BIOLOGY OF BONE MARROW TRANSPLANTATION
Robert Peter Gale, Organizer
February 17 - February 22, 1980

Plenary Sessions

Workshops & Poster Sessions

Current Status of Allogeneic Bone Marrow Transplantation in Man

001 BONE MARROW TRANSPLANTATION IN APLASTIC ANEMIA. R. Storb for the Seattle Marrow Transplant Team. Fred Hutchinson Cancer Research Center, Seattle, WA 98104.

One problem associated with high mortality after allogeneic marrow transplantation for aplastic anemia has been marrow graft rejection with a reported incidence ranging from 25 to 60%. A multifactorial regression analysis of data on the first 73 Seattle patients showed that 2 factors strongly predicted graft rejection. One was positive in vitro tests of cell mediated immunity indicating reaction of recipient lymphocytes against donor cells. The other one was a low number of donor marrow cells. Since most patients have been transfused before grafting, it was not possible to tell whether the in vitro immune reaction of the recipient against the donor was a manifestation of the basic disease or reflected sensitization to non-HLA antigens induced by transfused blood. Studies in DLA identical littermate dogs, as well as data in the first 37 untransfused patients grafted in Seattle after conditioning with a high dose of cyclophosphamide (Cy), strongly support the view that transfusion-induced sensitization is the major cause of graft rejection. Only 3 of the 37 untransfused patients (8%) showed failure of sustained engraftment and 81% are alive up to 6 years after grafting (median 20 months). We conclude, therefore, that the immunological mechanisms that result in graft rejection are, for the most part, iatrogenic (that is induced by previous blood transfusion) and not a manifestation of a pathogenetic mechanism of aplastic anemia. Initial attempts to avoid rejection in multiply transfused patients by conditioning with total body irradiation (TBI) combined with drugs instead of Cy alone were successful but survival was poor because of complicating graft-vs-host disease (GVHD) and infection. Hence, we discontinued the use of TBI. Other groups are still using TBI regimens, either at reduced doses or with lung shielding to avoid lung damage. Two centers have used Cy and total lymphoid irradiation with reduction in the rejection rate. More time is needed to evaluate the effect of these maneuvers on survival. Having demonstrated that graft rejection was less likely with a larger number of marrow cells, we turned to the donor's peripheral blood as an added source of hemopoietic stem and lymphoid cells. Forty-one patients were, therefore, conditioned with Cy followed by infusion of marrow and donor buffy-coat cells over a period of 5 days. 34 of the 41 patients (83%) accepted their grafts and 73% of all patients are surviving up to 3 1/2 years (median 16 months) after grafting. A multifactorial regression analysis confirmed the value of donor buffy-coat in reducing the rejection rate in multiply transfused patients. Other factors significantly associated with a reduced rejection rate were a high marrow cell dose, negative in vitro tests of sensitization and a female marrow donor. Over the last 3 1/2 years, the fatality rate from acute GVHD and interstitial pneumonia has dropped off sharply. Among 83 patients with sustained engraftment only 1 died with interstitial pneumonia and 6 (7%) with acute GVHD. Instead, chronic GVHD has emerged as a problem with a mortality rate of 8%. Chronic GVHD is more frequent with increasing patient age.

002 BONE MARROW TRANSPLANTATION IN LEUKEMIA. Robert Peter Gale, Transplantation Biology Unit, UCLA School of Medicine, Los Angeles, CA. 90024. There is no effective therapy for patients with leukemia who fail conventional treatment. Over the past 10 years several centers have studied bone marrow transplantation following high-dose chemotherapy and radiation in patients with resistant acute leukemia. These data indicate a 10-20 per cent two-year disease-free survival; results superior to alternative approaches. Leukemic relapse and graft-versus-host disease have been major problems. Recently, marrow transplantation has been evaluated in patients with leukemia in remission. This has resulted in improved survival in patients with acute lymphoblastic leukemia but leukemia relapse remains a major problem. Acute myelogenous leukemia patients transplanted in remission have a low rate of leukemic relapse and two-year disease-free survival rates exceeding 50 per cent. Recently, autologous bone marrow transplantation has also been considered in patients with acute leukemia. Results to date have been disappointing with a high relapse rate. Limited studies in patients with chronic myelogenous leukemia have also been reported. Transplantation during the acute phase is usually unsuccessful and is complicated by resistant leukemia and incomplete engrafment. Transplants during the chronic phase have produced more encouraging results. In summary, there is an evolving role for bone marrow transplantation in the treatment of patients with acute and chronic leukemia. A final evaluation of the utility approach awaits results of controlled clinical trials.

003 PROGRESS TOWARD CELLULAR, MACROMOLECULAR AND MOLECULAR ENGINEERING IN TREATMENT OF DISEASE, Robert A. Good, Ph.D., M.D., Sloan-Kettering Institute, New York, NY 10021

Major progress has been made in recent years towards understanding fully the nature of the immunologic systems and how they function in the body's defense. These efforts now enable us to begin to correct inborn errors of development of immunologic structure and function. Bone marrow transplantation from HLA-matched sibling donors to correct completely the cellular and functional defects in several genetically different forms of severe combined immunodeficiency disease (1-3) and fetal thymus transplantation to correct the immunologic defect in thymic aplasia (4) represent examples of the great potential of cellular engineering as a new therapeutic approach. The potential of bone marrow transplantation is being extended to employ HLA-matched relative donors or HLA-matched donors from the general population (5,6) and even fetal liver cells from non-matched donors (7). In the laboratory it has become possible to correct disease and limit disease potential by transplanting bone marrow and other tissues successfully and safely across major histocompatibility barriers (8,9). It seems likely that the techniques developed in these laboratory studies will be adaptable to the clinic, and that our current ability to cure some twenty (20) otherwise fatal diseases through cellular engineering (9-16) will be greatly extended in the years immediately ahead.

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Analysis of Clinical Problems in Allogeneic Bone Marrow Transplantation

004 CONDITIONING FOR MARROW TRANSPLANTATION USING CYCLOPHOSPHAMIDE (CY) AND TOTAL LYMPHOID IRRADIATION (TLI), John H. Kersey, Daniel A. Vallera, Phillip McGlave, Phyllis I. Warkentin, Mark E. Nesbit, William Krivit, Peter F. Coccia, William G. Woods, Chang W. Song, Tae H. Kim, and Norma K.C. Ramsay, University of Minnesota, Minneapolis, MN 55455.

Currently, humans with severe hematologic diseases who are transfused prior to marrow transplantation have a relatively high rejection rate (30-60% in various series) when conditioned for transplantation with CY alone. Conditioning with total body irradiation (TBI) is known to be associated with a significantly lower rejection rate; however, morbidity of TBI is significantly higher than that associated with CY alone. Current studies are in both animals and humans and are designed to evaluate new conditioning regimens that utilize CY in combination with TLI to reduce the risk of graft rejection (GR) and perhaps graft versus host disease (GVHD). C57BL/6 mice strain ($H2^b$) received skin and bone marrow from histoincompatible BALB/c mice ($H2^d$) following various conditioning regimens. Marrow graft survival was evaluated by chimerism, and skin graft survival by visual inspection. Long term skin and marrow graft survival (>100 days) was obtained when animals were preconditioned with either fractionated (17 days) TLI or with a combination of CY plus single dose TLI (2 days). CY alone and single or five dose TLI alone were relatively ineffective as pregrafting immunosuppressive combinations. Allogeneic marrow was necessary for long term skin graft survival. Allogeneic marrow transplantation resulted in approximately 15% more deaths than syngeneic transplantation with both CY plus TLI and fractionated TLI, suggesting that both are associated with a low but demonstrable incidence of GVHD. A high incidence of GVHD was observed when spleen plus bone marrow was used following conditioning, and this could be abrogated in a large part by treatment of donor marrow.

Studies in humans have utilized the combination of CY plus TLI prior to transplantation in previously transfused patients with aplastic anemia. A total of 13 patients have been treated with this combination. Eleven of 13 are alive; in one patient, death occurred from pre-existant candida albicans sepsis, and the other death occurred from GVHD in a patient with Fanconi's anemia. Median survival is now beyond one year. Life table analysis indicates a 85% one-year survival. GVHD has been seen in one of 11 patients and rejection has been seen in one of 11 patients in this group.

005 GRAFT-VS-HOST DISEASE (GVHD) IN ALLOGENEIC MARROW TRANSPLANTATION. P.L. Weiden and The Seattle Marrow Transplant Team, Fred Hutchinson Cancer Research Center and the University of Washington School of Medicine, Seattle, WA 98104.

GVHD is the syndrome which results when an immunocompetent graft encounters "non-self" histocompatibility antigens. Major challenges include the diagnosis, prevention and therapy of acute GVHD, the syndrome of chronic GVHD and the possibility of a graft-vs-tumor effect. The complexity of the course of marrow graft recipients may obscure both clinical and histologic criteria of GVHD. The skin, liver and intestine may also be affected by drugs, radiation, infection and underlying disease, or involvement may be masked by total parenteral nutrition. The recent observation that recipients of syngeneic (identical twin) transplants may exhibit similar findings emphasizes the difficulty of diagnosing acute GVHD.

Attempts to prevent acute GVHD have focused on 1) histocompatibility matching of donor and recipient, 2) post-grafting immunosuppression, 3) protective environment, and 4) in vitro treatment of the hematopoietic graft. Based on rodent and canine studies, most human grafts have involved HLA identical siblings and intermittent methotrexate post-grafting. Under these conditions, the incidence of acute GVHD among 123 patients with sustained engraftment transplanted in Seattle during 1970-74 was 72% (2/3 with grade II or more severe GVHD) while among 127 patients transplanted in 1977-78, the incidence was only 45% (1/2 with grade II or more severe). The reasons for these changes are currently under investigation. Specific attempts to decrease the incidence of GVHD with early administration of ATG or laminar air flow isolation and decontamination were not successful, however.

Treatment of established acute GVHD has proven to be difficult in both animals and man. In a recent randomized study, 20 patients were treated with corticosteroids and 17 with ATG. Both treatment modalities resulted in a mild decrease in severity of GVHD, but most patients required additional therapy and/or developed chronic GVHD.

Chronic GVHD has emerged as a major complication of allogeneic marrow transplantation. Clinical manifestations are protean, resembling those seen in autoimmune diseases. Although it can develop de novo, most patients have had acute GVHD. Treatment with corticosteroids and azathioprine has been well tolerated and favorably modified the course of chronic GVHD.

Recent analyses by several groups now suggests the presence of a graft-vs-leukemia effect in association with GVHD in man. Among patients transplanted in Seattle before 1978, the relative relapse rate was 2.5 times less in recipients with GVHD than in those without GVHD. More recently, GVHD has continued to be associated with a lower rate of leukemic relapse while the fatality rate of GVHD has decreased. The net result is that survival among patients with lymphoblastic leukemia is highest among recipients with GVHD. Thus, perhaps the greatest challenge of allogeneic marrow transplantation will ultimately be to utilize effectively the graft-vs-leukemia activity of the graft-vs-host reaction.

006 INTERSTITIAL PNEUMONIA AND OPPORTUNISTIC VIRAL INFECTION FOLLOWING MARROW TRANSPLANTATION, Paul E. Neiman, Joel Meyers and E.D. Thomas, Fred Hutchinson Cancer Research Center and the Department of Medicine, University of Washington, Seattle, Washington 98104

Interstitial pneumonia is a major and often (50-75%) fatal complication within the first 100 days following marrow transplantation. The incidence is high (approaching 50-60%) among end stage patients undergoing allogeneic transplantation for acute leukemia, somewhat lower (25-35%) both among recipients receiving allogeneic marrow grafts while in complete remission from acute leukemia and among recipients with aplastic anemia, and lowest (about 5%) among leukemic recipients of syngenic transplants. Aggressive diagnostic study employing early open lung biopsy revealed candidate pathogenes in about 60% of pneumonias while the remainder are apparently idiopathic. By far the most frequently observed candidate pathogen is cytomegalovirus (CMV) observed histologically and recovered by culture from 40 to 50% of such pneumonias. CMV is excreted by about half of marrow graft recipients during the first three months following transplantation. Failure to respond to CMV infection with a rise in complement fixing antibody titer is associated with a very high probability of lethal interstitial pneumonia. Mortality is reduced among recipients who respond serologically to CMV associated pneumonia. Graft vs. host disease is also associated with both an increase in the incidence and severity of interstitial pneumonia. The total body irradiation used to prepare patients for engraftment and post grafting methotrexate therapy are potential contributors to this syndrome, but the precise nature of their respective roles, if any, are not established. A number of interventions have been attempted without success to date. Both adenine arabinoside (Ara-A) and leucocyte interferon have been employed to treat CMV associated pneumonia without clear evidence of benefit. Ara-A has been given prophylactically in a controlled trial to marrow transplant recipients following engraftment without evidence of benefit. Initial trials with prophylactic CMV immune globulin have also failed to decrease either CMV infection or interstitial pneumonia.) A reasonably well tolerated dose of interferon has been established (in the treatment program) and a controlled prophylactic trial is planned. We will measure the effects of leucocyte interferon upon recurrent leukemia, interstitial pneumonia, opportunistic viral infection, and graft vs. host disease.

007 POST-TRANSPLANT IMMUNODEFICIENCY: SECONDARY TO THYMIC EPITHELIAL CELL DYSFUNCTION? Erwin W. Gelfand and Hans-Michael Dosch, Division of Immunology, Research Institute Hospital for Sick Children, Toronto, Canada. Allogeneic bone marrow transplantation is recognized as the treatment of choice for certain of the immunodeficiency diseases, aplastic anemia, and leukemia. In severe combined immunodeficiency disease administration of histocompatible bone marrow generally results in the rapid reconstitution of both B and T-cell compartments[1]. In addition, graft-versus-host disease, if present, is most often self-limited, only very rarely fatal in the immediate post-transplant period, and does not appear to result in chronic disease. In contrast, bone marrow transplantation for hematologic disorders in immunocompetent hosts is often complicated by infection and chronic graft-versus-host disease. Immunosuppressive agents are employed in the latter (but not in combined immune deficiency) diseases to permit engraftment. It is these conditioning regimens which may predispose the recipient towards developing "post-transplant immunodeficiency". The nature of the infectious agents resulting in disease in the post-transplant period suggest that it is cell-mediated immunity which is most severely affected. This has been confirmed in studies assessing antibody formation versus delayed type hypersensitivity following successful engraftment of hematopoietic elements. Further, graft-versus-host disease may also reflect abnormalities of T-cell differentiation and function with an imbalance in T-cell subset distribution[2].

Since the thymus and particularly the epithelial stroma are directly concerned with the acquisition of cell mediated immunity and the balance in various T-cell compartments, evaluation of T-cell subset distribution and the response to T-cell induction regimens[3] may delineate the nature of the post-transplant immunodeficient state. Application of this technology to patients in the post-transplant period has revealed abnormalities in T-cell maturation and differentiation, imbalances in T-cell subset distribution and responses to thymic epithelial cell derived factors. The reconstitution of immune function in vivo following implantation of thymus epithelium in selected patients with immunodeficiency supports this role of thymic epithelium in the induction of balanced T-cell differentiation. In certain instances post-transplant immunodeficiency may indeed reflect the effect of the various conditioning regimens on thymic epithelial cell function.

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Graft-Versus-Host Disease

008 PATHOGENESIS OF GRAFT VS HOST DISEASE, OR KNOTTING THE NET. William L. Elkins, Univ. of Pa., Phila., PA 19174. The intensity of graft vs host reaction (GVHR) is determined by the relative magnitudes of 2 opposed vectors. The magnitude of the pro-GVH vector depends upon undefined characteristics of the alloantigenic stimulus, its mode of presentation to donor lymphocytes, and the number and composition of T cells in the graft. The anti-GVH vector is determined by the capacity of the immune system, as a network, to resist the incursion of the exogenous alloaggressive cells and by suppressor activities of donor cells. GVHR and GVH disease are observed when the magnitude of the pro exceeds the anti-vector. The role of antigenic stimulation in determining the magnitude of the pro-GVH vector is axiomatic, but imprecisely characterized. The matter is complicated by synergistic effects, allelic differences, and host resistance(vide infra). The importance of the mode of "antigen presentation" has long been recognized. Special cells of hematopoietic origin(?macrophages,dendritic cells) may be required to effectively stimulate donor lymphocytes which generate GVHR. T lymphocytes in the graft initiate GVHR, and these may be subdivided into functional sub-classes, i.e. cytotoxic (Tc) and mediators of delayed type hypersensitivity(Tdhs) (1,2). The latter may be of particular importance in activation of macrophages to a state of non-specific cytotoxicity.

Even irradiated F_1 hybrid rodents possess the capacity to resist induction of GVHR by lymphocytes from inbred parental strain donors. There is evidence for both natural resistance to donor T cells, similar to that of exogenous hematopoietic stem cells, and highly specific adaptive responses mediated by host T cells against the clones of donor T cells responsive to the MHC of the host (3,4). Both forms of resistance can be viewed as manifestations of the network function of the immune system in the F_1. To the extent that network function is impaired by immunosuppressive Rx and disease, the anti-GVH vector is reduced, and GVHR facilitated. Thus relatively minor genetic differences may elicit GVHR under conditions of clinical transplantation.

Once established the GVHR can exert important influences on immunologic function in the chimera. There is inappropriate stimulation of B and T cells of diverse specificity (allogeneic effect) (5) which can lead to autoimmune responses and adds a further burden to network function. Suppressor T cells are activated during GVHR, and cause non-specific, non physiologic depression (6). The thymus is a target of GVHR (7), and may thus fail to function normally in reconstitution of the immune system in radiation chimera. Thus the GVHR is a major cause of the immunologic disease which commonly afflicts the transplant patient.

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009 IMMUNOLOGICAL BASIS OF GvH DISEASE

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The immunological nature of GvHD has been established many years ago (1, 2, 3) by immunogenetic and immunological methodology, as follows:

1) similarity of histopathological changes with other immunological diseases, e.g., lupus erythematodes, parabiosis disease, homologous disease, runting of newborn mice given large numbers of allogeneic lymphoid cells,
2) the occurrence of GvHD in parent $\longrightarrow$ F_1 donor-recipient combinations and its absence in $F_1 \longrightarrow$ parent donor-recipient combinations as well as in isogeneic combinations,
3) increase of severity of GvHD with increasing numbers of lymphoid cells grafted.

This concept has been subsequently confirmed and refined by identifying T lymphocytes as the immediate effector cells of acute GvHD, by the recognition that severity and incidence of GvHD in different species is determined by the proportional content of T lymphocytes in the bone marrow (high in primates, intermediate in dogs, low in rodents), by the mitigation or prevention of GvHD as a result of selective removal or inactivation of T lymphocytes from the bone marrow graft prior to transplantation (4, 5), by the effective treatment of existing GvHD with specific anti-T lymphocyte agents such as ALG (6), and perhaps Cyclosporin A.

GvHD is commonly accompanied by a state of immunodeficiency and immune desequilibrium which is ascribed to exhaustion of effector cells as a result of a persisting immunological reaction of donor type lymphocytes against recipient tissues. The resulting infections seem to intensify, sustain and under certain conditions even to initiate the GvHD, as evidenced by the beneficial results obtained with bacteriological decontamination of the bone marrow recipients (7, 8). The possible mechanism of this aggravating effect will be discussed. The various approaches which have been or are being developed for counteracting GvHD by manipulation of the immunological mechanisms and by donor selection (9) will be presented.

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010 PREVENTION AND TREATMENT OF GvHD BY ANTI-THYMOCYTE GLOBULIN

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Given after bone marrow transplantation (BMT) ATG can suppress an established acute GvH[1]. ATG does not reverse chronic GvH or prolong survival time.

Pretreatment of the donor's marrow with ATG requires pre-absorption of antibodies toxic for stem cells. Suppression of GvH in radiation chimaeras by absorbed ATG (aATG) has been studied in rodents[2-4], dogs[5] and patients[6]. aATG prevents GvH in H-2 compatible and in H-2 incompatible semiallogeneic (parent-to-F_1) mice by an opsonization of the donors' T cells. Rabbit aATG without its Fc fragment has no effect. C57BL/6 bone marrow pretreated with aATG differentiates T cells under the influence of the H-2 incompatible thymus of the (C57 BL/6 x CBA)F_1 recipients. C57BL/6 bone marrow pretreated with aATG did not repopulate T cell areas of lymphnodes in thymectomized (C57BL/6 x CBA)F_1 mice; it did so after the thymectomized recipients had been grafted under the kidney capsule with thymus of C57BL/6, (C57BL/6 x CBA)F_1 or CBA strains. Under these conditions humoral and cellular immunity against third party antigens resembled that of syngeneic controls. C57BL/6 spleen marrow pretreated with aATG and transplanted into irradiated (C57BL/6 x CBA)F_1 mice caused no GvH in secondary (C57BL/6 x CBA)F_1 recipients. C57BL/6 marrow pretreated with ATG caused no chronic mortality in CBA mice which had been rendered tolerant to C57BL/6.

Autologous bone marrow pretreated with aATG recovered in lethally irradiated dogs. T cell specificity of absorbed rabbit antidog thymocyte globulin was demonstrated immunohistochemically by selective staining of the thymus dependent interfollicular area of lymphnodes. aATG prevented GvHD in 40 % of DLA incompatible semiallogeneic dogs surviving as chimaeras now more than 2 years.

Human bone marrow pretreated with aATG did notshow reduction of proliferation of bone marrow in diffusion chambers nor of CFU-C. aATG did not interfere with hemopoietic engraftment in vivo. No GvHD was observed in leukemic patients grafted with HLA, MLC compatible sex-different marrow, the longest surviving now over 2.5years.

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011 PREVENTION AND TREATMENT OF GVHD BY DRUGS, George W. Santos and Peter J. Tutschka, Oncology Center, Johns Hopkins University, Baltimore, MD 21205

GVHD may contribute to mortality by acute destructive lesions as well as by its depressing effect on immune recovery. Animal studies indicate that following marrow transplantation there is a development of cytotoxic as well as suppressor T cells[1]. Treatment that favors the development of suppressor cells over cytotoxic cells would be expected to reduce the incidence and severity of GVHD. Extensive animal studies in the past indicated that cyclophosphamide (CY) and methotrexate administered after transplantation were quite useful in reducing the incidence and severity of GVHD[2]. These agents have been used in the clinic but results are far from perfect. Cyclosporin A administered immediately after transplantation in the rat is able to prevent GVHD presumably by blocking the induction of cytolytic T cells but allowing the development of specific suppressor cells[3]. At present this agent represents the most promise if one is going to give chemotherapy after transplantation. Treatment of established acute GVHD with anti-thymocyte serum or steroids and chronic GVHD with azathioprine and steroids has been useful but not entirely satisfactory. Manipulation of the marrow inoculum in an attempt to remove GVHD inducing cells has considerable attraction. Gradient fractionation, culture techniques and incubation with anti-sera show varying degrees of promise. Recent studies of our own have indicated that a short incubation of marrow with 4-hydroperoxycyclophosphamide, a congener of CY, will prevent GVHD in rats given MHC mismatched marrow. Similar results have been previously reported in mice when microsomal activated CY was used for incubation[4]. For many reasons the in vitro manipulation of marrow by a variety of methods including the use of chemotherapeutic agents would appear to be one of the most promising approaches for the prevention of GVHD.

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Supported by DHEW Grants CA-15396 and CA-06973

012 Specific transplantation tolerance to bone marrow allografts using total lymphoid irradiation (TLI), Shimon Slavin, Lola Weiss, Shoshana Morecki and Zvi Fuks, Hadassah University Hospital and School of Medicine, Jerusalem, Israel.

