RECENT ADVANCES IN BONE MARROW TRANSPLANTATION

Robert Peter Gale, Organizer

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Plenary Sessions

Poster Sessions

Is Bone Marrow Transplantation The Optimal Treatment of Acute Myelogenous Leukemia? A Forum

0105 IS BONE MARROW TRANSPLANTATION THE OPTIMAL TREATMENT FOR ACUTE MYELOGENOUS LEUKEMIA?, Karl G. Blume, Department of Hematology and Bone Marrow Transplantation, City of Hope National Medical Center, Duarte, CA 91010

Great strides have been made in the management of acute myelogenous leukemia during the past decade. After it was initially demonstrated that marrow ablation followed by allogeneic marrow transplantation from histocompatible sibling donors could cure a small but significant number of end-stage patients (the major reason for failure being leukemic recurrence), it was logical to utilize this treatment modality earlier in the clinical course, namely during complete first remission. Such studies with unselected patients in first remission under the age of 45 conducted since 1976 have resulted in reproducible rates of unmaintained disease-free long-term survival of 50-80%. Currently, over 250 patients have undergone allogeneic marrow transplantation during first complete remission of acute myelogenous leukemia. Younger patients appear to have better chances for successful outcome of marrow transplantation, possibly because of a reduced risk of graft-versus-host disease.

If marrow transplantation is carried out after a patient has suffered a leukemic relapse, chances for disease-free long-term survival drop to 10-40%, mostly due to an increase in recurrent leukemia. After first relapse of acute myelogenous leukemia, marrow transplantation represents the only curative treatment modality available.

Concurrent with advances in marrow transplantation, significant progress has been made in the area of conventional combination chemotherapy. Higher remission rates and longer duration of complete remissions are due to improved use of chemotherapeutic agents as well as more sophisticated supportive care. Remission rates of 60-85% are frequently being reported and 10-25% of patients who attain a complete remission appear to become long-term survivors. The use of maintenance chemotherapy does not seem to result in a prolongation of remission and improvement of survival.

Prospective studies on comparable groups of patients have shown a two to three-fold advantage in disease-free survival of transplanted compared to non-transplanted patients. Under the current circumstances, allogeneic marrow transplantation is the best possible therapy for patients under the age of 45 years who have a suitable donor.

Aplastic Anemia and Immune Diseases

0106 MARROW TRANSPLANTATION FOR APLASTIC ANEMIA. R. Storb, for the Seattle Marrow Transplant Team, Fred Hutchinson Cancer Research Center, 1124 Columbia Street, Seattle, WA 98104, USA.

One hundred seventy-five patients (pts) with severe aplastic anemia were treated in Seattle by cyclophosphamide (Cy) 50 mg/kg on each of 4 days, given marrow grafts from HLA-identical siblings, treated with methotrexate (MTX) postgrafting and observed for 2 3/4 to 11 1/2 years. Thirty-nine of these were untransfused before grafting and 136 had multiple prior transfusions. Forty-six of the 136 transfused pts received viable donor buffy coat cells in addition to the marrow inoculum and 90 did not. Long-term survival was 83% for untransfused pts, 70% for transfused pts given added buffy coat, and 45% for those without buffy coat. Graft rejection was the major problem in transfused pts occurring with a frequency of 32% in those not given buffy coat and 11% in those with buffy coat. Overall, 38 pts rejected; only 9 of these are surviving, 5 with successful second grafts and 4 with autologous marrow recovery. A binary regression analysis showed the following factors to have a significant association with a low risk for rejection: (1) high marrow cell dose, (2) negative relative response in mixed leukocyte culture, (3) added donor buffy coat cells, (4) female marrow donor, (5) absence of prior transfusions. A second major problem both in untransfused and transfused pts was graft-versus-host disease (GVHD). Of 130 pts with sustained 1st grafts who lived long enough to be evaluated, 55% had grade 0, 10% grade I, 10% grade II, 15% grade III and 10% grade IV acute GVHD. Thirty of the 34 pts with sustained 1st grafts who died had some form of GVHD. Not surprisingly, a proportional hazard regression analysis showed acute GVHD to carry the highest risk for death. Other factors with significant negative influence on survival were increasing pt age and the presence of refractoriness to random donor platelets at the time of grafting. Of note was that decontamination in a laminar airflow room increased survival from 68% to 86% ($p < 0.02$, log rank test). This was largely explained by a significant reduction in the incidence and delay of onset of acute GVHD among laminar airflow room pts even after stratifying for other risk factors. Chronic GVHD was more frequent among pts with acute GVHD, older pts, and pts given additional donor buffy coat. In addition to the data in Cy-conditioned and MTX treated pts, results will be reviewed with other approaches such as Cy in combination with total lymphoid irradiation, total body irradiation and thoraco-abdominal irradiation and the use of cyclosporine post grafting, either alone or in combination with MTX.

0107 CRITICAL ISSUES IN TRANSPLANTS IN APLASTIC ANEMIA

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Marrow transplantation employing genotypically identical HLA donors appears to be the treatment of choice in severe A. A. (1). Except in the patient who has a syngeneic donor, the ideal preparative regimen should be tolerable, induce sufficient immunosuppression to allow a high rate of complete lymphohematopoietic engraftment (2,3) and be relatively free of late sequella (4). Cyclophosphamide (CY) most closely approaches this ideal in the non-transfused patient, but a few graft rejections have been seen even in these otherwise ideal conditions.

In transfused patients marrow rejection may be seen in 30-50% of patients because of sensitization to the donor. The major cause of mortality was due to marrow graft rejection. A number of attempts were made to achieve a higher rate of engraftment. Higher engraftment rates were subsequently attained but survival was variable and in many cases the increased engraftment rate was balanced by increased mortality due to graft-versus-host disease (GVHD) and interstitial pneumonitis (I.P.) Series that have been reported as having high engraftment rates and long-term survival of 70% or above have been: CY and donor buffy coat (5), CY and total lymphoid irradiation (TLI) (6), CY and thoraco-abdominal irradiation with total lung shielding (7) and Cyclosporin A (CsA) used as pre- and post-treatment with the standard CY regimen (8). Unfortunately, most of these results have not been confirmed nor denied by other groups. Most workers have agreed that younger patients survive better than older patients but there is not uniform agreement as to why. Finally, some controversy exists as to whether a sex match or mismatch is an important determinant of survival. Clearly these are important issues when comparing various series. Since the number of centers for transplantation of aplastic anemia has markedly increased, it would appear that cooperative trials between institutions is at present a critical issue of prime importance. It will not, of course, be an easy matter to accomplish this where large centers are involved and where advances are continually being made to improve the complications such as GVHD or I.P. Without such an approach, however, I am somewhat pessimistic as to what the progress will be in the next few years.

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0108 IMMUNOSUPPRESSIVE THERAPY AND APLASTIC ANEMIA - ATG

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There is increasing evidence that many cases of aplastic anemia are immunologically mediated. These findings have resulted in studies of immunosuppressive therapy for marrow aplasia. Antilymphocyte sera/globulins (AL) have received the most extensive trials. Initial reports suggested that AL therapy markedly improved prognosis in aplastic anemia. However, these studies were difficult to interpret because of variable severity of aplasia, variable duration of aplasia, use of different antilymphocyte sources, short followup, and lack of appropriate control groups treated by standard medical means. Recently, the UCLA team reported that 8 of 15 patients with moderate to severe aplastic anemia who received antilymphocyte globulin (ATG) improved within three months. In contrast, none of the 17 patients who did not receive ATG responded within the same period (1). The Aplastic Anemia Study Group treated newly diagnosed severe aplastic anemia with antithoracic duct lymphocyte globulin (ALG), haploidentical marrow and androgen or with androgen alone (2). Life table survival of ALG treated patients plateaued at 74%; survival with androgens alone was 29%.

Although AL preparations increase survival in aplastic anemia, marrow recovery is usually incomplete. Additional problems regarding this treatment include: a) what is the target antigen(s)? b) do all AL preparations produce equivalent response rates? c) can toxicity be decreased by developing appropriate human monoclonal antibodies? d) can response be predicted? e) does the interval between diagnosis and treatment affect response? f) are haploidentical marrow or androgen necessary for optimal effectiveness? g) What are the appropriate indications for AL therapy vs. histocompatible bone marrow transplantation? Prospective, controlled trials are needed to answer these and related questions.

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0109 BONE MARROW TRANSPLANTATION IN HEMATOPOIETIC IMMUNODEFICIENCIES, Robertson Parkman, Joel M. Rappeport, Raif Geha, Jerome Ritz, Fred S. Rosen, and David G. Nathan, Children's Hospital Medical Center, Sidney Farber Cancer Institute, Boston, Massachusetts 02115.

Immunodeficiency states are due to abnormalities of the lymphoid stem cell, hematopoietic stem cell or both. Histocompatible bone marrow transplantation is an effective form of therapy for all three disease categories; however, the pre-transplant preparation of the patients varies with their primary disease. Lymphoid immunodeficiencies include severe combined immune deficiency (SCID), GPL-115 deficiency, etc. Patients with SCID have absence or defective lymphoid stem cells and require no pre-transplant preparation to permit histocompatible lymphoid stem cell engraftment. Patients with lymphoid immunodeficiencies characterized by the presence of abnormal lymphoid stem cells require chemotherapeutic preparation to eradicate their abnormal stem cells. Hematopoietic immunodeficiencies (chronic granulomatous disease, infantile agranulocytosis, actin deficiency) and diseases which involve both the lymphoid and hematopoietic stem cells (Wiskott-Aldrich syndrome) require preparation that can ablate their abnormal hematopoietic stem cells. Successful regimes have consisted of total body irradiation (800R), busulfan or dimethyl busulfan plus immunosuppression with anti-thymocyte serum and/or cyclophosphamide. In our experience total body irradiation has produced hematopoietic ablation in eight of eight recipients; however, four patients developed interstitial pneumonitis, all of whom died. Busulfan at the dose of 2 mg/kg x 4 days has produced sustained hematopoietic ablation in only six of nine patients necessitating the retransplantation of two patients following preparation with total body irradiation. No patients receiving busulfan have developed interstitial pneumonitis. The optimal regime for the ablation of abnormal hematopoietic stem cells is still unclear. Haploidentical transplantation of patients with lymphoid immunodeficiencies has been successful following the in vitro depletion of the donor bone marrow of immunocompetent donor T lymphocytes by treatment with monoclonal antibody and complement or lectin agglutination. Haploidentical transplantation for SCID has required chemotherapeutic preparation suggesting that non-immune mechanisms may be involved in graft rejection. The haploidentical transplantation of hematopoietic immunodeficiencies may be more difficult to achieve since the patients' normal lymphoid immunity will increase the likelihood of donor marrow graft rejection.

Acute and Chronic Leukemia

0110 ACUTE MYELOGENOUS LEUKEMIA, E. Donnall Thomas, Division of Oncology, The Fred Hutchinson Cancer Research Center, Seattle, WA 98104.

The Seattle Marrow Transplant Team has carried out a series of studies in patients with acute nonlymphoblastic leukemia (ANL) given marrow grafts from an HLA identical sibling. 1) Fifty-four patients with endstage refractory ANL were prepared with cyclophosphamide 60 mg/kg on each of 2 days (CY) followed by 1000 rad total body irradiation (TBI) and the intravenous infusion of marrow. Six patients continue in an unmaintained remission 7 to 11 years later. 2) Twenty-two patients with ANL in first remission were prepared with CY and 920 rad TBI. Twelve continue in unmaintained remission 5 to 7 years later. 3) In a prospective randomized study 53 patients in first remission were prepared with CY and either 1000 rad TBI single exposure or 200 rad on each of 6 days. Results were slightly better in the patients given fractionated irradiation ($P = 0.05$) with 26 patients living in remission 2 to 4 years later. 4) Sixty-two patients in first remission were prepared with CY and 1200 rad fractionated TBI. Post-grafting they were randomized to receive either Methotrexate or cyclosporine. The 1 year survival was 68% and 70% respectively with a plateau after that point extending out now to 2½ years. When all patients transplanted in first remission are considered together the actuarial relapse rate is only 16%. Death is principally related to graft-versus-host disease and opportunistic infections. Long-term survival, and presumed cure, is approximately 70% for patients under the age of 20, 55% for patients 20 to 30 and 35% for patients ages 30-50. 5) Seven of 37 of patients transplanted in first relapse are living in remission 2 to 6 years later. 6) Five of 25 patients transplanted in second remission are in remission after 2 to 6 years. These patients transplanted in relapse or in second remission show an approximate 50% probability of reucrrence of leukemia. These results, and the published results of other marrow transplant centers, will be discussed.

0111 ACUTE LYMPHOBLASTIC LEUKEMIA

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Treatment strategies for ALL have evolved into a 3-phase pattern consisting of remission induction, CNS prophylaxis and continuation therapy. The rationale for this approach has emerged from a series of clinical therapeutic trials with a relatively small biological base. The success of this approach, however, has been demonstrated and ALL is currently accepted as being curable in some cases (1). At this time there are 3 major areas of research emphasis. With increasing duration of survival, the problems of late consequences of disease and therapy have emerged. Current clinical studies must be designed to consider the potential for late effect and to make such an assessment an integral part of study design. Study of results to date must continue to identify all related factors. Surveillance for unsuspected problems must continue.

The current major obstacle for successful disease control is the emergence of drug resistant subpopulations of the original leukemic clone (2). Design of future therapeutic trials must focus on strategies to eliminate pre-existing resistant subpopulations and to prevent their development and emergence during therapy. One model which has been proposed (2) appears suitable for testing in ALL with appropriate modifications. Other models should be developed and designed for testing in clinical trials.

One of the most important developments of the past decade has been the growing number of clinical and laboratory features associated with specific clinical behavior (3). It is now understood that these prognostic factors are expressions of the biological characteristics of the leukemia cell population. The interrelationship of clinical features such as white blood count and laboratory characterization such as surface marker phenotype, proliferative activity and karyotype clearly indicate the need for more biological information. Of greatest importance is to determine the relationship of these known factors to the mechanisms by which drug resistance is acquired. On a clinical level, these prognostic factors improve our ability to identify patients most likely to benefit from specific therapeutic interventions such as bone marrow transplantation.

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0112 BONE MARROW TRANSPLANTATION FOR CHRONIC MYELOGENOUS LEUKEMIA (CML)

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Patients with CML have a poor prognosis. In the initial chronic phase (CP) of CML, patients can be symptomatically managed with chemotherapy. In nearly all patients, CML ultimately undergoes transformation to blast crisis (BC) which is highly resistant to treatment. Approximately half of patients develop an "accelerated phase" prior to BC. Survival has not been substantially effected by any conventional treatment.

We surveyed the results reported from 13 centers that have evaluated high dose chemoradiotherapy and bone marrow transplantation for CML. Three sources of hematopoietic cells have been utilized, autologous peripheral blood and marrow cells (auto Tx), marrow from an identical twin (twn Tx), or allogeneic marrow from an HLA identical sibling (allo Tx). Forty-two patients with BC received auto Tx with the intent of reestablishing a second chronic phase. Median survival was only 6 months largely due to relapse of BC. Sixteen patients with BC received twn Tx; the actuarial relapse rate is 63% and disease free survival (DFS) is 19% at 30 months. Forty-five patients received allo Tx while in BC, only 5 survived more than 1 year, actuarial DFS is 8%. Seventeen patients in CP received twn Tx, 3 relapsed and 13 remain in complete remission at 2 to 6 years with actuarial DFS 65%. Sixty-two patients received allo Tx while in CP; only one has relapsed, actuarial DFS is 75% at 2 years. These results must be interpreted and with caution since the median followup is only 8 months. The results have been less favorable in 50 patients receiving allo Tx during an accelerated phase. Only 3 have relapsed but actuarial DFS is 35% at 2 years. Fourteen patients who entered BC but achieved a second CP with intensive chemotherapy were transplanted at that time. Actuarial DFS is 71%.

These data indicate that high dose chemoradiotherapy and bone marrow transplantation from an identical twin or HLA identical sibling donor can produce prolonged complete remissions free of detectable Ph^1 chromosome positive cells in patients with CML. The optimal chance to control CML lies in marrow transplantation during the chronic phase of the disease.

0113 BONE MARROW TRANSPLANTATION IN LEUKEMIA: RECENT ADVANCES AND CRITICAL ISSUES, Robert Peter Gale, Transplantation Biology Unit, UCLA School of Medicine, Los Angeles, CA 90024 and The International Bone Marrow Transplant Registry, Milwaukee, WI 53201

Bone marrow transplantation is increasingly used to treat leukemia including ALL, AML and CML. Recently, there have been major advances with regard to transplant outcome; most important is a marked reduction in the rate of leukemia relapse when the transplant is performed before the leukemia is advanced. These data, summarized in the Table, indicate: (1) A low rate of relapse and ~50% survival in AML in 1st remission; (2) An extremely low risk of relapse & >60% survival in CML in chronic phase. Results in ALL are more difficult to interpret. Most data indicate high relapse rates & correspondingly poor survival in transplants in 2nd or later remission; some centers have reported favorable results in 2nd but not later remission. There are too few transplants to critically analyze results in ALL in 1st remission but recent data indicate ~50% survival.

How do these data compare to results of chemotherapy alone? Phase-3 prospective randomized trials are available only in AML in 1st remission. Results of the UCLA study in >75 patients indicates a decreased risk of leukemia relapse (20% vs 60%) and improved survival (50% vs 35%) in transplant recipients. Data from the CCSG & SKMI trials will also be reviewed. A transplant benefit in AML may be greatest in a subset of individuals ≤20yrs. Data from concurrent chemotherapy controls are highly variable & can be used to either prove or disprove the superiority of transplantation.

Results in ALL are even more difficult to interpret & no phase-3 studies have been reported. Transplants may have a higher salvage rate in ≥2nd remission or relapse. Critical data supporting transplants in 1st remission is lacking. In CML there is also a lack of controlled trials; because the disease is incurable with chemotherapy alone it should be possible to determine transplant efficacy if late relapses do not occur in current survivors. Hopefully, phase-3 trials in ALL & CML will be initiated to investigate these questions.

	AML		ALL			CML	
	1st	≥2nd*	1st	2nd	≥3rd*	Chronic	Acute
n	>300	>500	20	>75	>200	30	>75
Relapse°	15	> 60	<10	30-60	> 60	0	>60
Survival°	50	15	50	20-50	15	>60	15

*includes relapse °actuarial 1-2yr

0114 ALLOGENEIC BONE MARROW TRANSPLANTATION (BMT) IN ACUTE LEUKEMIA (AL) IN FIRST REMISSION IN PATIENTS WITH POOR PROGNOSIS FOR REMISSION DURATION.

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BMT has been introduced as an intensification treatment for patients with AL in remission. A significant fraction of patients with adult AL can achieve long term disease-free survival with chemotherapy alone. Therefore, BMT was done only in patients in early first remission with a poor prognosis for continuing complete remission (CR) at one year determined according to a logistic regression equation. Favourable characteristics consisted of abnormal cytogenetics (8-21 translocation or a single hyperdiploid clone), a high differentiation ratio, an increased precentage of eosinophiles in the bone marrow, acute promyelocytic leukemia, fast cytoreduction and the morphologic subtype of AML. BMT was carried out after conditioning with piperazinedione at a dose of 25 mg/m^2 twice and total body irradiation of a total dose of 1020 rads given in 6 fractions over three days. All patients were treated in laminar airflow units. Eight patients were transplanted in first remission. Seven patients are in continuous CR with a median follow-up of 16+ months (2+ to 25+). One patient died in CR, 18 months after bone marrow transplantation due to chronic graft versus host disease (GVH). Grades III to IV GVH occurred in four patients and responded to high dose methylprednisolone treatment in all cases. Interstitial pneumonia occurred in one patient. This transplant group was compared with 45 patients who did not have suitable donors and received chemotherapy only, consisting of AMSA, Vincristine, cytosine arabinoside and prednisone. Their median remission duration was 50 weeks. The difference of remission duration of the BMT group and the chemotherapy group is significantly different (P.04). The survival difference has not yet reached significance. Further patients will be entered into this program to confirm that allogeneic bone marrow transplantation is superior to conventional and experimental chemotherapy in a well defined patient population with a low probability of a significant remission duration.

Graft-Versus-Host Disease

0115 IMMUNE BASIS OF GRAFT-vs-HOST DISEASE (GVHD) IN MAN, M-S. Tsoi, S. Dobbs, E. Santos, T. Mori, E. D. Thomas, R. Storb. Fred Hutchinson Cancer Research Center, Seattle, Washington, 98104.

This report summarizes the results of our in vitro immunologic studies in patients (pts) given HLA-identical allogeneic marrow grafts for the treatment of leukemia and aplastic anemia. Following are the results and conclusions: (1) Dense cutaneous Ig and C' deposits were found in 86% of pts with chronic GVHD, and faint deposits in 39% pts with acute GVHD compared to faint deposits in only 11% of marrow donors or pts without GVHD. Results suggest that humoral immunity plays a role in GVHD. (2) Cells from the marrow donors did not respond to host cells in a mixed leukocyte culture (MLC). After transplantation cells from 27 of 76 (36%) pts (i.e. donor cells in the recipients) with chronic GVHD showed positive MLC responses to host cells compared to only 2 of 22 (9%) long-term pts without GVHD ($p < 0.02$). Results suggest an involvement of an anti-host cellular immune response in chronic GVHD. (3) Cytotoxic T cells directed specifically against host fibroblasts were found in 14 of 22 (64%) pts with acute GVHD compared to 3 of 13 (23%) pts without GVHD ($p < 0.05$). The time of testing was crucial in the detection of anti-host cytotoxicity. When tests were done within 1-2 mos of onset of GVHD, 13 of 15 (87%) pts with acute GVHD were found to have anti-host cytotoxic cells. Cytotoxicity against the host may be responsible for the manifestation of acute GVHD. (4) Nonspecific suppressor cells inhibiting MLC reactivity of donor cells to unrelated alloantigens were found in 47 of 95 (49%) pts with extensive chronic GVHD, in 5 of 22 (23%) pts with subclinical chronic GVHD ($p<0.05$) and in 4 of 54 (7%) long-term pts without GVHD ($p<0.00001$). They were rarely seen in short-term pts with (7 of 40 cases) or without (2 of 17 cases) acute GVHD. These cells may be involved in the immune deficiency associated with chronic GVHD. (5) Specific suppressor cells inhibiting MLC reactivity of donor cells to trinitrophenyl-modified host cells were found in 19 of 26 (73%) long-term healthy pts compared to 9 of 43 (21%) pts with chronic GVHD ($p<0.0005$). Results suggest that graft-host tolerance is maintained by an active cellular suppressor mechanism. (6) A recent study showed that most pts with acute or chronic GVHD had impaired cell-mediated lympholysis (CML) reactivity to unrelated target cells, while most pts without GVHD had normal reactivity. The impairment in pts with acute, but not in those with chronic GVHD, could be corrected by in vitro addition of interleukin 2 (IL-2). Results suggest that defective IL-2 production is the basis of the CML impairment in acute GVHD and that the impairment seen in chronic GVHD is related to a defect in the effector cells (or their precursors) or the presence of nonspecific suppressor cells. (7) Further studies revealed that cells from most pts with acute or chronic GVHD were defective in IL-2 production. The basis of such defect is being investigated.

