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ALLOGENEIC BONE MARROW TRANSPLANTATION: CURRENT OBSTACLES AND FUTURE PROSPECTS

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INTRODUCTION

Human bone marrow infusions, administered as part of unsuccessful therapy for aplastic anemia, were reported as early as 1939 (1). However, sustained scientific interest in bone marrow transplantation was first generated in the early 1950's by the observation that laboratory animals given supralethal irradiation could be fully reconstituted hematologically and immunologically by infusion of bone marrow cells from syngeneic donors (2). Between 1957 and 1962, a flurry of case reports appeared which described attempts to use bone marrow transplantation as therapy for radiation sickness, hematologic malignancies, aplastic anemia, or various congenital immunodeficiency states. Whereas successful bone marrow transplants with identical twins as donors were reported as early as 1959 (3), in all cases of allogeneic transplants either failure of engraftment or lethal graft versus host disease was observed (4). As measured by the number of cases reported in the medical literature, enthusiasm for this procedure markedly decreased in the mid-1960's. However, with the use of major histocompatibility complex (MHC) compatible donors for patients with immunodeficiency syndromes, the first successful allogeneic transplant was performed in 1968 (5), and a resurgence of clinical interest in this procedure was again seen. By the mid-1970's, therapeutic benefit of bone marrow transplantation in immunodeficiency syndromes and aplastic anemia was apparent (4,6). However, no clear benefit from bone marrow transplantation in treatment of acute leukemia was demonstrated until the late 1970's. In 1977, Thomas et al published the results of allogeneic bone marrow transplantation in 100 consecutive patients with "end stage" acute lymphoblastic or nonlymphoblastic leukemia (7). At the time of publication only 13% of patients remained alive and in complete remission 1-4 1/2 years post-transplant. Yet, these survival rates suggested that at least some patients could be cured of advanced leukemia with use of bone marrow transplantation.

Over the past decade, allogeneic bone marrow transplantation has been employed as part of the therapeutic regimen for an increasing number of malignant and non-malignant conditions. As of 1987 greater than 10,000 bone marrow transplants have been performed (approximately 25% by the Seattle group) of which more than 75% have been performed as therapy for malignancies (8,9). In Table I, the results currently being achieved in selected hematologic malignancies by this modality of therapy are summarized. It is readily apparent that impressive improvement in long term survival rates following allogeneic bone marrow transplantation have been achieved. As detailed in Figure 1, even more impressive survival rates are achieved when this therapy is

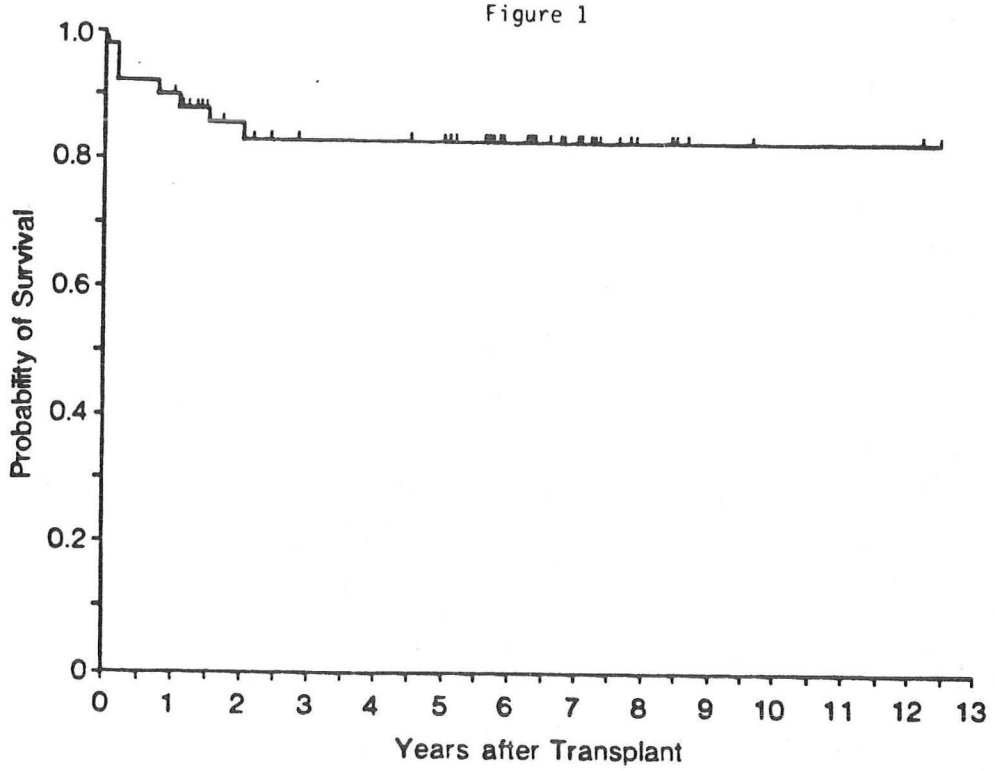
TABLE 1

Summary of Reported Results of Marrow Transplantation for Malignant Disease

	Probability of Survival*	Probability of Relapse†	Longest Time Since Transplant (Years)
End-stage acute leukemia	0.10–0.20	0.70	15
ALL in second remission	0.33–0.50	0.60	10
ANL in first remission			
Age < 20 yr	0.50–0.70	0.20	10
Age > 20 yr	0.35–0.50	0.20	10
CML: Blastic phase	0.10–0.15	0.80	10
Accelerated phase	0.20–0.40	0.60	7
Chronic phase	0.50–0.70	0.20	10
Advanced Hodgkin's and lymphomas	0.15–0.20	0.60	14

From E.D. Thomas, Amer. J. Med. Sci. 294:75

Figure 1



Kaplan-Meier percentage estimates of survival for 50 "untransfused" patients with severe aplastic anemia treated with cyclophosphamide and HLA-identical marrow grafts. Tick marks indicate surviving patients.

From Anasetti, et al, Ann. Intern. Med. 104:463

applied to selected patients with non-malignant disease. However, it should be noted that survival rates such as these detailed in Table I and Figure 1 have been achieved largely with use of MHC-compatible sibling donors and recipients <45 years of age and in "good clinical condition" (free of active infection or leukemic relapse). Furthermore, during the past decade, improved alternative forms of treatment have been developed for some of the diseases for which allogeneic bone marrow transplantation has been widely utilized. These include anti-thymocyte globulin therapy for aplastic anemia (10), improved chemotherapy regimens for acute nonlymphocytic leukemia (ANL) (11), and use of monoclonal antibody purged autologous bone marrow transplantation for refractory acute lymphoblastic leukemia (ALL) (12). Whereas allogeneic bone marrow transplantation still affords the best long-term survival in young patients with severe aplastic anemia and an HLA-identical donor (13), the advantage of this therapy in ANL in first remission (11), or in refractory ALL is not as clearly defined (12). Furthermore, as allogeneic BMT often entails far greater acute morbidity and cost than does conventional therapy, the decision to proceed with BMT clearly requires consideration of multiple factors. A detailed discussion of the precise indications for this form of therapy in each of the greater than 50 diseases in which it has been employed is clearly beyond the scope of this presentation. Rather, an attempt will be made to review the major obstacles which presently limit the use of allogeneic bone marrow transplantation and to consider prospective future uses of this therapeutic technique. An outline of the major disease categories for which allogeneic bone marrow transplantation is currently being employed is contained in Table II, and several recent general references reviewing the indications for this therapy in various disease categories are included.

TABLE II
DISEASES IN WHICH ALLOGENEIC BONE MARROW TRANSPLANTATION
HAS BEEN EMPLOYED AS THERAPY

Radiation injury
Malignancies of lymphoid or hematopoietic origin
Selected solid tissue malignancies
Aplastic anemia and other acquired non-malignant marrow disorders
Genetic disorders expressed in cells of bone marrow origin
    Immunodeficiency syndromes
    Hemoglobinopathies and other hematopoietic disorders
    Inborn errors in metabolism

General references:

Thomas, E.D. Marrow transplantation for malignant disease. 1987. Am. J. Med. Sci. 294:75-79
Krivit, W. and C.B. Whitley. Bone marrow transplantation for genetic diseases. 1987. New Engl. J. Med. 316:1085-1087.
Good, R.A. Bone Marrow Transplantation Symposium: Bone marrow transplantation for immunodeficiency disease. 1987. Am. J. Med. Sci. 294:68-74.
Parkman, R. Current status of bone marrow transplantation in pediatric oncology. 1986. Cancer 58:569-572.

Application of bone marrow transplantation as therapy for human disease is dependent upon a broad range of supportive care modalities. The improved survival rates demonstrated in Table I and Figure 1 were achieved in part because of advances in the use of intravenous hyperalimentation, platelet and granulocyte transfusion, protective isolation and antimicrobial therapy. Such supportive care is in large part similar to that which must be provided for any patient rendered transiently pancytopenic following various chemotherapy or radiotherapy regimens. However, several unique therapeutic challenges must be addressed in managing the recipient of an allogeneic bone marrow transplant. The leading obstacles which have faced human bone marrow transplanters from 1957 through 1987 are the dual problems of graft versus host disease and the host versus graft responses which lead to failure of allogeneic marrow engraftment.