Permanent and specific transplantation tolerance to bone marrow (BM) allografts can be established in different animal models following conditioning of recipients with high dose, fractionated total lymphoid irradiation (TLI) consisting of daily fractions of 150-200 rads to the major lymphoid organs including the thymus and spleen to a total of 2000-3500 rads. BM recipients develop no clinical signs of graft versus host disease (GVHD). The mechanism of the bilateral tolerance (host versus graft and graft versus host) is still uncertain, although antigen specific and nonspecific host and possibly donor suppressor cells were demonstrated using both in vivo and in vitro experiments, affecting both cell-mediated and humoral responses. Kinetic studies in mice on the relationship between the number of TLI fractions, the number of fractions per day, and the dose per fraction indicated that the efficient regimen was the long protocol, involving 17 fractions of 200 rads. We have now demonstrated that the TLI protocol could be shortened significantly using 8 daily fractions of 200 rads followed by one whole body irradiation (WBI). A dose of 200 rads WBI was efficient in semiallogeneic chimeras (parental → F_1), and 400 rads WBI was needed for complete allogeneic chimeras. These data suggest that (a) BM-space may be an important factor for establishing successful BM allografts and, (b) the cellular mechanisms responsible for the generation of tolerance to alloantigens are relatively radio-resistant (not eliminated by sublethal WBI). Establishing short conditioning protocols is essential in trying to apply TLI for clinical use for aplastic anemia and malignant hematological disorders.

New Concepts in the Regulation of Immune Responses

013 THE DISCRIMINATION BETWEEN SELF AND NON-SELF, William O. Weigle, Department of Immunopathology, Scripps Clinic and Research Foundation, La Jolla, CA 92037

The normal reaction of the body's immune system to its own self constituents is one of restraint, but occasionally this system reacts to self in a positive manner resulting in an autoimmune response which may be accompanied by disease. Once self recognition is achieved, the cellular events leading to destructive autoreactivity and its regulation are the same as those involved in the beneficial immune response to foreign antigens. The mechanism which results in self recognition, however, is controversial and may have several different pathways. In the present discussion the assumption is made that tolerance to self in both the T and B cells is the result of a central unresponsive state and not due to peripheral inhibition such as active suppression or antigen blockade (1). The various mechanisms that may be responsible for the loss of central unresponsiveness to self antigens can be divided into three general categories. First, of major consideration is the occurrence of abnormalities in the regulatory mechanisms that control the normal immune response. For example, genetic deficiencies in immune regulation may permit self recognition to proceed to an autoimmune response and possibly to disease. Second, a component of self that was once sequestered and nonimmunogenic may become exposed and presented in an antigenic form to the immune system. In this regard, factors generated during infection, trauma, etc., can either potentiate an immune response directly or do so indirectly by facilitating release of immunogenic levels of antigens. Third, a normally tolerated self component may for some reason lose its tolerated state and activate one or more arms of a normal immune system. In this latter case, the recognition of self may depend on the immune status of T and B lymphocytes which is controlled by the concentration of the autoantigens in the microenvironment of these cells. A second consideration is that maintenance of tolerance in B cells requires much higher levels of self antigens than do T cells. Depending on the immune status of T and B cells, self-recognition may result from direct activation of T cells, or triggering of B cells by either polyclonal activation or bypassing the specificity of, or need for, T cells (2). Although suppressor cells appear to play only a fail-safe role in self recognition, considerable data have recently been accumulated that suggest an inverse relationship between suppressor cell activity and self reactivity (3).

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014 MODULATION OF IMMUNE RESPONSES BY LECTINS. Benjamin Bonavida, T.P. Bradley, J. Fan, E.A. Grimm, J. Hiserodt and S. Wright, Department of Microbiology and Immunology, UCLA School of Medicine, University of California at Los Angeles, CA 90024.

Lectins have been used as a model system to study the activation and differentiation of cells involved in the immune response. Selective lectins trigger either T or B lymphocytes and thus activate lymphocytes to undergo division and differentiation. In addition, lectins induce various cells to produce immunoregulatory factors. These factors may amplify an immune response (e.g., helper factors, blastogeneic factor) or they may suppress the response (e.g., suppressor factor). Therefore, the activation of lymphocytes may involve cell to cell interaction and/or soluble factors.

In the last several years, our laboratory has examined several aspects of the role of lectins and mitogens in the induction and regulation of immune responses.* We have investigated (1) if lectins behave like antigens in the modulation of immune response; (2) the role of the antigen receptor and if lymphocytes possess more than one receptor which are involved in the triggering event; (3) the presence of lectin-like molecules on lymphocytes which may functionally mimic exogenous lectins in the activation of lymphocytes; (4) immunotherapeutic use of lectins in the absence of antigen.

Based on our studies and others, we propose that cell surface membranes on lymphocytes and macrophages may expose lectin-like molecules which recognize molecules which bear carbohydrates. Such cell interactions may, directly or indirectly via factors, stimulate and regulate the immune response. Supported by NCI CA12800.

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015 THE ROLE OF THE THYMUS IN T-LYMPHOCYTE EDUCATION, Michael J. Bevan, Center for Cancer Research and Department of Biology, Massachusetts Institute of Technology, Cambridge, MA 02139.

The process of T-lymphocyte differentiation from hematopoietic stem cells happens most efficiently in the thymus. Recent studies on congenitally thymusless nude mice have shown that T-cells can also mature without the thymus. The antigen reactivity of mature T-cells is influenced to a great extent by products of genes in the major histocompatibility complex (the H-2 complex in mice). The most profound influence is that of H-2 restriction of T-cell function; i.e. T-cells from an H-2A mouse immune to antigen X only recognize X when it is present on a cell membrane along with H-2A antigens. The same T-cells are apparently "blind" to X on H-2B or H-2C cells (1). This restriction to H-2 is learned by the population of immature cells during their stay in the thymus and is expressed by the mature population as a preference to react with antigen-plus-self H-2 better than to antigen-plus-nonself H-2. Work with bone marrow irradiation chimeras has shown that self H-2 in this case is dictated by the H-2 type of resident thymic epithelial cells. An H-2 heterozygous mouse (A/B) lacking its own thymus but grafted with an irradiated H-2A thymus responds much better to antigen-plus-A than to antigen-plus-B (2,3). Killer T-cells learn the thymic K and D antigens as self; helper cells learn thymic I antigens as self (4). I will review this work and discuss the following questions: 1) How absolute is this self-restriction? 2) What are the exceptions to the rule? 3) Does suppression play a role? 4) Do alloreactive T-cells learn a self-preference? 5) What are the implications for clinical transplants?

Nude mice which fail to reject allogeneic or xenogeneic skin grafts were shown to contain functional T killer cells in a paper by Gillis et al. (5). These were induced from nude spleen cells cultured with H-2-allogeneic stimulators and conditioned medium from Con A-activated normal spleen cells. We and others have confirmed this result and shown that the nude alloantigen-specific killer cell bears Thy-1 and that the precursor cell grom the mouse also bears Thy-1. These cells have obviously differentiated outside the thymus. We are presently studying these cells to ask whether they can make a self-plus-X response in addition to a response to foreign H-2. If they can make this response, do they have a self-preference in restriction?

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016 ROLE OF THE H-2 COMPLEX ON THE FUNCTION OF T CELLS SPECIFIC FOR MINOR HISTOCOMPATIBILITY ANTIGENS IN VIVO, J. Sprent and R. Korngold, Department of Pathology, School of Medicine, University of Pennsylvania, Philadelphia, PA 19104

Using a model in which CBA/J T cells cause lethal graft-versus-host disease (GVHD) in irradiated B10.BR mice (H-2-compatible mice expressing multiple minor histocompatibility antigenic (minor HA) differences), information was sought on whether the induction phase of GVHD to minor HA is H-2-restricted. When unprimed CBA/J ($\underline{H\text{-}2^k}$) T cells were recirculated from blood to lymph through irradiated H-2-compatible B10.BR or B10.K mice or through H-2-semiallogeneic (B10 x CBA/J)F_1 mice, the T cells underwent specific negative selection to the minor HA of the host, ie. the filtered T cells failed to cause GVHD after transfer to B10.BR mice. With filtration through totally H-2-different B10 ($\underline{H\text{-}2^b}$), B10.D2 ($\underline{H\text{-}2^d}$) or B10.S ($\underline{H\text{-}2^s}$) mice, by contrast, no selection occurred, ie. the filtered cells were unimpaired in their capacity to kill B10.BR mice. These data, together with the results of filtering T cells through the B10 H-2 recombinant strains, indicated that selection depended upon the donor and filtration host sharing determinants encoded by both the K- and D-ends of the H-2 complex; compatibility only in the I-region failed to cause demonstrable selection. These findings imply that, as for cytotoxic lymphocytes (CTL) generated to minor HA in vitro, negative selection in vivo does not reflect a response to free antigen but to antigen associated with H-2K/D determinants.

Using a different system in which selection was induced in situ rather than by adoptive transfer, evidence has been obtained that some form of antigen-processing controls T cell selection. In this sytem thoracic duct lymphocytes (TDL) from normal CBA/J mice were tested for their capacity to generate CTL against the minor HA of B10.BR target cells in vitro. Intravenous injection of CBA mice with large doses (2×10^8) of irradiated B10.BR spleen cells specifically abolished the capacity of TDL collected 1 day later to generate anti-B10.BR CTL. The spleen at this time contained heightened reactivity and TDL regained their responsiveness by day 5 post-transfer. Interestingly, negative selection of TDL at day 1 did not appear to be H-2-restricted, ie. selection of the CBA/J anti-B10.BR response was induced as effectively with irradiated H-2-different B10 or B10.D2 cells as with B10.BR cells. However, when the selecting cells were lightly fixed with glutaraldehyde before injection, selection became H-2-restricted, ie. minor HA-bearing cells failed to induce selection across H-2 barriers. These data are taken to imply that, at least in H-2-incompatible situations, the minor-HA-bearing cells must be "processed" by host cells, ie. to allow the antigens to become associated with "self" H-2 determinants. Circumstantial evidence from studies on the specificity of selection induced with glutaraldehyde-treated cells from mice of the B10 recombinant strains suggested that I-region-restricted T cells may control the induction of H-2K, D-restricted cytotoxic precursor cells.

Autologous Bone Marrow Transplantation

017 MONOCLONAL ANTIBODY TO ALL-ASSOCIATED ANTIGENS, Jerome Ritz, John M. Pesando, Luis Clavell, Stephen Sallan, Jean Notis-McConarty, and Stuart F. Schlossman, Departments of Medicine and Pediatrics, Harvard Medical School - Sidney Farber Cancer Institute, Boston, MA. 02115.

A monoclonal antibody designated J-5 has now been developed which is specific for leukemic cells from most patients with non-T cell ALL and some patients with chronic myelocytic leukemia (CML) in blast crisis.[1] Leukemic cells from 113 patients have now been tested for reactivity with J-5 antibody. Leukemic cells from 41 of 59 patients with non-T cell ALL and 5 of 7 patients with CML-blast crisis have been reactive. There was no reactivity with leukemic cells from 14 patients with T-ALL, 13 patients with CLL, 15 patients with AML, and 5 patients with stable phase CML. In addition, there has been no reactivity with normal hematopoietic tissues including peripheral blood cells, normal or regenerating bone marrow, fetal liver and fetal bone marrow. Immune precipitation studies have demonstrated that J-5 is specific for a cell surface glycoprotein with molecular weight of 95,000 daltons. This antigen has previously been identified by rabbit antisera to common ALL-antigen (CALLA).[2,3,4]

In vitro studies with rabbit complement have demonstrated that J-5 antibody is cytotoxic for CALLA positive cells at dilutions of 1:100,000. Clinical studies are now being designed to utilize this specific cytotoxic capacity of J-5 antibody for in vitro treatment of bone marrow prior to autologous transplantation. Clinical studies of specific immunotherapy in vivo have already been initiated and 3 patients with CALLA positive ALL in relapse have received intravenous infusion of J-5 antibody. In each patient, antibody mediated tumor cell lysis was limited by modulation of cell surface antigen in response to J-5 antibody. Antigenic modulation occurred with low doses of J-5 antibody (.1 mg/kg) in conditions of antigen excess as well as with high doses of J-5 antibody (5 mg/kg) in conditions of antibody excess. Antigenic modulation occurred in all detectable leukemic cells in peripheral blood and bone marrow but these same cells were able to reexpress common ALL antigen after clearance of J-5 antibody from patients' serum. Further studies to evaluate the cellular mechanism of antigenic modulation are in progress.

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018 LEUKEMIC HETEROANTISERA SUITABLE FOR THE IN VITRO TREATMENT OF MARROW PRIOR TO AUTOLOGOUS BONE MARROW TRANSPLANTATION, Ronald J. Billing, Department of Surgery, University of California, Los Angeles, CA 90024

The recent development of certain leukemic antisera has raised the possibility of their use in the treatment of leukemia bone marrow prior to autotransplantation. In choosing suitable antisera certain criteria have to be met. The most important of these is that the antiserum is cytotoxic for the leukemia cell but not cytotoxic for normal bone marrow stem cells. In addition, sufficient quantities of antiserum at high titers should be available. In our experience at least 10 ml of antiserum with a cytotoxicity titer of 1:64 are needed to treat cells from 600 ml of bone marrow from a single patient. This might preclude the use of some antisera that need extensive absorptions before they are specific. Because it has proven extremely difficult to satisfy these criteria, up to the present time a few suitable antisera have been available. An added problem is that definitive tests are not available for testing the antisera against certain rare stem cells that may be needed for normal hematopoieses. Therefore, at the present time it is not possible to determine if any antiserum is completely safe to use in immunotherapy. Considering this reservation we have raised two leukemic antisera in rabbits that are potential reagents for the treatment of leukemic bone marrow from terminally ill patients. One is an anti non B non T ALL antiserum which reacts with ALL cells from 70-80% of ALL patients (1). Without absorption this serum was cytotoxic to ALL cells to a titer of 1:128 but it did not kill normal CFU-C marrow cells or react with normal bone marrow cells by immunofluorescence tests. Remission bone marrow from a 17 year old patient with ALL was treated in vitro with the ALL heteroantiserum and rabbit complement. Following ablative chemotherapy and total body radiation the patient received an autotransplant of the treated marrow. He was in clinical remission for 4 months and showed good recovery of all blood cells. At this time he was readmitted to the hospital and died of acute herpes zoster pneumonia. Bone marrow sections taken at post mortem showed no evidence of leukemia but demonstrated active hematopoiesis of all cell lines. Our other potential immunotherapy serum was raised to AML cells and reacts with leukemia cells from 20% of AML patients (2). It has a titer of 1:32 and does not appear to react with normal cells. At the present in vitro immunotherapy with heteroantisera is virtually untried but it does offer a future alternative to allogeneic marrow transplantation.

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019 CURRENT STATUS OF AUTOLOGOUS BONE MARROW TRANSPLANTATION, A. Deisseroth, R. Abrams, U. Bode, J. Fontana, T. Holihan, and D. Wright, NCI, NIH, Bethesda, Md., 20014

Following the successful introduction of allogeneic marrow cells as a form of hematopoietic rescue in patients exposed to intensive combined modality therapy for leukemia, many cancer centers have begun to explore the use of cryopreserved autologous hematopoietic stem cell infusions as a support modality for the administration of intensive therapy for resistant neoplasms. Work in several laboratories using both animal models and clinical studies in man has shown that cryopreserved autologous stem cell infusions can effect the reconstitution of patients exposed to marrow ablative levels of combined modality therapy as well as accelerate hematopoietic recovery following levels of therapy of intermediate intensity. The availability of autologous stem cell reconstitution has permitted these workers to begin to report responses to intensive therapy in a small fraction of patients whose disease has been shown to be resistant to conventional doses of therapy. Currently, several cancer centers are using autologous stem cell reconstitution to permit the safe evaluation of single agent therapy delivered at high doses as a means of altering the poor response of neoplasms resistant to conventional dose therapy at the time of relapse. Autologous stem cell reconstitution is also being used to support patients through regimens in which intensive combined modality therapy is being tested in these centers as an adjuvant for neoplasms associated with a high probability of relapse even after the induction of complete clinical remission. Many potential alternatives to the marrow space are being evaluated as a source of hematopoietic stem cells. The continuous flow centrifuge is also being used for the collection of hematopoietic precursor cells from the peripheral blood from patients in whom circulating stem cell pools have been amplified. These preparations are being studied in several centers both in man and animal models to establish the reconstitutive capabilities of these preparations. The development of heteroantisera, which exhibit a complement dependent cytotoxic effect versus leukemic but not normal hematopoietic precursor cells,is under study as a means of reducing the leukemic cell burden of marrow cell preparations obtained from leukemic patients in complete remission for later use in the support of the intensive therapy of relapse.[1] Finally, many centers are attempting to develop methods of effecting hematopoietic reconstitution of patients afflicted with molecular diseases of hematopoietic cells with their own autologous stem cells following in vitro genetic modification in a manner which will be of benefit to such patients. The use of novel experimental systems, animal models, and clinical trials to study all of these questions, which is taking place in many centers throughout the world, will be reviewed so as to highlight the possible future directions which this rapidly developing field may take.

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020 RATIONALE AND PHARMACOLOGY OF SUPRALETHAL DOSES OF DRUGS IN AUTOLOGOUS BONE MARROW TRANSPLANTATION, Bruce A. Chabner Clinical Pharmacology Branch, National Cancer Institute, Bethesda, MD. 20014. Conventional chemotherapy regimens for the treatment of most malignant diseases are limited by host bone marrow toxicity and the unfavorable cytokinetic and biochemical characteristics of the neoplastic cell. Specifically, most malignant cells divide at a slower rate that the normal precursors of bone marrow and gastrointestinal epithelium, thus allowing only limited cell kill for the antimetabolite class of drugs (antifolates, purine and pyrimidine antagonists), which exert their effects on dividing cells. Increasing the concentration of drug or the duration of exposure leads to intolerable host toxicity. Secondly, due to the biochemical heterogeneity of most tumors, conventional chemotherapy favors the selection of resistant cell with altered ability to transport, activate, or degrade antitumor agents, or with increased ability to repair potentially lethal damage. In this situation, high dose chemotherapy, with autologous bone marrow rescue, offers significant advantages. It removes the unfavorable cytokinetic consideration for agents with predominant bone marrow toxicity, thus allowing for higher drug concentrations and longer durations of exposure. It assures distribution of drugs with low lipid solubility into the central nervous system, as in the case of methotrexate, and in general allows for increased penetration of poorly perfused tumor compartments by all classes of agents. Finally, high dose chemotherapy should extend the effectiveness of treatment to resistant cells with cytotoxicity thresholds above the concentration achieved by conventional drug regimens; this property should decrease the selection of resistant cell types. The primary disadvantages of high dose chemotherapy reside in the shift in dose limiting toxicologic effects to new sites, as observed with cyclophsophamide (heart, inappropriate ADH), nitrosoureas (liver, kidney, and lung), methotrexate (renal, gastrointestinal, skin), and other agents. Secondly, agents which produce dose-limiting toxicity to organs other than bone marrow, such as the anthracylenes and cis-DDP, can not be utilized in such regimens, despite an amelerioration of bone marrow toxicity. A new, and relatively untried, approach to enhancement of chemotherapy is the use of conventional doses of drugs in combination with normal metabolites or second agents which enhance the effect of the primary agent on dividing cells, without increasing toxicity to non-dividing tissues. Examples of such enhancement are the combination of cytosine arabinoside with thymidine, 3-deazauridine, or tetrahydrouridine, all of which markedly increase its toxicity to bone marrow and (selectively) to tumor cells.

021 HEMATOPOIETIC STEM CELL PROCUREMENT, SEPARATION AND CRYOPRESERVATION. J.R. Wells, Dept. of Medicine, University of California, Los Angeles, CA 90024.

Autotransplantation has been performed with nonfrozen bone marrow stored for 1-3 days, with cryopreserved buffy coat cells and with bone marrow frozen for over 18 months (1). Techniques for cryopreserved bone marrow cells employ whole bone marrow and cells isolated by differential and buoyant density centrifugation. DMSO is used in most reports at a concentration of about 10% as a cryoprotectant and freezing is usually accomplished using a programmed cooling rate whereby the heat of fusion is compensated for to maintain a constant temperature drop. Optimal cell recovery results from rapid thawing of the frozen cells in a 40°C water bath and the infusion of marrow follows immediately without washing to remove the DMSO. Cell recovery and stem cell assays have been employed as a measure of the proliferative potential of the returned marrow cells.

We have used the technique of density step isolation and cryopreservation to store marrow from 85 patients (2). Autotransplantation has been employed with 20 patients who had received intensive chemotherapy and radiation. The cryopreserved marrow was monitored to predict its capacity to restore hematopoiesis by thawing test vials and growing myeloid stem cells (CFU-C). The test vial values for cell recovery and CFU-C were compared to results from the mass thaw and the hematopoietic recovery of the patient (3). These data indicate that while complete reconstitution can be accomplished with as few as 1.2×10^7 separated marrow cells/kg body weight, higher doses are desirable to accelerate recovery. Other studies have demonstrated rapid restoration of immune reactivity when doses of more than 5×10^7 nucleated marrow cells/kg are given (4).

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Alternative Sources of Hematopoietic Stems Cells

022 PERIPHERAL BLOOD LEUKOCYTES AS A SOURCE OF HEMATOPOIETIC STEM CELLS. Theodor M. Fliedner, Department of Clinical Physiology and Occupational Medicine, University of Ulm, 7900 Ulm (Donau), Federal Republic of Germany

It has been shown for a number of species that there is in the peripheral blood a small but significant population of mononuclear leukocytes (MNC) that is capable of repopulating the hemopoietic tissues of animals rendered aplastic by means of whole body irradiation. The present status of research on the ontogenesis of hemopoiesis indicates that hemopoiesis occurs throughout development as a consequence of the seeding of hemopoietic stem cells into a cellular matrix. Evidence indicates a migration stream of stem cells from extraembryonic sites to the fetal "liver-anlage" at the onset of fetal hemopoiesis and from the liver to the many sites of bone marrow development. On this basis, the success of "bone marrow transplantation" efforts rests in the experience that a suspension of bone marrow derived cells contains a sufficient number of pluripotent hemopoietic stem cells (PHSC) that can migrate via the blood stream to tissues which are prepared to accept them for replication and differentiation. On this basis there is enough reason to attempt the collection of PHSC from any source where they might be found, such as in-vitro culture systems, fetal liver and the blood. However, several premises have to be considered. (1) There must be a dynamic equilibrium between PHSC in blood and bone marrow so that a sufficient number of cells can be collected from the peripheral blood without exhausting the available pools. (2) It must be shown that the stem cells collected from the blood are "pluripotent" in the sense that they can restore permanently all hemopoitic cell lineages, including those of the marrow and the lymphatic organs. (3) It is desirable to have technics available to store such blood derived stem cells for a long period of time in order to build up a "blood stem cell bank". (4) In order to avoid a "graft-versus-host-reaction" (gvh) in the allogeneic situation, it would also be desirable to "purify" the PMSC-population and to eliminate all mononuclear cells that may contribute to the establishment of a donor-type immunocompetent cell population in the recipient. Experimental evidence suggests, that blood derived PHSC have successfully been employed in the permanent restauration of hemopoiesis in irradiated mice, rats, dogs and monkeys. In human beings, there is as yet only limited experience. While there are sporadic observations that blood leukocytes have established a transient or permanent hemopoiesis in a suitable conditioned host, there are also reports on failures which may, however, been due to factors such as cell numbers or other special conditions. In studies on patients, given high doses of chemotherapy it has been demonstrated that the transfusion of autologous mononuclear cells has resulted in an enhanced hemopoietic recovery. Future work for the use of blood derived PHSC in patients will have to concentrate on technics to collect and store sufficient numbers of PHSC, on in-vitro test systems for the demonstration of PHSC in cell suspension and on the establishment of the validity of animal experiments for the human situation.