0116 PREVENTION OF GRAFT-VERSUS-HOST DISEASE, John H. Kersey, Lisa Filipovich, Ralph Quinones, Norma Ramsay, Richard Youle, David Neville and Daniel A. Vallera. Bone Marrow Transplantation Program, University of Minnesota, Minneapolis, MN and Laboratory of Neurochemistry, NIH, Bethesda, MD

Increasing evidence indicates that the T lymphocytes contaminating marrow grafts are a major cause of graft-versus-host disease (GVHD) in experimental animals. We have previously reported that the removal of T lymphocytes by monoclonal anti Thy 1 or Lyt 1 antibody plus complement ex vivo will permit marrow transplantation across the H-2 histocompatibility barrier without GVHD in the murine model. Recent evidence from our group indicates that Thy 1 antibody linked to the potent toxin, ricin, will permit successful marrow transplantation across the H-2 barrier without the need for complement.

Our studies in humans indicate that the human marrow aspirated for transplantation is contaminated with a significant number of T lymphocytes. We have utilized several means to remove and/or inactivate contaminating T lymphocytes. A randomized controlled study in HLA matched donor-recipient combinations demonstrated that combination of anti-T cell globulin with prednisone and methotrexate (mtx) post grafting was more effective than mtx alone when administered to patients in vivo. Donor T cell removal ex vivo using soybean lectin plus E rosette depletion has resulted in engraftment without GVHD in three cases of lethal immunodeficiency in which related but nonmatched donors were used. These results suggest that T cell removal or inactivation from donor marrow permits reduction in GVHD in both matched and mismatched donor-recipient combinations.

0117 REACTIVITY OF SYNGENEIC AND ALLOGENEIC GRAFTED CELLS AGAINST TUMORS. Philip D. Greenberg, Martin A. Cheever and Alex Fefer, Univ. of Washington, Seattle, Wa. Although in vitro killing of tumor cells by lymphocytes recognizing tumor-associated or histocompatibility membrane antigens is readily demonstrable, harnessing such antitumor reactivity for therapy of human tumors has been difficult. The recent demonstrations that some human tumors express antigens immunogenic to autologous lymphocytes and that lymphocytes present in an allogeneic marrow graft not only induce graft-versus-host disease but can also have an antileukemic effect in vivo have stimulated interest in adoptive transfer of syngeneic and allogeneic lymphocytes as a form of tumor therapy. Moreover, the development of animal models for treatment of advanced tumors has permitted elucidation of the requirements for such immunotherapy, the mechanisms of in vivo tumor lysis and the factors limiting efficacy.

Immunotherapy with autologous or syngeneic lymphocytes requires generation of cells specific for tumor-associated antigens and must overcome obstacles imposed by the large tumor burden. Murine models have been developed in which disseminated leukemias and lymphomas can be eradicated by a combination of cytoreductive chemotherapy plus transferred immune syngeneic cells. The effector cells operative in vivo are immunologically specific H-2 restricted $\overline{I}$- cells which must mediate a prolonged antitumor effect in the host. Infusion of the Lyt $1^{-}2^{-}$ subset of donor T cells, which contains amplifier and DTH effector cells but not cytotoxic cells, is nearly as effective as unfractionated T cells in tumor therapy, implying that the imperative donor contribution to tumor elimination is not a CTL [1]. The contribution of host T cells to tumor elimination appears limited, since the tumor-reactive cells detectable in the host even long after curative therapy are almost entirely of donor origin.

Therapy of human tumors by similar approaches which should be possible in the future will require generation of immune autologous cells. Technology now exists for selective expansion of even weak immune responses by in vitro sensitization followed by culture with the lymphokine, Interleukin 2. Such long-term cultured immune T cells have been shown to function in specific adoptive therapy of murine tumors. Moreover, the therapeutic activity of such IL 2 dependent T cells can be enhanced by in vivo administration of IL 2 after cell transfer [2].

Allogeneic cells, which recognize histocompatibility rather than more unique tumor-associated antigens, have therapeutic potential but can be toxic to and rejected by the host. H-2 identical, background-disparate donor T cells transferred into lethally irradiated hosts can persist and mediate an antitumor effect, but also can induce fatal GVHD. By contrast, similar allogeneic T cells infused into cyclophosphamide treated hosts induce an antitumor effect and minimal GVHD, but are noncurative presumably due to rejection. We are examining means for modulating T cells in vivo to facilitate the antitumor effect and diminish the GVHD.

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0118 BIOLOGY AND TREATMENT OF CHRONIC GRAFT-VS-HOST DISEASE (GVHD). K.M. Sullivan, R. Storb and E.D. Thomas. Fred Hutchinson Cancer Research Center, Seattle, WA, 98104. Chronic GVHD is an important late development affecting 25-40% of long-term survivors of allogeneic marrow transplantation. Clinical, pathologic and immunologic studies reveal features similar to several autoimmune diseases. Ninety-seven patients transplanted in Seattle between 1972 and 1979 developed chronic GVHD. Multivariate analysis has revealed several risk factors independently associated with the development of this syndrome: increasing patient age, unirradiated donor buffy coat infusions and prior acute GVHD. The diagnosis of chronic GVHD rests upon detection of typical clinical and pathologic lesions 100+ days after transplant. The most frequently involved tissues include: skin (dyspigmentation, erythema, poikiloderma, induration, contracture) liver (cholestasis), oral mucosa (atrophy, erythema, lichenoid changes), eye (sicca) and esophagus (stricture). Histologic diagnosis is most easily established by skin and oral biopsies which reveal inflammatory infiltrates, lichenoid reactions, eosinophilic bodies and fibrosis. Knowledge of expected target organ involvement permits the clinician to perform "screening studies" at day 100 to detect early subclinical skin, oral, liver or lacrimal GVHD and allows treatment to start before clinical deterioration. Two forms of chronic GVHD have been described. Patients with limited disease (skin and/or liver only) have a favorable outcome without treatment. In contrast, only 20% of patients with untreated extensive (multiorgan) disease survive free of disability. Early treatment with combinations of prophylactic antibiotics and immunosuppressive drugs (prednisone and azathioprine) appears to alter this adverse natural history and results in 70-80% long-term survival. However, certain features may be associated with poor outcome in spite of therapy and include: 1) early onset of chronic GVHD ($<$ day 120), 2) persisting thrombocytopenia ($<$ 100,000), and 3) "progressive" disease onset following acute GVHD. The pathogenesis of this syndrome is still poorly understood. Chronic GVHD patients have been found to lack "specific" suppressor cells that are thought to help mediate graft-host tolerance in healthy long-term survivors. In contrast, nonspecific suppression is observed in one-half of chronic GVHD patients and may account for the severe and prolonged immunodeficiency and frequent infections observed in these patients. Based on the increased risk for chronic GVHD in older adult marrow recipients and the findings of thymic gland atrophy and absent serum thymic factor levels in children with chronic GVHD, we asked if absent thymic regulation could be involved in the genesis of this syndrome. Initial attempts at prevention by transplantation of thymic tissues have not been successful and other approaches need to be explored. Of potential concern, however, is whether such

approaches may diminish the antileukemic effect observed in patients with chronic GVHD. The future challenge is to design methods to safely augment such a graft-vs-leukemia effect in patients at high risk for relapse.

Interstitial Pneumonia

0119 BIOLOGY OF NONBACTERIAL PNEUMONIA AFTER MARROW TRANSPLANTATION
Joel D. Meyers, Nancy Flournoy, Paul E. Neiman, E. Donnall Thomas
Fred Hutchinson Cancer Research Center, Seattle, WA 98104

Pneumonia due to causes other than bacterial or fungal infection has been common after both syngeneic and allogeneic marrow transplantation and has had substantial impact on the outcome of allogeneic transplant. Nonbacterial pneumonia occurs most commonly between 3 and 10 weeks after transplant, though cases continue to occur up to 6 months after transplant. The majority of cases after allogeneic transplant are due to either cytomegalovirus (46%) or "idiopathic" pneumonia (34%), with a smaller number due to other viruses (adenovirus, herpes simplex virus) and a decreasing number due to Pneumocystis carnii. Case fatality rate varies from approximately 60% for idiopathic pneumonia to 90% for biopsy-proven cytomegalovirus pneumonia. Review of 525 allogeneic transplant patients and 100 syngeneic transplant patients identified a series of risk factors for the development of pneumonia. The risk of pneumonia is significantly lower among syngeneic recipients (17%) compared with allogeneic recipients (41%), though interestingly the occurrence of idiopathic pneumonia is identical (11-12%). No cases of cytomegalovirus pneumonia have occurred after syngeneic transplant though these patients do acquire cytomegalovirus infection. The use of chemotherapeutic agents in addition to cyclophosphamide in twins with leukemia and the use of total body irradiation for conditioning among aplastic anemia patients receiving allogeneic transplants were associated with a significant increase in the risk of idiopathic pneumonia. These data strongly suggest that idiopathic pneumonia is due to pulmonary toxicity of chemotherapy and irradiation. The risk of cytomegalovirus pneumonia was increased significantly by the use of anti-thymocyte globulin or total body irradiation among allogeneic recipients with aplastic anemia, slightly by the occurrence of acute graft-versus-host disease among allogeneic recipients with leukemia, and by the occurrence of cytomegalovirus infection in general. The explanation for these findings is incomplete, but probably reflects combined effects of immunologic and virologic risk factors. The occurrence of idiopathic pneumonia should be influenced by reduction in the intensity of radiation or chemotherapy regimens while the occurence of cytomegalovirus pneumonia should be affected by reduction in rates of infection. Effective treatment for either condition remains to be established.

0120 ETIOLOGIC FACTORS IN TRANSPLANT-RELATED INTERSTITIAL PNEUMONITIS IN LEUKEMIA PATIENTS, Mortimer M. Bortin, International Bone Marrow Transplant Registry, Mount Sinai Medical Center, Milwaukee, WI 53201 and Alfred A. Rimm, Division of Biostatistics/ Clinical Epidemiology, Medical College of Wisconsin, Milwaukee, WI 53226

Comprehensive data reported to the International Bone Marrow Transplant Registry for patients with acute lymphocytic leukemia, acute myelogenous leukemia and chronic myelogenous leukemia treated with allogeneic bone marrow transplantation were analyzed for factors associated with interstitial pneumonitis. Both univariate and multivariate statistical methods were utilized. Included among the prognostic factors studied were age and sex of patient, age and sex of donor, sex match, disease, disease status, number of remissions, ABO match, HLA match, MLC match, number of pretransplant transfusions, interval from diagnosis to transplant, year transplanted, chemoradiotherapy regimen and severity of graft-vs-host disease (GVHD). Particular emphasis was placed upon factors previously reported to be associated with development of interstitial pneumonitis: dose of bone marrow cells/kg body weight, number of pretransplant transfusions, drug used to prevent or modify GVHD, total dose of total body irradiation (TBI), fractionated TBI, lung shielding and dose-rate of TBI. The focus of the presentation will be on those prognostic factors associated with development of interstitial pneumonitis which can be controlled by the referring physician and/or by the transplant team.

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0121 PREVENTION AND TREATMENT OF INTERSTITIAL PNEUMONIA ASSOCIATED WITH BONE MARROW TRANSPLANTATION, Drew J. Winston, Winston G. Ho, Richard E. Champlin, and Robert P. Gale, Department of Medicine, UCLA Center for the Health Sciences, Los Angeles, CA 90024.

Interstitial pneumonia occurs in approximately 40% of all patients undergoing allogeneic bone marrow transplantation and is fatal in at least 70-80% of the cases (1,2). Cytomegalovirus (CMV) has been the organism most often isolated from patients with interstitial pneumonia and is associated with approximately 60-70% of the cases. The other cases are mostly idiopathic and perhaps related to the effects of the radiation and chemotherapy used to prepare the patient for transplantation. Because CMV has been the candidate pathogen most often isolated from marrow transplants with interstitial pneumonia, most efforts to treat or prevent interstitial pnemonia have focused on CMV. Although recent studies of the restriction endonuclease patterns of CMV isolates have demonstrated that some post-transplant CMV infections are caused by pre-existing endogenous, pre-transplant CMV strains (3), many of these infections are likely related to the transfusion of exogenous latent virus present in blood products, including leukocyte transfusions (4). Attempts to treat established CMV interstitial pneumonia with antiviral agents (adenine arabinoside, partially purified leukocyte interferon derived from human cells, acyclovir, or combinations of these agents) have generally been ineffective. Thus, efforts have been made to prevent CMV interstitial pneumonia. Prophylactic adenine arabinoside or partially purified leukocyte interferon have been tried unsuccessfully and have been limited by their marrow toxicity. In contrast, initial studies at several transplant centers of CMV hyperimmune plasma or globulin have shown that passive immunization can effectively prevent severe CMV infections and interstitial pneumonia (5,6). Other ongoing studies using intravenous CMV hyperimmune globulin preparations are attempting to confirm these encouraging results. Similar studies evaluating the prophylactic effects of highly purified recombinant leukocyte interferon and of leukocyte-poor blood products from CMV-seronegative blood donors are needed. The effect of modifying the dose and rate of delivery of the pre-transplant total-body irradiation on the incidence of idiopathic interstitial pneumonia also needs further investigation in well-standardized trials (7).

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Immune Deficiency

0122 EXPERIMENTAL MODELS OF TRANSPLANT-RELATED IMMUNE DEFICIENCY, Erna Möller, Departments of Immunobiology and Clinical Immunology, Karolinska Institute Medical School, Wallenberglaboratory, S-104 05 Stockholm 50.

A summary of the basic mechanisms of graft-versus-host reactions will be given. Special emphasis will be on activation and suppression of host immune reactivity by transplanted immunocompetent cells. Furthermore, the presentation will include a review on the genetic disparities that may induce GvH reactions.
A discussion on specific immunological repertoirs of the T cell population, MHC restriction and its relevance for alloreactivity and reactivity against virus-infected syngeneic cells will be presented.

0123 IMMUNE DEFICIENCY AFTER HUMAN MARROW TRANSPLANTATION. R. Witherspoon, L. Lum, R. Storb, E. D. Thomas. Fred Hutchinson Cancer Research Center, Seattle, WA, 98104.
Since the first report of immunologic recovery after allogeneic human marrow transplantation in 1972, our understanding of the tempo of immunologic reconstitution has broadened through the work of many investigators. Studies using surface markers reveal that the peripheral blood is repopulated with surface immunoglobulin bearing cells by 2-3 months post-grafting. Compared to normal B cells, these B cells contain more μ determinants per cell suggesting that the B cells are immature early after grafting. Numbers of OKT4 T cells are lower than normal early after grafting and may remain low for more than 2 years. OKT8 cells rapidly recover to higher than normal levels and may remain high for more than 5 years regardless of clinical graft-versus-host disease (GVHD). Serum levels of IgG and IgM are low for 3-4 months and rise to normal therafter. IgA levels remain low for more than 1 year. Patients with chronic GVHD may have abnormally high IgG and IgM levels. Serum antibody responses to specific antigens bacteriophage, keyhole limpet hemocyanin and pneumonoccal polysaccharides are low in all patients in the first 3-4 months post-grafting. As patients become healthy long term survivors their responses normalize but responses in chronic GVHD patients remain low regardless of treatment for chronic GVHD. Skin testing to recall and neoantigens suggests that the cellular arm of the immune response is impaired for up to 4 years after grafting. These immunologic deficiencies suggest that chronic GVHD patients may be susceptible to bacterial illnesses and that all patients may be susceptible to viral infections for several years post-grafting. The pace of immunologic recovery after syngeneic marrow grafting closely resembles that of allogeneic recipients who do not develop GVHD. A number of workers have studied the mechanism of combined humoral and cellular immune deficiencies by using in vitro assays of lymphocyte proliferation and immunoglobulin secretion. Proliferation of transplant recipient lymphocytes is depressed in the early post-grafting period. The mechanism of this depression is unknown. Healthy long term survivors develop proliferation responses similar to those of the marrow donor. However, long term survivors with chronic GVHD have poor responses in proliferation assays due to suppressor cells. The mechanism of depressed antibody production in vitro early after grafting can be explained by a spectrum of abnormal lymphocyte functions of B cells, T helper cells and T suppressor cells. These functions normalize except in long term survivors with chronic GVHD. OKT4 and OKT8 cells do not predictably function in marrow graft recipients in classic helper and suppressor roles, respectively, described for normal people and certain congenital immunodeficiency patients. These data suggest that the combined humoral and cellular immunodeficiency after marrow grafting eventually recovers in healthy long term survivors but remains abnormal in patients with chronic GVHD. The deficiencies may result from the time required for lymphocytes to repopulate the host and develop mature regulatory functions.

0124 DIFFERENTIATION OF HUMAN T LYMPHOCYTES AND THEIR FUNCTIONAL REPERTOIRES: IMPLICATIONS FOR UNDERSTANDING IMMUNODEFICIENCY STATES. Ellis L. Reinherz, Stefan C. Meuer and Stuart F. Schlossman, Division of Tumor Immunology, Sidney Farber Cancer Institute, Boston, Massachusetts 02115.

Three discrete stages of thymic differentiation have been defined on the basis of reactivities of monoclonal antibodies to T lineage specific cell surface glycoproteins. During the process of differentiation, two independent T cell subset lineages are generated. One subset expresses the 62KD T4 glycoprotein whereas the second, mutually exclusive subset expresses the 76KD T8 glycoprotein. Clonal analysis of these cells indicates that the T4+ lymphocytes are triggered by class II (Ia) MHC molecules and provide inducer regulatory function whereas T8+ lymphocytes recognize class I (HLA) MHC molecules and mediate suppressor function. In this regard, the T4 and T8 molecules themselves serve as the associative recognition structures for the MHC gene products. In addition, all mature T cells express the 20KD T3 glycoprotein which is linked to a polymorphic antigen specific recognition structure. Examples of immunodeficiency disorders related to blocks of various stages of differentiation (i.e. severe combined immunodeficiency) as well as imbalances in the mature T cell subsets (i.e. acquired agammaglobulinemia) will be discussed.

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0125 THERAPEUTIC APPROACHES TO THE IMMUNODEFICIENT STATE AFTER MARROW TRANSPLANTATION. Kerry Atkinson, Department of Haematology, St. Vincent's Hospital, Sydney, Australia

Apart from bacterial and fungal infections most commonly associated with early post transplant neutropenia, the major infectious complications of marrow transplantation are cytomegalovirus (CMV) interstitial pneumonitis, varicella-zoster (VZ) infections and the late bacterial infections associated with chronic graft-versus-host disease (GVHD). Each of these three latter complications are due at least in part to the severe combined immunodeficiency which is a universal finding in marrow transplant recipients in the early period after grafting. Most of the parameters of this immune deficiency are uninfluenced by the genetic identity of the reconstituting marrow inoculum (syngeneic or allogeneic) or by the presence or absence of acute GVHD. The two major factors influencing immunological recovery are time post transplant and the presence of chronic GVHD. Given enough time and the absence of chronic GVHD, the new immune system of the marrow transplant recipient will mature normally. Chronic GVHD is the major clinical determinant for risk of late bacterial and fungal infections, while the non-specific suppressor cells found in approximately half the patients with chronic GVHD are responsible for an increased risk of late VZ infections. New therapeutic approaches to these infections include the use of CMV immune globulin for interstitial pneumonitis and human leucocyte interferon for VZ infections. Therapeutic approaches to the immune deficiency have met thus far with little success, although several observations pertinent to the possible causes of the slow development of T cell immunity have provided a rationale for a therapeutic approach. Firstly, the thymus was markedly atrophic in patients dying after marrow allografts. Furthermore, serum levels of the thymic hormone Facteur Thymique Serique (FTS) were low in all patients at three months and remained low in patients with chronic GVHD. Transplantation of allogeneic human thymic tissue, however, was unsuccessful in altering the pace of immune reconstitution or in preventing chronic GVHD. Administration of thymosin fraction V also failed to result in acceleration of immune reconstitution; the synthesis of FTS, thymopoietin pentapeptide and thymosin α_1 now makes further effort in this area possible. Interleukin-2 (IL-2) may also become available for therapeutic use; however, the poor responsiveness in vitro of purified patient T cells to exogenously supplied IL-2 argues against its likely therapeutic usefulness. The poor responsiveness was due, at least in part, to a low precursor frequency of T cells capable of responding. Finally, although the use of cyclosporin A was not associated with a faster pace of immune recovery than that associated with methotrexate, continuing efforts to decrease the incidence and severity of chronic GVHD, if successful, should result in an improvement in the rate of immune recovery.

Autotransplants In Cancer

0126 CRITICAL ISSUES IN AUTOLOGOUS MARROW TRANSPLANTATION. C. Dean Buckner, Fred Hutchinson Cancer Center, Seattle, WA 98104.

Methods for the cryopreservation of human marrow have been available for over 25 years. Effective therapeutic use of cryopreserved marrow has yet to be realized. Most of the problems that have inhibited progress in the past remain but there is more appreciation of the inherent difficulties of this approach by investigators in the field. The revival of enthusiasm for research in the area of autologous marrow transplantation comes primarily from the success of allogeneic and syngeneic marrow transplantation studies. This is appropriate but it is unlikely that results of autologous marrow transplantation will ever surpass the results of syngeneic transplants. However, the principles learned from allogeneic and syngeneic transplantation are germane to planning and evaluating studies of autologous transplantation. Marrow transplantation, in general, has been limited to the hematological malignancies as they are responsive to chemoradiotherapy regimens which are truly limited by marrow toxicity. It's no surprise that there is a dearth of reports of allogeneic and syngeneic transplants for solid tumors. This paucity of data results from the fact that curative transplant treatment regimens for patients with solid tumors that are clearly dose limited by marrow toxicity have not been developed. Progress in autologous transplantation for solid tumors must await the development of more effective treatment regimens. Hopefully more syngeneic transplants for solid tumors will be performed in the near future to help develop effective treatment programs.