Graft versus host disease: Clinical and pathological manifestations

In the mid-1950's, following the performance of splenic transplant rescue experiments after lethal irradiation, a syndrome variously designated as "secondary disease" or the "runting syndrome" was noted (14,15). It was appreciated that this syndrome was distinct from radiation sickness and occurred only in recipients of hematopoietic transplants. The hallmarks of this syndrome were failure to thrive, alopecia, emaciation, lymphoid atrophy, diarrhea and death (16).

TABLE III
ORGAN SYSTEMS INVOLVED IN GRAFT VERSUS HOST DISEASE

Primary targets of graft versus host response:
- Skin
- Liver
- Gastrointestinal tract
- Lymphoreticular system

Targets of synergistic effect of GVHR, bacterial or viral infection, chemo-radiotherapy:
- Lung
- All of the primary targets of GVHD

In humans, graft-versus-host disease is manifested by pathologic lesions of the skin, liver and gastrointestinal tract which are similar to those described following allogeneic bone marrow transplantation in the mouse, rat, rabbit, dog, horse, and non-human primates (15). Acute graft-versus-host disease (GVHD) in humans typically develops from 7 to 60 days after marrow transplantation and is characterized pathologically by inflammatory destruction of epithelial cells in the skin, liver, and gastrointestinal tract. The skin is usually involved with a pruritic, macular exanthem of the palms and soles with morbilliform lesions on the extremities, trunk, and face. In the most severe cases, generalized erythroderma, bulla formation, bacterial superinfection and exfoliation occur. Histologically, skin involvement is characterized by epidermal basal cell vacuolization, mononuclear cell infiltrates around superficial venules, ecsinophilic necrosis of individual epithelial cells in the basal or suprabasal layers, and in severe forms of focal areas of epidermal-dermal separation and bulla formation (15). Liver involvement (15,17,18) is characterized by cholestatic liver function abnormalities and occasionally by frank encephalopathy, ascites formation and coagulopathy. Histologically, acute hepatic GVHD is characterized by degeneration or paucity of small bile ducts and associated with lymphocytic infiltrates in both the portal tracts and around the central veins. Luminal endotheliolitis of portal and hepatic veins is also noted. Gastrointestinal involvement (15,17,18) is clinically manifested by watery diarrhea, crampy abdominal pain, anorexia, nausea and vomiting. In more severe forms, malabsorption, gastrointestinal hemorrhage and ileus are commonly present. Histologically, individual crypt cell necrosis and lymphocytic infiltration unaccompanied by widespread inflammation are said to be the hallmark findings of acute GVHD of the gastrointestinal tract. Such changes are most evident in rectal biopsies but have been described in all segments of the GI tract distal to the esophagus. Changes are localized to the bases of crypts in mild GVHD and extend to the surface epithelium in more severe disease. Severe disease is characterized by crypt abscesses, crypt dropout, and frank epithelial denudation.

Clinical and histological grading systems for acute GVHD as proposed by E. Donnall Thomas and coworkers (NEJM 292:895, 1975) are listed below.

TABLE IV
CLINICAL STAGE OF GRAFT-VERSUS-HOST DISEASE
ACCORDING TO ORGAN SYSTEM

Stage	Skin	Liver	Intestinal Tract
+	Maculopapular rash <25% of body surface	Bilirubin 2-3 mg/100 ml	>500 ml diarrhea/day
++	Maculopapular rash 25-50% of body surface	Bilirubin 3-6 mg/100 ml	>1000 ml diarrhea/day
+++	Generalized erythroderma	Bilirubin 6-15 mg/100 ml	>1500 ml diarrhea/day
++++	Generalized erythroderma with bullous formation and desquamation	Bilirubin >15 mg/100 ml	Severe abdominal pain with or without ileus

TABLE V
HISTOPATHOLOGIC STAGE OF GRAFT-VERSUS-HOST DISEASE
ACCORDING TO ORGAN SYSTEMS

Stage	Skin	Liver	Intestinal Tract
+	Basal vacuolar degeneration or necrosis (or both)	<25% abnormal (atypical degeneration or necrotic or both) small interlobular bile ducts	Dilatation of glands: single-cell necrosis of epithelial cells
++	+ and spongiosis, dyskeratosis and eosinophilic necrosis of epidermal cells	25-50%	+ and necrosis and dropout of entire glands
+++	+ + and focal microscopic epidermal-dermal separation	50-75%	+ + and focal microscopic mucosal denudation
++++	Frank epidermal loss	>75%	Diffuse microscopic mucosal denudation

TABLE VI
OVERALL CLINICAL GRADING OF SEVERITY OF GRAFT-VERSUS-HOST DISEASE

Grade	Degree of Organ Involvement
I	+ to ++ skin rash; no gut involvement; no liver involvement; no decrease in clinical performance
II	+ to +++ skin rash; + gut involvement or + liver involvement (or both); mild decrease in clinical performance.
III	++ to +++ skin rash; ++ to +++ gut involvement or ++ to ++++ liver involvement (or both); marked decrease in clinical performance
IV	Similar to grade III with ++ to ++++ organ involvement and extreme decrease in clinical performance

From Thomas, E.D., et al, NEJM 292:895, 1975.

Whereas acute GVHD is characterized by focal destruction of epithelial cells, chronic GVHD is characterized by increased collagen deposition resulting in fibrosis (15,19). Initial manifestations of the disease generally appear after day 100 but can appear as early as day 70. In most patients, these manifestations evolve from acute GVHD but in 20-30% of patients with chronic GVHD, a de novo late onset without prior acute GVHD is noted. Symptoms of chronic GVHD resemble selected manifestations of Sjogren's syndrome, polymyositis, lichen planus, scleroderma, and primary biliary cirrhosis with involvement of skin, liver, mouth, eyes and esophagus (15,19,20). Skin manifestations often occur in sun-exposed areas with a characteristic progression from erythema and insidious hyperpigmentation to lichen planus, dermal thickening, epidermal atrophy, and sclerosis. Liver abnormalities include those of progressive bile duct loss, cholestasis, and occasionally progressive fibrosis and cirrhosis. Oral involvement evolves as xerostomia, mucosal atrophy, lichenoid reactions on the buccal mucosa and tongue. Eye manifestations include symptomatic sicca which can progress to keratoconjunctivitis sicca. Histological involvement of the oral mucosa, salivary ducts, lacrimal ducts and conjunctiva commonly demonstrates mononuclear infiltrates with destruction of glands and ducts and interstitial fibrosis. Esophageal involvement can present as stricture formation or motility disorders, and in severely ill patients, extensive fibrosis of the submucosa and serosa of the GI tract occurs in the stomach, small intestine, and colon, and is associated with malabsorption and motility disorders. Bronchopulmonary sicca with occasional histologic demonstration of obliterative bronchiolitis have also been described as a manifestation of GHVD.

A proposed clinical classification system for chronic GVHD which tends to separate patients into good and poor prognostic groups is detailed in the table below.

TABLE VII
CLASSIFICATION SCHEME FOR PATIENTS WITH CHRONIC GVHD

Limited Chronic GVHD

Either or both:
(1) Localized skin involvement
(2) Hepatic dysfunction secondary to chronic GVHD

Extensive Chronic GVHD

Either:
(1) Generalized skin involvement; or
(2) Criteria for limited chronic GVHD with
    (a) Liver biopsy showing chronic active hepatitis or cirrhosis or
    (b) Abnormal Schirmer's test; or
    (c) Labial biopsy demonstrating salivary gland or oral mucosal involvement or
    (d) Involvement of any other recognized target organ

Adapted from Shulman, H.M. et al, Am. J. Med. 69:204, 1980.

Involvement of other organ systems

Aside from the propensity of GVHD to attack epithelial targets, the other common manifestation of GVHD in human and animal models is the presence of profound immunodeficiency. Whereas in rodent models lympadenopathy and splenomegaly are common manifestations of the graft versus host reaction, this is not a common finding in human GVHD. However, as has been described in murine models of GVHD, thymic atrophy and fibrosis have been found at autopsy in human chronic GVHD (19). By the very nature of bone marrow transplantation, a transient period of profound immunodeficiency is inevitable immediately after bone marrow transplantation. With the engraftment of donor marrow, normal circulating levels of neutrophils and mononuclear phagocytes appear within 1-2 months post-transplant, although neutrophil chemotaxis may be abnormal for up to four months (21). Natural killer cell (NK) number and functional activity can similarly normalize within 30-60 days post-transplant, whereas antigen-specific T and B cell responses may not normalize until >1 year post-transplant, even in the absence of GVHD (21,22). Assays of T helper function tend to remain abnormal for longer intervals than do any of the other measures of immune responsiveness in such patients (21-24). In the presence of GVHD, especially chronic GVHD, a prolonged inverted CD8/CD4 T cell ratio with the presence of circulating CD8(+) T cells with nonspecific suppressor cell function has been noted (24). Not surprisingly, in the face of the sicca-like syndrome of chronic GVHD, relative IgA deficiency is frequently observed (25,26). Of interest, selective IgG2a deficiency has also been seen in a fraction of such patients (26) and an increased incidence of autoantibodies has also been described (27). In general, during active acute or chronic GVHD, the incidence of a variety of opportunistic infections is high and is a leading cause of the excess morbidity and mortality associated with GVHD.

