphoid and fibroblast targets to the lytic signal are different.

0062 TIME-LAPSE VIDEOMICROSCOPY AND ULTRASTRUCTURE OF HUMAN NATURAL CELL-MEDIATED CYTOTOXICITY, Joseph H. Phillips, Andrew A. Amoscato, Kenji Nishioka, and George F. Babcock, Univ. Texas System Cancer Center, M. D. Anderson Hospital and Tumor Institute and the Univ. Texas Graduate School of Biomedical Sciences, Houston, TX 77030.

In the present study, human natural killer (NK) cells were isolated to 90% morphological homogeneity (large granular lymphocytes) on discontinuous gradients of Percoll. The purified NK cells were then combined with either K562 or HM39.5 tumor cells and natural cell-mediated cytotoxicity was analyzed by time-lapse videomicroscopy and electron microscopy. Results of the time-laspe videomicroscopy revealed several interesting features of NK cell-mediated cytotoxicity: (1) Upon contact with tumor cells, NK cells demonstrate increased mobility and numerous membrane changes; (2) The tumor target cells also show numerous morphological changes including cytoplasmic streaming at the areas of cell-cell contact, and zeiosis; and (3) NK cells survive the lytic interaction with the tumor target cells and can recycle to kill again. At the ultrastructural level, the initial contact of the NK cell with the tumor cell is characterized by the formation of discrete intercellular junctions between small microvilli of the NK cell and tumor cell plasmalemma. As the lytic process continues, a progression of ultrastructural events can be observed in both the NK cells and the tumor cells, including: (1) Invagination of the tumor cell by numerous NK cell cytoplasmic extensions; (2) A reorientation of several NK cell cytoplasmic structures; (3) Emperipolesis; and (4) Tumor blebbing and zeiosis. The ultrastructure of human NK cell-mediated cytotoxicity revealed several unique features, as well as features shared by both T cell and monocyte-mediated tumor cytolysis. This work supported in part by Grant CA27330 from NCI, DHEW.

0063 IN VIVO MEASURE OF THE DESTRUCTION OF ^{125}IUdR LABELLED LEUKEMIC CELLS INTRAPERITONEALY INJECTED IN SYNGENEIC HOSTS. S. Orbach-Arbouys, J. Lhéritier. Hôp. Paul-Brousse, ICIG, 94804 Villejuif Cédex, France.

10^7 ^{125}IUdR labelled gross virus induced E ♀ K_1 leukemic cells were injected intraperitonealy into BDF_1 mice, the radioactivity of which was counted in toto at various time intervals. The cumulative curve of the radioactivity remaining in the animals one day after the cell injection, indicates two types of responses : in one group of mice, the values stay close to the injected value and do not differ much one from the other. In the other group, the loss of radioactivity is greater and variable. The cumulative curve of the radioactivity loss per hour (RLH) measured from day 1 to day 3 shows also two types of responses : that of mice the RLH of which are low and comparable, and that of mice with higher and non homogenous RLH. The mice who belong to the group of greater RLH belong also to the group of greater immediate loss of radioactivity. They survive longer than the other animals. Such a correlation is even more evident when the mice are stimulated by an injection of low doses of cobra venom factor or inhibited by trypan blue. It is thus possible to admit that the measure of the RLH can be accepted as an evaluation of the host defense against the tumor. The existence of a mechanism responsible for the enhanced destruction in some mice is discussed.

0064 MONOCLONAL ANTIBODY BLOCKADE OF A SUBSET OF HUMAN NK CELLS, Walter Newman, Fred Hutchinson Cancer Research Center, Seattle, WA 98104

Antibody 13.1, a murine IgG1, was derived from mice immunized with an enriched population of human NK cells. The 13.1 antigen is expressed on all peripheral blood lymphoid cells. Lysis of the tumor target K562 by fresh perpheral blood lymphocytes is almost completely inhibited with 10 ug of antibody, and 50% inhibition of lysis is achieved with 1 ng antibody per 10^5 effector cells. Pretreatment of effector or target cells with antibody 13.1 revealed that inhibition was accomplished at the effector cell level only. Another murine monoclonal antibody, 34/28, which binds to NK cells and is an IgG1, failed to inhibit NK cell lysis. Cold target inhibition experiments suggested the antibody interfered with the effective binding of K562 targets to NK cells. Antibody 13.1 failed to inhibit the lysis of antibody coated Chang target cells (ADCC) and also failed to inhibit lysis by cytotoxic T lymphocytes from 6 day mixed lymphocyte cultures. Also, antibody 13.1 failed to inhibit lysis of K562 targets by the 'anomolous' cytotoxic effectors which develop in mixed lymphocyte culture. When fresh NK cells were tested on a panel of 17 NK susceptible targets, the lysis of only five of these was blocked by antibody 13.1, namely K562, HL-60, KG-1, Daudi and HEL, a newly derived human erythroleukemic cell line. The lysis of twelve additional human B and T cell line targets was not inhibited. In addition to the demonstration that the 13.1 antigen is a crucial cell surface structure uniquely involved in NK cell mediated lysis, a heterogeneity of target cell recognition has been revealed which argues for the proposition that individual NK cells have multiple recognitive capabilies.

0065 NATURAL KILLER CELL FUNCTIONS OF MARMOSET HERPESVIRUS-INDUCED T-CELL LINE LEUKEMIA, Donald R. Johnson[1] and Mikael Jondal[2], [1]University of Nebraska Medical Center, Omaha, NE 68105, [2]Department of Tumor Biology, Karolinska Institute, Stockholm, Sweden.

Herpesvirus ateles (HVA) and Herpesvirus saimiri (HVS) induced leukemias of marmoset monkeys or HVA transformed in vitro marmoset lymphocytes retain certain immunologic functions. We have demonstrated in a short term ^{51}Cr release assay, that they are cytotoxic against certain target cell lines and this cytotoxicity resembles that expressed by marmoset natural killer (NK) cells, and occurs without requiring lectins or antibodies attached to the target cells. Preliminary experiments using inhibitors of cell mediated lysis, indicate the cytotoxicity expressed by the HVA/HVS cell lines is subject to the same regulatory mechanisms as that of human or marmoset NK cells. These results indicated that HVA/HVS cell lines may be useful for characterization of the NK system and the mechanisms of lymphocyte mediated cytolysis.

0066 DISTINCTIVE PHENOTYPE OF A HUMAN NATURAL KILLER CELL CLONE, Neal Flomenberg, Jacki Kornbluth, and Bo Dupont, Memorial Sloan-Kettering Cancer Center, New York, NY 10021

Natural Killer (NK) cells are a small population of peripheral blood mononuclear cells identified by their constitutive in vitro cytotoxicity against a variety of tumor and viral infected cell lines. The lineage of NK cells remains uncertain, however. We have established a long term cloned line of mixed lymphocyte culture (MLC) activated human NK cells. This clone is phenotypically distinct from MLC effector T cells. While it reacts with a monoclonal antibody against the sheep red blood cell receptor (9.6), it fails to demonstrate the well defined pan-T cell antigens (Leu 4/OKT3 or Leu 1/10.2). It is similarly negative for markers of functional T cell subsets (Leu 3a/OKT4 and Leu 2a/OKT8) and for the OKM1 antigen present on some unstimulated or interferon activated but not MLC stimulated NK cells. This clone is positive, however, for the T cell/thymocyte antigen 3A1, which is also present on some SRBC+OKT3- peripheral blood cells. Morphologically and histochemically, it appears similar to the large granular lymphocytes which contain the bulk of NK activity in peripheral blood. Functionally, this clone is strongly cytotoxic against leukemic cell lines from several cell lineages (K562, MOLT-4, HSB, and Daudi). It does not exhibit allospecific cytotoxicity against the HLA antigens of the original stimulating cell. The growth of this clone is strictly dependent on IL2 containing conditioned medium, and absorption studies with the clone and with T cells suggest that IL2 is indeed the necessary growth factor. These data demonstrate that one human NK population is phenotypically unique and may either be related to the T cell population or descended from a distinct lymphoid lineage.

0067 A DIRECT EVALUATION OF THE ROLE OF THE NK CELLS IN RESISTANCE TO LYMPHOMA CELLS, Sylvia B. Pollack, University of Washington, Seattle, WA 98195

Previous studies had indicated that tumor resistance varies with the level of natural killer (NK) cell activity. We have developed a model to directly test *in vivo* functions of NK cells: C57Bl/6 (B6) mice were depleted of NK cells by *in vivo* treatment with antisera to the NK-associated alloantigen NK 1.1. When tested *in vitro* in the presence of complement, anti-NK 1.1 kills fewer than 5% of B6 spleen cells (SC), has no effect on cytolytic T cells or plaque forming cells. Anti-NK 1.1 alone does not affect NK activity. Injection of B6 mice with 25 µl of anti-NK 1.1 reduced the ability of their SC to lyse YAC-1 by 70% within 2 hr. NK activity gradually returned to control levels but still was significantly depressed at 48 hr. Decreased NK activity was comparable when the antiserum was injected i.p. or i.v. To test the requirement for NK cells in clearance of circulating lymphoma cells *in vivo*, B6 mice were pretreated with 25 µl anti-NK 1.1 and subsequently injected i.v. with 10^6 ^{125}IUdR (5-iodo-2'-deoxyuridine)-labeled YAC-1 or RBL-5 T lymphoma cells. Cpm in lungs, liver and spleen were determined 4 hr later. In a series of four experiments, tumor cell clearance was reduced 2-4 fold in the anti-NK 1.1 treated mice compared to injection controls. These results provide direct evidence that NK cells play an *in vivo* role in the elimination of circulating tumor cells. (Supported by CA18647.)

0068 CHARACTERIZATION OF LARGE GRANULAR LYMPHOCYTE (LGL) TUMORS IN THE RAT, Craig W. Reynolds and J.M. Ward, NCI-FCRF, Frederick, Maryland 21701

It has previously been demonstrated that a significant number of aged rats (> 2 years old) will spontaneously develop a metastasizing splenic leukemia with characteristic azurophilic granules in the cytoplasm. The nature and origin of these tumors, however, is not clear. Recently, we reported that a subpopulation of normal rat lymphocytes, large granular lymphocytes (LGL), also have these characteristic granules. We were therefore interested to investigate whether any relationship exists between LGL and these spontaneous leukemias in Fisher (F344) rats. The results demonstrate that about 20% of these splenic leukemias are similar to LGL in the following respects: 1) they are similar in both size and morphology, 2) they share some cell surface antigens such as leukocyte-common (L-C) and W3/13, and 3) they are both able to function as effectors in natural killer (NK) and antibody-dependent cell mediated cytotoxicity (ADCC). As in previous studies using purified LGL, the cytotoxic activity of these tumor cells could be augmented by pretreatment with interferon. In addition, experiments using discontinuous Percoll gradients to isolate LGL leukemias from normal spleen cells suggested that LGL tumor cells have a similar density to normal LGL. These studies demonstrate that spontaneous LGL leukemias exist in aged rats. The use of these tumors should help to identify the hematopoietic origin of their normal LGL counterparts. In addition, we have observed that a significant percentage of these tumors maintain very high cytotoxic activity. The use of these cytotoxic LGL leukemias may be extremely useful in obtaining a large number of functionally active cells for future analysis.

0069 CHARACTERIZATION OF FIVE CLONED MURINE CELL LINES SHOWING HIGH CYTOLYTIC ACTIVITY AGAINST YAC-1 CELLS. Colin G. Brooks, Kagemasa Kuribayashi, George E. Sale and Christopher S. Henney, Fred Hutchinson Cancer Research Center, Seattle, WA 98104.

To provide a selection procedure for the long-term growth and subsequent cloning of NK cells, mouse lymphocytes were treated with monoclonal anti-Thy-1 antibody and complement, incubated for 24 h with poly I:C to boost residual NK activity, and placed in growth medium containing supernatant from Con A stimulated spleen cells. Following cloning, 5 clones were isolated which had very high cytotoxic activity (100-1000x greater than normal spleen cells) against YAC-1 cells. All the clones bore Thy-1, Ly-5, and asialo-GM1, 4 of the clones bore NK1.2, and all lacked Ig, Lyt-1, and Fc receptors. This antigenic phenotype was consistent with them belonging to the NK lineage. In addition, cytotoxicity by these cloned lines was blocked by anti-Ly-5 antiserum, a reagent which inhibited lysis by splenic NK cells but not by cytotoxic T cells (CTL). Morphologically, the cells were very large and contained prominent cytoplasmic granules. Although YAC-1 cells were always the most susceptible targets, the specificity of the cloned killer cells differed markedly from splenic NK cells. In particular, cytotoxicity by one clone tested on a panel of 45 target cells showed no significant correlation with the specificity of splenic NK cells. Most strikingly, there was a complete lack of cytotoxicity against either solid tumor derived targets and against xenogeneic targets. Although 3 of the cloned lines were Lyt-2 negative by FACS analysis, 2 of the lines bore high quantities of this antigen. These cloned lines thus had properties characteristic of both NK cells and CTL, and suggest that there may be a close relationship between these two lineages of cells.

0070 ASSOCIATION OF NATURAL KILLER CELL FUNCTION AND VIRAL INFECTION IN PATIENTS UNDERGOING BONE MARROW TRANSPLANTATION. Clara Ching,* William Lau, Young Paik, Livingston Wong and the Hawaii Bone Marrow Transplantation Group. St. Francis Hospital, Cancer Center of Hawaii,* University of Hawaii School of Medicine. Honolulu HI 96813 USA.

Natural killer cell function(NK) as an immunosurveillance mechanism in viral infections and cancer, was evaluated in prospective bone marrow transplant recipients with hematopoietic malignancies. The nature of the NK cell was studied against a herpesvirus infected fibroblast NK(HSV-1); uninfected fibroblast NK(FS); a chronic myelogenous leukemia cell NK(K562). NK function was deficient in all 7 patients with acute leukemia in relapse, 3 with aplastic anemia(AA), normal in 2 in remission. Six patients received BMT from an HLA identical sibling. Five patients who developed graft-versus-host disease (GVHD) had low pre-transplant NK except for one in complete remission. NK post-BMT was normal with one exception(controls:NK(HSV-1) 38%±10,mean±SD,N=40; NK(K562) 55%±13,N=27 at E/T ratio 50/1). The nature of the NK cells at the time of GVHD in an AML patient differed biologically from the pre-transplant NK cell. NK was normal in patients surviving viral infections(disseminated herpes zoster, cytomegalovirus (CMV) pneumonitis, adenoviral hemorrhagic cystitis). In the patient with complications of CMV interstitial pneumonitis post-BMT and receiving high dose methyl prednisolone,NK function examined one day prior to death, was low:NK(HSV-1)6%, NK(FS)1%, NK(K562)20%. Only NK(K562) increased from 20 to 33% by treatment of his lymphocytes with IFN. This patient had 1% large granular lymphocytes (LGL) (control LGL:9%±3,N=10). These studies suggest a biological role for NK function in resistance to viral infections in BMT recipients. (Supported by NIH AI 17403, Straub Foundation)

0071 GENETIC CONTROL OF NK CELL ACTIVATION IN SM/J MICE Edward A. Clark and Nancy Windsor Dept. of Genetics, University of Washington, Seattle, WA 98195

The SM/J mouse strain has both abnormally high NK and K cell activity and elevated responses to B cell mitogens (Engel et al J. Exp. Med. 154:726, 1981; Clark et al. J. Immunol. 127: Dec. 1981). Splenic NK cell reactivity against YAC-1 lymphoma cells (100:1 E:T ratio) was dramatically higher in SM/J mice (71%) than other strains such as B6(27%) or H-2^v congenic B10.SM controls(35%) but was not abnormally high against other targets such as L5178 c127v or P 815. The hyper NK activity in SM/J animals may be due to a form of chronic NK cell activation since 1) hyper NK activity was present even in older SM/J mice; 2) a high proportion of the SM/J cells binding to YAC-1 targets had lytic activity; 3) spleen cells from SM/J mice had significantly more IFN units/10^6 cells than did cells from B6, B10.SM, or A/J mice. The spleen cells from SM/J mediating killing were NK-1^+, Thy-$1^\pm$, Qa-5^-, Qa-4^-, and Lyt-2^- but H-2^v-identical B10.SM NK cells were Qa-5^+ Qa-$4^\pm$. This at first suggested that SM/J NK cells belong to an activated Qa-5^- subset. However, NK cells even from very young SM/J mice are Qa-5^- although they are not very cytolytic, and NK cells from hyperactive (B6xSM/J)F_1 are Qa-5^+. Thus, it appears that non-H-2 SM/J genes may influence the expression of Qa antigens. The SM/J NK phenotype is under polygenic non-H-2 gene control. Using the cross-intercross method of Snell, we have begun to develop an A.SM "NK high" (>40% activity) congenic line (currently at gen. 6) in order to better examine gene control of NK activation (supported by NIH grant CA 26713 and NIH core grant RR 00166 and Genetic Systems Corp).

0072 CLONED YAC-1 TARGET CELLS SELECTED FOR THEIR RESISTANCE TO NATURAL KILLER CYTOTOXIC FACTORS (NKCF) ARE ALSO RESISTANT TO NK CELL MEDIATED CYTOTOXICITY, Susan C. Wright and Benjamin Bonavida, UCLA School of Medicine,Los Angeles, CA 90024

Previous work has suggested that natural killer cytotoxic factors (NKCF) released from normal murine spleen cells may be involved in the lytic mechanism of NK cell mediated cytotoxicity (NKCMC) (J. Immunol. 126:1516, 1981). If NKCF are the lytic mediators of NKCMC,it would be predicted that NK target cell clones which have been selected for their resistance to NKCF would also be resistant to NKCMC. YAC-1 cells which had been cultured in the presence of dilute NKCF for 35 days became resistant to lysis by NKCF and were cloned. Nineteen clones were isolated which were all resistant to lysis by NKCF and NKCMC. Two of these clones were subcloned for further studies which demonstrated that the resistant subclones were recognized and bound by NK effector cells, were effective in activating the NK effector cell to release NKCF, but were ineffective target cell competitors in a 4 hr. ^{51}Cr-release assay. Resistance to lysis was restricted to the NK system since the subclones were sensitive to lysis by alloimmune cytotoxic T cells.Adsorption experiments demonstrated that the subclones were unable to deplete NKCF from supernatants, unlike the parental sensitive target, YAC-1. Altogether, these results provide further support for a common mechanism of target lysis by NKCF and in NKCMC reactions. Furthermore, the ability of the resistant clone to stimulate effector cells to release NKCF but its inability to bind NKCF suggest that different membrane sites are involved in triggering release of NKCF and target cell lysis. (Supported by CA-12800 from NCI)

0073 A Cloned Cell Line Mediating NK Cell Function Suppresses Ig Secretion, Gary J. Nabel, Jeffrey Allard and Harvey Cantor*

We have previously described a cloned cell line which combines information for a unique display of cell surface antigens and specialized function similar to activated NK cells. In addition to conventional cellular targets such as the YAC-1 and MBL2 lymphomas, this cloned line also lysed LPS-activated B lymphocytes. To determine whether NK cells could inhibit B cell function, we have tested the ability of NK clones to suppress Ig secretion in vitro and in vivo. These cloned cells suppressed Ig secretion when they constituted as few as 1% of total cells in culture. In addition, Ig secretion is inhibited in a non-H-2 restricted fashion. We suggest that NK -like cells may suppress Ig secretion through the recognition of specific molecules other than H-2 expressed on the cell surface of activated B lymphocytes.

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0074 NATURAL KILLER CELL ACTIVITY IN TUMOR BEARING MICE, Alfred E. Brown and Nabil Hanna, Smith Kline & French Laboratories, Philadelphia, PA 19101

The effect of a progressively growing tumor on the expression of natural killer (NK) cell activity and on the ability of biologic response modifiers to activate NK cells in vivo and in vitro was investigated. The NK cell-mediated cytotoxicity of C3H mice bearing a relatively small (< 1.0 cm; Stage I) UV-2237 fibrosarcoma was comparable to that observed in age-matched controls. Mice bearing larger tumors (1.5-2.0 cm; Stage II) exhibited a marked inhibition of NK cell-mediated cytotoxicity, which could be elevated to control levels by removal of the nylon wool adherent cells prior to in vitro testing. At terminal stages (tumor size > 3.0 cm; Stage III), however, the depressed NK cell activity persisted even after selective elimination of nylon wool adherent cells. In vitro incubation with the interferon inducing agent poly I.C.failed to stimulate NK cell-mediated cytotoxicity in spleen cells obtained from tumor bearing mice (Stages II and III). All of the suppressive activity at Stage II was mediated by nylon wool adherent cells and could be partially reversed by in vivo treatment of mice with indomethacin. Conversely, treatment of mice bearing Stage III tumors with indomethacin or removal of nylon wool adherent cells in vitro did not enhance the activation of NK cells by poly I.C. Elucidation of the mechanism of NK cell suppression during tumor progression may contribute to a better control of tumor growth and dissemination.

0075 INDUCED NATURAL KILLER ACTIVITY IN HODGKINS AND NON-HODGKINS LYMPHOMA, David B. Jones, Kathleen Higginson and Dennis H. Wright, University Department of Pathology, General Hospital, Southampton, SO9 4XY, UK.

This abstract reports the results of a preliminary study of induced NK activity in Hodgkin's disease (HD) and non-Hodgkin's lymphoma (NHL).

Mononuclear cells prepared from 15 HD spleens showed a significant increase in NK activity at a range of effector target ratios when compared with 20 controls. In HD, the presence of histologically recognisable tumour in the spleen or the presence of 'B'-symptoms (weight loss, night sweats, pruritis) was associated with NK levels which did not differ significantly from normal. Five spleens obtained from patients with NHL gave values for cytotoxicity against NK sensitive targets significantly below the normal values. The induction of cytotoxicity by co-culture with mitomycin C treated lymphoblastoid cells, polyclonal activators which induce active NK cells via the production of Interferon gave comparable results. Thus, Hodgkin's spleens were easily induced to give high levels of cytotoxicity over controls cultured in human AB serum. NHL spleens could not be induced. Spleen mononuclear cells from HD and NHL were also tested for their ability to lyse antibody coated chick red blood cells and Chang liver cells. The results paralleled those obtained with the K562 target. The data presented indicates that reactive populations present in the spleen in HD and NHL are qualitatively different. Elevated natural killing in HD spleen is significant with regard to the role of polyclonal lymphocyte activation in the disease pathogenesis.

0076 HUMAN NATURAL KILLER (NK) CELLS MAINTAINED IN CONTINUOUS CULTURES WITH IL-2, John R. Ortaldo, Tuomo T. Timonen, and Jose A. Alvarez, LID, NCI, Bethesda, MD 20205
Recently NK and K cells, which mediate antibody-dependent cell-mediated cytotoxicity (ADCC), were found to have a characteristic morphology of large granular lymphocytes (LGL) with indented nucleus, high cytoplasmic: nuclear ratio, and azurophilic granules in the cytoplasm. A major limitation for the detailed analysis and characterization of NK cells has been that they represent a small proportion of lymphoid cells in the peripheral lymphoid organs. We present here direct evidence that human NK cells as well as typical T cells can be cultured in the presence of IL-2 and these cultured cells possess distinct cytotoxic capabilities. Highly enriched populations of LGL, which are virtually devoid of mature typical T lymphocytes (as enumerated by morphological and surface antigen analysis using monoclonal antibodies, e.g. OKT3), and of T cells (greater than 95% sheep erythrocyte-forming and devoid of LGL and NK/K activities) were both propagated in the presence of crude or partially purified IL-2. Cultures of LGL could be initiated consistently even in the absence of lectins and the cultured LGL retained their characteristic morphology and cytotoxic activity. However, within 7-10 days after initiation, the cultured LGL changed in surface phenotype to become similar to cultured T cells, reacting with OKT3 and losing reactivity with OKT10 and OKM1.

Thus, we have demonstrated that highly purified NK cells, in addition to T cells, can be expanded on IL-2. These results rule out the possibility that small numbers of T cells were responsible for growth or cytotoxicity in NK cells. Our demonstration of the lack of stability of surface markers during culture makes the analysis of NK cells in cultures from a mixed population very difficult.

0077 ROLE OF NK CELLS IN ANTITUMOR AND ANTIMETASTATIC DEFENSES. Elieser Gorelik and Ronald B. Herberman, Laboratory of Immunodiagnosis, NCI, Bethesda, MD 20205 U.S.A.
The possible participation of NK cells in the antitumor immune surveillance was studied in two experimental models: urethane-induced carcinogenesis in the lungs and x-irradiation-induced leukemogenesis. We have found: a) the chemical carcinogen urethane (1mg/g), or fractionated leukemogenic doses of irradiation (179 R x 4), induced profound and prolonged suppression of NK reactivity of mice; b) young mice were more sensitive to both the carcinogenic and NK-suppressive effects of urethane or radiation; c) sensitivity (or resistance) of mice of different genotypes to the carcinogenic and NK-suppressive effects of urethane was positively correlated; d) transplantation of normal bone marrow or spleen cells into urethane-treated or irradiated mice could inhibit carcinogenesis in these mice. Further, using a radioisotopic technique, we have found that NK cells may participate in the elimination of tumor cells from the local site of transplantation. Selective suppression of NK reactivity of mice by treatment with anti-asialo GM1 serum suppressed the elimination of tumor cells from the blood stream and dramatically increased the number of metastatic foci that developed in the lungs. Suppression of NK reactivity of C57BL/6 mice bearing 3LL tumor substantially increased the growth of postoperative spontaneous metastasis in these mice. These data indicated that NK cells may participate in defense against the development of primary tumors and the metastatic spread of tumors.

0078 UNI- OR MULTI-POTENTIALITY OF CYTOTOXIC CELLS STUDIED AT THE SINGLE CELL LEVEL USING THE TWO-TARGET CONJUGATE ASSAY. Thomas P. Bradley and Benjamin Bonavida, Dept. of Microbiology and Immunology, UCLA School of Medicine, Los Angeles, CA 90024

Human peripheral blood lymphocytes (PBL) mediate several different cytotoxic reactions (i.e., Natural Killing (NK), Antibody Dependent Cellular Cytotoxicity (ADCC), and Lectin Dependent Cellular Cytotoxicity (LDCC)). Studies at the population level, using the chromium release assay, made it difficult to resolve the question of whether the effector cells involved in each of these cytotoxic reactions are distinct, or whether certain effector cells can mediate more than a single cytotoxic reaction. Thus, the two-target conjugate assay involving two different targets bound simultaneously to a single effector cell (described in J. Immunol. (1981) 126:208) was used to directly solve the question of uni- versus multi-potentiality of effector cells. PBL enriched on Percoll gradients to yield large granular lymphocytes (LGL) were used as a source of cytotoxic cells. K562 or Molt were used as NK targets, antibody (rabbit anti-RAJI) treated RAJI for ADCC targets, and Con A, PHA or Periodate-treated RAJI as LDCC targets. Results with two-target conjugates show that single effector cells lyse both NK and ADCC targets. However, NK effectors which kill K562 did not kill LDCC targets. These results unequivocally demonstrate that NK and ADCC cytotoxic reactions are mediated by the same effector cells, whereas the LDCC effector cell is distinct. The multi-potential activity of certain effector cells suggests that multiple receptors are involved in activating the lytic pathway of cytolysis.

(Supported by grant CA 12800 from the NCI, USPHS)

Clinical Classification of Tumors

0079 EXPRESSION OF T-CELL DIFFERENTIATION ANTIGENS BY CUTANEOUS T-CELL LYMPHOMA AND NORMAL AND NEOPLASTIC CELLS OF MONOCYTE/MACROPHAGE AND LANGERHANS LINEAGE, Gary S. Wood, Noel L. Warner, David G. Deneau, Richard A. Miller, Ronald Levy, Richard T. Hoppe and Roger A. Warnke, Stanford University, Stanford, CA 94305 and Becton Dickinson Monoclonal Center, Mountain View, CA 94043.

Monoclonal antibodies were used to immunohistologically characterize cellular antigen expression in 25 patients with cutaneous T-cell lymphoma. Although all cases expressed the Leu-2^-/Leu-3^+ phenotype characteristic of helper T cells, four subtypes were defined based upon variable expression of Leu-1 and Ia. In individual patients, the phenotype was constant irrespective of body compartment sampled or interim therapy. Ia^+ non-T cells typically constituted one third of the cellular infiltrate. Along with neoplastic cells, $Ia^+/T6^+$ dendritic cells were observed within Pautrier microabscesses, dermis and individually throughout the epidermis. Dendritic cells and macrophages were also observed to express Leu-3. Subsequent studies documented expression of Leu-3 by normal and neoplastic cells of monocyte/macrophage and Langerhans lineage including the U937 cell line and the cells of histiocytosis X. While monocytes and macrophages were $T6^-$, Langerhans cells and the cells of histiocytosis X were strongly $T6^+$.

0080 STUDIES ON THE PHENOTYPE OF NON-HODGKIN'S LYMPHOMAS. Maria Scouros, Francis Davis, James Reuben, and Richard Ford. University of Texas, M.D. Anderson Hospital, Houston Texas, 77030.

Studies were performed on a spectrum of T cell and B cell type Non-Hodgkin's lymphomas (NHL) obtained from lymph node biopsies and spleens from untreated patients. Conventional cell membrane markers (CM) and a repertoire of monoclonal antibodies (MCA) were compared with the nucleolar antigen (NA) on population of neoplastic cells depleted of accessory cells by various separation procedures. Results of these studies indicated that the T or B cell surface phenotype determined by CM usually corresponded to the presence of a number of normal T or B cell associated antigens by MCA. However, many of the B cell type NHL also contained various antigens associated with normal thymocytes, T cells, or MØ. These studies suggested that more heteroneneity is present within the histopathologically-defined NHL subtypes than has been recognized. In addition, the NA which has been shown to be useful in identifying malignant cells in other types of non-lymphoid tumors was also present in the NHL cells but not in normal or reactive lymphoid cells present in the lymphatous lesions. This antigenic marker is very useful not only for identifying lymphoma cells in mixed lymphoid populations, but also for associating cell surface antigens and receptors with an identifiable non-surface marker associated with the tumor cells. The use of techniques such as these should allow for better lymphoma cell characterization in the future.

0081 IMMUNOHISTOLOGY OF B CELL LYMPHOMAS, H. Stein, J. Gerdes, K. Lennert, D.Y. Mason, M. Naiem, A. Ziegler, and P. Wernet, Institute of Pathology, Univ. of Kiel, Germany, Nuffield Dept. of Pathology, Univ. of Oxford, U.K., and Dept. of Internal Medicine, Univ. of Tübingen, Germany

To improve the systems for classification of B-type non-Hodgkin's lymphomas (NHL), frozen and paraffin sections of more than 150 cases of NHL were stained with a large panel of monoclonal antibodies (MCA) characteristic, or even specific for lymphoid cells, lymph node framework cells, and macrophages. Cells of B-type chronic lymphocytic leukemia (B-CLL) usually showed positive staining for surface (S) IgM and SIgD with only one light chain type and for C3RTo15 (B cell specific), TÜ1, Leu1, HLA-DR, and C3 receptors, but not for monotypic cytoplasmic (C) Ig and OKT11, OKT4, OKT8, TÜ14, or CALLA. Hairy cell leukemia reacted for SIg with one light chain type and for HLA-DR, but not for TÜ1, Leu1, C3 receptors, T-cell antigens, or CALLA. Follicular centroblastic-centrocytic lymphoma (CB-CC) was characterized by the expression of SIg with one light chain type, C3RTo15, TÜ1, HLA-DR, C3b receptors, and CALLA and the absence of Leu1, TÜ33, and SIgD (the latter in nearly all cases). Centrocytic lymphoma (CC) reacted for SIg, C3RTo15, HLA-DR, C3 receptors, and TÜ33, but not for TÜ1, Leu1, or CALLA. Follicular CB-CC and CC were found to have a specific feature: follicle-like (CB-CC) or less sharply defined (CC) collections of dendritic reticulum cells as revealed with the MCA R4/23. Lymphoblastic lymphomas of Burkitt and non-Burkitt B type were shown to be positive for C3RTo15 and CALLA and positive or negative for SIgM, but constantly negative for TÜ1, Leu1, TÜ33, and C3 receptors. The large cell lymphomas without T markers were positive or negative for SIg and HLA-DR and constantly expressed C3RTo15, but not TÜ1, Leu1, TÜ33, or CALLA; three showed J chains.