Even when discussing "curative treatment regimens" for acute leukemia it must be kept in mind that with the single exception of patients with ANL in 1st remission, the majority of patients with leukemia and lymphoma who receive allogeneic or syngeneic marrow transplants are doomed to relapse if they survive the nonleukemic complications. This makes the evaluation of marrow purging techniques very difficult as these trials will necessitate large numbers of patients. Effective "ex vivo" techniques to eradicate tumor in cryopreserved marrow may well be developed in the near future. The difficulty of evaluating the effectiveness of such techniques will be great. First it has not been demonstrated that tumor cells in cryopreserved remission marrow contribute to post transplant relapses. In addition, most patients will relapse because of residual host tumor cells making better patient selection or the development of more effective means of preventing host relapses mandatory. It is possible that we may soon have 2 technologies available, marrow cryopreservation and "ex vivo" purging of contaminated marrow, neither of which can be utilized effectively without a radical change in our ability to eradicate malignant cells in the patient or without improving patient selection.

New Approaches To Recipient Without Donor

0127 MARROW TRANSPLANTATION FROM DONORS OTHER THAN HLA GENOTYPICALLY IDENTICAL SIBLINGS, John A. Hansen, Reginald A. Clift, Patrick G. Beatty, Eric M. Mickelson, Brenda Nisperos and E. Donnall Thomas, Medical Oncology, Fred Hutchinson Cancer Research Center, Puget Sound Blood Center, and the Department of Medicine, University of Washington, Seattle, WA 98104

As of June 30, 1982, 78 patients in Seattle have received marrow grafts from donors other than HLA genotypically identical siblings for the treatment of a hematological malignancy. Thirty-one patients had ALL, 36 had ANL, 8 had CML, 1 had Burkitt's lymphoma, and 2 had preleukemia. Thirty-five were in remission and 43 in relapse at the time of transplant. Forty-two transplants were between a parent and child (41 parent to child, 1 son to father), 1 was from an uncle, 1 from a great aunt, 33 from a sibling and 1 from an unrelated donor. In all cases where the donor and recipient were related (N = 77), donor and recipient were genotypically identical for one haplotype ("haploidentical"). Ten of 77 related patients received marrow from a donor who in addition was phenotypically identical for antigens of the nonshared haplotype, and 67 from a related donor who was haploidentical but incompatible for some HLA antigens of the nonshared haplotype. In 15 of the latter cases, donor-recipient incompatibility occurred as a result of intra-HLA recombination. One additional patient received marrow from an unrelated donor who was HLA phenotypically identical. As of October 1, 1982, 19 of 78 patients (24%) are surviving from 95 to 1763 (median, 800) days. Of the 43 patients transplanted in relapse, 4 (9%) are alive from 95-1163 (median, 691) days. By contrast, of the 35 patients transplanted in remission, 15 (43%) are alive from 113 to 1763 (median, 800) days. The relapse patients who are surviving include 0 of 15 patients with ALL (0%), 2 of 17 with ANL (12%), 1 of 8 with CML (13%), 0 of 1 with Burkitt's (0%), and 1 of 2 with preleukemia (50%). The remission patients who are surviving include 7 of 16 with ALL (44%) and 8 of 19 with ANL (42%). Results between HLA phenotypically identical and HLA "incompatible" transplants were not different: 4 of 11 phenotypically identical (36%) versus 15 of 67 (22%) "incompatible" recipients are alive. Eight patients were HLA-D,DR incompatible with their donor because of HLA-B/D recombination, and two (25%) are alive. The causes of death in this group included leukemia in 3 (days 64, 135, 241), interstitial pneumonia in 2 (days 28, 96) and veno-occlusive disease in 1 (day 7). None of the patients had GVHD with a grade of more than II (2 patients grade 0, 3 patients grade I, 1 patient grade II, and 1 patient not evaluable). For the entire series of 78 patients, the most common causes of death were leukemia (29%) and interstitial pneumonia (27%), while 13 of 62 deaths (21%) were attributable to GVHD. Graft rejection occurred in 3 of 78 (4%) patients. Although there is considerable heterogeneity among the patients in this study, the results of transplantation do not appear to be significantly different from those expected for patients receiving marrow grafts from HLA genotypically identical siblings.

0128 MONONUCLEAR CELL COLLECTIONS FROM THE PERIPHERAL BLOOD: POTENTIAL FOR USE IN AUTOTRANSPLANTATION, RA Abrams, G Messerschmidt, and AB Deisseroth, Medical College of Wisconsin, Milwaukee, WI 53226; Wilford Hall Medical Center, Lackland, TX 78236; University of California, San Francisco, CA 94100

Hematopoietic reconstitution following hematopoietically ablative levels of systemic antineoplastic therapy has been demonstrated with allogeneic, syngeneic, and autologous marrow infusions. In the autologous setting the ability to collect marrow from the bony pelvis may be compromised by clinically evident tumor involvement of the pelvic bones, prior pelvic irradiation, or chronic treatment related marrow hypoplasia. The ability to effect autologous hematopoietic reconstitution through the use of infusions of peripheral blood mononuclear cells could in theory overcome these difficulties either by obviating the need for bone marrow harvesting or by augmenting limited marrow availability. An accumulating body of data including the demonstration of multiple types of hematopoietic progenitor cells in the peripheral blood of both animals and man, technical advances in leukopheresis techniques, and success with peripheral blood hematopoietic reconstitution in canine models has suggested that stem cell numbers adequate to effect hematopoietic reconstitution might well be achieved clinically by harvesting circulating mononuclear cells in large numbers from the peripheral circulation. However, limited clinical trials involving identical twins have in two cases failed to demonstrate hematopoietic reconstitution from peripheral blood mononuclear cells. In order to explore further the possibilities for hematopoietic reconstitution in association with peripheral blood mononuclear cell infusions we have utilized a well established canine model to study the reconstitutive significance of clinically observed, chemotherapy induced, expansion of circulating hematopoietic progenitor numbers and the ability of peripheral blood mononuclear cells to augment the reconstitutive potency of quantitatively inadequate autologous marrow collections. In this canine model our data demonstrate: (a) a substantial augmentation in the reconstitutive potency of peripheral blood mononuclear cells alone when such cells are collected at times of cyclophosphamide induced expansion of circulating progenitor (CFUgm) numbers, and (b) that either with or without cyclophosphamide induced expansion of progenitor numbers, peripheral blood mononuclear cells permit hematopoietic reconstitution when infused in conjunction with marrow collections that would otherwise be quantitatively inadequate. Whether this latter effect is mediated through augmented hematopoietic progenitor numbers per se, synergistic cellular interaction between marrow hematopoietic stem cells and non stem cell mononuclear cells from the peripheral blood, or some other mechanism remains to be determined. These results suggest that peripheral blood mononuclear cells may augment both the opportunity and possibility for autologous hematopoietic reconstitution and once again confirm the importance of preclinical animal models for furthering clinical studies of hematopoiesis and hematopoietic reconstitution.

0129 USE OF LECTINS IN BONE MARROW TRANSPLANTATION, Yair Reisner. Weizmann Institute of Science, Rehovot, Israel.

Studies in murine models of marrow transplantation have demonstrated that graft-versus-host (GVH) disease is initiated by thymus-derived T lymphocytes of donor origin. Transplantation of allogeneic hematopoietic tissues dificient in T lymphocytes, such as early fetal liver and adult spleen or marrow cells from mice thymectomized in the neonatal period, have resulted in stable chimeras without GVH disease. Such transplants have regularly produced immunologically vigorous, long-lived chimeras when donor and recipient are haploidentical (e.g., parent $\rightarrow F_1$). Production of long-lived chimeras has been less consistent when fully allogeneic transplants are performed. This may be due to a reduced incidence of engraftment, as well as to genetic restriction of cooperation between donor lymphoid cells and host elements in the generation of an effective immune response. Several techniques have now been developed which are effective in depleting alloreactive T lymphocytes from mouse marrow or spleen cells. In one approach we demonstrated that cells producing GVH reactions can be removed from mouse bone marrow by differential agglutination with the galactose-binding lectins soybean agglutinin (SBA) and peanut agglutinin. Subsequently this approach was developed in a primate model and in man to fractionate marrow cells in four steps: (i) selective removal of red blood cells by gravity sedimentation in Hetastarch solution; (ii) agglutination with SBA and differential sedimentation of the agglutinated (SBA+) cells; (iii) removal of E-rosette forming T cells from the SBA- cell fraction by centrifugation over Ficoll hypaque (SBA-E-); and (iv) repetition of the E-rosetting step using neuraminidase-treated sheep red blood cells to eliminate residual rosetting cells (SBA-E-E_N-). Whereas the final recovery of mononuclear cells (SBA-E-E_N-) averages approximately 5% of the starting fraction, more than 80% of the colony forming cells are usually retrieved. This technique has recently been applied as an experimental transplantation approach in a restricted group of patients with severe combined immune dificiency, and advanced acute leukemia, who lacked an HLA-identical sibling donor. Early results indicate that the use of SBA-E-E_N- haploidentical parental bone marrow cells can prevent the development of GVH disease in these patients without prophylactic treatment with methotrexate. Tolerable cytoreductive regimens, in excess of that provided by cyclophosphamide and high-dose (>10 Gy) total body irradiation, may be necessary to achieve sustained engraftment of T cell-depleted marrow in patients receiving histoincompatible grafts.

0130 TOTAL LYMPHOID IRRADIATION AND BONE MARROW TRANSPLANTATION, S. Slavin, S. Morecki, M. Weigensberg and L. Weiss. Immunobiology Research Laboratory, Department of Medicine A., Hadassah University Hospital, Jerusalem, Israel.

Establishment of stable BM chimeras depends on adequate repopulation of cytoreduced, immunoincompetent host with immunocompetent BM inoculum. In MHC incompatible combinations prevention of host versus graft (HVG) reactivity is difficult to accomplish even after lethal conditioning. Sufficient degree of suppression of HVG can be accomplished using sublethal, fractionated high dose TLI, in several animal models including primates and man. In mice, TLI results in depletion of cells bearing θ, Thy-1, LyT-1, LyT-2 and Ig determinants in the blood, lymph nodes, thymus and spleen. Tolerance to heterologous protein antigens and alloantigens can be induced upon completion of TLI. Post-TLI period, spleen cells appear large and immature with increased CFUs and CFUc capacity and can efficiently block ($\leq$100%) T cell-mediated responses including MLR, CML and mitogen-induced proliferation. TLI-induced suppressor cells can also block GVHD by allogeneic BM cells in adoptive transfer experiments, as well as T-dependent and T-independent antibody responses. This suggests that the suppressor cells play a role in the induction of tolerance. These cells can be agglutinated by peanut and soybean lectins, but show no Ig, Thy-1, LyT-1, LyT-2, TL or asialo GM_1 cell determinants and cannot be eliminated by binding to plastic surfaces, nylon wool or G-10. Suppressive capacity is extremely radioresistant ($\geq$3000 rads) and is thymus independent since it can be generated in mice thymectomized prior to TLI. Similar radioresistant cell subsets were documented in fetal livers, BM and spleen of nude mice and in normal BM. We hypothesize that tolerance induction in TLI-treated hosts results from enrichment of immature cells which may operate as suppressor cells. Thus self-recognition may be an ongoing process reexpressed by the stem cell reservoir present in adult BM. BM-induced GVHD can be suppressed in TLI-treated rodents inoculated with few T-cells but transfer of mixtures of BM and spleen cells invariably results in GVHD. Nevertheless TLI-treated recipients show 3-5 fold increased survival compared to mice treated with whole body irradiation. In man, the increased T-cells present in bone marrow will probably require stem cell purification. We speculate that transplantation across MHC might become feasible in man by proper manipulations leading to enrichment of host and donor immature/suppressor cell subsets. In this presentation we will also review current results of TLI in human transplant recipients of both HLA-identical and mismatch marrow grafts.

Future Applications of Bone Marrow Transplantation

0131 BONE MARROW TRANSPLANTATION AS A PRELUDE TO ORGAN TRANSPLANTATION, Samuel Strober, Shigeki Okada, Allan Oseroff, Department of Medicine, Stanford University, Stanford, CA 94305

Adult mice and rats treated with total lymphoid irradiation (TLI) accept allogeneic bone marrow (BM) grafts and do not develop graft versus host disease. BM recipients are specifically tolerant to tissues of the BM donor, and long-term survival of donor skin and heart allografts has been observed (1). In the absence of BM transplantation, skin and heart allograft survival is prolonged, but grafts are ultimately rejected (1).

We studied the mechanism of tolerance induction in this model system, and found antigen-nonspecific suppressor cells in the spleens of TLI-treated recipients before BM transplantation, and antigen-specific suppressor cells after BM transplantation. The relationship between the nonspecific and specific suppressor cells was investigated in the mixed leukocyte reaction (MLR). Ordinarily, both suppressor and cytolytic cells are generated in the MLR. However, the addition of spleen cells from TLI-treated mice to the MLR blocked the generation of cytolytic cells and enhanced the generation of antigen-specific suppressor cells (2). Thus, the nonspecific suppressor cells in TLI-treated BM recipient may block the ability of both donor and host cells to generate cytolytic cells and enhance the generation of antigen-specific suppressors which maintain mutual tolerance. A similar process occurs in neonates given BM transplants (3).

We have propagated the nonspecific suppressor cells in vitro for more than 8 months, and have recently cloned them. The cultured suppressor cells inhibit the MLR and generation of cytolytic cells at a responder to suppressor cell ratio of 10,000 to 1. In addition, the suppressor cells secrete a factor which inhibits the MLR and generation of cytolytic cells at dilutions of at least 1,000 to 1. The surface phenotype of the propagated cell is Thy-1^+, Lyt-2^+ and Ig^-. We are currently studying the ability of the propagated cells and their secreted products to induce tissue transplantation tolerance in the context of BM transplantation.

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0132 CORRECTION OF GENETIC DISEASES IN THE FETUS, Beatrice Mintz, Institute for Cancer Research, Fox Chase Cancer Center, Philadelphia, PA 19111

The genotype of hematopoietic cells may be changed prenatally in mice, either by replacing defective hematopoietic stem cells with normal ones, or by introducing recombinant DNA into the fertilized egg. The former has been demonstrated in mice of the W-series mutant genotypes, whose macrocytic anemia varies in severity depending upon the allele; the defect apparently originates in the hematopoietic stem cells. The disease is prevented by microinjection of normal fetal liver cells into mutant recipients at 11 days of fetal life via an efferent placental blood vessel in utero (1). Successful engraftment occurs by cell selection at the totipotent hematopoietic stem cell (THSC) level and begins with seeding of the recipient's liver. In some cases, very small numbers of THSC—probably single ones—are responsible for prolonged self-renewal and differentiation into definitive myeloid and lymphoid lineages (2). Allogeneic combinations are accepted with impunity; this will be compared with injections of adult bone marrow cells. Adult bone marrow cells do not re-express a fetal-specific erythrocyte antigen despite return to a fetal environment (3). An alternative route to genetic change is the introduction of recombinant DNA (e.g., cloned globin genes) into mice by microinjection into a pronucleus of the zygote. The sequences are transmitted to progeny in a Mendelian fashion (4).

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Clinical Bone Marrow Transplantation

0133 IDENTIFICATION OF A CMV ANTIGEN AND CMV NUCLEIC ACID IN STUDIES OF PNEUMONITIS AND HEPATITIS IN MARROW TRANSPLANT RECIPIENTS, Robert C. Hackman, Joel D. Meyers, Steven C. Springmeyer, David Myerson and the Seattle Bone Marrow Transplant Team, Fred Hutchinson Cancer Research Center, Seattle, WA 98104

Cytomegalovirus (CMV) infections are a frequent and often fatal complication of marrow transplantation. Culture isolation of CMV is slow and histologic diagnosis by inclusion identification may lack sensitivity. We have retrospectively evaluated immunofluorescent(IF) staining of 52 consecutive, coded, open lung biopsies with a monoclonal antibody designated 6-C5 (Genetic Systems, Inc. Seattle, WA) to an 80,000 dalton late protein antigen of CMV. The results of IF analysis correlate well with those of viral culture. Both techniques are more sensitive than histological diagnosis. Preliminary CMV nucleic acid hybridization studies confirm the IF and culture results. The high sensitivity and specificity of IF staining with this antibody allow the identification of individual infected cells, many of which lack definite CMV inclusions. This suggests that CMV pneumonia can be rapidly diagnosed by IF analysis of pulmonary cells obtained without thoracotomy. We are evaluating this possibility in a prospective comparison of open lung biopsy, bronchoalveolar lavage, transbronchial biopsy, and needle aspiration of lung. Five of the first ten patients have had CMV pneumonia on open lung biopsy. In two of these five, IF staining has demonstrated CMV infection of lavaged cells, indicating value in this rapid, nonsurgical approach to pneumonia diagnosis. Preliminary IF studies of liver with antibody 6-C5 have demonstrated Kupffer cell and hepatocyte infection in several marrow recipients with liver function abnormalities and disseminated CMV. This approach may aid in the diagnosis and understanding of posttransplant liver dysfunction.

0134 ALLOGENEIC BONE MARROW TRANSPLANTATION (BMT) IN PATIENTS WITH LEUKEMIA: BENEFIT OF HIGHER-DOSE FRACTIONATED RADIOTHERAPY, MJ Bozdech, PM Sondel, RE Exten, M. Trigg, R Steeves, and R. Hong, University of Wisconsin, Madison, WI 53792

The use of BMT following high-dose chemoradiotherapy in patients with poor-prognosis leukemias offers a greater potential for cure than any alternative; but the side effects of infection, GVHD, and interstitial pneumonia (IP) as well as relapse of leukemia are major obstacles. We tested a higher total dose of TBI to decrease leukemic relapse, divided into fractions (5-8) to reduce lung toxicity. Marrow recipients in our hospital receive simple infection precautions. Preparation for BMT consists of CY 60 mg/kg on d -7 and -6, 1T MTX 12 mg on d -8 and -5, and 1200 Gγ of TBI in fractions on days -4 through 0. Empiric use of oral TMP-SMZ and ketoconazole is followed by parenteral antimicrobials for fever. WBC transfusions are not given prophylactically. We have performed 13 transplants in 12 patients with acute leukemia. The overall survival is 6/12 = 50%. Median age was 31.5y (11 adults and 1 child). Survivors and their diagnoses are: AML not in remission: 0/2; AML-PR: 0/2; AML-R1: 2/4; AML-R4: 0/1; ALL-R1: 2/2; ALL-R2: 2/2. Survival for AL-CR is 6/9 = 66%; for ALL-CR, 4/4 = 100%; and for AML-CR, 2/5 = 40%. Acute GVHD has occurred in 6/12 and resolved in all; and 3/6 have developed cGVHD, which has also resolved. Four patients had sepsis or pneumonia (2 fatal) due to S. epidermidis, which represents a major pathogen in this population. Deaths have been from bacterial, fungal or mixed pneumonia in 3 patients (1 not in remission; 1 in 4th R; 1 in CR); 1 myocardial infarction; and relapse in 2 patients transplanted in PR. No patients in CR have relapsed after BMT. Only 1 patient receiving fractionated TBI has developed IP and this occurred on d275 in association with cGVHD and resolved with CY and prednisone.

0135 COMBINATION THERAPY OF MEGA-DOSE CHEMOTHERAPY AND AUTOLOGOUS MARROW TRANSPLANTATION IN PATIENTS WITH ADVANCED SOLID TUMOR. Fukumi Morishige, Sajio Sumida, Eiji Kimoto and Yoshimoto Katsura. Tachiarai Hospital, National Fukuoka Central Hospital, Fukuoka University Chemistry Department and Kyoto University Serology Department, Japan

Four hundred ml or more of bone marrow ($5.0\pm2.6\times10^9$ nucleated marrow cells) was obtained by aspiration from the anterior to posterior iliac crests under general anesthesia. Cryopresevation was performed by controlled rate freezing at -1 C/min to -80 C in the presence of a 10% Me_2SO in medium 199 and transferred into liquid nitrogen. Recovery of hemopoietic stem cells was 60% or more of the prefreeze level. After the marrow was cryopreserved, the patients received the mega-dose chemotherapy of 5FU 1000-3000 mg + Mitomycin C 10-30 mg + Adriamycin 100-300 mg (or Cyclophosphamide 1000-3000 mg) as one shot infusion. The cryopreserved marrow was thawed and infused intravenously 72-96 hr after completion of chemotherapy. During the period of profound marrow aplasia intense use of systemic antibiotics was required in an aseptic tent. If necessary, the 2nd and 3rd shot were applied by the same protocol. 106 patients were applied of our combination therapy. 60 cases showed subjective and/or objective remission or improvement and 33 are now in observation. No side effect was seen except each one of febril reaction, ulticaria and nausea.

0136 IN VITRO TREATMENT OF DONOR BONE MARROW WITH ANTI-E ROSETTE ANTIBODY AND COMPLEMENT PRIOR TO TRANSPLANTATION. Michael Trigg, Ronald Billing, Paul Sondel, Chris Erickson and Richard Hong, University of Wisconsin, Madison, WI 53792 and UCLA, Los Angeles, CA 90024

Allogeneic bone marrow transplantation (BMT) has been used with increased frequency in the treatment of acute leukemia. Graft-versus host disease (GVHD), a major complication of BMT, is the major cause of death in the early post transplant period. In vitro treatment of donor bone marrow with an anti-E rosette receptor antibody (CT 2) and complement (C') was initiated at the University of Wisconsin in 1982 to prevent GVHD following BMT. CT2 is an IgM murine monoclonal antibody directed to a 50,000 dalton E-rosette antigen. Baby rabbit sera is used as the C' source. The first study patient, an 8-year-old with ALL in remission following a simultaneous bone marrow/CNS relapse, received HLA -MLC compatible bone marrow treated in vitro with CT2 and C'. Pretransplant conditioning included Cytoxan 60 mg/kg x 2 and 1320R TBI over 4 days. No Methotrexate or other anti-GVHD therapy was given post BMT. Total WBC reached 1000 per mm^3 within 14 days and platelet count was 100,000/mm^3 by day 50. There was no evidence of GVHD and the rapid engraftment correlated with the enhanced hematopoietic colony formation in vitro. The in vitro treatment removed >99% of the E-rosette positive cells and >98% of the T3 positive cells. However, T cells from the donor were evident in the peripheral circulation within 14 days following BMT. Additional patients are now under preparation for BMT with bone marrow depleted of E-rosette positive cells. Successful BMT without GVHD in this setting may extend the application of BMT using histoincompatible bone marrow.