Despite the fact that such organs as the heart, kidney and pancreas induce florid allorejection responses following orthotopic organ transplantation, these organs are not apparent targets for the graft versus host response (GVHR) following human allogeneic bone marrow transplantation (15,19,28). The brain (29) and the major endocrine organs are similarly uninvolved (15). The degree of lower respiratory involvement by GVHD alone has been difficult to define. Whereas the lung is not a prominent target of GVHD in uninfected animal models of the graft versus host reaction (15,16), in human bone marrow transplantation, interstitial pneumonitis is one of the leading causes of morbidity and mortality. Two syndromes which appear to be potential GVHD manifestations in the lung are the syndromes of lymphoid interstitial pneumonia (3) and of obliterative bronchiolitis. However, as discussed in a later section, the etiology of these syndromes is clearly multifactorial (15,32).

Immunologic and Genetic Basis of GVHD

More than 2 decades have passed since Billingham (33) postulated that three criteria are essential for generation of GVHD:

(1) Genetically determined histocompatibility differences between donor and recipient
(2) Immunocompetent cells in the graft able to recognize and mount an immunologic reaction against foreign histocompatibility antigens of the host
(3) Inability of the host to recognize and mount an immunologic reaction against the graft

With progressively improved histocompatibility typing techniques, the role of the human MHC antigens in induction of GVHD has become readily apparent. As detailed in Figure 2, when recipients of HLA-A,B and C identical-sibling bone marrow were compared to recipients of haploidentical donor cells disparate at one or more loci, a statistically significant difference in incidence of acute GVHD was noted (34).

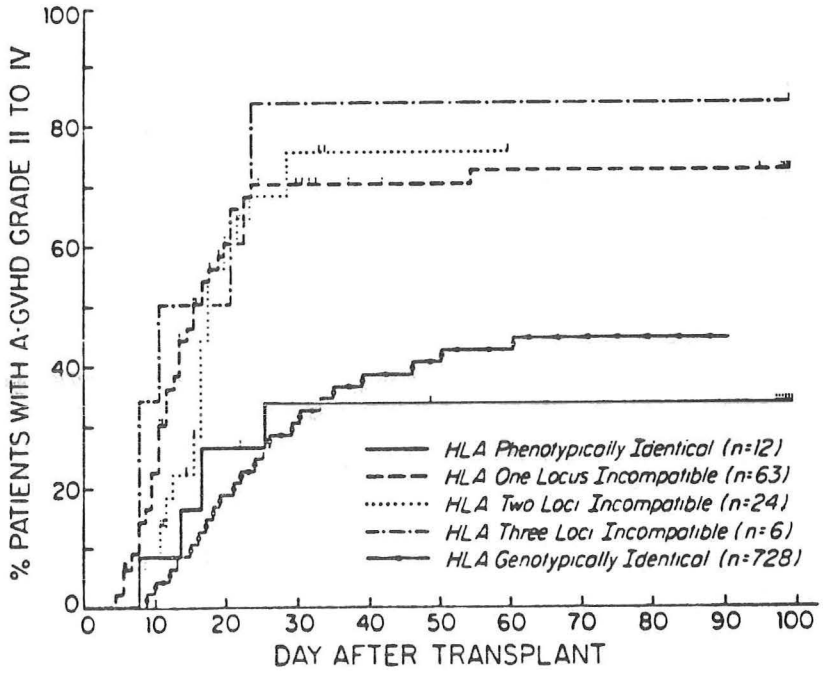
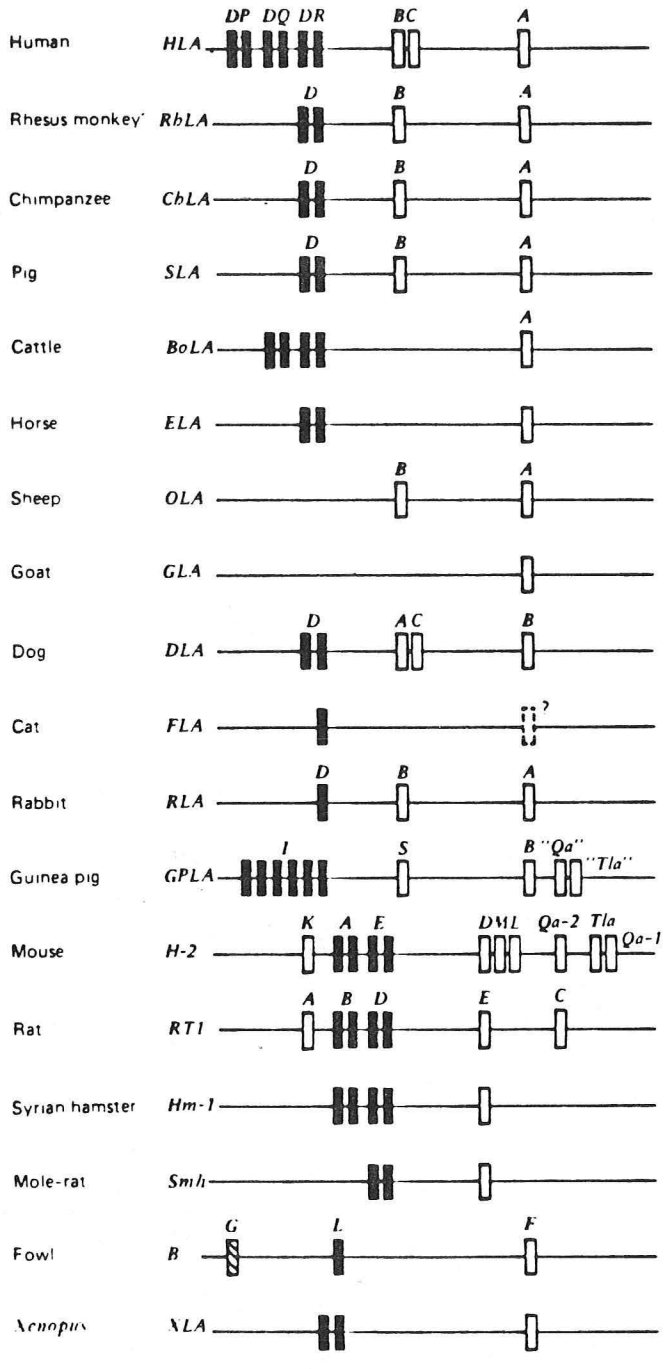


Figure 2

From Beatty, P.A., et al , NEJM. 313:767

In the study outlined in Fig. 2, the increased risk for acute GVHD associated with class II MHC (HLA-D region) mismatches was equal to that associated with mismatch at class I MHC (HLA-A or B) loci. Whereas the role of MHC antigens is demonstrated by such observations, it is also clear that even when transplants have been performed between fully HLA-matched siblings, a 20-40% incidence of acute GVHD is seen in spite of routine post-transplantation immunoprophylaxis with methotrexate or other agents (35). Furthermore, in another study from the Seattle group, it was noted that when post-transplant immunoprophylaxis was omitted after HLA-matched allogeneic BMT for leukemia, 100% of patients developed hyperacute GVHD (36, median onset day 8). As shown in Fig. 3, the human MHC antigens are encoded by genes closely grouped on a single chromosome as are the MHC genes in all other species examined to date (37). The exceedingly high incidence of GVHD in HLA-matched sibling donor-recipient pairs vs. the almost complete absence of this syndrome in twin transplants suggests that genetic differences at loci not closely linked to the known regions of the human MHC must be involved. In murine transplantation models, multiple such non-MHC transplantation antigens have been identified by capacity to induce allograft rejection. Functionally, major histocompatibility antigens have been defined by capacity to induce first set rejection of grafts in <3 weeks, whereas minor histocompatibility antigens (minor HA) have been defined as those which induce first set graft rejection after 3 weeks posttransplantation. Whereas all major loci in the mouse map to a single chromosome region termed the major histocompatibility complex (MHC or

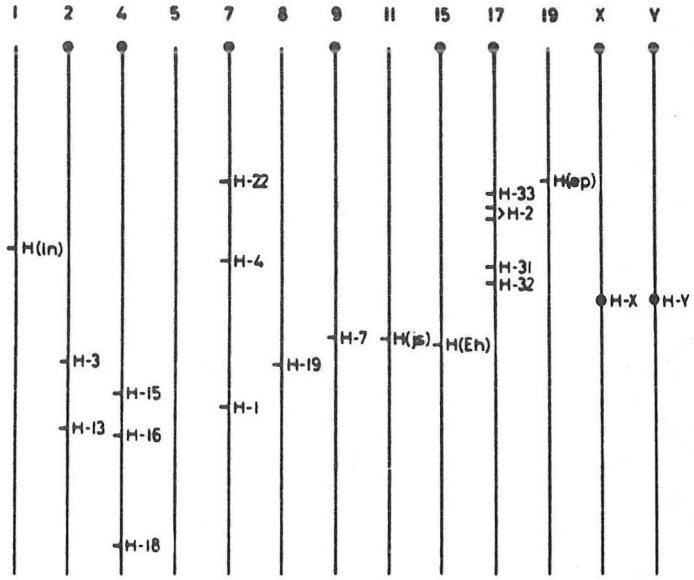
Figure 3



Genetic maps of MHC in different species. Open rectangles are class I genes, closed rectangles class II genes.