0082 ANTIGENIC PROFILE OF DENDRITIC RETICULUM CELLS (DRC) AND THEIR OCCURRENCE IN NON-HODGKIN'S LYMPHOMAS (NHL) AS REVEALED WITH MONOCLONAL ANTIBODIES (MCA), J. Gerdes, H. Stein, K. Lennert, M. Naiem, D.Y. Mason, A. Ziegler, and P. Wernet, Institute of Pathology, Univ. of Kiel, Germany, Nuffield Dept. of Pathology, Univ. of Oxford, U.K., and Department of Internal Medicine, Univ. of Tübingen, Germany

Some years ago, DRC were demonstrated in follicular lymphoma. In non-malignant lymphoid tissue, DRC were found to occur only in B-cell areas, i.e., the follicular mantle, germinal center, and primary follicle. In the past, DRC could be demonstrated reliably only by electron microscopy or enzyme cytochemistry. Recently, we succeeded in preparing an MCA, called R4/23, that reacts nearly selectively with DRC. The R4/23 antibody was used in the present study for two purposes: (1) to trace DRC during suspension attempts and (2) to immunostain sections of normal lymphoid tissue and NHL. The R4/23+ DRC suspended from hyperplastic tonsil tissue showed a surprising morphology: they were giant cells, usually with more than 10 nuclei. They proved to be strongly positive for μ, γ, α, C3b, and C3b receptors and weakly positive for HLA-DR, but negative for δ, T-cell antigens (OKT11,4,8) myeloid cell antigens (TÜ5,6,9), macrophage antigens (OKM), and antigens of interdigitating reticulum cells (NA1/34). This indicates that the DRC is a unique cell type. Among the NHL, only follicular centroblastic-centrocytic lymphoma and diffuse centrocytic lymphoma and a few cases of centroblastic lymphoma showed a more or less dense meshwork of DRC, whereas all other types of NHL contained only solitary or no DRC. This confirms the view that the presence of a meshwork of DRC in NHL is a sign of the germinal center cell (GCC) derivation of the tumor cells. In contrast to DRC of normal lymphoid tissue, DRC of GCC lymphomas proved to be negative for μ, γ, α, and C3b; all other antigen reactions were the same.

0083 FLOW CYTOMETRY: A NEW APPROACH TOWARDS CHARACTERIZING LYMPHOMAS, Raul C. Braylan, Neal A. Benson and Virginia A. Nourse, Department of Pathology, University of Florida College of Medicine, Gainesville, FL 32610

Correlated analysis of surface antigens, DNA and light scatter of cells from 22 human B-cell lymphomas was performed by flow cytometry. These lymphomas are defined by the presence of B-lymphocytes bearing predominantly a single surface immunoglobulin light chain (SIg). In addition, these tumors contain varying numbers of non-neoplastic cells (mainly T-cells). SIg and T-cell antigens were labeled with fluorescein-conjugated antibodies. DNA was stained with propidium iodide after ethanol fixation. Analysis was performed with a modified FACS II linked to a microcomputer. By use of SIg and T-cell antigens as markers to discriminate in the same tissue between neoplastic and non-neoplastic cells, increased DNA fluorescence (flow aneuploidy) was observed in the neoplastic cells of 86% of the tumors. B-cell lymphomas contained varying numbers of cells in the synthetic phase of the cell cycle (S cells). In lymphomas containing numerous dividing cells (8 cases), most S cells were SIg-bearing cells. However, in one case there was a high percentage of T-cell antigen-bearing S cells. In 4 cases, discrete populations of diploid and near tetraploid cells bearing the same SIg were observed, most likely indicating that 2 neoplastic clones were present in these tumors. Alternatively, the aneuploid cells may represent cells arrested in the cell cycle. In 8 cases, two subpopulations of cells were detected by light scatter. The presence of discrete populations of small and large cells in these tumors was also observed by independent Coulter volume analysis and confirmed by histology. In all instances, the larger (high scatter) cells were SIg-bearing aneuploid cells.

Immunotherapy

0084 THE IMMUNOTHERAPEUTIC EFFECT OF THE PEPTIDE TUFTSIN ON THE B-CELL LYMPHOMA CELL. George F. Babcock, Jospeh H. Phillips, and Kenji Nishioka, The University of Texas Cancer Center, M. D. Anderson Hospital and Tumor Institute, Department of Surgery, Surgical Research Laboratory, and The University of Texas Health Science Center, Graduate School of Biomedical Sciences, Houston, Texas 77030.

Tuftsin is a physiological tetrapeptide which has been shown to possess immunopotentiating properties including the stimulation of macrophage and granulocyte phagocytosis, migration, bactericidal, and tumoricidal activities. We have also shown that this compound possesses in vivo immunologically mediated anti-tumor activity. In this study, we investigated the effect of tuftsin on the B-cell lymphoma, CH1, grown in syngeneic B10H-2^aH-4^bp/wts (2^a4^b) mice. All mice received 10^6 CH1 cells ($100LD_{50}$) i.v., followed daily in injection tuftsin beginning 24 hr after receiving the tumor. Control animals received the tumor and daily injections with normal saline. Daily treatment with 0.001 or 0.01 µg had no effect while a minimal effect was observed with mice receiving 0.1 µg. Mice receiving 1, 10, or 100 µg of tuftsin daily survived significantly longer than control animals (28±2, 32±3, and 30±1, respectively for tuftsin treated animals compared with 22±1 days for controls). In addition to a massive ascites, all the animals which received CH1 tumor cells, i.v., developed subcutaneous (SC) tumors in several areas. Tuftsin treatment was also shown to significantly reduce the number of mice which developed these SC tumors. Daily treatment with 100, 10 or 1 µg reduced the incidence of SC tumors from 100% to 20, 30, and 60%, respectively. Lower dosages of tuftsin had no effect on the development of SC tumors. Supported in part by grant CA27330 from NCI, DHEW.

0085 LEVAMISOLE EFFECTS IN ATAXIA-TELANGIECTASIA: IN VITRO AND IN VIVO, Richard A. Gatti, Elena Boder and Michael A. Medici, Dept. of Pathology, UCLA Center for the Health Sciences and Dept. of Pediatrics, Cedars-Sinai Medical Center, Los Angeles, CA, 90024

A one-hour incubation (37°C) of lymphocytes from patients with ataxia-telangiectasia (AT) with levamisole (Lev) (10^{7}--10^{9}M) increased the proportions of neuroaminidase-treated E-rosetting cells. Similar effects have been noted by others using thymosin and thymopoietin pentapeptide. Although such effects are also observed with lymphocytes from normal persons, the effects of Lev incubation were much more dramatic in AT patients. In vitro dose response curves suggested that previous therapeutic trials with Lev involved excessive, less than optimal, dosages. On this basis, we treated five AT patients for several years with Lev, using between 0.75 and 2.5 mg/Kg in intermittent six-month cycles. E-rosetting lymphocyte levels and PHA responses improved in association with changes in Lev dosages. Patients had less frequent infections and their neurologic deterioration appeared diminished to parents, neurologists and teachers. When Lev was discontinued, neurologic symptoms once again worsened. Due to the insidiousness of this deterioration and the small numbers of patients available, it may not be possible or practical to objectively document this clinical improvement. Improvement of laboratory parameters has been maintained for over 1½ years, suggesting that Lev may have permanently induced in vivo maturation of a long-lived T-cell population. The mechanism for this may involve cyclic nucleotide metabolism and/or factors which stabilize the movement of cell surface receptors, both of which we have shown to be perturbed in AT and can be influenced by Lev.

0086 ANTIBODY DIRECTED AT T LYMPHOCYTE DIFFERENTIATION ANTIGENS INHIBITS T LEUKEMIA PROLIFERATION IN VIVO AND IN VITRO, Mary E. Kirch, Joan Abbott, Theresa Lee, and Ulrich Hammerling, Sloan-Kettering Institute, New York, NY

To establish a model to study passive immunotherapy using monoclonal antibody (McAb) we have transplanted murine leukemias into hosts congenic for Thy 1. McAb directed at the lymphocyte differentiation antigen was then tested for its effect on tumor growth. Studies with the A strain-derived tumor ASL.1 (Thy 1.2) transplanted into A/Thy 1.1 hosts have yielded the following information: 1. Anti-Thy 1.2 McAb eradicated tumor inocula of 500 times the LD_{50} when the antibody was administered within 24 hours of the tumor; cell numbers in excess of this responded briefly to therapy, but ultimately killed the host. 2. Between 24 and 48 hours, the tumor becomes refractory to therapy, a fact that is not attributable to differences in the number of tumor cells present. 3. Although IgG_3 McAb routinely cured 80-100% of mice, the effect of IgM antibody was to prolong host survival without curing. 4. Animals reinoculated with tumor cells after successful therapy died within the same time span as previously untreated controls.

ASL.1 tumor cells that have been established in tissue culture were used to evaluate direct effects of antibody in the absence of host influences. As we observed in vivo, IgG_3 anti-Thy 1.2 inhibited cell proliferation in vitro. The inhibition was cell density dependent, and did not appear to be related to variant selection.

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0087 BINDING OF PARENTERALLY ADMINISTERED ANTI-DNP TO ITS IN VIVO TARGETS. Veronica Yakoleff Greenhouse and Carlos Larralde, Instituto de Investigaciones Biomedicas, U.N.A.M., Mexico, D.F., 04510 Mexico.

To determine quantitatively the efficiency with which parenterally administered antibodies bind to their in vivo targets and the effect of compartmentalization on the binding, dinitrophenylated silk thread and untreated silk were implanted into selected organs of guinea pigs which were subsequently injected i.v. with tracer amounts of purified ^{125}I-antiDNP. In the kinetic studies (subcu. and i.p.), at various time intervals, plasma samples were taken and the threads were retrieved. After surgical implantation into the internal organs, the plasma samples were taken at the time of sacrifice, 6 or 24 hr. post-injection.

Specific binding ($CPM_{silk\text{-}DNP}$ - CPM_{silk}) was obtained in all organs. The kinetic studies showed that the binding reached a maximum of 10^{-3}% dose injected at 24 hr. which was maintained for 4 days and declined thereafter. At 24 hr. this same low level of binding was obtained in the internal organs. A 10-fold increase in the amount of antibody administered also produced a binding of 10^{-3}% dose injected.

From these data, it appears that binding of parenterally administered antibodies is not continuously accumulative and that methods to increase access of antibody to the tumor are necessary to improve binding.

Tumor Associated Antigens

0088 MURINE T LYMPHOMA "IgT" IS IDENTICAL TO LEUKEMIA VIRUS GP70, Stephen M. Baird, Veterans Administration Medical Center, La Jolla, CA 92037 and William C. Raschke, La Jolla Cancer Research Foundation, Cancer Research Center, La Jolla, CA 92037.
Chicken anti-mouse immunoglobulin sera have been reported to react with molecules in detergent extracts of radioiodinated mouse T cells that resemble immunoglobulin molecules. These molecules, after thiol reduction, have molecular weights of about 70,000 daltons and cross-react with IgM heavy chains. A light chain of about 20,000 daltons is sometimes found also. One type of mouse T cell that consistently exhibits these molecules recognized by chicken anti-mouse immunoglobulin is the thymic lymphoma cell. Thymic lymphoma cells also express a large amount of mouse leukemia virus envelope glycoprotein, gp70. Using well characterized chicken anti-mouse immunoglobulin and rabbit anti-gp70 sera and two-dimensional gel electrophoresis we have precisely defined the molecules precipitated from extracts of radioiodinated WEHI-22 cells by these two antisera. The two patterns of the proteins with their multiple spots due to glycosylation are identical in the 70,000 dalton region. Normal rabbit serum and rabbit anti-mouse immunoglobulin precipitate no molecules in this molecular weight region. Cell surface IgM from B cell lymphomas are clearly distinct from the gp70 of WEHI-22. These observations conclusively demonstrate that the molecules on WEHI-22 cells recognized by chicken anti-mouse immunoglobulin serum are not immunoglobulin heavy chains, but instead viral gp70. These results further show that the two antisera used in this study recognize different determinants on the same gp70 molecule and imply that gp70 on WEHI-22 cells and mouse IgM share antigenic determinants.

0089 MONOCLONAL ANTIBODIES TO LEUKEMIA ASSOCIATED ANTIGENS· Ronald Billing, Chun Deng, Paul Terasaki; Dept. of Surgery, University of California, Los Angeles, CA 90024

We have produced 9 monoclonal antibodies to leukemia associated antigens that are useful for classifying leukemias into clinical subgroups. 1. CALL1 reacts with CALL and CML-blast crisis lymphoid type. 2. CALL2 reacts with T ALL. 3. CAML1 reacts with 90% of AML cases but not CML or lymphoid leukemias. 4. CBL1 reacts with leukemia blast cells but not CLL. 5. CSZ reacts with Sezary cells and some T leukemias but not normal cells. 6. CG1 reacts with certain myeloid leukemias and myeloid lines such as HL60, K562 and U937 and mature granulocytes but not non myeloid cells. Three other monoclonal antibodies are described that recognize antigens present on either normal B and T lymphocytes that are also useful in subclassify leukemias into T and B subgroups. An antibody raised to Sezary cells reacts with all T lymphocytes and T leukemias tested but appears to be different from other pan T monoclonal antibodies previously reported. Two B cell monoclonal antibodies are described, one CIA is anti Ia (gp 27,35), the other CB1 reacts with B lymphocytes and some Ia positive leukemia cells but does not react with the Ia gp 27,35 antigen. Serological testing of these antibodies agaisnt a panel of normal blood cells and leukemia cells and molecular characterization of the antigens detected will be described.

0090 STUDIES OF HOST-TUMOR INTERACTIONS DURING THE PROGRESSIVE GROWTH OF MURINE LYMPHOID TUMORS, Lionel A. Manson, The Wistar Institute, Philadelphia, PA 19104
We have been studying the tumor-host interaction during the growth of two lymphoid tumors from small inocula (10^3 cells) in their host of origin as a model for the events taking place during the growth of an autochthonous tumor. Both tumors are immunogenic in the host of origin (P815Y in DBA/2 mice and EL-4 in C57BL/6 mice). Yet 10^3 cells inoculated i.p. will result in the death of the animal after 3-4 weeks. In previous studies, killer T cells were found in the expanding ascites mass on day 10 after tumor inoculation that would kill the tumor cells obtained from the same ascites. On day 16 the tumor cells had modulated and were resistant to these in vivo killer cells. In parallel we have found an immunoglobulin (Ig) accumulating on these tumor cells during in vivo growth. Day 16 tumor cells, which are "killer cell" resistant, have a maximum amount per cell of this Ig firmly bound to the cells. This Ig prevents the cells from taking up additional amounts of H-2K/D antibodies. This Ig reacts positively with an ^{125}I-anti-Fab, and an ^{125}I-anti-IgM, but not with an ^{125}I-anti-IgA nor with an ^{125}I-anti-IgG. This Ig can be eluted from the in vivo grown tumor cells with 3M NaSCN. The eluted Ig preparation shows similar binding specificities to the specificity of the "killer T cells", i.e., it appears to bind to cells sensitive to the killer cells and not bind to tumors resistant to the killer cells. It is not an anti-H-2K/D antibody. Data will be presented suggesting that the tumor-specific antigen(s) in such systems is a gene product that associates with the H-2K and D gene products in the surface membranes of the cells.

0091 TRANSFERRIN RECEPTOR EXPRESSION IN CONCANAVALIN A STIMULATED RAT LYMPHOCYTES IS IL-2 DEPENDENT, Thomas A. Hamilton, St. Jude Children's Research Hospital, Memphis, TN 38101

The serum iron carrying glycoprotein transferrin is required for maximal cellular proliferation in vitro and the cell surface receptor for transferrin is expressed on all proliferating cells in culture and on mitogen stimulated but not resting normal lymphocytes. We have examined the kinetics of transferrin receptor expression in Concanavalin A (Con A) stimulated rat lymphocytes as determined by the binding of radiolabeled transferrin and by the detection of the 95 k dalton transferrin binding cell surface protein. While quiescent lymphocytes had no detectable receptor activity, Con A treatment led to acquisition of the receptor with kinetics which coincided with the expression of DNA polymerase α activity and the associated ^{3}H thymidine incorporation into DNA. Both transferrin receptor expression and DNA synthetic activity peaked at 48 hrs following stimulation and by 96 hrs had declined to near the original levels. DNA replication and cellular proliferation could be restimulated in such cultures by the addition of partially purified interleukin 2 (IL-2). Under these conditions, both transferrin binding activity and the expression of receptor protein were also reacquired. Thus, transferrin receptor expression was independent of blastogenesis but dependent upon the cellular proliferation produced by the interaction of blast cells with IL-2.

0092 A MONOCLONAL ANTIBODY TO THE COMMON ALL ANTIGEN WHICH DOES NOT MEDIATE ANTIGENIC MODULATION IN VITRO, Tucker W. LeBien, Daniel R. Boue', J. Garrett Bradley, and John H. Kersey, University of Minnesota, Minneapolis, MN 55455

Immunization of mice with the pre-B ALL cell line NALM-6, followed by spleen cell fusion with NS-1 myeloma cells, resulted in the production of a monoclonal antibody designated BA-3. Extensive side-by-side serologic comparisons of BA-3 with the anti-common acute lymphoblastic leukemia-associated antigen (CALLA) monoclonal antibody J-5 (provided by J. Ritz, Sidney Farber Cancer Inst.) was undertaken. Cells examined by indirect immunofluorescence included established leukemic cell lines, malignant cells from patients with newly diagnosed leukemia/lymphoma, and normal hematopoietic tissues (bone marrow, liver, spleen, thymus, and peripheral blood). In all experiments the cellular distribution of the antigens recognized by BA-3 and J-5 were identical when analyzed by microscopy and the FACS. Iodination of NALM-6 cells, followed by radioimmuneprecipitation and SDS-PAGE, indicated that BA-3 and J-5 both precipitated glycoproteins of Mr 100,000 daltons. Furthermore, competitive binding studies using ^{125}I-labelled BA-3 indicated that BA-3 and J-5 were probably binding to the same epitope on CALLA. However, additional studies with BA-3 failed to demonstrate antigenic modulation of CALLA in vitro. Cells tested in antigenic modulation experiments included NALM-1, NALM-6, DAUDI, REH, and malignant cells from two newly diagnosed ALL patients. Whether this difference between BA-3 and J-5 is explainable on the basis of subclass (BA-3 = IgG2b, J-5 = IgG2a) and/or antibody affinity is not known. This finding may warrant a reassessment of the efficacy of anti-CALLA reagents in serotherapy of human leukemia. (Supported by NIH grants)

0093 ISOLATION AND CHARACTERIZATION OF H-2 SOMATIC CELL VARIANTS OF AN ERYTHROLEUKEMIA CELL LINE. T.A. Potter, T.V. Rajan, and T.H. Hansen. Dept. of Cell. Immunology, Merck Sharp and Dohme Research Laboratories, Rahway, N.J. 07065 and Dept of Pathology, Albert Einstein College of Medicine, Bronx, N.Y. 10461

H-2 variant cell lines isolated from a (BALB/c x BALB.B)F_1 ($\underline{H\text{-}2}^{d}/\underline{H\text{-}2}^{b}$) in vitro erythroleukemia fall into one of 3 classes: 1) cells which fail to express any of the products of the chromosome on which the gene coding for the antigen selected against resides, i.e., Haplotype loss variants; 2) cells which lack the entire molecule which bears the antigenic site selected against, but express other closely linked genes of that haplotype, i.e., Molecular deletion variants; 3) cells which have lost the antigenic site with which the selecting monoclonal antibody reacts, but have retained the expression of specificities present on the same molecule and recognized by other monoclonal antibodies and/or conventional alloantisera. These "structural" variants are only obtained after mutagenesis with agents such as ethylmethane sulfonate.

Quantitation of the levels of expression of H-2 antigens coded for by the unselected chromosome and the H-2K linked enzyme, Glyoxalase 1, suggests that some of the haplotype loss variants have become homozygous for the unselected chromosome.

Further data on the mechanism by which the H-2 variants arise, and characterization of the structural variants will be presented.

0094 BIOCHEMICAL DIFFERENCES BETWEEN THE H-2K AND H-2D GENE PRODUCTS AND THE H-2 RESTRICTED KILLING OF MOLONEY VIRUS-INDUCED LYMPHOMAS, Marco A. Pierotti, Dario Ballinari, Mario P. Colombo and Giorgio Parmiani, Istituto Nazionale per lo Studio e la Cura dei Tumori, Via G. Venezian 1, 20133 Milan, Italy.

In the H-2 restriction of the killing of Moloney virus-induced lymphomas the active molecule is the D-coded when H-2^b tumor cells are employed and the K-coded in the case of H-2^d lymphomas. We have first studied the in vivo biosynthesis of H-2 and viral env gene products expressed by the Moloney virus-induced lymphoma cells and the role of the carbohydrates in their cell surface expression. The results indicated a different glycosilation for the H-2K and H-2D antigens of the H-2^b and H-2^d haplotypes. In particular K^d and D^b, the H-2 molecules active in the restriction mechanisms with Moloney virus-induced tumors, were characterized by three carbohydrate prostetic groups at variance with K^b and D^d which have been shown to carry only two carbohydrate chains. The kinetics of the processing of the different antigens were also investigated. K^d and D^b molecules were processed more slowly than the products of the homologous H-2 regions and their maturation appeared coincident with that of the env viral gene precursors. The biochemical findings were considered in the light of the different susceptibility of the lymphoma cells, grown in presence of tunicamycin, to restricted anti-tumor and to alloimmune T-lymphocytes. These latter experiments showed that alloimmune CTL were still able to lyse the tumor cells whereas the reactivity of syngeneic CTL to lymphomas was abolished.

0095 THE STRUCTURAL GENES FOR THE HEAVY CHAINS OF THYMUS-LEUKEMIA ANTIGENS ARE NOT REITERATED, EDWARD P. COHEN, RYSZARD SLOMSKI AND CHRISTINE MARTENS, UNIVERSITY OF ILLINOIS, CHICAGO, IL 60612

Thymus-leukemia (TL) antigens are on the surface membranes of some but not all mouse leukemias and "immature" mouse T cells. At the membrane, the molecule consists of a light chain of 12,000 daltons and a glycosylated heavy chain of 47,000 daltons forming TL antigenic determinants. We obtained mRNA specifying the heavy chain of TL antigens by isolating poly(A)-containing RNA from polysomes of ASL-1 cells, a TL(+) mouse leukemia cell line. Polysomes forming TL antigens were immunoprecipitated with antiserum specific for TL 1,2,3 antigenic determinants. The antiserum was produced in strain A TL(-) congenic mice injected with TL(+) thymus cells from strain A mice. The mRNA obtained directed the synthesis in a reticulocyte lysate system of protein of 42,000 daltons, the non-glycosylated heavy chain. ^{32}P-labeled cDNA was prepared from the mRNA with reverse transcriptase. In gel electrophoresis, cDNA migrated as a predominant 17S species equivalent to TL-mRNA. High molecular weight DNA from ASL-1 cells was digested with EcoRI, Bam I or Hind III restriction endonucleases. The fragments were separated by gel electrophoresis before hybridization with cDNA for TL antigens. Using the Southern "blot" technique, cDNA for TL antigens hybridizes with two separate DNA segments in each restriction digest. Using solution hybridization methods, cDNA for TL antigens hybridizes with mouse DNA according to single copy kinetics. The experimental approaches used reveal the presence of one, or perhaps two genes for this integral membrane protein. Supported by USPHS grant number Ca 27579-02.

0096 LOSS OF $D^{\underline{s}}$ EXPRESSION ON SJL/J LYMPHOMAS: EFFECTS ON TUMOR CELL PROPERTIES, J. Lightbody, K.W. Beisel and S.P. Lerman, Wayne State Univ., Detroit, MI 48201.

Investigations have been undertaken to examine variations in H-2 $K^{\underline{s}}$ and H-$2D^{\underline{s}}$ antigenic expression in SJL/J lymphomas (reticulum cell sarcomas) transplanted into SJL/J mice. A panel of appropriate H-2 alloantisera and monoclonal antibodies were used in both serological and immunochemical assays. All tumors were freed of contaminating host cells by passage into $(C57BL/10 \times SJL/J)F_1$ mice and by treatment with appropriate antisera and complement. The H-$2K^{\underline{s}}$ glycoproteins were detected in all tumors analyzed. The H-$2D^{\underline{s}}$ molecules were undetectable on the membrane in the long term transplantable tumors, SJL/SJL and RCS5. Shorter term transplantable tumors RCSW1, RCSW8 and RCSW20 lacked serologically detectable $D^{\underline{s}}$ glycoprotein. However, immunochemical techniques demonstrated the presence of $D^{\underline{s}}$ molecules in these three lines. Interestingly, in the RCS5 tumor monoclonal anti-$D^{\underline{s}}$ antibodies precipitated a protein which was not bound by the lentil lectin column. Loss of expression of $D^{\underline{s}}$ occurred gradually during the first several transplants from the original spontaneous tumor. Those tumors which lacked $D^{\underline{s}}$ expression grew more vigorously as indicated by increased lymphoid organ weights and shorter intervals between transplantations. We are hypothesizing that during passage of the tumors a population of cells has developed which is unable to insert the D molecule into the tumor cell membrane, even though it is still capable of synthesizing this antigen. This new population appears to have a selective growth advantage over H-$2D^{\underline{s}}$ positive cells.
(Supported in part by NIH CA 27047, CA 29111 and Childrens' Luekemia Foundation of Michigan).

0097 SEPARATION AND CHARACTERIZATION OF MULTIPLE HUMAN Ia-LIKE ANTIGENS, Deborah Shackelford and Jack Strominger, Harvard University, Cambridge, MA 02138

Multiple Ia-like antigens expressed on human B lymphoblastoid cell lines have been separated using human alloantisera and monoclonal antibodies. On cell lines which type homozygous for DR2 or DRw6, three Ia-like antigens have been identified. One of the three has been identified as the DC1 (MT1, MB1, LB12) antigen using alloantisera and the monoclonal antibody Genox 3.53. This antigen represents only 10-20% of the total Ia-like antigens on B cell lines. The DR allodeterminants are expressed on one or both of the other two molecules. These two molecules appear to have the same heavy chain but different light chains. These two antigens from DRw6 positive cell lines can be separated using the monoclonal antibodies, L203(L243), L227, and LKT111. Both the heavy and light chains of the DC1 molecule differ from the DR subunits as assessed by two-dimensional gel electrophoresis and peptide mapping. The differences are not due to glycosylation. Thus, in total, these B cell lines express at least 2 heavy chains and 3 light chains. Like the DR antigen, the DC1 heavy chain appears invariant between cell lines whereas the light chain is structurally polymorphic. Another antigen, MB2, which may be allelic to DC1, has been identified on another cell line. The DC1 molecule can be distinguished functionally as well as structurally from the DR molecule and may represent the human equivalent of the murine I-A antigen.

0098 SEROLOGICAL ANALYSIS OF IE-LIKE Ia MOLECULES ON SPONTANEOUS RETICULUM CELL SARCOMAS OF SJL/J MICE, Stan Wilbur, Ornella Marelli and Benjamin Bonavida, Dept. of Microbiology and Immunology, UCLA School of Medicine, Los Angeles, CA 90024

SJL/J mice mount a T-lymphocyte proliferative response to syngeneic spontaneous reticulum cell sarcomas (RCS) in mixed lymphocyte tumor cultures (MLTC). Previous cellular studies from this laboratory examined the antigens on the RCS which stimulated this syngeneic tumor response. It was found that the tumor expresses antigens which mimic normal allogeneic Ia molecules of the IA/IE hybrid type. Normal SJL/J cells do not synthesize the IE component, so consequently they fail to express this Ia molecule. It was postulated that the RCS can make this component and this allows for the expression of a seemingly allogeneic Ia molecule of the $IA^{S}IE^{?}$ haplotype (J. Exp. Med. 153:501). Monoclonal anti-Ia.7 antibodies to the IE derived polypeptide are used in the present study to identify this specificity on the RCS in both lytic and direct binding assays. The RCS could absorb lytic activity of the anti-Ia.7 antibodies, but not monoclonal antibody against an unrelated IA subregion molecule. Normal SJL/J lymphocytes were totally ineffective. In addition, co-incubation with RCS, but not normal SJL/J lymphocytes, inhibits the lysis of CBA lymphoid cells by anti-Ia.7. Direct binding was assessed by measuring ^{125}I-protein binding to cells pretreated with anti-Ia.7. Significant binding was demonstrated on RCS, yet not on host SJL/J lymph node cells. These data provide further evidence that an allogeneic-like hybrid Ia molecule is expressed on the membrane of the RCS, but not on normal SJL/J tissues.

(Supported by CA-19753 and CA-24314 from NCI)

0099 ROLE OF HYBRID IA/IE Ia ANTIGENS ON RETICULUM CELL SARCOMAS (RCS) IN THE INDUCTION AND REGULATION OF THE SJL/J MIXED LYMPHOCYTE TUMOR INTERACTION (MLTI), Elizabeth Wayner, Stanley Wilbur and Benjamin Bonavida, Department of Microbiology and Immunology, UCLA School of Medicine, Los Angeles, CA, 90024. Cells derived from SJL/J RCS induce pronounced in vivo and in vitro proliferation of syngeneic $Ly1^{+}$ T lymphocytes. Hybrid Ia antigens of the IA/IE subregion which are not expressed by SJL/J, in particular the α chain Ia.7 specificity, have been implicated in vitro SJL/J T lymphocyte recognition and binding of RCS (J. Exp. Med.,153: 501, 1981). In this study the role of these antigens in the induction and regulation of the MLTI was examined. Removal of CFe phagocytic but not Thy 1.2^{+} stimulators inhibited the MLTI. This could be greatly enhanced at high responder:stimulator ratios,CFe treatment of responders and could be reconstituted by adherent RCS derived cells. Addition of monoclonal anti-Ia.7 also significantly inhibited MLTI proliferation. Further, Thy 1.2^{+}, CFe non-phagocytic suppressor cells were generated in the MLTI, the action of which was mediated by soluble factor. Suppression could be enhanced by increasing the number of stimulators in the test MLTI which suggested some interaction between suppressors +/or factor and antigens present on the RCS. In fact, MLTI suppressors were not RCS specific,but exhibited the same cross-reactivity with Ia.7 bearing cells as sensitized T lymphocytes in cell binding assays. These findings implicate RCS derived CFe phagocytic cells and IA/IE antigens in the in vitro MLTI T lymphocyte activation. These studies also provide clear evidence for regulation of the anti-RCS response during the MLTI which may also involve antigens of the IA/IE subregion of the murine H-2.

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0100 MODULATION OF MEMBRANE (mb) TUMOR ASSOCIATED ANTIGENS (TAA) REGULATES THE TUMOR PLASMACYTIC (P) SECRETION FUNCTION, Clara G. Bell and Edward Andrews, U of I and CRC. P amplification, blockade & transplantation resistance (TpR), like B cell maturation to clonal dominance & B & T cell interaction & function, are mediated by cell mb antigens (A); loss of ability to maintain normal cellular interaction reflecting a basic difference between the mb of a P cell, expressing TAA & its normal B counterpart, expressing mb A. Mb phenotype similarities of mouse BALB/c P $\alpha(1\rightarrow3)$-epitope-specific ($\alpha3$) antibody (Ab) clones, 104 E($\alpha\lambda_1$) & J-558 ($\alpha\lambda_1$), which express shared V_H (IdX) & individually unique D_H (Id1) Ig idiotypes (Id), acting as TpR TAA, & BALB/c $\alpha3$ normal B cell counterpart, which express cross-reactive IdX & Id1 mb A, were studied in relation to function at the single cell & population levels. A FACS analysis delineated similar mb A, at dissimilar densities, on the P & normal clones. In clonally adoptive differentiation to monoclonality, the V_H-D_H gene encoded mb A were expressed functionally in normal-, were diminished in germ-free-, & were first elevated than diminished in auto anti-IdX or Id1-, or passive anti-IdX or Id1 administered BALB/c. Earliest life treatment resulted in longest mb A diminuition & arrest in the B- differentiation to functional Ab secretion & to altered response profiles with transition to non-IdX-Id1 expression, albeit the functionally secreting normal B clones differentiate from mb A(-) to mb A(+) to a loss of mb A with the acquisition of secretory immunocompetence. FACS sorted P according to mb A (TAA) densities exhibited on cloning heritable differences in their mb A TAA which corresponded with their rates of myeloma protein (MP) Ab secretion. When transplanted to auto anti-IdX/Id1 reactive BALB/c each evoluted to intermediate or non-expressing sublines of diminutive or no MP Ab secretion but none to λ_1 secretion. Mb A & secretion function concomitantly on P but not on normal B.