023 FETAL LIVER TRANSPLANTATION, Bob Löwenberg, Dept. of Hematology, Erasmus University, Rotterdam.

Broad interest in fetal liver as an alternative of bone marrow was raised when allogeneic fetal liver grafts were shown to induce no or only moderate graft versus host disease (GvHD) in strongly histo-noncompatible donor-recipient pairs in rodents and larger animals. These observations have been a logical impetus to clinical efforts of fetal liver transplantation.
Human fetal liver grafts have been employed mainly in patients with severe combined immunodeficiencies (SCID), and occasionally in others. In general, these transplants were associated with a notably low incidence of GvHD, even though they were MCH-mismatched. The majority of these attempts however did not result in maintained reconstitutions. A limited number of patients have been recorded with engraftment with recovery of immunological functions. The difficulties to achieve full and consistent immunological reconstitutions were and have remained the major stumbling block of fetal liver grafts ever since their introduction.
It is the subject of this presentation to analyse the accumulated clinical data on fetal liver transplantation and to consider prognostic factors and methodological aspects. The analysis will address the question why certain grafts were succesful while so many failed. The impact of e.g., age of embryo donor, cell dose, cell handling, route of administration, use of cryopreserved cells, will be assessed. Animal data are particularly helpful to add information to our incomplete understanding of clinical failures and successes. Throughout the last decennium a wide body of experimental data have been collected which have furnished a coherent insight into the unique transplantation properties of fetal liver cells. It has been established that the proliferative capacities of embryo liver and bone marrow cells differ substantially and these are related to qualitative as well as quantitative cellular factors.
Clinical and preclinical data will be discussed in the context of the present human fetal liver experience and will be put into the perspective of future strategies of fetal liver transplantation in man.

024 LONG-TERM CULTURES OF BONE MARROW - A SOURCE OF STEM CELLS WITHOUT GvHD?
T. Michael Dexter, Elaine Spooncer and Ian Toogood, Paterson Laboratories, Christie Hospital, Withington, Manchester M20 9BX, England

Proliferation of murine stem cells (CFU-S) can be maintained *in vitro* for many months. Differentiation into granulocyte/macrophage (GM-CFC), erythroid (BFU-E) and megakaryocyte precursor cells is occurring during this time and further maturation into functionally mature granulocyte and megakaryocyte end cells is seen. However, mature B or T lymphocytes cannot be detected using immunological techniques. The stem cells produced in these cultures are functionally normal and will protect potentially lethally irradiated mice from haemopoietic death. Essentially 100% survival is seen in such radiation chimeras using cultured syngeneic bone marrow cells. Since mature T cells present in bone marrow inocula are thought to be responsible for eliciting GvHD, and since T-cells are absent in long term cultured marrow, we investigated the capacity of the cultured cells to reconstitute irradiated semi-allogeneic (i.e. $H\text{-}2^{b} \longrightarrow H\text{-}2^{b/d}$) recipient mice. Using freshly isolated marrow, less than 10% long-term survivors were seen, whereas cultured marrow cells regularly gave 90-100% survival in semi-allogeneic chimeras, with no evidence of $GvHD_a$. Using cultured cells to reconstitute irradiated allogeneic recipients (ie. $H\text{-}2^{b} \longrightarrow H\text{-}2^{d}$ or $H\text{-}2^{b/d} \longrightarrow H\text{-}2^{k}$) gave uniformly poor survival, with a majority of animals dying within 4-6 weeks post reconstitution. However, the cause of death was difficult to ascertain, since at autopsy the spleen and marrow showed extensive myelopoiesis and no evidence of GvHd was observed. Moreover, very few lymphocytes could be found. The data as a whole indicate that for successful reconstitution, some H-2 compatibility is necessary.

Studies with long-term human marrow cultures have shown a situation distinct from that of mouse in that there is a prolonged survival of T-lymphocytes. Various culture methods for human cells will be described and the efficiency of various treatments in eliminating immunoreactive T-cells will be discussed.

025 LYMPHOID STEM CELLS IN IN VITRO CULTURE SYSTEMS

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In normal animals, large numbers of lymphocytes are continuously generated. Little is known however about either the cells from which these lymphocytes arise, or, about the factors which regulate their production. The in vitro study of lymphopoiesis offers many advantages for the study of the cellular stages and regulation of differentiation.

Using the Dexter long-term bone-marrow tissue culture system, we have demonstrated the in vitro proliferation of cells able to repopulate irradiated animals with functional T and B lymphocytes. An important question is whether the cells responsible for lymphoid repopulation are a special population of lymphoid stem cells. We have demonstrated that terminal deoxy-nucleotidyl transferase positive cells are being generated in these cultures and regard these cells as candidate prothymocytes or T stem cells. More recently we have shown that Thy.1 positive T cells can be generated. Attempts are also being made to demonstrate the presence of direct precursors of B cells in the long-term cultures. Thus the Dexter system appears to be a useful in vitro method for the analysis of the early stages of lymphopoiesis.

In vitro studies are also giving information on the regulation of lymphopoiesis. They suggest that the activated T cell and the macrophage are involved in the production of factors regulating lymphocyte differentiation, effects being demonstrable on cells in the lymphoid differentiation sequence ranging from CFU-s to mature lymphocytes. We have recently demonstrated that a T cell hybridoma produces factors with such a range of activities, an observation that clearly indicates that the T cell itself can produce these factors. Biochemical analyses are revealing that the factors affecting mature lymphocytes, differ from those affecting CFU-s. This means that specific factors may be obtained and used for promoting the in vitro expansion of one cell population in bone-marrow, e.g. CFU-s, without promoting the growth of other cells, for example mature T cells, capable of producing GVH disease.

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026 INTERACTION OF T LYMPHOCYTES AND HEMOPOIETIC STEM CELLS

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In the middle to late sixties, when the thymus was coming into its own in immunology, we made observations in P→F1 (parental into hybrid offspring) radiation bone marrow (BM) chimeras suggesting that thymocytes also play a role in hemopoiesis (1). Early attempts to demonstrate an effect in isogenic transplantation were not really successful. Although a positive, augmentative affect of thymocytes was reported by Lord and Schofield (2) in an isogenic situation, the transplanted marrow had been compromised by irradiation. More recently studies in the genetically anemic W/WV mouse (3) have led to further investigation of this relationship between thymus-derived or-dependent (T) lymphocytes and hemopoietic stem cells. In vitro studies have produced data to indicate that thymocytes at different T:BM ratios can either increase or decrease erythropoiesis (4,5). In an effort to study the interaction in vivo, under isogenic conditions that might reasonably be comparable to physiological, we are manipulating both donor and recipient with respect to T cell content in transplantation experiments. Preliminary results suggest that recipients of BM depleted of T cells show an unusually great CFU-S renewal in their regenerating marrow compartment. This implies a lack of regulatory control referable to the transplant itself. Moreover, data from a single experiment so far suggest that the presence of a thymus in the regenerating chimera has little bearing on reconstitution of BM CFU-S within the first month after transplantation. Experiments are currently in progress to explore the possible role of circulating T cells in feedback regulation of committed BM progenitors.

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Virus Lymphocyte Interrelationships and Interstitial Pneumonia

027 ANIMAL MODELS OF LATENT CMV INFECTION--RELATIONSHIP TO HUMAN DISEASE, Michael B. A. Oldstone, Department of Immunopathology, Scripps Clinic and Research Foundation, La Jolla, CA 92037

A puzzling and challenging biological problem in medicine concerns the behavior of cytomegalovirus (CMV) infection. A DNA virus in the herpes virus family, CMV infects humans, causes significant birth defects as well as clinical disorders following transplantation manipulations. CMV persists in the host it infects despite the host's vigorous immune response. Thus, CMV in man is a model system par excellence for studying virus latency, persistence and reactivation. Host-specific CMVs have been found in several species including man, monkey, mouse, hamster, rat and guinea pig. As CMV infection in mice (MCMV) is very similar to that in man, abundant interest has focused on the use of a murine model to provide clues for which cells or tissues harbor CMV and the mechanism(s) whereby it becomes activated.

Several models of murine CMV have been used. Employing either mouse passed virus (virulent) or tissue culture passed virus (attenuated), acute, chronic and latent virus infections have been studied as well as the mechanisms whereby latent virus can be activated. Using tissue culture passed virus, my colleagues and I were able to show that MCMV is harbored in spleen lymphocytes, peritoneal macrophages and male germ line cells of latently infected mice. We noted that activation of MCMV from spleen lymphocytes, predominantly B lymphocytes, depended on at least three factors: (1) appropriate antigenic stimulation, (2) activation of responding cells carrying the genetic capacity to express viral information, and (3) the need for a feeder layer of cells that were susceptible to infection and permitted the virus to replicate, once activated. Studies with peritoneal macrophages demonstrated that whereas both resident and activated macrophages contained viral genetic information, infectious virus was shed from activated macrophages frequently but resident macrophages rarely. Other studies show that MCMV replicated in reproductive tissue of male mice infected with virus. Hybridization studies localized viral DNA to immature and mature sperm cells. Murine CMV was harbored in testes during both acute and latent infections.

Recent interest has focused on the study of reactivation of MCMV from differentiated cells in vitro. Thus, MCMV was found to replicate in differentiated cells, but not at all in several undifferentiated cells tested. Further, the undifferentiated pleuri-potential PCC4 cell line, following infection with MCMV, failed to express MCMV antigens or release infectious virus, although small amounts of viral DNA per cell was detected. When such PCC4 cells were induced to differentiate by treatment with N, N-dimethylacetamide, they now expressed MCMV antigens, released infectious virus and showed a four to five-fold amplification of the amounts of viral DNA contained per cell (4-5 gene copies/cell). This work was done in collaboration with Drs. F. Dutko, A. Brautigam, L. Olding and D. Kingsbury.

028 THE BIOLOGY OF CYTOMEGALOVIRUS. David J. Lang, Division of Pediatric Infectious Diseases, Duke University Medical Center, Durham, NC 27710

The cytomegaloviruses (CMV), species-specific Herpesviruses capable of establishing persistent and latent infections, are relatively fragile and require close interpersonal contact or transfer of infected material for transmission. CMV infections occur worldwide, though the age-specific acquisition is variable. CMV has been recovered from most organs and cells, as well as from urine, saliva, blood, stool, milk, cervix, and semen. CMV-associated illness in normal hosts ranges from sub-clinical infection to heterophile negative mononucleosis, mild hepatitis, interstitial pneumonia, and some hematologic and neurologic syndromes. Pre-natal acquisition of CMV infections is relatively common (1% or more of births) and is an important cause of sensorineural dysfunction. Transplacental transmission may follow reactivation of latent maternal CMV as well as primary infection although the relative contribution to fetal injury of these infections remains to be determined. Shedding of CMV may persist for variable periods of time especially following pre- or perinatal infection, and may recur in pregnant women, cancer patients, and immunosuppressed individuals. Transmission of CMV can occur with organ transplants or cells including blood, which implies the existence of asymptomatic carriers. Nevertheless, recovery of CMV from the blood of healthy carriers has been largely unsuccessful and localization of this virus in blood remains uncertain. In immunosuppressed hosts (especially in allograft recipients), CMV infections may be severe, progressive and even fatal and clinical syndromes include pneumonitis, hepatitis, febrile leukopenia, and retinitis. It has been proposed that primary and even reactivated latent CMV infection may accelerate renal graft rejection. It is also plausible that the rejection process reactivates latent virus. To prevent transmission of CMV the concept has been proposed of "protective matching" of donors and recipients. The foremost problem relevant to CMV infection among bone marrow transplant recipients has been the relatively late occurrence of a serious and often fatal interstitial pneumonia. Anti-viral treatment of CMV infections has been largely unsatisfactory. Prophylactic administration of leukocyte interferon to renal transplant patients yielded delays in CMV shedding and a decrease in viremia. Trials are currently underway in bone marrow recipients of passive immunization using high-titered anti-CMV globulin. Prototype CMV vaccines are currently under study and preliminary data indicate that the vaccine induces antibody and CMI, and does not reactivate after immunosuppression. Vaccine-induced immunity does not, however, appear to prevent subsequent CMV infection.

029 ANTIVIRAL THERAPY--CHEMOTHERAPY AND INTERFERON. Martin S. Hirsch, Massachusetts General Hospital, Harvard Medical School, Boston, MA 02114

Recent clinical trials suggest that certain antiviral agents may eventually prove useful in the control of herpes virus infections among marrow transplant recipients. Immune modulators such as vaccines and hyperimmune globulin, nucleoside derivatives that interfere with viral DNA synthesis, and the broad spectrum antiviral and antiproliferative agent, interferon, may all be of value.

Live virus vaccines against cytomegalovirus (CMV) induce both humoral and cellular immunity to CMV antigens and are currently undergoing trials in renal transplant recipients. Varicella-zoster (VZV) vaccine has proven effective in the prevention of varicella among leukemic Japanese children. Hyperimmune globulin to varicella-zoster virus (VZIG) can also prevent or moderate clinical varicella when administered to high risk seronegative recipients early after exposure. CMV immune globulin is undergoing prophylactic trials against CMV interstitial pneumonitis in bone marrow recipients.

Several nucleoside derivatives exhibit inhibitory activity against herpes virus DNA synthesis, but few have had acceptable therapeutic/toxic ratios in man. Adenine arabinoside (Ara-A) reduces both morbidity and mortality from herpes simplex encephalitis and also appears to be effective against VZV infections among immunosuppressed hosts and against neonatal disseminated HSV infection. However, attempts at Ara-A prophylaxis or therapy of CMV pneumonitis in marrow transplant recipients have been disappointing. Acycloguanosine (Acyclovir), the newest of promising nucleoside derivatives, inhibits herpes viruses that produce their own thymidine kinase (HSV,VZV). In vitro and animal studies suggest a high therapeutic/toxic ratio for this agent and human studies in herpetic infections are presently underway.

Interferons are species-specific, broad-spectrum antiviral mediators produced by virus-infected cells; immunologically stimulated T lymphocytes also produce interferons with different physico-chemical characteristics than those produced by virus-infected cells. Interferons not only inhibit translation of viral messenger RNA into viral protein, they also have cellular antiproliferative and immunomodulatory effects. Interferon has shown therapeutic activity against zoster among immunosuppressed patients and is prophylactic against CMV and EBV infections in high risk renal transplant recipients. Studies in renal transplant recipients indicate that for a maximal antiviral effect, interferon depends on the integrity of the host immune response. Consideration of interferon's use in marrow transplant recipients must include understanding of its potential myelosuppressive, immunomodulatory and antiviral effects.

030 GENETIC CONTROL OF VIRUS-LYMPHOCYTE INTERACTIONS, Peter C. Doherty, The Wistar Institute, Philadelphia, PA 19104

Virus-immune cytotoxic T cell responses are restricted by the major histocompatibility complex. In general, cytotoxic T cells generated in mice interact only with H-2K or H-2D compatible target cells infected with the same virus. The same is true for HLA-A and HLA-B in man, at least in the response to influenza A viruses and to Burkitt's lymphoma. Recent experiments indicate that the capacity of a T cell to respond to virus presented in the context of a particular histocompatibility determinant depends critically on the lymphocyte having encountered that histocompatibility determinant on radiation-resistant thymus epithelium during physiological development. However, such manipulations cannot be shown to modify situations where there is a total failure of response associated with a particular H-2 allele.

Some exceptions have been found to the generally applicable model of restriction of T cell responsiveness to thymic H-2 type. Results from studies where T cells have been negatively selected to remove alloreactive precursor lymphocytes indicate that, for instance, H-2^b T cells can respond to vaccinia virus presented in the context of H-$2K^k$. The ramifications of these experiments will be discussed in detail, particularly with respect to the effects of adult thymectomy, and previous exposure to the virus or to the histocompatibility antigen in question. The possible involvement of T-T help and suppression in immune response gene effects will also be considered.

Transplantation in Man

031 CURRENT STATUS OF KIDNEY TRANSPLANTATION, P.J. Morris, University of Oxford, Nuffield Dept. Surgery, Oxford, U.K. Transplantation of the kidney has become not only an accepted therapy for end-stage renal failure, but is now the treatment of choice in most instances with the present emphasis being directed at cadaveric transplantation. Recent years have seen a dramatic improvement in patient survival (patient survival after cadaveric transplantation in Oxford is 91% at 2 years), but graft survival although variable remains relatively constant in most units (cadaveric graft survival in Oxford is 63% at 2 years). Irreversible rejection and complicatiions arising from therapy for rejection remain the major problems of renal transplantation. Developments in several areas promise to reduce the significance of these problems. For example matching for HLA-DR can improve cadaveric graft survival significantly (1 year survival of OR compatible cadaveric grafts in Oxford =83%), and better definition of specific donor presensitisation allows many previously "non-transplantable" patients to receive a transplant. Better immunosuppression appears to be associated with blood transfusion before transplantation and new approaches to immunosuppression such as the use of Cyclosporin A, TL1, and pretransplant thoracic duct drainage, may become established clinical practice. These developments suggest that substantial improvements in the results of renal transplantation will be seen over this coming decade.

032 CURRENT STATUS OF LIVER TRANSPLANTATION, Thomas E. Starzl, Department of Surgery, University of Colorado Health Sciences Center, Denver, CO 80262

Orthotopic liver transplantation (liver replacement) was done in 164 consecutive patients at the University of Colorado Health Sciences Center from March, 1963, until the end of 1978. Among the first 111 patients only 31 lived for as long as one year. Of the 31 one-year survivors, 15 are still alive after 3 1/2 to almost 10 years.

Thirty more patients have liver replacement from the summer of 1976 until January, 1978. Fifteen of the 30 lived for at least one year and 13 are still alive after 1 1/2 to 3 years.

The still unsatisfactory one-year survival of 50% has not been maintained in a small, subsequent series of patients of whom a number do not have a follow-up of a full twelve months. Further improvements will require better immunosuppression. Striking increases in graft survival have been made in renal recipients who were pretreated with thoracic duct drainage (TDD). However, the excessive lymph losses in patients with liver disease has made this form of pretreatment dangerous for prospective liver transplant recipients. For this reason, we are currently attempting pre-transplantation conditioning of liver recipients by removal of lymphocytes from peripheral blood. The rationale and prospects of this approach (lymphapheresis) will be discussed.

033 CURRENT STATUS OF CARDIAC TRANSPLANTATION, Bruce A. Reitz, Philip E. Oyer, Charles P. Bieber, Edward B. Stinson, Norman E. Shumway, Dept. Cardiovascular Surgery, Stanford University School of Medicine, Stanford, CA 94305

Human cardiac transplantation is now entering its second decade of clinical application. Although the initial clinical experience was discouraging, progress in the early diagnosis of acute allograft rejection and better monitoring of the adequacy of immunosuppression has led to significantly improved patient survival (1). A total of 180 patients have undergone transplantation at Stanford University Medical Center with 72 patients presently surviving. Survival ranges from one month to ten years, and at the present time the one-year survival rate is approximately 66% with an expected five-year survival of 50%. These results have been attained by improved patient selection, early diagnosis of rejection by transvenous endomyocardial biopsy, routine use of rabbit antihuman thymocyte globulin in the early post-transplant period, better control of chronic allograft rejection, and the liberal use of cardiac retransplantation in the event of uncontrollable rejection or the late development of graft coronary artery disease. The availability of donor hearts has improved in recent years with the institution of distant heart procurement. Rehabilitation of surviving patients has been excellent with more than 90% returning to full activity. The major factor contributing to morbidity and mortality following cardiac transplantation is the necessity for long-term generalized immunosuppression with its associated complications. It would appear that on the basis of this improved survival, and the continuing need for means of treating otherwise terminal cardiac disease, programs in heart transplantation are warranted at other centers.

Ref.1: Baumgartner, WA, Reitz, BA, Oyer, PE, Stinson, EB, Shumway, NE: CARDIAC HOMOTRANSPLANTATION. Current Problems in Surgery, vol. XVI, no. 9, September 1979.

Clinical Bone Marrow Transplantation

034 SUPPRESSOR CELLS IN PATIENTS AFTER HLA-IDENTICAL MARROW GRAFTING. M.S. Tsoi, R. Storb, S. Dobbs and E.D. Thomas. Fred Hutchinson Cancer Research Center, Seattle, WA 98104. Seventy-two human long-term patients with or without chronic graft-versus-host disease (C-GVHD) after marrow grafts for aplastic anemia or leukemia were studied for circulating non-specific suppressor cells. Patient mononuclear cells (of donor origin) were tested for their ability to suppress the responses of lymphocytes obtained from their marrow donors to allo-antigens in mixed leukocyte culture and/or to Concanavalin A. Cells from only 2/36 long-term patients without C-GVHD showed suppression, whereas cells from 21/36 patients with C-GVHD showed more than 30% suppression of donor responses. Suppressor cells were nylon-wool non-adherent and radiosensitive. A soluble suppressor factor was detected in the culture supernatant. The suppressor activity could be abrogated by amplifying factor(s) obtained from one day mixed leukocyte culture supernatant. Four patients whose C-GVHD resolved lost their suppressor cell activity in vitro. Only 5/23 patients with acute GVHD and 1/14 short-term patients without GVHD had nonspecific suppressor cells. The nonspecific suppressor cells may be involved in the severe combined immunodeficiency seen in patients with C-GVHD. - Patient mononuclear cells were also tested for their ability to suppress donor lymphocyte responses to cryopreserved Trinitrophenyl-modified host lymphocytes. In contrast to the findings with non-specific suppressor cells, cells from 4/4 patients with C-GVHD did not inhibit that response whereas cells from 7/7 long-term patients without C-GVHD suppressed the donor cell responses to Trinitrophenyl-modified host (but not unrelated) lymphocytes. These results may have implications for the maintenance of the stable chimeric state after marrow grafting.

035 TOTAL LYMPHOID IRRADIATION (TLI) AND CYCLOPHOSPHAMIDE (CPM) AS PRECONDITIONING FOR BONE MARROW TRANSPLANTATION (BMT) IN SEVERE APLASTIC ANEMIA (AA), Norma K.C. Ramsay, Philip B. McGlave, Taehwan H. Kim, William Krivit, Mark E. Nesbit, Peter F. Coccia, William G. Woods and John H. Kersey, University of Minnesota, Minneapolis, MN 55455

Although BMT is the therapy of choice for patients with AA, methods to improve survival by decreasing rejection rates and morbidity are presently being explored. From 1977-1979 at the University of Minnesota, 13 previously transfused patients with severe AA were transplanted utilizing a preparative regimen consisting of CPM 50 mgm/kg I.V. x 4 days and single dose TLI (750 rads at 26 rads/min. using the linear accelerator). The patients ranged in age from 1-30 years (median = 12 years). All patients received major histocompatibility complex genotypically identical sibling marrow. The median number of cells was 3.9 x 10^8/kg with a range of 2.4 - 5.2 x 10^8/kg. Patients were randomized to receive one of two forms of graft versus host disease (GVHD) prophylaxis, methotrexate alone or methotrexate plus prednisone and anti-thymocyte globulin. Post-transplant complications consisted of 1 episode of interstitial pneumonitis and 6 episodes of sepsis. 2/13 patients died in the first month following BMT. The remaining 11 patients (85%) are surviving with a median follow-up of 11 months. (Range < 1-23 months). Rejection has not occurred in this group of patients. One patient developed acute GVHD. Our experience suggests that the combination of TLI and CPM is an effective conditioning regimen with minimal morbidity for previously transfused patients with severe AA.

036 BONE MARROW TRANSPLANTATION (BMT) FOR ACUTE LEUKEMIA DURING COMPLETE REMISSION, Karl G. Blume and Wayne E. Spruce for the City of Hope Bone Marrow Transplant Team, City of Hope National Medical Center, Duarte, Cal. 91010.