From Klein , J. Natural History of the Major Histocompatibility Complex, P-75

Figure 4

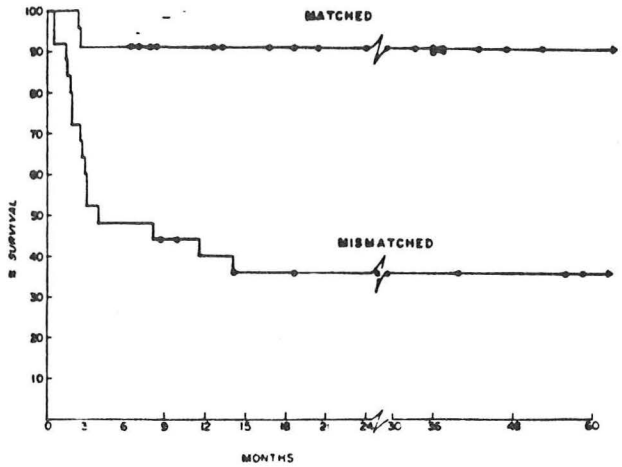


Distribution of histocompatibility loci in the mouse genome.

From Klein J., The Mouse in Biomedical Research, 1:120

H-2), as shown in Figure 4, the minor HA loci identified thus far map to multiple chromosomes (38) or in one case to extrachromosomal mitochondrial DNA (39). Not only have such minor HA been shown to be capable of inducing GVHD in murine BMT, but the presence of multiple HA disparities has been shown to synergistically amplify GVHD and skin graft rejection (40,41). Of note, development of anti-sera against minor HA has proven quite difficult in murine models, and in unprimed mice, primary mixed lymphocyte culture responses also have proven of little value in detection of such minor HA. Thus, present inability to clearly define such minor HA in man cannot be taken as credible evidence against their existence. Indeed, several investigators have reported the presence of donor T cells reactive against host non-MHC cell surface antigens in the setting of human GVHD (42,43). The only putative minor HA for which humans can be readily phenotyped is the H-Y antigen (44) encoded by the Y chromosome. A Y chromosome encoded antigen has been shown to be the target antigen for class I restricted cytotoxic T cells isolated from a female patient previously infused with HLA-matched male sibling donor cells (45). Independent of recipient sex, use of female donors and especially of multiparous female donors has been shown to be associated with increased risk of acute GVHD (35,46). In addition to this risk which is probably related to prior priming against a host of largely sex-independent histocompatibility antigens, several studies have noted a significant effect of donor/recipient sex mismatch on survival after BMT for aplastic anemia (47,48, see Figure 5). Such findings have been interpreted as resulting from the negative effects of female versus male responses on male donor marrow engraftment in female recipient/male donor combinations and on induction of GVHD in male recipient/female donor combinations (48). Certainly the preponderance of data obtained with bone marrow or other organ transplants between HLA-matched siblings suggests that in the human as in the mouse, multiple histocompatibility antigen loci with no discernible linkage to the HLA-locus must be involved in induction of GVHD or orthotopic allograft rejection. Even if such loci could be precisely

Figure 5



Survival of 47 patients with severe aplastic anemia who had successful and sustained marrow grafts from HLS identical siblings. The survival of 22 patients with sex matched donors is compared to that of 25 patients whose donors with sex mismatched.

From Storb R, et al J. Clin. Invest. 59:625

phenotyped in man, the chance of full matching at multiple non-linked loci would appear to be a statistically rare event, and thus efforts at further reduction in incidence of GVHD have focused on the effector mechanism in this immune response.

Whereas a secondary role for antibody mediated or NK cell mediated responses in GVHD has been suggested, the primary effector cell responsible for initiation of GVHD has been clearly demonstrated to be a post-thymic mature T cell in a variety of animal models (14,35). Multiple recent studies have shown that T cell purging of donor marrow has similar benefit in preventing development of human GVHD (49-51). Figures 6 and 7 schematically outline current understanding of the nature of histocompatibility antigen recognition by T cells.

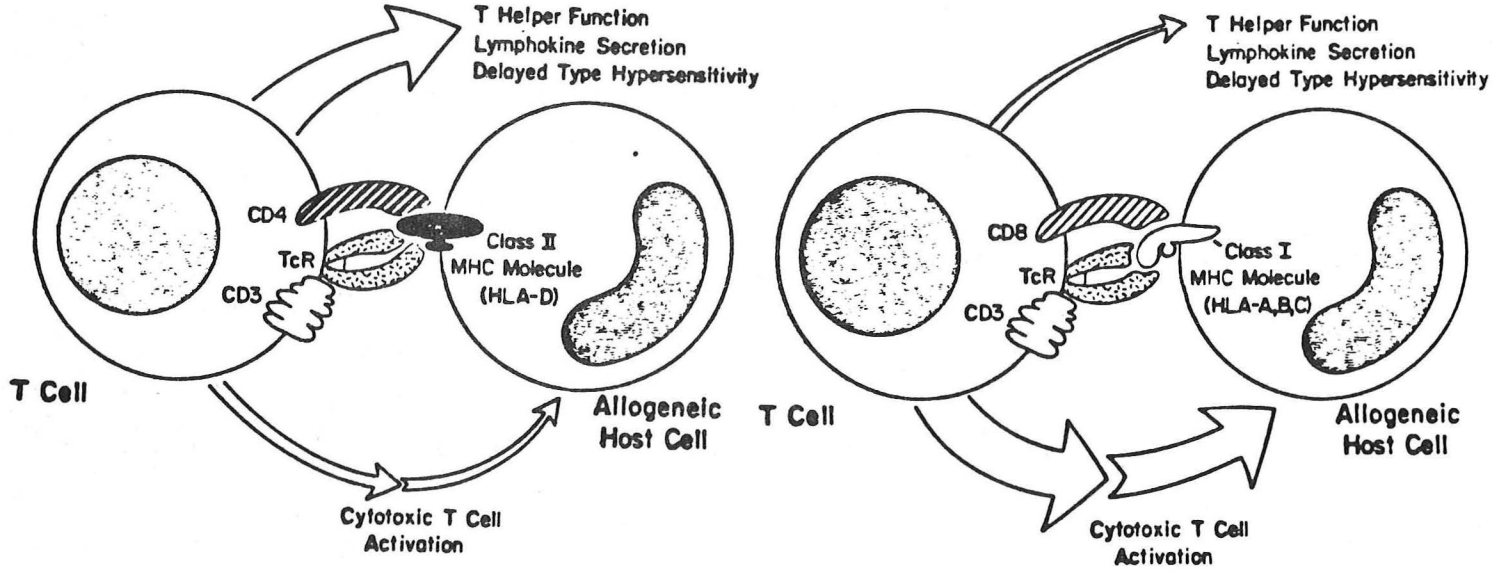


Figure 6

The CD4 (OKT4, L3T4) and CD8 (OKT8, Lyt2) markers on human and murine T cells were originally noted to correspond with the capacity for mediating T helper and T cytotoxic responses, respectively. Subsequently it has been suggested that the true function of these T cell surface proteins is related to class II restriction of CD4(+) T cell responses and the class I restriction of CD8(+) T cell responses. Certainly, with respect to allospecific responses both T helper and T cytotoxic responses have been elicited from the CD4(+) T cell subset against class II (I-A, HLA-DR) alloantigens, and both T helper and T cytotoxic responses have similarly been elicited from CD8(+) T cells stimulated with class I (H-2K,D, HLA-A,B,C) alloantigens (52-55).