0137 Oral Manifestations of Chronic Graft-Versus-Host-Disease, Mark M. Schubert, Ken Izutsu, Keith Sullivan, Edmond Truelove, Rainer Storb, and E. Donnall Thomas, University of Washington and Fred Hutchinson Cancer Research Center, Seattle, WA 98195

To determine if oral soft tissue changes observed after allogeneic marrow transplantation correlated with active chronic graft-versus-host disease (GVHD), we studied 23 aplastic anemia and 37 leukemia patients 180-500 days after transplant. One observer (MMS) studied patients without knowledge of the GVHD status. Lips, buccal and labial mucosa, gingiva, tongue, palate, and salivary glands were evaluated for changes in color, texture, keratinization, form, vascularity, and function. Also scored were dryness and stomatitis. Findings were rated on a scale of 0 (normal) to 3 (severely abnormal). P-values were calculated by chi-square. The following were associated with the presence of chronic GVHD: Buccal-Labial Mucosa - Atrophy and Erythema ($p<0.000$), Lichenoid changes ($p<0.003$); and Gingival Atrophy ($p<0.003$); Tongue - Atrophy and Lichenoid changes ($p<0.025$); Palate - Erythema ($p<0.02$); Stomatitis ($p<0.002$); and Xerostomia ($p<0.02$). Positive (+PV%) and negative (-PV%) predictive values were calculated to determine if findings could aid in diagnosis of active chronic GVHD. Data are expressed as the ratio of +PV% to -PV%: Lips - Increased White (100/50), Lichenoid (83/48) and Angular Stomatitis (100/47); Buccal-Labial Mucosa - Lichenoid (100/56) and Gingival Atrophy (88/58); Tongue - Atrophy (75/55), Lichenoid (91/53) and Edema (100/46); Palate - Inflamed Minor Salivary Glands (100/47); Stomatitis (88/65) and Xerostomia (80/57). These manifestations are useful in diagnosing chronic GVHD and may be of value in assessing disease activity and response to therapy.

0138 AUTOLOGOUS MARROW TRANSPLANTATION (AMT) IN PATIENTS WITH ACUTE NONLYMPHOCYTIC LEUKEMIA (ANL) DURING FIRST REMISSION. Patricia Stewart, C. Dean Buckner and E.Donnall Thomas, Fred Hutchinson Cancer Research Center, Seattle, Washington.

Ten patients (pts), ages 14 to 38 years, with ANL in first remission without matched donors had marrow aspirated and cryopreserved a median of 86 days (range 27-405) after achieving remission. They were then treated with cyclophosphamide 120 mg/kg, total body irradiation (1000-1200 rads) and AMT. In an attempt to eradicate residual leukemic cells, 4 pts received intravenous human leukocyte interferon (HLI) and 6 pts intravenous Methotrexate (MTX). HLI was administered in an escalating dosage beginning at 3.3×10^4 units/kg every third day following engraftment until day 80. The treatment was discontinued in two pts due to marrow toxicity. One pt expired from cytomegalovirus pneumonitis on day 116. The remaining 3 pts relapsed 8-10 months following AMT. Six pts received MTX at a dose of 15 mg/m^2 on day 1 and 10 mg/m^2 on days 3,6,11 and 18 at which time it was discontinued in all patients due to slow engraftment. Two pts are too early to evaluate. In the remaining 4 the median day to achieve 500 granulocytes/cu.mm was 38 (range 34-45) and a self sustaining platelet count of 20,000/cu.mm was 73 (range 39-116). All 4 pts remain in an unmaintained CR 4,6,26 and 31 months following AMT and 10,13,30 and 39 months following diagnosis.

0139 CLINICAL PROBLEMS WITH THE USE OF CYCLOSPORINE IN MARROW TRANSPLANT. Michael S. Kennedy, Fred Hutchinson Cancer Research Center, Seattle, WA 98104

Cyclosporine (CSP) used for prophylaxis or treatment of graft-versus-host disease (GVHD), presents problems of drug delivery and how to monitor for efficacy and toxicity. Oral absorption showed significant inter and intrapatient (pt.) variability. Peak serum values (by RIA) varied 8-fold after a 6.25 mg/kg dose and occurred at 2-6 hours. Clearance of the drug was affected by hepatic dysfunction (68% decrease in pts. with bilirubin $\geq$ 2 mg%). Toxicity, manifest by a rise in serum creatinine (cr.) to double the baseline value was observed in $\geq$ 90% of pts who received the drug prophylactically for 30 days (n = 47). In most pts, the cr. rise was gradual and in those with $\leq$ 100% rise, trough CSP values were lower (med.164 ng/ml) than those with $\geq$ 100% rise (med 422 ng/ml $p \leq .01$). Of 160 pts at risk, 23 have required at least one dialysis treatment, 12 became independent of dialysis but only 5 were discharged from hospital. Renal failure risks included HLA non-identical grafts, second transplants, veno-occlusive disease of the liver, sepsis treated with aminoglycosides + or - amphotericin-B. The med. CSP trough level (433 ng/ml) was not different than those with the reversible rise ($p \geq .1$). Elimination kinetics in 36 pts with normal hepatic function revealed a $t_{\frac{1}{2}}$ of 6.3 hours. This may explain the findings in 15 of 18 pts who developed new or recurrent manifestations of GVHD within 4 days (med) after CSP was withheld. Nine of the 15 received only more CSP and symptoms improved in 8. CSP trough levels in the week before, during and after the dose changes were 250, 60 and 250 ng/ml respectively. These data suggest a therapeutic range of 100-300 ng/ml.

0140 PULMONARY CHANGES IN LONG-TERM SURVIVORS OF MARROW TRANSPLANTATION. S.C. Springmeyer, N. Flournoy and K.M. Sullivan, R. Storb, E.D. Thomas, Fred Hutchinson Cancer Research Center, Seattle, WA 98104.

Successful marrow transplantation for aplastic anemia and hematologic malignancy has resulted in increased numbers of long-term survivors. These patients are experiencing late complications which include chronic GVHD, bacterial and viral infections. Since 1979 we began to prospectively evaluate pulmonary function in transplant recipients at yearly intervals to assess late pulmonary complications. Pulmonary function testing has included spirometry, lung volumes, and a diffusion study (DLCO). Both obstructive and restrictive lung disease has occurred. Severe airflow obstruction has complicated primarily patients with chronic GVHD and has an incidence of 10%. Restrictive ventilatory changes have not been as apparent clinically, and the mean loss in total lung capacity (TLC), vital capacity (VC), and DLCO compared to pretransplant testing is shown in the table.

Time post Transplant	n	TLC (liter)	VC (liter)	DLCO (ml/min)
1 year	26	-.84*	-.64*	-8.5*
2 year	8	-.42	-.31	-8.5*
3 year	9	NC	NC	-3.1

* $p < .02$ NC= no change

When the patients are separated into subgroups, these restrictive pulmonary changes appear to be predominately in those patients with the complication of chronic GVHD, rather than those patients who had received prior total body irradiation. We conclude that there are significant obstructive and restrictive pulmonary complications occurring in long-term survivors of marrow transplantation and these warrant further investigation.

0141 AUTOLOGOUS BONE MARROW TRANSPLANTATION FOR END-STAGE LYMPHOMAS. William Bensinger, C. Dean Buckner, Patricia Stewart, Fred A. Appelbaum and E. Donnall Thomas, Fred Hutchinson Cancer Research Center, Seattle, Washington.

Eleven patients with advanced lymphomas and extensive prior therapy received autologous bone marrow transplants after conditioning with total body irradiation and cytoxan or other chemotherapy. Four patients had Hodgkin's disease of 1 to 14 years duration. Three had failed combination chemotherapy and irradiation while one had relapsed after combination chemotherapy. Of the 7 patients with non-Hodgkin's lymphoma, five had diffuse histiocytic lymphoma, 1 nodular mixed lymphoma and 1 malignant histiocytosis. Three patients were transplanted after failing to achieve a complete remission with chemotherapy and 4 patients were transplanted in their first or second relapse. Five of 7 patients had received prior radiation therapy. Marrow was cryopreserved a median of 11 months from diagnosis and infused a median of 3 months after storage. Four patients died prior to day 20 of interstitial pneumonitis (IP)(3) or IP plus renal failure (1) and are not evaluable for engraftment. Five of the remaining 7 patients had recovery of granulocytes to $500/mm^3$ between 18 and 30 days. Six patients died by day 53 without recovering platelet function. One became platelet independent by day 35. Causes of death in these six patients included infection (4), interstitial pneumonia (5), organ failure (2) and hemorrhage (1). Of 3 patients evaluable for response, 2 achieved PR and 1 a CR. These data indicate that heavily treated refractory patients with lymphoma do poorly after autologous bone marrow transplant.

0142 PREINCUBATION OF DONOR MARROW WITH A COMBINATION OF MURINE MONOCLONAL ANTI T CELL ANTIBODIES (WITHOUT COMPLEMENT) DOES NOT PREVENT GRAFT-VERSUS-HOST DISEASE, Paul J. Martin, John A. Hansen, E. Donnall Thomas and The Seattle Bone Marrow Transplant Team, Fred Hutchinson Cancer Research Center, University of Washington, Seattle, WA 98104

The donor marrow for 9 marrow transplant patients was treated with a combination of 8 McAb (6 IgG, 2 IgM) specific for 7 different T cell antigens. Patients were between 30-54 (median 37) years old, were genotypically HLA-identical with their donors, received cyclophosphamide and total body irradiation (1200-1575R) and methotrexate post-grafting for prophylaxis of GVHD. The marrow was incubated with saturating concentrations of the McAb for ½ hr. at room temperature and was not washed before infusion. The mean nucleated cell dose was 2.2 x 10^8/kg, containing an average of 21% T cells. In all patients the marrow infusion was accompanied by fever. Prompt engraftment occurred in 8 patients with > 100 polys within 12-19 (median 15) days. Five patients are alive from 38-108 (median 82) days after transplantation. Two patients died on days 23 and 38 following pulmonary hemorrhage; 1 died on day 17 of veno-occlusive disease and candida sepsis without evidence of engraftment; and 1 died on day 58 of hepatic failure with GVHD. Of 6 evaluable patients surviving at least 40 days with sustained engraftment, 4 had GVHD (1 grade I, 2 grade III, and 1 grade IV). Onset of GVHD occurred on days 15, 30 and 35 in patients with grade III-IV disease. We conclude that in these patients who have received high dose chemoradiotherapy, preincubation of donor marrow with McAb does not prevent or delay acute GVHD. Either the RE systems in these patients is incapable of removing a sufficient number of McAb-coated T cells, or simple blocking of T cells with McAb is not adequate to prevent GVHD.

0143 PURE RED CELL APLASIA INDUCED BY HUMORAL INHIBITION OF CFU-E IN ANGIOIMMUNOBLASTIC LYMPHADENOPATHY, Yoshihito Yawata & Masakiyo Mannoji, Kawasaki Med. Sch., Kurashiki, JAPAN 701-01

Pure red cell aplasia (PRCA) was developed in a patient (71 y.o. male) with a 4 month history of angioimmunoblastic lymphadenopathy (AILD), which was established by biopsies of the patient's lymph nodes. Severe anemia (RBC $148\times10^4/\mu l$, reticulocytes 0%) and erythroid aplasia in the bone marrow with normal granulopoiesis and thrombopoiesis were noted. White cell counts and platelet counts were 9,100/µl and $12.2\times10^4/\mu l$, respectively. A diagnosis of PRCA was established. Serum IgG was markedly increased (5,082mg/dl) with slight elevation of IgM and IgA (640 and 340mg/dl). Cold agglutinin titer was 1:8,192. Other serological tests were negative, such as cryoglobulin, Paul-Bunnell test, monotest, rheumatoid factor, LE cells, anti-nuclear antibodies, anti-DNA titer, thyroid test and microsome titer. CFU-E was markedly decreased in the patient's bone marrow (19.8 colonies/10^5 bone marrow nucleated cells, compared to normal;72.9). The addition of the patient's serum inhibited CFU-E activities by 40.1% of normal control. IgG prepared by DEAE column chromatography from the patient's serum also inhibited normal CFU-E (29.8%). Cytotoxic effects of the patient's lymphocytes per se were not observed. Treatment was started with prednisolone (60mg/day), azathioprine (50mg/day) and vincristine (1.0mg/week). At the 12th hospital week, reticulocytes appeared with erythroid hyperplasia in the bone marrow with a regression of AILD. CFU-E activity of the patient was normalized (332 colonies/10^5 BM nucleated cells) after the treatment. In summary, it appears that a humoral factor (IgG) produced by the lymph nodes of the patient with AILD inhibited CFU-E of the bone marrow, resulting severe anemia (PRCA). After the regression of AILD, the patient was completely recovered from PRCA.

0144 SUSCEPTIBILITY AND TREATMENT OF VIRAL INFECTIONS IN SEVERE COMBINED IMMUNODEFICIENCY DISEASE, (SCID), E.W.Gelfand, D.McCurdy, W.R.Jarvis and P.J.Middleton, Divisions of Immunology and Virology, Research Institute, Hospital for Sick Children, Toronto, Ontario.

In patients with SCID, infections of viral etiology are common with pulmonary and gastrointestinal infections being most frequent. We have done a prospective analysis of 12 patients over a 6 year period. The etiologic agents were usually viruses common to the general pediatric population and only occasionally, by a virus seen almost exclusively in immunosuppressed individuals such as cytomegalovirus or papovavirus. The duration of symptoms and viral shedding were very prolonged unless immunoreconstitution was achieved. Respiratory pathogens, particularly parainfluenza virus Type 3 have been invariably associated with progressive respiratory failure and death in these affected infants. The necessity for effective drugs in the treatment of viral pneumonitis is underscored by the numerous failures in the past. We have recently successfully treated two patients with SCID suffering from progressive hypoxia; one patient was infected with parainfluenza virus Type 3 and the other with respiratory syncytial virus. We used continuous aerosolization of the drug Ribavirin which resulted in a rapid decrease in oxygen requirements, numbers and appearance of virus particles and ultimate elimination of the pathogen. The advent of such agents and the ability to deliver continuous small particle aerosolization may significantly affect the pre and post-transplant courses of patients with primary or secondary immunodeficiency suffering with life-threatening viral pneumonitis.

0145 MISMATCHED BONE MARROW TRANSPLANTATION (BMT): THE MINNESOTA EXPERIENCE (UMH), A.H. Filipovich, N.K.C. Ramsay, P. McGlave, R. Quinones, C. Winslow, K.J. Heinitz, J.H. Kersey, for the BMT team, University of Minnesota, Minneapolis, MN 55455

BMT from related donors not genotypically MHC identical with their recipients (i.e.mismatched) is often complicated by an increased risk of rejection or GvHD compared with BMT among MHC identical siblings. Twenty patients at UMH have received mismatched BMT. In Group I, 14 pts. (ages 1-32 yrs.) received BM from related donors with 2,3 or 4 HL-A matches with absent or low MLC reactivity. All 14 received methotrexate, ATG and prednisone post BMT for GvHD prophylaxis. 6/14 pts. had severe aplastic anemia (AA); only 1 pt. is surviving d.+2000 with limited chronic GvHD. 3 AA pts. died without engraftment 47-97 d. post BMT, 1 pt. died at d. 31 of unknown causes, and 1 pt. with IP (interstitial pneumonia) and extensive chronic GvHD. 8/14 pts. with various diagnoses were conditioned with TBI and other chemotherapy. 4/8 developed IP and 3 died with GvHD and infections. The remaining 4 pts. (ages 6-32) are currently alive 154-690 d. post BMT, 1 without GvHD and 3 with limited chronic GvHD. In summary 9/11 (82%) engrafted patients developed significant GvHD which contributed, with IP, to mortality in 5/11 cases (45%). Group II: Subsequently 6 pts. with immunodeficiencies pretreated with Cytoxan (Cy) alone or Cy and TBI with lung shielding have received T cell-depleted mismatched BM (5 haploidentical parents, 1 half-sibling). In the 3 patients who are evaluable and engrafted, no GvHD has been documented 75-330 d. post BMT and 2/3 pts. are alive and out of the hospital. We propose that successful mismatched BMT can be accomplished by optimizing pretransplant conditioning to ensure engraftment, and in vitro T cell depletion from BM for prevention of GvHD prior to BMT from "best matched" related donors.

0146 BONE MARROW TRANSPLANTATION (BMT) FOR RELAPSED STAGE IV NEUROBLASTOMA (NBL/IV). Charles S. August, Fredric T. Serota, Penelope A. Koch, Edith D. Burkey, Harvey Schlesinger, Giulio J. D'Angio and Audrey Evans, Children's Hospital, Philadelphia, PA 19104

Nine children, 3-13 years old, with relapsed NBL/IV received supralethal chemotherapy, local radiation therapy (LRT) and total body irradiation (TBI). Rescue with autologous (5) or allogeneic (4) bone marrow followed. Patient (PT) 1 was treated with LRT, cyclophosphamide(CY) 200 mg/kg, vincristine, VM-26, 1000 rad TBI, and HLA-identical sibling marrow, achieved complete clinical remission (CCR) but died of interstitial pneumonia 3 months (mos) post BMT with only 1 focus of microscopic NBL. The 2nd PT received cis-platinum, nitrogen mustard, vindesine and fractionated (F) TBI (333 rad x 3), followed by infusion of cryopreserved autologous marrow (CAM). Marked drug and radiation toxicity followed. She died with recurrent tumor 9 mos post BMT. We attempted to treat the others with VM-26 (180 mg/m^2 x 2), adriamycin (ADR) (45 mg/m^2 x 2), L-phenylalanine mustard in 2 doses (140 mg/m^2 + 70 mg/m^2) and FTBI. PT's 6 and 9 received no ADR because of the high doses received previously and/or cardiotoxicity. PT's 3, 4, and 6 received HLA-identical sibling marrow. PT's 5, 7, 8, and 9 received CAM. All had severe mucositis. Two PT's experienced extreme toxicity, developed disseminated fungal infections and died 23 days post BMT. One of these failed to clear his tumor. Two allograft recipients remain in CCR 41 and 23 mos post BMT, and 3 autograft recipients survive 22, 9, and 1.5 mos post BMT. PT 3 developed chronic graft-versus-host disease. Conclusions: relapsed NBL/IV may be controlled by supralethal combinations of chemotherapy and TBI, coupled with either autologous or allogeneic bone marrow rescue.

0147 MARROW TRANSPLANTATION FOR ACUTE LYMPHOBLASTIC LEUKAEMIA IN REMISSION. Barrett,A.J.,Kendra,J.R.Ingram,L., Joshi,R., Rogers,T.R., Phillips,R., Barrett,A. James, D.C.O., Hugh-Jones,K., Hobbs,J.R.,Saleem, N.Westminster Hospital, Dean Ryle Street, London, England.

The results of 44 BMT performed in Acute Lymphoblastic Leukaemia in remission using HLA and MLC compatible family donors is presented. All patients receive Total Body Irradiation (TBI) to 950 rad using either a 60mev linear accelerator (23pts) or a cobalt source (21pts). Dose rates were 4.5 and 2.3. r. per min. respectively. In addition to TBI patients received either Cyclophosphamide, Vincristine, Methyl Prednisolone and intrathecal Methotrexate (VIPER), or Daunorubicin, Cytosine arabinoside, Methyl Prednisolone, VM26 and intrathecal Methotrexate (V.RAPID). The actuarial disease free survival for all patients is 45% at 1409 days post BMT with a median disease free survival of 373 days. There were 8 deaths due to complications of the transplant procedure and 9 relapses. BMT in first remission for poor prognosis ALL results in a high chance of cure: of 10 first remission grafts there were no relapses but 1 patient died of septicaemia. Patients in second remission have a higher relapse rate (5 out of 15 evaluable, 33%) and the results are similar for third remission cases. Females have a low relapse rate following BMT in first or second remission, while males only have a high chance of cure when grafted in 1st remission.The results obtained withV.RAPID & VIPER regimes will be compared together with the effectiveness of TBI from 2 sources.

0148 EXPERIENCE WITH INTERSTINAL PNEUMONIA FOLLOWING TOTAL BODY IRRADIATION IN MARROW TRANSPLANTATION. W.B. Rybka, J.F. Prchal, T.H. Kim, representing The Cooperative Group for Marrow. Transplantation, Montreal, Canada.

Since 1980, a total of 25 patients with leukemia (10 ALL, 14 AML, 1 CML) have undergone bone marrow transplantation at McGill University hospitals. All patients received TBI, 9 Gy, single fraction, one day proceeding transplantation. A set of 2 mm thick lead cutouts were placed on the patient's chest to reduce the lung dose to the prescribed tumor dose.

The first group of 13 patients was treated using a Co-60 unit with a dose rate of 5 to 6.5 cGy/min. Six patients have developed interstitial pneumonitis. The clinical course of 3 patients was mild and they recovered completely: 2 were idiopathic and 1 drug induced. The other 3 patients had a severe course Pneumocystis carinii was identified in 2 patients and CMV in 1. One recovered but 2 died.

The second group of 12 patients was treated on a 4 MeV linear accelerator. The patient is positioned on the floor and the beam head sweeps over the patient at a constant speed of 25 seconds per sweep. This method delivers a total tumor dose over the same length of time as on a Co-60 with the average dose rate for each patient being 5.9 to 6.6 cGy/min. However, the instantaneous dose rate to the exposed portion of the body ranges from 20.7 to 23.4 cGy/min. Seven patients have developed interstitial pneumonitis, 5 of them idiopathic (4 died). CMV was identified in the other two patients (both died).

It appears that the instantenous dose of radiation is an important determinent in lung toxicity.

0149 BUSUFAN AND CYCLOPHOSPHAMIDE AS PREPARATION FOR SIBLING BONE MARROW TRANSPLANTATION FOR CHRONIC MYELOGENOUS LEUKEMIA, Gerald J. Elfenbein, John Graham-Pole, Samuel Gross and Roy S. Weiner, University of Florida, Gainesville, FL 32610

Three patients with poor prognosis (n=1) or accelerated (n=2) chronic myelogenous leukemia (CML) received an allogeneic bone marrow transplant (BMT) from genotypically HLA identical siblings and one patient with an identical twin received a syngeneic BMT for CML within 3 months of diagnosis. Preparation for cytoreduction and engraftment was oral busulfan (BU) and intravenous cyclophosphamide (CY) as described by the Hopkins team for acute myelogenous leukemia. One patient (the syngeneic recipient) is alive and free of disease beyond 6 months after BMT. Another patient is alive but undergoing chemotherapy for blast crisis relapse (with pulmonary involvement) which occurred during the 13th month after BMT. This patient originally was Philadelphia chromosome negative and had other poor prognostic findings of CML including splenomegaly and pulmonary infiltrates. Immediate and delayed complications of the BU-CY regimen include hepatic veno-occlusive disease (2 cases/0 fatalities), interstitial pneumonitis (IP; 2/1), severe graft-versus-host disease (GVHD; 2/1), prolonged thrombocytopenia (6-10 months; 2/0) and relapse (1/0). Deaths were due to disseminated CMV in the patient with IP and polymicrobial sepsis in the patient with GVHD. BU-CY was designed for the older patient population with CML who were not candidates for total body irradiation. From our small experience with BU-CY for CML, it does not appear that the regimen will be generally applicable. Similar experience with BU-CY for CML at Hopkins confirms this observation.