Since December, 1976, 20 patients with acute leukemia underwent BMT from HLA-identical sibling donors (19 allogeneic, 1 syngeneic) while being in complete 1st, 2nd or 3rd remission (CR). Those patients who were transplanted during 1st CR were 1-9 months (mean = 3) after successful induction therapy. Nine patients (ages 11-36 yrs., mean = 24) had acute myelogenous leukemia (AML), 6 of them were in 1st CR, 2 in 2nd CR and 1 in 3rd CR. All AML patients transplanted in 1st CR are alive and in continued remission without further maintenance therapy. Three AML patients died, 1 with leukemic relapse, 1 with CMV pneumonia and 1 with intramyocardial bleeding. Eleven patients (ages 14-48 yrs., mean = 25) had acute lymphoblastic leukemia (ALL), 2 were in 1st CR, 8 were in 2nd CR and 1 in 3rd CR. Eight of the ALL patients are alive but 1 of them has developed leukemic relapse; 3 patients died, 1 with sepsis, 1 with acute graft-vs.-host disease (GVHD) and CMV pneumonia and 1 with leukemic recurrence.

Actuarial 35 month survival for all 20 patients entered on the study is 64%; no patient was excluded from evaluation. Acute GVHD was observed in virtually all patients but responded well to methotrexate and prednisone in all but one patient; antithymocyte globulin was not used. None of the surviving patients has developed chronic GVHD but one is suffering from leukoencephalopathy. It appears noteworthy that all patients who have relapsed had a pre-transplant history of extramedullary leukemia. We conclude that early BMT offers a promising alternative for patients with acute leukemia who are at high risk of relapse if treated with conventional chemotherapy only.

037 BONE MARROW TRANSPLANTATION FOR PEDIATRIC PATIENTS WITH ACUTE LEUKEMIA IN REMISSION, Maura O'Leary, Norma K.C. Ramsay, Mark E. Nesbit, William Woods, Peter Coccia, Taehwan Kim, William Krivit and John H. Kersey, University of Minnesota, Minneapolis, MN 55455

At the University of Minnesota, 17 pediatric patients have received bone marrow transplantation for acute leukemia while in remission. All patients received a cytoreduction regimen of cyclophosphamide 60 mg/kg for two days followed by 750 rads of total body irradiation at 26 rads per minute utilizing a linear accelerator. This was followed by an intravenous infusion of HLA-MLC identical marrow from healthy sibling donors. Following transplantation, all patients received graft versus host disease (GVHD) prophylaxis with methotrexate alone or methotrexate, Minnesota anti-thymocyte globulin and prednisone. Eight patients had acute non-lymphocytic leukemia and ranged in age from 11 to 17. They were all in first remission. 5 of 8 (62.5%) are alive at 3 to 36 months. Three patients are dead: 2 of bacterial sepsis at 2 weeks and 1 month, and one of relapse at 2 years. Life table analyses indicates a 71% survival at one year. Nine patients, ages 4 to 22, had acute lymphoblastic leukemia and were in second or subsequent remissions. 6 of 9 (67%) are alive from 4 to 16 months. Three patients have died: 1 of relapse at 3 months, 1 of interstitial pneumonitis and GVHD at 5 months, and 1 of interstitial pneumonitis at 8 months. One patient is alive after bone marrow relapse. Life table analysis indicates a 60% survival at 1 year with the ALL group. All 10 survivors are at home and completely active. One patient has mild chronic skin GVHD. Early bone marrow transplantation for acute non-lymphocytic leukemia in first remission has provided an acceptable alternative to conventional therapy in this poor prognostic group of patients.

038 BONE MARROW TRANSPLANTATION FOR 229 PATIENTS WITH SEVERE APLASTIC ANEMIA, Mortimer M. Bortin and Alfred A. Rimm for the Advisory Committee of the International Bone Marrow Transplant Registry (Fritz H. Bach, Chairman, Dirk W. van Bekkum, Robert P. Gale, Eliane Gluckman, Robert A. Good, Walter Hitzig, Humphrey E.M. Kay, Jon J. van Rood, George W. Santos, Kenneth W. Sell and Bruno Speck), Mount Sinai Medical Center, Milwaukee, WI 53233. Analyses of data reported to the International Bone Marrow Transplant Registry will be presented regarding 229 patients with severe aplastic anemia who were treated with allogeneic bone marrow transplantation. Emphasis will be on those pretransplant factors which were found to be associated with no engraftment, unsustained engraftment, autologous recovery of hematopoiesis, sustained engraftment, quality of hematopoietic restoration, intensity and type of graft versus host disease, occurrence of interstitial pneumonitis and survival.

039 DEMONSTRATION BY Y-BODY ANALYSIS OF THE BONE MARROW ORIGIN OF OSTEOCLASTS AND CULTURED MACROPHAGES BUT NOT OSTEOBLASTS, Peter F. Coccia, Jaroslov Cervenka, Phyllis I. Warkentin, John H. Kersey, William Krivit, Mark E. Nesbit, Norma K.C. Ramsay, and David M. Brown, University of Minnesota, Minneapolis, MN 55455.

A 5-month old female with severe autosomal recessive osteopetrosis received a bone marrow transplant (BMT) from her 5-year-old HLA-MLC identical brother after preparation with cyclophosphamide and modified total body irradiation. Engraftment was documented by chromosomal analysis of bone marrow and PHA-stimulated peripheral blood cells. Progressive and complete correction of hematologic abnormalities occurred. Pre-BMT, increased numbers of osteoclasts were seen in bone biopsies, but osteoclastic activity could not be demonstrated. Normal osteoclast function post-BMT was demonstrated by progressive bony remodeling, new non-sclerotic bone formation, and histologic evidence of bone resorption and medullary cavities containing abundant marrow elements. Fluorescent Y-body analysis was performed on osteoclasts and osteoblasts obtained by imprinting Jamshidi bone biopsies on glass slides, and on macrophages obtained from liquid culture of peripheral blood and bone marrow. Prior to BMT, Y-bodies were not identified in any cell line. On 5 occasions 2-22 weeks post-BMT, a total of 42 osteoclasts with 248 nuclei were studied; intranuclear fluorescent Y-bodies were present in 116 nuclei of 34 osteoclasts. No Y-bodies were present in 166 osteoblasts studied 2-22 weeks post-BMT. Mononuclear and multinucleated macrophages studied at 6 and 22 weeks post-BMT had Y-bodies in 30% of 140 and 35% of 80 nuclei respectively. These studies provide evidence for the bone marrow stem cell origin of osteoclasts but not of osteoblasts, and further evidence for the bone marrow origin of macrophages.

040 ABO INCOMPATIBLE BONE MARROW TRANSPLANTATION (BMTX) WITHOUT PLASMA EXCHANGE. Steven N. Wolff, Gordon L. Phillips, and Geoffrey P. Herzig, Div. of Hematology-Oncology - Washington Univ. Sch. of Med., St. Louis, MO 63110.

HLA matched, ABO incompatible BMTX is feasible provided acute hemolysis of donor RBC's by recipient isohemagglutinins is prevented. Most successful transplants have been accomplished by multiple recipient plasma exchanges to reduce isohemagglutinin titre. We describe a simple technique of BMTX accomplished by donor marrow RBC depletion to avoid hemolysis without the use of expensive and cumbersome cell-separators. An O+ recipient with relapsing leukemia (anti-A titre of 1:16) was prepared for BMTX by a multi-drug TBI regimen. The donor (A+) marrow was placed in 600ml Fenwall blood bags, centrifuged at 4000 RPM for 10 minutes at room temperature and 80% of the plasma was expressed. The resulting cell suspension was placed in siliconized 16x150mm glass tubes and centrifuged at 2500 RPM for 15 minutes at room temperature. The superficial fat layer was removed and discarded. The buffy coat layer was then carefully aspirated using 18 gauge spinal needles and 10cc syringes, pooled and adjusted to a final volume of 150ml with donor plasma. The final concentrate contained 9.9 ml of RBC's (98% reduction) and $42.2x10^9$ nucleated cells (<10% reduction). The RBC depleted marrow was infused over 15 minutes. Sustained marrow engraftment rapidly occurred with WBC >1000, platelets >40,000 and all marrow cells donor karyotype by day +14 after infusion. Severe GVHD occurred, and the patient expired at day +63 of disseminated CMV and fungal infection. Successful engraftment of ABO incompatible marrow may be accomplished by a simple and quick technique of donor marrow RBC depletion avoiding the complicated process of recipient plasma exchange.

041 USE OF HLA NON-IDENTICAL DONORS IN BONE MARROW TRANSPLANTATION (BMT) FOR SEVERE APLASTIC ANEMIA (AA), Phyllis I. Warkentin, Norma K.C. Ramsay, Peter F. Coccia, Taehwan H. Kim, William Krivit, Mark E. Nesbit, William G. Woods and John H. Kersey, University of Minnesota, Minneapolis, MN 55455

Five pts. ages 2-16 yrs. with AA of 2-5 months duration received bone marrow transplants from HLA 1 or 2 antigen mismatched, related donors. In mixed lymphocyte culture (MLC), 4/5 demonstrated low but significant bidirectional stimulation (range=6-38%); 1 was non-reactive. D-locus typing demonstrated identity in 3/4. Cell mediated lympholysis showed no significant killing in 5/5. Pts. received 2.9-6.9 X 10^8 cells/kg; those with subsequent rejection received the lowest cell dosages: 2.9 and 3.2 X 10^8 cells/kg. All pts. received post-BMT graft vs. host (GVH) prophylaxis with weekly Methotrexate X 100 days, and antithymocyte globulin (ATG) and corticosteroids on days 7-21. Pre-BMT, 1 of 3 preparative regimens was used. Two pts. received Procarbazine 15 mg/kg/day X 3, ATG 15 mg/kg/day X 3, and 750 rads total body irradiation. Both engrafted. One died (day 47) with graft rejection and sepsis. One developed mild acute GVH which resolved with Prednisone; she is well > 31 months post-BMT. Two pts. received cyclophosphamide (CPM) 50 mg/kg/day X 4 and 750 rads single dose total lymphoid irradiation (TLI). Both engrafted; 1 rejected the graft and died of sepsis (day 51); the other died 293 days post-BMT with chronic GVH and infection. A 5th pt. received CPM and TLI as above plus Procarbazine and ATG, each 15 mg/kg/day X 3. Although she died suddenly on day 31 of unexplained electrolyte imbalance, she had chromosomal evidence of engraftment without GVH or rejection. These pts. demonstrate that BMT for AA can be expanded beyond HLA-MLC identical siblings; further studies are needed to determine optimal conditioning regimens.

042 A CANINE MODEL FOR APLASTIC ANEMIA: PRELIMINARY RESULTS OF BONE MARROW TRANSPLANTATION, Katherine A. Stitzel, H. M. Vriesendorp*, and Floyd D. Wilson, Laboratory for Energy-Related Health Research, University of California, Davis, CA 95616

Dogs of 50 or more days of age that are chronically whole body irradiated at an average dose rate of 11 R/day consistently develop an aplastic anemia at a total dose of 700 R or greater. Most animals become severely ill between 900 and 2000 R, but some are able to survive up to 4000 R. All animals first manifest with a severe thrombocytopenia which causes few clinical problems and in a few animals may regress. Animals then present with a rapidly declining hematocrit and finally with a leukopenia. Few animals have shown evidence of severe clotting problems, and none have thus far developed infections from the leukopenia, probably because this is a very terminal event. Two of these animals have been transplanted with bone marrow from DLA, MLC matched siblings and both had evidence of a take. One animal survives today, and the second died from severe septicemia but with a demonstrable take. 750 R of irradiation was used as a conditioning regime. These animals are a model for the study of the mechanisms of induction of anemia and its treatment as well as basic studies on bone marrow stem cells.

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043 B CELL FUNCTION IN HUMAN MARROW TRANSPLANT RECIPIENTS, Olle Ringdén Robert P. Witherspoon, Rainer Storb, Elsie Ekelund and E. Donnall Thomas, Fred Hutchinson Cancer Research Center, Seattle, Wash. 98104.

B cell function was studied in 65 healthy controls and 80 individuals who were recipients of bone marrow transplants for aplastic anemia (n=24) or hematologic malignancies (n=56). Lymphocytes were stimulated in vitro by staph. aureus bacteria (Cowan 1). Antibody secretion was measured as direct plaque forming cells (PFC) against fluorescein isothiocyanate coupled sheep red blood cells (FITC-SRBC) or in an indirect assay using protein A coupled SRBC and antisera against human IgG, IgA and IgM. Lymphocytes from patients studied during the first three months (short-term patients) showed a mean of 12 ± 4 (SE) PFC/10^6 cells against FITC-SRBC which was statistically significantly lower ($p<0.01$) compared to 300 ± 91 in the controls. In patients studied more than a year after transplantation the mean PFC 10^6 against FITC-SRBC was 310 ± 222. In the healthy controls, the direct assay only detected about 4% of the IgM producing B cell found in the indirect assay. Using the protein A SRBC assay the PFC responses for IgG, IgA and IgM after stimulation with staph. aureus were significantly lower in short-term patients compared to normals ($p < 0.001$). Patients with acute GVHD had unusually high numbers of IgG PFC after staph. aureus activation and this stimulation was significantly higher ($p < 0.001$) than in patients without GVHD. Increased IgG secretion was also associated with infections and HLA non-identical donors. More than a year after transplantation, healthy patients have normal B cell responses, but patients with chronic GVHD have deficient IgM stimulation.

Graft-Versus-Host-Disease: Clinical

044 IN VITRO TESTS CORRELATING WITH PRESENCE OR ABSENCE OF GRAFT-VERSUS-HOST DISEASE IN DLA NONIDENTICAL CANINE RADIATION CHIMERAS. K. Atkinson, R. Storb, P.L. Weiden, H. J. Deeg, and E.D. Thomas. Fred Hutchinson Cancer Research Center, Seattle, WA 98104.

Twenty random bred canine littermate pairs were selected for marrow transplantation on the basis of nonidentity at the major canine histocompatibility complex including reactivity in mixed leukocyte culture (MLC). Recipient (host) lymphocytes were cryopreserved before transplantation for subsequent use as stimulator cells in MLC. Recipients were conditioned with 1200 R total body irradiation and given a hemopoietic graft from their respective littermates. All recipients received Methotrexate for 102 days post-transplant in an attempt to ameliorate graft-vs-host disease (GVHD). Ten recipients developed fatal acute GVHD and blood mononuclear cells (of donor origin) from all 10 animals retained their reactivity in MLC to preserved host lymphocytes. Six dogs developed delayed fatal GVHD although five had a long period of clinical good health prior to its onset. During this period of good health, chimera cell reactivity in MLC to host lymphocytes was markedly diminished, but strong reactivity was detectable with the onset of delayed GVHD. Chimera cells reactive to host antigens were thus detectable in all chimeras during acute and delayed GVHD. Four dogs did not develop GVHD and survived 142 days, >485, >577 and >1005 days post-transplant. Cells from all 4 had virtually absent reactivity to host lymphocytes while retaining good reactivity to third party lymphocytes. Chimera lymphocytes cytotoxic to host cells could not be detected in cell-mediated lympholysis. The relative MLC unresponsiveness of these healthy long-term chimeras was not mediated by circulating suppressor cells nor by serum blocking factors. The mechanism of complete operational tolerance in these chimeras is compatible with the concept of clonal abortion.

045 GvHD SCORING SYSTEM TO PREDICT SURVIVAL AND SPECIFIC MORTALITY IN BMT RECIPIENTS Chaim Hershko and Robert P. Gale, UCLA Marrow Transplant Unit and Department of Hematology, Hadassah University Hospital, Jerusalem.

Evaluation of GvHD in BMT recipients is complicated by the lack of specific tests for early diagnosis and objective criteria to define severity. In 115 patients transplanted for aplastic anemia (AA) and resistant acute leukemia (AL), the following variables correlated best with survival and with specific (GvH or non-GvH) mortality; Bilirubin>5mg/dl, diarrhea> 1000 ml/d, serum albumin<2.5 gm/dl and skin rash duration>1 week. The outcome of BMT in patients with 0 to 4 of the above findings at any time during their post transplant hospital course was as follows:

Score	0	1	2	3	4
(n)	(39)	(30)	(26)	(10)	(10)
Surviving (%)	36	30	8	10	0
GvH death (%)	5	10	38	60	100
Non-GvH death (%)	59	60	54	30	0

In patients with AA the chance of fatal GvHD was 25% in grade 1-2 and 89% in grade 3-4. The corresponding figures in AL were 11% and 83% respectively. This scoring system is based on simple quantitative clinical and laboratory parameters allowing re-evaluation on a daily basis without resorting to tissue biopsies. It allows the prediction of survival and specific mortality with a reasonable degree of accuracy ($P<0.00001$). It provides an objective basis for comparison of results obtained at different transplantation centers, and for the evaluation of new methods designed to modify the severity and outcome of GvHD in BMT recipients.

046 TREATMENT AND EARLY DETECTION OF CHRONIC GRAFT-VS-HOST DISEASE (C-GVHD). K. Sullivan, H. Shulman, P. Weiden, R. Storb, M.S. Tsoi, and E.D. Thomas. Fred Hutchinson Cancer Research Center, Seattle, WA 98104.

C-GVHD is a major complication of allogeneic marrow transplantation. Of 11 untreated patients (pts) with extensive C-GVHD, 5 died and only 2 (18%) survive with Karnofsky scores ≥70%. Treatment with ATG (7 pts) or steroids (20 pts) showed little benefit. Of 21 pts treated with combinations of steroids and cytotoxic agents, 16 (76%) are currently alive without disability >2 years post-grafting. Such results encouraged attempts to detect and treat C-GVHD before clinical deterioration. Over the past 3 years we have screened pts at day 100 for evidence of disease with skin physical exam (PE) and biopsies for light microscopy (LM) and direct immunofluorescence (DIF), oral biopsy, liver function tests (LFTs), and tear tests (TT) for ocular sicca. 70 pts have been followed >1 year:

Subsequent course	Skin PE		Skin LM		Skin DIF		TT		LFTs		Oral LM	
	Abn	Nl	Abn	Nl	Abn	Nl	Abn	Nl	Abn	Nl	Abn	Nl
C-GVHD (40)	15	25	25	15	24	7	21	10	22	18	19	1
No C-GVHD (30)	5	25	2	28	8	19	1	13	5	25	12	3

Thus, >90% of pts with either abnormal TT or skin LM ultimately developed C-GVHD. Of note, 14 pts with rash and positive LM at day 100 all developed C-GVHD; while of 13 pts with no rash at day 100 but positive LM, 11 subsequently displayed disease. While skin PE, DIF, and LFTs may have some predictive screening value, oral LM by current criteria does not. Conversely, 73% of pts with a normal skin DIF remained clinically well after 1 year follow-up.

047 HOST DETERMINED FUNCTION, REFLECTED BY NATURAL KILLER CELL ACTIVITY NK(HSV-1), CORRELATES WITH GVHD AFTER BONE MARROW TRANSPLANT. Dahlia Kirkpatrick, Clara Ching, Richard J. O'Reilly, Carlos Lopez; Memorial Sloan-Kettering Cancer Center, New York, N.Y.10021

Natural killer cell activity against herpes simplex infected targets, NK (HSV-1) was studied prospectively in 22 patients undergoing bone marrow or fetal tissue transplant at the Memorial Sloan-Kettering Marrow Transplant Unit to see if any correlations existed between NK function and the subsequent development of graft versus host disease (GVHD) following transplant. Three patients with severe combined immunodeficiency (SCID), five with Aplastic Anemia, two with osteopetrosis, two with Wiskott-Aldrich Syndrome, and ten with acute leukemia showing sustained evidence of engraftment were included in the group studied. Ten out of ten patients having normal NK function (± 2S.D.) developed GVHD, and the twelve remaining patients with low NK (greater than 2 S.D. below mean) failed to develop GVHD. These results were independent of the mode of preparation for transplant or the source stem cells transplanted. In five patients studied serially post transplant, GVHD was diagnosed at the time when NK function was returning to normal. This evidence would suggest that host factors independent of the mode of preparation for transplant are important in triggering the GVH response following transplant.

048 INDUCTION OF FUNCTIONAL T CELL DEFICIENCY BY GVH ASSOCIATED THYMIC INJURY. Wayne S. Lapp, John G. Gartner, Mona Seddik and Thomas A. Seemayer, McGill University, Montreal, Quebec, Canada H3G 1Y6

The graft-versus-host reaction induces a permanent state of suppression of both cell-mediated and humoral immune responses. We have shown that early suppression is mediated by macrophages and various suppressor cells. The precise mechanism(s) responsible for the permanent immunosuppression has not been determined. Earlier studies suggested that a deficiency of some thymic component was responsible, at least in part, for the suppression. Our more recent light and electron microscopic studies have demonstrated consistent morphological changes in the thymus as a consequence of a graft-versus-host reaction. The cellular alterations consist of an effacement of the normal cortico-medullary demarcation, a progressive disappearance of Hassall's corpuscles and injury to medullary epithelial cells. These changes occur in adrenalectomized animals and thus are not simply manifestations of stress. We therefore tested the ability of thymocytes obtained from thymuses demonstrating epithelial cell injury during graft-versus-host reaction to reconstitute T cell function in T cell deprived mice (adult, adrenalectomized, thymectomized, irradiated, bone marrow reconstituted). The thymocytes were theta positive but failed to reconstitute allograft reactivity and T cell mitogen responses; in contrast thymocytes from normal mice restored these functions. Therefore, immunosuppression which is often associated with the graft-versus-host reaction in man and mouse could be due, in part, to an arrest of T cell maturation as a consequence of thymic medullary epithelial cell injury.

049 CORRELATION OF LABIAL SALIVA [Na] WITH SIALADENITIS IN BONE MARROW TRANSPLANT RECIPIENTS, S. Oberg, K. Izutsu, H. Shulman, M. Schubert, K. Sullivan, T. Morton, G. Sale and E. Truelove. University of Washington & Fred Hutchinson Cancer Res. Ctr. Seattle, Washington.

We recently found that the average whole saliva sodium concentration ([Na]) of patients with GVHD was six fold higher than transplant recipients without GVHD (Izutsu et. al., J. Dent. Res. in press). As a result, we wondered if a similar [Na] change also occurs in minor gland saliva and whether such a change correlates with certain labial gland lesions often observed in these patients. Labial saliva was collected from the upper lip and eluted from collection strips by shaking for 1 hr in 15mM $LiCl_2$. [Na] was determined by flame photometry. The mean [Na]'s were not significantly different in either GVHD (42.9±7.3, n=12) or asymptomatic patients (35.7±8.7, n=7), whereas both patient groups had significantly higher [Na]'s than the nontransplant control group (15.1±1.6, n=9). Also there was a correlation between elevated [Na]'s and minor gland sialadenitis: labial biopsies of all allogeneic marrow recipients (both with GVHD and asymptomatic) with [Na]'s $\geq$ 29mM had sialadenitis and two patients (both with GVHD) who did not have any sign of sialadenitis had relatively normal [Na]'s (i.e., [Na]'s of 16.5 and 19.3mM, respectively). These results show (1) that minor gland [Na]'s are a useful, albeit nonspecific, indicator of minor gland sialadenitis, (2) that this simple assay may provide a useful diagnostic parameter to be considered in patients refusing labial biopsies, since unexplained elevated [Na]'s may be a harbinger of chronic GVHD.

Supported by NIH grants DE 02600, CA 15704 and CA 18029.