0101 STUDIES ON THE BIOSYNTHESIS AND PROCESSING OF Leu-1, A HUMAN T LYMPHOCYTE ANTIGEN, Yehudit Bergman and Ronald Levy, Stanford University, Stanford, CA, 94305
In this study, the biosynthesis, processing and intracellular transport of Leu-1 T lymphocytic antigen were analyzed. The Leu-1 antigen is present on the surface of human mature T lymphocytes and leukemia T lymphocytes. Matabolically labeled cells yield multiple forms of intracellular Leu-1 molecules. The various Leu-1 forms were characterized according to their kinetics of metabolic labeling, their carbohydrate structures and their rate of intracellular transport. The antibiotic tunicamycin and the enzyme Endo-H were used. A 58,000 MW polypeptide backbone is initially glycosylated with high mannose type oligosaccharides, giving the molecule an AMW of 62,000. The sugar chains are progressively modified to complex type carbohydrates within 20 minutes. The mature 67,000 MW glycoprotein arrives at the cell surface over a period of 30 minutes after synthesis. Similar results and time course to ours were obtained for other cell surface glycoproteins including HLA and vesicular stomatitus virus glycoprotein.

0102 THE RELATIONSHIP BETWEEN TRANSFORMING GENES AND TUMOR ANTIGENS, Jeffrey A. Drebin, Ben Zion Shilo*, Robert A. Weinberg* and Mark I. Greene, Harvard Medical School, Boston, MA 02115; *Department of Biology, Massachusetts Institute of Technology, Cambridge, MA 02139.

Transformed cells express a variety of cell surface structures which can be recognized by discrete subsets of immune T cells. We have studied the relationship between transforming genes and the appearance of a tumor specific transplantation antigen (TSTA) by evaluating the capacity of cloned DNA obtained from a methylcholanthrene tumor to both transform NIH3T3 cells following transfection onto cell monolayers, and to result in the appearance on such cells of TSTA line products. Using a methylcholanthrene (MCA) tumor of C3H($H\text{-}2^k$) origin, a cloned DNA segment was transfected into NIH3T3 cells. A clone of transformed NIH3T3 cells which developed was found capable of immunizing for specific immune responses to the MCA tumor. Moreover transfected fibroblasts which received transforming DNA from an irrelevant tumor (human colonic carcinoma) were unable to immunize against the MCA tumor. MCA primed mice could reject tumors which resulted from transfection of the relevant DNA but could not reject tumors which resulted from transfection of irrelevant DNA. Collectively these results indicate a linkage between the transforming element and the genetic capacity to encode TSTA. Whether or not the information for TSTA is directly encoded in the transfected DNA is not yet known.

Monoclonal Tumor-Specific Antibodies

0103 UNEXPECTED CELL SURFACE MARKERS ON CHRONIC LYMPHOCYTIC CELLS by *Frances S. Ligler.Central Research & Dev. Dept.,E.I.du Pont de Nemours & Co., Glenolden,PA,19036

Mononuclear cells from patients with chronic lymphocytic leukemia (CLL) were analyzed for both markers on the tumor cells and numbers of residual normal T cells and macrophages using monoclonal antibodies and the fluorescence activated cell sorter. All the CLL cells were monoclonal with respect to light chain type and were HLA. DR-positive. Approximately 2/3 of the CLLs were stained using PI153.(1)Approximately 1/3 of the CLLs stained with two antibodies characteristic of monocytes,OKM1 (2) and 63D3 (3),and/with the antibody J-5 against the common acute leukemia antigen(4) There was no correlation between the presence of these surface markers and clinical stage by the Rai Classification,white blood count,period since initial diagnosis,or therapy administered. Exposure to other mouse antibodies of the same heavy chain subclass did not produce similar fluorescence. If these markers are products of gene derepression typical of tumor cells,great care must be taken in using monoclonals as for diagnosis and typing of lymphoid tumors.

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0104 MONOCLONAL ANTIBODY TO X-RAY LYMPHOMA: USE IN CHARACTERIZATION OF ANTIGEN AND IN IMMUNOTHERAPY. James P. Allison, Bradley W. McIntyre, David Bloch and James Irvin, Univ. of Texas Science Park, Smithville, TX 78957, Univ. of Texas, Austin, TX and S.W. Texas State Univ., San Marcos, TX.

A panel of hybridomas was constructed by fusion of P3 myeloma cells with spleen cells from a Balb/c mouse immunized with C6XL, an X-ray induced lymphoma of C57BL mice. One of the hybridomas, 124-40, was found to secrete an antibody reactive with C6XL lymphoma cells, but not with normal C57BL/6 adult thymus, spleen, lymph node, or bone marrow cells, fetal spleen or thymus, or unrelated lymphoid tumors (as assessed by radioimmune binding assays, radioimmune precipitation, and flow microfluorometry). The antigen was found to be structurally and immunologically unrelated to murine leukemia virus env or gag proteins. The reactive antigen was isolated from C6XL lymphoma cells by radioimmune precipitation with McAb 124-40 and found to be a glycoprotein with two disulfide-bonded subunits of 39KD and 41KD. A molecule with similar properties could be detected by diagonal electrophoresis of extracts of surface-labeled normal lymphoid cells and thymomas unreactive with McAb 124-40. The reactive antigen may thus be a clonally expressed epitope of a common cell surface component. McAb 124-40 (IgG_1) was not active in complement-dependent cytotoxicity. Administration of the McAb to tumor-bearing mice resulted in a slight increase in survival time. A specific immunotoxin was constructed by conjugation of McAb 124-40 with the cellular toxin pokeweed antiviral protein (PAP). The conjugate was found to inhibit the growth of lymphoma cells in vivo. (Supported in part by NCI Grant CA 26321 and R.A. Welch Fdn. Grant AI605.)

0105 BIOSYNTHETIC AND STRUCTURAL STUDIES ON A MONOCLONAL ANTIBODY DEFINED HUMAN MELANOMA - ASSOCIATED ANTIGEN. T.F. Bumol and R.A. Reisfeld. Scripps Clinic and Research Fdtn. La Jolla, California 92037

The monoclonal antibody 9.2.27,which demonstrates a high specificity for human melanoma cell surfaces, has been used as a molecular probe in a combination of biosynthetic, enzymatic, and structural studies to define an antigenic complex consisting of a 250 kilodalton N-linked glycoprotein and a high molecular weight proteoglycan (HMW-C) with an M_r > 480 kilodaltons. The 250K glycoprotein reveals Endoglycosidase H sensitive precursors in pulse-chase biosynthetic studies and shows a lower apparent M_r after treatment with neuraminidase indicating its sialoglycoprotein nature. The biosynthesis of HMW-C occurs concurrently with acquisition of Endoglycosidase H resistance of the 250K antigen and is inhibited by exposure of M21 cells with $^{35}SO_4^=$, is sensitive to β-elimination in dilute base, and is degraded by both chondroitinase AC and ABC lyases suggesting strongly its chondroitin sulphate nature. Descending chromatography analysis of chondroitinase ABC digests of 9.2.27 immunoprecipitates demonstrates the presence of characteristic chondroitin sulphate disaccharides and tryptic peptide analysis comparing the 250K and HMW-C indicates many similar peptides suggesting that the 250K component recognized by the 9.2.27 monoclonal antibody is a core glycoprotein of a chondroitin sulphate proteoglycan at the cell surface/extracellular matrix of human melanoma cells.(Supported by ACS M218 and NIH CA 28420. T.F.B. is supported by DRG-432 from the Damon Runyon-Walter Winchell Cancer Fund)

0106 MONOCLONAL ANTIBODY AGAINST HUMAN GRANULOCYTIC LEUKEMIC CELL LINE (KG-1). Amanullah Khan, Fumio Aota, N.O. Hill, Wadley Institutes of Molecular Medicine, Dallas,Tx 75235

A monoclonal antibody was raised against KG-1 cell line which is derived from acute granulocytic leukemia. Balb/C mice were immunized by injecting 1 X 10^7 cells, every 10 days, i.v. X 3. Four days after the last injection, spleen cell suspension was obtained and fused with NS-1 cells using 50% polyethylene glycol. Supernates from 50 hybridomas were tested for antibody against KG-1 cells by indirect immunofluorescence, using goat anti-mouse serum conjugated with FITC as the second antibody. Seven hybridomas were found to be positive. Following further cloning, one stable clone, designated as WI-5 was further characterized. It produced anti-KG-1 antibody which was IgG. The antibody was also tested against 18 other cell lines (HL-60, K-562, NAll-1, NAll-16, Reh, NALM-1, NALM-6, RPMI-1788, RPMI-4098, RPMI-6140, MOLT-4, RPMI-8402, Namalwa, GM-1500, GM-2132, U-266, RPMI-8226 and U-937). These cell lines are derived from acute lymphocytic leukemia of nul cell, T cell, and B cell origin, acute granulocytic leukemia, transformed B cells, Burkitt's lymphoma, multiple myeloma, histiocytic lymphoma derived monocytoid cell line, acute promyelocytic leukemia, and acute granulocytic leukemia and failed to react with the antibody. These results suggested that the antibody had high degree of specificity against acute granulocytic cell line (KG-1). The antibody was also found to be cytotoxic by the microcytotoxicity technique of Teresaki against KG-1 cells.

This research was made possible by a grant from the Wadley Guild Research for Leukemia Fund.

0107 MONOCLONAL ANTIBODIES AGAINST HUMAN B CELLS, Ann J. Feeney and Donald E. Mosier, Institute for Cancer Research, Philadelphia, PA 19111

In order to more precisely define the differentiation pathway of human B lymphocytes, we have produced a panel of monoclonal antibodies with different B cell binding characteristics. Mice were immunized with human peripheral blood lymphocytes (PBL) depleted of E-rosetting T cells. Three weeks later, the mice were boosted with the B-lymphocyte enriched PBL, and after 3 days, the splenocytes were fused with Sp 2/0. Hybridoma supernatants were screened by binding to E-rosette depleted PBL followed by staining with FITC-conjugated anti-mouse Ig and analysis on the FACS. 98/1380 wells showed staining of the B cell-enriched PBL and 40 anti-B cell hybridomas have been cloned so far. Double staining of PBL with rhodamine-conjugated anti-human Ig as well as with the hybridoma supernatants and fluorescein-conjugated anti-mouse Ig showed that most of the cloned hybridomas stained subsets of B cells (from 8-76% of B cells) and 5 hybridomas also stained some fraction (9-44%) of non-B cells. We are currently screening these hybridomas on human B and T cell lines, including the T cell ALL lines 8402 and HSB2, and the corresponding B cell EBV-transformed lymphoblastoid lines from the same individuals. The preliminary results show 5/31 monoclonal antibodies bind strongly to both B cell lines and fail to react with the T cell lines. The majority of the antibodies bind to neither cell line, suggesting that they recognize distinct stages of B cell differentiation.

0108 A MONOCLONAL Ab WITH REACTIVITY FOR LYMPHOID CELLS TRANSFORMED OR ACTIVATED BY EBV, G.E. Marti and T.J. Kindt, LIG,NIAID,NIH,Bethesda, Md. 20205

Balb/c mice were immunized with an EBV transformed, homozygous typing cell line (TRAL) in adjuvant. Hybridoma clones were screened in a solid phase ELISA against glutaraldehyde fixed TRAL and Daudi cells. One selected clone (33.1) which produced an IgG_1 product was strongly positive on TRAL and weakly positive on Daudi cells. Microscopic surface immuno-fluorescence (sIF) revealed that PBLs and B cell enriched fractions were essentially negative when compared to EBV transformed B cells. All EBV transformed PBLs were brightly positive with 33.1 including those derived from autologous negative PBL samples. Negative cells included the Marmonset line H95-8, NALM, Ramos, and human T cell lines HSB, Molt-4, and CEM. CLL lymphocytes are positive. A FACS-II analysis confirmed visual fluorescence in all cases. An RIA using ^{125}I labelled 33.1 antibody revealed that binding to EBV transformed B cell lines was 1000 times greater than to PBLs while CLL lymphocytes bound 33.1 at an intermediate level. Stimulation of PBLs with EBV as a polyclonal, T cell-independent activator led to the appearance of 33.1 positive cells at days 5, 7, and 14. Two dimensioanl PAGE analysis of immunoabsorbent eluted material gave an Ia-like pattern. It appears that 33.1 recognizes an Ia-like antigen that is absent or in very low amounts on normal PBLs but is related to B cell activation and/or differentiation.

Bone Marrow Transplantation

0109 GRAFT-VS-LEUKEMIA REACTION IN MICE FOLLOWING TRANSPLANTATION OF H-2 MATCHED MARROW, James P. OKunewick, Ruby F. Meredith, Mary J. Buffo and Deborah Jones, Cancer Research Laboratories ASRC, Allegheny General Hospital, Pittsburgh, PA 15212
Previous studies have documented the existence of a GvL effect in both mice and men following allogeneic bone marrow transplantation. In the early mouse studies it had been assumed that this GvL effect was an aspect of Graft-vs-Host reaction and dependent on H-2 differences. However, other studies have indicated that the severity of the H-2 determined GvH reaction is not predictive of the level of GvL activity of the transplanted marrow. To test the postulate that the GvL effect might be controlled by other minor histocompatibility genes (MiHL), we carried out experiments using Rauscher leukemic SJL/J ($H\text{-}2^s$) mice as recipients and comparing the GvL effects of donor marrow from the normal SJL/J, the C57BL/10 ($H\text{-}2^b$), and its congenic B10.S ($H\text{-}2^s$) derivative strain which has the MiHL type of the C57BL/10. The recipients were prepared for transplantation by exposure to 950R of x-ray. This left a median of 41 surviving endogenous splenic hematopoietic colony formers per mouse, and hence was not a total marrow ablative dose. The syngeneic SJL/J marrow produced no GvL effect. However, a GvL effect resulting in approximately 50% survival at 4 months after transplant was demonstrable with both the semi-allogeneic B10.S as well as the totally-allogeneic C57BL/10 donors. Additional transplants were done using F_1-hybrids of the B10.S and the C57BL/10 with the SJL/J. These also indicated that significant GvL activity could be obtained independent of H-2 differences, but possibly under the control of the MiHL alleles contributed by the allogeneic parent. Other experiments using monoclonal anti-T-cell antibody suggest that those T-cells which mediate acute GvH reaction do not contribute significantly to this GvL effect. (NCI supported.)

0110 ALLOIMMUNIZATION AND ADOPTIVE IMMUNOTHERAPY OF T CELL LEUKEMIA. Chiu-Yang Shih, Robert L. Truitt, and Mortimer M. Bortin, Winter Research Laboratory, Mount Sinai Medical Center, P.O. Box 342, Milwaukee, WI 53201 U.S.A.

Allogeneic bone marrow transplantation is often used for the treatment of acute leukemia that is refractory to conventional therapy. In both experimental and clinical transplants a beneficial antileukemic effect of the marrow transplant is usually associated with unacceptable graft-vs-host (GVH) reactivity. We found that preimmunization of the donor with normal tissues from allogeneic mice induced graft-vs-leukemia (GVL) reactivity against the H-2-compatible acute T lymphoblastic leukemia of AKR mice (Nature 281:490, 1979). More importantly, alloimmunization did not cause any significant increase in GVH reactivity of the donor cells when measured in independent assays. Alloimmunization-induced GVL reactivity could not be attributed to H-2 or Mls disparity between the donor and immunogen. GVL reactivity appeared to be mediated by a donor $Thy\text{-}1.2^+$,$Lyt\text{-}2^+$ cell(s) which persisted in the donor for as long as eight weeks after alloimmunization. Adoptively transferred cells required at least five days residence in the leukemic host before any therapeutic benefit was noted. Using limiting dilution microcytotoxicity assays we have quantified the GVL and GVH reactive T cell populations in transplanted leukemic AKR mice. A significantly higher number of leukemia-reactive cells could be recovered from AKR mice given alloimmunized donor cells. The levels of cytolytic cells was greatly influenced by the presence of leukemia in the AKR hosts.(Supported by USPHS CA 20484)

0111 EXPRESSION OF THE MURINE IgD HEAVY CHAIN COMPLEX IN PLASMACYTOMAS, B CELL LYMPHOMAS, AND NORMAL SPLEEN, Leona Fitzmaurice,* James Owens,+ Frederick Jacobsen,* and J. Frederic Mushinski,+ *LI, NIAID, and +LCB, NCI, NIH, Bethesda, MD 20205.
IgD is found on the surface of most splenic B cells; its role in B cell differentiation and function is poorly understood but probably complex. The IgD heavy chain (δ) gene is comprised of multiple exons situated in the genome between the genes for IgM and IgG constant regions. Based upon the co-expression of IgM and IgD by spleen cells and the structural relationship of the μ and δ genes, it has been proposed that μ and δ mRNAs may result from differential polyadenylation and/or splicing of a single primary transcript. Furthermore, both μ and δ are expressed in membrane and secreted forms; mRNAs for these two forms of protein differ at their 3' termini. We have examined δ chain mRNA synthesis in plasmacytomas, B cell lymphomas, and normal BALB/c spleen. We have identified at least five different δ mRNAs (1.75, 2.1, 2.65, 2.9, and 3.2 kb) which are encoded by different exons. We have shown that most, if not all, of these δ mRNA species appear to be mature, translatable mRNAs. Our studies indicate that the plasmacytomas and B cell lymphomas provide models not only for secreted but also for membrane δ production. These model systems are allowing us to better define the multiple δ mRNAs we have observed and their protein products and to better understand the regulation of δ gene expression during B cell differentiation.

0112 BONE MARROW TRANSPLANTATION FOR THE TREATMENT OF SPONTANEOUS T LYMPHOBLASTIC LEUKEMIA IN AKR MICE. Robert L. Truitt and Chiu-Yang Shih, Winter Research Laboratory, Mount Sinai Medical Center, P.O. Box 342, Milwaukee, WI 53201 U.S.A.

The purpose of these studies was to evaluate the adoptive immunotherapeutic effect of H-2-incompatible bone marrow transplantation on the acute T lymphoblastic leukemia which develops spontaneously in AKR ($H\text{-}2^k$) mice. AKR mice diagnosed as bearing advanced leukemia were given remission-induction chemotherapy, pre-transplant immunosuppression, then transplanted with bone marrow and lymph node cells from unprimed SJL ($H\text{-}2^s$) donors. In order to avoid secondary disease complications, antibiotic decontamination and isolation in laminar airflow protective environments were used. Use of allogeneic cells in combination with gnotobiotic techniques resulted in significant proportions of mice surviving six months post-transplant as compared to conventionally housed animals or decontaminated mice given syngeneic cells. Factors found to significantly influence survival included (a) interval of pre-transplant decontamination, (b) use of both decontamination and protective environments rather than either alone, (c) antileukemic and antihost reactivity of donor cells, and (d) interval of time post-transplant before reconventionalization. Leukemia relapse in donor cells was noted in some long-term chimeras. The results of these studies indicate the immunotherapeutic potential of marrow transplantation for chemoradiotherapy-resistant T lymphoblastic leukemias and the importance of host microflora control in the success of such therapy. (Supported by USPHS Grants CA 18440 and CA 26245 and the Allen-Bradley Foundation; RLT is a Scholar of the Leukemia Society of America, Inc.)

0113 SUPPRESSION OF CFU-S DEVELOPMENT BY SYNGENEIC LYMPH NODE CELLS. Tsuyoshi Tange and Harley Tse. Department of Immunology, Merck Sharp & Dohme Research Laboratories, Rahway, New Jersey, 07065.

Evidence has accumulated indicating that the development of pluripotent stem cells is under immunological regulation. This aspect has important implications particularly in bone marrow transplantation. We have studied the effects of normal lymph node cells on the development of stem cells in the spleen colony forming assay(CFU-S). When lymph node cells together with bone marrow cells were injected intravenously into lethally irradiated syngeneic hosts, the number of spleen colonies were reduced especially at the lower doses of lymph node cells. Of the 6 strains of mice tested so far, only C57BL/6 mice showed such a suppression phenomenon. In vivo pretreatment of lymph node cell donors with cyclophosphamide abrogated the suppressor activity. Furthermore, cell-cell interactions between lymph node cells and CFU-S precursor cells seem to be essential as delaying injection of lymph node cells by 24 hours failed to generate any suppression of CFU-S. The nature of the suppressor cells in lymph node cells was next investicated. Treatment of lymph node cells with monoclonal anti-Thyl.2 antibodies plus complement did not affect the suppressor activity whereas nylon-wool-column-passed non-adherent lymph node cells were not suppressive. It is also interesting to note that irradiation of lymph node cells at 2,200 rads greatly enhanced their suppressor activities. In other experiments, suppression was also enhanced when the recipient host was allogeneic to the bone marrow and lymph node cells. Thus, the suppressor cells appear to be Thyl.2-negative, radioresistant and cyclophosphamide -sensitive. Whether this could be a pre-T cell or macrophage is currently under investigation.

0114 AUTOLOGOUS BONE MARROW TRANSPLANTATION IN T-CELL MALIGNANCIES: USE OF IN VITRO MONOCLONAL ANTIBODY TREATMENT OF REMISSION MARROW TO ELIMINATE TUMOR CELLS. Herbert Kaizer[1], Ronald Levy[2], and George W. Santos[1], The Johns Hopkins University[1], Baltimore, MD 21205 and Stanford University[2], Palo Alto, CA

The primary obstacle to wider exploration of the use of bone marrow transplantation in the treatment of lymphoproliferative malignancies is the presence of occult tumor cells in remission marrow. The presence of T-cell specific antigens in the T-cell leukemias and lymphomas provides a target for in vitro immunologic attack on such occult tumor cells. We have an ongoing program using monoclonal antibodies to treat autologous remission bone marrow prior to cryopreservation. Three patients have received marrow treated with a monoclonal anti leu-1 antibody using conditions developed in an in vitro model which produce lysis of all antigen bearing cells. Marrow was reinfused in each instance after supralethal doses of total body irradiation (TBI). Hematologic reconstitution occured successfully in all three patients and its time course was indistinguishable from patients receiving untreated cryopreserved marrow. The one patient whose follow-up has exceeded 12 months remains free of recurrent clinical infection. He displays normal delayed cutaneous hypersensitivity and normal lymphocyte proliferative responses to alloantigens and mitogens. Serum immunoglobulin levels were low in this patient prior to transplant, but IgA and IgM have returned to normal levels. Although all patients remain in remission at this time, it is too early to assess the therapeutic efficacy of this approach.

0115 RADIATION AND BONE MARROW TRANSPLANTATION (BMT), H.M. Vriesendorp and P.M. Johnson, Northwestern University, Chicago, Illinois 60611.

Radiation is the most effective single agent in preparing animals or human patients for BMT. Radiation creates enough space in the host bone marrow, suppresses the Host vs Graft reaction and has activity against most types of tumors. A patient with a non-malignant disorder can be conditioned for bone marrow from a major histocompatibility complex (MHC) identical donor by radiation only. Data in dogs indicate that 5.0 Gy of whole body irradiation is sufficient for space formation. Effective methods of Graft vs Host prevention and the use of MHC mismatched donors decrease the engraftment rate and require the use of additional radiation. In cancer patients radiation alone cannot achieve complete tumor eradication. A low toxicity radiation protocol has to be combined with agents with a better anti-tumor response than radiation. Insufficiently explored variables of radiation are field size, number of fractions and dose rate. Animal studies will only be helpful if conditions are chosen that can be extrapolated to human BMT, e.g., a low concentration of hemopoietic stem cells and a high concentration of T lymphocytes in donor bone marrow cells. The success of Total Lymphoid Irradiation (TLI) in mice cannot be extrapolated yet to human application because murine bone marrow contains an approximately 10 fold higher concentration of stem cells and very few T lymphocytes. TLI in dogs and rhesus monkeys is less successful. Another important consideration in designing more effective conditioning regimens for BMT is the reciprocal interference of Host vs Graft and Graft vs Host reactions. Many modifications that influence one of the two mirror reactions will have the opposite effect on the other reaction. For optimal results both reactions have to be suppressed.

0116 IMMUNHISTOLOGICAL ASSESSMENT OF GvHD MEDIATED SKIN LESIONS BY MONOCLONAL ANTIBODIES, C. Müller, K. Schüch, K. Wilms, A. Ziegler, P. Wernet, Med. University Clinics , Tübingen FRG

Cutaneous lesions of seven patients presenting with acute and chronic GvHD after bone marrow transplantation were investigated in the immunoperoxidase technique on frozen tissue sections with selected monoclonal antibodies against HLA-class I and II antigens and different T-cell determinants, in order to characterise immunologically mediated dermal damage. All patients presented with varying amounts of T-cell infiltrates positive for HLA-ABC and -D region products in the upper dermis. Many of these T-lymphocytes , which carried the phenotype OKT 4^-, OKT 8^+, penetrated the basal cell layer. They seemed to destruct epidermal cells and to cause an irregular turnover of epithelial cells, which could result in Ia antigen expression on keratinocytes observed in one patient with chronic GvHD.Except this patient HTA 1^+, Ia^+ dentritic cells of the epithelium, so called Langerhans cells, were reduced in all other investigated skin biopsies. Further examination and comparison of dermal biopsies presenting with different stages of GvHD may prove, wether these Langerhans cells can actively or passively participate in the immunological process of GvH reactivity of the skin. This approach offers new means for an early and accurate diagnosis of GvHD mediated skin lesions. Similar studies can be performed on other critical sites GvH reactivity, such as liver, gut , and lung.

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0117 MANIPULATING OF ACUTE GVHD IN ALLOGENEIC BONE MARROW CHIMERAS, Wolfgang Heit, Hildegard Heit, Hans Rodt (+) and Bernhard Kubanek University Ulm, and Institut of Hematology, GSF, (+) Munich

Allogeneic marrow transplantation of germfree whole body irradiated recipients (C57BL → CBA, 950 R) results in long-term hemo-lymphopoietic chimerism (>1 year) without lethal GvHD. The prognosis and histopathological pattern of GvHD were found to be strictly dose related to the number of lymphocytes contaminating the graft. 3×10^5 spleen lymphocytes were compatible with long-term survival but caused sustained tissue damage in germfree allochimeras. The addition of 10^6 lymphocytes resulted in lethal GvHD within 60 to 70 days. Increasing cell numbers caused further reduction of survival time to a minimum of 15 to 17 days (3×10^7 spleen cells). Acute GvHD could successfully be controlled by preincubation of the graft with ATG (rabbit anti-θ-globulin). High lymphocyte concentrations (3×10^7/graft) required exposure to complement. Once, chimeras recovered from acute GvHD, a second load of allogeneic or isogeneic spleen lymphocytes (3×10^7, C57BL or CBA) was ineffective of reinduction of GvHD or graft rejection. The data will be discussed with particular concern to T-suppressor functions in GvHD and transplantation tolerance.

0118 THE RECONSTITUTION OF HAEMOPOIETIC PRECURSORS AFTER BONE MARROW TRANSPLANTATION, Bernhard Kubanek, Renate Arnold, Wolfgang Heit and Hermann Heimpel, for the Bone Marrow Transplantation Group of the University Ulm, Ulm, F. R. Germany

Clonal assays of haemopoietic precursors (CFUc, BFUe and CFUe) in connection with regeneration pattern of the peripheral blood allow the descriptive analysis of the reconstitution of haemopoiesis after bone marrow transplantation (BMT). CFUc, BFUe and CFUe were followed regularly up to 4 years after BMT in 4 patients with acute myeloid leucemia (AML) and 2 patients with severe aplastic anemia (AA). There was no evidence of recurrent disease judging from all parameters including chromosome analysis throughout the observation period. Although all patients accomplished an initial recovery of haemopoietic precursors and peripheral blood counts in the first 4 weeks after BMT different patterns of reconstitution developed thereafter. In the majority of the patients the incidence of early haemopoietic precursors did not reach normal values, although the peripheral blood counts normalised. A dichotomy as has been observed in AA with compensated haemopoietic production. This inadequate longterm reconstitution of haemopoietic precursors is accompanied by other features like a low density CFUc population which was observed during remission before BMT and consistently for several month thereafter. Host factors have to be discussed for these defective recovery rather than an inefficient graft. In this context the effect of immunosuppressive agents used for the treatment of GvHD on the recovery of haemopoietic precursor will be discussed, particularly the effect on a heteroantiserum against T cells which has a specificity for erythroid, but not for pluripotent stem cells.

0119 SUPPRESSION OF LEUKEMOGENESIS IN B CELL LEUKEMIA (BCL_1)-BEARING MICE FOLLOWING ALLOGENEIC BONE MARROW TRANSPLANTATION, Shimon Slavin, Ellen S. Vitetta*, Shoshana Morecki and Lola Weiss, Hadassah University Hospital, Jerusalem, Israel.

Experimental as well as recent clinical observations suggest that immunological mechanisms responsible for alloreactivity may also exert beneficial graft vs. leukemia (GVL) effects. 75% of BALB/c x C57BL/6 hybrid (F_1) mice inoculated with 10^7 BCL_1 cells, conditioned with total lymphoid irradiation 200 rads x 8 (TLI) and treated with cyclophosphamide (CY) 200mg/kg I.V. followed by infusion of 3 x 10^7 incompatible A/J or C57BL/6 bone marrow (BM) cells, showed no signs of leukemia at 18 months following tumor inoculation, whereas 100% of compatible (BALB/c or F_1) BM recipients showed leukemic relapse (≤6 months). The lack of clinically overt GVHD suggests that GVL by incompatible BM allografts may be independent of GVHD. Although residual BCL_1 cells were undetectable in the blood and in the spleen of the BM chimeras by cell counts, morphological criteria and lack of positive cell surface staining with tumor-associated anti-idiotypic antibody by fluorescence activated cell sorter, tumor developed in about 50% of naive BALB/c recipients of 10^6 spleen cells obtained from chimeras showing no overt BCL_1 (60-100 days following inoculation). On the other hand, spleen cells obtained from cured A/J→F_1 or C57BL/6→F_1 chimeras delayed or prevented (>180 days) the onset of leukemia upon coinjection with a challenge of 10^2-5 x 10^5 BCL_1 cells in naive BALB/c mice. Thus the "cure" of leukemia post BM transplantation may result from cell mediated suppression of the proliferative cytokinetic potential of residual tumor cells or from complete elimination of tumor cells in some cases.

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Immunosuppression

0120 IMMUNOSUPPRESSION IN THE MURINE B CELL LEUKEMIA (BCL_1). Judith E. Layton, Andre van der Hoven, Jonathan W. Uhr and Ellen S. Vitetta, Department of Microbiology, University of Texas Health Science Center at Dallas, Dallas, Texas 75235.

The murine B cell leukemia, BCL_1, resembles in many respects the prolymphocytic variant of human chronic lymphocytic leukemia and, therefore, may serve as a useful model in which to analyse the immunodeficiency associated with this disease.

These studies show that both humoral and cell-mediated primary *in vivo* responses are suppressed in BCL_1-bearing mice. The cell mediating *in vitro* suppression, previously shown to be Ig^- and Thy 1.2^+, was found to be phagocytic as well as G-10 adherent. Suppression was unaffected by indomethacin, indicating that it was not mediated by prostaglandins. In addition, normal T cells were required for suppression of *in vitro* T-independent responses.

0121 REGULATION OF HYBRIDOMA ANTIBODY PRODUCTION BY COCULTURE WITH ANTIGEN SPECIFIC OR IDIOTYPE SPECIFIC IMMUNE SPLEEN CELLS, James J. Gibbons, Jr., Young Tai Kim and Gregory W. Siskind, Cornell University Medical College, New York, NY 10021

We have studied antibody production by hybridoma cell line formed by fusion between TNP-ficoll immune C57BL/6 spleen cells and P3NP (BALB/c) hybridoma cell line. The hybridoma line (B.22) has been carried continuously in culture for eight months and secretes IgM anti-TNP antibody. We have tried to regulate antibody production by coculture of hybridoma cells with either antigen specific or idiotype specific suppressor cells generated by CB6F spleen cells under appropriate conditions. Previously we have shown that a four-day pre-culture of spleen cells with antigen (DNP-polyacrylamide) induces both antigen specific and nonspecific suppressor cells which suppress the heterogeneous *in vitro* response to DNP by normal cells. When these precultured cells are added to B.22 hybridoma cultures we have observed a 40-80% depression in the number of TNP specific PFC. The degree of depression was variable from animal to animal and greatest at day three-four of coculture. Thereafter, the number of PFC rose to approach control levels. Affinity purified hybridoma (B.22) protein was coupled ($CrCl_3$) to $CB6F_1$ spleen cells and injected into syngeneic animals in order to induce idiotype specific suppressor cells. When immune spleen cells were then precultured in the presence or absence of B.22 antibody for four days and added to hybridoma cell cultures we observed an 85% depression of PFC as compared to normal spleen cells or TNP-BSA coupled spleen cells injected and cocultured in the same way. We have thus demonstrated that the antibody production by hybridoma cells can be modulated by immune cells.