0150 HIGH-DOSE CYTOREDUCTIVE THERAPY AND BONE MARROW TRANSPLANTATION IN PATIENTS WITH REFRACTORY LYMPHOMA, N. Tannir, G. Spitzer, A. Zander, S. Jagannath, M. Kanojia, L. Vellekoop, P. McLaughlin, F. Hagemeister and K. Dicke, M. D. Anderson Hospital and Tumor Institute, Houston, Texas 77030

Eight adult patients with refractory Hodgkin's disease (HD) and non-Hodgkin's lymphoma (NHL) were treated with high-dose combination chemotherapy (cyclophosphamide, BCNU, and VP-16) or with cyclophosphamide and fractionated total-body irradiation (TBI), followed by bone marrow transplant (BMT). Six patients received autologous and two patients allogeneic BMT. Five patients achieved complete remissions, and three of them (two with undifferentiated lymphoma, one with lymphoblastic lymphoma) are alive and free of disease 4-18+ months after BMT. The other two complete responders died of opportunistic infections 2 and 5 months, respectively, after BMT. One patient with HD achieved partial remission and is alive 18+ months after BMT. Two patients were considered failures: One developed leptomeningeal disease 24 days after BMT, and the other died of progressive lymphoma 7 months after BMT. Engraftment and prompt hematologic recovery occurred in all patients. The major toxicity included two fatal infections and one case of diffuse idiopathic interstitial pneumonitis. High-dose chemotherapy with or without TBI followed by BMT appears to produce a high response rate and, although associated with toxicity, it demonstrates the potential for salvaging patients with refractory lymphoma who otherwise would have a dismal prognosis.

0151 SURGICAL PROCUREMENT OF BONE MARROW FOR TRANSPLANTATION. E.F. Saunders, W.P. Bobechko M.H. Freedman, S. Weitzman, L.J.A.C. Chang. Div of Hematology and Orthopedic Surgery, Hosp for Sick Children, Toronto, Ontario.

We obtained marrow surgically from 12 HLA-MLR matched sibling donors (ages 6 to 22 yr). Via a window in the cortical bone of one iliac wing, as much marrow containing spongy bone as possible was removed. A cell suspension was obtained by passing the marrow through a bone mill, crushing the particles in a garlic press, washing in heparinized TC199 and filtering. Final volume was 200 ml. Red cell contamination was minimal (RBC 0.06 x $10^6/mm^3$). We obtained a mean of 1.1 x 10^8 marrow cells (range 0.6 to 2.0)/Kg of donor, allowing administration of 1.3 x 10^8 cells (range 0.7 to 2.3)/Kg of recipient. Hematopoietic stem cells were assayed in the marrow using a mixed colony assay (CFU-GEMM). Mean concentration of total colonies was $174/10^5$ cells plated (range 118-263) from surgically obtained marrow, in contrast to a mean of 122 colonies/10^5 (range 75/163) from aspirated marrow. One pt. (of 9 with acute leukemia) failed to engraft due to an early death. One pt. (of 3 with aplastic anemia) rejected the marrow on 2 occasions after documented engraftment. Five pts. are alive and well (no GVH) with a median follow-up of one yr. Mean time until: engraftment -14 da (range 8-20); 1000 polys/mm^3-36 da (26-58); 50,000 platelets/mm^3-35 da (23-57); discharge from hospital - 47 da (29-74). These data compare favourably to the results of 25 transplants using aspirated marrow. Obtaining marrow surgically has several advantages: speed of the procedure (1.5 hr); minimal red cell contamination, small marrow volume, increased stem cell concentration; and decreased T-cell contamination. Disadvantages include mildly increased post-op morbidity, and a surgical scar.

0152 SEVERE APLASTIC ANEMIA: TREATMENT WITH ANTITHYMOCYTE GLOBULIN (ATG) WITH OR WITHOUT HAPLOIDENTICAL BONE MARROW. R. M. Bukowski, J. S. Hewlett, S. A. Rothmann. Cleveland Clinic Foundation, Cleveland, Ohio 44106.

Thirteen patients with acute onset (<3 mos.) severe aplastic anemia were treated with ATG (ATGAM, Upjohn Company) receiving total doses from 50 to 420 mg/kg. One additional patient received ATG (105 mg/kg) followed by haploidentical bone marrow infusion (2.1 x 10^8 cells/kg). The median age of these patients is 30 yrs. (range 5-74 yrs.). The etiology of the bone marrow aplasia included: idiopathic 11, post-viral infection 1, hepatitis 2. Toxicity of ATG included fever, chills, and urticaria in 14/14 patients. Serum sickness occurred in all patients, was usually mild, and responded to prednisone (20-30 mg/day). In one instance, however, severe serum sickness occurred with moderate renal failure developing. Therapy in this instance included plasma exchange. Of the 13 patients receiving only ATG, 8/13 survive with median predicted survival of 42.0+ mos. (range 1.0-48.0+ mos.). In these 8 patients, hemoglobin concentration is normal in 8/8 without transfusions, mild neutropenia persists in 4/8 (1.3-1.9 x 10^9 neutrophils/L), and mild thrombopenia in 5/8 (25-96 x 10^9 platelets/L). Three of eight patients in this group continue to require prednisone (5-20 mg/d) for maintenance of blood counts. Recently, one patient received ATG at a dose of 15 mg/kg day 1-7, and haploidentical bone marrow on d8. Complete hematologic recovery of recipient cells occurred by day 28, with normalization of the blood count. The patient remains normal at 4.0+ mos. Overall, 9/14 (64%) patients with severe aplastic anemia appear to have responded to ATG ± haploidentical bone marrow. Continued investigation of ATG therapy, including haploidentical bone marrow infusion, is indicated.

0153 IS IgE ELEVATION AN INDICATION OF AN ACUTE GRAFT-VERSUS-HOST REACTION? Olle Ringdén, Ulla Persson and S.G.O. Johansson, Div. of Transplantation Surgery, Huddinge Hospital and Department of Clinical Immunology, Karolinska Hospital, Stockholm, Sweden.

In 21 bone marrow transplant recipients, three with aplastic anemia and 18 with hematologic malignancies, IgE levels were studied regularly by conventional radio-immuno-assay (PRIST, Pharmacia, Sweden). A marked increase in serum IgE (from 2 to 748 fold) compared to previous posttransplantation levels was observed from day 13 to day 34 after transplantation. Increased IgE levels appeared in 19 of 21 patients: 12 with and 7 without acute graft-versus-host disease (GVHD). In patients with GVHD there was a significant correlation between the timing of the IgE increase and appearance of clinical GVHD ($p<0.01$). The highest IgE level (8000 kU/l) was noted in a recipient of a syngeneic graft. During the IgE peak the serum from this patient contained low concentrations of IgE reacting with several tested allergens as well as for the hapten TNP, which indicated polyclonal activation. In a patient with a known allergy to animal danders, RAST tests were positive against dog and cat before and 6 weeks after total body irradiation and transplantation with marrow from a non-allergic donor. A slight increase in the amount of allergen-specific IgE antibodies was seen during the increase in total IgE. A non-allergic patient was transplanted with marrow from a donor allergic to timothy. Timothy-specific IgE antibodies were detected immediately after transplantation, but they disappeared within a few days and could not be detected during the period of increase in total IgE.

The IgE elevation seen in bone marrow transplant recipients is probably a polyclonal response and there is indirect evidence that this occurs in host B cells.

0154 MARROW TRANSPLANTATION FOR STABLE PHASE CHRONIC GRANULOCYTIC LEUKEMIA, J Armitage, L Klassen, S Patil, R Gingrich, J Kugler, G Ahmann, M Fyfe, H Tewfik, Univ of Iowa, Iowa City, IA 52242 and the Univ of Nebraska Med Center, Omaha, NE 68105. Ten adults ranging in age from 18 to 45 years with chronic granulocytic leukemia (CGL) in the stable phase underwent allogeneic (9 cases) or syngeneic (1 case) bone marrow transplantation (BMT). Marrow cytogenetics on all patients prior to BMT revealed the Philadelphia chromosome without other abnormalities. The patients were prepared for BMT with cytarabine 5 mg/kg I.V. on day -7 and day -2, cyclophosphamide 90 mg/kg on day -5, and 900 R total body irradiation on day 0. After BMT all patients received prophylactic methotrexate. All patients had complete hematopoietic engraftment with recovery beginning from day +16 to day +29 after BMT and repeat chromosome studies showing the normal chromosome complement of the donor. The period of aplasia was complicated by fever in 7 patients, but only 2 patients had culture proven infections. Acute GVHD appeared in 7 patients and was severe in 3. One of these patients died from multiple organism septicemia on day +43. Three patients have developed chronic GVHD. Nine patients are currently surviving from 34 days to 637 days (median 273 days) following BMT. Karnofsky scores of the surviving patients vary from 70 to 100. BMT is capable of eliminating the abnormal clone of myeloid cells in patients with stable phase CGL. Because of the pernicious, albeit sometimes prolonged, natural history of CGL, BMT in stable phase is a reasonable therapeutic option for young patients with HLA identical siblings.

0155 HIGH DOSE INTENSIFICATION WITH AUTOLOGOUS BONE MARROW TRANSPLANTATION IN LIMITED DISEASE SMALL CELL LUNG CANCER (SCLC), Peter Farha, Gary Spitzer, Manuel Valdivieso, M.D. Anderson Hospital, Houston, TX 77030.

Twenty-one patients (pts) with limited disease SCLC received three courses of induction chemotherapy at regular doses of drugs, with either vincristine, adriamycin, and Ifosfamide, (10 pts), or with vincristine, adriamycin, cytoxan and VP-16 (11 pts). Intensive consolidation chemotherapy followed, with 2 courses of cytoxan 1.5 gm/m^2 dl-3, VP-16 200 mg/m^2 dl-3, and vincristine 1.5 mg dl,3 in the first 10 pts, with the addition of high dose methotrexate 1.5 gm/m^2 with citrovorum rescue in the last 11 pts. This treatment was followed by the infusion of the autologous bone marrow on day 6. There were 9 CR (43%) and 10 PR (48%) after induction. Following the intensive consolidation, there were 14 CR (67%) and 7 PR (33%) with 2 of the PR patients later achieving CR with radiation therapy. The median survival of the pts with CR before intensification is 16+ months (range 5.5-25+), whereas those who achieved a <CR before consolidation was 10 m (8.5-22+). Toxicities included some myelosuppression, but prompt recovery occurred in 3-4 weeks. There were no treatment related deaths, but there were 19 episodes of fevers of unknown origin and 7 pneumonias. All documented infections occurred in the patients who received high dose methotrexate, along with a high incidence of stomatitis and skin reactions. Intensification at high doses did not help those whose response is less than CR to regular doses of treatment, but may be beneficial for complete responders. High dose methotrexate added a lot of toxicity to the program.

0156 IMMUNOLOGICAL RECOVERY AFTER ALLOGENEIC AND AUTOLOGOUS BONE MARROW TRANSPLANTATION, Mikio Ueda, Shintaro Shiobara, Kosei Matsue, Mine Harada, Ken-ichi Hattori and Kanazawa University Bone Marrow Transplant Team, Kanazawa 920, Japan

We studied immune reactivity of patients who achieved unmaintained complete remission after allogeneic or autologous bone marrow transplantation. Three allotransplant and 3 autotransplant patients were studied at 6-48 mo after transplantation. All patients were conditioned with CY-TBI regimen and received similar supportive cares. Tests for evaluating immune reactivity included absolute number of PBL, T cell subsets defined by monoclonal antibodies and proliferative responses to mitogen or alloantigens. In allotransplant patients, absolute number of PBL increased to 4,600-5,400/mm^3 whereas their responses to mitogen were markedly depressed at 6-48 mo posttranplant. When their T cell subsets were measured by OKT-3, 4, 8 monoclonal antibodies, they were characterized by marked increase of OKT-8 positive cells, which resulted in reversed T4/T8 ratio. Proliferative responses of allotransplant patients were much lower than those of autotransplant patients. In autotransplant patients, absolute number of PBL ranged from 1,300 to 1,800/mm^3 but their proliferative responses were moderately depressed at 24-26 mo posttransplant. Interestingly, the T4/T8 ratio was also reversed in autotransplant patients though it was not so remarkable as that observed in allotransplant patients. These observations suggest that the difference in immune reactivity between allogeneic and autologous marrow transplants may be reflected by the T4/T8 ratio. It is unknown why depressed immune reactivity and relative increase of T8-positive cells were persistent even in autotransplant patients.

0157 DECREASED SEVERITY AND MORTALITY OF ACUTE GVHD AFTER BONE MARROW TRANSPLANTATION, Mine Harada, Hisashi Funada and Ken-ichi Hattori for Kanazawa University Bone Marrow Transplant Team, Kanazawa 920, Japan

Eighteen patients with leukemia were treated with allogeneic marrow transplantation. All patients were conditioned with CY-TBI regimen and received bone marrow cells from their HLA-A,B,D identical sibling donors. Infection prevention included isolation in an LAF room, gut sterilization by nonabsorbable antibiotics(GM, VCM & NYS) and sterile food. Prophylactic methotrexate was given as scheduled. Of 16 patients surviving for more than 60 days posttransplant, 7 developed mild acute GVHD. Although the incidence of acute GVHD was not low, the severity of the disease was Grade-I in 6 and Grade-II in one of them. Interestingly, none of the patients experienced gastrointestinal GVHD when the disease was evaluated on the basis of diarrhea. Mild acute GVHD was successfully treated or subsided without therapy. Compared with results by others, the severity and mortality of acute GVHD was markedly decreased in our series of patients. One of the possible explanations for this observation is successful complete suppression of the intestinal microflora by total intestinal decontamination. Strict isolation procedures and total intestinal decontamination are also effective for decreasing the chance of bacterial infection and requirement of granulocyte transfusions.

0158 HIGH DOSE CYTOSINE ARABINOSIDE (ARA-C) AND FRACTIONATED TOTAL BODY IRRADIATION (F-TBI) AS PREPARATION FOR BONE MARROW TRANSPLANTATION (BMT) FOR CHILDHOOD ACUTE LEUKEMIA IN REMISSION--A PRELIMINARY REPORT, Peter F. Coccia, Sarah E. Strandjord, Erlinda M. Gordon, Louis J. Novak, Donald C. Shina, and Roger H. Herzig, Case Western Reserve University, Cleveland, Ohio 44106.

Thirteen children (3.5-18 years old), 10 with ALL in second remission and 3 with ANLL in first remission, were prepared for BMT with ARA-C 3000 mg/m^2 Q 12 hrs X 12 doses followed by 200 rads TBI administered at 15-20 rads/min Q 12 hrs X 6 doses. Marrow infusion was 12 hours post F-TBI. The preparative regimen was extremely well tolerated. Transient photophobia, conjunctivitis, and mild erythematous skin rashes, but no cerebellar toxicity, developed after ARA-C infusions. Donors were HLA-MLC identical siblings (10), partial mismatched relatives (2), and an identical twin (1). All patients engrafted within 16 days. Five have died 1.0 to 2.5 months post-BMT of interstitial pneumonitis (2), acute GVHD (2), and sepsis (1). Eight are alive and well in complete remission 2+, 4+, 5+, 8+, 9+, 15+, 17+, and 20+ months post-BMT. No relapses have occurred; and actuarial event-free survival is 58%. High dose ARA-C + F-TBI is a tolerable and effective preparative regimen for BMT in childhood acute leukemia.

0159 HIGH DOSE MELPHALAN (Melph) AND AUTOLOGOUS BONE MARROW SUPPORT (AMS) IN REFRACTORY GERM CELL NEOPLASMS. D.D. Hurd[1], P.I. Warkentin[1], H.M. Lazarus[2], and R.H. Herzig[2], Univ. of MN[1], Mpls, MN 55455 & Case Western Reserve University[2], Cleveland,OH 44106

Recent advances in chemotherapy for germ cell tumors suggest that 50-60% of patients (pts) with advanced stage disease may be cured using combination chemotherapy that includes vinblastine, bleomycin and cis-platinum (VBP). For pts who relpase after such therapy or fail to achieve a complete remission the prognosis is extremely poor. Since germ cell neoplasms infrequently metastasize to the bone marrow, intensive chemo/radiotherapy with AMS provides another treatment approach for these refractory pts. As part of a larger Phase I study, 3 pts with end stage germ cell neoplasms were treated with Melph (60mg/m^2/dx3d) and AMS. The pts had all failed multiple previous chemotherapeutic trials including VBP. All pts had elevated tumor markers (AFP, +/- βHCG) as part of their measurable disease. A single course of Melph with AMS was given to 2 pts and 2 courses to 1 pt. All 3 pts responded as demonstrated by a marked decrease in tumor markers.

	Pre-Therapy Level		Nadir Post-Therapy (day)	
	AFP	βHCG	AFP	βHCG
Patient 1	3600	63,760	188(+20)	93(+20)
Patient 2	51	28	<4(+8)	3(+29)
Patient 3	32,560	--	197(+64)	--
	4560	--	362(d+36)	--

We would conclude that high dose Melph may be a promising agent for refractory germ cell neoplasms. Additional studies with Melph and AMS would seem indicated.

0160 HIGH DOSE MELPHALAN AND AUTOLOGOUS BONE MARROW TRANSPLANTATION FOR RESISTANT NEUROBLASTOMA AND EWING'S SARCOMA, J Graham-Pole, S Gross, R Herzig, H Lazarus, R Weiner, and P Coccia, Depts of Pediatrics and Medicine, Univ of FL, Gainesville, FL 32610, and Case Western Reserve Univ, Cleveland, OH 43210

We conducted a phase II study of high dose melphalan (L-PAM: 120-210 mg/m^2 IV in 3 daily divided doses) and autologous bone marrow transplantation (BMT) in children (ch) with neuroblastoma (NB) and Ewing's sarcoma (ES) resistant to multi-agent chemotherapy. L-PAM was followed by infusion of previously cryopreserved normal BM (10-15 ml/kg). 2nd courses were given to ch who had complete (CR) or partial (PR) responses.

Conclusions: (1) L-PAM is more effective against disseminated NB than ES: (2) non-osseous respond better than osseous metastases; (3) greater toxicity in ES than NB ch is probably due to age rather than higher drug dosage; (4) phase III studies of L-PAM are indicated for NB but additional agents are needed for ES.

Results:	NB	ES
Patient number	10	7
Age-median (range)	3 yr (2-18)	15 yr (9-20)
Dose (mg/m^2)-120/180/210	5/5/0	1/5/1
Responses-CR/PR/NR	5/2/3	0/5/2
Duration -median (range)	7 mo (2-9+)	3 mo (2-6)
Nucleated cell dose-median	1.2 x 10^8/kg	1.3 x 10^8/kg
Days → White cells > 1000	22 (14-30)	25 (20-35)
Neutrophils > 500	25 (14-35)	28 (20-42)
Platelets > 20,000	28 (29-56)	35 (28-112+)
GI toxicity mild/severe	8/2	3/4

0161 TREATMENT OF CYTOMEGALOVIRUS PNEUMONIA AFTER MARROW TRANSPLANTATION, James C. Wade and Joel D. Meyers. Fred Hutchinson Cancer Research Center, Seattle, WA 98104

Cytomegalovirus (CMV) pneumonia is a major cause of death after marrow transplantation. Five consecutive treatment trials utilizing vidarabine (Ara-A), leukocyte interferon (IF), the combination of ARA-A plus IF, high-dose acyclovir (ACV), and ACV plus IF have been performed in 45 marrow transplant patients with biopsy proven CMV pneumonia. All 5 studies used an escalating dose treatment scheme with daily doses of the antiviral agents ranging from 2.5-15mg/kg for Ara-A; 1500-3600mg/m^2 for ACV; and 2-64x10^4u/kg for IF. Results of the trials are shown below and compared to 22 patients with biopsy proven CMV pneumonia who received no treatment.

Regimen	Number Treated	Survivors	Median Survival (Days)	Toxicity	
				Marrow	CNS
No Treatment	22	2(10%)	19	-	-
Ara-A	9	2	23	3	1
IF	8	0	13.5	3	0
Ara-A+IF	7	1	17	4	2
ACV	8	1	24.5	2	1
ACV+IF	13	3	11	5	2
Total	45	7(16%)		20	6

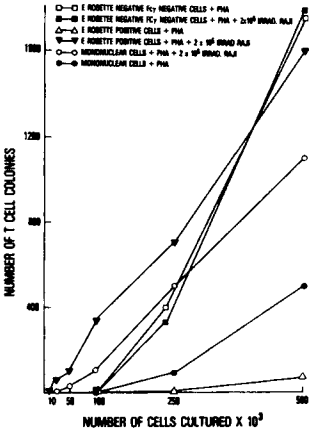
Quantitative CMV titers in lung tissue obtained at biopsy and autopsy from 29 of 38 patients with fatal pneumonia showed no consistent antiviral effect. Therapy with these five antiviral regimens was associated with important toxicity and no clear efficacy against CMV.

0162 **INTENSIVE VP-16-213 CHEMOTHERAPY FOR ADVANCED REFRACTORY GERMINAL CELL TUMORS**, Steven N. Wolff, Vanderbilt University, Nashville, TN 37232

VP-16-213, a congener of podophylotoxin, is an active agent against germinal cell tumors. Standard dose of VP-16-213 has been defined by modest myelosuppression to be approximately 300mg/m^2 - 500mg/m^2 given at 2-3 week intervals. In a previous Phase I study (AACR,23:134, 1982) VP-16-213 was dose escalated to 2400mg/m^2 when reconstitution of hematopoiesis was assured by autologous bone marrow transplantation (ABMTX). Severe mucositis prevented the administration of doses >2400mg/m^2. This present study reports a Phase II trial of high-dose VP-16-213 for patients (pts) with refractory germ cell tumors. From 12/81 through 7/82, 11 pts with platinum-resistant germ cell tumors were treated with high-dose VP-16-213. Seven pts had prior VP-16-213 therapy given at standard dose; 4 had no prior VP-16-213 exposure. From 1-5 courses of VP-16-213 were administered at doses of 1200-2400mg/m^2. ABMTX was used only for pts treated at 2400mg/m^2. One pt died of progressive disease during the VP-16-213 therapy. Of 10 evaluable pts, 9 responded (1 CR, 8 PR) with a disease-free duration of 1-7 months (median=2 months). Of 5 VP-16-213 resistant pts, augmented dose VP-16-213 resulted in 4 PRs. Some responses to high-dose VP-16-213 were of greater magnitude than with initial platinum-containing regimens. In summary, high-dose VP-16-213 has a high response rate in refractory germ cell tumors including some pts who had failed prior standard-dose VP-16-213, suggesting a dose-response relationship. High-dose VP-16-213 should be evaluated as part of the initial management of poor prognosis germinal cell tumor pts.