050 DEPRESSED SALIVARY IGA; AND SODIUM AND FLOW CHANGES IN C-GVHD PATIENTS, K. Izutsu, S. Oberg, H. Shulman, M. Schubert, K. Sullivan, T. Morton, G. Sale, and E. Truelove. University of Washington, Seattle, Washington 98195 (NIH grants DE 02600, CA 15704 CA 18029)

Stomatitis and xerostomia were previously reported in bone marrow transplant (BMT) patients who developed chronic GVHD(C-GVHD) [Gratwhol et al, Ann. Int. Med. 87:703, 1977] and we previously reported salivary electrolyte and flow changes in these patients that were similar to changes observed in patients with Sjogren's Syndrome [Izutsu et al, J. Dent. Res., abstract in press]. We now report sodium and flow data from additional patients and we report apparently depressed salivary IgA levels in patients with C-GVHD. Timed whole saliva samples were collected while chewing Parafilm. Na was measured by flame photometry and IgA levels by radial immunodiffusion (Kallestad).

n	*group*	*Na (mM/l)*	*P*(Na)*	*F.R. (ml/min)*	*P*(F.R.)*	*IgA***
16	C-GVHD BMT patients	45.8 ± 9.8	.01	.48 ± .10	.001	10
13	asymptomatic BMT patients	17.2 ± 3.0	.01	.74 ± .13	.02	0
8	normal controls	12.9 ± 2.0	---	1.38 ± .20	---	0

*P=level of significance compared with normal group (2 tailed t-test),**number with undetectable IgA levels.

10/16 patients with C-GVHD were found to have undetectable salivary levels of IgA (i.e. < .7 mg/dl), while every patient in the two control groups had detectable IgA levels. These population ratios were different at better than the .05 level by the Chi-square test. Since IgA is thought to be the principle antimicrobial protein of the mucosal surfaces, the results might explain the high incidence of respiratory infections in C-GVHD patients.

Graft-Versus-Host-Disease: Experimental

051 CYCLOSPORIN A (CyA) IN MARROW TRANSPLANTATION IN DOGS. H.J. Deeg, R. Storb, P.L. Weiden, E.D. Thomas. Fred Hutchinson Cancer Research Center, Seattle, WA 98104.

Dogs were prepared for transplantation by 1200 R total body irradiation. All 76 dogs given marrow from DLA nonidentical unrelated donors and no immunosuppression post-grafting died with graft-versus-host disease (GVHD) with a median survival of 11 days and only 2 dogs living longer than 25 days. Those given post-grafting methotrexate (MTX) (46 dogs) had a median survival of 25 days and 15% became long-term survivors. Six dogs were given CyA, 25 mg/kg/d, on days 5-25 and 10 dogs on days 11-25 in addition to MTX; their median survivals were 13.5 and 17.5 days respectively. Problems appeared to be related to drug toxicity: dogs died with intussusception (5), infection (5), GVHD (3) and other causes (3). Six dogs were given CyA, 20-25 mg/kg/d, on days 0-25 and no MTX. Three are alive, without GVHD, on days 14,21, and 84; two died with rejection (d.16,17), one with extensive GVHD of sudden onset (d.40). - Dogs given DLA identical littermate grafts and no immunosuppression (67 dogs) had a median survival of 134 days and 48% became long-term survivors. When MTX was added post-grafting 16 of 17 dogs became long-term survivors. Twelve DLA-identical littermates were given CyA, 25 mg/kg/d (instead of MTX) on days 0-25 or 0-100. Eight are alive, 40 to 193 (median 110) days after grafting and four died. Deaths were due to intussusception (d.9), pulmonary hemorrhage (d.14), unknown causes (d.74), wasting and liver GVHD (d.88). - Although these results are still preliminary, we conclude: 1) CyA added to MTX is toxic (intestinal tract); 2) CyA can prevent acute GVHD in littermate and unrelated transplants; 3) In DLA identical littermate transplants it may be less effective than MTX in inducing stable graft-host tolerance.

052 EXPONENTIAL RELATIONSHIP BETWEEN SPLEEN CELLS AND LETHAL GvH RESPONSE DEVELOPMENT AFTER TRANSPLANTATION OF SPLEEN/MARROW CELL MIXTURES, James P. OKunewick and Ruby F. Meredith, Cancer Research Unit, Allegheny General Hospital, Pittsburgh, PA 15212

SJL/J mice when transplanted with marrow cells from C57BL/10J mice normally show only a moderate GvH response, with less than 35% fatalities. However, when C57BL/10J spleen cells are used instead of marrow GvH response is severe, with 100% fatalities within 11 days. In an attempt to achieve controlled and predictable levels of GvH disease studies were conducted in which small, measured, percentages of C57BL/10J spleen cells were added to marrow cells engrafted into irradiated SJL/J recipients. It was found that predictable levels of fatal GvHD could be achieved, ranging from 30% to 100%. Also, both the incidence of and the median time for fatal GvHD were found to be *exponentially* related to the amount of spleen cells in the donor cell preparation. Kinetic analysis of the data has indicated that doubling the spleen cells in the preparation decreases the median survival time by 9 days and reduces the number of survivors by 17%. More importantly, there was also an apparent *time-limit* for the development of fatal GvHD of approximately 55 days. Of a total of 215 mice given all concentrations of spleen cells used in these studies 163 suffered fatal GvHD. 94.5% of these died before day 55, while only 5.5% died between day 56 and 120 after transplant. A total of 24% of all the animals survived more than 4 months after transplantation. The data suggests that there may be a component among spleen cells capable of programming another cell found in the marrow that may become the actual effector-cell for GvHD, and which has a population doubling-time of 9 days, but a limited survival time of 55 days, after which fatal GvHD is not likely to occur. (Supported by NIH-NCI Grant 1 ROI Ca22512.)

053 EVIDENCE FOR A ROLE OF CIRCULATING LYMPHOID CELLS IN THE GENERATION OF GRAFT VERSUS HOST DISEASE BY ALLOGENEIC BONE MARROW. Rudolph Almaraz, David H. Sachs, and Steven A. Rosenberg. NIH, Bethesda, MD. 20205.

Studies were undertaken in mice to evaluate the factors causing lethal graft versus host disease (GVHD) in whole body irradiated (WBI) allogeneic bone marrow chimeras. C57BL/6 mice (6-8 wk) underwent lethal WBI (750R) and immediate reconstitution with 3.0×10^7 BALB/c bone marrow cells i.v. Marrow donors were lightly anesthetized with ether and were exsanguinated by laceration of both jugular veins. Twenty four C57BL/6 mice undergoing lethal WBI followed by reconstitution with these donor cells showed a 92% survival at 26 wks. In contrast, twenty three C57BL/6 mice undergoing the same irradiation and reconstitution but with bone marrow cells from unbled BALB/c donors showed only a 23% survival at 26 wks ($p<.01$). In a second experiment survival of mice reconstituted with marrow from bled donors also exceeded that from mice reconstituted with marrow from unbled donors ($p<.05$), while in two other experiments no significant difference in survival was seen. Further studies were undertaken to define the cells responsible for the increased incidence of lethal GVHD in those animals reconstituted with cells from unbled donors. Addition of 3.0×10^4 to 10^6 BALB/c peripheral blood lymphocytes to 3.0×10^7 bone marrow cells from exsanguinated donors caused a significant decrease in survival of the chimeras in three of three experiments ($p<.05$). Thus, contamination of allogeneic marrow with circulating allogeneic lymphocytes may be responsible for lethal GVHD. Careful exsanguination of donors may therefore have a significant impact on the survival of mice undergoing allogeneic reconstitution after lethal WBI.

054 LYMPHOID CELL-TARGET REPLACEMENT AND REFRACTORINESS TO FATAL GRAFT-VERSUS-HOST DISEASE (GVHD), David Steinmuller and Jane Shelby, Mayo Clinic, Rochester, MN 55901

Stable, H-2^k haploidentical mouse radiation chimeras, with no gross sign of GVHD, are produced routinely in our laboratory by the iv inoculation of lethally irradiated C3H/HeJ hosts with 50×10^6 normal CBA/J spleen cells, but the inoculation of even fewer CBA anti-C3H immune spleen cells invariably is fatal. In contrast, the inoculation of CBA anti-C3H spleen cells rarely is fatal in established CBA-to-C3H chimeras; i.e., the hosts are refractory to GVHD. However, less than 50% of C3H hosts reconstituted with (C3H x CBA)F_1, as opposed to CBA, spleen cells survive subsequent inoculation of CBA anti-C3H spleen cells, showing the importance of hemopoietically-derived targets in systemic GVH reactions. This also is demonstrated by the finding that fatal GVHD frequently results from the inoculation of C3H anti-CBA immune spleen cells into established CBA-to-C3H chimeras. In this situation the GVH-effector cells are syngeneic to the hosts, and hemopoietically-derived cells (i.e., passenger leukocytes) are the only possible alloantigenic targets. The prevalent explanations for the refractoriness to GVHD that develops in bone marrow chimeras include the induction of suppressor T cells of donor origin, or of anti-idiotype responses to donor T cell receptors. Our experiments suggest the simple replacement of host lymphoid-cell targets by donor lymphoid cells should be considered as an additional, non-mutually exclusive explanation for the refractoriness to the induction of GVHD that often develops in hosts that survive initial systemic GVHD.

055 NON-SPECIFIC ACTIVATION OF GvH REACTIVE CELLS BY IN VITRO INCUBATION. Hans W. Grünwald and Robert Likoff, Queens Hospital Center Affiliation of Long Island Jewish-Hillside Medical Center, Jamaica, N.Y. 11432 and the State University of New York School of Medicine at Stony Brook.

We have investigated methods of modifying the Graft-vs-Host Reaction (GvHR) by in vitro manipulation of cells employed to reconstitute lethally irradiated mice. Two-month old C3D2F1/J hybrids given $4x10^6$ parental (C3H or DBA2) cells, intravenously, after lethal irradiation with 950r are usually fully protected and survive indefinitely. If, however, one adds spleen cells (as low as $1x10^5$ for C3H cells or $5x10^5$ or more for DBA2 cells), the reconstituted F1 hybrids usually develop a GvHR, leading to death in 25 to 70 days. When the spleen cells in the reconstituting cell mixture are preincubated for 24 to 48 hours in McCoys' 5A tissue culture medium, the animals develop an accelerated and exceedingly intense reaction and are usually dead by the 12th day after irradiation, most often even prior to the death of unreconstituted lethally irradiated controls. Cytologic examination of the incubated spleen cells reveals many clumps of transformed lymphoblasts in the midst of isolated small lymphocytes, the latter occasionally showing signs of nuclear pyknosis. We believe the above described findings represent non-specific stimulation and activation of GvH reactive cells by the in vitro incubation. These observations may provide clues to develop a simple system to eliminate or modify the GvHR in bone marrow transplant recipients, if lack of alteration of the hemopoietic repopulating cells by these incubations can be demonstrated.

056 HUMORAL AND CELLULAR PHAGOCYTIC DEFENSES DURING GRAFT VERSUS HOST DISEASE, Elena R. Reece, Wayne S. Lapp, Montreal Children's Hospital, McGill University, Montreal, Quebec H3H-1P3. Studies during human graft versus host disease (GVHD) post marrow transplantation demonstrate in vitro depressions of neutrophil and monocyte chemotaxis, as well as neutrophil bacterial ingestion and killing. Human GVHD secondary to maternal cell engraftment was also found to be associated with profound depressions of yeast opsonization and alternative pathway hemolytic complement, both of which improved with plasma infusions. Humoral and cellular phagocytic defenses were further studied using the murine F1 hybrid model of GVHD. Bone marrow phagocytic cell random migration was depressed and became undetectable by day 10 of chronic GVHD. Chemotactic responses to endotoxin activated mouse serum were abnormal from day 10 onward. Opsonins, including immunoglobulins, hemolytic complement, immunoreactive C5 and the ability to opsonize yeast for phagocytosis were elevated or unchanged during acute GVHD. However, in chronic GVHD, there was a progressive decline in total immunoglobulins and C5 after days 10-14. Yeast opsonization was profoundly depressed in some animals from day 17. This did not correlate with classical hemolytic complement. Thus, abnormalities in humoral and cellular phagocytic mechanisms occur in human and murine GVHD. In the murine model, the abnormalities correlate temporally with the onset of T-cell and thymic morphologic and humoral dysfunction, as well as B-cell dysfunction.

057 LONG TERM SURVIVAL OF MURINE ALLOGENEIC BONE MARROW CHIMERAS: EFFECT OF ALS AND BONE MARROW DOSE. Allen J. Norin, Eugene E. Emeson and Frank J. Veith. Montefiore Hospital Albert Einstein College of Medicine, New York, N.Y. 10467.

Failure of donor stem cells to engraft permanently and graft versus host disease (GVHD) are two major obstacles to successful bone marrow (BM) transplantation in man. In this study we established several conditions for obtaining permanent engraftment of histoincompatible BM in the mouse without GVHD. Our criteria for engraftment is that >90% of the blood lymphocytes have the H-2 type of the donor (complete chimeras) at 100 days after receiving supralethal total body irradiation (TBI). Only 30% of A/J ($H\text{-}2^a$) mice given antilymphocyte serum (ALS) before TBI and receiving $2x10^6$ C57BL/6J (B_6, $H\text{-}2^b$) BM cells were complete chimeras at 100 days. However, >90% of ALS-TBI treated A/J mice receiving $>6x10^6$ BM cells ($B_6 \rightarrow A/J$) were complete chimeras. The in vivo ALS treatment was highly effective in preventing GVHD. Between 80% to 100% of ALS-TBI treated mice given $2x10^6$ or $1x10^7$ H-2 different BM cells survived in good health for >150 days. Over 95% of the mice receiving TBI and $1x10^7$ allogeneic BM cells without ALS succumbed to lethal GVHD in 24 to 50 days. The survival of TBI treated mice receiving $2x10^6$ BM was 35% to 60%. Spleen and BM cells from $B_6 \rightarrow A/J$ chimeras were not effective in producing acute GVHD in lethally irradiated A/J mice but they were effective in causing lethal GVHD in irradiated CBA ($H\text{-}2^k$) mice suggesting that the non-reactivity is H-2 specific. In view of the concept of H-2 restriction in the killing of virus infected cells it is interesting that many chimeras survive in good health over 275 days after ALS-TBI. Supported by USPHS grant HL17417 and the Manning Foundation.

058 GRAFT VERSUS HOST DISEASE (GVHD) IMPAIRS NEONATAL RAT BRAIN DEVELOPMENT, W.S.T. Griffin, J.R. Head and M. Morrison. University of Texas Health Science Center at Dallas, TX 75235

The grafting of mature lymphocytes into allogeneic neonatal rats causes a variety of systemic symptoms collectively known as GVHD. This disease is dependent on the immunoincompetent status of the host and can be induced experimentally, as here, or naturally, by maternal-fetal transfer, or iatrogenically, by clinical use of fetal or neonatal transfusions and bone marrow transplants. Even though much of the grafting occurs during postnatal neuronal cell proliferation, migration and maturation, nothing has been reported previous to our studies on the effect of GVHD on brain development. We have found that in the developing rat cerebellum DNA content, the cell number of specific neuronal populations, and RNA content, synthesis and translational capacity are all decreased as a consequence of systemic GVHD. In addition, protein content and the amount of specific proteins produced are altered. Differentiation as assessed by Purkinje cell dendritic growth is modified significantly with regard to dendritic branching patterns and orientation within the molecular layer. Immunotherapy by injection of specific alloantisera into animals with induced GVHD results in a return to near normal values in spleen, thymus, cerebellum and body weights. In addition, the cerebellar RNA, DNA and protein contents as well as the ability to incorporate ^{3}H-thymidine into the DNA fraction is returned to near control levels at postnatal day 14, after 7 days of serum immunotherapy. We conclude that the effects of GVHD on neuronal cell mitosis, migration and differentiation in the developing cerebellum can be ameliorated by passive transfer of specific antisera.

Supported by NIH AI 14663, USPHS CA 21602 and the Leland Fikes Foundation, Inc.

059 HOST VERSUS GRAFT (HVG) DISEASE - ANOTHER ALLOGENIC KILLER, Richard C. Hard, Jr., Medical College of Virginia, Richmond, Va. 23298

Fatal HVG disease has so far been observed in 6 inbred strains of mice perinatally inoculated with related F_1 spleen cells. Manifestations of the full-blown syndrome include thrombocytopenia, hyperfibrinogenemia, intestinal hemorrhage, hepatic infarcts, hyperglobulinemia, membranous glomerulonephropathy, and lymphosplenomegaly with thymic atrophy. Deaths in acute HVG syndrome are due to the rapid formation of immune complexes, which cause the severe glomerular disease and trigger disseminated intravascular coagulopathy.

The central lesion seems to be destruction of all peripheral T-cell subsets studied so far. In the B-cell system we see the precocious development of germinal centers and plasma cells, accompanied by the early appearance of IgA and IgM. Later, marked plasmacytosis is accompanied by hyperimmunoglobulinemia.

Prevention of the disease by suppression of host immunocompetence suggests a HVG rather than a F_1-versus-parent reaction is the cause. Thus far, there has been no obvious correlation between susceptibility and H-2 alleles.

060 INHIBITION OF GRAFT VERSUS HOST REACTION BY SULFATO-trans-(-)-1,2 DIAMINOCYCLOHEXANE-PLATINUM(II), Amanullah Khan, Susan Hogan, J.M. Hill and Cindy Kiker, Wadley Institutes of Molecular Medicine, Dallas, TX 75235.

Platinum Coordination complexes, a new class of antitumor agents, have been shown to be immunosuppressive. Sulfato-trans(-)-1,2 diaminocyclohexane platinum(II) (neo-SHP) is one of the newest analogs in this series. neo-SHP has been shown to be immunosuppressive for both humoral and cellular immunity (Cancer Research 39:3476-3478, 1979). The present study extends and confirms the initial results suggesting inhibition of graft-versus-host reaction (GVHR) by this drug. GVHR was produced in 3 week-old AKD_2F1 hybrid mice by injecting 2 x 10^7 AKR/J spleen cells intraperitoneally (i.p.). The spleens were removed on day 8 and splenic indices were recorded as a measure of GVHR. neo-SHP in doses of 7.5, 5, 2.5, and 1 mg/kg was given i.p. on day 0. A group of animals also received 1 mg/kg neo-SHP given daily for 5 days starting with day 0. The control group had a mean splenic index of 2. The animals receiving neo-SHP 7.5, 5, 2.5, 1 mg/kg and 1 mg/kg x 5 gave mean indices of 0.9, 1.2, 1.4, 1.4, and 0.5 respectively. These results represent means from 2 different experiments and confirm the initial results that neo-SHP is a potent inhibitor of GVHR. Work is in progress to see if neo-SHP will inhibit established GVHR.

This work was supported by the Hillcrest Foundation

061 ALLOGENEIC BONE MARROW AND SPLEEN CELL GRAFTING IN GNOTOBIOTIC MICE, W. Heit, H. Heit, H. Rodt, F. Porzsolt, B. Kubanek, University Ulm and Inst. of Hematology, G.S.F., Munich

The presentation will contribute to the following aspects of b. marrow grafting in allogeneic recipients (C57BL into CBA, 800 rad, WBI): 1. The role of the gnotobiotic state for long term surviving of allochimeras (10^7 BM-cells): no lethal GvHD, survival of >80 %/10 weeks of gnotobiotic recipients. 2. The pattern of recovery of lympho-hemopoietic functions. 3. The transplantation induced selective tolerance of chimeric spleen and bone marrow cells towards histocompatibility antigens of CBA mouse origin. 4. The reactivity of spleen cells of germfree mice in the MLC and CML in vitro: impaired stimulatory activity of germfree mouse derived lymphocytes as an indication of differences in the GvHD-induction by conventional and germfree allogeneic recipients. 5. The influence of ATG on the course of the acute GvHD in germfree allorecipients: no abrogation but a significant delay of lethal GvHD.
In view of these observations possible mechanisms involved in GvHD will be discussed.

062 BONE MARROW ANTIGENS, TRANSPLANTATION, AND GRAFT VERSUS LEUKEMIA EFFECT IN EXPERIMENTAL AML, R. Michael Williams and D.E. Singer, Northwestern University, Chicago, Illinois 60611

Transplantation (T) of $<10^3$BN Myelocytic Leukemia(BNML) cells is fatal to BN and Lewis X BN F1 hybrids, but Lewis (L) rats are resistant to $>10^8$cells. Leukemic rats show clinical features of AML in man. Our purpose was to determine whether there were bone marrow (BM) alloantigens shared by BNML which could be exploited for graft versus leukemia (GVL) effects after BMT. Growth of BN BM in lethally irradiated recipients was quantified by %uptake of 125IUdR(I). Only BN and BNDAF1 rats "accepted" the BN BM. When normal animals were injected with 10^5 BNML only L rats survived but LBNF1 and WFBNF1 rats lived significantly longer (MSTs>40 days) than BNDAF1 or BN (MSTs<30). Thus, F1 hybrid resistance to BNML parallels resistance to BMT. The effect of preimmunization on day -7 with 10^7 BM on survival of LBNF1 rats (n=10)given BNML was specific for BN BM (II). Immunization with BN lymph node cells was ineffective (MST=26.9±2.4). Finally, LBNF1 animals were irradiated (1000rad) and reconstituted with BN,L, or LBNF1 BM. The lymphohematopoietic system of the recipients was verified to be of donor origin. The animals were healthy, had no clinical GVH, and were shown to be unresponsive to L,BN, or LBNF1 in MLC. After 10^5BNML,L→F1 chimeras lived 30.5 days compared to 25.5 for BN→F1 and 26.5 for F1→F1. Matching donor and recipient at the MHC and mismatching at the BM alloantigen loci might confer enhanced GVL effects without increasing GVH.

I.BM	%uptake
BN	.40±.01
BNDAF1	.36±.01
WFBNF1	.03±.02
LBNF1	.03±.01
Lewis	.01±.01

II.BM	MST(SD)	P
none	27.5(3.0)	-
LBNF1	28.6(2.7)	>.1
Lewis	29.0(5.8)	>.1
BN	36.5(3.5)	<.001

063 INDUCTION OF ANTILEUKEMIC REACTIVITY WITHOUT AUGMENTATION OF GVH REACTIVITY FOLLOWING ALLOIMMUNIZATION OF MHC COMPATIBLE MICE, R.L. Truitt, M.M. Bortin, A.A. Rimm and C-Y. Shih, Winter Research Laboratory, Mt. Sinai Medical Center, Milwaukee, WI, 53233.

One approach to resolve the problem of recurrent leukemia following supralethal chemoradiotherapy and allogeneic bone marrow transplantation is the use of marrow with antileukemic reactivity to obtain a graft-vs-leukemia (GVL) effect. GVL reactivity of major histocompatibility complex (MHC) compatible cells in man and animals is weak or absent and efforts to induce GVL reactivity in experimental animals often resulted in augmentation of graft-vs-host (GVH) disease. We found that alloimmunization of MHC compatible mice induced a population of cells which, when transplanted into leukemic AKR mice, led to destruction of disseminated leukemia cells. Furthermore, there was no augmentation in the mild GVH disease observed when cells from unimmunized donors were used. Alloimmunization could be accomplished with a single skin graft or injections of normal allogeneic lymphoid cells. Although individual MHC compatible and incompatible strains varied in their ability to induce GVL reactivity, pooled allogeneic cells were very effective. No GVL effect was observed if cells from alloimmunized mice were treated with antitheta serum plus complement. Chemoradiotherapy plus adoptive immunotherapy using cells from alloimmunized, MHC compatible CBA donors was evaluated in AKR mice bearing advanced spontaneous T cell leukemia. Only 7 of 35 leukemic AKR mice (20%) given chemoradiotherapy plus unimmunized CBA bone marrow and lymph node cells survived 90 days post-transplant; whereas, 19 of 26 mice (73%) given cells from alloimmunized CBA mice were alive on day 90.