0122 CHARACTERIZATION OF θ^-, Ly 2,3$^-$ HAPTEN SPECIFIC SUPPRESSOR CELLS, Rosemarie DeKruyff, Carol Clayberger, James Aisenberg and Harvey Cantor, Harvard University, Boston , MA 02115.

Cells specific for the haptens 4-hydroxy-3-nitrophenyl acetyl (NP) and trinitrophenol (TNP) were derived from the spleens of hyperimmune mice. These cells were selected on either TNP-KLH or NP-OVA coated Petri dishes and were subsequently treated with either anti θ or anti Lyl plus rabbit complement. The antigen binding cells (ABCs) were cultured with irradiated syngeneic spleen cells and antigen. ABCs proliferate specifically to hapten, as measured by thymidine incorporation. Morphologically, these cells are round, nonadherent, and contain prominent basophilic granules, as demonstrated with Wright's stain. Their phenotype is θ^-, Ly 23$^-$. These cells are unresponsive to Interleukins 1 and 2 but are activated by Interleukin3. The growth of these cells is optimized by the addition of irradiated Lyl$^+$ T cell clones, or by the supernatants of antigen-activated Lyl$^+$ T cell clones. Irradiated antigen binding cells suppress the proliferation of T cell clones which share their hapten specificity. However, they do not suppress the proliferation of T cell clones specific for other antigens. This indicates that suppression is mediated by θ^-, Ly 23$^-$ cells as well as θ^+, Ly 23$^+$ cells.

0123 ABROGATION BY RETROVIRUSES OF LYMPHOCYTE MITOGENESIS: REVERSAL BY T CELL GROWTH FACTOR, Mark A. Wainberg and Evelyne Israel, Jewish General Hosp., Montreal, Canada.

Avian leukosis viruses induce B cell lymphomas in chickens after latency periods of 7-10 months, following neonatal infection. Viral replication occurs throughout this period and the development of the disease involves a viremic phase, during which time the animals are usually immunosuppressed. We have researched the mechanisms which may underlie such immunosuppression by examining the effects which co-incubation of avian leukosis viruses with peripheral blood lymphocytes may have on the ability of the latter to respond to T and B cell lectins and to alloantigenic stimuli. The results indicate that the viral presence can be inhibitory to the usual mitogenic responses by as much as 90%. Similar results can be obtained using UV-inactivated virus particles, indicating that active infection of the responder cell populations is not involved. Viruses can be added to the cell-lectin mixture as late as 28 hours after culture initiation and still achieve significant inhibition of responsiveness. In vitro transfer experiments indicate that the inhibitory effect is apparently mediated by macrophages which are stimulated to produce a soluble inhibitor of mitogenesis. The latter is not interferon. Finally, the addition to such cultures of exogenous T cell growth factor (TCGF) causes a dose-dependent reversal of the usual inhibitory effect. Indeed, little or no TCGF activity can be detected in the fluids of virus-coincubated lymphocytes that have been exposed to T cell lectins. These results may help to explain the immunosuppression that accompanies viremia during many types of infection in general and during the induction phase of B cell lymphomas in particular. (Supported by the Medical Research Council of Canada).

0124 USE OF A HUMAN "WHOLE-BLOOD" COLONY TECHNIQUE AS A SENSITIVE BIOASSAY FOR DETECTION OF IMMUNOTOXICITY AND IMMUNOLOGICAL DISORDERS, F. D. Wilson, R. Graham, G. Fisher, L. Mulne, S. Davidson, S. Knox, Battelle Columbus Laboratories and Children's Hospital, Columbus, Ohio.

We previously described a "whole-blood" method for the growth of T-lymphocyte colonies for humans (1, 2). The method does not require gradient-enrichment, uses minimal quantities of peripheral blood and is more sensitive for detecting radiation effects than either colony methods requiring gradient-enrichment or ^{3}HTdr-incorporation techniques (1,2,3). We have also demonstrated abnormalities in radiation sensitivity of colonies in patients with Fanconi's anemia and other hematological disorders including leukemia (2,3). We have investigated the utility of the whole-blood colony method as a sensitive indicator of immunotoxicity produced by trace elements. The results of studies using Se, Zn, V and Ni indicate that the method is extremely sensitive for the detection of trace elemental immunotoxicity. Fifty percent suppression of colony formation occurred at 0.03 ppm Se. Thus, the whole-blood T-lymphocyte colony technique can be utilized as a sensitive bioassay for the detection of immunotoxocity, the detection of enhanced toxicity in patients with genetic hematopoietic disorders, and potentially for demonstrating clonal abnormalities in patients with lymphoid tumors. Wilson, F. D. et al., Exp. Hemat. 8:1031, 1980; Knox, S. J., Wilson, F. D. et al., Experimental Hematology Today, 1981; Knox, S. J., Wilson, F. D., Blood 57:1043, 1981; Wilson, F. D. et al., Exp. Hemat. 8:802, 1980. Work supported in part by U.S. EPA and in part by Electric Power Research Institute.

0125 THE EFFECT OF INDOMETHACIN ON THE GENERATION AND EFFECTOR FUNCTION OF SUPPRESSOR CELLS FROM TUMOR-BEARING MICE, Barbara L. Pope, Dalhousie University, Halifax, Nova Scotia, Canada B3H 4H7.

The spleens of mice bearing large M-1 fibrosarcomas contain two distinct populations of suppressor cells which have been identified as macrophages and T cells. Both the activation and effector function of the suppressor macrophages appear to be independent of prostaglandin synthesis. This has been demonstrated by experiments in which a) indomethacin treated tumor-bearing mice developed suppressor macrophages in association with progressive tumor growth and b) indomethacin did not reverse the macrophage associated suppression observed in vitro using mixing assays. In contrast, mice treated with indomethacin from the day of tumor cell inoculation eventually developed large tumors, but without the concomitant activation of suppressor T cells. Indomethacin treatment of mice bearing large tumors for one week prior to assay or the addition of indomethacin to in vitro mixing assays did not reverse the suppressor T cell activity. Marbrook vessel experiments in which different populations of cells were separated by dialysis membranes demonstrated that tumor-bearer spleen cells seceted a soluble factor which activated suppressor T cells from normal populations. This activation was totally blocked by indomethacin. These data are consistent with the hypothesis that tumor-bearer spleen cells secrete prostaglandins which activate suppressor T cells. However, the effector functions of both the suppressor macrophages and the suppressor T cells appear to be independent of prostaglandin synthesis. (Supported by the National Cancer Institute of Canada).

0126 IMMUNOSUPPRESSIVE EFFECTS OF ANTILYMPHOCYTE ANTIBODIES, Stefan Thierfelder, Hans Rodt, Udo Kummer, Inst.f.Hämatologie, GSF, Munich, FRG

Mono- and/or polyclonal antibodies against T and/or B lymphocytes were tested in vitro and in vivo for their immunosuppressive effects on humoral and cellular immunity in mice, rats and leukemic patients. Injection into mice of 5 mouse or rat monoclonal antibodies with high cytotoxic titers for lymphocytes or subtypes of lymphocytes failed to inhibit humoral antibody formation or delay skin graft rejection with one exception. Unabsorbed polyclonal mouse, rat or rabbit antilymphocyte antibodies were all immunosuppressive in both indicator systems. In vitro incubation of mono or polyclonal anti T but not of anti-Ia suppressed secondary disease. A follow-up of 20 ALL patients grafted in Munich and Tübingen with marrow pretreated with cytotoxic anti-T revealed no delay of engraftment. The importance of in vitro lymphocyte lysis of the hemopoietic graft before bone marrow transplantation in order to prevent secondary disease is demonstrated.

Phenotyping and Properties of T and B Tumors

0127 IMMUNOLOGICAL ANALYSIS OF NODULAR (FOLLICULAR) LYMPHOMA, John A Habeshaw, Dennis Bailey and Alfred G Stansfeld, St Bartholomew's Hospital, London EC1A 7BE

Nodular (follicular) non-Hodgkin lymphomas are the most common form of non-Hodgkin lymphoma in Europe and North America. An immunological study of 10 cases by suspension and frozen section phenotyping reveals (1) the presence of C-ALL antigen detected by the monoclonal antibody J5 in the tumour nodules, (2) B lymphocyte subset heterogeneity within these tumours demonstrated by the differential expression of the surface membrane Ig heavy chain isotype, binding of peanut agglutinin and reactivity with monoclonal antibodies LeuI/OKT1, (3) in 3 cases, absence of light chain class restriction in the coronal B lymphocyte population, (4) differences in the proportions and distribution of T helper/inducer and cytotoxic/suppressor subsets compared with 5 cases of control reactive follicular hyperplasia.

These results show that nodular (follicular) lymphomas have some features in common, but that the immunological typing of these lesions reveals considerable heterogeneity.

0128 ESTABLISHMENT OF B-CELL HYBRIDOMAS WITH B-CELL SURFACE ANTIGENS, Teruaki Hamano, K. Jin Kim, William M. Leiserson, and Richard Asofsky, NIAID, NIH, Bethesda, MD 20205

M12.4, one of several B-lymphoma cell lines from BALB/c mice, was mutagenized with ethyl methanesulphonate, selected in the presence of 6-thioguanine (6-TG) and cloned by limiting dilution. M12.4.1, a subline of the mutants, showed resistance to 6-TG and was sensitive to hypoxanthine-aminopterin-thymidine selective medium. M12.4.1 cells were fused with normal splenic B cells of C57BL/6 mice in the presence of polyethylene glycol (m.w. 4,000) and dimethyl sulfoxide. Surface antigens of the hybridomas were characterized by flow microfluorometry and a C-mediated cytotoxicity assay. The results showed that these hybridomas express some of the typical B-cell surface antigens, such as μ-chain, Ia^b and Ia^d, indicating that they are the hybrids with B-cell surface antigens. We examined next whether these B-cell hybridomas can exhibit some B-cell functions. These cells were stimulated with bacterial lipopolysaccharide (LPS) as well as T-cell replacing factor (TRF), then IgM secretion in the culture supernatant was determined by a solid-phase radioimmunoassay. The TRF used in this study was obtained by gel filtration of the culture supernatant from spleen cells primed with keyhole limpet hemocyanin. The TH2.5 hybridoma, one of the B-cell hybrids studied, released significant amounts of IgM into the culture medium after stimulation with LPS. In addition, TRF alone could induce polyclonal IgM secretion by TH2.5 cells. On the other hand, parental M12.4.1 did not secrete any IgM under the same conditions. These findings show that we have been able to establish the hybridoma with B-cell surface antigens which can exhibit some B-cell functions in vitro.

0129 IMMUNOLOGICAL PHENOTYPES AND CLINICAL CORRELATES IN ADULT ALL: A PROSPECTIVE STUDY FROM CALGB, Ivor Royston, Oliver Glidewell, and Rose R. Ellison, presented for Cancer and Leukemia Group B, University of California, San Diego, 92093, Mt. Sinai School of Medicine, New York, N.Y. 10029, and Columbia University, New York, N.Y. 10032

Since October, 1980, we have been prospectively determining by flow cytometry the cell surface phenotypes of newly diagnosed adult cases of acute lymphocytic leukemia (ALL) using a panel of monoclonal antibodies and conventional markers. In addition, nuclear terminal transferase and cytoplasmic IgM have been measured by immunofluorescence microscopy. Fifty-five cases have been evaluated and extensive heterogeneity has been observed. The clinical manifestations, response to therapy and survival of the patient are being analyzed and correlated to the observed phenotypes and will be presented at the Symposium.

0130 THE FUNCTIONAL CHARACTERIZATION OF MALIGNANT LY1$^+$ T CELL CLONES ISOLATED FROM B CELL LYMPHOMAS. Carol L. Reinisch, Amy P. Sing and John L. Troutner. The Department of Comparative Medicine, Tufts University School of Veterinary Medicine, Boston, 02111.

C57B16(B6) mice injected with MSV-MuLV-Moloney as weanling animals develop plasmacytoid lymphocytic (B cell) lymphomas in the mesenteric lymph node(MLN) two years after virus injection. This lymphoma can be transferred into normal B6 mice using lymphoid cells from the MLN, unseparated spleen cells or theta plus complement treated B cells. However, cells from the tumor cell population cultured at limiting dilutions(0.3 cells/well) on a syngeneic feeder layer are all Ly1$^+$ Ly2$^-$ Thy 1.2$^+$. When injected into normal B6 mice, the Ly1$^+$ T cells cause rapidly proliferating immunoblastic T cell sarcomas. When treated with 10000 rad prior to injection, the cells enhance specific cytolytic reactivity to allogeneic tumor cells and boost both the IgM and IgG response to SRBC. When incubated with unfractionated spleen cells or B cells in culture,the Ly1$^+$ T cells stimulate Ig secretion in a non-antigen stimulated system(as measured by reverse hemolytic plaque assay). Preliminary experiments show that the factors isolated from the T cells support the growth of malignant B cells derived from the lymphoma in vitro. These experiments show that the malignant lymphoma induced by MSV consists of at least two neoplastic populations, the B cell which causes a slowly proliferating plasmacytoid lymphocytic lymphoma and the Ly1$^+$ T cell which causes immunoblastic T cell sarcomas. The Ly1$^+$ T cell clones which have been repeatedly isolated from MSV-induced B cell lymphomas are functional and may selectively interact with a B cell subset to maintain lymphomagenesis in vivo. Supported by NIH Grant CA 21100 and ACS Grant IM 3000.

0131 FURTHER STUDIES OF THE BB-1 HUMAN B LYMPHOBLAST ANTIGEN, Takashi Yokochi, Edward A. Clark, and Marshall Kadin, University of Washington, Seattle WA 98195

A B lymphoblast antigen (BB-1) was detected with a mouse IgM monoclonal antibody and indirect IF or cell sorter analysis. BB-1 was expressed on both human B lymphoblastoid (B-LCL) or Burkitt's lymphoma (BL) cell lines and pokeweed mitogen or Epstein-Barr virus (EBV) activated Ig+ B cell blasts. However, BB-1 was not detectable on normal lymphoid tissues, T cell blasts, myeloma cell lines, or T cell, pre-B cell, or erythroblastic leukemia cell lines. A second lymphoblast antigen, LB-1, unlike BB-1, was present on both T and B cell blasts and was on virus-transformed T- and B-LCL (Yokochi et al., J. Immunol. 128: Feb.1982). Peripheral blood lymphocytes from 6 acute infectious mononucleosis patients, unlike normal subjects, had 2-3% LB-1+ cells, but lymphocytes from all but one patient (1% BB-1+ cells) were BB-1$^-$. BB-1 was expressed on a B lymphoblastoid leukemia and LB-1 was expressed on a T prolymphocytic leukemia, but neither BB-1 or LB-1 were detected on the Ia+ non T, non B acute lymphocytic leukemias (n=8), chronic lymphocytic leukemias (n=2), or chronic myelocytic leukemias (n=2) we have tested. BB-1 was expressed on the Jijoye BL but not on a virus producer subline of Jijoye, P3HR-1. Results bearing on the relationship between BB-1 antigen expression and EBV lytic cycle and EBV receptor expression will be discussed (Supported by American Cancer Society grant IM-260 and NIH Core grant RR00166).

0132 ALTERED EXPRESSION OF LYMPHOCYTE DIFFERENTIATION ANTIGENS ON IN VIVO AND IN VITRO AKR/J LYMPHOMAS. A.L.Howell and E.R.Richie, M.D. Anderson Hosp.,U. Texas,Houston,TX,77030.

Phenotypic diversity among individual spontaneous lymphomas from AKR/J mice was investigated by determining TdT activity and expression of lymphocyte surface antigens. Monoclonal antibodies to Thy 1.1, IA^k, Lv1, Ly2 and Ly5, and heterologous antisera to the p30 viral core protein, surface Ig, and PNA were used in an indirect immunofluorescent (IF) assay. Each lymphoma expressed a distinctive cell surface and TdT phenotype indicating extensive inter-tumor heterogeneity. To investigate phenotypic stability and the extent of intra-tumor heterogeneity, four lymphomas were adapted to in vitro growth. The in vitro cell lines expressed surface Thy 1.1, Ly5, p30 and PNA receptors, and were negative for IA^k as were the original spontaneous tumors from which they were derived. Also, H-2K^k antigens were detected on all cell lines. In contrast, the Ly1 antigen was not detectable on any of the four cell lines, even though the original tumors were positive for this antigen. Neither the addition of excess anti-Ly1 nor increasing the high voltage gain of the cytofluorograf resulted in detection of Ly1 surface antigen. The Ly1 IF pattern was not changed by treating the cells with neuraminidase in an attempt to expose masked antigenic determinants. Whereas three of the spontaneous tumors were Ly2 positive, only one of the cell lines expressed detectable levels of this antigen. Although Ly1 and Ly2 were not detectable on the cell surface by IF, we have not ruled out the possibilities that they could be detected by immunoprecipitation, or are present in the cytoplasm. Clonal derivatives of these cell lines will be used to further investigate the apparent loss of Ly1 and Ly2 surface antigens. Factors to be investigated that could contribute to this phenomenom include chromosomal loss, cell-cycle effects, in vitro culture conditions, and in vivo regulation.

0133 DISCORDANT EXPRESSION OF LY 2 ON THYMIC AND SPLENIC LYMPHOMA CELLS FROM A SINGLE AKR/J MOUSE. E.R. Richie and A.L. Howell, M.D. Anderson Hosp., U. Texas, Houston, TX 77030

Several spontaneous AKR/J lymphomas were characterized for expression of lymphocyte differentiation markers. Although there was extensive phenotypic diversity among individual lymphomas, thymic and splenic tumor cells derived from the same animal generally displayed identical phenotypes. However, in one animal the thymic lymphoma cells were Thy-1+,Ly1+,Ly2+,Ly5+ and PNA+ while the splenic lymphoma cells had a similar phenotype except that they were Ly2 negative. When the spontaneous thymic tumor cells were passaged i.p. into young syngeneic AKR/J recipients, the tumor infiltrated both the thymus and spleen as determined by morphological assessment and expression of p30 viral core proteins on the surface membrane of the tumor cells. As in the original tumor-bearing mouse, the thymic lymphoma cells from the P1 host were Ly1+,Ly2+ while the splenic tumor cells were Ly1+Ly2-. There are at least two hypotheses to explain the discordant Ly antigenic phenotype of lymphoma cells recovered from thymus and spleen of the same animal. It is possible that there were two distinct neoplastic cell populations in the original host, one (Ly1+,2+)that localized predominantly in the thymus, and the other (Ly1+,2-) that localized predominantly in the spleen. An alternative possibility is that a monoclonal Ly1+,2+ lymphoma population originating in the thymus underwent phenotypic alteration in the splenic microenvironment resulting in loss (or masking) of the Ly2 surface antigen. One approach to distinguish between these possibilities is to separate lymphoma cells into highly purified Ly2+ and Ly2- populations by cell sorting or panning techniques for passage into AKR (Jax X Cum)F1 recipients. Splenic and thymic lymphoma cells from F1 hosts will be separated from residual normal host T-cells by anti-Thy1.2 plus C' treatment and analyzed for Ly antigens.

0134 PATHOLOGY AND PHENOTYPE OF AN UNUSUAL PAROSTEAL HUMAN LYMPHOBLASTIC LYMPHOMA. R. G. Smith and J. Ritz, University of Texas Health Science Center at Dallas, Dallas, TX 75235, and Sidney Farber Cancer Center, Boston, MA 02115

We established an unusual pre-B cell line (SMS-SB) from the leukemic blood cells of a patient with acute lymphoblastic leukemia (ALL) (J. Immunol. 126:596, 1981). This patient's leukemia began as a paraspinal lymphoblastic lymphoma with lytic lesions in adjacent vertebra and foci of bone marrow involvement, but without generalized marrow replacement or leukemia. This pattern of disease is strikingly reminiscent of lymphomas induced in certain strains of mice by the Abelson murine leukemia virus (A-MuLV) and differs from most lymphoblastic lymphomas, which originate in anterior mediastinal lymphatic tissue and involve bone only late in the course of the disease, if at all. The phenotype of the leukemic cells is very similar to the major subpopulation of normal bone marrow pre-B lymphocytes and to many A-MuLV-induced murine lymphoma lines (ibid.). We now report a more detailed analysis of the surface marker of SMS-SB cells, using a panel of monoclonal antibodies. The cells react with the following antibodies: J-2, binding a 26,000 MW protein found on many ALL cells but not mature lymphocytes; Ia and B-1, B lymphocyte differentiation markers; T-1 and T101, recognizing antigens present on T lymphocytes and most chronic lymphocytic leukemias (CLL) of the B cell type. The cells do not react with J-5, which binds the common ALL antigen (cALLA), or with a monoclonal antibody to the sheep erythrocyte receptor found on T cells. This phenotype is very unusual amongst ALL cells. Terminal deoxynucleotidyl transferase (TdT) is found in 2-8% of SMS-SB cells by the immunofluorescence assay. The absence of cALLA and infrequent expression of TdT are features characteristic of the major subpopulation of marrow pre-B cells. In preliminary experiments, surface immunoglobulin expression can be induced after exposure to 5-azacytidine. Thus, SMS-SB cells may provide a useful model of early human B lymphocyte precursors.

0135 THE ISOLATION OF B AND T CELL SPECIFIC GENES, Mark M. Davis, David, I. Cohen, Ellen A. Nielsen, and William E. Paul, LI, NIAID, NIH, Bethesda, MD 20205

An important addition to our understanding of lymphocyte differentiation and function involves a more extensive knowledge of the genes involved. Towards this end we have measured the differences in gene expression between B and T cell lymphomas and developed procedures for systematically isolating the genes which make up these differences. Specifically we have found that B and T cell tumors differ by about 2% of the mass of their mRNA and that this corresponds to approximately 200 different genes. We have also shown the cDNA probes corresponding to this 2% difference (either B*-T or T*-B): 1) are representative of genes expressed in normal tissues (spleen and thymus); 2) can be used to screen recombinant libraries and isolate B or T cell specific cDNA clones; and 3) can be cloned directly into plasmids, resulting in whole libraries of B or T cell specific genes. The characterization of an initial group of 10 B-cell specific genes shows significant shifts in gene expression between pre-B, B-cell and plasma cell stage tumor lines, as well as possible subset related differences between the B cell stage tumors.

0136 B Cell Lymphoma Lines from NFS Mice. Wendy F. Davidson and Torgny N. Fredrickson. Laboratory of Microbial Immunity and Laboratory of Viral Diseases. NIAID, NIH Bethesda, Maryland 20205

Old NFS mice congenic for ecotropic murine leukemia virus (V) loci have a high incidence of non-thymic lymphomas. By flow microfluorometric analysis (FMF), a high proportion of these lymphomas appear to be of B cell origin. Cells from splenic lymphomas from 4 old NFS V-congenic mice were cultured in vitro and after 4 weeks of incubation 3 cultures yielded continuous B cell lines, and the other culture a continuous B cell line and an actively growing macrophage line which survived for 10 weeks. The 4 B cell lines were lymphoblastoid in morphology and had the following cell surface phenotype; $sIgM^+$, $sIgG1^-$, $I\text{-}A^{k+}$, $2C\text{-}2^+$, $H\text{-}11^+$, $F\text{-}11^+$, $Ly1^+$ and $Thy1^-$. All 4 lines also appeared to be weakly $sIgD^+$ and all were polyploid with 41 or 42 chromosomes. When tested for infectious murine leukemia virus production, the B cell lymphomas were found to make variable amounts of ecotropic, xenotropic and recombinant mink cell focus - inducing viruses. Following I.P. injection into NFS V-congenic mice, the B cell lines grew out rapidly in both the spleen and lymph nodes. Spleens weighing approximately 1gm were removed from mice 14 days after inoculation. Cells from these spleens were morphologically and phenotypically identical to the original inocula. These lines therefore represent neoplastic clones of B cells derived from spontaneous murine lymphomas.

0137 IMMUNOLOGIC CLASSIFICATION AND CLINICAL FEATURES OF CHRONIC LYMPHOCYTIC LEUKEMIA, Robert O. Dillman, Ivor Royston, University of California San Diego, La Jolla, CA 92093

Peripheral blood lymphocytes from 82 patients with a diagnosis of chronic lymphocytic leukemia were analyzed for E-rosette receptor (E), surface immunoglobulin (sIg), and T cell (T65) and Ia (Ia) antigens. The last two were detected by murine monoclonal antibodies. In addition sIg^+ cells were analyzed for κ and λ light chains μ, γ, α and δ, heavy chains, and cytoplasmic immunoglobulin. Use of these markers allowed identification of a number of phenotypes; patient records were reviewed for clinical correlations with these phenotypes. Variables analyzed included age, clinical stage, WBC, presence of albuminuria or serum paraprotein, time from diagnosis to first treatment and survival. Paraproteinemia was associated with the $sIg^+T65^-E^-Ia^+$ phenotype. Proteinuria was present in a high proportion of patients with the $sIg^-T65^+E^-Ia^+$ phenotype. The most common phenotype, $sIg^+T65^+E^-Ia^+$, was seen in 57 patients. Clearcut differences in survival were not seen. 17 patients have been repeatedly tested from 2-9 times over 2-24 months. The phenotypes of their CLL cells have not changed, although the specific malignant population has been decreased with therapy. Hairy cell leukemia and lymphosarcoma cell leukemia also have the $sIg^+T65^-E^-$ phenotype. Definitive clinical correlations will require a prospective study, and it is liekly that use of additional monoclonal antibodies will delineate more subgroups which may have clinical relevance.

0138 CHARACTERIZATION OF A MULTIPOTENTIAL HEMATOPOIETIC CELL LINE, Margrit P. Scheid, Paul W. Kincade, Thomas M. Dexter*, Memorial Sloan-Kettering Cancer Center, New York, NY 10021; The Paterson Laboratories, Christie Hospital and Holt Radium Institute, Withington, Manchester M20 9BX, England*

This cell line was derived from a long-term culture of $(C57BL/6xDBA/2)F_1$ female bone marrow cells injected with Friend leukemia virus and characterized in its stem cell characteristics previously (T.M. Dexter et al. 1978). We phenotyped the 416B line as $Ly5^+$, $Thy\text{-}1^+$, $Lyb\text{-}2^-$, 14.8^-, $Mac\text{-}1^{+/-}$. Subsequently we reconstituted irradiated F_1 animals [including $(B6xBALB/c)F_1$, $(DBAxB6\text{-}Ly5.1)F_1$, $(DBAxB6\text{-}Lyb\text{-}2.1)F_1$] with 416B cells and determined the percentage and subset phenotype of T and B lymphocytes derived from the stem cell line using the differential Lyt-1, Lyt-2, Lyb-2 or Ly5 alloantigens as discrimanatory marker for host and donor cells. The representation of $Thy\text{-}1^+$ cells in the chimeric mice was low (between 10-17%). After pre-sensitization of the chimeric spleen suspensions with monoclonal α-Thy-1.2 and fractionation on P11-PA-SRBC plates, $Thy\text{-}1^+$ cells were enriched to 61% in the adherent fraction. Subsequently phenotyping of both adherent and supernatant (B cell enriched fraction) with alloantisera directed against both allelic determinants of Lyt-1, Lyt-2, Lyb-2 and Ly5 respectively, clearly indicated that > 90% of the T cells and approximately 70% of the B cells detected were of donor origin.

0139 TCGF-DEPENDENT LEUKEMIC T-CELL GROWTH, Kendall A. Smith, Dartmouth Medical School, Hanover, New Hampshire, 03755.

Detailed studies have shown that T-cell mitosis is mediated by T-cell growth factor (TCGF), a 15,000 (Mr) sialoglycoprotein that interacts with specific membrane receptors. To examine a possible role of TCGF in neoplastic T-cell growth, the opposing effects of TCGF and glucocorticoid hormones on T-cell proliferation were examined. Advantage was taken of a TCGF producing T-leukemic cell line (MLA-144). MLA-144 was found to absorb TCGF activity, express saturable TCGF binding sites, and to respond to the growth promoting properties of TCGF. As with normal T-cells, glucocorticoids suppressed MLA-144 cell growth primarily by an inhibition of TCGF production. The response to Glucocorticoids by MLA-144 cells was heterogeneous: isolated clones were found to differ in their levels of glucocorticoids receptors, the levels of TCGF produced and the magnitude of the glucocorticoid suppression of proliferation. However, there was a direct correlation among MLA-144 clones between the magnitude of suppression of TCGF production and the extent of inhibition of proliferation. As well, the magnitude of the glucocorticoid effect on both of these parameters was glucocorticoid concentration - dependent. Finally, TCGF supplementation antagonized the glucocorticoid effect in a concentration-dependent manner. Thus, our results establish that some leukemic T-cells proliferate in response to their own specific growth factor, and they support the notion that glucocorticoids suppress such cells by perturbing this mechanism of neoplastic growth regulation.

0140 HETEROGENEITY OF NON-HODGKIN'S LYMPHOMAS (NHL) AS DEFINED BY A PANEL OF MONOCLONAL ANTIBODIES. K.J. Gajl-Peczalska, C.D. Bloomfield, G. Frizzera, J.H. Kersey and T.W. LeBien, University of Minnesota, Minneapolis, MN 55455.

We correlated immunologic phenotype of 76 ML with histologic groups A-K of the International Working Formulation (IF). Cell suspensions and frozen sections of involved tissues were analyzed with standard cell markers and a panel of monoclonal antibodies (BA-1, BA-2, BA-3/J5, HLADR (Ia), T101, OKT11/9.6, OKT6, OKT8, OKT4/Leu3, OKM1/OKM2). Results for IF groups are:

B cell(Sig&/orCig+,Ia+,T antigens-,E-,TdT-)						T cell(E+, T11/9.6+, Sig-, Cig-, Fc-)								
Histology	C'+	T101+	BA-1+	BA-2+	BA-3+	IF	C'	TdT	T3	T4	T6	T8	Ia	BA1,2	J5
A (n=16)	100%	75%	100%	31%	0	A(n=3)	0	0	3	3	0	2	0	0	0
B-D(n=28)	79%	0	79%	14%	29%	F(n=4)	0	0	4	4	0/2	4	3	0	0
G,H (n=9)	71%	0	33%	25%	0	I(n=4)	2/2	2/2	1/2	4	3/3	4	1/3	2	2
J (n=3)	0	0	100%	100%	100%	K(n=1)	0	0	1	1	0	0	0	0	0

"Null" cell(n=8). Positive: Ia(8),C'(5),BA-1(2),Fc(1). Negative: Sig,Cig,E,T antigens,TdT,BA-2, BA-3. Our data show a marked heterogeneity of phenotypes within ML histologic and immunologic types. No monoclonal antibody is specific for one ML type, but patterns occur. Among "B" ML, only small lymphocytic (A) were T101+ and they were BA-3-, while all follicular ML (B-D) were T101- and 29% were BA-3+. All Burkitt's (J) were BA-1+, BA-2+, and BA-3+. All T ML except lymphoblastic (I) were TdT-. Two T ML cases (one A and one mycosis fungoides) were "helper" (T4+/T8-). Among T ML, only lymphoblastics were C'+,TdT+,T6+,BA-1+,BA-2+,BA-3+; they thus seem immunologically more immature than other T ML. While these findings are important in understanding ML biology, their clinical relevance remains to be defined.

0141 E-ROSETTE FORMING CELL COLONIES (ERFC-C) WITH MALIGNANT MARKERS IN B CELL NEOPLASMS AND MYELOID LEUKEMIA. R.Nogueira Costa*, F.Davis*, C.Kusyk*, D.S.Verma and G. Spitzer. M.D.Anderson Hospital and Tumor Institute, Houston, TX 77030.