0163 5 AZACYTIDINE SELECTIVELY CAUSES INCREASED FETAL HEMOGLOBIN SYNTHESIS IN PATIENTS WITH β THALASSEMIA AND SICKLE CELL DISEASE. T. J. Ley, J. DeSimone, C. Noguchi, P. Turner, A. Schechter, P. Heller, and A.W. Nienhuis. National Institutes of Health, Bethesda, MD and University of Illinois College of Medicine, Chicago, Ill.
5-Azacytidine (5-AzaC) is a cytidine analogue that causes hypomethylation of DNA and is capable of "activating" repressed genes in tissue culture cells. Initial studies in anemic baboons (PNAS:79; 4428, 1982) revealed a prompt, striking, and reproducible increase in Hb F synthesis with 5-AzaC administration. Two patients with severe β^+ thalassemia and two patients with sickle cell disease were treated with 2mg/kg/day of 5-AzaC given as a continuous IV infusion for 7 days. No significant GI or hematologic toxicity was observed. All patients demonstrated a 6-7-fold increase in γ globin synthesis for 1-2 weeks after treatment. Increased γ globin synthesis improved the α/non-α chain imbalance in the thalassemic patients, causing more effective erythropoiesis. In patients with SS disease, β^S production decreased in proportion to the increased γ synthesis, thus α/non-α synthesis remained balanced. Peripheral blood Hb F increased from 6.5 → 15.9% in one SS patient and from 1.6 → 8.9% in the second. Stractan gradient analysis revealed a dense RBC population with high MCHC before treatment; the proportion of cells in this population decreased markedly following 5-AzaC. The γ, ζ, and ε regions of bone marrow DNA became hypomethylated after treatment. Despite significant hypomethylation of the ε gene region, only 5-10 copies of ε mRNA were present in bone marrow cells at the time of peak drug effect, implying specific "activation" of the γ genes. The mechanism of the "reverse switch" from β to γ globin production may involve hypomethylation of DNA and/or a shift to erythroid progenitors that produce erythroblasts making more Hb F.

0164 HIGH-DOSE AMSA AND AUTOLOGOUS BONE MARROW TRANSPLANTATION IN PATIENTS WITH REFRACTORY METASTATIC BREAST CANCER, G. Spitzer, N. Tannir, F. Schell, A. Zander, M. Kanojia, K. Dicke and G. Blumenschein, M. D. Anderson Hospital and Tumor Institute, Houston, Texas 77030
Sixteen patients with refractory metastatic breast cancer were treated with high-dose AMSA (200-250 mg/m^2/day intravenously on three consecutive days, and repeated at three-week intervals), followed by autologous bone marrow transplant (ABMT). All patients had extensive prior chemotherapy, with a median of 2.5 (range: 1-6) treatment regimens. Two patients had partial remissions for 11 months and 7 months, respectively; two had stable disease for 7 months and 4 months, respectively, and twelve had progressive disease. The two responders are still alive 24+ months and 13+ months, respectively, from the time of AMSA treatment, while all the nonresponders are dead, with a median survival of 6 months (range: 2-14). High-dose AMSA therapy produced severe myelosuppression, with platelet counts remaining less than 20,000/μl for a median of 4 days (range: 1-16), and absolute granulocyte counts less than 500/μl for a median of 13 days (range: 5-24). During 25 courses of treatment, there were four episodes of documented sepsis (three bacterial and one fungal) and ten episodes of fever of unknown origin associated with neutropenia. The dose-limiting toxicity however, was mucositis which was more frequent and more severe at the higher dose (750 mg/m^2 total dose per course). Other toxicity included nausea and vomiting, and diarrhea. Engraftment was prompt, with platelet recovery (>100,000/μl) occurring at a median of 17 days (range: 11-27) and neutrophil recovery (>1000/μl) at a median of 24 days (range: 15-31) post ABMT. It appears that, in this group of patients, high dose AMSA has a minimal activity and is associated with significant toxicity.



0165 COMPARISON OF IMMUNO SUPPRESSION AND BONE MARROW TRANSPLANTATION FOR SEVERE APLASTIC ANEMIA. Gluckman E. for the European group of bone marrow transplantation (EGBMT).

The EGBMT working party on SAA has analyzed more than 450 patients treated either with I.S. or BMT. The actuarial one year survival is identical in both groups (60 %). Prognosis factors have been defined = patients with less than $0,2 \times 10^9$/l granulocytes and less than 10×10^9/l reticulocytes are unlikely to respond to I.S. In contrast, patients with a female donor, infected and older than 16 have a poor outcome after BMT. The use of Cyclosporin A seems to improve the prognosis of BMT in abrogating rejection and mitigating GVHD : the expected long term survival will be of at least 70 %. The recomendation of the EGBMT is to transplant as early as possible patients less than 40 years old if they have an HLA identical sibling. I.S. is mandatory if there is no donor. Preliminary results of a prospective protocol using high dose Methyl-Prednisolone and ATG will be presented.

0166 BONE MARROW TRANSPLANTATION IN SEVERE APLASTIC ANEMIA : A CONDITIONING REGIMEN USING CYTOXAN AND THORACO-ABDOMINAL IRRADIATION. Gluckman E;*, Devergie A.* and Dutreix J.**
*Bone marrow transplant unit - Hospital Saint-Louis - PARIS - FRANCE - and
** Radiotherapy department - I.G.R. VILLEJUIF - FRANCE -.

28 consecutive patients with SAA received a conditioning regimen for BMT using Cytoxan 50 mg/kg/day given on days -6,-5 and -4 followed on day -1 by a 6 Gy thoraco-abdominal irradiation (TAI) with total lung shielding at the dose rate of 5cGy/min. Their age ranged from 6 to 33 (median 15). They were transplanted with an HLA identical sibling and three were transplanted accross a major ABO incompatibility. After transplant, 19 patients received MTX as prophylaxis of GVHD and 9 patients received Cyclosporin A (CY A). Currently, 23 out of 29 patients are alive with a follow-up ranging from 112 days to 867 days and a one year actuarial survival of 78 %. One patient only rejected his graft, all the others had a permanent take. Among the group of patients treated with MTX, grade III-IV GVH was observed in 5 cases and was the cause of death of 2 patients. Chronic GVH was observed in 10 cases and was the cause of death of 2 patients. In the CY A group, one patient died on day 29 of A. GVH and infection, all the others survived. Severe A. GVH was observed in one case, C. GVHD in 4 cases.

In conclusion, CYT and TAI abrogate rejection, CY A seems to decrease the incidence and severity of GVH.

0167 MURINE MONOCLONAL ANTI-HUMAN T CELL ANTIBODIES FOR TREATMENT OF ACUTE GRAFT-VS-HOST DISEASE (GVHD), John A. Hansen, Paul J. Martin, Kathleen Remlinger, Kris C. Doney, Anajane Smith, H. Joachim Deeg, Keith Sullivan, Rainer Storb and E. Donnall Thomas, Fred Hutchinson Cancer Research Center, Seattle, WA 98104

In order to determine the potential utility of monoclonal T cell antibodies mcAb for the treatment of acute GVHD, we have begun a clinical phase I study to evaluate feasibility and toxicity. At the present time, at least 8 distinct T cell surface molecules have been defined. To date, fifteen patients with severe grade II-IV steroid resistant GVHD have received intravenous infusions of four different anti-T cell mcAb. Antibodies 9.6 (IgG2a) and 35.1 (IgG2a) bind Tp50, antibody 10.2 (IgG2a) binds to Tp67, and antibody 12.1 (IgG2a) binds to Tp100. A total of 151 infusions were given ranging in dose from one to 20 mg, each administered over 1-4 hr. One patient received a total of 259 mg mcAb over a period of 45 days. Six of 151 infusions (4%) in two patients were associated with fever or chills. Although most of the patients treated with monoclonal antibodies required platelet support, this was not significantly different from similar patients not receiving monoclonal antibodies. None of the five patients receiving low dose (1-2 mg/day) antibody therapy showed any evidence of improvement, while in 6 of 10 patients receiving intermediate to high doses (5-20 mg) there was evidence of partial improvement in GVHD in at least one involved organ system. None of the patients became immunized to mouse immunoglobulin. Antibodies specific for other T cell surface molecules will be similarly evaluated in phase II/III trials in order to identify the reagents most likely to be effective.

0168 BASIS OF CELLULAR IMMUNE DEFICIENCY ASSOCIATED WITH GRAFT-vs-HOST DISEASE (GVHD)
M-S. Tsoi, T. Mori, S.D. Brkic, E. D. Thomas and R. Storb, Fred Hutchinson Cancer Research Center, Seattle, Washington, 98104.
This study was designed to investigate the mechanism of immune deficiency in 40 patients (pts) given allogeneic marrow, mostly HLA-identical, for the treatment of hematologic malignancy or aplastic anemia. Peripheral blood mononuclear leukocytes (PBL) from pts were tested post grafting for indirect cell-mediated lympholysis (CML) reactivity against PBL from unrelated individuals and results were compared to those with cells from their marrow donors. An impairment of CML was found with cells from most pts with acute and chronic GVHD while cells from most short-term and long-term pts without GVHD had reactivity comparable to that of cells from the marrow donors. The impaired immune reactivity of cells from most short-term pts with acute GVHD, but not that of cells from most pts with chronic GVHD, could be restored to normal levels by the addition of interleukin 2 (IL-2) to the cultures. These results suggest that the cellular deficiency of pts with acute GVHD is due to defective production of IL-2 and that of pts with chronic GVHD is related to defect of effector cells (or their precursors) or to the presence of suppressor cells. We then attempted to document whether cells from pts with GVHD indeed had a defect in IL-2 production. PBL from pts and donors were stimulated in vitro for IL-2 production. Culture supernatants were assayed for their ability to induce proliferation of lymphoblasts. Cells from nearly all pts without GVHD produce IL-2 at a level of $\geq$70% of those from the donors. Cells from most pts with acute or chronic GVHD, however, produce significantly less IL-2 than those from the donors. The basis for defective IL-2 production is being investigated.

0169 BONE MARROW TRANSPLANTATION IN CHRONIC MYELOGENOUS LEUKEMIA, Hans A. Messner, John E. Curtis and Mark Minden, Ontario Cancer Institute, Toronto, Ontario, M4X 1K9.
A pilot study was performed to explore the efficacy of bone marrow transplantation in 23 patients with CML in various clinical stages. Patients were prepared with Cytosine Arabinoside 100 mg/m^2/day x 5 days given as continous infusion, Cyclophosphamide 60 mg/kg/day x 2 days and 500 rads total body irradiation midplane dose delivered at 50 to 80 rads per minute. Splenectomy was not routinely performed. The age of this group of patients varied from 18 to 46 years. The mean interval between diagnosis and transplant was 735 days. Fourteen of the 23 patients are currently alive between 2 to 40 months post-BMT with a median followup of 13 months. These include all 6 patients transplanted in chronic phase, 5 out of 11 transplanted in accelerated phase, 1 out of 4 in blast crisis and 2 out of 2 in second chronic phase. Three patients transplanted with grade IV - V myelofibrosis demonstrated complete resolution of reticulin. Relapse of the Philadelphia chromosome positive clone was only observed in one patient transplanted in blast crisis. The other deaths occurred due to transplant-related complications such as infections and graft vs. host disease. Cytogenetic analysis of the other patients revealed Philadelphia chromosome negative karyotypes in all examined metaphases. Besides status of disease, age of the patient appeared to be important as risk factors. Eight out of 10 patients transplanted below the age of 30 are currently alive, 6 out of 10 between 30 and 40. No survivors were observed above the age of 40.
In conclusion, this pilot study demonstrates that bone marrow transplants can successfully be performed in patients with CML. Younger patients and patients early on in their course of the disease appear to have the best prognosis.

Experimental Bone Marrow Transplantation

0170 SELECTIVE IMPAIRMENT OF IMMUNOGLOBULIN (Ig) SECRETION RESPONSES MAY REFLECT MATURATIONAL STAGES OF HUMAN T AND B CELLS AFTER MARROW GRAFTING. L.G. Lum, M.C. Seigneuret, N. Orcutt-Thordarson, T. Froelich, and R. Storb. Fred Hutchinson Cancer Research Center, Seattle, WA, 98104.
The function of T and B-cells from 12 long term healthy patients (pts) was studied after HLA-identical marrow grafting for aplastic anemia or hematologic malignancy from 360 to 1812 days after transplant. An indirect plaque assay was used to assess Ig secretion after stimulation with pokeweed mitogen (PW), herpes simplex type 1 virus (HSV), tetanus toxoid (TT), or Epstein-Barr virus (EBV). The results show: co-cultures of T and B cells failed to secrete Ig ($<$20% of control) in response to PW in 7 of 12 pts, to HSV in 6 of 12 pts, and to TT in 10 of 12 pts. B cells failed to respond to PW in 6 of 12 pts, to HSV in 4 of 12 pts, to TT in 6 of 12 pts, and to EBV in 3 of 6 pts. Impaired T cell helper activity ($<$ 20% of control) occurred in response to PW in 3 of 12 pts, to HSV in 4 of 12 pts, and to TT in 7 of 11 pts. T cells actively suppressed Ig synthesis by normal (T+B) cell cultures $>$ 80% in the PW system (3 of 11 pts), in the HSV system (0 of 11 pts), and in the TT system (2 of 11 pts). In summary: 1) B cells from a given pt can exhibit variable responses to different stimulants of Ig synthesis; 2) T cells from a patient can lack helper activity in response to one but not to other stimulants; 3) similarly, T cells from a particular patient could suppress Ig production induced by one stimulant but not by other stimulants. Selective impairment of specific functional responses may reflect different maturational stages of T and B cells that develop after marrow transplantation.

0171 IN VITRO MODEL OF GRAFT VS HOST REACTION AND ITS ANTILEUKEMIA EFFECT. William L. Elkins and Giuliana Pierson, Children's Hospital, Philadelphia, PA 19104

In order to determine whether Tc cells with GVH specificity were present in blood of 3 engrafted recipients (ER) of HLA matched sibling marrow, we set up the following mixed lymphocyte cultures (responder + primary stimulator + secondary stimulator): 1) ER + parents + host (pre-BMT), 2) ER + host + host. Media containing Il-2 was added with the secondary stimulator cell, and the secondary stimulus was renewed weekly to maintain the growth of the effectors. When sufficient effectors were present they were tested in a 4 hr. cell-mediated cytotoxicity assay against CR-51 labelled target cells. These included mitogen induced T cell blasts of donor and host origin and leukemic blasts, obtained from the host at diagnosis and cryopreserved. Strong killing at low killer: target ratios was seen against both normal and leukemic targets of the host in 2 cases where the engrafted recipient had acute GVHD. There was no cytoxocity to the donor-type targets. Thus killing displayed allogeneic GVH specificity. No such killing was generated in parallel cultures in which the responding cells were obtained directly from the marrow donor. Thus the donor derived cells from the ER, which killed host targets, were probably derived from precursors generated in the GVH reaction in vivo. The effector population was 90% Leu 4 +/ve, 56% Leu 2 +/ve by FACS analysis. Experiments with the 3rd patient, who had no manifestations of GVHD, did not yield killers with GVH specificity. We conclude that the donor T cells that are responsive to foreign minor antigens in the host comprise a detectable population in the blood of ER with GVHD, and that these target antigens are expressed both by lymphocytes and leukemic blasts of the host at least in some cases.

0172 GRAFT VERSUS LEUKEMIA (GVL): HLA IDENTICAL AML BLASTS AND T CELL GROWTH FACTOR (TCGF) CAN ACTIVATE MINOR LOCUS REACTIVE T CELLS IN VITRO. Paul M. Sondel, Jacquelyn A.Hank, Thad Wendel, Bridget Flynn and Marek Bozdech. University of Wisconsin, Madison, WI 53792

A GVL response is suggested by the inverse correlation of GVH with relapse after BMT. To study this in vitro, lymphocytes (Ly) from an HLA-MLC identical BMT donor (D) were stimulated for 7 days with her sib's irradiated AML blasts (alone or with allogeneic irradiated Ly). No cytotoxic T cells (Tc) against the AML blasts were detected. Culturing for 7 more days in TCGF induced Tc that killed the AML blasts, but not D's Ly. These Tc were grown in TCGF and repeatedly destroyed AML blasts, and Ly from mother and father, but not from D or an unrelated. A Tc clone was obtained from these cells by limiting dilution. Tc from this clone were cytotoxic to AML blasts, (see table below) and Ly from father, but not mother or D, and were inhibited by PA 2.6 (a monoclonal anti-HLA Class I antibody*). Thus AML cells plus TCGF induced Tc recognition of HLA restricted minor locus antigens by D. That this patient remains in remission 13 mo post BMT, having recovered from moderate GVH, is consistent with this in vitro "GVL" response.

CYTOTOXICITY BY CLONED Tc

	Target cells (12 Tc per Target)			
Anti HLA Ab*	AML Blasts	Father's Ly	Mother's Ly	D's Ly
None	30.1%	28.8%	2.0%	2.0%
1.0mg/ml	8.2%	0.6%	0.8%	—

0173 Monoclonal Antibody Specific for Myelogegenous Leukemia, Andrew J. Malcolm, Patricia M. Logan and Julia G. Levy, University of British Columbia, B.C., Canada.

Previous and ongoing work in our laboratory has permitted the purification of a cell surface protein from human acute myelogeneous leukemia (AML) cells, which is apparently myeloid leukemia specific and relatively easy to isolate. Spleens from immunized Balb/c mice were fused to NS-1 myeloma parental cells and a myelogeneous leukemia specific monoclonal Ab was selected from the hybrid colonies produced. This monoclonal Ab, as well as a rabbit anti-AML-serum previously reported (Malcolm et al., J. Immun. 128:2599, 1982) has been used to help with diagnosis of myelogeneous leukemia in the fluorescence-activated cell sorter (FACS IV). In addition, this monoclonal also demonstrates positive fluorescence binding to HL-60 (a promyelocytic leukemia cell line) and negative binding to lymphocytic leukemia cell lines (CCRF-SB-ALL-B and CCRF-CEM-ALL-T cell lines). The monoclonal has been shown to be specific with the enzyme linked immunosorbent assay (ELISA) and to isolate the AML Ag by employing an immunoadsorbent column.

Bone marrow transplant patients have been FACS analyzed with the monoclonal for the malignancy marker. A preliminary study has indicated that it will be possible to predict relapse in bone marrow transplant patients. (Supported by the B.C. Health Research Foundation and the National Cancer Institute of Canada).

0174 DIMINUTION OF GRAFT-VERSUS-HOST (GVH) DISEASE FOLLOWING INCUBATION OF EFFECTOR CELLS WITH NON-INFECTIOUS VIRUS, Mark A. Wainberg and Evelyne Israel, Jewish General Hospital, Montreal, Canada

Previous studies have shown that co-incubation of UV-inactivated retroviruses with murine or human lymphoid cells inhibits the ability of the latter to respond to antigenic and mitogenic stimuli. We have now investigated the effect of pre-exposure to inactivated Friend leukemia virus (FLV) on the ability of mouse spleen cells to participate in acute GVH reactions. These studies were performed using combinations of Balb/c, C3H and C57Bl6 mouse strain F_1 hybrids, and the injection of 5 x 10^6 parental splenocytes into 2-3 day-old recipients. The effect of virus on effector cell activity was assessed by the pre-incubation of parental spleen cells with virus (10^7 virus particles/10^6 cells/ml) for varying periods at 37°C. The results showed that acute GVH disease was eliminated in 50% of cases (i.e. 15 of 30) in which viral pre-incubation was carried out for 2-4 hr, and significantly reduced in a further 25% of cases tested. Viral co-incubation for shorter periods or the use of virus:cell ratios less than 3:1 were far less effective in inhibiting acute GVH reactions. Cells which were virus co-incubated and unable to respond to T cell mitogens (PHA and Con A) remained viable; ability to respond to lectin was restored if exogenous T cell growth factor (TCGF) was added to the medium. Addition of TCGF to cultures of virus co-incubated spleen cells did not, however, restore ability to participate in GVH reactions. These studies show that FLV can exert time- and concentration-dependent immuno-regulating influences through mechanisms other than active infection, and that this can be manifested both in vitro and in vivo. Supported by the Medical Research Council of Canada.

0175 IMMUNOSUPPRESSIVE ACTIONS OF 2-PYRIDINECARBOXALDEHYDE-2'-PYRIDYL-HYDRAZONE-COPPER(II) ON SPONTANEOUS LYMPHOCYTE TRANSFORMATION AND SKIN GRAFT REJECTION. Loren Pickart, William Goodwin and William Burgua. Virginia Mason Research Center, Seattle, WA 98101.

The aroylhydrazone-copper complex, 2-pyridinecarboxaldehyde-2'-pyridyl-hydrazone-copper(II) (PCPH-Cu), is a structural analog of the growth-modulatory Gly-His-Lys-Copper(II) complex, and a potent mitotic inhibitor with antineoplastic and immunosuppressive actions (Pickart, Lymphokines 8:425-446 (1983)). In vitro: When added to culture medium, PCPH-Cu gives 50% inhibition of the spontaneous transformation (visual microscopic quantitation) of mixed splenic lymphocyte cultures from Balb/C and C57 Bl/6J mice after 96 hours at 0.94 ng/ml. In contrast, bleomycin sulfate gives 50% inhibition at 32 ng/ml. Measurement of the ^{3}H-thymidine incorporation into DNA in mixed lymphocyte cultures in the presence and absence of PCPH-Cu gave similar results. In vivo: PCPH-Cu delayed skin graft rejection in mice. Treatment of male Balb/C mice twice per week with 100 µg PCPH-Cu extended the mean life of skin grafts from male C57 Bl/6J mice from 13.7 to 21.0 days. Also, the skin grafts in treated mice showed markedly less retraction from graft margins. Aroylhydrazone-copper complexes similar to PCPH-Cu may represent a new class of immunosuppressive compounds. (Supported by NIH grants CA 28858 and RR 05588).