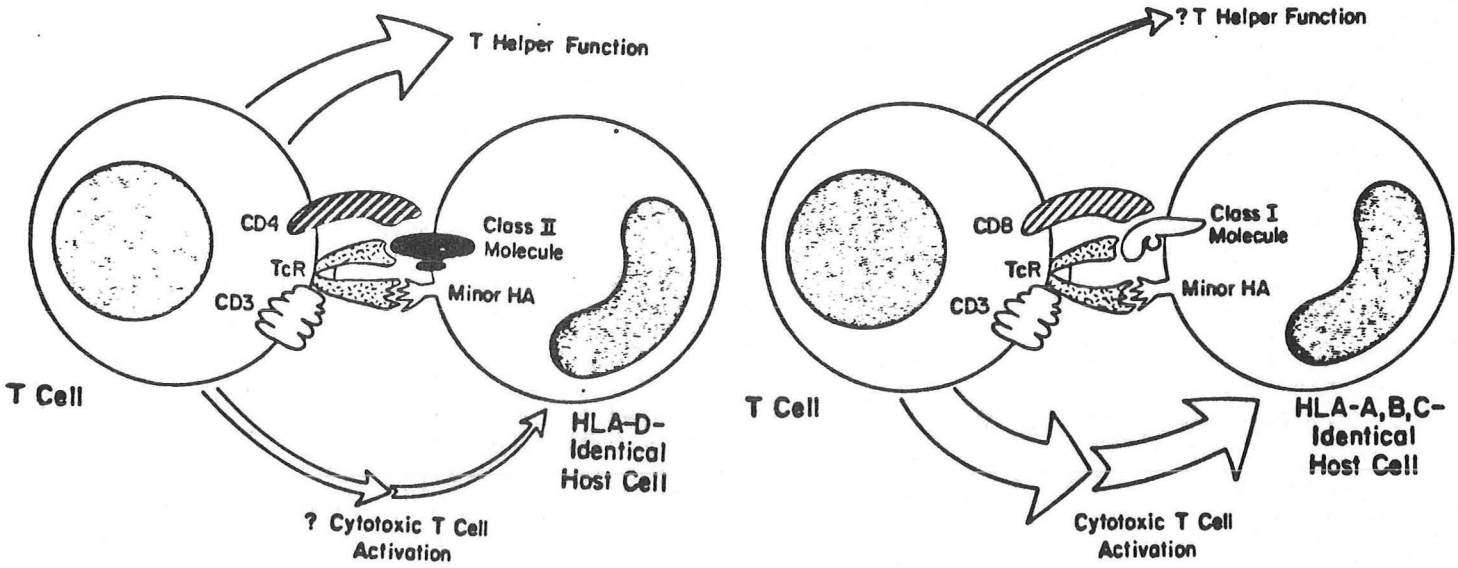


Figure 7

With respect to minor HA, in murine models such antigens have always been shown to be recognized in the context of "self" MHC antigens (40), much as in the case of self-MHC restriction of xenoantigen recognition. For some minor HA, it has been demonstrated that requisite T helper function was mediated by the L3T4 (CD4)(+) T cell subset and T cytotoxic function by the Lyt2 (CD3)(+) T cell subset (56). However, results of recent studies in MHC-identical mouse strains indicate that in transplants performed across multiple minor HA barriers, transfer of either highly purified L3T4 (CD4) or Lyt2 (CD8)(+) T cell subsets can independently induce lethal graft versus host disease in selected strain combinations (57). Similarly, when BMT in mice are performed across isolated class I or class II MHC barriers, highly purified Lyt2 (CD8+) or L3T4 (CD4+) T cell subsets, respectively, have been shown to be independently capable of inducing acute GVHD or skin graft rejection (58). A small study has suggested that CD4(+) cells alone can induce GVHD in human MHC-matched allogeneic recipients (59). Thus, it appears likely that currently available T cell subset markers will be inadequate as selective agents for the purging of GVHD inducing cells from donor marrow.

The separate roles of T cell lymphokine production and cell-mediated cytotoxicity in GVHD has been difficult to define. A variety of studies have suggested that the sclerotic aspects of chronic GVHD were related to effects of T cell lymphokines whereas aspects of acute GVHD were largely mediated by cytotoxic T cells (35,60). Such studies have been subsequently questioned because interpretations were based on monoclonal antibody phenotyping of cell function. However, recent data generated through use of a biochemical technique to deplete cytotoxic T cells have suggested that selective removal of cytotoxic T cell precursors from donor cell inoculum can greatly reduce mortality from acute GVHD (61) while not preventing all manifestations of skin and intestinal GVHD (62). Thus, it may be possible to ascribe different aspects of GVHD to selected T cell lineages or functions and, hopefully, in the future permit more selective approaches to ablating donor T cells responsible for GVHD. Such efforts to better understand and hopefully more selectively direct therapy against GVHD have become of increased interest in light of recent observations that pan-T cell depletion of donor marrow has not proven of benefit in improving survival following MHC-matched bone marrow transplantation for acute leukemia.

In contrast to the experience with transplant of other allogeneic organs, transplantation tolerance, defined as specific unreactivity of an immunologic system towards a set of foreign antigens with full immunocompetence against all other third party antigens, is achieved with relative ease in allogeneic bone marrow transplantation (63-69). Indeed, much of the early interest in bone marrow transplantation was generated by observation of this phenomenon (70). In experiments such as that detailed in Figure 8, it was noted that if bone marrow was transferred from an allogeneic donor to an irradiated recipient, the resulting chimera accepted skin grafts (or other organ grafts) of donor or recipient type while rejecting third party grafts.

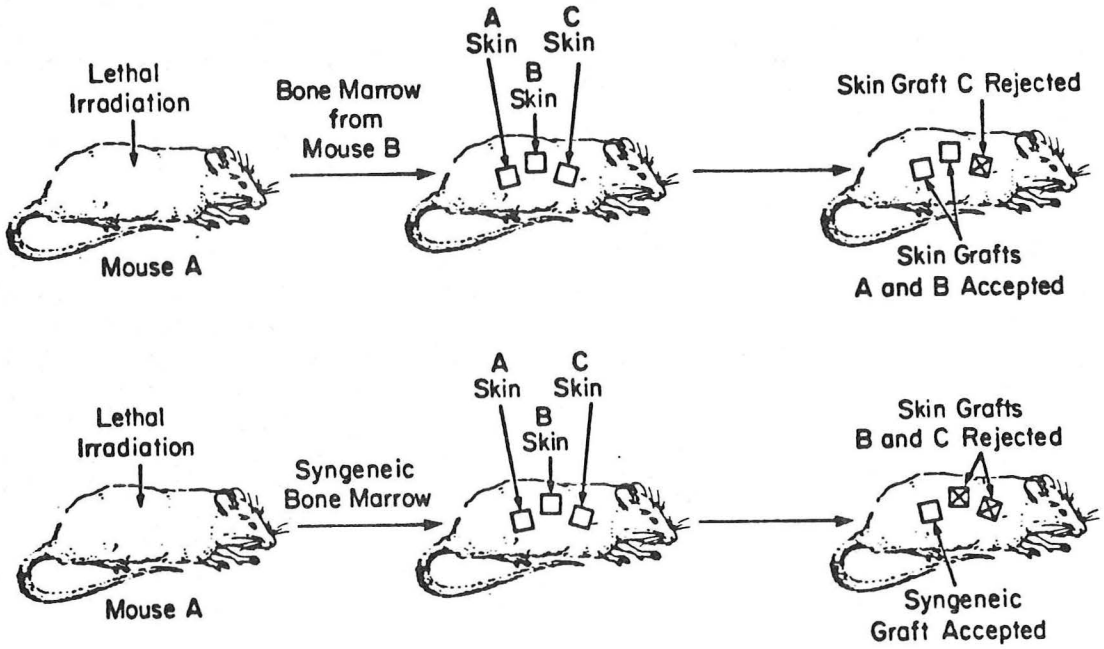


Figure 8

This ideal result is only achieved if any initial GVHD is nonlethal and has resolved, or if GVHD is prevented by depletion of mature T cells from the donor inoculum. Furthermore, this model for induction of transplantation tolerance works best if donor and recipient are at least haploidentical at MHC loci. If T-depleted bone marrow is infused into a fully MHC mismatched irradiated recipient, allogeneic tolerance is observed, but in most cases, immunocompetence does not develop, and the recipient typically dies from opportunistic infection (66,67). The immunoincompetence observed in fully MHC incompatible chimeras appears to result from failure of appropriate MHC restricted immune cell interactions (67).

These observations, coupled with additional experiments using thymus transplants between radiation chimeras, have demonstrated that the host thymus influences the specificity of the developing T cell repertoire by selecting for further differentiation only those T cell precursors which express appropriate specificities. In the "normal" situation, "thymus-educated" T cells contain no autoreactive T cells (or in the case of a chimera, no T cells reactive with donor or host thymus antigenic phenotype). Furthermore, the reactivity of T cells against foreign antigens is preferentially restricted to recognition in context of the self MHC types common to the T cell and the host thymus. This model of T cell differentiation explains both the development of transplantation tolerance in radiation chimeras as well as the typically short-lived or finite nature of acute GVHD (14,35). Thus, unlike the situation in solid organ transplants where newly matured alloreactive T cells are constantly being generated from the host thymus, in bone marrow transplantation only a finite number of alloreactive T cells is introduced. Of note, in human as well as animal models of acute GVHD, either early death from complications of this syndrome or an eventual resolution, which may be independent of any immunosuppressive therapy which is administered, are noted. In sharp contrast to the situation with kidney, heart or liver transplants, 2 to 5 years after unpurged bone marrow transplantation, 70%-90% of survivors receive no immunosuppressive therapy and are free of any active GVHD (35,71). A small minority, however, do have chronic GVHD which remains active beyond the 2nd or 3rd year post-BMT and indeed may result in death as late as 10 years post-transplant (72).