064 SIMULTANEOUS INDUCTION AND EXPRESSION OF ANTIGEN DEPENDENT (ADCR) AND ANTIGEN INDEPENDENT (AICR) CYCLOPHOSPHAMIDE RESISTANT SUPPRESSOR CELLS. Stan Wilbur and Benjamin Bonavida, University of California, UCLA School of Medicine, Los Angeles, CA 90024
Mice alloimmunized and given cyclophosphamide (Cy) (100 mg/kg) 6-7 days following sensitization have in their spleen and lymph nodes suppressor cells (ADCR) which can inhibit the induction of cell mediated immune responses both in vivo and in vitro. Furthermore, they also can suppress mitogenic responses. The suppressors of cell mediated lympholysis are T dependent, irradiation resistant, antigen nonspecific and H-2 unrestricted. Spleen cells from mice given Cy alone (AICR) could not suppress CML, but suppressed mixed lymphocyte and mitogenic responses. The AICR cells were adherent and probably macrophages. Comparison of both the ADCR and the AICR suppressor systems revealed the following differences: (1) The kinetics of induction are overlapping yet different. (2) A suppressor factor has been identified in the ADCR, but not in the AICR suppressor system. These results suggest that in the ADCR system, two types of suppressor cells develop which can act simultaneously but independently. In vivo, the ADCR suppressor cells express functional activity. Normal mice given ADCR suppressor cells showed no generation of cytotoxic cells after priming with allogeneic tumor. Allogeneic skin graft rejection was also depressed in animals which received suppressor cells prior to grafting. Preliminary experiments have shown that suppressor cells can inhibit graft-versus-host reactions. Because of the lack of both H-2 restriction and antigen specificity of the suppressor cells, they could be useful in alleviating GvH reactions without eliminating immunocompent or hematopoietic stem cells. Supported by NIH Grant CA12800.

065 GRAFT VS. HOST ASSOCIATED SUPPRESSION OF CELL MEDIATED LYMPHOLYSIS TO TRINITROPHENYL-SELF: GENETIC AND MECHANISTIC PARAMETERS. Gene M. Shearer, Richard P. Polisson and Matthew W. Miller. Immunology Branch, National Cancer Institute, Bethesda, MD 20205. F_1 hybrid mice injected with parental spleen cells exhibited severely depressed T-cell mediated lympholysis potential to syngeneic cells conjugated with the trinitrophenyl hapten (TNP-self). In (bxa)F_1, (bxk)F_1, and (bxd)F_1 mice, depressed responses were observed only when non-$\underline{H\text{-}2}^b$ parental spleen cells were injected. Further studies indicated that the failure of $\underline{H\text{-}2}^b$ parental spleen cells to induce depressed immunity was due to radiosensitive F_1 anti-parent resistance mechanism. Genetic studies indicated that the $\underline{H\text{-}2}^b$ parental determinant recognized by the F_1 in resistance to parental induced suppression mapped to H-2D. Co-culture experiments involving mixtures of spleen cells from normal and parental injected F_1 mice indicated that the phenomenon was due to suppression. Analysis of the suppressor mechanism indicated that a major portion of suppression could be accounted for by cytotoxic activity of parental cells against the alloantigens expressed by the F_1, although an additional suppressive effect by F_1 cells is possible.

066 IMMUNOGENETIC AND OTHER FACTORS AFFECTING BONE MARROW TRANSPLANTATION IN IRRADIATED MICE. Delta E. Uphoff, National Cancer Institute, Bethesda, MD 20205.
My previous research involved the immunogenetic factors involved in establishing viable permanent chimerism in irradiated mice. My recent work has involved almost exclusively the effect of the quality of the irradiation as it effects survival and bone marrow transplantation. This conference will review and update the progress in resolving the problems encountered in both clinical and experimental bone marrow transplantation. The information will be valuable in extending my present research program into other areas with greater clinical application.

067 ROLE OF SUPPRESSOR CELLS IN RECOVERY FROM EXPERIMENTAL ALLERGIC ENCEPHALOMYELITIS, R.H. Swanborg, A.M. Welch, and J.H. Holda, Wayne State University, Detroit, MI 48201.
Lewis rats are very susceptible to experimental allergic encephalomyelitis (EAE), a T cell-mediated autoimmune disease. Ten-to-twelve days after challenge with myelin basic protein in Freund's complete adjuvant, these rats develop severe paralysis. Most animals recover by day 18 and are subsequently resistant to the disease. We have observed an association between the disappearance of disease-inducing effector T cells and the appearance of suppressor cells that correlates with the recovery phase of EAE. Thus, lymph node cells (LNC) obtained from challenged donor rats prior to, or at time of onset of EAE, will transfer disease to normal syngeneic recipients. In contrast, LNC and spleen cells (but not thymocytes) protected recipients against EAE when transferred after the donors had recovered from the disease, as demonstrated by inability to induce EAE in recipients by challenge with antigen. This suppression was antigen-specific, since recipients of LNC from donors given unrelated antigen + adjuvant were susceptible to EAE when challenged. Suppressor cell activity was lost when the LNC from rats which had recovered from EAE were passed through glass wool or nylon fiber columns (i.e., depleted of Ig^+ cells and other adherent cells). In contrast, LNC which adhered to nylon wool (the Ig^+ enriched population) were found to possess suppressor activity. Our findings suggest that the activation of nylon-adherent, antigen-specific suppressor cells may be implicated in recovery from EAE.

Supported by NIH grant NS-06985, and National Multiple Sclerosis Society grant 1073-B-5.

068 EFFECT OF PLATINUM ON HEMATOPOIETIC STEM CELLS. P. Paul, S.A. Rothmann, R.R. Reimer, A.S. Gordon. Cleveland Clinic, Cleveland, OH and New York Univ., New York, NY.

Platinum coordination complexes have been introduced as cancer chemotherapeutic agents with considerable success. Although the myelosuppressive effects of these agents are usually mild, some patients exhibit markedly decreased erythropoiesis. Because platinum is being utilized in an increasing number of chemotherapeutic regimens, we sought to determine the effect of this agent on committed stem cells after in vitro and in vivo exposure. 10^6 bone marrow cells from humans or C57 Bl/6 mice were incubated for one hour at 37°C with cisdiamino, dichloroplatinum (Platinol, Bristol) at concentrations ranging from 5-100 ug. Mannitol in saline was used as a control. Cells were washed twice in alpha media prior to plating in methyl cellulose cultures. In both species, CFU-e, BFU-e and CFU-c were significantly reduced after exposure to 5 ug/10^6 cells and virtually abolished after 10 ug/10^6 cells. Preliminary experiments using BM obtained 5 days after a single intraperitoneal injection of 10 mg/kg in C57 Bl/6 mice revealed a reduction in the number of CFU-c and CFU-e and no significant difference in BFU-e as compared to vehicle injected controls. Serum erythropoietin was not elevated which may reflect platinum-induced nephrotoxicity. This could explain why CFU-e were not measurable whereas BFU-e were similar to vehicle treated animals. The results suggest that platinum-induced anemia may be due to a direct effect on bone marrow stem cells as well as an effect on erythropoietin-producing tissue.

Immune Regulation

069 LACK OF GVHD IN MICE AND DOGS GIVEN TOTAL LYMPHOID IRRADIATION (TLI) AND ALLOGENEIC BONE MARROW TRANSPLANTATION, Michael S. Gottlieb and Samuel Strober. Stanford University School of Medicine, Palo Alto, CA

Preparation of adult recipients with TLI facilitates consistent engraftment of allogeneic bone marrow (BM) without clinical GVHD in both inbred rodents and mongrel dogs. BALB/c mice ($H\text{-}2^d$) received 3,400 rads as 17 fractions of 200 rad each to the major lymph nodes, spleen and thymus followed by an infusion of 30 x 10^6 untreated BM cells from the C57BL/Ka strain ($H\text{-}2^b$). 48/51 (94%)showed chimerism in the peripheral blood (mean of 68% donor-type cells) when tested after 40 days with a microcytotoxicity test using BALB/c anti-C57BL/Ka antiserum. The chimeras did not develop GVHD and remain clinically well 80-140 days after BM transplantation. 12 adult mongrel dogs were given TLI over 3-4 weeks as 18 fractions of 100 rad each (total dose-1,800 rads). The day after the 18th fraction recipients were transfused with BM from a mismatched donor. All 12 dogs were found to have cells of donor origin (by karytype analysis) in serial BM aspirates after transplantation, and donor-type red blood cells were detected in peripheral blood samples in the majority of dogs where a DEA marker was available. Clinical or biochemical signs of GVHD have not been observed over 4-15 months followup. The absence of GVHD in allogeneic chimeras prepared with TLI remains unexplained. Spleen cells from healthy C57BL/Ka-BALB/c chimeras have a potent non-specific suppressor effect on the mixed lymphocyte reaction (MLR), regardless of the strain of origin of the responder or stimulator cells, when tested at greater than 120 days after BM transplantaion. The generation of non-specific suppressor cells in the altered lymphoid tissues of the host could contribute to the observed protection from GVHD.

070 LACK OF SUPPRESSOR CELLS IN NON-MHC STABLE BONE MARROW CHIMERAS. Brian L. Hamilton, and Robertson Parkman, Division of Immunology, Children's Hospital Medical Center, and Department of Pediatric Oncology, Sidney Farber Cancer Institute, Boston, MA 02115.

In spite of matching at the major histocompatibility complex (MHC), graft-versus-host disease (GVHD) is still a significant limitation in human allogeneic bone marrow transplantation, probably due to non-MHC antigenic differences between donors and recipients. We have established a murine model for non-MHC GVHD. LP donors - C57BL/6 recipients ($H\text{-}2^b$) and B10.D2/nSN donors - BALB/cJ recipients ($H\text{-}2^d$) were used. The GVHD produced is clinically and histologically similar to human acute GVHD. The tempo, but not the severity, of GVHD is proportional to the number of peripheral lymphoid cells infused. Using a chromium release assay, cytotoxic T lymphocytes (CTL) are detectable in animals with GVHD, but not in similarly transplanted animals without GVHD. We have looked for evidence of suppressor cells in stable chimeras using both in vivo and in vitro methods. Spleen cells from stable chimeras inhibit neither the generation nor function of CTL, nor are suppressor cells generated in vitro from chimeric spleen cells. GVHD to non-MHC antigens appears to be due to donor T lymphocytes infused at the time of transplant. Transplantation tolerance to non-MHC antigens in this model may be due to a functional clonal deletion mechanism.

071 EFFECT OF ABSORBED ANTI-T-CELL-GLOBULIN ON GvHD AND HEMOPOIETIC PRECURSOR CELLS: H.Rodt, B. Netzel, H.J. Kolb, R.J. Haas, Ch.Bender-Götze, B.Emmerich, S.Thierfelder, Institut für Hämatologie der GSF und Arbeitsgemeinschaft KMT, München.

Many experimental and clinical attempts have been made to abrogate the GvH-potential of bone marrow grafts. In earlier experiments we could show that GvHD could be suppressed by incubation of incompatible donor marrow in vitro with an anti-T-cell globulin, freed of toxicity against hemopoietic stem cells by a specific absorption and purification procedure. These experiments were performed in mice and dogs. The results in the animal models suggested an application in humans, provided that suitable antisera could be produced for the clinical situation. Absorption of anti-human thymocyte serum with liver-kidney homogenate, B-CLL cells and LCL followed by a purification procedure revealed an anti-human T cell globulin which is specifically reactive against T-cells in several indicator systems, and no longer inhibitory to human CFUc and bone marrow growth in diffusion chambers. This antibody preparation was applied to clinical BMT performed in 7 patients with ALL. 6 patients were transplanted with HLA-identical bone marrow. In one case the donor marrow was HLA-D different. 5 patients got sex different donor cells. The donor marrow was concentrated using an IBM cell separator to about 200 ml, incubated with anti-human T-cell globulin in a final concentration of 1:200 and transferred to the recipient, conditioned with an antiproliferative regimen and a total body irradiation of 1000 rad. The patients tolerated the incubated marrow without side effects and an uneventful hemopoietic engraftment in 6 out of 8 cases. So far no symptoms of GvH were observed in all transplanted patients. 3 patients are still alive, the longest surviving now over 2.5 years.

072 SUPPRESSOR CELL MECHANISMS IN TLI-TREATED MICE: SUPPRESSION OF THE MLR AND OF GRAFT-vs-HOST DISEASE, Donna P. King and Samuel Strober, Department of Medicine, Stanford University School of Medicine, Stanford, CA

Total lymphoid irradiation (TLI) in rodents consists of 17 daily fractions of 200 rads delivered to the major lymphoid organs. TLI results in the presence of a population of cells in the spleens of irradiated Balb/c mice which is capable of suppressing both the in vitro mixed lymphocyte reaction (MLR) and the in vivo induction of graft-vs-host disease (GvHD). Suppression is non-specific and we are currently investigating the suppressor cell surface characteristics. The cell is not depleted from the spleen by adherence to plastic or passage over a glass wool column. Suppressor activity appears to wane following completion of irradiation and disappears within 30-40 days.

Stable C57BL/Ka→Balb/c chimeras were made by treatment of Balb/c mice with TLI followed by administration of 1 x 10^9 C57BL bone marrow (BM) cells/kg body weight. Non-specific suppressors of the MLR could be demonstrated in a series of chimeras studied up to 120 days following BM transplantation, suggesting that the suppressor cells persist in chimeric mice and may be responsible for absence of GvHD. In addition, suppressor cell activity can be detected in mice given fewer than 10 fractions (2000r) TLI, whereas greater than 14 fractions (2800r) were required to achieve BM chimerism. This suggests that both changes in the immune status of the animals and creation of marrow space may be necessary to allow for permanent BM engraftment.

073 IMMUNOSPECIFIC DEPLETION OF GRAFT-VERSUS-HOST REACTIVE LYMPHOCYTES USING SENSITIZED SYNGENEIC INITIATOR T LYMPHOCYTES, Arie Belldegrün and Irun Cohen, Department of Cell Biology, The Weizmann Institute of Science, Rehovot, Israel

We investigated a model of a lethal graft-versus-host (GVH) reaction with the aim of depleting donor spleen cells of immunospecific GVH-reactive lymphocytes. In previous studies of the recruitment of effector T lymphocytes by sensitized syngeneic initiator T lymphocytes (ITL) we found, using a local GVH reaction, that precursors of specific GVH-reactive lymphocytes were recruited to a draining lymph node. In the present study, adult F_1 hybrid mice were lethally irradiated and reconstituted with 2×10^6 syngeneic bone marrow cells and varying numbers of spleen cells from parental strain mice. To deplete donor spleen cells of GVH-reactive lymphocytes, parental strain mice were injected to the hind foot pads 6 days earlier with syngeneic ITL that had been sensitized in vitro against allogeneic fibroblasts. We found that injection of ITL sensitized against the relevant allogeneic antigens led to a marked decrease in the specific GVH potential of donor spleen cells. These findings show that GVH-reactive lymphocytes can be depleted selectively by activating their recruitment to particular lymph nodes, using syngeneic ITL.

074 PRELIMINARY STUDIES OF 31 ANTISERA THAT IDENTIFY NON-HLA HISTOCOMPATIBILITY ANTIGENS, Wilma B. Bias and Kathryn Pollard. The Johns Hopkins University School of Medicine, Baltimore, Maryland 21205.

All instances of graft rejection or graft-versus-host disease in bone marrow transplant patients and rejection of HLA-identical renal allografts can be attributed to non-HLA histocompatibility systems. In order to identify non-HLA antibodies we have screened platelet-absorbed sera from patients hyperimmunized by transfusion.

The two-color fluorescent cytotoxicity technique permits simultaneous identification of T or B-cell specific reactivity. Sera that exhibit "extra" reactions with HLA-D homozygous typing cells (HTC) or that give discrepant results with two HTC's of the same specificity are then screened on the entire HLA-DR typed panel. This procedure has led to the identification of 31 sera with reactivity to T cells, B cells or both that segregate in families independent of HLA. The segregation patterns revealed two or more non-HLA antibodies in several of the sera.

These antisera have been tested for reactivity against red cells of various ABO and Lewis phenotypes. The Lewis genotype is known for many of the families used in the segregation studies and rule out specificity for the Lewis system.

Additional individuals and family studies are required to determine whether any of the sera recognize the same or allelic antigens. Typing of genetic markers in the informative families is in progress in order to map the non-HLA loci.

075 THE FORMATION OF HUMAN LYMPHOCYTE COLONIES IN SEMISOLID CULTURES IN RESPONSE TO ALLOGENEIC MIXED-LYMPHOCYTE STIMULATION, Floyd D. Wilson, Christine B. Whaley, Moshe Shifrine, Jacqueline A. Dyck, Augustina R. Carbonell (UCD School of Medicine), and Dave Hinds, Laboratory for Energy-Related Health Research, School of Veterinary Medicine, University of California, Davis, CA 95616

A technique is described for the growth of human lymphocyte colonies in semisolid culture systems in response to allogeneic lymphocyte stimulation. Colonies did not form to any major extent using autologous lymphocyte stimulation. Both one-way and two-way mixed-lymphocyte reactions were investigated. Ultrastructurally, such colonies are composed of cells with lymphoblastic and lymphocytic morphology. The majority of the lymphoid elements composing the colonies were T-cells based on their ability to rosette with sheep red blood cells. Our studies suggest that the colonies are clonogenic in origin and therefore the technique offers the potential for isolation of specific clones, or subpopulations of lymphocytes involved in allogeneic reactions and characterization of their function. Studies directly comparing the stimulation indices achieved with standard mixed lymphocyte cultures utilizing ^{3}HTdr-incorporation to the colony-forming assay indicate that the cloning technique produces higher stimulation indices for allogeneic/autologous reactions and produces less autologous (background) response than the ^{3}HTdr incorporation technique. In addition to lymphocyte colonies, we also observed colonies of surface-adherent populations of macrophages, including multinucleated giant cells. Thus, the technique appears to provide a new and potentially more sensitive method for the study of transplantation immunology and cell-mediated immunity in humans.

076 SYNERGY IN GRAFT VERSUS HOST ACTIVITY OF NON-LYTICALLY SEPARATED Lyt2^{+} and Lyt2^{-} LYMPHOCYTES, Michael Mage, Bonnie Mathieson*, Susan Sharrow, Louise McHugh, and Ulrich Hammerling**, NCI and *NIAID, Bethesda Md., and **Sloan-Kettering Memorial Cancer Center, New York, N.Y.

In studies of mouse T lymphocytes, elimination experiments with anti-Lyt 1 do not recover the entire Lyt2^{+} population, because Lyt1^{+}2^{+} as well as Lyt1^{+}2^{-} cells are killed. We report a non-lytic preparative separation. Thymus, spleen, or lymph node cell suspensions were incubated with a monoclonal antibody to Lyt2.2 at 0°, washed, and placed in polystyrene tissue culture dishes precoated with anti-immunoglobulin. Nonadherent populations were depleted of Lyt2^{+} cells, as measured by flow-microfluorometry, and were essentially devoid of cytotoxic T lymphocyte (CTL) precursors, in 5 day mixed lymphocyte cultures with semi-allogeneic stimulators. Adherent populations were enriched for Lyt2^{+} cells and for CTL precursors. The GVH activity of separated Lyt2^{+} and Lyt2^{-} cells in the Simonsen spleen assay was less than that of unfractionated cells, but the activity of re-mixed Lyt2^{+} and Lyt2^{-} cells was equal to that of unfractionated cells, and higher than the sum of the contributions of Lyt2^{+} and Lyt2^{-} cells tested separately. This synergy, interpreted in the light of elimination experiments from other laboratories, indicates that Lyt1^{+}2^{+} cells play a role in the generation of GVH splenomegaly. Use of monoclonal antibodies in non-lytic plate separations may be useful for preparative scale isolation or removal of cell subpopulations.

077 GENERATION AND INHIBITION OF CYTOTOXIC LYMPHOCYTES BY PURIFIED H-2 ANTIGENS INCORPORATED INTO LIPOSOMES, Duane W. Sears and Pamela H. Wilson, UCSB, Santa Barbara, CA.

Histocompatibility antigens appear to be involved in both the production of cytotoxic lymphocytes (CTL) and the recognition by CTL of target cells. However, it has not yet been proven that H-2 antigens themselves are directly responsible for these two antigen recognition processes. Thus, highly purified H-$2K^b$ and H-$2D^b$ antigens, isolated from EL4 tumor cells, have been incorporated into phospholipid vesicles (H-2^b-liposomes) and tested in vitro for their ability to 1)generate and 2) inhibit secondary CTL. Six weeks after primary immunization of BALB/c(H-2^d) mice with C57BL/10(H-2^b) splenocytes, the immune H-2^d anti-H-2^b splenocytes were cultured in vitro for six days with either X ray irradiated B10 splenocytes or H-2^b-liposomes. Secondary CTL, as assayed by ^{51}Cr release from EL4 targets, were generated in both cases; no secondary CTL were produced without stimulation. For the same killer-to-target ratio, H-2^b-liposome stimulated CTL achieved a level of lytic activity which was as much as 50% the level achieved by splenocyte stimulated CTL. In addition, H-2^b-liposomes were found to specifically and strongly inhibit the lytic activity of H-2^b-liposome stimulated CTL but, interestingly, not splenocyte stimulated CTL. These phenomena are currently being investigated with CTL generated across a more limited range of genetic differences; specifically, B6(H-2^b) splenocytes are being used to immunize Hzl(H-2^{bml}) mutant mice and mice from other congenic resistant strains. Our finding that H-2-liposomes with highly purified H-2 antigens are effective in generating and blocking secondary CTL in vitro opens the way for a closer examination of the molecular mechanisms involved in cytolysis by immune CTL generated either against H-2 antigens themselves or against other antigens whose recognition is restricted by H-2 antigens.

078 TRANSPLANTATION TOLERANCE: CLONAL DELETION, ACTIVE SUPPRESSION OR BOTH?
R.S.Gruchalla and J.W.Streilein, Univ.Tex.Health Science Center, Dallas, Texas 75235

Neonatal transplantation tolerance is a classical method used to study immune discrimination between Self and Nonself. The tolerant state has been ascribed mechanistically to (1)deletion/inactivation of antigen reactive cells (ARC); (2)suppression of ARC by T lymphocytes or (3) blocking of ARC by serum factors. In the latter two postulated mechanisms,specific ARC are present in tolerant animals. Recently,studies have shown that alloantigens encoded by different regions of the murine major histocompatibility complex (MHC) are not equally able to induce tolerance, suggesting that more than one mechanism may be operative, depending upon the nature of the inducing MHC alloantigens. To examine this possibility, lymphoid cells from tolerant mice were studied in cell mediated lympholysis (CML) and mixed lymphocyte culture (MLC) assays. Lymphoid cells from animals tolerant of the following types of H-2 disparities-isolated K or I; Central I+D regions; entire H-2 segment-failed to respond to the tolerated alloantigens in CML and MLC, while reactivity to third party alloantigens was vigorous, suggesting that specific deletion of ARC had taken place. However, upon co-culturing of lymphoid cells from animals tolerant of I region disparities with normal responder lymphoid cells, and with stimulator cells bearing the tolerated antigens (mixed lymphocyte co-cultures-MLC-C),normal reactivity was diminished significantly. That is,"tolerant" lymphoid cells were able to suppress syngeneic normal cells from responding in MLC-C to the appropriate tolerated I region antigens. No comparable suppression was found when tolerated alloantigens were of the isolated K region type. These results suggest that neonatal tolerance induced by Class I K region alloantigens occurs primarily via clonal deletion, whereas Class II (Ia) alloantigens evoke a suppression mechanism.