ERFC-C grown in agar probably represent a subset of T+/pre-T cells. We investigated the nature of ERFC-C (90%-100% E-rosette positive) cloned from lymph nodes, bone marrow and pleural effusions in cases of lymphomas(4); lymph node, peripheral blood and bone marrow in cases of AML (6) and bone marrow in a case of Hairy cell leukemia(1). The assay contained media prepared from T-lymphocytes incubated with 1% V/V PHA. As a marker for malignancy we used the human malignant associated nucleolar antigen (HMANA) assay and in some instances, cytogenetic analysis. Beyond the reported specificity of HMANA (Davis,F.M. et al:PNAS 76; 892-896,1979), further controls were performed. ERFC-C grown from histologically normal nodes, nodes involved by nonhematological neoplasms and normal blood, marrow and spleen were all negative for HMANA, including one patient with a normal marrow which had been previously involved by lymphoma. Plating efficiency ranged from 0.006 to 0.75%. In four patients with B cell lymphomas ERFC-C were positive for HMANA; simultaneous cytogenetic analysis revealed a group Dq+ chromosome abnormality on ERFC-C in one case. The patient with Hairy cell leukemia exhibited HMANA positive cells both in the T-cells from peripheral blood and ERFC-C cloned from bone marrow. ERFC-C cloned from a chloromatous lymph node, bone marrow(3) and peripheral blood(2) of patients with AML showed again a high percentage of HMANA positive cells. Cytogenetic analysis on the chloromatous ERFC-C showed a 47XY +C abnormality which was also present in the bone marrow specimen. These studies document that there are markers of malignancy in ERFC-C in a certain number of B-cell neoplasms, and myeloid leukemia.

Cytotoxic T Cells and Macrophages

0142 STUDIES ON A NON-H-2 RESTRICTED TUMOR ASSOCIATED ANTIGEN OF PLASMACYTOMAS: DISSOCIATION OF LYT-2 EXPRESSION AND CYTOTOXICITY, J.V.Giorgi, J.A.Zawadzki, and N.L.Warner, University of New Mexico School of Medicine, Albuquerque, New Mexico 87131.

Lymphocyte clones derived from cytotoxic T lymphocyte lines (CTLL) have been used by a number of investigators to study the role of Lyt differentiation antigens in the interaction of cytotoxic T cells with their target antigens. In most cases, the T cell response studied was toward Class I, i.e., H-2 encoded or H-2 restricted antigens. In these cases, Lyt-2 appeared to be essential for recognition of the target cells by the mature cytotoxic T cells. In other cases, when the T cell response was to Class II, i.e., I region encoded antigens, Lyt-1, but not Lyt-2, appeared to be involved. In studies which we have carried out to examine the cell surface phenotype of CTLL directed against a non-H-2 restricted tumor associated antigen (TAA) found on murine plasmacytomas, CTLL which had been generated from spleen cells sensitized in vitro to this antigen were examined. Our results indicated that neither Lyt-2 nor Lyt-1 expression was essential for the expression of the cytotoxic potential of these cells. Although these results do not exclude the possibility that Lyt molecules play an essential role in the differentiation and maturation process which tumor-directed lymphocytes undergo when they respond to antigen, they do suggest that these molecules may not be required for mature cells to carry out their differentiated function. Attempts are now being made to generate CTLL against other TAA of plasmacytoma, and to determine the role of Lyt molecules in the T cell response to these antigens. (Supported by NIH CA-22268 and CA-05921)

0143 SELECTIVE LOCALIZATION OF RADIOLABELED IMMUNE LYMPHOCYTES INTO SYNGENEIC TUMORS: CELL SURFACE PHENOTYPES OF TUMOR-LOCALIZING LONG-LIVED LYMPHOCYTES, James J. Mulé, Department of Biological Structure, University of Washington Medical School, Seattle, Washington 98195

Using a cell separation technique to isolate mononuclear leukocytes from solid tumors in conjunction with combined radioautography and two-color immunofluorescence, I have characterized the surface markers of ^{3}H-labeled, immune long-lived small lymphocytes (LLSL) that selectively localize into chemically induced sarcomas. When small tumor pieces were implanted into immune mice whose LLSL had been radiolabeled, the LLSL that selectively localized into those tumors to which the hosts had been immunized were predominantly Thy-1^{+}, Lyt-1^{+}2^{-}. Localization into the appropriate tumor was also detected upon adoptive transfer of immune LLSL to tumor-bearing mice, especially when these mice had, before transfer, received 400 rads whole body irradiation. These ^{3}H-labeled LLSL were also found to be predominantly Thy-1^{+}, Lyt-1^{+}2^{-}. The selective localization of immune LLSL, however, did not prevent tumor growth, with initially relatively small pieces of transplanted tumors continuing to grow in spite of the fact that specifically immune LLSL homed into them. (Supported, in part, by ACS grants IN-26U and IM-43L, by NCI grant CA-20823, and by DOE Contract 79EV10270).

0144 ACTIVATED MACROPHAGES BLOCK TARGET CELL PROLIFERATION IN THE G1 PHASE OF THE CELL CYCLE, Marvin Fishman, Thomas A. Hamilton, Gail Crawford and A. Thomas Look, St. Jude Children's Research Hospital, Memphis, TN 38101

Lymphokine-LPS activated thioglycollate elicited murine peritoneal macrophages inhibit the proliferation of both normal (Con A stimulated rat spleen cells) or neoplastic (G-1 lymphoma) cells. We have examined the ability of macrophage-inhibited target cell populations to proceed through individual phases of the cell cycle. Spleen cells or lymphoma cells were first separated by centrifugal elutriation into populations highly enriched for G1, S or G2/M phases of the cell cycle and cultured in the presence of non-activated or activated macrophages for periods of 2, 4, or 6 hours. The recovered nonadherent target cells were then analyzed by flow cytometry after staining with propidium iodide. Macrophage contamination of target cell populations was insignificant under these conditions. The results show that macrophage mediated cytostatic activity resulted in an absolute block of the transition of cells in G1 phase into S phase. This blockade was dependent upon prior in vitro activation of the macrophage preparation. Cells already in S phase were able to continue DNA replication although the rate was appreciably slowed relative to control cells. There was no inhibition of the passage of cells through G2 phase or mitosis. There was no difference between normal or neoplastic cells with respect to these criteria. G1 blockade can be seen by as early as 2 hours of macrophage target cell co-culture. Therefore the macrophage mediated biochemical injury must involve molecular events restricted to G1 to S phase transition.

0145 MITOGEN-INDUCED CLONAL EXPANSION OF T CELLS UNDER LIMITING DILUTION CONDITIONS: A NEW APPROACH TO STUDY THE REPERTOIRE OF CTL-PRECURSORS, K. Pfizenmaier, M. Röllinghoff and H. Wagner, University of Mainz, Germany

The enumeration of precursors of antigenspecific cytotoxic T lymphocytes (CTL-P) as a probe to analyse the repertoire of T cells was so far limited due to the fact that only those antigens which elicit a primary CTL-response in vitro could be used for stimulation. We have therefore asked, whether mitogenic activation of T-cells would result in clonal expansion such that also those T cells which cannot be induced by antigenic stimulation under in vitro conditions, can now be detected. A limiting dilution system similar to that described by Larsson et al.*, was used in which mouse spleen and lymphnode cells were stimulated byT-cell mitogens in the presence of T cell growth factors and irradiated feeder cells. With this experimental system, we found about 1 in 7 lymphnode cells and 1 in 16 spleen cells to be cytotoxic in a lectin-dependent cytolysis assay. We then applied the above protocol to determine frequencies of alloantigen-, hapten- and virusspecific CTL-P. We noted that this experimental system is, with respect to the detection of specific CTL-P, apparently very sensitive. Thus, upon mitogenic stimulation, higher frequencies of specific CTL-P were found compared to antigenspecific stimulation protocols. We are currently using this approach to enumerate virus- and tumorspecific CTL-P in different mouse strains.

*(J. Immunol. 127: 1081, 1981)

0146 ANALYSIS OF CYTOTOXIC T CELL ACTIVATION USING CLONED CYTOTOXIC T CELL TUMOR LINE AGAINST SYNGENEIC TUMOR IN THE MOUSE, Masaru Takata, Shigeyoshi Fujimoto, Department of Immunology, Kochi Medical School, Kochi, Japan.

We have previously defined that the cytotoxic T cells against methylcholanthrene-induced sarcoma,S1509a in A/J mouse can exert their killing function with the aid of macrophage-like accessory cells. This evidence was further confirmed by using a cloned cytotoxic T cell tumor line against S1509a , which was found to be transformed to a functioning tumor of the cytotoxic T cell in vitro by restimulating immune spleen cells with mitomycin C-treated S1509a and cultivating them with T cell growth factor(TCGF). The established cytotoxic T cell tumor clone only showed specific killing to S1509a in the presence of a small number of accessory cells such as peritoneal exudate cells(PEC) or T cell depleted normal spleen cells. These results suggest that accessory cells are essentially required for the activation of the cytotoxic T cell to manifest its cytotoxic killing function at the effector phase. Furthermore, accessory function of PEC can be substituted by soluble factor(s) from PEC which was activated by culture supernatant of the cytotoxic T cell clone together with target tumor cells. These results,taken together, suggest that not only specific antigen recognition of cytotoxic T cells but also a cytotoxic T cell activation signal(s) derived from activated accessory cells are essentially required for the manifestation of the cytotoxic killing function of the primed cytotoxic T cells.

0147 EFFECTOR CELL SPECIFICITY OF BCL_1 TUMOR REJECTION IN ALLOTYPE CONGENIC MICE. Lee A. Henderson, Richard P. Ciavarra and James Forman, Dept. Microbiology, University of Texas Health Science Center at Dallas, 5323 Harry Hines Blvd., Dallas, TX. 75235

A previous report has demonstrated that an sIg^+ bearing B cell leukemia (BCL_1) that arose in a BALB/c ($Ig\text{-}1^a$) mouse is rejected by the Ig congenic strain, C.B-20 ($Ig\text{-}1^b$); whereas, tumor mortality occurs when injected into BALB/c or the Ig heavy chain recombinant strain BAB-14 ($Ig\text{-}1^a_V$, $Ig\text{-}1^b_C$), (Ciavarra and Forman J. Immunol. (1981) 126:54). Immunity is T cell mediated since adoptive transfer of spleen cells from C.B-20 tumor rejectors protects sublethally irradiated C.B-20 recipients that are challenged with tumor. Identical adoptive transfer experiments of C.B-20 rejector spleen cells into BALB/c recipients results in mortality, indicating that BALB/c mice express an antigen shared by the tumor cells and recognized by effector cells. BAB.14 mice, however, are protected by immune cells from C.B-20. Taken together these results indicate that a difference at both the $Ig\text{-}H_V$ and $Ig\text{-}H_C$ region is needed to induce an anti-tumor response, but once generated effector cells are specific for antigens controlled by the IgH_C region. Further experiments using various Ig recombinants will be discussed to define the role of Ig linked loci in tumor rejection.

0148 MOLECULAR INTERACTIONS IN HUMAN T CELL-MEDIATED CYTOTOXICITY TO EPSTEIN-BARR VIRUS, Constantine D. Tsoukas, Robert I. Fox, Sherman Fong, Dennis A. Carson and John H. Vaughan, Scripps Clinic and Research Foundation, La Jolla, CA 92037

The mechanism of lysis of virally transformed target cells by human cytotoxic T lymphocytes is poorly understood. We have utilized a well defined system for the generation of HLA-restricted human cytotoxic T cells specific for autologous Epstein-Barr virus (EBV) transformed targets to study killer-target cell interactions. The cytotoxic function was inhibited by monoclonal antibody OKT3 (pan-T) and its $F(ab')_2$ and Fab fragments in the absence of complement. Inhibition was not due to antigenic modulation since incubation with antibody OKT3 did not decrease detectably the amount of antigen on the cell surface. Inhibition could be bypassed by the addition of Concanavalin A. To elucidate the mechanism of inhibition by antibody OKT3, we defined conditions which allow the dissection of the cytolytic process into two discrete temperature and time dependent steps. The first step, killer-target adhesion formation, begins after incubation for 5-10 minutes at 37°C. Adhesion formation will also proceed at 20°C, but 5-fold less efficiently. The second step, programming for lysis (PFL), follows adhesion formation and commits the target to lysis. This step proceeds only at 37°C, and not at all at 20°C. It is completed after 20 minutes of incubation. Antibody OKT3 inhibits cytotoxicity only when added before the initiation of the PFL step. These results indicate that antibody OKT3 inhibits directly either killer-target adhesion formation or the PFL step itself. OKT3 may bind to a structure intimately connected with, or identical to, the receptor for human cell mediated cytotoxic reactions.

0149 REGULATION OF CYTOTOXIC T CELLS: REQUIREMENT FOR HELPER ACTIVITY DURING IN VIVO PRIMING. Jo-Ann Keene and James Forman, UTHSCD, 5323 Harry Hines, Dallas, TX. 75235

B6.T1a^a (Qa-1^a) mice that are primed in vivo and restimulated in vitro with Qa-1 congenic cells from B6 (Qa-1^b) animals are unable to generate anti-Qa-1 cytotoxic T lymphocytes (CTL). The inability to generate anti-Qa-1 CTL is not due to an absence of anti-Qa-1 CTL precursors (CTL.P) since these mice generate anti-Qa-1 CTL when primed in vivo with Qa-1 and H-Y alloantigens (females primed with male cells) or Qa-1 and minor H-alloantigens (primed with sex matched A.BY cells). Rather, the inability to generate anti-Qa-1 CTL is due to a lack of helper or accessory antigens on B6 immunizing cells. Helper determinants are obligatory during in vivo priming.

In contrast, although it is necessary to restimulate the in vivo sensitized anti-Qa-1 CTL.P during a five day mixed lymphocyte culture, helper determinants are not required for this in vitro restimulation. Thus, different helper requirements exist for unprimed CTL.P in vivo versus primed CTL.P in vitro.

Further, the generation of anti-Qa-1 CTL is under IR gene control since F_1 mice, obtained by crossing responder A/J with nonresponder B6.T1a^a animals, generate CTL to the Qa-1^b alloantigen when presented on B6 spleen cells. Progeny testing of backcross mice further demonstrated that the IR gene(s) is linked to the H-2 complex.

These data indicate that an H-2 linked IR gene controls the recognition of helper determinants required for CTL priming in vivo. These helper determinants can be distinguished from CTL determinants and both must be recognized together for successful priming of CTL.P.

0150 THE EFFECTS OF CYCLOPHOSPHAMIDE ON IN VITRO CYTOTOXIC RESPONSES TO A SYNGENEIC TUMOUR, Elizabeth J. Hancock and Douglas G. Kilburn, Department of Microbiology, The University of British Columbia, Vancouver, B.C., V6T 1W5.

We have studied the effects of treating DBA/2 mice with cyclophosphamide, upon their subsequent ability to generate cytotoxic cells in vitro against syngeneic tumour antigens or alloantigens. Low doses of cyclophosphamide (25-50 mg/kg body weight) appeared to enhance both responses probably by removing suppressor cells from the responding population. Higher doses (75-200 mg/kg) eliminated the response to both antigens. The addition of normal DBA/2 thymocytes into these cultures restored the response to allogeneic cells but not to tumour cells. The anti-tumour response could be restored by the addition of IL2 to the cultures.

0151 MACROPHAGE MEDIATED TUMORICIDAL ACTIVITY TOWARD MALIGNANT TUMOR VARIANTS, T.K. Huard, A.M. Kalynych and A.F. LoBuglio, University of Michigan, Ann Arbor, MI 48109

We have initiated studies to examine the cytotoxic potential of macrophages to syngeneic tumor cells that vary in metastatic potential. This model is being developed to evaluate the in vivo effects of biological response modifiers with potential in cancer therapeutics. We have investigated the capacity of macrophages to kill syngeneic variants of a 3-methylcholanthrene induced fibrosarcoma in normal C57BL/6 mice. Killing was assessed in a ^{3}H-thymidine release assay at 48,74 and 96 hr post culture initiation. The tumor cells used as targets in these studies varied in malignant potential including: a) the parent tumor line which induced tumors in 100% of syngeneic mice and elicited pulmonary metastases in 20-45% of the recipients, b) a highly malignant isolate from pulmonary nodules that caused tumors in 100% of recipients and lung nodules in >80% of recipients, and c) a low malignant cell line adapted for growth in human serum in vitro that caused tumors in 25% of recipients and pulmonary lesions in <5%. Since these tumor variants selectively metastasize to the lungs pulmonary alveolar macrophages (AM) were used as effector cells. The AM were isolated by pulmonary lavage. Cell recovery was 0.4-1.2x10^{6} cells/mouse and the cells were >95% pure by morphology, >92% nonspecific esterase (+) and >95% viable. Direct AM cytotoxicity to 1x10^{4} ^{3}H-TdR labeled tumor cells was assessed at effector/target cell ratios (E/T) of 0.5:1 to 10:1. Significant AM cytotoxicity to these malignant variants was observed at E/T of 1:1 to 10:1. The cytotoxicity increased with time of incubation and was evident against all three malignant variants. Alterations in the cytotoxic potential of AM from mice stimulated in vivo with agents known to activate macrophages and/or induce interferon will be discussed.

0152 LYT-2 NEGATIVE CYTOTOXIC T LYMPHOCYTE HYBRIDOMAS, Yael Kaufmann and Zelig Eshhar, The Weizmann Institute of Science, Rehovot, Israel

Lyt-2 alloantigen has been detected in most murine cytotoxic T lymphocytes (CTL) and was implicated in target cell (TC) recognition or lysis. The relevance of Lyt-2 to the lytic activity of CTL was examined here using CTL-hybridomas which specifically lyse allogeneic leukemic TC in vitro (Y. Kaufmann, G. Berke & Z. Eshhar, Proc. Natl. Acad. Sci. US 78: 2502, 1981). The cytotoxicity of the CTL-hybrid clones was not affected by monoclonal anti Lyt-2 antibodies, even at 30-fold the concentration which caused 50% inhibition of TC killing by CTL in a mixed lymphocyte culture (MLC). In contrast to anti Lyt-2, several other antibodies, which were generated against CTL or the CTL-hybridomas, inhibited the CTL hybridoma mediated lysis. The expression of Lyt-2 was examined by flow cytofluorometric analysis using the same monoclonal anti Lyt-2. Although the hybridomas' parental CTL populations (peritoneal exudate lymphocytes and MLC) expressed considerable amounts of Lyt-2, the CTL-hybridomas were Lyt-2 negative. The absence of detectable Lyt-2 from CTL hybridoma surfaces and the inability of anti Lyt-2 to inhibit their specific killing activity suggest that Lyt-2 molecules are not obligatory for CTL-mediated TC lysis.

0153 HIGHLY PURIFIED POSITIVELY SELECTED Lyt2^{+} T CELLS FROM NORMAL MOUSE SPLEEN HAVE AN OBLIGATORY REQUIREMENT FOR Lyt2^{-} HELPER CELLS OR IL2 FOR CTL GENERATION IN VITRO. [1]Michael Mage, [2]Bonnie Mathieson, [1]Louise McHugh, [3]Susan Sharrow, and [4]John Farrar, [1]Lab. of Biochemistry, NCI, [2]Lab. of Microbial Immunity, NIAID, [3]Immunology Branch, NCI, and [4]Lab. of Microbiology and Immunology, NIDR, NIH, Bethesda, Md. 20205.

Generation of CTL from precursors in MLC has previously been reported to require helper cells (inferred to be Lyt2^{-} and Ly7^{+} by negative selection experiments with antibody plus C), or of their putative product, the lymphokine IL2 (Shaw et al. J. Immunol. 124:2231, 1980). The recent development of a general cell separation procedure that permits nonlytic preparative separation of Lyt2^{+} and Lyt2^{-} T cells has allowed us to directly examine the requirement for help for CTL generation. Semiallogeneic B cell stimulators (not irradiated) were purified by specific adherence and release from anti-Ig coated tissue culture dishes (Mage et al. J. Imm. Methods 15:47, 1977). Lyt2^{+} and Lyt2^{-} responders were purified by binding of anti-Lyt2 coated T cells to anti-Ig coated tissue culture dishes (Mage et al. Eur. J. Immunol. 11:228, 1981). We have found that in primary MLC, the Lyt2^{+} CTL precursors are exquisitely sensitive to the presence of Lyt2^{-} helper cells, without which no CTL activity is generated, but that this requirement for help is fully met by the substitution of IL2. Further, when the stimulators are depleted of accessory cells, addition of IL2 but not of helper cells restores the ability of the MLC to generate CTL. This finding is consistent with the postulated role of macrophage-derived IL1 in the stimulation of helper T cells to produce IL2. Generation of CTL in an MLC between purified Lyt2^{+} responders and B cell stimulators can be used as a highly sensitive assay system for helper cells or for IL2.

0154 IMMUNOREGULATORY PROPERTIES OF SPLENIC SUPPRESSOR MACROPHAGES, Bruce C. Veit, St. Jude Children's Research Hospital, Memphis, TN 38101.

Studies of the suppressive activity of splenic macrophages (MØ) indicate that suppression is reversible and does not result in the destruction of immunocompetent progenitor cells. Splenic MØ cultured for 3 to 5 days in the presence of T cells acquired an increased capacity (per cell) to suppress the development of a secondary cytotoxic T lymphocyte (CTL) response to a Gross virus induced lymphoma. When such cultured MØ were added to constitute 1 to 2% of total cells, complete suppression of the CTL response resulted. Suppression was brought about by the synthesis and release of prostaglandins (PG) as evidenced by inhibition of involvement of the suppressive activity with inhibitors of PG synthesis (aspirin, Indomethacin, and d, 1-6-chloro-2-methyl carbazole-2-acetic acid). Events which occurred within the first 24 to 48 hours of the CTL response were inhibited by MØ since normal responses were obtained if MØ were added later than Day 2 or if Indomethacin was present during the first 48 hours of culture. Two processes of lymphocyte activation, namely blast transformation and DNA synthesis, were inhibited in the presence of MØ. However, suppression of these events did not result in the loss of CTL progenitor cells since CTL responses that were inhibited in the presence of suppressive MØ proceded normally following their removal. These findings indicate that lymphocyte activation which results from antigenic stimulation can be regulated by MØ through the production of PG without eliminating immunocompetent progenitor cells. Therefore, an immune response to a particular immunogen can be effectively suppressed while the normal repertoire of antigen reactive cells is maintained. (Supported by ALSAC)

0155 CHARACTERIZING THE TWO SIGNAL REQUIREMENT FOR GENERATION OF CTL RESPONSE, Steven H. Herrmann, Ofra Weinberger, Steven J. Burakoff, and Matthew F. Mescher, Harvard Medical School, Boston MA 02115

The requirements for generation of a secondary allogeneic CTL response have been examined using purified H-2K^k incorporated into liposomes (signal 1) and a concanavalin A supernatant from rat lymphocytes (T_HF, signal 2). The alloantigen signal could be delivered by mixing liposomes and primed spleen cells for a short period of time followed by removal of >90% of the alloantigen by several cycles of centrifugation. The timing for addition of the T_HF in relation to the alloantigen signal was examined by incubating primed spleen cells with T_HF before or after they were pulsed with antigen. This demonstrated that the CTL will not respond to T_HF (signal 2) until 12-24 hrs after interacting with antigen (signal 1). Conditions for delivery of the first signal were examined by pulsing spleen cells with antigen followed by the addition of an optimal amount of T_HF 24 hrs later. We found that recognition of antigen takes place in a time and concentration dependant manner. Pulsing with liposomes for 30 min at 4°C provided a maximal first signal to spleen cells depleted of adherent cells. The response could be blocked by preincubating the liposomes with antisera directed against the alloantigen prior to pulsing the spleen cells. Addition of the antisera after liposomes and spleen cells were mixed did not significantly reduce the CTL response. These and other data indicate that pre-CTL can directly recognize alloantigen in liposomes at 4°C as the first signal and must then be incubated at 37°C for about 24 hrs before becoming responsive to the second signal (T_HF).

0156 ANTIGEN-SPECIFIC CLONED T CELLS, Paul L. Black and Ian A. Mac Neil, Temple University School of Medicine, Philadelphia, PA 19140

We generated alloreactive cytotoxic T cells by immunizing C57BL/6J mice (H-2^b) with BALB/c (H-2^d) spleen cells. Cytotoxic T lymphocytes (CTL) were maintained in supernatants obtained from mitogen-stimulated rat spleen cell cultures for 2 months, at which time the CTL were cloned twice successively by limiting dilution. One resultant Interleukin 2 (IL2)-dependent cloned line, CTL2.A4.E6, recognizes specificities encoded by H-$2K^d$. Since the generation of this and other cloned CTL lines, we have pursued 3 areas of investigation. 1) We have evaluated alternate sources of IL2 and have isolated a BALB/c thymoma which secretes IL2 constitutively. This thymoma has been subcloned to isolate high producers, and a biochemical analysis of the secreted proteins is currently underway. In addition, the supernatant is being used to maintain our cloned CTL in culture. 2) Interleukin 1 (IL1), derived from the supernatants of lipopolysaccharide-activated macrophage tumor cells (RAW 264.7), has a synergistic effect with IL2 on the proliferation of cloned CTL. This synergistic effect resides in both low (12-16,000) and middle (25-35,000) molecular weight fractions of IL1. 3) We have attempted to generate IL2-independent cytotoxic hybridomas by fusing thymomas with IL2-dependent cloned CTL lines. Various thymomas and several fusion procedures have been evaluated, and a reliable method has been developed.

0157 SPECIFIC ALLORECOGNITION OF AN MHC GENE PRODUCT DERIVED BY DNA-MEDIATED GENE TRANSFER, Jerold G. Woodward, Anders Orn, Richard C. Harmon, Robert S. Goodenow, Leroy Hood, and Jeffrey A. Frelinger, University of Southern California, Los Angeles, CA 90033

Mouse L cells co-transformed with the herpes virus tk gene plus a genomic clone containing the H-2L^d gene were shown to function as targets for H-2L^d specific cytotoxic T lymphocytes (CTL). Gene transfer and immunoselections were used to identify an L cell clone (8-5) expressing the L^d gene product (Goodenow et al. Science, in press). High levels of H-2L^d-specific cell mediated cytotoxicity were demonstrated on 8-5 target cells in a 4 hour chromium release assay. No H-2L^d specific CTL activity was detected on L cells transfected with the HSV tk gene only. CTL specificity was demonstrated by 1) the use of CTL with restricted specificities, 2) cold target inhibition, and 3) monoclonal antibody inhibition. We also demonstrated that 8-5 cells could function as targets for antibody plus complement-mediated cell lysis. Again, H-2L^d specificity was confirmed using 3 different L^d specific monoclonal reagents. These experiments demonstrate that a genomic clone of an MHC gene product can be functionally expressed in a foreign cell and mediate immunologically specific cellular interactions. With the capabilities now in hand to specifically modify genes in vitro, specific structure-function relationships of MHC molecules are now possible.

(Supported by NIH Grants CA22662 and GM06965. JGW is a recipient of a National Arthritis Foundation Postdoctoral Fellowship)

0158 MOLECULAR CHARACTERIZATION OF LYT-2/3 ANTIGENS EXPRESSED BY CTL CLONES, Claude Bron, Oresto Acuto, Andrew L. Glasebrook, Jean-Charles Cerottini, H. Robson MacDonald, Department of Biochemistry, Université de Lausanne; Department of Immunology, ISREC; Ludwig Institute for Cancer Research; CH-1066 Epalinges (Switzerland).

Antibodies against Lyt-2/3 antigens inhibit cytolytic activity, antigen-specific proliferation and lymphokine secretion of certain cloned T cells. This suggests that these surface molecules may play a role in antigen recognition and/or binding. However a number of CTL clones have been derived which were insensitive to antibody inhibition. In order to further investigate the possible function of Lyt-2/3 antigens, we compared the surface expression and structure of these molecules on inhibited and non-inhibited clones. No quantitative differences in the surface density of Lyt-2/3 antigens could be detected by flow microfluorometry. Immunochemical analysis revealed a single broad band of 40-42 Kd apparent MW which differs from the Lyt-2 antigens expressed by thymocytes. However no obvious structural differences could be found which could account for the different susceptibility of CTL clones to inhibition by anti-Lyt-2/3 antibodies. More detailed biochemical analysis using 2-dimensional gel electrophoresis and peptide mapping is currently in progress in an attempt to demonstrate structural heterogeneity in Lyt-2/3 antigen expression.

0159 HIGH AFFINITY CONCANAVALIN A (Con A) BINDING GLYCOPROTEIN ON THE CYTOTOXIC T LYMPHOCYTE CELL SURFACE IS INVOLVED IN CTL-TARGET INTERACTIONS, M.V. Sitkovsky, M.S. Pasternack, and H.N. Eisen, Massachusetts Inst. of Technology, Cambridge, MA 02139

This study was undertaken to test the idea that exposure of cytotoxic T lymphocytes (CTL) to certain lectins at low concentration would lead to selective interaction with a small number of surface molecules, among which there might be a structure required for recognition or lysis of target cells. Of 5 lectins tested, Con A seemed to meet the objective: at 1-10 μg/ml it profoundly blocks antigen-specific lysis of target cells by CTL. Separate pretreatment of CTL and target cells showed that the effect is exercised primarily on CTL. Several trivial explanations could be eliminated: e.g., reversibility of the effect with α-methylmannoside, and the use of succinylated Con A and subagglutinating concentrations of Con A, ruled out 1) CTL-CTL self-killing by a nonspecific lectin-dependent mechanism and 2) agglutination preventing recycling of CTL among target cells. It appears that subagglutinating concentrations of Con A block CTL activity by binding to and interfering with a CTL surface structure that is critically involved in recognition or lysis of target cells. A preliminary observation (with James Lugo), involving immunoprecipitation of ^{125}I-surface-labeled lymphocytes with Con A and anti-Con A antibodies, followed by SDS-PAGE, suggests that a critical protein with high affinity for Con A is part of the T-200 complex. (Supported in part by NCI training grant (T32CA09255) and NCI research grants (CA15472 and CA28900)).

0160 ROLE OF HAPTEN MODIFIED Ia ANTIGENS ON TARGET B LYMPHOCYTES IN THE CYTOLYTIC T LYMPHOCYTE MEDIATED SUPPRESSION OF ANTI-HAPTEN ANTIBODY RESPONSES. Steven J. Burakoff, Gina Moser and Abul K. Abbas. Sidney Farber Cancer Institute, Harvard Medical School and Brigham and Women's Hospital, Boston, MA 02115

Addition of 2, 4, 6-trinitrophenyl (TNP) reactive cytolytic T lymphocytes on day 3 to syngeneic, TNP-hemocyanin primed spleen cells stimulated with TNP-hemocyanin leads to marked inhibition of IgM and IgG anti-TNP PFC measured 1 day later. The cells mediating this effector phase suppression possess properties characteristic of CTL, are hapten-specific and H-2 restricted. Moreover, the targets of the suppression appear to be B cells which are recognized by CTL via antigen bound to specific immunoglobulin receptors on their surface. The suppression can be blocked by adding monoclonal antibodies directed against I-A but not I-E or H-2K encoded determinants on the target B cells prior to adding CTL. Therefore, it appears that TNP-specific CTL recognize receptor-bound antigen only in association with I-A encoded molecules on the surface of B cells. These findings suggest that: 1) receptor bound antigen on B cells tends to preferentially associate with I-A and not H-2K/D encoded determinants and 2) the small subpopulation and TNP-reactive CTL which recognizes hapten-modified Ia antigens mediates this suppression and may therefore have a role in immune regulation. (Supported by NIH grants AI16349 and AI17258 and the American Cancer Society).

0161 FREQUENCIES AND REGULATION OF TRINITROPHENYL-SPECIFIC CYTOTOXIC T PRECURSOR CELLS, Ute Hamann, Klaus Eichmann, Peter H.Krammer, Institute of Immunology and Genetics, German Cancer Research Center, Im Neuenheimer Feld 280, D-6900 Heidelberg, Fed.Rep.Germany.

The precursor frequency and the regulation of trinitrophenyl(TNP)-specific cytotoxic lymphocytes in unimmunized and immunized T cell populations was investigated. T cell populations from normal non-immune mice or from mice immunized by skin painting with pycrilcloride, subcutaneous injection with TNP-coupled syngeneic cells, or from spleen cells primed with TNP-coupled cells in vitro were activated by Concanavalin A for two days and then plated in limiting dilution microcultures in the presence of irradiated peritoneal exudate filler cells and medium supplemented with TCGF. Cells expanded after a growth period of 7 days were tested for their cytotoxic activity on normal and TNP-coupled syngeneic target cells. This analysis established the presence of several sets of CTL precursors independently regulated by suppressive mechanisms. Similar sets of CTL precursors were observed in normal, non-immune cell populations and in cell populations early after immunization. Several days after priming only the frequent set of CTL precursors could be observed which was of similar frequency as the CTL precursor set found in normal, non-immune cell populations.

Functional Properties of T Cell Tumors

0162 SEZARY SYNDROME: EVIDENCE FOR MALIGNANT PROLIFERATION OF T CELLS PRODUCING INTERLEUKIN-2 (T-CELL GROWTH FACTOR). W.Solbach, S. Barth, M.Röllinghoff and H. Wagner, Inst.f.Med.Mikrobiologie, University Mainz,W.-Germany.