Whereas the majority of the data collected in human bone marrow transplantation suggest that GVHD is initiated entirely by a pool of post-thymic donor T cells which is transferred with the bone marrow inoculum (35,49-51), there appear to be certain exceptions to this model of GVHD induction. Following both autologous and syngeneic (identical twin) human bone marrow transplants, cases of apparent GVHD have been reported (73-75). In a series of over 100 patients from Johns Hopkins, 8% of autologous or syngeneic BMT were associated with clinical and histologic GVHD (75). Whereas most such cases have had transient Grade II or less skin GVHD, some patients have had histologic and clinical involvement of the liver and GI tract as well, and some have required immunosuppressive therapy. In both the mouse and rat, irradiation, followed by autologous or syngeneic bone marrow reconstitution and a 3-6 week course of cyclosporin results in development of autologous GVHD upon cyclosporin withdrawal (76-79). In these animal models, both irradiation of the thymus and administration of cyclosporin were found to be important in the development of this syndrome (76,77,79). Of note, cyclosporin administration was found to markedly reduce the number of Ia (Class II MHC)-positive cells in the thymus and to lead to rapid depletion of medullary thymocytes (77). In rats with syngeneic GVHD, development of cytotoxic T cells with apparent polyclonal anti-Ia specificity have been observed (78). These findings

suggest that damage to Class II MHC antigen positive thymus cells which normally play an important role in selection of the class II restricted T cell repertoire may be the basis for this syndrome. Of note, damage to thymic epithelium has been noted to occur in human and animal models of allogeneic GVHD (15,19,80). Furthermore, L3T4 (CD4+) T cells reactive with "public" epitopes of both donor and host class II MHC antigens have been isolated from animals with chronic allogeneic GVHD. Thus, allogeneic stimulation of donor T cells might well initiate acute GVHD which, through damage to the thymus, might serve as a risk factor for a secondary syndrome of immune dysregulation akin to syngeneic GVHD or certain autoimmune diseases. Indeed, it has long been noted that certain aspects of chronic GVHD bear a close resemblance to the histologic abnormalities in such putative autoimmune diseases as primary biliary cirrhosis, Sjogren's syndrome, scleroderma or mixed connective tissue disease (20,81,82).

Extrinsic risk factors for graft-versus-host disease

In addition to the primary role of donor T cells and host histocompatibility antigens in the induction of GVHD, a variety of additional extrinsic factors have also been shown to play a role in GVHD induction. Table VIII itemizes a number of factors shown to increase the risk of developing GVHD after BMT. The underlying mechanisms explaining some of these factors such as the role of MHC antigens, the H-Y antigen and induction of increased allogeneic responses in multiparous women have already been discussed. The T cell content of buffy coat infusions almost certainly explains the propensity of this therapy for induction of increased GVHD. The reasons why the latter four items in Table VIII are associated with an increased incidence of GVHD are not as immediately obvious.

TABLE VIII
RISK FACTORS FOR GVHD

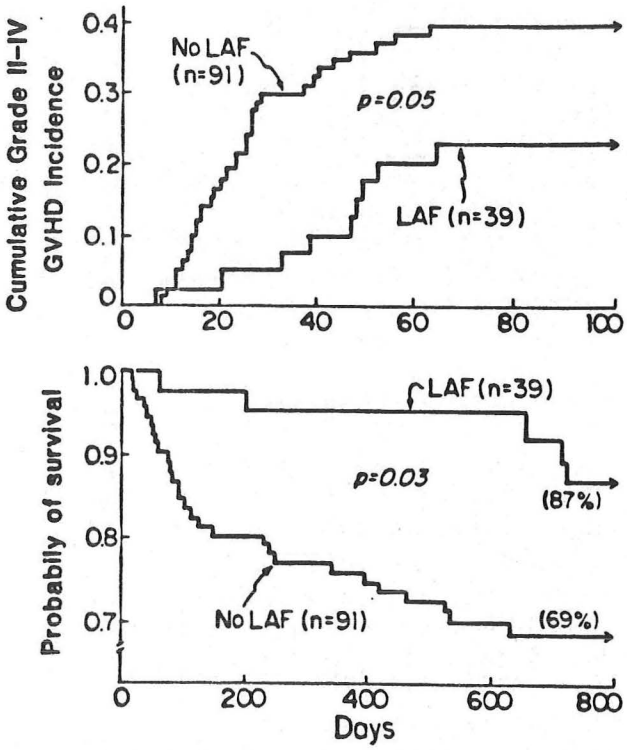
MHC mismatch > MHC match, allogeneic >> Syngeneic (34,75)
Female donor (46)
Donor/Recipient sex mismatch (47,48)
Infusion of donor buffy coat cells (83,84)[b]
Recipient bacterial colonization/infection (85)
Donor immunity to CMV, HSV (86,87)
Presence of active CMV infection post-BMT (84,88,89)[b]
Donor/Recipient age (34,83,84,90,91)

[b]Risk factor for development of chronic GVHD only; all other factors listed in the table have been shown to be independent risk factors for development of acute GVHD alone or for both acute and chronic GVHD.

The synergistic effects of microbial infection and GVHD are well demonstrated in a variety of animal models. The observation that mortality following allogeneic BMT was greatly delayed or reduced in germ free mice was initially interpreted as demonstrating the role of sepsis as a cause of GVHD related mortality (92). However, subsequent experiments (93) which utilized sterile fetal gut implants into allogeneic radiation chimeras demonstrated that histologic damage to intestinal epithelium remote from the site of any bacterial colonization was greatly increased in severity in conventional animals as compared to germ free chimeras. These findings suggested that simultaneous bacterial infection and GVHD caused true amplification of the GVHD reaction through either the nonspecific augmentory effects of monokines or lymphokines generated in

response to bacteria or through the induction of antigen specific T cells which recognize bacterial antigens and alloantigens in a cross-reactive fashion (94,95). A decreased incidence of GVHD has been noted in human recipients transplanted in germ-free isolation (see Figure 9). Unfortunately, this effect on reduction in GVHD incidence was most dramatic in those maintained in laminar airflow rooms for 50 days. The cost and psychological impact of such prolonged isolation has limited its use. However, since even brief intervals of gut decontamination and isolation also tend to improve survival after BMT through reduction of systemic opportunistic infections, these approaches are of value above and beyond any reduction in incidence or severity of GVHD.

Figure 9



Cumulative incidence of grades II through IV acute graft-versus-host disease (Top Panel) and estimated survival (Bottom Panel) in 39 patients treated in laminar-airflow rooms, compared with 91 patients treated outside such rooms.

From Storb R., et al, NEJM 308:305

The correlation between incidence of GVHD and level of donor immunity to cytomegalovirus or herpes simplex virus may in part be due to the fact that the T cell repertoire reactive against foreign antigens in association with self MHC encoded molecules and that portion of the T cell repertoire directed against alloantigens tend to overlap (94,95). Thus, it has been demonstrated in animal models that priming with a foreign antigen induces a subpopulation of T cell clones which fortuitously are cross-reactive with alloantigens (94). When subsequently challenged with alloantigens, an amplified secondary immune response might be induced from such cross-reactive clones in individuals with multiple prior viral infections. Alternatively, bone marrow from an individual seropositive for a latent viral infection might serve as a source of infection for a seronegative recipient. Certainly, active CMV infection in the post-transplant period is a major cause of morbidity and mortality. Both renal graft rejection in renal transplant recipients and chronic GVHD in bone marrow transplant recipients is increased in frequency following active CMV infection in the post-transplant period (84,88,89,96,97). The complex interrelationships between viral infection and GVHD is an especially major factor in induction of pulmonary disease in such patients. Interstitial pneumonitis is a leading cause of death following allogeneic BMT. CMV is isolated by culture or identified histologically in approximately half of such cases, but clearly the etiology of this syndrome is multifactorial as detailed by the list of risk factors contained in Table IX (32,98,99).