079 EFFECTS OF ATG ON PRECURSOR CELLS OF HUMAN MARROW AND RECOGNITION OF NON-HLA ANTIGENS
Mine Harada and Robert Peter Gale, Transplantation Biology Unit, UCLA School of Medicine, Los Angeles, CA 90024

Horse anti-human thymocyte globulin (ATG) was absorbed with human fetal liver cells or granulocytes and tested for antihemopoietic activity and immunosuppressive potential including immunity to non-HLA antigens using TNP-modified cells. Its effect on precursors of T cells was also tested. Absorbed ATG did not inhibit growth of granulocyte-macrophage precursors (CFU-C). Precursors of T cells inducible by thymic factors were inhibited by ATG treatment. Absorbed ATG also inhibited E-rosette formation, primary and secondary proliferative responses to alloantigens and TNP-modified autologous cells, and HLA-non-restricted cytotoxicity against TNP-modified cells. These data provide an experimental basis for the use of ATG in human bone marrow transplantation.

080 IMMUNE SUPPRESSION OF ALLOANTIGEN REACTIVE T CELL PROLIFERATION IN VITRO. Susan Solliday Rich and Robert R. Rich, Department of Microbiology and Immunology and Howard Hughes Med. Inst. Lab., Baylor College of Medicine, Houston, Texas 77030
Alloantigen-activated murine T cells suppress proliferative responses of alloreactive T cells in mixed leukocyte reaction (MLR). Interaction between suppressor (MLR-Ts) and target responding cells is controlled by MHC genes in the I-C, rather than I-J, subregion of H-2. We have investigated the regulatory role of I-C gene products by structural studies of the secreted T cell suppressor factor (MLR-TsF) that expresses I-C determinants, and by defining expression of I-C molecules on suppressor and responding cells. MLR-TsF was biochemically characterized by gel filtration chromatography; major suppressive activity resided in 40,000-60,000 MW fractions, with additional activity associated with 75,000 and 25,000 MW fractions. All fractions retained I-C restricted interaction with MLR responder cells. Preparative isoelectrofocusing fractionated suppressor activity into a major peak at pH 5.0, associated with molecules of 75,000-80,000 MW, and a second peak at pH 7.0-8.0 To investigate I-C molecules on MLR-Ts,in vivo alloantigen-activated spleen cells were exposed to anti-I subregion sera and C before in vitro culture for MLR-TsF production. Elimination of $I\text{-}J^+$, as well as $I\text{-}C^+$ cells prevented suppressor activity, while removal of $I\text{-}A/B^+$ cells had no effect. Admixture of anti-I-C and anti-I-J serum-treated cells failed to reconstitute suppressor activity. The data thus suggest that both I-C and I-J molecules are expressed on a single population of MLR suppressor T cells, although MLR-TsF expresses only I-C determinants. Pretreatment of MLR responder cells with anti-I subregion sera in blocking or cytotoxic protocols, before addition to MLR with MLR-TsF, failed to prevent MLR suppression. Thus, I-C molecules do not appear to play a role in MLR suppression at the target responding cell level.

081 HUMAN LYMPHOID CELL SURFACE ANTIGENS, Ronald Levy, Stanford University School of Medicine, Stanford, California, 94305
We have prepared hybridoma monoclonal antibodies to human leukemia cells of T cell type. Included in our panel of hybridomas are some which recognize T cell differentiation antigens as well as some which recognize leukemia associated antigens (Levy, et al, Proc. Nat'l. Acad. Sci., in press, 1979). We are now exploring the use of these antibodies for clinical disease monitoring and considering their therapeutic potential as well.

082 AUTOANTI-IDIOTYPIC ANTIBODIES IN THE REGULATION OF THE IMMUNE RESPONSE Göran Möller and Carmen Fernandez. Karolinska Institute, Stockholm, Sweden
The immune response to the alpha 1-6 epitope of dextran is determined by one gene linked to the heavy chain locus. High responder strains, such as CBA make only IgM antibodies, whereas the high responder strain C57BL produces both IgM and IgG antibodies. After a primary immune response to dextran, all high responder strains fail to give a secondary response for up to 20 weeks. This antigen-specific immunosuppression affects only the response to dextran. Suppression could be passively transferred with serum from primed animals into untreated mice, also when antibodies against dextran had been removed by absorption. Dextran absorbed sera from dextran primed mice specifically suppressed the development of plaque-forming cells against dextran in vitro, but not those against other antigens. Sera from dextran primed CBA mice only suppressed plaque-forming cells of CBA, but not C57BL origin, and the reverse. The suppressive principle was an IgG antibody directed against the variable region of anti-dextran antibodies. This autoanti-idiotypic antibody was responsible for the prolonged period of immunosuppression, but did not in other ways influence the primary immune response.

083 *INHIBITION OF THE "LETHAL HIT" BY XENOGENEIC ANTISERUM MADE AGAINST "ACTIVATED" ALLOIMMUNE CYTOTOXIC T LYMPHOCYTES.* John C. Hiserodt and Benjamin Bonavida, UCLA School of Medicine, Department of Microbiology and Immunology, Los Angeles, CA 90024
Xenogeneic antisera have been prepared in rats by ip injection of alloimmune thymus derived cytotoxic T lymphocytes (CTL) which have been "activated" on monolayers of PHA-P coated L-929 fibroblasts. The serum is a potent inhibitor of murine CTL mediated lysis of P815 target cells in the absence of added C'. Analysis of mechanism of antiserum inhibition revealed the following characteristics: 1) it did not affect CTL-target cell conjugate frequencies; 2) it was not cytotoxic by itself to effector T cells; 3) it could inhibit ^{51}Cr release from P815 targets when added to ongoing CTL reactions at a time when either EDTA or EGTA could not inhibit lysis. Thus, the serum could inhibit the "lethal hit" after "programming for lysis" has occurred; and 4) it did not inhibit lysis of P815 targets by immune rat or human effector cells. Exhaustive absorption of antisera on P815 targets, normal C57 spleen cells, thymocytes, or even alloimmune T cells could not reduce the inhibitory activity of this serum. However, absorption on "activated" alloimmune T cells or on CTL-P815 conjugates fully removed the blocking activity. Finally, control rat antisera made against normal C57 T cells could not inhibit CTL lysis after the target cell binding step. These data collectively suggest that cytotoxic T lymphocytes possess a lytic mechanism which is "cryptic" (not reactive with inhibitory serum) but becomes exposed when activated by interaction with specific target cells or with lectins. Furthermore, it appears once the "lethal hit" has been delivered to the target it is possible to prevent cytolysis during the lymphocyte independent phase. Supported by NIH Grant CA12800.

084 SEPARATION OF HUMAN MYELOID COLONY FORMING CELLS BY FLUORESCENCE ACTIVATED CELL SORTING AND COMPLEMENT MEDIATED CYTOTOXICITY WITH MONOCLONAL ANTIBODIES TO LEUCOCYTE SURFACE ANTIGENS, Peter C.L. Beverley and David Linch, Imperial Cancer Research Fund Human Tumour Immunology Group and Department of Clinical Haematology, University College Hospital Medical School, University Street, London WC1E 6JJ.

Human haemopoietic pluripotent stem cells and the progenitors of the myeloid, erythroid and lymphoid lineages have not yet been satisfactorily identified. We have used two monoclonal antibodies to human leucocytes to characterise myeloid progenitor cells. Fluorescence activated cell sorting of bone marrow stained with anti-human leucocyte antibody (anti-HLE-1) yields three major fractions containing cytologically distinct myeloid, erythroid and lymphoid populations. All the CFU-GM activity is found in the myeloid population. A second cytotoxic monoclonal antibody kills all morphologically distinct granulocytic series cells. Cell sorting with this antiserum shows that CFU-GM lack or are extremely low in the antigen recognised by this antiserum. Thus combinations of antisera allow identification and separation of bone marrow progenitor cells.

085 HEMATOPOIETIC STEM CELL ANTIGENS, Kenneth A. Foon, John H. Fitchen, Michael B. Belzer, David W. Golde and Martin J. Cline, UCLA School of Medicine, Los Angeles, CA, and VA Wadsworth Medical Center, Los Angeles, CA.

We studied the antigenic characteristics of human myeloid and erythroid progenitor cells using bone marrow culture techniques. Normal bone marrow or peripheral blood cells were incubated with alloantisera, heteroantisera raised in rabbits, or hybridoma-derived monoclonal antibodies in a complement-dependent cytotoxicity assay. After treatment with immune reagents of various specificities, cells were cultured to assay for CFU-C, CFU-D, and CFU-E. Complement-dependent inhibition of colony formation by a particular immune reagent was taken as evidence that the corresponding antigen is expressed by the progenitor cell being assayed. Results are given in the table (+ indicates antigen present).

Progenitor	HLA-A,B			*HLA-DR	Thymocyte	cALL	AML	PMN
Cell	Allotype	*Heavy chain	$\beta_2\mu$					
CFU-C	+	+	+	+	-	-	-	+
CFU-D	+			+	-	-	-	+
CFU-E				-	-		-	-

(*indicates test done with monoclonal, hybridoma-derived antibody)

Knowledge of the antigenic make-up of hematopoietic stem cells and the development of immune reagents with restricted cellular specificity has potential importance in bone marrow transplantation.

086 IDENTIFICATION OF LEUKOCYTE-ENGRAFTMENT AND -DIFFERENTIATION WITH NEW MONOCLONAL ANTIBODIES AFTER BONE MARROW TRANSPLANTATION.

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A group of six well defined monoclonal antibodies derived from murine and rat plasmocytoma fusions,specifically directed against: human thymocyte antigen (HTA1),HLA-ABC common,HLA-DRw common, a monocyte antigen (M1/70.15.2), a leukocyte antigen (YD1/23.1.2) and a lymphocyte subpopulation antigen (YD1/48.13.12) were employed in addition to most classical surface markers for leukocytes in indirect immunofluorescence to assess and monitor distinctly different steps of hemopoietic engraftment and the sequential differentiation of myelocytes,monocytes and lymphocytes in two pediatric patients with acute a) T-cell and b) myelocytic leukemia after successful sibling donor bone marrow transplantation. Having used these reagents previously for leukemia classification,for T cell growth factor propagated MLC and PLT lymphoid clones as well as for the assessment of progentitor cell differentiation in normal human bone marrow, these new tools allow a more precise insight into the kinetics of leukocyte maturation after bone marrow transplantation and help to establish meaningful correlations to functional hematological and immunological in vitro assays.

Supported by the DFG-Forschergruppe "LEUKÄMIEFORSCHUNG" Tübingen,W.-Germany

087 TWO-DIMENSIONAL GEL ELECTROPHORETIC ANALYSIS OF HLA-D ANTIGENS WITH MONOCLONAL ANTIBODIES, D.J. Charron and Hugh O. McDevitt, Stanford University, Stanford, CA 94305.

A group of monoclonal antibodies directed against the HLA-D region associated molecules (human Ia) was produced by somatic cell hybridization. These antibodies are cytotoxic to every B cell tested (B27 lymphoblastoid cell lines, 15 individual peripheral blood B lymphocytes) and binds in radioimmunoassay and immunofluorescence (Fluorescence Activated Cell Sorter) to Con A stimulated T cells. The two-dimensional polyacrylamide gel electrophoresis (2D-PAGE) pattern from NP-40 B lymphocyte extracts immunoprecipitated by these monoclonal antibodies revealed a very heterogeneous set of spots within a 26,000 to 33,000 dalton molecular weight range, spread from the basic to the acidic end of the gel. When 2D-PAGE profiles from different HLA-D homozygous B cell lines are compared, reproducible genetic polymorphisms are observed. The basic end of the gel shows a major electrophoretic polymorphism in the lower molecular weight area (26,000 to 28,000 d) which varies according to the HLA-D type of the cell extract. The lower molecular weight acidic area also reveals a second polymorphic pattern which is more difficult to analyze because of minimal dispersion of the spots. These 2D-PAGE patterns represent allele specific "fingerprints" of different HLA-D genotypes. A pulse chase labeling experiment reveals four precursor chains. Non-glycosylated chains (from tunicamycin treated cells) also display a polymorphic pattern. Moreover, there are striking similarities with respect to number, size, and charge spectrum between HLA-D polypeptides and the murine I-A and I-E subregion polypeptides, suggesting a similar genetic organization and molecular complexity in both species.

Autologous Bone Marrow Transplantation

088 AUTOLOGOUS BONE MARROW TRANSPLANTATION FOR TREATMENT OF HEMATOLOGIC MALIGNANCY, G.P. Herzig, G.L. Phillips, J. Fay, C. NaPombejara, and S. Wolff, Washington University, St. Louis, MO and Duke University, Durham, NC

Bone marrow was obtained from 18 patients with hematologic malignancy (10 acute leukemia, 8 lymphoma) and preserved by freezing in 10% DMSO. When refractory to conventional therapy, patients were treated with a combination of high dose cyclophosphamide (60 mg/kg/day x 2 days) and lethal total body irradiation (1000 rads at 5-10 rads/min) followed by transplantation with their own cryopreserved marrow. Recovery of marrow function occurred in all patients and was similar to that reported for allogeneic marrow transplantation after the same regimen (neutrophils >500/µl in 28 days, platelets >10,000/µl in 34 days). Recovery was more rapid for patients with lymphoma rather than leukemia. There were no deaths due to hemorrhagic or infectious complications. Pneumonitis developed in 3 of 10 patients at risk for >3 mos but was fatal in none. All 10 patients with acute leukemia (treated in relapse) were rendered disease-free with a median duration of 4 mos (range 2.5-6.5 months). No relation was found between the interval from marrow storage to relapse and the subsequent duration of remission after marrow transplantation. Five of 8 patients with malignant lymphoma achieved complete remission; one relapsed after 1 mo., while 4 remain free of disease without further therapy for 25+, 20+, 10+, and 1+ months.

089 INTENSIVE 1,3-BIS(2-CHLOROETHYL)-1-NITROSOUREA (BCNU) AND AUTOLOGOUS BONE MARROW TRANSPLANTATION (BMTX) FOR REFRACTORY MALIGNANCY: A PHASE I STUDY. GL Phillips, JW Fay, SN Wolff, C. Karanes, RH Herzig and GP Herzig; Washington U., St. Louis 63110, Duke U., Durham 27710, and Case Western Reserve, Cleveland 44106.

55 patients with refractory malignancies underwent cryopreservation of their own bone marrow followed by intensive therapy with BCNU (200-950mg/m2/dailyx3d.) and autologous BMTX 7 d. after completing BCNU. Hematopoietic toxicity was evaluable in 38 patients who survived 28 d.; 37/38 rapidly recovered marrow function (pmn 500/ul and platelets 10-20,000/ul) by d. +15.

While hematopoietic toxicity was successfully prevented by marrow autografting, BCNU doses $\geq$400mg/m2/x3d. resulted in severe liver toxicity in 8/18 evaluable patients (7 fatal), and fatal CNS toxicity in 3/19 patients. Fatal liver or CNS toxicity was not observed at BCNU doses of$\leq$400mg/m2/x3d. Pulmonary fibrosis occurred in 2 patients and was not obviously dose related. Renal toxicity was not seen among 33 evaluable patients. Objective tumor responses were seen in 19/36 patients at all dose levels.

In conclusion, autologous BMYX can limit the hematopoietic toxicity of BCNU and thereby permit the safe use of doses of 400mg/m2/x3d; this represents a 4-fold increase above the usually tolerated dose of BCNU. Further dose increases are prevented by liver and CNS toxicity. While the full antitumor activity of intensive BCNU remains to be evaluated, the high response rate in a variety of resistant malignant neoplasms is encouraging.

090 AN IN VITRO ASSAY FOR THE EFFECT OF CHEMOTHERAPY ON BONE MARROW STROMAL CELL SUPPORT OF DONOR HEMOPOIETIC STEM CELL PROLIFERATION, by Gary I. Cohen, George P. Canellos, and Joel S. Greenberger, Department of Medicine, Sidney Farber Cancer Institute and Joint Center for Radiation Therapy, and Harvard Medical School, Boston, MA. 02115.

A modification of the long-term bone marrow culture technique of T.M. Dexter has been developed into an assay for in vitro study of the role of stromal cells in bone marrow transplantation (BMT). 10^{-7}M hydrocortisone supplemented cultures of NIH Swiss mouse bone marrow grown in 25% horse or fetal calf serum generate CFUc and CFUs for periods of over 20 weeks with no required marrow recharging (Greenberger, et.al., Virology, 95:317-333, 1979). In contrast, establishment of cultures in fetal calf serum without corticosteroid results in a confluent stromal cell monolayer with no detectable generation of CFUc or CFUs by day 28. These steroid-deprived culture flasks were used as "recipients". Nonadherent cells from 40 or 50 day old corticosteroid supplemented cultures (donor) were transferred to recipient stromal cultures at day 28 in serial 2-fold dilutions from 5 x 10^7 to 5 x 10^6 cells. Colonies of "cobblestone" and round hemopoietic cells formed on the recipient stroma in a direct dose-response fashion. These colonies generated immature granulocytic cells and CFUc for over 8 weeks. In contrast, transfer of (donor) nonadherent cells to empty flasks produced only mature granulocytes for less than 2 weeks and no CFUc. Treatment of recipient cultures with chemotherapeutic agents or x-ray decreased engraftment of normal donor cells. This system should be valuable for study of bone marrow stromal cell damage and recovery following in vitro administration of cytotoxic agents used in BMT.

091 EFFECT OF 4-HYDROPEROXYCYCLOPHOSPHAMIDE (4-HC) ON HEMOPOIETIC STEM CELLS, Martin Korbling, Peter Tutschka, Michael Colvin, and George Santos, Oncology Center, Johns Hopkins University, Baltimore, MD 21205

4-HC recently has been shown to eliminate tumor cells from the marrow in a rat model of acute myelogenous leukemia by short-term incubation with 17.5 µg/ml of 4-HC prior to infusion of the autologous marrow graft. This study attempts to identify a safe dose range of 4-HC for incubating marrow by comparing the granulocyte colony formation (CFUc) with the in vivo potential restoring hemopoiesis. When human marrow was incubated with 1 µg/ml of 4-HC throughout the culture period (10 days) CFUc formation was reduced by 80%. When drug incubation was limited to 30 min at 37°C followed by twice washing the cells the formation of CFUc in human marrow was dependent on the dose of 4-HC as well as the number of target cells: a normal marrow suspension of 10^7 mononuclear cells incubated with 80 µg/ml 4-HC showed a 50% decrease of CFUc formation, whereas 10^7 red cell depleted mononuclear cells showed a 50% decrease of CFUc when incubated with 5 µg/ml. A similar response curve could be established with rat bone marrow. When, in addition, normal rat marrow was incubated with graded doses of 4-HC and infused into lethally irradiated syngeneic recipients, a 100% survival of the recipients at 21 days was seen with as high a dose as 80 µg of 4-HC per ml marrow inoculum. The study suggests that although 4-HC at higher doses decreases significantly the formation of CFUc it still permits the establishment of an autologous marrow graft. This must be taken into account in clinical studies where CFUc is used to indirectly monitor the potential stem cell toxicity of marrow incubated with 4-HC.

092 TREATMENT OF NEOPLASTIC DISEASE METASTATIC TO THE CENTRAL NERVOUS SYSTEM (CNS) WITH HIGH-DOSE 1,3-BIS(2-CHLOROETHYL)-1-NITROSOUREA (BCNU) AND AUTOLOGOUS MARROW TRANSPLANTATION (AMTX), Joseph W. Fay, Gordon L. Phillips, Geoffrey P. Herzig and Roger H. Herzig, Duke University, Durham, North Carolina, Washington University, St. Louis, Missouri, Case Western Reserve University, Cleveland, Ohio

17 patients with neoplastic disease metastatic to the CNS were treated with high-dose BCNU and AMTX. The dose of BCNU ranged from 900 to 2850 mg/M^2 over 3 days followed in 7 days by AMTX. As shown in the table, 10/17 patients had partial (≥50% regression) or complete regression of disease based on cranial axial tomography or spinal fluid examination. 7 patients had no objective response. 5/17 patients are alive from +67 to +389 days following therapy. The patient CNS leukemia was refractory to cranial radiation and intrathecal therapy. 2 patients died secondary to myelosuppression, 5 died due to BCNU extramedullary toxicity, and 5 died of prgressive disease. These data suggest that high-dose BCNU with AMTX may be a useful therapeutic modality in the treatment of neoplastic disease metastatic to the CNS in man.

Diagnosis	Patient Number	Dose (mg/M^2)	CR	PR	NR
Melanoma	8	1200-2850	1	3	4
Renal ca.	2	1200-1500	0	0	2
Lung ca.	3	1200-1500	0	2	1
CNS leukemia	1	900	1	-	-
Thymoma	1	1200	-	1	-
Testic. ca.	1	2850	-	1	-
Unkn. 1°	1	1200	-	1	-

093 HIGH DOSAGE BCNU WITH AUTOLOGOUS MARROW RESCUE, Tak Takvorian, Leroy Parker, Fred Hochberg, Nicholas Zervas, Emil Frei III and George Canellos, Sidney Farber Cancer Institute, Massachusetts General Hospital, Boston, Massachusetts 02115

15 patients with glioblastoma, recurrent post-surgical decompression and radiation therapy, and 2 patients with melanoma were treated with a single course of high dosage BCNU with non-cryopreserved autologous marrow support: 7 patients who had former nitrosourea therapy received a fixed dosage of 800 mg/m^2 whereas the other patients received doses between 600 mg/m^2→1400 mg/m^2. Bone marrow obtained from the iliac crests under general anesthesia was stored in ACD-heparin at 4°C and re-infused 48 hours after its procurement (24-36 hours after treatment with BCNU). Toxicity was severe but acceptable. Biopsy-proven interstitial pneumonitis was seen in one patient, 4 months after treatment at 1200 mg/m^2, which is resolving on high-dosage steroids. Fatal centrilobular necrosis attributed to BCNU developed in one melanoma patient, 6 weeks after treatment at 1200 mg/m^2. One patient died of Clostridia sepsis 9 days after treatment at 1400 mg/m^2 and on autopsy had complete denudation of his GI tract. No renal toxicity has been noted. Myelosuppression was earlier and more profound in patients treated at higher dosages and in the patients who had former nitrosourea therapy, but infection and hemorrhage were not problems. As noted above, 2 patients died of drug-related toxicity at 9 days and 2 months. Three patients died of tumor progression at 2.5, 3.5 and 5.0 months. Three patients have relapsed at 7.0, 7.5 and 8.0 months. The 9 remaining patients are alive, without evidence of disease progression, at 1+ month to 7.5+ months. Future studies will continue to explore dose-response relationships of activity and toxicity, and treatment pre-radiation therapy.

094 THE EFFECT OF LITHIUM CARBONATE ON HEMATOPOIETIC RECOVERY FOLLOWING AUTOLOGOUS MARROW INFUSION. A. Robert Turner, M. Joan Allalunis, Locksley E. McGann, and Jean-Michel Turc, Cross Cancer Institute, University of Alberta, Edmonton, Alberta, Canada, T6G 1Z2.

Lithium carbonate (LC) induces a neutrophilia in hematologically normal patients and patients with neutropenia. It also has been reported to attenuate myelosuppressive effects of chemotherapy and to shorten the aplastic period following therapy of acute myelogenous leukemia. Severe cytopenias follow high dose chemotherapy and/or irradiation which preceed autologous hematopoietic stem cell infusion. This study was designed to see if LC would shorten the duration of that cytopenia. Marrow cells were collected from twelve mongrel dogs and stored at 4° C. Immediately after collection of marrow, 60 mg/Kg cyclophosphamide was given intravenously. Twenty-four hours later 5 x 10^7 nucleated autologous marrow cells/Kg were administered. Seven animals were treated with LC, 300 mg twice daily for the course of the experiment. Five animals served as controls. White blood counts (WBC) and platelet counts were obtained daily. No significant difference in the nadir of WBC or platelets was noted. However, recovery of WBC above 1000 cells/min^3 occurred one day earlier in dogs taking LC. Platelet recovery was not affected. 5/7 LC dogs survived compared with 2/5 controls. Serum lithium levels in treated animals ranged between 0.1 and 0.6 mmol. Lithium carbonate administration appears to have had only a minimal effect on the cytopenia following autologous marrow infusion.