0176 POTENTIAL APPLICATIONS OF RADIONUCLIDE THERAPY TO BONE MARROW TRANSPLANTATION W.D. Bloomer, W.H. McLaughlin, S.J. Adelstein, Harvard Medical School, Boston, MA 02115 and A.P. Wolf, Brookhaven National Laboratory, Upton, NY 11973

Radionuclides that decay by alpha (helium nuclei) or beta (electron) emission have the potential to increase the efficacy of bone marrow transplantation programs. If covalently bound to monoclonal antibodies, radionuclides should increase the magnitude of antibody-directed tumor cell killing in vitro and possibly in vivo in the bone marrow of patients being prepared for autotransplantation. If part of a bone marrow-seeking radiocolloid, these same radionuclides may supplement and reduce external beam dose requirements in all patients receiving bone marrow transplantation, thus minimizing normal tissue damage in critical organs such as the lung.

On the basis of energy deposition per unit path length and experimental studies in vitro and in vivo comparing tumor cell killing and normal tissue toxicity, alpha emitters possess a greater lethal efficiency than the beta emitters phosphorus-32, yttrium-90 and dysprosium-165. The prototype for such therapy, astatine-211 is an almost pure alpha emitter with a 7.2 hour physical half-life. Nonetheless, short-lived beta-emitters may be more than adequate adjuncts in a multimodality approach. Radionuclide therapy warrants further careful investigation in bone marrow transplantation. Supported by grants CA 12662 and CA 30043 from NCI.

0177 IDENTIFICATION AND PARTIAL PURIFICATION OF A HUMAN VIRUS THAT INHIBITS HEMATOPOIESIS IN VITRO, Neal Young, R. Keith Humphries, Jeffrey Moore, and Philip Mortimer, National Institutes of Health, Bethesda, MD 20205

Transient aplastic crisis in children with sickle cell disease has been linked epidemiologically to infection with a parvovirus-like virus (SPLV). This virus inhibits human hematopoiesis in vitro. 10^{-2} dilutions of sera containing this virus, obtained from five blood donors and two patients with brief febrile illnesses, were incubated with normal bone marrow cells for 4 hours at 4°C; the cells were then cultured in methylcellulose to assay erythroid (CFU-E and BFU-E) and myeloid (CFU-C) progenitors by colony formation. In comparison to sera from laboratory personnel, 6 of 7 sera containing SPLV showed more than 50% inhibition of CFU-E derived colony formation; three of 7 sera also inhibited erythroid colony formation from the more primitive progenitor, the BFU-E, and did not inhibit myeloid colony formation. Fresh serum obtained from a child with hereditary spherocytosis during the acute phase of an aplastic crisis almost completely abolished CFU-E derived colony formation; inhibition was evident at dilutions of 10^{-4}. Convalescent phase sera from two patients with febrile illnesses partially or completely neutralized the suppressive effect of acute phase sera. Acute phase sera obtained from patients with other viral illnesses, including hepatitis A, hepatitis B, cytomegalovirus, herpes simplex, Varicella, and Echovirus II infections, did not inhibit hematopoiesis in vitro. Using a 10 to 40% sucrose gradient, SPLV colony inhibitory activity was present in the virus-particle containing fraction and separated from IgG and IgM containing fractions. These results provide evidence that one form of hematopoietic failure is the result of a direct, cytotoxic effect of virus on bone marrow progenitors.

0178 IMMUNOTOXINS IN HUMAN BONE MARROW TRANSPLANTATION (BMT): I. REAGENTS SELECTIVITY REACTIVE WITH CELLS OF T CELL LINEAGE WITH POTENTIAL USE FOR ALLOGENEIC OR AUTOLOGOUS BMT FOR T CELL ALL. D. Vallera, R. Ash, R. Youle, E. Zanjani, D. Neville, R. Quinones and J. H. Kersey. University of Minnesota, Minneapolis, MN; NIMH, Bethesda, MD

In theory monoclonal antibody (MoAb) plus complement (C) treatment should be useful for purgation of human BM for either allogeneic of autologous BMT. In fact, human protocols are limited by technical problems associated with the use of C. We are examining the alternative approach of linking MoAb directly to the potent toxin ricin and using this reagent for ex vivo treatment of BM. The natural binding site of ricin is blocked by lactose rendering the conjugate antibody specific. We have employed a technique whereby ricin is covalently linked by a thioether bond to MoAb specifically reactive to different determinants on human T lymphocytes and T lymphoblasts. Included is TA-1, an IgG2a MoAb recognizing most peripheral blood T cells, monocytes, natural killer cells, and T cell ALLs. Human PB mononuclear cells were pretreated, washed, and then evaluated for anti-T cell activity in PHA and MLR assays. Reactivity was antibody-specific as it could be blocked during pretreatment with cold TA-1 antibody and not control myeloma antibody. Differential selectivity was demonstrated in the fact that "immunotoxin" killed antigen-positive, but not antigen-negative leukemic cell lines suggesting potential value for these reagents in autologous BMT. Most importantly, TA-1-ricin eliminated anti-T cell activity at concentrations (500-1000 ng/ml) which did not effect pluripotent human stem cells measured in CFU-GEMM assays. Our findings indicate that immunotoxins display appropriate selectivity for use in purging bone marrow of T cells or their malignant counterparts.

0179 IMMUNOTOXINS IN HUMAN BONE MARROW TRANSPLANTATION (BMT): II. REAGENTS SPECIFICALLY REACTING WITH CYTOTOXIC T LYMPHOCYTES (CTL) IN HUMAN MARROW, WITH POTENTIAL FOR GVHD PROPHYLAXIS. R. Quinones, J. Kersey, R. Youle, D. Neville, R. Ash, E. Zanjani, D. Vallera. Univ. of Minnesota, Minneapolis, MN. NIMH, Bethesda, MD.

Experimental and clinical evidence indicate that depletion of immunocompetent T cells from bone marrow (BM) inoculums can prevent GVHD; however, there is a need for a practical methodology for large scale application of T cell depletion. Immunotoxins, pan-T-cell monoclonal antibodies covalently linked to whole ricin toxin, offer a method for rapid, selective elimination of functional T cells, while preserving hematopoeitic stem cells. We have found generation of CTLs to be a reliable, specific assay for T cell function in BM and may serve as an in vitro model of the in vivo phases of generating target-specific effector T cells involved in GVHD. We have compared effects of immunotoxin on BM T cells and stem cells simultaneously. Anti-T-cell immunotoxins were incubated with peripheral blood (PB) or BM mononuclear cells in the presence of lactose,which competatively blocks the native lectin-like binding by the ricin B chain. One hybrid, TA1-ricin, at doses of 500 ng/ml eliminated generation of CTLs from precursors in PB or BM to <1% or <5% of controls, respectively. Stem cell activity, as assessed by the CFU-GEMM assay, was preserved at these levels of immunotoxin, evidence of the selectivity conferred by the monoclonal antibody. Further evidence of binding being mediated by the antigen-specific binding site of the antibody is that blocking of an immunotoxin's cytotoxic effect is only by the parent antibody. Anti-T-cell immunotoxins are potential reagents for practical trials of GVHD prevention in MHC matched allogeneic BMT and possibly in mismatched transplants.

0180 EXPERIMENTAL HOST VERSUS GRAFT (HVG) SYNDROME AS A MODEL OF TRANSPLANT INDUCED DISEASE OF ALTERED IMMUNITY. Richard C. Hard, Jr., Sue S. Cross, Joel Mahler, and Peter H. Bick, Med. Col. of Va., Richmond, VA 23298

HVG syndrome is the fatal disease of altered immunity which has thus far been observed in 6 strains of inbred mice following the perinatal inoculation of semiallogenic spleen cells. Histopathologic and functional studies have shown that the primary lesion is the severe depletion of peripheral T-lymphocytes of both F_1 donor and parental host. This correlates with poor primary responses to thymic dependent antigen. In contrast, there is marked hyperplasia of B-cells with hyperglobulinemia. Recent work has suggested that F_1 donor B-cells are spared from the allogenic HVG attack which destroys T-cells. The present work tests for the contributions of $(T_6 \times RFM)F_1$ donor cells to serum Ig levels in $RFM/(T_6 \times RFM)F_1$ chimeras with HVG disease. F_1 spleen cells presensitized to SRBC and to horseradish peroxidase (HRP), and spleen cells from F_1 mice which express murine leukemia virus (MuLV) and produce antiviral antibody are inoculated into RFM perinates. Plaque forming cells and hemolytic antibodies are found in $RFM/(T_6 \times RFM)F_1$ mice without further stimulation with SRBC. No rise in hemolysin titers is seen between 18 and 25 days. Similarly, HRP-binding plasmacytoid cells are seen in other HVG mice. This suggests that the allogenic HVG reaction stimulates B-memory cells to proliferate and mature. Mice tested for MuLV may be due to additive effects of: 1) antigen and the allogenic effect on persensitized, F_1 donor B-cells; or 2) host responses. It appears that the high levels of serum Ig's in HVG mice with poor primary responses to T-dependent antigens is due in part to F_1 donor B-cells.

0181 FATE OF INTRAUTERINE FOETAL BONE MARROW GRAFTS, Ronald D. Barnes and Bruce Pottinger, Harrow, Middlesex.

Head of Multi-disciplinary Group primarily involved in foetal development. Major efforts have been recently made investigating the possibility of correcting certain inherited diseases by transuterine foetal grafting. In this context we have concentrated upon following the fate of bone marrow grafting.

Working with mice, rabbits, rhesus monkeys and baboons - all prior to extrapolation to man, we have shown following transuterine injection of both xenogeneic or allogeneic viable bone marrow cells:

a) that tolerance to (i) cellular and (ii) soluble, antigens can be induced,
b) that tolerance is permanent,
c) chimaerism can be established even leading to the grafted cells producing their "normal"
d) deficiency states have been "corrected"
e) moreover all without risk to foetal well being - graft versus host disease never having been detected.

0182 CAMPATH 1 - A HUMAN COMPLEMENT FIXING MONOCLONAL ANTIBODY FOR REMOVING MLR REACTIVE CELLS FROM DONOR TRANSPLANT MARROW, Stephen P. Cobbold, Gill Chumbley, Geoffrey Hale, Trang Hoang, Donald Metcalf, Alan J. Munro, David Swirsky and Herman Waldmann, Department of Pathology, University of Cambridge, England.

A rat IgM monoclonal antibody has been produced which is strongly cytotoxic for human lymphocytes with autologous complement. Treatment of peripheral blood mononuclear cells with antibody and human complement removed > 99% of the lymphocytes (T and B cells) and > 98% of the cells which respond in a mixed lymphocyte culture. Under these conditions, marrow colony forming cells are unaffected. Repeated CAMPATH 1 administration (up to 100 mg i.v.) to cynomolgus monkeys and two human patients (with non-Hodgkin's lymphoma and chronic lymphoblastic leukaemia) was non-toxic, utilised serum complement, and caused a rapid, partially reversible reduction in circulating lymphocytes, without antigenic modulation. CAMPATH 1 is currently on clinical trial for eliminating graft versus host disease in mismatched bone marrow transplants (aplastic anaemia and chronic granulocytic leukaemia) by pretreatment of donor marrow.

0183 DONOR-RECIPIENT MATCHING WITH THE MIXED LYMPHOCYTE-EPIDERMAL CELL REACTION (MLECR) David Steinmuller and Mark R. Pittelkow, Mayo Medical School and Mayo Clinic, Rochester MN 55905

Like Sontheimer and Gilliam (Fed.Proc.40:1165, 1981) we have found that human epidermal cells (EC) are more efficient than peripheral blood mononuclear cells (PBMC) in stimulating allogeneic lymphocytes to blastogenesis. Irradiated EC obtained by trypsinization of suction-blister skin biopsies from 10 normal subjects were tested in parallel with irradiated autologous PBMC for their ability to stimulate a panel of lymphocytes from 5-8 unrelated donors. 5×10^4 responder cells were cultured with an equal number of stimulator cells for 5 days; then terminally pulsed for 6 hrs with 1 µCi of ^{3}HTdR. Under these conditions, EC almost invariably were more stimulatory than autologous PBMC, sometimes several-fold so. In 2 of 4 cases EC of leukemia patients were less able than autologous PBMC to stimulate the lymphocyte panel, possibly reflecting abnormalities in Langerhans cells (the bone marrow-derived, DR-stimulatory cells of the epidermis) induced by chemotherapy. Like Sontheimer (J.Invest. Derm. 78:337, 1982) we also have found an individual whose EC very significantly stimulate lymphocytes of his HLA-identical, MLR non-reactive sibling. However, this may be a rare phenomenon because it has been observed in only 1 of 9 HLA-identical sibling pairs tested to date in our laboratory. Given the ease with which human EC suspensions can be prepared from simple, benign, suction-blister biopsies and the fact that the skin is a prime target-organ in graft-versus-host disease, the MLECR may be a useful adjunct to the MLR in matching donors and recipients for bone-marrow transplantation. Supported by USPHS research grant AM 26783 and the Robert H. Kieckhefer Fund.

0184 HUMAN MONOCLONAL ANTIBODIES WHICH REACT WITH ACUTE MYELOGENOUS LEUKEMIC CELLS, BUT NOT WITH REMISSION BONE MARROW, Christopher L. Reading, Meena Chandran and Lijda Vellekoop, Department of Tumor Biology and The Bone Marrow Transplantation Center, The University of Texas System Cancer Center, M.D. Anderson Hospital and Tumor Institute, Houston, TX 77030

We have produced human monoclonal antibodies reactive with human acute myelogenous leukemic (AML) cells by Epstein-Barr Virus (EBV) transformation. A leukemic patient in complete remission was found to have serum antibodies which were reactive with his own AML cells which had been stored at presentation, but unreactive with his own bone marrow cells stored after he reached remission. Peripheral blood mononuclear cells (PBMC) were obtained from this patient, and sheep red blood cell rosette forming cells (E_r) and adherent cells were removed. The partially purified B cell population was absorbed onto a monolayer of his own immobilized remission bone marrow cells, and the non-adherent cells, and the non-adherent cells were removed after 1 hr and absorbed onto a monolayer of his own immobilized leukemic cells. After 1 hr, the non-adherent cells were removed and the adherent cells were transformed with EBV. After 24 hr, the cells were plated in 192 microwells with PBMC from another donor. Transformed B cells grew in every well and when the cultures were dense, the medium was tested for reactivity with the patients AML cells. 44/192 (23%) of the cultures produced antibodies reaction with the AML cells. Cell lines were established from seven of these cultures, and all continued to produce the antibodies. Only one of these antibodies reacted with the patient's remission bone marrow cells in the ELISA assay.

0185 DEVELOPMENT OF IMMUNOCOMPETENCE IN THE FETAL DOG, Otto Prümmer, Wenceslao Calvo and Theodor M. Fliedner, Department of Clinical Physiology and Occupational Medicine, University of Ulm, D-7900 Ulm, Fed. Rep. of Germany

In the murine system fetal liver cells have successfully been used as an alternative source of hemopoietic stem cells, restoring hemato- and lymphopoiesis without acute graft-versus-host disease in an allogeneic adult recipient. Attempts at transferring these results to other species have to consider interspecies differences with respect to contaminating immunocompetent cells in grafts of different gestational periods. In the present study the development of immunocompetence was followed in the liver, thymus, and spleen of dog fetuses by means of in vitro lymphocyte transformation by different lectins (PHA, ConA, PWM) and allogeneic mononuclear cells of adult origin (MLC). SIg^+ cells were determined by immunofluorescence and T cells as $ANAE^+$ cells. No response could be elicited in the fetal liver by lectins up to the 21st after delivery. In contrast, as soon as the 34th gestational day MLC reactions were positive, transiently disappearing after day 41 and reappearing at day 57 of gestation. Early MLC reactions, however, were brought about by proliferating hemopoietic cells after an initial rise of CFU-C in culture. Substantial MLC reactivity could be detected in thymus and spleen at day 53 of gestation. The frequency of sIg^+ cells was about 1 % in liver, 11 % in spleen, and less than 0.1 % in thymus at day 53. Fetal liver grafts taken from fetuses younger than 53 days of age may prove appropriate for transplantation purposes in the dog.

0186 SECONDARY TUMORS AFTER HIGH-DOSE CHEMO-RADIOTHERAPY PRECEDING BONE MARROW TRANSPLANTATION. Barbara Lopes Cardozo, Anton C.M. Martens and Anton Hagenbeek, Radiobiological Institute TNO, Rijswijk, The Netherlands.

High-dose chemoradiotherapy followed by bone marrow transplantation has become standard treatment for (hematological) malignancies and aplastic anemia in various centers. Since the number of longterm disease-free survivors steadily increases, late effects due to the initial high-dose treatment become increasingly important. In particular, a significant number of secondary tumors might be expected. As the latency period in man possibly ranges from 5-20 years, studies in the rat were initiated with the emphasis on the incidence of malignancies after high-dose cyclophosphamide and total body irradiation.

Brown Norway rats were given a supralethal combination of cyclophosphamide (100 $mg.kg^{-1}$) and total body irradiation (either flash or fractionated high dose), followed by autologous bone marrow transplantation. Out of 110 long-term survivors ($>$ 100 days) 31 (28%) rats died from a secondary malignancy with a median post-treatment latency period of 15.0 months (range 3.3 - 24.8). Non-treated control rats show a spontaneous tumor incidence of about 1% at a comparable age which is rising to higher levels ($\lesssim$ 30%) during the aging process. A striking observation found in this study was the strongly shortened latency period as compared to control values.

Although these figures are not directly comparable to the clinical situation, the combination of cyclophosphamide and total body irradiation is clearly carcinogenic in the Brown Norway rat.

The work was supported by the Queen Wilhelmina Fund (grant IKR 82-8).

0187 CELL SEPARATION STUDIES IN AUTOLOGOUS BONE MARROW TRANSPLANTATION. Anton Hagenbeek and Anton C.M. Martens, Radiobiological Institute TNO, Rijswijk, The Netherlands.

The successful application of autologous bone marrow transplantation in acute leukemia depends on (1) complete eradication of residual disease in vivo, and (2) elimination of clonogenic leukemic cells from the graft in vitro. Studies in a rat model for human acute myelocytic leukemia (BNML) will be presented dealing with various methods of separation of leukemic cells from normal hemopoietic stem cells. The efficacies of both biophysical methods (velocity sedimentation, density gradient centrifugation, free-flow cell electrophoresis), pharmacological means (in vitro treatment with derivatives of cyclophosphamide) and immunological procedures (monoclonal antibodies) will be compared. So far, by combining two methodologies, effective separation was achieved with mixtures containing up to 1 clonogenic leukemic cell per 200 normal bone marrow cells (0.5% contamination). A sufficient number of normal stem cells was harvested to repopulate lethally irradiated recipients.

Supported in part by the Queen Wilhelmina Fund of the Dutch National Cancer League (grant RBI 80-1).

0188 EFFECTS OF MONOCLONAL ANTI-Lyt-1 ON ACUTE GvH REACTION ACROSS MAJOR AND MINOR BARRIERS, James P. OKunewick, Mary J. Buffo and Deborah L. Jones, Cancer Research Laboratories, ASRC, Allegheny General Hospital, Pittsburgh, Pa. 15212

Using a mouse model the effects of Lyt-1.2 monoclonal antibody directed against the T-helper cell subset was compared to that of Thy-1.2 monoclonal pan-T antibody. GvH reaction across major histocompatibility barriers was evaluated using the C57BL/10 ($H\text{-}2^b$) as the donor, and across minor barriers using its congenic B10.S ($H\text{-}2^s$) derivative. The recipients were ($H\text{-}2^s$) SJL/J. Being congenic, the B10.S and C57BL/10 differed in an identical way from the SJL/J at the minor histocompatibility determinants. At the major determinants the B10.S was identical to, and the C57BL/10 different from, the SJL/J. To maximize acute GvH reaction spleens were used as the only source of donor cells. These cells were exposed to the antibody in the presence of complement before being infused into the recipients. Anti-Lyt-1 had little effect in GvH reaction across major barriers, extending median survival by only a single day, with no survivors. However, the effect across minor barriers was highly significant extending median survival by more than a month, with 25% survivors, all of which later developed chronic GvHD. This contrasts sharply with our earlier results in which anti-Thy-1.2 prevented fatal GvH reaction across minor barriers in 85% of the recipients with no chronic GvHD development. Across major barriers the same anti-Thy-1 eliminated acute GvHR but had no effect on delayed GvHR. The results suggest that the anti-Lyt-1 may be acting on a helper-cell subset that plays a significant role in minor barrier GvH reaction, while in contrast major barrier GvH reaction may not be highly helper dependent. (Supported by H.R.S.F., United Way of Pittsburgh and by NCI/NIH).

0189 A MODEL FOR AUTOLOGOUS AND ALLOGENEIC MARROW TRANSPLANTATION IN LEUKEMIA/LYMPHOMA, Bruce C. Veit, Otto Schofer, F. Leonard Johnson and Patrick J. Buckley, St. Jude Children's Research Hospital, Memphis, TN 38101.

A model was established for the study of autologous and allogeneic bone marrow transplantation in rats that exhibit a rapid and progressive intra- and extra-medullary growth of leukemia/lymphoma. Wistar Furth rats were irradiated with 1000 rads and injected i.v. with $5x10^7$ syngeneic bone marrow cells containing 0.2% ($1x10^5$) G-1 tumor cells (syngeneic T cell lymphoma). At days 16-17, hind-leg paralysis was observed in all animals treated with tumorous marrow or with tumor alone but not in animals that received irradiation alone or irradiation + nontumorous marrow. At autopsy of paralyzed animals, tumor was found in bone marrow (90-100% blasts), blood (4-6000 WBC/mm^3, 25-70% blasts), thymus and associated lymph nodes, inside the spinal canal, and as solid nodules in the chest wall and along the spine. Paralysis was attributed to a compressive outgrowth of vertebral tumor. In a similar series of experiments, rats were inoculated with cells from two established human T cell leukemia lines, MOLT-4 and PEER. Animals treated with PEER cells developed paraplegia and exhibited the associated pathology in an identical manner to that observed with the syngeneic T cell lymphoma. However, animals treated with MOLT-4 cells did not develop paraplegia but did develop solid tumors in the neck (presumably of lymph node origin) at days 20-25 post irradiation. Tumor cells were also seen in the blood (1-2000 WBC/mm^3, 30-40% blasts) and bone marrow (40-60% blasts). This model provides an opportunity to study (1) immunologic and chemotherapeutic approaches to the in vitro treatment of autologous marrow containing subdetectable numbers of tumor cells, and (2) the biology of graft vs. leukemia in allogeneic transplants.