The observation that malignant peripheral blood cells (PBL) from patients with Sézary-syndrome (Sézary cells) are identical in their surface phenotype with a subset of normal T helper cells ($OKT3^+$, $T4^+$, $T8^-$)suggests that malignant Sézary T cells are derived from mature, well differentiated T cells. Therefore, their functioning as helper cells in T-T-cell collaboration was investigated. In particular we studied, whether Sézary cells were able to release a T cell growth factor, Interleukin-2 (Il-2), which in healthy individuals is produced by activated $OKT4^+$-T cells and is essential for the development of cytotoxic T lymphocytes. Both, freshly drawn PBL and Sézary cells cultured in vitro produced high levels of Il-2 upon stimulation with alloantigens, PHA and phorbol esters (PMA).(PMA-stimulation of healthy controls never resulted in Il-2 release.).In addition, PBL from Sézary patients proliferated in response to exogeneously added Il-2 in long term cultures (4 wks)in vitro.
These studies suggest, that malignant Sézary T cells are well differentiated, functionally competent T helper cells. Moreover, if the Il-2 producer Sézary T cell is identical to the cell responding to Il-2, this lymphokine may be important for the self-perpetuation of the malignant process.

0163 DIFFERENTIAL RESPONSES OF MOUSE LYMPHOID TUMOR CELL LINES TO X-IRRADIATION, Alan W. Harris and John W. Lowenthal, The Walter and Eliza Hall Institute of Medical Research, Royal Melbourne Hospital Post Office, Victoria 3050, Australia

For almost all types of mammalian cell, death after X-irradiation follows transit through one or more post-irradiation cell divisions. Mitogenically activated lymphocytes also follow this pattern, but normal resting lymphocytes, unlike other non-cycling cells, die rapidly after irradiation without passing through mitosis. We have tested a number of murine lymphoid tumor cell lines for response to X-irradiation in vitro as part of a program exploring ways in which such cell lines retain normal lymphocyte characteristics and can therefore be used to study the cellular physiology of those characteristics.

After an X-ray dose of 1000 rad, cells of 7 T lymphoma and 3 pre-B lymphoma lines died as rapidly as irradiated normal lymphocytes, with viability declining through 50% in 6-12 hr. Cells of another 7 T lymphoma and 2 pre-B lymphoma lines, as well as a total of 20 B lymphoma, myeloma and nonlymphoid cell lines, lost viability in a delayed fashion with 50% viability-loss times ranging from 23 to about 100 hr. Cells of the first group died without passing through mitosis whilst those of the second group showed a mitotic delay and then resumption of mitosis before substantial cell death occurred.

Although divergence of cell properties might develop during prolonged growth in culture, it is possible that the differential responses of lymphoid tumor cell lines to irradiation are representative of different physiological states of the lymphocytes at the time of their neoplastic transformation.

0164 A T-CELL CHRONIC LYMPHOCYTIC LEUKEMIA WITH SUPPRESSOR EFFECTOR CELL FUNCTION, Philip S. Crosier and Mike E.J. Beard, Departments of Medicine and Pathology, Christchurch Clinical School of Medicine, Christchurch Hospital, Christchurch, NEW ZEALAND.

A patient will be discussed who presented with a T-cell chronic lymphocytic leukemia. Analyses revealed that the T-cells functioned in vitro as suppressor effector T-cells. The T-cells expressed HLA-DR, OKT3 and OKT8 surface antigens. The cells were incapable of responding or stimulating in mixed lymphocyte culture. The cells functioned in a genetically non-restricted manner by regulating MLC proliferative responses in all donors tested. The T-CLL also markedly inhibited the generation of alloreactive cytotoxic T-cells. The T-cell leukemia was not directly cytotoxic to allogeneic lymphocytes. Both the suppression of MLC proliferation, and the generation of cytotoxic T-cells, was cell dependent, and could not be achieved using T-CLL culture supernatants. No interleukin-2 activity was detectable in the supernatants. The results could be interpreted as perhaps representing the malignant clonal expansion of an immunocompetent T-suppressor cell clone.

0165 THE RESPONSE OF MURINE THYMOMA CELLS TO GLUCOCORTICOIDS: ANALYSIS OF CELL CYCLE PHASES. Carol L. MacLeod and Suzanne Bourgeois, Regulatory Biology Laboratory, The Salk Institute, San Diego, California.

The murine thymoma cell line WEHI-7.1 (W7) is sensitive to the cytolytic action of glucocorticoids, and this response requires the presence of functional glucocorticoid receptors. The cellular events which occur immediately following nuclear translocation of the receptor-glucocorticoid complex are unknown. It is possible that the cell cycle phase has an effect on the hormone responsiveness of these suspension cultured cells. In an effort to identify gene products which may be regulated by dex, we developed a method to isolate purified populations of cells in each of the major cell cycle phases in the hope of enriching for responding cells. To avoid the use of toxic synchronizing agents, we have employed the DNA specific Hoechst 33342 vital stain to obtain synchronized cell populations. The G1, S and G2 phases of the cell cycle are separated using a dual parameter fluorescence activated cell sorter (FACS). We have established staining conditions in which growth and progression through the cell cycle proceed without delay following FACS. Flow microfluorimetry of the sorted fractions show they are more than 95% pure. Cells treated with hormone for six hours and subsequently sorted retain their cellular integrity and incorporate ^{35}S-methionine into protein. Work is now in progress to detect specific hormone induced changes in protein synthesis. The sorted fractions from dex-treated and control cell populations are pulse-labeled with ^{35}S-methionine and the radiolabeled proteins are displayed on high resolution 2-dimensional polyacrylamide gels for analysis. Supported by grants from NIH, Whitehall Foundation and National Leukemia Assoc. C.M. is a Leukemia Society of America Special Fellow.

0166 GENERATION, SPECIFICITY, AND FUNCTION OF HAPTEN-SPECIFIC T CELL CLONES, Carol Clayberger, Rosemarie DeKruyff, and Harvey Cantor, Harvard University, Boston, MA 02115

T cell clones specific for the haptens 4-hydroxy-3-nitrophenyl acetyl (NP) and trinitrophenol (TNP) were derived from spleens and lymph nodes of immunized mice. These clones were cultured with irradiated syngeneic spleen cells, antigen, and Interleukin 2. The clones recognize either haptenated carrier protein or haptenated spleen cells. Two types of clones which recognize soluble antigen were generated. TNP specific clones respond to TNP on a variety of carrier proteins; NP specific clones respond to NP only when presented on the appropriate carrier. Hapten specific proliferation requires identity at the I-A locus. The phenotype of all clones is Θ^+, Lyl^+, $Ly2,3^-$. The clones produce Interleukin 2 when activated by either antigen plus irradiated spleen cells or Concanavalin A. Other factors produced by these cells after activation promote hapten specific B cell proliferation and antibody secretion. After stimulation with antigen, cells were internally labelled with ^{35}S-methionine and supernatants were affinity purified on NP or TNP Sepharose columns. Hapten binding proteins were separated by SDS-PAGE. Fluorography of these gels shows that 4-5 proteins bound specifically to the affinity columns.

0167 ALKALINE PHOSPHATASE ACTIVITY OF MURINE LYMPHOID CELL LINES. Judith Rae Lumb*, Allen Silverstone and Margrit Scheid, Memorial Sloan-Kettering Cancer Center, 1275 York Avenue, New York, New York 10021

Alkaline phosphatase is found in a layer of cells surrounding lymphoid follicles in the normal mouse spleen, in thymic lymphomas of C57BL mice and in the fetal thymus up to sixteen days gestation in C57BL mice. Alkaline phosphatase activity can be induced by mitogens in lymph node cells, is increased in the spleen of nude mice over that in the spleen of nu/+ littermates and is increased in the regional lymph node after treatment with oxazalone. These data suggest that the alkaline phosphatase-positive cell is a pre-T cell. This report describes a survey of alkaline phosphatase activities in murine lymphoid cell lines which have been characterized according to other markers to determine their level of differentiation. It is concluded that alkaline phosphatase is found in cell lines with pre-T characteristics, in cell lines with pre-B characteristics and in cell lines with both pre-T and pre-B characteristics. Alkaline phosphatase was not found in the cell lines tested which had mature T-cell, macrophage or stem cell characteristics. It is concluded that alkaline phosphatase is a characteristic of cell lymphocyte differentiation prior to the differentiation into T- and B-cells and that it is repressed in the T-cell lineage but not in the B-cell lineage. It must be noted, however, that these conclusions are based on data from cell lines which are inherently immortal. Normal cells must also be tested. *Current address: Biology Department, Atlanta University, Atlanta, Georgia

0168 IR-GENE RESTRICTED INDUCTION BY LYMPH NODE T CELLS OF LYMPHOKINE SYNTHESIS IN IRRADIATED LYMPHOMA CELLS OF SJL/J MICE. N.M. Ponzio, T. Hayama, C. Nagler, I.R. Katz, J. Vilcek and G.J. Thorbecke. CMDNJ-New Jersey Medical Sch, Newark, N.J. and N.Y.U. Med Ctr, NY.

Previous studies have shown that the reticulum cell sarcomas (RCS) of SJL/J mice fall within the B cell lineage, presumably arrested at a pre-B cell stage of development, since they are sIg^-, $I\text{-}A^+$, $Lyb\text{-}2^+$. Co-culture of irradiated tumor cells (X-RCS) and normal, syngeneic lymph node (LN) cells results in marked proliferation of $Lyt\text{-}1^+$ T cells and lymphokine production, without development of cytotoxic T cells. When compared to allogeneic MLR, the lymphokine production and T cell proliferation in this syngeneic response is faster and at least 2-fold higher in magnitude. Studies using SJL F_1 hybrid LN cells indicate a dominant negative effect of IR regions of certain H-2 haplotypes. Analysis of supernatant fluid (SN) from cultures of LN + X-RCS revealed the presence of IL-2, interferon (IFN-γ), thymocyte stimulating activity and antibody enhancing activity (TRF). IL-2 and TRF activities were both removed after absorption of crude SN with the IL-2-dependent assay cell line, suggesting that they are the same, or that IL-2 is a required component for TRF activity. Fractionation of LN cells and limiting dilution analysis of the cellular participants in LN + X-RCS cultures indicate that as few as 10^5 X-RCS cells suffice for lymphokine production. After pretreatment of X-RCS with an irreversible metabolic inhibitor (Pactamycin), prior to co-culture with LN cells, little, if any lymphokine activity was detectable in SN. Similar pretreatment of LN cells had less effect, suggesting that the tumor cells produced at least part of the lymphokine activity. (Supported by USPHS Grants CA22544, AI12948 , CA14462 and the Foundation of the College of Medicine and Dentistry of New Jersey)

0169 PHORBOL ESTER-INDUCED PROLIFERATION AND INTERLEUKIN-2 PRODUCTION IN T-CELL MALIGNANCIES, F.A. Vyth-Dreese, H.J. v.d. Reijden and J.E. de Vries, Division of Immunology, The Netherlands Cancer Institute, 1066 CX Amsterdam, The Netherlands

The mitogen reactivity of peripheral blood and bone marrow cells from 5 patients with T-cell chronic (T-CLL) and 2 patients with T-cell acute (T-ALL) lymphoproliferative disease was investigated. Proliferative responsiveness to phytohemagglutinin (PHA) generally was found to be reduced or completely absent. Stimulation with PHA in the presence of 12-O-tetradecanoyl phorbol-13-acetate (TPA) however, resulted in the induction of proliferation in 5/8 PHA non responsive cultures and augmentation of proliferation in 6/6 cultures that were already reactive to PHA itself. Under identical conditions a similar induction and enhancement of mitogenesis was found with normal thymocytes and mature T cells respectively, the latter however only at suboptimal PHA doses. TPA-induced mitogenesis of the group of T-CLL cell populations was comparable to that of normal, mature T cells, whereas the phenotypically more immature T-ALL cell populations showed the same reactivity pattern as normal thymocytes. In addition, TPA itself was weakly mitogenic only for T-CLL and normal mature T cells. The identical triggering conditions of mitogenesis for neoplastic and normal T-cell populations depending on their state of maturation, suggest that TPA induces neoplastic T-cell populations to differentiate into a functionally more mature state. In general, a correlation was observed between the induction and enhancement of proliferation and the generation of Interleukin-2 (IL-2) in the supernatant of PHA + TPA stimulated cultures. Therefore, failure to produce sufficient amounts of IL-2, essential for normal T-cell proliferation, might be the basis of the impaired responsiveness in T-cell lymphoproliferative disease.

0170 STIMULATION OF PHAGOCYTOSIS BY A T-CELL LYMPHOMA DERIVED LYMPHOKINE, E.S. Jaffe, C.R. Simrell, G.R. Crabtree, A.S. Fauci and J. Cossman, NIH, Bethesda, Md. 20205

Certain patients with peripheral T-cell lymphoma develop a syndrome which mimics malignant histiocytosis (MH). It is suspected that a pathogenetic mechanism of this phenomenon may involve a lymphokine produced by the neoplastic T-cells which can stimulate mononuclear phagocytes. To test this hypothesis, neoplastic cells from malignant lymphomas were placed in culture and twenty-four hour supernatants were tested for the presence of a phagocytosis inducting factor (PIF) using the human macrophage-like cell line U937. Phagocytosis was assayed using IgG coated OX-RBC. Fc receptors on the surface of U937 cells were assayed by measuring the specific Fc receptor dependent binding of ^{125}I-IgG. Control studies showed PIF activity in supernatants from normal peripheral blood mononuclear cells (MNC) activated in mixed leukocyte culture (MLC) or by concanavalin-A; unstimulated supernatants were negative. Neoplastic cell culture supernatants from three of fourteen patients with malignant lymphomas of T-cell origin also contained PIF activity. One of these three patients died with the MH-like syndrome. A supernatant from one case of T-cell acute lymphoblastic leukemia was also positive. None of these supernatants induced any concomitant increase in Fc receptors. Supernatants from eleven cases of non-Hodgkin's lymphoma of B-cell origin and two cases of Hodgkin's disease were negative for PIF. Two lymph nodes with reactive follicular hyperplasia and two normal spleens also failed to yield any activity, although MNC from a reactive pleural fluid containing 50% T-cells did. We conclude that some neoplastic T-cells, as well as normal antigen or mitogen activated T-cells, can produce a factor which stimulates the phagocytic activity of human macrophages.

Growth of Normal and Malignant Cells

0171 A SPECIFIC DEFECT IN THE L-SYSTEM AMINO ACID TRANSPORT PATHWAY IN CHRONIC LYMPHOCYTIC LEUKEMIC (CLL) LYMPHOCYTES, George B. Segel and Marshall A. Lichtman, University of Rochester, Rochester, New York 14642

Fundamental changes in membrane function have been associated with neoplastic transformation. We have compared the three main pathways of amino acid transport, the A, ASC and L-systems in CLL-lymphocytes (80% B-cells), normal blood lymphocytes (14% B-cells) and tonsillar lymphocytes (40% B-cells). L-system transport was measured by BCH, a synthetic amino acid whose transport is limited to the L-system. BCH transport at 0.1 mM was markedly decreased in CLL-lymphocytes, 57 compared to 443 in blood or 317 μmol/L cell water/min in tonsillar lymphocytes. CLL-cells had an L-system maximal velocity that was less than 15% that of blood or tonsillar lymphocytes and a very low L-system substrate affinity (high Km) (Table). Further, in CLL-cells, leucine uptake by the L-system was less than 15% of that in blood or tonsillar lymphocytes, and total leucine uptake was sustained at 40% of normal only by its transport through the alternative ASC-system. Thus, the L-system of amino acid transport, a hallmark of normal T and B-lymphocytes, is vestigial in CLL-cells. This key functional membrane defect is more closely related to the neoplastic nature of CLL-cells than to their immunologic origin.

L-SYSTEM (BCH) TRANSPORT	CLL	BLOOD	TONSILS
$Vmax^1$	134	1025	1058
Km^2	360	131	175

[1] (μmol/L cell H_2O/min); [2] (μM)

0172 B CELL CLONES OF KNOWN SPECIFICITY: INTERACTIONS AFFECTING GROWTH AND DIFFERENTIATION, David W. Scott, Margaret Piper and P.S. Pillai, Duke Med. Ctr., Durham, N. C. 27710.
Our previous studies have shown that fluorescein (FL)-specific B cell colonies (CFU-B), grown in soft agar for 6 days, can be triggered at high frequency (10-50%) with FL-coupled thymus-independent (TI) or thymus-dependent (TD) antigens to differentiate into anti-FL PFC. We have further shown that the inclusion of a known tolerogen (FL-sheep gamma globulin) in the agar inhibits a portion of B cell colonies in their growth, and that the resistant colonies are inhibited in their differentiation upon antigenic stimulation. This inhibition occurs only with those colonies which are grown with the potentiators, LPS and SRBC in agar, but not with those grown with SRBC or LPS alone. We have now compared the effects of anti-immunoglobulin reagents on the growth and subsequent differentiation of these hapten-specific CFU-B. The addition of affinity purified goat anti-mouse-μ or monoclonal anti-δ (10-4.22) each inhibits a portion of hapten-specific B cell colonies in their growth in this agar cloning system. Interestingly, those colonies which are able to grow in the presence of anti-δ show no diminution in their ability to be triggered by FL-polymerized flagellin as the immunogen. However, the CFU-B which are able to grow in the presence of anti-μ have received a negative signal since they are unable to respond to antigen on subsequent restimulation. The results with anti-μ are similar to those observed with tolerogen. Thus, our studies with hapten-specific B cell colonies allow us to establish the differential effect of tolerogen, anti-μ, or anti-δ on the growth and differentiation of the progeny of single hapten-specific B cells. Moreover, these studies help to delineate the existence of distinct B cell subpopulations. (Supported by USPHS grant no. AI-10716-10).

0173 DEVELOPMENT OF NORMAL HUMAN T CELL LINES AND HUMAN T CELL HYBRIDOMAS WHICH RECOGNIZE TETANUS TOXOID ANTIGEN, Elaine C. DeFreitas, Stefano Vella, Alban Linnenbach, Carlo M. Croce and Hilary Koprowski, The Wistar Institute, Philadelphia, PA 19104
We have developed continuous lines of normal human T cells from peripheral blood which recognize tetanus toxoid in vitro. These lines are propagated in the presence of tetanus toxoid, autologous irradiated peripheral blood lymphocytes (PBL) and interleukin 2 (Il-2). T cells grown in this manner proliferate specifically to tetanus toxoid in the presence of irradiated autologous PBL and provide helper activity to autologous B cells in vitro resulting in the production of anti-tetanus toxoid antibody. Populations of these tetanus-reactive T cell lines were fused in vitro with the HGPRT$^-$ mutant of Jurkat, a human T cell lymphoma. Clones of T cell hybrids were screened for their antigen specificity in two ways: (1) by their ability to produce Il-2 in the presence of tetanus toxoid and autologous irradiated accessory cells, and (2) by their ability to bind to autologous monocytes pre-pulsed with tetanus toxoid. Several hybrids were found which showed both characteristics. One of these, SH-2, has been subcloned and examined for karyotype, surface phenotype, and for the HLA restricting elements involved in the recognition of tetanus toxoid.

0174 GROWTH CHARACTERISTICS OF A T-CELL LYMPHOMA ARISING IN A MOUSE TOLERIZED AT BIRTH TO MHC ALLOANTIGENS, Denise L. Johnson, Jack Kettman, and J. Wayne Streilein, UTHSCD 5323 Harry Hines Blvd., Dallas, Texas 75235
A T-cell lymphoma was found in a B10.S adult mouse made tolerant to (B10.Sx9R)F_1 lymphoid cells at birth. The original tumor had the following surface phenotypes using FACS: Thy 1.2+, Ly.1+ and Ly.2+. Two cell lines have been derived from the original tumor. One cell line requires TCGF for growth and the other cell line is TCGF independant. Both cell lines are tumorogenic. Using limiting dilution analysis, we have isolated single cell clones whose cell surface phenotype is Ly.1+, Ly.2-. These clones are also tumorogenic. Absolute growth restriction is demonstrated when TCGF dependant tumor cells are cultured at low cell density on semi-allogeneic adherant irradiated BAO treated bone marrow, peritoneal, or splenic feeder layers. The cloning effiency of the tumor depends on the genotype of the feeder layer. B10.S x 9R and B10.S x 7R feeder layers shows the highest cloning effiency. The B10.S, 9R, B10.S x B10.M, B10.S x B10.HTT have intermediate cloning effieciency. B10.HTT and B10.S(10)9R feeder layers will not support tumor growth. Tumor growth in vivo shows an identical pattern to the cloning effienciency data. We can then predict which MHC genes are permissive or are restrictive for tumor growth.

0175 MOLECULAR ANALYSIS OF ANTIGEN-SPECIFIC B CELL TRIGGERING: SEPARATE PROLIFERATIVE AND DIFFERENTIATIVE SIGNALS, M.A. Jaworski and Erwin Diener, MRC Group on Immunoregulation, University of Alberta, Edmonton, Alberta.

The proliferative and differentiative signals controlling the in vitro IgM response by unprimed, affinity-enriched B cells were studied using conditions under which as few as 2000 B cells, stimulated by antigen specific, I-A positive, allogeneically restricted T cell derived helper factor (Hf) or the polyclonal activator lipopolysaccharide (LPS) yielded on the average 400 antibody forming cells (AFC) by direct plaque assay. Antigen (Ag) alone induces neither B cell proliferation nor differentiation into AFC. B cell proliferation, but not differentiation, is induced in the presence of Ag and Hf or LPS; Hf, in contrast to LPS, induces proliferation which is Ag-specific. Reconstitution of a fully differentiated AFC response requires the additional presence of non-antigen specific, radioresistant, accessory (A) cells or of their secreted product(s). In the absence of Hf or LPS, B cells, even those which have bound Ag, are not receptive to the differentiative signal originating from A cells. The differentiative signal originates from a cell population which includes Thy 1.2 bearing cells. In the case of the LPS-, but not the Hf-mediated response, A cells can be substituted by using supernatant derived from an interleukin-2 secreting T lymphoma cell line (EL4); this indicates that Hf-induced triggering of B cells has A cell requirements which are either different from, or supplemental to, those needed for LPS-induced triggering. Taken together, these findings case doubts on the generally assumed functional homology between T cell-derived and LPS-derived triggering signals.

0176 CLONED LINES OF INTERLEUKIN-2 PRODUCER HUMAN T CELLS. Ronald Palacios.Karolinska Institute,Wallenberglaboratory, Department of Immunobiology,S-10405,Stockholm,Sweden.

Cloned lines of Interleukin-2 producer T cells have been raised from peripheral blood of normal human volunteers. The studies were performed with two subclones designated RP1aa and CF2a3 respectively. Both RP1aa and CF2a3 cells possess the phenotype $OKT3^{+}4^{+}8^{-}DR^{+}$ which has been preserved by these cells throughout the last 9 months. RP1aa and CF2a3 cells synthesized IL-2 after their stimulation by PHA,ConA,PWM,OKT3 antibody and phorbol myristate acetate as determined in three different assay systems, namely, support of grow of a human cytotoxic T cell clone, promotion of PHA-initiated thymocyte proliferation and help to murine thymocytes to express cytotoxicity against allogeneic target cells. Neither RP1aa nor CF2a3 constitutively produced IL-2. The helper factor responsible for the above biological effects was found to have a m.w. of 15000-17000 daltons, resistant to temperature of 56^{0}C for 1h, stable at PH 2.0 to 8.0 and sensitive to trypsin.
RP1aa and CF2a3 cells did not proliferate to IL-2 stimulation and could not absorb this T cell growth factor, indicating that these IL-2 producer cells lack receptors for IL-2. Both RP1aa and CF2a3 cells have been growing in culture in medium supplemented with human serum lacking exogenous factors and in the absence of filler cells. None of the IL-2 producer T cell clones were sensitive to IL-1, alfa or gamma interferon. However, both RP1aa and CF2a3 clones proliferated under the stimulus of Interleukin-3 obtained from WEHI-3 cells.In addition , IL-3 as well as several T cell mitogens increased their growth rate (from 18-24 h to 13-15 h).Finally, both IL-2 producer T cell clones assisted macrophages to synthesize IL-1 stimulated by either Con A or the OKT3 antibody.

0177 DETECTION OF HUMAN B-CELL GROWTH FACTOR ACTIVITY BY USING HUMAN B LEUKEMIC CELL AND ANTI-IMMUNOGLOBULIN STIMULATED NORMAL B CELLS, K. Yoshizaki, T. Nakagawa, T. Kaieda, M. Okada and T. Kishimoto, Osaka University Medical School, Osaka, Japan.

Assay systems for the B cell growth factor (BCGF) activity have not yet been established, since there are as yet no cloned B cell line that can respond to BCGF. We have established three assay systems for the measurement of BCGF activity. One assay system was the induction of proliferation of anti-idiotypic antibody-stimulated monoclonal human B leukemic cells (B-CLL cells), which showed the proportional proliferation to the amount of BCGF in culture medium. The second assay system was the BCGF-mediated augmentation of proliferation of an established B cell line which was derived from B-CLL cells and grew slowly in RPMI-1640 with 10% FCS medium without BCGF. The third assay system was the augmentation of the proliferation of anti-μ-stimulated normal human B cells. BCGF and IL-2 activities of the culture supernatants obtained from PHA-stimulated T cells or from an IL-2-dependent helper T cell line were examined. Those culture supernatants were fractionated on Sephadex G-100 and BCGF and IL-2 activities in each fraction were measured. IL-2 activity was detected only in the fraction with the molecular weight between 15,000 and 20,000, while BCGF activity was present in the fractions with the molecular weight of 45,000 and of 15,000 to 20,000. The fraction with the molecular weight of 45,000 did not show any IL-2 activity. The fraction with the molecular weight of 15,000 to 20,000 was further fractionated by chromatofocusing but IL-2 and BCGF activities were not separated. However, the activity to induce differentiation of B cells, TRF activity, could be isolated from IL-2 and BCGF activities by chromatofocusing.

0178 PROLIFERATION AND DIFFERENTIATION OF NEOPLASTIC HUMAN B CELLS MEDIATED BY INTERLEUKINS. Richard J. Ford, John Morgan, Shashi Mehta, Lawrence Lachman, & Abby L. Maizel. University of Texas, M.D. Anderson Hospital, Houston, Texas, 77030, & Duke University Medical School, Durham, NC.

Non Hodgkin's lumphomas (NHL) and lymphocytic leukemias (CLL) were studied from freshly obtained biopsies or peripheral blood from untreated patients. The neoplastic cells were enriched by T cell and MØ depletion and characterized by both conventional cell membrane markers and monoclonal antibodies, revealing monoclonal B cell populations with < 5% contamination with non-neoplastic accessory cells. We have demonstrated that purified IL-2 preparations contain B cell growth factors for normal human B cells (Nature 294: 261, 1981), so these preparations were tested on a spectrum of NHL and CLL. Our studies showed that IL-2 stimulated ^{3}H-Tdr incorporation and growth in a variety of the neoplastic cells. IL-1, however, did not stimulate proliferation alone, but stimulated Ig secretion in neoplastic cells when either autologous or allogeneic purified T cells were present. Our findings suggest that some human neoplastic B cells are sensitive to immunoregulatory factors from normal accessory cells that may play a role in the in vivo biology of the tumors. Since many of the lymphomas of the same histopathologic type show considerable heterogeneity when phenotyped with monoclonal antibodies, it is possible that functional heterogeneity also exists within morphologic subtypes of human lymphoma. Thus the identification of cell surface phenotype and proliferative and differentiation capabilities may provide a new methodology for characterizing human lymphomas.

0179 DYNAMICS OF mIgD, mIgM and mIa EXPRESSION ON MITOGEN-STIMULATED MURINE B LYMPHOCYTES. John G. Monroe and John C. Cambier. Duke University Medical Center, Durham, NC 27710.

Analyses of cell cycle state by two-parameter cytofluorometric analysis of acridine orange stained cells and cell diameter by cytometric "time of flight" measurement indicates a correlation between murine B lymphocyte diameter and position in the cell cycle. This correlation allows assignment of B lymphocytes with diameters of 4.5-5.5μm as G_0 cells. Cells in early G_1 (G_{1A}) reside in a population 5.5-7.0μm in diameter. Late G_1 (G_{1B}) and S phase cells have diameters of 7.0-10μm. Lastly, G_2 and M cells are found exclusively in a 10-12μm population.

In view of this correlation, we have investigated expression of surface IgD, IgM and Ia antigen on mitogen-stimulated B cells by immunofluorescence relative to cell diameter using the flow cytometer. Results demonstrate an abrupt decrease in mIgD expression of approximately 80% as cells transt G_1. MIgM expression, on the other hand, remains constant throughout the cell cycle. MIa antigen expression increases by roughly 3-fold during G_1. However, during early S phase, there is a 10-fold decrease in its expression. This decrease is observable only on an individual cell basis. Mean Ia expression within the population as a whole, appears elevated relative to unstimulated B lymphocytes. Data derived from analyses involving cell cycle blocking agents confirm these results. The modulation in expression of these markers suggests that mIgD and mIa may be important early in B cell activation. However, in view of its persistance throughout the cell cycle, mIgM may function throughout the proliferative phases of the immune response. (Supported in part by NIH Grant #AI 16128).

0180 ACCESSORY CELL DERIVED FACTORS REQUIRED FOR ANTI-Ig INDUCED B CELL PROLIFERATION, Maureen Howard, Kenji Nakanishi and William Paul, Laboratory of Immunology, NIAID, NIH, Bethesda, MD 20205

To identify the signals required to activate small B lymphocytes and maintain their continuous cultivation, we have developed a variety of short-term co-stimulator assays which measure the proliferation of small numbers of highly purified B lymphocytes. Three distinct signals appear to be required for induction of DNA synthesis by a particular subset of small B lymphocytes: a signal delivered by antibodies specific for the IgM receptor expressed on the B cell membrane; a signal delivered by a T cell derived factor of Mr 18,000 (BCGF), which is distinct from IL-2; and a signal delivered by a macrophage derived factor of Mr 10,000-20,000 (M15). BCGF and M15 are not mitogenic for resting B cells, and act on anti-Ig activated B cells in a non-antigen specific, non H-2 restricted, synergistic manner. Their requirement is not observed at high B cell numbers, presumably reflecting accessory cell contamination and endogenous factor production at such cell densities. The B cell activation induced when these three signals are saturating shows single hit kinetics and is restricted to proliferation without the production of antibody-forming-cells.

0181 ANALYSIS OF REQUIREMENTS FOR ANTIGEN ACTIVATION OF ISOLATED HAPTEN SPECIFIC B LYMPHOCYTES, John C. Cambier, John G. Monroe and Melinda J. Neale, Duke Univ. Med. Ctr., Durham, NC 27710

Studies of antigen, T cell and macrophage requirements for stimulation of entry of resting (G_0) hapten specific B cells into cell cycle (G_1) are reported. Isolated TNP specific cells ($\geq$97% B cells) were prepared using gelatin coated plates to which hapten had been coupled via a cleavable crosslinking reagent (Cambier and Neale, J. Immunol. Meth., In Press) and cultured under varied conditions in microtest wells. Entry into cell cycle was assessed by flow cytofluorometric analysis of acridine orange stained cells. Results indicate that as many as 60% of isolated Hapten specific cells can be activated by the thymus independent antigen TNP-Brucella abortus in the apparent absence of T cells and macrophages or their products. Ability of TNP-Ba to activate cells at very low cell density suggests that antigen presentation is not essential in this system. Activation is strictly antigen specific and dependent on cell surface immunoglobulin expression. Antigen exposure for as brief as 3 hours is sufficient to cause activation. Capping of cell surface immunoglobulin appears unnecessary for activation. Under conditions described above, significant activation does not occur when TNP-SRBC, TNP-OVA or TNP-HgG are used as antigens. Ability of antigen specific help and nonspecific factors to augment stimulation is under study and will be discussed. (Supported by NIH grant AI 16128)

0182 T CELL HELP FOR IGA RESPONSES, Julia M. Phillips-Quagliata, Margaret Arny, Patricia Kelly-Hatfield and Michael E. Lamm, NYU Medical Center, New York, NY 10016

IgA-specific T helper cells have been postulated to be at least partially responsible for the predominance of IgA in antibody responses initiated in mucosa-associated lymphoid tissue. To examine this possibility, we have compared populations of carrier-primed T cells from peripheral lymph nodes (PN), mesenteric lymph nodes (MN) and Peyer's patches (PP) in their capacity to help the IgA anti-2,4 dinitrophenyl (DNP) antibody response. Lyt-2$^-$ T cells were titrated into a fixed number of DNP-primed splenic B cells (plus macrophages) and the mixtures were cultured for 4 days in the presence of appropriate DNP-carrier conjugates. IgM, IgG and IgA plaque forming cells were then enumerated. When sheep red blood cells (SRBC) were the carrier, all T cell populations were successfully primed and T cells from the three sources did not differ in their ability to help IgM, IgG and IgA responses. When keyhole limpet hemocyanin (KLH) was the carrier, priming of MN and PP Lyt-2$^-$ T cells was not very reproducible, but PN T cells were reproducibly primed. When SRBC-primed and KLH-primed PN T cells were compared, the titration curves for the three isotypes were quite different. Much lower ratios of IgA to IgM and IgG PFC were stimulated by optimal numbers of KLH-primed T helpers than by optimal numbers of SRBC-primed T helpers. Our results are compatible with the idea that there are two pools of precursors of IgA PFC in primed splenic B cell populations. One responds to non-specific factors known to operate in systems involving SRBC as carrier, the other requires cognate and perhaps isotype-specific interaction. We plan to use the system to evaluate cloned T cell populations. Supported by NIH Grants CA 20045, AI 15071, CA 23885 and CA 09161.