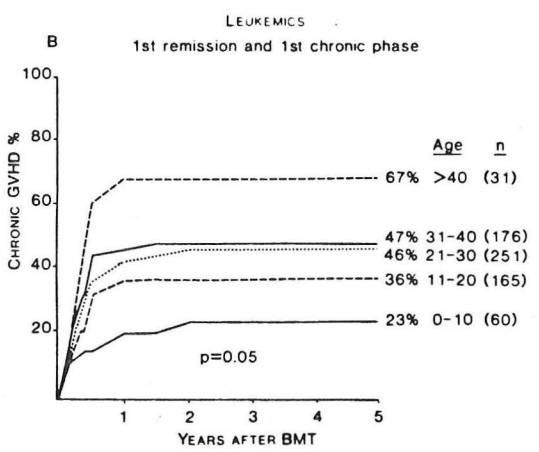
TABLE IX
RISK FACTORS FOR DEVELOPMENT OF INTERSTITIAL PNEUMONITIS
AFTER BONE MARROW TRANSPLANTATION

Allogeneic donor
Presence of acute GHVD
Pre-transplant chemotherapy
Older recipient/donor age
Lung (i.e. TBI) irradiation
High dose cyclophosphamide conditioning
Use of methotrexate immunoprophylaxis

Ascribing cause and effect relationships to the association between active CMV infection and GVHD is very difficult. Of note, a high frequency of cases of active CMV infection are preceeded by acute GVHD (100,101). Yet in three studies in which intravenous immune globulin therapy was used as prophylaxis for CMV infection, not only was the incidence of symptomatic CMV infection and interstitial pneumonitis found to be decreased, but in addition, in both studies the incidence of acute GHVD was significantly reduced in recipients of high titer anti-CMV immune globulin (102-104). In animal models in which interstitial pneumonitis is not seen after challenge with either CMV or a GVH reaction alone, simultaneous challenge with CMV and a GVH reaction causes lethal interstitial pneumonitis (105). Of additional interest, after syngeneic BMT for leukemia an 11% incidence of "idiopathic" pneumonitis has been reported, but no cases of CMV pneumonia were noted in review of 100 identical twin transplant recipients in comparison to the 19% incidence of CMV pneumonitis (and 13% incidence of idiopathic interstitial pneumonia) in recipients of allogeneic BMT who received similar levels of pre-transplant radiation and chemotherapy (34). Thus, there is good evidence that chemotherapy/radiation injury, infection and graft-versus-host reactions play synergistic roles in induction of lung injury following human allogeneic BMT. This triad of factors are likely to have synergistic effects as well on the level of tissue injury seen in the other target organs of the GVH reaction.

The risk for development of both acute and chronic GVHD increases with increasing recipient age. This effect is most dramatically seen when the age related incidence of chronic GVHD is examined (see Figure 10). As most series consist largely of sibling donor/recipient pairs, donor and recipient age tend to increase in tandem. Some series have actually revealed that GVHD risk correlates more closely with increasing donor age than with recipient age (84). Two mechanistic explanations have been proposed for this phenomenon.

Figure 10



From Ringden P., et al, Transplantation Today, 9:2603.

Beyond age 20, thymic atrophy is observed (106). In light of the critical role which the thymus plays in induction of specific transplantation tolerance in radiation chimeras, it has been proposed that defective thymic function is the cause of chronic graft versus host disease through mechanisms previously discussed with respect to syngeneic or autologous GVHD (107,108). Use of thymic transplants from unrelated human donors has not proven of benefit in preventing GVHD (108). However, currrent concepts of intrathymic T cell education would predict that only thymic transplants from young donors syngeneic to the recipient would be of uniform benefit, and thus this hypothesis is unlikely to ever be properly tested. It is also apparent that with increasing age, humans acquire latent viral infections (and immunity to same) with increasing frequency. Thus, the previously observed risks related to donor immunity to CMV or HSV infections or to the reactivation of such infections in the post-transplant period could also explain the age-related risk of development of GVHD. The relationship between donor/recipient age and GVHD is one of the major factors which limits use of allogeneic BMT to only a fraction of patients with certain malignancies.

Treatment and prevention of graft-versus-host disease

Treatment of established GVHD poses a difficult problem. All immunosuppressive regimens used thus far in treatment of acute GVHD have been associated with a 30-60% remission rate (109), but unfortunately infections and subsequent development of chronic GVHD continue to limit 1-2 year survival after development of acute GVHD to 25% or less. Attempts to further intensify immunosuppressive regimens for acute GVHD have been met with an increased incidence of fatal infections and actual decreases in overall survival rates (35). Combinations of prednisone and cytotoxic drugs have had more favorable effects on survival in patients with chronic GVHD (110), but many treated patients continue to have significant long-term morbidity. Therefore, major efforts have focused on prevention of GVHD. Attempts to decrease prophylactically the frequency and severity of GVHD have taken several forms. As detailed in Table X, the approaches taken thus far fall into several broad categories and include the alternation of risk factors, where possible, by selecting HLA-matched or identical twin donors, combining antimicrobial decontamination of skin, gut and respiratory tract with patient isolation procedures and use of prophylactic ISG and antimicrobial agents. Prophylactic immunosuppressive therapy has also been shown to decrease the incidence of acute GVHD and improve survival following allogeneic BMT.

TABLE X
PROPHYLACTIC MEASURES PROVEN OF BENEFIT IN DECREASING FREQUENCY AND SEVERITY OF GVHD

HLA matching between donor and recipient (34)
Bacterial decontamination and protective isolation (85)
Prophylactic trimethoprin/sulfamethoxazole (111)
Immune serum globulin therapy (high titer anti-CMV) (102-104)
Post-transplant methotrexate alone (versus no immunosuppression) (36)
Methotrexate plus cyclosporin (versus either agent alone) (112,113)
T cell depletion of donor marrow (50,51)

The improved survival rates achieved after allogeneic BMT in recent years can largely be attributed to the use of expanded supportive care (transfusion therapy, hyperalimentation, expanded armamentarium of anti-microbial agents) and the use of the first six approaches detailed in Table X. Unlike the striking success of donor T cell depletion techniques permitting establishment of stable chimeras in animal models, the recent application of this technique to human bone marrow transplantation has been a major disappointment. Based on animal models and some human data demonstrating a dose-dependent correlation between numbers of mature T cells contaminating donor bone marrow and the frequency and severity of GVHD (114), most trials aimed at preventing GVHD in man have been devised to achieve maximal T cell depletion while preserving stem cell function. A summary of the techniques used to deplete T cells prior to human bone marrow transplantation is given in Table XI.

TABLE XI
TECHNIQUES USED TO DEPLETE MATURE T CELLS FROM HUMAN BONE MARROW

Sheep erythrocytes rosetting (115)
Lectin agglutination (116)
Counterflow centrifugation (117)
Anti-T cell globulin + complement (118)
Anti-T cell monoclonal antibody opsonization (119)
Anti-T cell monoclonal antibody(s) + complement (49,50,51,120)
Immunotoxin-linked anti-T cell monoclonal antibody(s) treatment (121)

In the setting of haploidentical parent bone marrow transplants to children with SCID, such approaches appear to have been of significant benefit (see Table XII).

TABLE XII
RESULTS OF MHC-INCOMPATIBLE BMT FOR SCID

Series	T Cell Depletion[a]	Survival at one year
Cumulative 1968-1977 (122)	-	0/31
Reisner et al, New York, N.Y. (116)	+	2/3
Cowan et al, San Francisco (123)	+	5/9
Friedrich et al, Germany (124)	+	8/12
Buckley et al, Durham, N.C. (125)	+	9/11

[a]T cell depletion accomplished by lectin agglutination (soybean agglutinin) and sheep erythrocyte rosette fractionation.

Of note, however, whereas none of these small series noted significant GVHD after such T cell depleted transplants, several problems with the outcome of such T cell depleted haploidentical transplants have been noted. Unlike SCID patients transplanted with non-T cell depleted MHC compatible bone marrow in which development of full chimerism is usually noted, a form of mixed chimerism characterized by stable engraftment of donor T cells with persistence of host B

cells has been the most common outcome after T cell depleted haploidentical transplants (123-125). In addition, development of fatal EBV induced B cell lymphoproliferative disorders which is occasionally seen in untransplanted SCID patients may occur more frequently in the immediate post-transplant interval in recipients of haploidentical T cell depleted bone marrow (35,125-128).

In contrast to the rewarding results of donor T cell depletion prior to allogeneic BMT in SCID patients, the outcome of trials examining benefits of donor marrow T cell depletion prior to adult allogeneic BMT for leukemia have been thoroughly unimpressive with respect to any beneficial effect on long-term survival.

TABLE XIII
PATIENT OUTCOME FOLLOWING DONOR BONE MARROW T CELL DEPLETION PRIOR TO MHC-MATCHED BMT FOR LEUKEMIA[a]

Trial	T Cell Depletion	Incidence of Grade II-IV acute GVHD	Incidence of chronic GVHD	Failure of Engraftment	Leukemia Relapse	Relapse-Free Survival 2 Years
UCLA (50)	-	65%	25%	0%	10%	35%
	+	15%*	5%*	25%*	60%*	30%
France (51)	-	42%	29%	0%	8%	63%
	+	6%*	2%*	28%*	30%*	49%

[a]Both studies included patients with ALL, AML and CML; *$p<0.05$.