095 SEPARATION OF NORMAL HEMOPOIETIC STEM CELLS FROM LEUKEMIA CELLS BY LONG-TERM CULTURE OF MOUSE BONE MARROW, E.F. Hays and L. Hale, Warren Hall UCLA, Los Angeles, CA 90024.

AKR mice with early and advanced spontaneous and virus-accelerated T cell lymphoma (leukemia) were studied. Bone marrows which contained 50-95% lymphoma cells were placed in long-term cultures and evaluated at weekly intervals for 1-12 weeks for persistence of lymphoma cells, totipotent stem cells (CFUs), granulocyte-monocyte progenitors (CFUc) and granulopoiesis. The contents of a femur and tibia were flushed into 10cc of Fischer's Medium with 25% horse serum and hydrocortisone 0.5 μg/ml. The cultures were incubated at 33°C, 5% CO_2. At weekly intervals 1/2 of the medium was removed and replaced with fresh medium. At week 4, 25% fetal bovine serum replaced the horse serum. Cell counts, cell morphology and stem cell assays were performed on selected samples. After 2 weeks in culture no leukemic cells could be seen. The cultures consisted of attached cells with progranulocytes and maturing granulocytes in suspension. In the non-adherent cells at week 6-7 CFUc (500-700/ml) and CFUs (15-17/ml) were present. Cell counts revealed that proliferation was occurring. There was a peak in cell number at week 5-6. The spleen colonies formed were erythroid, granuloid or mixed. No lymphoma cell colonies were seen and 10^6 cells from 6-week cultures did not produce lymphomas when inoculated into syngeneic mice. Preliminary studies suggest that inoculations of stem cells from 5, 6, & 7 week cultures could reconstitute 950 r treated syngeneic animals. These experiments show that the culture conditions used favor normal hemopoietic stem cell proliferation and granulocyte maturation. They selectively deplete leukemic blasts thus providing normal hemopoietic progenitor cells for transplantation to syngeneic hosts.

Viral Infection and Interstitial Pneumonia

096 FETAL LIVER A SOURCE FOR HAEMOPOIETIC RECONSTITUTION WITHOUT GvHD? Bernhard Kubanek, Wolfgang Heit and Franz Porzsolt, University Ulm, Ulm, F. R. G.

Human fetal haemopoietic tissue was examined in respect to its potential for haemopoietic recovery and to its cellular immune reactivity in vitro to define it as a source for transplantation of bone marrow failure. CFU-C, BFU-E, CFU-E and the growth potential in long term cultures was examined from fetal liver, bone marrow, spleen and thymus of 9 human foetuses, 15 to 24 weeks of gestational age. T cell functions were investigated by E-rosseting, by their PHA response and in mixed lymphocytes cultures (MLC) and by the cell mediated lysis (CML). The CFU-C-content of a 20 g fetal liver at the age of the 18th to the 20th week is approximately 1 x 10^7 CFU-C, which is equivalent to the average recommended dose for achieving a graft with adult bone marrow. If BFU-E are taken as an indicator for the haemopoietic potential of the graft, fetal liver should be superior to adult bone marrow since the BFU-E concentration in fetal liver is approximately three times higher than in adult bone marrow.

There seems to be an age dependent stimulatory function of fetal liver cells in the MLC, which is always weaker than fetal bone marrow and adult controls. There are no effector cells (T cells) in fetal liver judging from the cell mediated lysis (CML). This indicates that fetal liver may be an alternative (better?) candidate as adult bone marrow for transplantation in respect to GvHD.

097 DEPRESSED SPECIFIC CELLULAR IMMUNITY TO CYTOMEGALOVIRUS AND DECREASED LYMPHOKINE PRODUCTION IN BONE MARROW TRANSPLANT RECIPIENTS. Y. Bryson, S. Gard, B. Ank, E.R. Stiehm, UCLA School of Medicine, Los Angeles, Ca.

Because of the increased susceptibility to viral infection particularly cytomegalovirus (CMV) in bone marrow transplant patients (BMT), we assessed CMV antibody, CMV transformation, monocyte migration inhibition factor (MIF) and interferon (IF) production in 38 BMT serially and 35 normals. Separated mononuclear cells were stimulated with CMV-antigen, phytohemagglutinin (PHA), newcastle disease virus (NDV), and supernatants were assessed for MIF by an agarose technique and for IF by a microtiter assay using human diploid cells and encephalomyocarditis virus challenge. PHA induced MIF production was 70% of controls 0-3 mos. post transplant and returned to normal by 12 mos. PHA induced (immune) and NDV induced (classical) IF were initially markedly depressed and increased slowly with time. Geometric mean PHA IF titers from 0-3 mos. were 8.3±5 SD IU, and from 3-6 mos. were 51±3 SD IU (normal 200±2 SD IU). Geometric mean NDV IF titers at 0-3 mos. were 4.8±6 SD IU and at 3-6 mos. were 48±2 SD IU (normal 160±2 SD IU). In CMV antibody positive BMT's CMV transformation was depressed for 0-3 mos. post transplant, mean index 3.6 vs. 14 in normals. Low CMV transformation indices were associated with CMV viral excretion early (0-3 mos.) post transplant. BMT recipients surviving CMV infection showed increased transformation to CMV antigen 4-12 mos. post transplant. CMV CMI and lymphokine production in BMT are severely compromised in the early post-transplant period and correlate with the time of greatest risk of severe CMV infection.

098 CELLULAR IMMUNITY TO HERPES SIMPLEX VIRUS, CYTOMEGALOVIRUS AND VARICELLA-ZOSTER VIRUS AFTER MARROW TRANSPLANT, Joel D. Meyers, Nancy Flournoy and E. Donnall Thomas, Fred Hutchinson Cancer Research Center and the University of Washington School of Medicine, Seattle Washington 98104

Nearly 50% of marrow transplant (MT) patients become infected with herpes simplex virus (HSV), cytomegalovirus (CMV) and varicella-zoster virus (VZV) within the first year after transplant. Fatal infection has occurred with all three viruses. The cellular immune response to these viruses was measured by the lymphocyte transformation response (LTR). Responses of MT patients were followed serially and compared with the responses of normal persons. Before transplant, MT patients with a history or serology consistent with previous infection had responses lower than normal, due primarily to patients with leukemia in relapse. Patients with aplastic anemia or with leukemia in remission were not different from normal. Responses to CMV and VZV were depressed in most patients for the first 100 days after transplant. In contrast, responses to HSV among seropositive patients recovered earlier. Recovery of the LTR to HSV was related in most instances to recurrent infection. Responses were lower among patients with leukemia and among patients receiving antithymocyte globulin, but the occurrence of graft-versus-host disease and the pretransplant donor response were less important factors. At one year, responses among most seropositive patients were again normal, associated with the lower frequency of virus infection by one year among long-term survivors.

099 PULMONARY FUNCTION AFTER MARROW TRANSPLANTATION, Steven C. Springmeyer, Ronald Silvestri, W.Kosanke, Darwin Petersen, Nancy Flournoy, E.Donnall Thomas, Fred Hutchinson Cancer Research Center, Seattle, Washington.

Pulmonary complications after allogeneic bone marrow transplantation (BMT) remain a significant problem. Interstitial pneumonia (IP) is the major cause of respiratory failure. We prospectively examined pulmonary function in patients referred for BMT during a two year period. The pulmonary function tests (PFT) included spirometry, lung volumes and a diffusion study (DLCO). Sixty three patients (20 with aplastic anemia, 43 with hematologic malignancy) were studied on admission and approximately every other week during hospitalization (mean of 6 studies per patient). Neither diagnosis nor conditioning regimen affected the PFT during the study period (mean of 66 days). Twenty five patients developed IP and these patients developed a decreasing DLCO which was generally less than 80% of their admission DLCO by week 3 after BMT. Additionally, the mean DLCO was significantly decreased more than a week prior to the diagnosis of IP. Eighteen patients studied developed graft-versus-host disease (GVHD). Their functional residual capacities (FRC) and residual volumes (RV) were increased compared to their admission values. The mean FRC and mean RV were greater than 120% of their admission values 2 weeks prior to the diagnosis of GVHD. Although airflow obstruction was not detected by spirometry, these increased volumes are most compatible with a compensatory mechanism for increased airways resistance. We conclude that the DLCO may be a sensitive test to identify patients developing IP and that patients with GVHD have alterations in pulmonary function that are suggestive of increased airways resistance.

Supported by Grant Number CA 18029, National Cancer Institute, DHEW.

100 STIMULATION OF ERYTHROID AND MYELOID COLONY FORMATION FROM HUMAN FETAL LIVER BY ACTIVITIES DERIVED FROM A T CELL LINE, E. Niskanen, A. Lusis, R. Champlin, A. Oki, M.J. Cline, R.P. Gale, and D.W. Golde, Department of Medicine, University of California School of Medicine, Los Angeles, CA 90024, and the Department of Internal Medicine, University of Virginia School of Medicine, Charlottesville, VA 22908, USA.

Human fetal liver cells were cultured in agar for myeloid colonies (CFU-C), in methycellulose for both erythroid colonies (CFU-E) and bursts (BFU-E), and in diffusion chambers implanted intraperitoneally in mice for myeloid colonies (CFU-DG). In all categories we were able to stimulate colony formation with conditioned medium obtained from human T cell line cultures (Mo-CM) (Golde et al, Blood 52:1068, 1978). Increments in CFU-C, CFU-F and BFU-E numbers indicated that Mo-CM contained both CSA and erythropoietin like activity (EPA). Following boiling in a water bath and gel filtration chromatography, it was possible to test the effect of these two partially purified factors separately. Surprisingly, a four fold increase in CFU-DG numbers could be achieved after intraperitoneal administration of the EPA fraction devoid of CSA. The results indicate that the two myeloid precursors, CFU-C and CFU-DG, can be differentiated by their responsiveness to EPA. The effect of EPA on primitive myeloid and erythroid precursors suggests that it in fact is a general hemopoietin.

101 FETAL HEMATOPOETIC CELLS PRODUCE ADULT HEMOGLOBIN WHEN THEY ARE TRANSPLANTED IN THE ADULT ANIMAL. Philip B. McGlave, E.D. Zanjani, A. Bhakthavathsalan and G. Stamatoyannopoulos, University of Minnesota, Minneapolis, MN 55455. Ohio Regional Perinatology Center, Toledo, OH. University of Washington, Seattle, WA.

In order to examine the role of the hemopoietic environment on the fetal to adult hemoglobin switching, we transplanted hemopoietic cells from fetal sheep into adult irradiated recipients, and examined the hemoglobins synthesized. The donor cells were from fetuses homozygous for Hb A, while the recipients were homozygous for Hb B (Hb A and Hb B are alleles at the Hb B locus). Donor cells were obtained from 100 gestational day fetuses; 95-98% of the Hb at this gegestational stage is fetal. Three BB recipients were supralethally radiated (920 rads) and each received 107 bone marrow and liver cells (per kg body weight). No matching was attempted. Two of the animals died (postransplant day 1 and 11) while one survived for 45 days. In this animal, WBC counts returned to normal by day 28, and there was reticulocytosis (peak at day 35). Hb A was electrophoretically detectable at 7 postransplant day and composed 36% of the animal's Hb by day 45. Hb F appeared at 7 day and it amounted to 4-6% at day 45. Similar Hb F levels were observed in a supralethally radiated Hb BB sheep transplanted with autologous bone marrow cells. The findings demonstrate that: a) fetal hematopoietic stem cellls are capable of repopulating the adult marrow of the sheep and b) the engrafted fetal stem cells produce erythroid progency which has switched to adult Hb formation. We conclude that the switch from fetal to adult hemoglobin id due to factors that operate throughout the postnatal period, and it is influenced by the cellular or humoral hematopoietic environment in which the progenitor cells differentiate.

102 THE ROLE OF INTERFERON ON HUMAN LYMPHOCYTE HISTOCOMPATIBILITY ANTIGEN EXPRESSION, I. Heron, Marianne Hokland, G. Garotta and K. Berg. The Institute of Medical Microbiology, University of Aarhus, Denmark.

Mononuclear cells from the blood of healthy normal humans were kept in cultures under nonstimulating conditions for 16 hours in the presence or absence of human interferon. The relative quantities of HLA- and β2-microglobulin on the cultured cells were determined by quantitative immunofluorescence using a fluorescence activated cell sorter, by the capacity of cells to absorb out cytotoxic antibodies against the relevant antigens and by direct radiolabelling employing monoclonal antibodies. Interferons of different origin and purities including completely pure human leukocyte IF enhanced the expression of HLA- and β2-microglobulins, whereas membrane immunoglobulins and antigens recognized by antiserum raised human brain and T-cells were the same on interferon treated and control cells. Similar interferon effects were observed on an EBV negative Burkitt lymphoma cell line.

The enhanced expression of histocompatibility antigen subsequent to interferon treatment was observed on B- and T-enriched lymphocyte population and was found dose dependent with optimum within "physiological" concentrations of interferon.

103 OBSTRUCTIVE PULMONARY DISEASE AFTER BONE MARROW TRANSPLANTATION (BMT), David D. Ralph, Steven C. Springmeyer, Keith M. Sullivan, and E. Donnall Thomas, Fred Hutchinson Cancer Research Center, Seattle, Washington, 98104.

Four patients (pts) developed chronic graft-versus-host disease (C-GVHD) and severe obstructive pulmonary disease after allogenic BMT for aplastic anemia (3 pts) and acute myelogenous leukemia (1 pt). Pts ranged in age from 15 to 31 years and were non-smokers. None had prior clinical evidence of obstructive lung disease and pulmonary function tests (PFTs) pretransplant were normal in the two pts tested. Extensive C-GVHD involved the skin, oral mucosa, and lacrimal glands (sicca) in all pts. Two had hepatic disease and three had esopageal involvement (dysphagia, reflux, or web formation). Recurrent bacterial infections (sinusitis and pneumonia) occurred in two pts, and immunologic deficiencies of delayed hypersensitivity were seen in all four. C-GVHD was treated with either prednisone alone (2 pts) or combinations of prednisone and cytotoxic agents (cyclophosphamide or azathioprine). PFTs obtained between day 270 and 709 post BMT showed mean one-second forced expiratory volume (FEV_1) was 34% of predicted; mean FEV_1 to vital capacity ratio was 46%. Bronchoscopy and biopsy in two pts revealed acute and chronic bronchitis with an increased percentage of neutrophils in the bronchoalveolar lavage fluid. Open lung biopsy in one pt showed minimal chronic pneumonitis. Pathologic mechanisms leading to obstructive pulmonary disease in C-GVHD may include bronchial sicca with impaired mucleiliary transport, indolent sinopulmonary infections, or recurrent airway damage from aspiration of gastric contents in pts with esophageal disease.

104 IN VIVO & IN VITRO X-RAY STUDIES ON CLONOGENIC HUMAN HEMATOPOIETIC AND LYMPHATIC CELLS S.L. Seagren, P.M. Calabro-Jones, J.E. Byfield, University of California, La Jolla, CA 92093

Whole body and half-body X-ray exposures are clinically useful in man for both bone marrow transplantation and in the palliative treatment of advanced cancer. Because quantitative information on the radiation sensitivity of clonogenic human lymphocytes is sparse and because little is known as yet as to the kinetics of human hematopoietic stem cells trafficking, we have studied these phenomena both in vivo and in vitro. In vivo studies have examined the effects of large dose half-body X-ray exposures (800-1,000 rads) on circulating CFU-c's assayed using leukocyte feeders in a modification of the technique of Iscove, et.al. (1971).

All such studied were done on patients with advanced cancer heavily pre-treated with chemotherapeutic agents. In all cases studied to date (N=6) there was a significant reduction in trafficking peripheral blood CFU-c's following X-ray exposure with a nadir (sometimes below measurable numbers) reached of a median of about 9 days followed by a recovery towards control values. Retreatment would appear feasible at about 21 days but has not been attempted as yet. The radiobiological properties of human T-cells were studied using an agar colony forming assay employing PHA stimulation. Human cells are being studied using a modified assay with PWM. Human T-cells show a typical radiation survival curve with a small shoulder (D_q=160 rads), a D_o of 160 rads, and an extrapolation number, N=2.7. Although radiosensitive human T-cells have a demonstrable capacity to repair sub-lethal X-ray damage, when evaluated for cycling related changes in cell radiation sensitivity no changes were found. This would suggest that T-cells are not rendered more radiosensitive by induction into cycling.

105 A STUDY ON THE AGING HEMOPOIETIC MICROENVIRONMENT, Kim I. Matthews and David A. Crouse, University of Nebraska Medical Center, Omaha, Nebraska 68105

Numerous investigations performed to study the effects of aging on the hemopoietic system have mainly focused on the functional capacity of aged hemopoietic stem cells (CFUs) rather than on the ability of aged marrow hemopoietic microenvironments (HM) to support such CFUs. The experiments described here examine in vitro the ability of aged versus young marrow derived HM to promote maintenance, proliferation and differentiation of young stem cells.

Young and aged male and female mice were sacrificed, their femurs flushed and the bone marrow cells inoculated into culture flasks. After three weeks an adherent monolayer became established and a second marrow inoculum from young donors was introduced into each flask. The nonadherent cell suspension was evaluated at various time points for cellularity, morphology and CFUs content.

Results of these experiments may be summaried as follows: a) aged HM supported significantly greater numbers of CFUs than young HM, with cultures derived from aged females being more supportive than those from males; b) cellularities were higher in the young and old female cultures than in the male cultures; c) one consistent morphological difference was the presence of enhanced megakaryopoiesis in cultures derived from aged animals.

One can conclude from these results that there are differences in the aged and young marrow in vitro hemopoietic microenvironments in their ability to support CFUs (the aged HM being more supportive than the young HM) and this in turn may affect the functional capacity of CFUs in aged animals. Supported by N.I.A. Grant AG 01455.

106 SUPPRESSION OF HUMAN MARROW CFU-E BY AUTOLOGOUS T LYMPHOCYTES. S.A. Rothmann, J.H. Finke, C.M. Varelzes, M. Krause, and J.K. Weick. Cleveland Clinic, Cleveland, OH.

To evaluate the role of suppressor T lymphocytes in the regulation of erythropoiesis, bone marrow (BM) was aspirated from 4 hematologically normal individuals, and separated on ficoll-hypaque to obtain a mononuclear cell preparation. This was depleted of T lymphocytes by rosetting with sheep red cells. Cells were cultured for CFU-e in microtiter plates with methylcellulose and erythropoietin (Ep). No CFU-e were present in the T cell fraction. T cell-depleted BM showed no decrease in CFU-e compared to whole BM, with 150 ± 36 CFU-e/10^5 cells. Addition of an equivalent number of T cells resulted in a 70% reduction in CFU-e to $46 \pm 23/10^5$ cells. An analysis of 16 consecutive cultures of normal BM samples, showed that $19 \pm 11\%$ T cells were present in 14 BM which grew CFU-e, whereas $56 \pm 3\%$ T cells were present in 2 BM which did not have CFU-e growth. In addition, we have found evidence for lymphocyte suppression of CFU-e in patients with aplastic anemia. BM from 11 patients with aplastic anemia was incubated with antithymocyte globulin (ATG) in the presence of Ep. Before ATG addition there was no growth of CFU-e in 9 BM and 8 and 50 colonies in 2 BM. After ATG addition normal CFU-e growth was observed in BM from 5/6 pediatric patients and in 1/5 adult patients. Together, these results suggest that suppressor T cells exert a normal regulatory function for erythropoiesis, at least at the level of CFU-e, and that some cases of aplastic anemia may be a result of overproliferation of this cell population. The ability to eliminate this cell population may be important for the success of bone marrow transplantation in such patients.

107 REGULATION OF PRE-B CELL GROWTH, Anthony R.Hayward,Paolo Paolucci,Lorna Layward & Nicholas Rapson University of Colorado Health Sciences Center & Institute of Child Health, London England.

Pre-B cells,identified by immunofluorescence as bone marrow cells containing small amounts of cytoplasmic IgM but lacking surface IgM are the least mature members of the B cell series whose regulation is accessible to study. $5 \pm 2\%$ of bone marrow cells from patients who had completed antileukemia treatment more than 3 months previously were pre-B cells compared with $1.2 \pm 1\%$ in controls. Both the percentage and absolute number of pre-B cells approximately doubled when control or post treatment marrow samples were cultured in RPMI 1640+20% FCS for 24 hours but significant numbers of B cells were not produced. The rise in pre-B cell numbers was blocked by BUdR,colchicine,cycloheximide and cytochalasin: the latter resulted in the appearance of binucleate pre-B cells. Addition of 10μg/ml of Con A to marrow cultures prevented the rise in pre-B cell numbers, as did heterologous Con A stimulated,but not control, T cells. Con A activated T cells treated with Mitomycin C did not prevent a rise in the number of pre-B cells. These results provide the first evidence that pre-B cells continue to divide during short term in vitro culture and that division alone is not sufficient for the production of B cells. They also indicate that pre-B cell division may be at least partly suppressed by T cells: this observation provides a possible explanation for the acquired deficiency of pre-B cells in hypogammaglobulinemic thymoma patients, in whom increased suppressor activity has been reported. Partly supported by grants from the Leukaemia Research Fund and the American Cancer Society.

108 T CELL INDUCTION OF ERYTHROID PROLIFERATION IN LEUKEMIC MICE. Eta Rena Bacon and Carol L. Reinisch, Sidney Farber Cancer Institute, Boston, MA 02115

Erythroid differentiation is normally regulated by T cells which enhance both CFU-E and BFU-E proliferation. To investigate how erythropoiesis is maintained in the malignant state, the influence of T cells on CFU-E maturation in experimentally induced lymphocytic leukemia was examined. Balb/c mice were inoculated with murine sarcoma virus (MSV) at three weeks. By 8 months, 100% of the mice developed leukemias bearing the TL, Thy 1.2 and Ly differentiation antigens. The leukemic spleens contained high levels of erythropoietin (epo) dependent CFU-E's (up to 16x that of control spleens) despite the fact that the spleen was the primary site of immunoblastic T cell proliferation. To determine if T cells from leukemic animals increased CFU-E differentiation, which could account for the elevated numbers of CFU-E's, the effect of purified T cells on CFU-E growth was assessed. For example, 5×10^4 purified T cells from normal or MSV leukemic mice were mixed with 5×10^4 normal bone marrow cells and cultured in the presence of epo on plasma clots for 48 hours. Without T cells, 36 CFU-E per 5×10^4 bone marrow cells grew out. However, in the presence of normal splenic T cells, 66 CFU-E/5×10^4 nl bone marrow cells were found. When MSV splenic T cells were added, 126 CFU-E/5×10^4 normal bone marrow cells grew. The Thy 1.2^+ tumor cells did not become CFU-E nor did they stimulate CFU-E differentiation, suggesting that T cells but not tumor cells enhance CFU-E differentiation. Furthermore, while the spleen had elevated CFU-E, levels of CFU-E in the bone marrow of the same animal were severely depressed. Our data suggest that the focus of CFU-E maturation in the leukemic animal shifts from the bone marrow to the spleen and that CFU-E differentiation is amplified by T cells in the splenic microenvironment.