0183 ISOLATION AND CHARACTERIZATION OF HUMAN B CELL MITOGENIC FACTOR. Abby Maizel, Chintaman Sahasrabuddhe, Shashikant Mehta, John Morgan, Lawrence Lachman, & Richard Ford. M.D. Anderson Hospital, Dept. of Pathology, Houston, Texas, 77030.

Recent studies (Nature 294: 261, 1981) have demonstrated the ability of purified normal human B cells to undergo G_1 phase cell cycle progression and subsequent DNA synthesis upon exposure to factor(s) present in lectin (PHA and/or PWM) stimulated mononuclear cell conditioned media (CM). We have presently isolated that factor responsible for inducing normal human B cell proliferation. 72 hour lectin stimulated CM, prepared in serum-free conditions, was fractionated by ammonium sulfate precipitation, DEAE-sepharose chromatography, p30 and p100 gel filtration. During purification the proliferative activity of the column fractions was assayed simultaneously on purified human T cells and purified human B cells. Both T cell (TCGF) and B cell (BCGF) stimulatory factors present in the initial CM were found to copurify through p 30 gel filtration. However, separation of these two factors was achieved after p100 gel filtration. Analytic PAGE of [^{3}H]-formaldehyde labelled TCGF and BCGF fractions demonstrated distinct, separate bands of 14-16000 m.w. for TCGF and 12-14000 m.w. for BCGF. The BCGF will induce B cell proliferation in the absence of any detectable Ig (IgM or IgG) secretion. Conversely, coculture systems utilizing purified normal B cells, T cells and monocyte derived Interleukin 1 will induce human B cell IgM secretion without significant proliferation (supported by NIH grant CA 21927, ACS grant IM-296, and a grant from the King Faisal Foundation).

0184 REQUIREMENTS FOR THE ACTIVATION AND REPLICATION OF ANTIGEN-SPECIFIC INTERLEUKIN 2 PRODUCING T-CELLS, Ronald B. Corley, Duke Medical Center, Durham, NC 27710

Interleukin 2 (IL2) is a T lymphocyte-derived soluble factor that is an obligatory requirement for the growth of T cells. In order to more fully understand the regulation of T cell growth, the requirements for stimulating the growth of antigen-specific IL2 producing T cells and the production of IL2 by these cells has been evaluated. T cells from carrier (keyhole limpet hemocyanin)-primed mice, which provide a conventional population of helper T cells, were also found to be an ideal source of IL2 producing T cells. The activation (to IL2 production) and replication of these IL2 producing lymphocytes are antigen specific events and depend on H-2 (I-A) restricted interactions with antigen-presenting cells. These IL2 producing T cells can be maintained in culture for months without addition of exogenous IL2 and with a 2 to 10-fold net increase in cell number each week. Soluble macrophage-derived factors released as a result of T cell-macrophage interaction do not appear to be sufficient, even in the presence of antigen, to promote the growth of IL2 producing T cells. This suggests that the direct interaction of IL2 producing T cells with antigen-presenting cells is obligatory for their activation and growth. The results indicate that T cell proliferation, and as a consequence the magnitude of all T cell mediated immune responses, is limited by the availability of "appropriately" presented antigen to IL2 producing T cells. The role of IL2 in the growth of IL2 producing T cells at the population and clonal level is currently under investigation.

0185 PERSISTANCE AND FUNCTION OF $(T_6 \times RFM)F_1$ B-CELLS IN YOUNG RFM/$(T_6 \times RFM)F_1$ MICE WITH HOST VERSUS GRAFT (HVG) DISEASE. Richard C. Hard, Jr., Med. Col. of Va., Richmond, VA 23298.

HVG syndrome is the fatal disease which may follow the perinatal inoculation of semiallogenic F_1 hybrid cells into related susceptible inbred strains of mice. Histopathologic studies have shown that there is generalized T-cell depletion, B-cell hyperplasia and the development of non-thymic lymphomas. Recent cytogenetic studies have shown that large numbers of replicating donor F_1 cells persist in the lymph nodes, but only a few in the spleens of RFM mice with HVG disease following the perinatal inoculations of $(T_6 \times RFM)F_1$ spleen cells [RFM/$(T_6 \times RFM)F_1$ mice]. To test the hypothesis that donor F_1 B-cells persist and function in RFM hosts, $(T_6 \times RFM)F_1$ mice were sensitized to sheep RBC's (SRBC's) or horseradish peroxidase (HRP) prior to inoculation but received no subsequent antigenic challenge. RFM mice which had received spleen cells from F_1 donors immunize against SRBC's, had hemolytic antibody titers ranging from 1/40 to 1/160. Cells producing antibody to HRP were found to average $70/10^6$ nucleated cells in the nodes and $7/10^6$ cells in the spleens. One of $5 \; T_6/(T_6 \times RFM)F_1$ chimeras had anti-HRP antibody producing cells in the spleen (average = $1/10^6$) but none were detected in the nodes. Because donor B-cell proliferation and function were probably not antigen driven, it seems likely that the allogenic donor T-T host cell interaction provided the stimulus. These early disruptions of the T-and B-cell systems may favor the later malignant transformation of host and persisting donor B-cell lymphocytes.

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0186 CELLULAR INTERACTIONS IN T COLONY FORMATION, Alan Winkelstein and Susan A. Walker, University of Pittsburgh, Pittsburgh, Pennsylvania 15213.

The interactions of cells involved in the generation of T cell colonies were evaluated in these investigations. Human mononuclear cells, isolated by ficoll-hypaque centrifugation and stimulated with optimal quantities of PHA, yielded a mean of 6,103 $\pm$ 539 colonies/plate. Following separation of these cells by Sephadex G-10 filtration, neither the non-adherent (NA) nor adherent (ADH) cells showed significant colony forming activity. However, combinations of NA + irradiated ADH cells restored reactivity. Using a 2:1 ratio of NA and ADH colony formation averaged 4,993 $\pm$ 497 colonies/plate. Direct cell to cell contact was not required; ADH cells, plated in the hard underlayer, were capable of inducing growth. Furthermore, soluble factors obtained from cultures of ADH cells promoted colony formation. The activity of ADH cells could be supplied by either irradiated Raji cells, a B lymphoma cell line, or U937 cells, a monocytoid cell line. Raji cells were even more active than ADH cells; at a 1:1 ratio, the number of colonies averaged 10,865 $\pm$ 1,097. Soluble factors from these lines were also capable of inducing T colony growth. By contrast, K562, an erythroleukemic cell line, xenogenic YAC cells and sheep red cells were not active. Furthermore, T cell growth factor could not replace the soluble factors. Cells from one patient with acute myeloblastic leukemia also promoted colony formation. Studies characterized this growth promoting factor are now in progress. This system should prove useful in delineating factors controlling T cell lymphopoiesis.

0187 INHIBITORY AND CONTRAINHIBITORY COMPONENTS IN NORMAL MOUSE SERUM (NMS) WHICH REGULATE NONSPECIFIC ACTIVATION OF A MALIGNANT B CELL LINE, Sharyn M. Walker and William O. Weigle, Scripps Clinic and Research Foundation, La Jolla, CA 92037.

B cells can be activated into proliferation and antibody synthesis either specifically with antigen or nonspecifically with various B cell activators. Recently our laboratory showed that normal mouse plasma and serum contain an inhibitory substance, called NMS-In, which inhibits nonspecific B cell activation; specific activation of B cells appears not to be affected (J. Immunol. 126:1852,1981). Current study has found that activation of a malignant B cell line, the BCL_1, by bacterial endotoxin (ET) into $[H]^3$-thymidine incorporation is also inhibited by NMS-In. However, in contrast to normal B cells, whole NMS does not inhibit activation of the BCL_1; rather, NMS must be subjected to S-300 chromatography to separate out the 200,000 m.w. inhibitor. The failure of whole NMS to inhibit the BCL_1 is due to another substance found in NMS in the 50 to 75,000 m.w. region which stimulates the BCL_1. When the 200,000 m.w. inhibitory region is mixed with the 50 to 75,000 m.w. region, inhibition of the BCL_1 is not observed. This contrainhibitory substance has no effect on the capacity of NMS-In to inhibit activation of normal B cells by ET. Thus, it is postulated that the responsiveness of the BCL_1 to a contrainhibitory substance in plasma circumvents its control by a natural plasma regulator. A means to increase either the levels of NMS-In or to reduce the amount of contrainhibitor in plasma could possibly lead to control of growth of the B cell tumor *in vivo* without leading to immunosuppression of specific B cell responses.

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0188 A SELECTIVE CULTURE SYSTEM FOR GENERATING TERMINAL DEOXYNUCLEOTICYL TRANSFERASE POSITIVE CELLS IN VITRO, Irving Goldschneider and Jun Hayashi, University of Connecticut Health Center, Farmington, CT. 06032

Terminal deoxynucleotidyl transferase (TdT) is a useful marker for identifying immature lymphopoietic cells and for classifying lymphocytic leukemias and lymphomas. Our studies have indicated that many TdT-positive bone marrow cells in rats and mice are prothymocytes, and that some serve as targets for leukemic transformation by the Gross leukemia virus. Here we describe a xenogenic culture system which selectively promotes the growth of TdT-positive bone marrow cells in vitro. In this system, rat bone marrow cells are cultured on mouse bone marrow feeder layers using a modified Dexter culture technique. TdT-positive cells of rat origin are generated exponentially from cortisone-resistant precursors over a period of 14 days. Unlike the classical Dexter culture system, our system does not support the growth of pluripotent hemopoietic stem cells or of myeloid or erythroid cell populations. Hence, the culture system appears to be entirely selective for immature lymphoid cells. Optimal growth of TdT-positive cells is obtained in vitro when the culture medium is supplemented with charcoal-extracted fetal calf serum, fibroblast growth factor and guinea pig bone marrow extract. The TdT-positive cells that are generated in vitro have the same antigenic and enzymatic profiles, size, morphology, and cortisone-sensitivity as do their counterparts in normal rat bone marrow; and they do not cause leukemia when injected into irradiated recipients. The ability of these cultures to generate lymphocyte precursors without simultaneously inducing differentiation offers the unusual opportunity to study the mechanisms which control these processes during the earliest stages of lymphopoiesis and leukemogenesis.

0189 A T CELL DERIVED B CELL GROWTH FACTOR, Marilyn L. Thoman, Edward L. Morgan and William O. Weigle, Scripps Clinic and Research Foundation, La Jolla, CA 92037.

Several cytokines are known to be released by murine spleen cells upon mitogen or antigen stimulation. Isolation and purification of a T cell growth factor, Interleukin 2, from supernatants of such activated cells was made possible by a rapid, quantitative proliferative assay utilizing factor-dependent T cell lines. Identification of similar B cell growth inducing substances produced by T cells has been more difficult in part due to the lack of an appropriate assay system. We have used the B cell tumor line, BCL_1, as the target for putative B cell growth promoting factors, as these cells represent an inducible population which displays the phenotypic and functional characteristics of immature B cells. B cell growth factors (BCGF) can be identified in culture supernatants from mitogen-activated murine and human spleen cells, and the T cell line, LBRM-33. The LBRM-33 derived BCGF copurifies with IL-2 by AcA54 chromatography and isoelectric focusing. However, phenylsepharose chromatography separates these two activities; the IL-2 being bound to the matrix, while the BCGF is present in the effluent. The IL-2 can be recovered by elution of the column with ethylene glycol. This BCGF does not induce proliferation of T-depleted splenic B cells, although BCL_1 cells are stimulated. This indicates that normal B cells must be triggered to express receptors for BCGF, but the BCL_1 are apparently activated by transformation to display the receptor.

Idiotypic Markers

0190 POLYCLONALLY DISTRIBUTED LOW AFFINITY PC-BINDING MOLECULES ON B CELLS, Mark A. Bach, Heinz Köhler and Daniel Levitt, LaRabida University of Chicago Inst.,Chicago, IL 60649

The response of the Balb/c mouse to the common bacterial antigen phosphoryl choline is primarily of the TEPC15-HOPC8(H8) idiotype. Two types of monoclonal antibodies (MAB) raised in A/He mice against H8 were studied. The binding of one, 4C11, to H8 is inhibited by free or protein conjugated antigen in RIA. 4C11 co-caps with cell surface μ and binds 0.13% of normal Balb/c μ^+ splenic lymphocytes by immunofluorescence. A second type, F6-3, does not recognize the antigen binding site, does not co-cap with μ on most B cells and binds 3.9% of Balb/c splenic lymphocytes. The F6-3 MAB stains the same percentage of cells as high concentrations (10^{-4} M PC) of PC-BSA while 10^{-5} M 4C11 MAB stains a slightly smaller percentage of spleen cells than low concentrations (10^{-5} M PC) of PC-BSA. The correlation between F6-3 staining and high dose PC staining (low affinity PC receptor) and between 4C11 and low dose staining (high affinity PC receptor) is found in 2 other strains (Table). These data suggest the existence of a polyclonally distributed low affinity PC binding molecule on murine B cells which may crossreact with a non-binding site H8 idiotypic determinant. In addition, the high affinity clonally distributed PC binding Ig molecules would possess both binding site and non-site H8 idiotopes. The low affinity molecules may represent a class of receptors responsible for polyclonal regulation of lymphocytes by specific antigens. Supported by NIH 5-T32-GMO-7281-07 and NCI 5-PO1-CA1-9266-05.

Strain	F6-3*	10^{-4} M PC*	4C11*	10^{-5} M PC*
Balb/c	3.9	3.6	0.15	0.12
NZB	11.8	11.9	0.08	0.19
WBB6F$_1$ w/w^v	0.46	0.61	<0.05	<0.05

*% staining among μ^+ splenic lymphocytes

0191 IDIOTYPE CROSS-REACTIVITIES BETWEEN SURFACE IMMUNOGLOBULINS OF DIFFERENT CH LYMPHOMAS. Larry W. Arnold, Christopher A. Pennell and Geoffrey Haughton. UNC Medical School, Chapel Hill, N. C. 27514.

Xenogeneic anti-idiotype sera have been raised in rabbits against affinity purified membrane IgM of seven independently derived B cell lymphomas of B10.H-2^aH-4^bp/Wts (2^a4^b) mice, namely CH-1, CH-2, CH-5, CH-6, CH-8, CH-10 and CH-12. Each serum has been absorbed with immobilized myeloma protein, cells of other CH tumors and normal spleen cells. The absorbed sera do not react detectably in indirect immunofluorescence tests with normal lymphoid cells of 2^a4^b mice, but each reacts with cells of the immunizing tumor. Each antiserum has been tested for reactivity with the cells of up to 18 other independently derived B lymphomas of the CH series. Three reactivity patterns have been seen. One serum, anti-CH-12, reacts only with cells of the immunizing tumor and reactivity cannot be removed by absorption with cells of any other tumor. Two sera, anti-CH-1 and anti-CH-6, react with the immunizing tumor and one other (CH-9 and CH-13 respectively) but absorption with the cross-reactive tumor does not remove all activity for the immunizing tumor. The other four sera react with the immunizing tumor and one or more others; absorption with any member of the cross-reactive group removes reactivity for all other members. No tumor falls into more than one of the six cross-reactive groups so far identified. Cross-reactive tumors differ one from another by light chain class, induction protocol, growth characteristics and/or spectrum of cell surface differentiation markers expressed. These findings have interesting implications regarding the etiology and therapy of this series of tumors.

0192 ANALYSIS OF AN IgA B CELL LEUKEMIA CLONE USING MONOCLONAL ANTI-IDIOTYPE AND ANTI-V_H ANTIBODIES, M. Mayumi, H. Kubagawa, G. A. Omura, W. E. Gathings, J. F. Kearney, and M. D. Cooper, University of Alabama in Birmingham, Birmingham, AL 35294

A monoclonal anti-idiotype antibody to $IgA_{1\kappa}$ molecules on leukemic B cells from patient WF was made by fusing Ag8.653 myeloma variant cells with lymph node cells from mice immunized with the leukemia cells. A heterohybridoma, made by fusing Ag8.653 cells with WF leukemia cells, secreted the $IgA_{1\kappa}$ molecules used to detect clones producing monoclonal anti-WF Id antibody. The selected monoclonal anti-WF Id detected an idiotope formed by the combined heavy and light chain. Immunofluorescent studies revealed that 94% of the patient's blood mononuclear cells (MNC) expressed surface $IgA_{1\kappa}$ with the WF Id. About 10% of IgM B cells in his bone marrow also expressed the WF idiotype. Three percent of $IgA_{1\kappa}$ leukemia cells in blood expressed γ chains in their cytoplasm. Approximately 0.1%, 1% and 10% of bone marrow MNC respectively expressed μ chains, γ chains and α chains in their cytoplasm, but no light chains or surface Ig. These cells had the convoluted nucleus and narrow cytoplasm characteristic of normal pre-B cells. Eleven percent and 63% of $sIg^-c\gamma^+$ pre-B cells respectively coexpressed μ and α chains. One of four monoclonal anti-V_H antibodies stained these pre-B cells and the surface α and cytoplasmic γ chains of the leukemic B cells in blood. These data suggest that the μ^+-, γ^+-, and α^+-pre-B cells, IgM^+Id^+ B cells and $IgA_{1\kappa}^+Id^+$ B cells all belong to the same clone of leukemic cells, and imply precommitment to isotype switches and light chain expression at a pre-B cell level.

0193 INHIBITION OF MOPC-315 IMMUNOGLOBULIN BIOSYNTHESIS BY IDIOTYPE INDUCED AND SPECIFIC T SUPPRESSOR CELLS. Gary L. Milburn and Richard G. Lynch, University of Iowa College of Medicine, Iowa City, IA 52242.

Balb/c mice immunized with the IgA anti-TNP protein (M315) produced by plasmacytoma MOPC-315 develop T suppressor cells specific for the idiotype (Id^{315}). The suppressor cells directly inhibit M315 secretion from MOPC-315 cells both in vivo and in vitro. Results from co-culturing Id^{315}-immune T cells with MOPC-315 cells in vitro have demonstrated the T cells are $Ly+\ I^{-}2^{+}$ that bear surface membrane receptors that recognize idiotypes on M315. Preliminary studies indicate the suppressor T cells are specific for V_H^{315}. The secretory inhibition is: 1) T cell dose-dependent; 2) macrophage independent at the effector level; 3) mediated by a soluble factor; 4) reversible and 5) virtually complete 24 hours after co-culture. Secretory inhibition occurs without any detectable effect on MOPC-315 growth, viability or surface membrane M315 expression. Inhibition of M315 secretion results from a selective inhibition of M315 biosynthesis within the myeloma cell. The mechanism of this highly selective suppression of secreted, but not membrane expressed M315 is currently being investigated at the mRNA level using Northern blot analysis. In addition, an anti-Id^{315} T cell hybridoma has been constructed and should be useful in analyzing the mechanism of T-B cell recognition and interaction.

These studies clearly demonstrate direct regulation of antibody secreting cells by idiotype specific T cells and may lead to a better understanding of B cell regulation in precise molecular terms. (Supported by CA-09118 and CA-23217.)

0194 THE USE OF MONOCLONAL ANTI-IDIOTYPE ANTIBODIES IN THE ANALYSIS AND GREATMENT OF B CELL LYMPHOMA. David G. Maloney, Richard A. Miller and Ronald Levy, Medicine/Oncology, Stanford University, Stanford, CA, 94305

Cell surface immunoglobulin was obtained from the tumor cells of a patient with nodular lymphoma by hybridization to mouse myeloma cells. This immunoglobulin was used as an immunogen to produce murine monoclonal anti-idiotypes. These antibodies were used as probes to identify idiotype positive tumor cells in the patient's blood and tissues and to quantitate the plasma levels of idiotype bearing immunoglobulin secreted by the tumor cells. Both the number of circulating idiotype positive cells and the level of circulating idiotype were found to correlate with the patient's clinical status. The low level of free idiotype protein was not affected by plasmapheresis and eventual disappearance of detectable serum idiotype. The patient experienced a dramatic tumor response to the anti-idiotype with regression of lymphadenopathy, scalp tumors, hepatosplenomegaly and resolution of anemia and thrombocytopenia. There was no acute or chronic toxicity to the treatment and no host anti-mouse antibody was produced. This remission has been sustained for greater than six months without further therapy. A murine B cell tumor system has been developed to help define the parameters and mechanisms of anti-idiotype therapy.

0195 IMMUNOCHEMICAL AND BIOCHEMICAL CHARACTERIZATION OF β-ADRENERGIC RECEPTORS. A. Donny Strosberg, P. Olivier Couraud, O. Durieu-Trautmann, C. Delavier-Klutchko and A. Schmutz-I.R.B.M.-C.N.R.S. and University Paris VII, Place Jussieu 2 - 75251 Paris Cedex t, France.

Affinity chromatography on alprenolol-agarose was used to purify turkey erythrocyte β-adrenergic recpetors which retain all the binding properties of the membrane-bound catecholamine binding sites. These receptors were injected in mice and yielded polyclonal antibodies. After fusion between splenocytes and the SP2 myeloma derived vector line, monoclonal anti-receptors antibodies could also be obtained. By immunofluorescence, these antibodies specifically stained β_1 and $\beta_2$adrenergic receptor-bearing cells but did not recognize receptor-deficient cells. The polyclonal antibodies immobilized alprenolol-receptor complexes on pretein A Sepharose and could be used to immunoprecipitate the affinity purified receptor. The antibodies were shown to stimulate the membrane-bound catecholamine-sensitive adenylate cyclase.

A second type of immunological receptor-specific reagent was prepared by raising anti-iodiotypic antibodies directed against the active site of antiβ-adrenergic ligand antibodies. These anti-idiotype antibodies displayed the same receptor-recognition properties than the antibodies raised against the receptor. In addition they inhibited the binding of the hormone or its antagonists to the membrane-bound receptor. Both types of antibodies are presently used to characterize various properties of the -adrenergic complex.

0196 IDIOTYPIC PRODUCTION BY HUMAN NEOPLASTIC B LYMPHOCYTES: ITS USE IN MONITORING CHEMOTHERAPY AND IMMUNOTHERAPY, F.K. Stevenson, T.J. Hamblin, A.L. Tutt and G.T. Stevenson, Tenovus Research Laboratory, General Hospital, Southampton SO9 4XY, England.

Idiotypic immunoglobulin (Ig) appears to be secreted in small amounts by the majority of human neoplastic B lymphocytes in culture (1). Chronic lymphocytic leukemia (CLL) has been investigated most extensively, but export of Ig appears to occur also in cells of non-Hodgkin's lymphomas (NHL). The level of export and class of Ig is variable and may depend on maturity of cell type involved (2). Cells in CLL usually secrete idiotypic IgM and sometimes IgD, the latter only from cells of the λ light chain type.

Serum levels of idiotype can be monitored by radioimmunoassay and one patient has been studied during three separate courses of chemotherapy with chlorambucil. After a few days delay chemotherapy caused a marked fall in blood lymphocyte count accompanied by a rise in serum idiotype levels to 2-3 times the initial values. Cell destruction appeared to increase idiotype levels which then fell over the following week.

During immunotherapy with anti-idiotype however, serum idiotype levels paralleled blood lymphocyte counts closely. Plasmapheresis was used to remove circulating idiotype before antibody administration, but levels were reduced even further by anti-idiotype at the same time as other parameters showed massive destruction of tumor cells.

1. F.K. Stevenson, T.J. Hamblin, G.T. Stevenson and A.L. Tutt. J. Exp. Med. 152: 1484-1496, 1980.
2. A.C. Hannam-Harris, J. Gordon and J.L. Smith. J. Immunol. 125: 2177-2181, 1980.

Tumor Metastasis

0197 CHANGE IN METASTATIC PROPERTIES OF THYMOMA CELLS INDUCED BY THEIR HYBRIDIZATION TO NORMAL LYMPHOCYTES, Zelig Eshhar and Raya Vekstein, The Weizmann Institute of Science Rehovot 76100, Israel.

To study the process and mechanism of spread of tumor cells into specific sites in the host,we generated somatic cell hybrids between the BW5147 (an AKR/J spontaneous thymoma) and lymphocytes of (BALB/c x DBA/2)F1 mice and followed the distribution patterns of these cell lines by macroscopic and microscopic analysis. Most of the hybrid lines were more tumorogenic than the BW5147 parental line. Few of the hybridomas proliferated in organs like liver, spleen,kidneys and meninges which were rarely invaded by the BW cells after i.v. inoculation. One of the hybrid lines (8-4) which grew preferentially in the meninges and eventually cause paralysis of mice, was isolated from the mininges, cultured and reinoculated into mice. After such 5 sequential passages, a stable clone (C12) was selected that metastesized preferentially to the meninges. The affinity of C12 cells towards the meninges was not dependent on the route of injection and the amount of cells inoculated. This affinity was persistent although less pronounced when C12 cells were injected into various histocompatible strains and into irradiated mice. BW and C12 cells differ in their morphology, chromosome number, surface membrane fluidity and the presence of Fc receptors. No differences were observed in the binding ability of various lectins to the cells. The relevance of these differences to the alterations induced in the thymoma cell and the meninges predilection of the hybrid clone is still ambiguous, but the different hybrid lines and subclones derived provide us with a powerful tool for comparative and analytic studies of the cellular components that influence the process of preferential metastases spread.

0198 RELEASE OF DEGRADED GLYCOCONJUGATES TO MEDIUM BY HUMAN OVARIAN CARCINOMA CELLS, H.J. Allen, M.S. Piver, M. Gamarra and E.A.Z. Johnson, Roswell Park Memorial Institute, Buffalo, New York 14263.

Human ovarian carcinoma cells were isolated from effusions and incubated in vitro for 14 to 156 hours in the presence of radiolabeled glycoprotein precursors. The cell-free culture media were collected and analyzed. Mesothelial cells and histiocytes prepared from malignant effusions were used as controls. SDS-PAGE analysis of the non-dialyzable fraction of culture media from carcinoma cells revealed the presence of a continuous molecular weight spectrum of glycoconjugates whereas purified mesothelial cells released to culture media a discrete glycoconjugate peak of 70000MW. Similar electrophoretic profiles of labeled glycoconjugates from carcinoma cells were obtained when cells were incubated in the absence of protein supplement, in the presence of heat-inactivated FCS, in the presence of heat-inactivated cell-free effusion, and in the presence of soybean trypsin inhibitor. Analysis of the dialyzable and non-dialyzable incorporated radioactivity present in culture media obtained from cells labeled with ^{3}H-mannose showed that 67% of the incorporated label was in non-dialyzable components for medium from mesothelial cells whereas only 19% was in the corresponding fraction from carcinoma cells. These and other results suggest that ovarian carcinoma cells release to culture medium glycoconjugates that are randomly cleaved by proteases. Non-malignant cells from effusions did not exhibit this phenomenon.

0199 ENHANCEMENT OF TUMOR METASTASIS BY β-ESTRADIOL TREATMENT. Nabil Hanna, Smith Kline & French Laboratories, Philadelphia, PA 19101

The effect of β-estradiol-induced depletion of natural killer (NK) cells on the development of tumor metastases in syngeneic mice and allogeneic nude mice was investigated. Mice treated with β-estradiol exhibited selective inhibition of NK cell activity against YAC-1 lymphoma, UV-2237 fibrosarcoma, K-1735 and B16 melanoma target cells in vitro. Poly I.C, a potent NK cell stimulator, failed to activate NK cells in β-estradiol-treated mice. However, treatment with free or liposome-encapsulated poly I.C was effective in stimulating the tumoricidal activity of alveolar and peritoneal macrophages in both normal and hormone-treated groups. The frequency of experimental and spontaneous pulmonary metastasis of UV-2237, B16 and K-1735 tumors was markedly higher in mice treated with β-estradiol than that observed in age-matched controls. Moreover, injection of poly I.C before i.v. tumor challenge inhibited the formation of lung tumor colonies in normal recipients, whereas it was ineffective in mice treated with β-estradiol. Nude mice exhibit high levels of NK cell activity and strong resistance to tumor metastasis. Treatment with β-estradiol suppressed NK cell activity and rendered adult nude mice susceptible to the development of metastases of allogeneic tumors. Such in vivo model may prove valuable for studies of metastases of human neoplasms.

0200 CELL SURFACE VARIANTS AND METASTASIS, Tien-wen Tao Wiedmann, Department of Radiology, Stanford University Medical Center, Stanford, Ca 94305

Surface variants of mouse B-16 melanoma cells were selected with the use of lectins. Two different types of selection procedure were used to produce different groups of variants: (1) "negative selection" to obtain (a) variant clones resistant to toxicity of certain lectins, e.g. wheat germ agglutinin (WGA), ricin, and (b) revertants of WGA-resistant variants, and (2) "positive selection" to obtain variants positive and negative for binding of specific lectins, e.g., peanut agglutinin (PNA) - with the use of fluorescein-labelled PNA and Florescein-Activated-Cell-Sorter (FACS). Some of the surface variants showed altered metastastasizing capacity and tumorigenicity *in vivo*. Certain *in vitro* properties which may play a role in metastasis were studied, e.g., homotypic and heterotypic adhesiveness, microfilament organization and surface sialic acid. Attempts were made to correlate the *in vitro* properties with the *in vivo* behaviour.

0201 MONOCLONAL ANTIBODIES TO HLA-DR INHIBIT THE GROWTH OF HUMAN MELANOMA CELLS IN SOFT AGAR. J.R. Harper and R.A. Reisfeld. Scripps Clinic and Research Fdtn., LaJolla,CA 92037

The ultimate step in a successful metastatic event following survival in the circulation and invasion of vascular and stromal tissue is tumor regeneration.

Using a double agar clonogenic assay as an *in vitro* model of this process, we have studied the involvement of antigenic determinants coded for by the human major histocompatibility complex in tumor regeneration of human malignant melanoma. Cultured human melanoma cells (M14) which express HLA-A,B,C and HLA-DR antigens have been treated with saturating, non-cytotoxic amounts of monoclonal antibody to framework determinant of HLA-DR (Q5/13) then cultured in the clonogenic assay. When compared to cells treated with irrelevant monoclonal IgG (MOPC 21) or monoclonal antibodies to HLA-A,B, and C (W6/32), the soft agar cloning ability of the Q5/13 treated cells was drastically inhibited. This effect appears specifically attributable to the determinant recognized by Q5/13 and not to antibody binding, *per se*, since W6/32 does not inhibit soft agar cloning. These data suggest that HLA-DR antigens expressed on human melanoma cells may play a role in cell-cell communication necessary for tumor regeneration. The clonogenic assay system allows us to further investigate the involvement of numerous monoclonal antibody defined cell surface antigens in a process believed to be important in tumor metastasis.