0190 HISTOPATHOLOGY OF GRAFT-VS-HOST SKIN DISEASE IN MICE DUE TO MINOR HISTOCOMPATIBILITY ANTIGEN DISPARITY. M.R. Charley, C.A. Elmets, B.L. Hamilton, J.N. Gilliam and R.D. Sontheimer, Dermatology Department, Southwestern Medical School, University of Texas, Dallas,Texas and Department of Biol. Str., School of Medicine, University of Washington, Seattle, Washington. The skin is a major target organ in both acute and chronic graft-vs-host disease (GVHD), yet little is understood regarding the patho-physiology of the cutaneous injury. A mouse model of GVHD with skin involvement would offer several advantages over other laboratory animals, including the availability of more inbred strains and more immunologic reagents. The murine strains C57Bl/6J and LP/J are both $H\text{-}2^b$ and are mutually non-reactive in mixed lymphocyte culture. They both develop cutaneous GVHD when used as irradiated recipients of the others bone marrow and spleen cells. The LP/J's sequentially develop a lichenoid pattern of epidermal and follicular injury (7 days) which resolves and is followed by a progressive dermal and subcuticular sclerosis (17 days). In addition the parotid and submandibular , but not the sublingual glands show a round cell infiltrate followed by acinar destruction. The C57Bl/6J's have a similar initial lichenoid pattern (12d) but, unlike the LP/J's, do not resolve their interface dermatitis. They neither develop sclerosis nor salivary gland involvement. Syngeneically transplanted, irradiated LP/J and C57Bl/6J controls do not show skin changes. Thus, in less than one month, this murine model offers the opportunity to study and manipulate both the acute and chronic injury patterns of GVHD in the skin.

0191 ROLE OF NON-H-2 ALLOANTIGENS IN THE INDUCTION OF ANTILEUKEMIA REACTIVE CELLS IN H-2-COMPATIBLE DONORS, R.L. Truitt, C-Y. Shih, A.V. LeFever and M.M. Bortin, Winter Research Laboratory, Mt. Sinai Medical Center, P.O. Box 342, Milwaukee, WI 53201 USA.

Alloimmunization of donor mice with lymphoid cells from normal allogeneic mice induced graft-vs-leukemia (GVL) reactivity against H-2-compatible T cell leukemia in AKR mice. In some, but not all, cases induction of GVL reactivity was independent of an increase in graft-vs-host (GVH) reactivity. Clarification of the mechanism involved might facilitate clinical application of this approach for increasing the antileukemia reaction without increasing the antihost reaction in recipients of allogeneic bone marrow. In vivo assays were used to measure GVL and GVH reactivity. The GVL reaction was mediated by an $Lyt\text{-}1^+2^+$ donor T cell(s) which persisted in the donor for as long as 8 weeks after alloimmunization. NK-specific alloantiserum had no effect on the GVL reaction. Alloimmunization of H-2-compatible donors with non-H-2 identical and F_1-hybrid cells established the importance of non-H-2 alloantigens in the induction of GVL reactivity. H-2-incompatibility of the alloimmunogen sometimes caused a decrease in the GVL reactivity induced by non-H-2 alloantigens. Failure to induce GVL reactivity in some donor-immunogen combinations was due to a lack of shared alloantigens between AKR leukemia cells and the immunizing strain and/or alloantigenic compatibility between the spleen cell donor and the immunogen relative to the antigens on AKR leukemia. The GVL reaction was found to be donor cell dose dependent in those donor-immunogen combinations in which a relatively high or low GVL reaction was observed, but not in the non-GVL reactive combinations. (Supported by USPHS CA 20484 and CA 26245, the Evan and Marion Helfaer Foundation, the Rexnord Foundation and the Leukemia Society of America, Inc.)

0192 IS LYMPHOID CHIMERISM IN RLA-MATCHED RABBITS RESTRICTED TO B CELLS? Louise T. Adler, Michelle M. LeBeau and Frank L. Adler, St. Jude Children's Research Hospital, Memphis, TN and University of Chicago, Chicago, IL

The transfer of spleen, lymph node or bone marrow cells from adult rabbits to newborn recipients results in the establishment of lasting (>1 yr) B cell chimerism if unrelated donors and recipients are matched for major histocompatibility antigens (RLA). Presence and function of donor B cells have been conveniently monitored by deliberate mismatches for Ig allotypes. To trace T cells of donor origin, cells from male or female recipients which had received cells from a donor of the opposite sex were stimulated in vitro with Concanavalin A and metaphases were karyotyped. Donor-derived T cells could not be found in recipients of RLA-matched donor cells. Appropriately designed mismatches led to fatal GvH in most instances, presumably due to the establishment of donor T-effector cells responding to the recipient's foreign RLA type. However, some recipients of incompatible donor cells lived and showed a total replacement of their lymphoid system by donor B and T cells. These findings, together with relevant findings on the establishment of lasting B cell chimerism reported separately, lead us to suggest that chimerism in our noninbred rabbit model results largely, if not entirely, from the establishment and expansion of committed B (and sometimes T) cells, and that stimulation of such committed cells by specific extrinsic or intrinsic antigens plays an important or decisive role in this process. The apparent failure of donor T cells to colonize histocompatible newborn recipients may result from lack of "space" in the T cell compartment compounded by insufficient antigenic stimulation or, alternatively, could reflect inadequacies in the RLA typing procedure.

0193 SELECTIVE IMMUNOCOMPETENCE OF DONOR B CELLS IN CHIMERIC RABBITS, Frank L. Adler and Louise T. Adler, St. Jude Children's Research Hospital, Memphis, TN 38101.

Transplantation of lymphoid cells from adult rabbits to newborn recipients results in lasting chimerism when donors and recipients are matched for major histocompatibility antigens. Mismatching for Ig allotypes permits quantitation of donor-derived B cells and their products. The serial transfer of donor B cells to secondary recipients has been demonstrated. Although chimeric recipients may have 20-50% donor derived Ig, the donor B cells and their progeny appear to be immunoincompetent in that they fail to participate in antibody responses to TI or TD antigens injected into the recipients. Evidence to be presented supports the hypothesis that the apparent immune deficiency results from a restriction in the B cell repertoire of donor cells stemming from colonization of the recipient by a finite number of committed B cells and their selective clonal expansion. When chimerism is induced by the transfer of spleen cells from rabbits primed with OVA, donor cells are able to participate in anti-OVA responses of the 6 month old recipient. Further, the transfer of spleen cells from hyperimmune (anti-OVA) donors to newborn recipients, followed by injections of OVA, results in strong anti-OVA responses entirely made by donor cells. These data show that committed B cells of donor origin can expand clonally, can function as antibody producers, and can respond to regulation by antigenic stimulation. The model of noninbred rabbits which accept permanent grafts of lymphoid cells from histocompatible unrelated donors and the selective expression of immunocompetence by donor B cells and their progeny may be useful in the study of immune deficiency in human bone marrow transplant recipients and in future efforts to manipulate the quality of engraftment.

0194 ANALYSIS OF CYTOTOXIC T LYMPHOCYTES (CTL) IN MURINE GVH DUE TO MINOR-H ANTIGENS Brian L. Hamilton, University of Washington, Seattle, WA 98195

A murine model of GVH induced in response to Minor histocompatibility antigens (Minor-HA) has been developed which includes the sclerodermatous changes of chronic GVH. GVH develops in response to Minor-HA when sufficient numbers of Thy-1+ spleen cells are transplanted into lethally irradiated, H-2 identical recipients. The role of CTL as an effector mechanism of Minor-HA-GVH was analyzed using this model. Two strain combinations were studied: C57BL/6 (B6) and LP (both H-2^b) and B10.D2/nSN and BALB/cJ (both H-2^d). In reciprocal transplantation experiments both recipients in the H-2^b combination (B6→LP and LP→B6) developed GVH whereas in the H-2^d combination only the BALB recipients of B10.D2 cells (B10.D2→BALB) developed GVH. Spleen cells were obtained from transplanted mice 10-14 days after lethal irradiation and transplantation (10^7 BM plus $5x10^7$ spleen cells) and tested for cytotoxic activity on a panel of Con A blast cells in a 4 hr chromium release assay. Spleen cells from each of the four transplanted strains were specifically cytotoxic to their respective recipient strain target cells, including the BALB→B10.D2 recipients which did not develop GVH. The CTL were Thy-1+, Lyt-2+, with about half the response attributed to Lyt-1+ cells. Spleen cells from mice without GVH after transplantation with anti-Thy-1 treated donor cells were not cytotoxic to recipient strain target cells in any of the four strains tested. The lack of correlation between the presence of splenic CTL and GVH in these mice suggests that CTL are not the significant in vivo effector mechanism of Minor-HA-GVH. Alternatively, CTL may be necessary, but not sufficient for GVH to develop in response to Minor-HA.

0195 USE OF DONOR ALLOGENEIC CULTURED CELLS IN BONE MARROW TRANSPLANTATION. Peter Mauch, Jeffrey Lipton, Brian Hamilton and Samuel Hellman. Joint Center for Radiation Therapy Boston, MA 02115

These experiments were devised to see if the loss of θ-positive cells in culture would allow for transplantation with cultured marrow cells without development of acute graft verses host disease (GVHD). Both major and minor histocompatibility complex (MHC) differences were tested. Less than 2% of fluorescence labeled Thy 1 positive cells survive after 6 days in culture. Evidence for permanent donor engraftment was obtained by hemoglobin electrophoresis and synthesis. Fresh and cultured (3 day-4 week) marrow from C57Bl/6($H\text{-}2^b$) mice was transplanted into 1200 rad (split dose) irradiated C3H/HeJ($H\text{-}2^k$) mice. Recipient animals developed evidence for acute GVHD when transplanted with fresh or cultured allogeneic marrow. However, the 50% survival time for animals receiving cultured allogeneic cells was 44-54 days as compared to 33 days when fresh bone marrow cells were used. In separate experiments, fresh and cultured marrow from LP/J($H\text{-}2^b$) mice was transplanted into irradiated C57Bl/6($H\text{-}2^b$) mice. Transplantation with fresh donor marrow plus $5x10^6$ spleen cells resulted in greater than 90% of recipient animals dead of acute GVHD by 10 weeks. Eighty-nine percent of recipient animals were alive without GVHD at 10 weeks when 3 day cultured donor cells were used in place of fresh cells. These experiments demonstrate a rapid loss of θ positive cells in the bone marrow culture system without loss of stem cells required for long term engraftment and animal survival. The use of donor cultured marrow resulted in a delay in the development of acute GVHD in MHC different animals. When non-MHC differences were tested the majority of animals survived without GVHD. This may have implications for human bone marrow transplantation.

0196 CELL SEPARATION ON THE BASIS OF MONOCLONAL ANTIBODY COATED COLLOIDAL GOLD PARTICLES AND MAGNETIC DEXTRAN PARTICLES, L. Vellekoop, C.L. Reading, C.H. Poynton, M. Chandran, C.M. Hickey and K.A. Dicke, The Bone Marrow Transplantation Center and the Department of Tumor Biology, M.D. Anderson Hospital, Houston, TX 77030

We have investigated novel ways of <u>in vitro</u> treatment of bone marrow to remove mature lymphocytes (allogeneic transplantation) or tumor cells (autologous transplantation). To model the effectiveness we used OKT3 on bone marrow specimens, mixed with peripheral blood, of healthy volunteers. Immunogold separation: After incubation with OKT3, bone marrow was incubated with colloidal gold particles which were previously coated with goat-anti-moust IgG. The density of antibody-reactive cells is increased by the indirect immunogold binding and centrifugation on a ficoll-hypaque (d=1.077) gradient separates OKT3 positive cells from nonreactive cells. In six experiments the mean PHA response of the final cell suspension was 14±10% (mean±SD) of the control. Magentic separation: Magnetic dextran particles (Carl Borrebaeck, Pure and Applied Biochemistry Center, Lund, Sweden) were coated with goat-anti-mouse IgG and incubated with bone marrow cells previously incubated with OKT3. When placed in a magnetic field, cells binding magnetic particles are drawn to the side of the tube, leaving nonreactive cells in solution. In six experiments mean PHA response of the final cell suspension was 32±9% (mean±SD) of the initial suspension. Improvements in strength of the magnetic field and size of the particles are being made. Neither immunogold treatment nor magnetic separation affected bone marrow precursor cells as measured by CFC-GM, BFU-E and CFC-GEMM growth <u>in vitro</u>. Experiments using K562 cells will be discussed.

0197 MURINE GRAFT-VERSUS-HOST REACTION IS ASSOCIATED WITH THE PRODUCTION OF AUTOANTIBODIES TO HEMATOPOIETIC DIFFERENTIATION ANTIGENS ENCODED BY ENDOGENOUS MuLV, John L. Portis and William J. Britt, NIH, NIAID, Rocky Mountain Laboratories, Hamilton, MT 59840

(C57BL/6 X DBA/2)F_1 recipients of spleen cells from either parent produced antibody to MuLV envelope antigens first detectable by day 14 after adoptive transfer. Hybridomas were recovered from the spleens of recipient mice. These cells lines were of F_1 origin and produced antibodies reactive with a wide variety of MuLV. The viral specificities of antibodies recovered from F_1 recipients of D2 cells was different than that from recipient of B6 cells. Several of these GVHR antibodies immunoprecipitated gp70 expressed at the membrane of normal adult spleen cells and cells from embryonic liver, the site of hematopoiesis in the fetus. One antibody reacted with a subgroup of xenotropic viruses and bound to a viral-like envelope determinant expressed by cells of the erythroid lineage. Another antibody reacted with a gp70 determinant shared by many MCF viruses of diverse origin but failed to bind to either xenotropic or ecotropic viruses. This MCF-specific antibody also reacted with a gp70 expressed by a small but reproducible population of cells in embryonic liver. Thus, antibodies produced by mice undergoing graft-versus-host reaction bound to MuLV envelope determinants which appeared to be restricted to particular hematopoietic cell lineages. Since acute graft-versus-host reaction is manifested by hematopoietic depression involving the lymphoid, erythroid and myeloid series, we are studying the potential relevance of these antibodies to the genesis of GVHR.

0198 COMPARISON OF THE REPOPULATING POTENTIAL OF STEM CELLS DERIVED FROM BLOOD AND BONE MARROW: AUTOTRANSPLANTS IN DOGS. Aruna Raghavachar, Otto Prümmer, Theodor M. Fliedner and Karl-Heinz Steinbach, Dept. of Clinical Physiology and Occupational Medicine, University of Ulm, D-7900 Ulm, F.R.G.

The kinetics and pattern of hemopoietic recovery after supralethal total-body irradiation (TBI) were compared after transfusion of cryopreserved autografts derived from peripheral blood and bone marrow. Fractionated TBI was given in 3 doses of 6 Gy each at intervals of 48 hrs. Grafts of peripheral blood mononuclear cells (MNC) were collected by means of continuous-flow leukapheresis and using the mobilizing agent dextran sulphate. Autografts were adjusted to contain equal numbers of hemopoietic progenitor cells (CFU-c). Dogs grafted with blood derived MNC (group I) and with MNC from bone marrow (group II) all received about 1×10^5 CFU-c/kg b.w.. In all dogs consistent hemopoietic engraftment was achieved. Comparing the pattern of regeneration of the granulocytes, group I dogs showed a significant regeneratory advantage over group II dogs, in particular during the first 20 days after transplantation. Lymphoid recovery was more rapid in group I until day 14. In both groups blood lymphocytes remained below normal values beyond day 100. The regeneration patterns of platelets and reticulocytes revealed no significant differences. Our results are in agreement with the hypothesis that there are differences in the relationship between CFU-c content and hemopoietic potential of autografts from different sources. Attempts are being made to simulate by computer models a pattern of hemopoietic reconstitution and to derive models for the regeneration of the hemopoietic stem cell pools in the bone marrow.

0199 IN VIVO AND IN VITRO ACTIVITY OF ALLOIMMUNIZATION-INDUCED ANTILEUKEMIA REACTIVE CELLS, C-Y. Shih, R.L. Truitt, A.V. LeFever, L.D. Tempelis and M.M. Bortin, Winter Research Laboratory, Mt. Sinai Medical Center, P.O. Box 342, Milwaukee, WI 53201 U.S.A.

Increased reactivity against T cell leukemia of AKR mice (AKR-L) in vivo was observed after adoptive transfer of immunocompetent cells from H-2-compatible donors alloimmunized with normal lymphoid cells. The present studies were designed to examine this antileukemia reaction in vitro and to establish whether there was a correlation with the antileukemia effect in vivo (i.e., with the graft-vs-leukemia or GVL effect). Using limiting dilution cytotoxicity assays (lda) the frequency of leukemia-reactive precursor and committed cytotoxic lymphocytes (CLs) in the spleens of unprimed and alloimmunized $H\text{-}2^k$ mice was estimated and compared with the proportion of leukemia-free mice given the same spleen cells in vivo. There was a direct and linear association between the total number of CLs transplanted and the proportion of leukemia-free mice; however, the number of in vitro CLs associated with elimination of leukemia in vivo varied greatly with the alloimmunized donor strain used. Cells from unprimed $H\text{-}2^k$ donors, despite the transfer of large numbers of precursor CLs were ineffective in eliminating all the leukemia in vivo. Cold target inhibition studies showed that the target antigens on AKR-L cells were non-H-2 antigens shared by the alloimmunizing cells and/or normal AKR cells. Cytotoxicity in vitro was H-2 restricted and mediated by an $Lyt\text{-}1^+2^+$ donor cell. CLs derived under lda conditions (so that the probability of clonality was <0.05) provided direct evidence for a cell population(s) which was leukemia (GVL?) specific and not antihost (GVH?) reactive.
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0200 FETAL LIVER CELL TRANSPLANTATION (FLCT) IN DOGS. R Champlin, K Stitzel, E Branam, and RP Gale, Division Hematology/Oncology UCLA and UC-Davis.

Fetal liver is a rich source of hematopoietic stem cells, capable of producing complete hematologic reconstitution when transplanted into irradiated rodents. The ensuing graft versus host disease (GVHD) is mild even if the donor and recipient are mismatched for MHC antigens. Application of FLCT to large animals and man has been largely limited by failure of engraftment. We report the first successful examples of FLCT in dogs. Litters were aborted on day 53-55 of gestation and a single cell suspension of fetal liver cells was obtained. Recipients received total body irradiation followed by infusion of $0.9\text{-}2.6 \times 10^8$ fetal liver cells/kg (1.6 to 6.6×10^4 CFU-C/kg). Selected dogs received methotrexate post transplant at a dose of 0.25 mg/kg d +1,+3,+6, and 0.5 mg/kg d+11 and weekly until d+101.

N	DLA Match donor→recipient)	Radiation (Gy x no. fractions)	Methotrexate	Engraftment	GVHD	Survival (m)
4	AA→AA	8 x 2	-	+	-	5+,16+,16+,23+
2	AA→AB	8 x 2	-	+	-	13+, 14+
4	AB→AA or CC	8 x 2	-	reject	-	.5, .5, .5, .5
2	AB→AA	8 x 2	+	+	-	4+, 1
2	AB→AA	8,6,6	-	+	-	1, 4+ (hepatotoxicity)

9 animals survive and are clinically well, free of infection with normal peripheral blood counts. These data indicate that sustained engraftment can be achieved in haploidentical recipients without GVHD by the use of intensive radiation and methotrexate.

0201 IMMUNOSUPPRESSIVE EFFECTS OF ALLOGENEIC AND XENOGENEIC MONOCLONAL ANTI-THY-1 ON GVHD AND SKINGRAFT REJECTION, S. Cobbold[1], S. Thierfelder[2] and H. Waldmann[1], University of Cambridge, Dep. of Pathology, Cambridge, UK
[2] GSF, Inst.f. Hämatologie, Landwehrstr. 61, München, FRG
8 monoclonal anti-Thy-1 antibodies (3 murine and 5 rat) of IgM, IgG2a, IgG2b, IgG2c class were used for in vitro incubation of C57BL/6 donor spleen plus bone marrow cells before transfusion into H-2 incompatible CBA mice. All antibodies showed considerable suppression of graft-verus-host disease (GVHD). When injected in vivo only one anti-mouse-Thy-1 (of rat IgG2b origin) clearly delayed the rejection of H-2 incompatible C57BL/6 and H-2 compatible AKR/J skin grafts of CBA mice. Its complement-fixing capacity was not greater than that of the other antibodies. It is unclear why many monoclonal antibodies against the pan-T antigen Thy-1 do not suppress cellular immunity when injected into mice. It is of interest to note that the one exception we have seen so far is also particularly good in suppressing GVHD.

0202 ALLOREACTIVE AND CYTOTOXIC LYMPHOCYTES IN DLA NON-IDENTICAL CANINE RADIATION CHIMERAS. H.J. Deeg, R. Raff, F. Appelbaum, E.D. Thomas and R. Storb. The Fred Hutchinson Cancer Research Center, Seattle, Washington, 98104.
Dogs were DLA typed by serology, mixed leukocyte cultures (MLC) and cell-mediated lympholysis assays (CML). Recipient (host) lymphocytes were stored for in vitro use. Recipients were given 9 Gy total body irradiation and hemopoietic grafts from DLA non-identical unrelated donors (N=9, Group 1) or one haplotype identical littermates (N=10, Group 2). Methotrexate was given on days (d) 1, 3, 6 and 11 and cyclosporine on d 0-100. Results, in vivo, Group 1: 5 dogs died 103-517 d after grafting. Four dogs (2 chronic GVHD) survive at 480-779 d. Group 2: 3 dogs died on d 55, 107, 203 and 7 (2 chronic GVHD) survive at 53-336 (median 280) d. In vitro, Group 1: chimera cells responded in MLC to control but never to host cells both in dogs with and without chronic GVHD. Nylon wool adherent (NWA) chimera cells suppressed the response of donor cells to host and (less) to control cells. In CML chimera cells failed to show cytotoxicity to host and control targets. Group 2: In MLC chimera cells were unresponsive to host cells in healthy chimeras but responded in dogs with chronic GVHD. Cells from all dogs responded to control. NWA chimera cells suppressed donor cell responses to host and (less) to control cells. In CML chimera cells showed cytotoxicity to control but not host targets. Results 1) are consistent with previous findings that tolerance is mediated by clonal abortion but data in unrelated chimeras are more complex, 2) show nonspecific suppressor cells of unknown significance in all chimeras, 3) indicate that generation of cytotoxic cells, thought to be involved in antiviral defense, requires sharing of one haplotype between donor and recipient (DLA-restriction?).