(Supported by NIH CA 28420 and ACS M218)

0202 AUGMENTATION OF TUMOR-SPECIFIC IMMUNITY BY T-T CELL INTERACTION AND ITS POTENTIAL APPLICATION TO TUMOR METASTASIS INHIBITION, Toshiyuki Hamaoka, Kiyoshi Takatsu and Hiromi Fujiwara, Inst. Cancer Res., Osaka University Medical School, Osaka, Japan

In our previous studies, the concept of T-T cell interaction in the induction of cytotoxic T lymphocyte activity was applied to the area of induction of tumor-specific immunity and conditions were established under which augmentation of killer T cell activity against tumor-associated transplantation antigens was obtained. This involved induction of hapten-reactive or tuberculin (PPD)-reactive helper T lymphocytes, followed by immunization with hapten- or PPD-coupled syngeneic myeloma or leukemic cells. The resulting augmented killer T cell activity was very effective to inhibit the growth of challenged viable tumor cells and to suppress the growing tumors. In our another approach, we observed enhancement of tumor-metastasis by suppressing concomittant killer T cell-mediated immunity in the same tumor system. In this system mice received i.v. injection of 7,000R X-irradiated tumor cells 3 times at 4 day intervals and rendered tolerant to tumor-associated transplantation antigens (TATA). These pretreated mice could not develop TATA-specific killer T cell response even after an appropriate immunization procedure and allowed enhanced metastasis formation from intradermally inoculated site into lymphoid organs. The above T-T cell interaction system was applied to the prevention of metastasis formation. It was found that almost complete tumor-metastasis inhibition was induced by preventing tolerance induction through an appropriate T-T cell interaction. The results will be discussed in the context of future applicability of this system to the specific immunotherapy in human.

0203 DIFFERENT INVASIVE CAPACITIES OF A PAIR OF MURINE LYMPHOMAS WITH DIFFERENT METASTATIC POTENTIAL, Catherine A. Waller and Volker Schirrmacher, German Cancer Research Center, Heidelberg, FRG.

The invasive capacities of the paired DBA/2 lymphoid tumors Eb (low metastasis) and ESb (high metastasis) were investigated using a 3-dimensional in vitro invasion system. The assay involved measuring the movement of ^{75}Se-methionine prelabelled tumor cells into 400 µm rotation aggregates of newborn DBA/2 brain in a 24 hr, 1 ml rotation culture. Using this assay, it was found that 18.5 ± 7.8% of the Eb tumor cell-bound counts were present in the normal tissue after 24 hr, compared with 57.5 ± 7.6% of the ESb tumor cell-bound counts. Treatment of the cells with 1 µg/ml of the microtubule inhibitor Nocodazole reduced the number of bound counts to 12.9% (Eb) and 19.7% (ESb). The differing abilities of these tumors to bind to, and penetrate normal tissue could therefore to partly responsable for the differences in metastatic capacity.

0204 A SPONTANEOUS LOW METASTATIC VARIANT SELECTED FROM A HIGH METASTATIC TUMOR LINE BY ADHERENCE TO PLASTIC, Mina Fogel, Peter Altevogt and Volker Schirrmacher, German Cancer Research Center, Heidelberg, FRG.

ESb is a high metastatic subline of the methylcholanthrene induced DBA/2 lymphoma L5178Y which after s.c. transplantation into syngeneic mice produces widespread internal metastases. The tumor cells can be cultured in special culture medium in Falcon flasks and grow in suspension. Continuous culture of ESb cells for several months with removal of only non-adherent cells resulted in the selection of a plastic surface adherent ESb variant designated ESb-M1.

ESb-M1 cells, when tested in vivo and compared to original ESb cells, were tumorigenic but had a highly reduced metastatic capacity. ESb-M1 cells were found to express similar cell surface markers as ESb cells, including a specific tumor antigen (TATA), the Lyt 1 T lymphoid differentiation antigen and the Fc_{γ} receptor. ESb-M1 cells were also similar to ESb cells in expression of membrane glycoproteins as revealed by galactose oxidase/NaB$^3H_4$ labeling and lectin binding studies. Differences were detected between ESb-M1 and ESb cells when comparing total cell surface proteins labeled by lactoperoxidase catalyzed iodination.

The comparison of plastic adherent variant cells to non-adherent high metastatic parental type cells could be a useful general method to study relationships between cell surface characteristics and metastatic behavior.

0205 ANALYSIS OF TUMOR CELL LECTIN RECEPTORS USING A HPLC-ENZYME ASSAY. Christopher L. Reading, M.D. Anderson Hospital and Tumor Institute, 6723 Bertner, Houston, TX 77030.

We have developed a simplified method for the analysis of tumor cell lectin receptors based on lectin binding of glycoenzymes. After denaturing in sodium dodecylsulfate (SDS) and 2-mercaptoethanol (2-ME), the glycoproteins were separated according to their molecular weightr on a TSK-4000 gel-permeation high pressure liquid chromatography (HPLC) column. Fractions were collected, air dried onto polyvinyl microtitration plate wells, and washed with ice cold 10% acetic acid containing 25% isopropanol to fix the proteins and remove the SDS. The plates were coated with 1% polyvinylpyrolidone to prevent non specific binding, washed and incubated with Lens culinaris hemagglutinin. After incubation, the plates were washed and horseradish peroxidase was added. After incubation and washing, the colorless substrate 2,2'-azino-di-(3-ethylbenzathiazoline sulfonic acid) diammonium salt (ABTS) and hydrogen peroxide were added. The colored enzyme product was evident in wells which contained immobilized lectin receptors. The analysis of leukemic cell surface lectin receptors and other lectin-glycoenzyme pairs will be discussed.

0206 CHARACTERIZATION OF A SPECIFIC INTERACTION BETWEEN B AND T LYMPHOMA CELLS AND HIGH ENDOTHELIAL VENULES. W. Michael Gallatin, Eugene C. Butcher and Irving L. Weissman, Stanford University, Palo Alto, Calif. 94305

Murine B and T cell tumours were characterized for their capacity to adhere to the high endothelial venules (HEV) in lymphoid tissue. As with normal lymphocytes, organ specificity was demonstrated. Some tumours adhered only to peripheral node HEV while others bound only to Peyer's patch HEV. Following immunization of rats with a peripheral node-specific B cell tumour, 38C-13, a monoclonal antibody, MEL-14, was produced which blocked the tumour cell-HEV interaction in vitro. This blockade was specific in that pretreatment of 38C-13 cells with saturating amounts of control antibodies directed against mouse IgM, H-2, T-200(B-220) and 38C-13 idiotype did not block HEV adherence. Non-specific inhibition due to cell death or agglutination was not observed with either MEL-14 or the control reagents. While virtually all normal lymphocytes were positive for the MEL-14 marker, pretreatment with this antibody blocked only their adherence to peripheral node HEV and not binding to Peyer's patch HEV. With the cell populations tested so far the MEL-14 marker was positively correlated with the ability to adhere to peripheral node type HEV. It's expression on cells which do not bind to HEV was either very weak or undetected. Studies on the nature of the structure(s) recognized by MEL-14 are in progress.

This work was supported in part by NIH-AIO-9072

0207 ESTABLISHMENT AND CONTROL OF THE L5178Y T-CELL LYMPHOMA TUMOR DORMANT STATE IN DBA/2 MICE. E. Frederick Wheelock, Department of Pathology, Hahnemann Medical College, Philadelphia, PA 19102

A murine model of tumor dormancy has been developed in which small numbers of highly tumorigenic methylcholanthrene-induced L5178Y cells persist under growth restraint in clinically normal syngeneic DBA/2 mice for a prolonged period, with little increase in the size of the tumor cell population. Establishment of the tumor dormant state is mediated by cytotoxic T lymphocytes (CTL). During the maintenance of tumor dormant state, growth restraint on tumor cells consists of the cytolytic activity of CTL and macrophages acting independently and a synergistic cytolytic activity produced by the interaction of T lymphocytes and macrophages. Memory CTL are present in tumor dormant mice and can be stimulated to become CTL by endogenous tumor cells. These CTL may then lyse the proliferating tumor cells thereby maintaining the dormant state. Some tumor dormant mice also contain suppressor cells which prevent the generation of CTL from memory CTL, and may thereby permit the progressive growth of tumor cells and formation of a recurrent tumor. Once the tumor dormant state is established, mice are not spontaneously cured. However, active specific and active nonspecific immunotherapy have each been effective in eliminating tumor cells from approximately 50% of tumor dormant mice. This murine model will be presented within the framework of tumor dormant states in other animal models and in man. (USPHS CA-32575, USPHS CA-32577)

0208 ROLE OF CELL SURFACE ANTIGENS IN MURINE LYMPHOMA/LYMPHOSARCOMA METASTASIS TO SPECIFIC SITES, Garth L. Nicolson, Department of Tumor Biology, The University of Texas System Cancer Center, M.D. Anderson Hospital and Tumor Institute, Houston, Texas 77030

Metastatic variant sublines of murine RAW117 lymphosarcoma were selected in vivo by repeated sequential selection for enhanced blood-borne colonization of lung or liver or in vitro by sequential selection for lack of binding to the immobilized-lectins Con A, RCA_I, PNA or WGA. Cell clones were obtained from these sublines, some of which were shown to be phenotypically unstable in tissue culture with time. Cell surface analysis of various RAW117 sublines and clones indicated a good correlation between loss of cell surface RNA tumor virus envelope glycoprotein gp70 and metastatic potential. Colonization of organ sites was also related to antigen content. Liver-colonizing RAW117 sublines express an antigen cross-reacting with fetal liver cells. Using anti-murine embryonic liver antibodies the liver selected sublines expressed more of this antigen than parental or lung-selected sublines. Treatment of a liver-selected subline with Fab' antibody blocked blood-borne colonization of liver (G.L. Nicolson, J.J. Mescali and E.J. McGuire, Oncodevelop. Biol. Med., in press) indicating that cell surface antigens are involved in the qualitative and quantitative aspects of metastasis to specific sites. Supported by NCI grant RO1-CA-29571.

0209 INTERACTION OF METASTATIC AND NON-METASTATIC DBA/2 LYMPHOMA CELLS WITH THE VASCULAR ENDOTHELIUM AND ITS UNDERLYING BASAL LAMINA, Israel Vlodavsky[1] and Volker Schirrmacher[2], Dept. of Clinical Oncology, Hadassah University Hospital, Jerusalem, Israel[1], German Cancer Research Center, Heidelberg, FRG[2].

Invasion of tumor cells through blood vessels and the capillary bed of different organs provides a major pathway for the dissemination and escape of neoplastic cells into the extravascular tissues where they establish metatasis. The DBA/2 T lymphoma Eb and its highly metastatic variant line ESb were investigated for differences in ability to invade a confluent monolayer of cultured vascular endothelial cells and to degrade its underlying basal lamina. Both the Eb and ESb lines exhibited a much faster and firmer attachment when placed directly on the subendothelial basal lamina than on top of the endothelial cell layer. Whereas the Eb cells retained their spheroidal shape when attached to the subendothelium, the ESb cells adopted a more flattened morphology and often extended a long pseudopod. Similarly, adherance to the endothelial cell layer was associated with pseudopod formation, tumor cell invasion and endothelial cell retraction to a greater extent with the ESb than the Eb cells. Interaction of the highly metastatic ESb cells with the subendothelium was associated with degradation of its sulfated proteoglycans as revealed by a release of labeled degradation products upon incubation with a metabolically $^{35}SO_4$-labeled basal lamina. In contrast, there was little or no degradation activity observed with the non-metastatic Eb cells when incubated under similar conditions. A much higher degradation activity associated with the ESb than the Eb cells was also revealed by SDS gel electrophoresis of iodinated degradation products released from a basal lamina that was subjected to a lactoperoxidase catalyzed iodination.

Monoclonal Lymphoid Populations and Their Products

0210 Ontogeny of TI and TD α(1-3) Dextran Precursors: Kappa light Chain-Expressing Precursors Mature Before Lambda Precursors. R. E. Ward, J. F. Kearney, and H. Kohler. La Rabida-Univ. of Chicago Inst., Chicago, IL 60649 and Univ. of Alabama, Birmingham, AL 35294. The maturation of B-cell precursors responding to the α(1-3) linkage determinant of bacterial dextran was analyzed using the splenic fragment culture assay. Liver and spleen cells were taken from neonatal Balb/c mice of different ages and transferred to irradiated, hemocyanin primed and unprimed, NBF_1 male hosts. Splenic fragment cultures were immunized with T-independent Dextran B1355S or T-dependent Dextran-hemocyanin. Responding clones were analyzed for light chain isotype, and heavy chain idiotype using monoclonal anti-idiotypic antibodies. Compared to the syngeneic hosts, the immunodefective NBF_1 host-environment maximizes the expression of kappa-producing precursors in both TI and TD dextran responses. The α(1-3) dextran-specific idiotypes, J558 IdI, M104E IdI, and IdX, are not associated with the kappa light chain-expressing precursors. The results show that kappa dextran-specific precursors are expressed earlier in development than lambda dextran-specific precursors. Furthermore, TD precursors appear earlier than the TI precursors . Finally, the IdI idiotype appear earlier than the IdX idiotype . These findings support the conclusion that antibody gene rearrangement and expression during ontogeny is an ordered and regulated process.

0211 ROLE OF INTERLEUKINS DERIVED FROM T CELL LINES AND CLONES IN THE PROLIFERATION AND DIFFERENTIATION OF B CELL SUBSETS, Susan L. Swain and Richard W. Dutton, University of California, San Diego, La Jolla, CA 92093

The roles of IL2 and (DL)TRF in the proliferation and differentiation of B cell subsets was analyzed. The differentiation of purified B cells to Ig secretion required synergy between IL2 and (DL)TRF. Using a wide variety of sources of IL2 it was found that effectiveness in this assay was always correlated with units of IL2 determined in the TCGF assay. IL2 also supported B cell proliferation of anti-μ or LPS stimulated B cells. A separate factor was also found in several sources of IL2 which caused proliferation of B cells in the absence of other stimulation. (DL)TRF and TRF from several other sources were compared for activity in the differentiation and proliferation of B cells from normal and B cell defective mice. (DL)TRF synergized with IL2 to cause B cell Ig secretion in response to SRBC in B10 congenic mice, in DBA/2J, DBA/2Ha, CBA/J, CBA/CaJ but not in CBA/N mice. (DL)TRF also caused some B cell proliferation which was additive or synergistic with that caused by IL2. We conclude that (DL)TRF acts directly on one subset of B cells to cause proliferation and/or differentiation. The role of IL2 in the B cell response appears to be to cause proliferation after activation by antigen or mitogen but it may be that this effect is either indirect through residual T cells or their precursors or may work on a separate subset of B cells.

0212 ANTIGEN SPECIFIC MURINE T CELL LYMPHOMAS IMMORTALIZED BY THE RADIATION LEUKEMIA VIRUS (RadLV), Eitan Yefenof, Shlomo Z. Ben-Sasson, Eli Kedar, Yehudit Azar and Rina Guy, The Lautenberg Center for General and Tumor Immunology, The Hebrew University-Hadassah Medical School, Jerusalem 91010, Israel.

Ovalbumin (OVA) specific T lymphomas of C57BL/6 origin were established by transformation of enriched, OVA specific immune T cells with RadLV. These lymphoma lines could provide the carrier specific help required for primary and secondary anti hapten antibody responses in normal and preimmunized mice, respectively, as well as in a cooperative in vitro PFC assay. Cloning by limiting dilution revealed that about 30% of the clones had OVA specific helper activity. None of the lymphoma lines nor their descendant clones could induce antigen specific DTH reaction. Helper activity was also detected in supernatants collected from OVA specific cloned lines. Antiserum was raised in C57BL/6xBALB/c F1 mice that could specifically react with OVA committed T lymphocytes and lymphoma cells. Upon exposure to OVA, supernatants obtained from several helper clones contained Interleukein-II-like activity and could boost NK activity in vitro. The interrelationship between the functional properties, heterogenicity and antigenic specificity of the lines and their cloned derivatives is currently being studied.

0213 Products from Monoclonal T Cell Immune Reactions Induce Differentiation by B Cell Tumor Lines, Charles L. Sidman, Christopher J. Paige and Max H. Schreier, Basel Inst. for Immunology, Basel, Switzerland.

When cloned T helper cells encounter antigen presented by macrophages of the proper I-A type, soluble mediators are produced which affect the differentiation and activation of normal B lymphocytes and cell lines of the B lineage. Exposure to such T cell culture supernatants causes two effects in the 70Z/3 cell line, which represents a pre-B stage of differentiation. These cells begin to synthesize Ig L chains, and gain membrane Ig which is detectable by immunofluorescence. Two other effects are seen after similar treatment of the WEHI-279.1 cell line, which represents a mature, Ig(+) B cell. These cells shift the ratio of μ chains produced from mostly membrane to mostly secretory type, and begin to secrete large amounts of IgM. These results show that products from T cell immune reactions exert multiple effects on B cell development and activation, at several stages of the B cell developmental pathway. The observed changes range from nuclear processes, including gene transcription and RNA splicing, to such post-translational aspects as protein processing, catabolism, and membrane architecture. Current work, using the defined B cell lines as models, and products of monoclonal T cell cultures, is aimed at defining the molecular nature, source, and mechanism of action of these products.

0214 STUDIES OF AN ANTI-SELF TNP SPECIFIC T CELL CLONE WITH BOTH SUPPRESSIVE AND CYTOTOXIC activity, M.A. Cooley, A.M. Schmitt-Verhulst, Centre d'Immunologie INSERM-CNRS de Marseille-Luminy - case - 906 - 13288 - Marseille - Cedex 9 - France.

Clone 3.1 was obtained from limiting dilution culture of CBA lymp nude T cells primed in vivo with trinitrochlorobenzene (TNCB) and restimulated in vitro with trinitrobenzene sulphonate (TNBS)-modified syngeneic spleen cells. Expansion was performed in the continual presence of Con A induced TCGF containing supernatant. The clone showed an absolute requirement for TCGF for growth (^{3}H-Thymidine incorporation) but growth was doubled in the presence of specific, $H2^k$ TNP stimulating cells. This stimulation was napped to H-2 K^k-TNP by use of recombinants. Clone 3.1 is also cytotoxic, and though specific for H-2 K^k TNP, will not kill tumor (RDM-4) or Con A blast targets, only LPS blast cells. A possible role for Ia determinants in this restriction is being studied. In addition, 3.1 irradiated 1000R suppresses specifically induction of anti CBA-TNBS CTL, as opposed to anti-alloantigen (B10) CTL in vitro. This suppression is not due to death os stimulating cells since (a) a third-party response is also suppressed in the presence of CBA-TNP, and (b) a subclone of 3.1, 3.1.24 which is cytotoxic, does not suppress CTL generation.

0215 A T CELL DERIVED GROWTH PROMOTING ACTIVITY ACTS ON SINGLE B CELLS, Gayle D. Wetzel, Susan L. Swain and Richard W. Dutton. Univ. Ca. San Diego, La Jolla, Ca. 92093

T cell potentiation of murine B cell proliferation was examined using a B cell cloning system which allows observation of responses by a single input cell. Frequencies of B cells induced to clonal expansion and clone sizes were determined in the presence and absence of crude and semi-purified products of a cloned T cell line C.C3.11.75. Such preparations were previously shown to contain T cell replacing factor activity (DL)TRF. Monoclonal T cell mediators increased, by as much as an order of magnitude, the proportion of B cells induced to clonal expansion. This occurred when B cells were stimulated with fetal bovine serum, lipopolysaccharide, dextran sulfate, LPS+DXS and anti-Ig. B cells of mice which did not respond to these mitogens did not respond to the B cell growth promoting activity (BCGP) from the T cell line suggesting the factor acts on B lymphoblasts. B cells were pulsed with mitogen, separated by a cell sorter and subcultured. BCGP factor was not sufficient to allow clonal expansion of subcultured blasts and the presence of mitogens was required. This suggests that BCGP is not a classical growth factor but that it acts as a costimulator of proliferation. Analysis revealed that while BCGP increased the proportion of B cells induced to division, the clone sizes of responding cells were unaltered. The single cell nature of these experiments provides unequivocal proof that T cell products can act directly on B lymphocytes to enhance proliferative responses.

0216 EFFECT OF LYMPHOKINES DERIVED FROM MONOCLONAL T CELLS ON Ig SECRETION AND ISOTYPE SWITCHING BY NORMAL AND NEOPLASTIC B CELLS. Ellen Puré, Peter Isakson, Peter Krammer and Ellen Vitetta, University of Texas Health Science Center, Department of Microbiology, Dallas, Texas 75235.

Studies of B cell activation indicate that soluble T cell products can induce polyclonal Ig secretion by B cells. We have studied the effect of soluble products of three monoclonal T cell populations: a cloned T cell line (7.1.1a), a hybridoma (FS7) and a thymoma (EL-4) on the differentiation of B cells to Ig secretion. These studies have led to the definition of two T cell-derived B cell differentiation factors (BCDF). Supernatants from these lines: 1) induce IgM secretion by normal B cells and BCL_1 leukemic cells ($BCDF_\mu$); 2) some of these supernatants enhance LPS driven IgG secretion of normal B cells by specifically enhancing switching to the IgG_1 subclass ($BCDF_\gamma$). By several criteria, we have shown that $BCDF_\mu$ and $BCDF_\gamma$ are distinct from IL-2, IL-3, TRF or other known lymphokine activities.

0217 ESTABLISHMENT AND CHARACTERIZATION OF HUMAN T HYBRID CELLS SECRETING IMMUNOREGULATORY MOLECULES, Masaji Okada, Norio Yoshimura, Takeji Kaieda, Yuichi Yamamura and Tadamitsu Kishimoto, Osaka University Medical School, Osaka, Japan.

Hybridization of human T cells with an azaguanine-resistant human T leukemic cell line (CEM-AG^R) gave rise to T hybrid cell lines secreting immunoregulatory molecules. Analyses of karyotypes, HLA-phenotypes, and other surface phenotypes, such as T cell specific antigens or receptors for SRBC and the patterns of mitogen responsiveness confirmed that the HAT-resistant cell lines were human T-T hybridomas.

One of the established hybrid clones (24-A) secreted human Interleukin-2 (IL-2). The culture supernatants induced the proliferation of Con A-stimulated murine T cells and supported the proliferation of an IL-2-dependent human cytotoxic T cell line. IL-2 production from the clone, 24-A, was augmented by stimulation with Con A alone, or Con A and PMA. Not only Con A-induced production of IL-2 in a hybrid clone, 24-A, but also macrophage-induced production of IL-2 in another hybrid clone, 38-B, was shown. In 38-B clone which did not show any IL-2 activity in culture supernatants, the addition of macrophages induced IL-2 production in the presence of PHA, suggesting that Interleukin-1 (IL-1) induced IL-2 production in T hybrid cells. Hybrid cells secreting killer helper factor were also established. The culture supernatants from this cell line, 55-A, helped the induction of cytotoxic T cells in the primary MLC by using UV-treated human B-blastoid stimulator cells, but did not show any IL-2 activity, suggesting 55-A hybrid cells produced killer helper factor distinct from IL-2.

0218 SUPPRESSION OF T CELL MEDIATED RESPONSES TO SYNGENEIC SARCOMAS BY A MONOCLONAL T CELL SUPPRESSOR FACTOR, Karen A. Nelson, Fred Hutchinson Cancer Research Center, Seattle, WA 98104 USA.

Thymus derived (T) lymphocytes control the immune response to syngeneic methylcholanthrene (MCA)-induced sarcomas in BALB/c mice. In order to define the basis of their suppression of other T cell mediated responses to these tumors, T cell hybridomas were established from thymocytes of tumor-bearing mice and BW5147 thymoma cells. The product of one line, I82K54, has been shown to suppress the induction of delayed-type hypersensitivity(DTH) to the immunizing tumor, MCA-1490, and to suppress rejection of MCA-1490 by adoptively transferred, specifically immune lymph node cells. The I82K54 factor prevented the induction or proliferation of the Lyt 1$^+$ T cells which transfer DTH responsiveness and also suppressed the elicitation of the DTH reaction. In assays of tumor rejection, binding of the I82K54 factor to immune nonadherent Lyt 1$^-$2$^+$ T cells resulted in suppression of tumor rejection. In both of these assays, responses to other MCA-induced sarcomas were not suppressed by the I82K54 factor. It also did not appear to induce suppressor cells in syngeneic mice in the absence of MCA-1490 antigens. The I82K54 factor was bound by immunoabsorbents of MCA-1490 cells, Lens culinaris lectin and antibody to rat T cell receptors. The I82K54 factor appears to be a T cell suppressor factor which is specific for an antigen of a chemically-induced sarcoma and acts both in the induction and effector phases of the immune response to that sarcoma. (Supported by grants CA 26116 from NIH-NCI and IM 43M and IM 305 from ACS.)

0219 PRODUCTION AND CHARACTERIZATION OF A T CELL HYBRIDOMA THAT SECRETES A B CELL DIFFERENTIATION FACTOR, Karen Elkins and John Cambier, Duke University Medical Center, Box 3010, DUMC, Durham, N.C. 27710

In order to investigate the role of soluble T cell products during B cell antibody responses, we have fused a population of helper T cells, prepared as described by Julius and Augustin (Eur. J. Immunol. 9:671, 1979) with the T cell lymphoma BW5147. Here we report the activity of the supernatant of one hybrid derived from this fusion which has the ability to promote antibody production in cultures containing B cells, sheep red blood cells as antigen, and limiting amounts of Interleukin 2 (IL 2). This clone, designated I4G3, acts exclusively in antigen nonspecific promotion of B cell maturation, having no detectable IL2 activity, B cell growth factor activity, or antigen binding activity. The hybrid has been subcloned by limiting dilution; 88% of the subclones tested were positive for factor production, and one subclone, I4G3.1F2, used in all subsequent studies. The functional activity of the I4G3.1F2 supernatant is strictly antigen dependent. Although some generation of plaque forming cells is seen when the hybridoma product is added to cultures containing B cells and antigen alone, it is optimally effective when small amounts of IL 2 are also present. The product appears to act late in the response, promoting antibody production when added on day two of a four day culture period, similar to the T cell replacing factor activity of mixed lymphocyte culture supernatants described by Schimpl and Wecker (Nature New Biol. 237:15, 1972). Cell surface markers on the hybrid studied by immunofluorescence indicate that it is negative for immunoglobulin, Lyt 1, Lyt 2, Thy 1.1, Thy 1.2, and T200, and positive for H-2^d and H-2^k.

0220 TRF-RESPONSIVE B CELLS COMPRISE OF A SUBPOPULATION DISTINCT FROM OTHER B CELL POPULATION, Kiyoshi Takatsu, Yasuhiro Hashimoto, Noboru Hashimoto, Yoshimi Sano and Toshiyuki Hamaoka, Inst. Cancer Res., Osaka Univ. Med. Sch., Osaka, Japan

Recently, we demonstrated that the DBA/2Ha mouse carries an X-linked recessive defect, reflected in part by the low responsivity of its B cells to the helper T cell signal mediated via T cell-replacing factor (TRF), whereas the B cell activity expressed by cognate interaction with T cells was within normal range. Taking advantage of the lack of a TRF acceptor site(s) on the B cells of DBA/2Ha mice, we raised an alloantiserum in (DBA/2Ha x BALB/c)(DC)F_1 male mice (TRF low-response animals) by immunizing them with antigen-primed B cells from parental BALB/c mice (TRF-high responders). In the present study we addressed ourselves to the question of whether B cells bearing TRF acceptor site(s) and responsive to TRF constitute a subpopulation distinct from B cells which receive T cell signals through cognate cell interaction. We utilized TRF derived from T cell hybridoma (B151), which contains only TRF without any other lymphokine activities such as IL-1, IL-2, TCGF or BCGF. This B151 T cell hybridoma produces TRF spontaneously without any further antigen- or mitogen-stimulation. We demonstrated that injection of DCF_1 male anti-BALB/c-B cell antibody i.p. into neonatal mice selectively suppressed the development of B cell activity of the former type responsive to TRF, whereas activity of the latter B cell type was not affected significantly. We also demonstrated that DNP-primed splenic B cells treated with biotinated DCF_1 anti-BALB/c-B cell antibody followed by treatment with avidin-ricin A conjugates did not respond to TRF, whereas the activity of the latter B cell type was again intact. Thus, the B cell subpopulation responsive to TRF is dinstinct from the other B cell subpopulation cooperating with helper T cells.

0221 CHARACTERIZATION OF A HUMAN IL-2 PRODUCING T LYMPHOMA (JURKAT) AND VARIANTS DEFICIENT IN HPRT, Ellen Lakow, Amnon Altman and Dennis A. Carson, Scripps Clinic and Research Foundation, La Jolla, CA 92037

Interleukin 2 (IL-2) is essential for T cell proliferation and function. Gillis and Watson (JEM 152:1709, 1980) have described a human T cell lymphoma, Jurkat, that secretes IL-2 in vitro after stimulation with phytohemagglutinin (PHA). The purpose of the present experiments was (a) to characterize the surface antigen phenotype of the Jurkat cells, (b) to define optimal conditions for IL-2 secretion, and (c) to select and characterize variants deficient in hypoxanthine phosphoribosyltransferase (HPRT) suitable for human T-T hybridization. A mycoplasma-free clone of Jurkat has maintained a helper T cell phenotype during continuous growth in our laboratory for six months. Thus, the Jurkat cells bind monoclonal antibodies against the Leu 3a and OKT3 antigens, but not antibodies against the Leu 2a and Ia antigens. Following stimulation with 1% PHA, Jurkat cells secrete IL-2 into the medium over a 48 hour period, as measured by ^{3}H-thymidine uptake by a murine IL-2 dependent cell line. Normal peripheral blood T lymphocytes and Jurkat cells produce approximately equivalent amounts of IL-2. Mutants of Jurkat resistant to 8-azaguanine, and deficient in HPRT, were selected and cloned by limiting dilution. The mutant T cells secrete IL-2 in amounts equivalent to the parent cell line. In preliminary experiments, HPRT deficient Jurkat cells have yielded successful somatic cell hybrids after fusion with normal T lymphocytes. Because HPRT deficient Jurkat cells have a helper phenotype, secrete IL-2, and clone efficiently, they are ideal for the production of functional human T-T hybridomas.

0222 IS LYB 2, A B-LYMPHOCYTE SURFACE ANTIGEN, A TARGET FOR T CELL DERIVED FACTOR?, B. Subbarao and D. E. Mosier, Institute for Cancer Research, Fox Chase Cancer Center, 7701 Burholme Avenue, Philadelphia, PA 19111

We have prepared a monoclonal antibody to Lyb 2.1 molecule, which is a surface antigen present on all B lymphocytes. The monoclonal anti-Lyb 2.1 antibody (m-anti-Lyb 2.1) inhibits in vitro plaque forming cell (PFC) responses to the TI-2 antigen TNP-Ficoll and to the T-dependent antigen, sheep red blood cells (SRBC) in Mishell-Dutton cultures. This antibody does not affect the PFC responses to the TI-1 antigens TNP-Brucella abortus and lipopolysaccharide. The m-anti-Lyb 2.1 induced inhibition of the PFC responses to SRBC can be overcome by the 24 hour supernatant from Concanavalin A activated mouse spleen cells (Con A Sup). In T-cell depleted spleen cell cultures, the m-anti-Lyb 2.1 antibody inhibits the PFC response to SRBC obtained in presence of interleukin 2 but not that obtained with the help of Con A Sup. Further, in serum free cultures of B cells, m-anti-Lyb 2.1 induces blast transformation and proliferation with little or no antibody secretion. Thus it appears that a factor in Con A sup antagonizes the action of m-anti Lyb 2.1 antibody and the antibody partially mimics the action of such a factor in inducing blast transformation without antibody secretion.

0223 REGULATION OF CYTOTOXIC CAPACITY OF HUMAN NEUTROPHILS AND EOSINOPHILS BY T-LYMPHOCYTE PRODUCTS, Mathew A. Vadas, Ian Clark-Lewis, William Sewell, Pat Mottram, John Schrader and Nicos Nicola, The Walter and Eliza Hall Institute of Medical Research, Royal Melbourne Hospital P.O., Victoria 3050, Australia

Antibody dependent cell-mediated cytotoxicity (ADCC) of purified human neutrophils and eosinophils is greatly enhanced by colony stimulating factor (CSF)-like molecules. The receptors for CSFs differ between neutrophils and eosinophils as only one sub-species of human CSF, CSF-α, has the capacity to stimulate eosinophils, whereas both sub-species (CSF-α and CSF-β) stimulate neutrophils. The secretion of molecules from mouse cell-lines with the capacity to stimulate ADCC by human granulocytes was studied. Only thymomas and T-cell lines and T-cell clones secreted substances that stimulated eosinophils but their production did not parallel that of any other lymphokine, suggesting that a new class of substances was identified. Furthermore a sub-line of a myelomonocytic leukemia (WEHI-3FA) secreted a substance that stimulated neutrophil- but not eosinophil-mediated ADCC. These findings suggest that (i) T-cell (and rarely other cell) products critically alter the functional capacity of granulocytes and (ii) there exists a cellular specialization in the secretion of three factors that provides a mechanism for the selective activation of only a sub-set of granulocytes.