Table XIII summarizes the results of two randomized trials comparing standard allogeneic BMT protocols (with post-transplant immunosuppression) to protocols in which donor marrow was treated with anti-T cell monoclonal antibodies and complement prior to transplant (two separate antibody cocktails were employed in the French trial and have been combined in the table). Both trials showed significant reduction in GVHD with reciprocal increase in incidence of leukemic release and graft failure resulting in no benefit in disease free survival. A host of uncontrolled trials of donor marrow T cell depletion prior to BMT for leukemia have also now been reported (summarized in references 9 and 35). Virtually every trial uses different monoclonal antibodies with most employing a cocktail of up to 8 antibodies (49). All include at least one antibody reactive with all mature T cells and some have included antibodies which also react with mature NK and/or B cells. Fortunately for our ability to interpret results obtained with such disparate reagents, the outcome of such trials has proven to be amazingly similar. A low incidence of clinically significant GVHD has been uniformly reported with an overall incidence of 7%, even in the absence of post-transplant immunosuppression (35). This is quite impressive when compared to the 65-100% incidence of this complication in MHC-matched recipients of untreated allogeneic bone marrow who receive no post-transplant immunosuppression. However, virtually all such trials have noted a discouraging number of cases in which graft failure occurred (9). Of note, most such graft failures occur after initial evidence of early donor marrow engraftment - a phenomenon rarely seen after non-T cell depleted BMT. In series in which attempts were made to compare such regimens to conventional therapy, an apparent increased risk of leukemia was

seen (50,51), and several authors also report the development of EBV related lymphoproliferative disorders following T cell depleted BMT for adult leukemia, much as has been previously reported in SCID patients (35).

Thus, the primary desired outcome of donor marrow T cell depletion, reduction in incidence of GVHD, has been achieved by regimens developed in a large number of transplant centers. Yet, no improvement in overall survival has been noted because of a reciprocal increase in other obstacles to successful outcome of allogeneic BMT for adult leukemia.

Marrow Graft Failure

Failure of engraftment was a major obstacle to initial efforts to utilize BMT as therapy for leukemia or aplastic anemia. The role of host versus graft reaction in mediating allogeneic marrow resistance was clearly recognized in early animal studies (14,33) and was a major consideration in devising improved techniques for achieving successful BMT therapy for aplastic anemia in the 1970's (129). Allogeneic BMT for aplastic anemia initially was complicated by a 30-60% graft failure rate. Risk from graft failure was found to correlate with presence of frequent pre-transplant transfusions, with the presence of pre-transplant recipient versus host mixed lymphocyte responses, and with a low dose of donor marrow (35,46-48). Three strategies devised to deal with these risk factors - early performance of BMT prior to multiple transfusions, use of pretransplant immunosuppressive therapy (cytoxan, total body irradiation and/or total lymphoid irradiation) and supplementation of donor marrow with donor buffy coat cells were found to be of benefit in achieving a higher frequency of stable engraftment. Initial interpretations of these results included an appreciation for the role of host alloreactive T cells in destruction of donor marrow and the induction of enhanced host anti-donor responsiveness by prior immunization by the leukocytes contained in blood transfusions. One of the initial rationales for use of donor buffy coat cells was based on the observed presence of a small frequency of circulating stem cells in human peripheral blood and the hope that any attempt at increasing bulk numbers of donor stem cells would in some way aide in overwhelming host resistance to engraftment (35). However, recent experience with T cell depleted BMT in patients with leukemia suggests that the large number of donor T cells contained within buffy coat infusions may play a primary role in the efficacy of this adjuvant therapy in achieving successful engraftment.

In the absence of donor T cell depletion, the radiation and chemotherapy regimens used to reduce host tumor burdens have generally proven adequate to permit virtually uniform success with respect to donor marrow engraftment (35,50,51). The failure of T cell depleted allogeneic marrow grafts in identically prepared recipients can be explained by a variety of mechanisms. The most obvious initial concern is that exposure to the reagents used in T cell depletion or the multiple incubations and washing procedures inherent to such techniques might in some way damage donor stem cells. Although a modest decrease in total number of bone marrow cells as well as number of functional stem cells present in such T cell depleted infusions has been documented (49), incidence of graft failure does not correlate with the total number of donor cells infused or number of myeloid colony-forming units (CFU-GM) or erythroid blast forming units (BFU-E) infused (130,131).

Certainly subtle forms of stem cell damage or removal of an accessory or helper cell (such as the T cell itself) could still be evoked as partial explanation for the increased frequency of graft failures following T cell depletion of donor marrow. However, a substantial number of observations in human and animal models of allogeneic BMT suggests that active host resistance of donor marrow is the critical mechanism which explains this phenomenon. In Table XIV, a number of features of the graft failure observed after infusion of human recipients with T cell depleted marrow are listed.

TABLE XIV
FEATURES OF BONE MARROW GRAFT FAILURE NOTED AFTER T-CELL DEPLETION OF DONOR MARROW

(1) Most graft failures occur "late" after initial engraftment (49-51)
(2) Incidence decreased after increased host irradiation (49,132)
Incidence greater for immunocompetent adult than for similarly conditioned SCID recipients (35)
Identical T cell depletion techniques do not decrease autologous bone marrow engraftment (35)
Host T cells cytotoxic for donor lymphocytes have been isolated after graft failure (133)
Increases in MHC disparity between host and donor increase graft failure rate (134).

These features suggest that much as has been found in animal models, extensive T cell depletion of donor marrow increases vulnerability to host resistance factors. In animal models, two major types of genetically restricted mechanisms for bone marrow rejection have been delineated. One mechanism appears to be the host versus graft counterpart of the graft versus host reaction. Radioresistant host T cells have been shown to respond to donor cell MHC antigenic differences and mount an antigen specific response which eventually destroys donor marrow cells (135). The other major radioresistant host lymphoid cell capable of resisting donor marrow grafts in rodent models is the NK cell. This form of marrow graft resistance was initially recognized following observation of semisyngeneic F_1 recipient resistance to homozygous parental marrow engraftment and has been referred to as "hybrid resistance" (136).

The mediation of hybrid resistance by T cell deficient athymic mice (137) and by SCID mice (138) which lack both T and B cell functions as well as the ablation of such resistance by anti-NK antibody treatment of the recipient (139) has demonstrated that such hematopoietic resistance is mediated by NK cells. Such hematopoietic resistance in mice is genetically restricted in a fashion quite different from that of classical T cell mediated transplant rejection in that the donor Hh antigens recognized by host NK cells are not inherited in a co-dominant fashion as are classical histocompatibility antigens, and therefore are expressed by homozygous but not heterozygous cells (136). In vitro data obtained from human NK cell/bone marrow stem cell cocultures suggest that by other non-genetically restricted mechanisms, NK cells can suppress stem cell proliferation (*140). Such mechanisms have been offered as explanation for host resistance of syngeneic (identical twin) bone marrow grafts in aplastic anemia patients in whom coculture of donor marrow with host NK cells demonstrated evidence of such NK cell suppression of CFU-GM (141). Such syngeneic marrow graft resistance was overcome

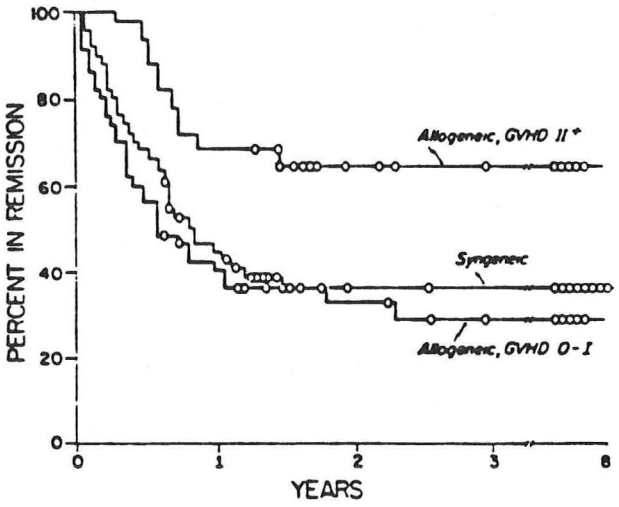
by pretransplant cyclophosphamide, an agent inhibitory of NK function, but not by pre-transplant anti-thymocyte globulin therapy (141). It has also been suggested that the occasional failure of T cell depleted donor marrow engraftment in human SCID recipients without apparent T or B cell function might also be mediated by NK cells. However, such human NK cell mediated marrow graft resistance is unlikely to be the major explanation for late graft failure in leukemia recipients of T cell depleted allogeneic bone marrow.

Such late graft failure has been found to correlate with the degree of donor/recipient MHC disparity and to be associated with the presence of host anti-donor cytotoxic T cells (133,134). In murine models of NK cell mediated graft resistance, marrow rejection occurs early in the post-transplant period and does not respond to priming, whereas T cell mediated graft rejection occurs later in the post-transplant period (135,136,142). Thus, the markedly delayed rejection of human T cell depleted allogeneic bone marrow is most consistent with a delayed T cell-mediated antigen-specific response to histocompatibility antigen disparities expressed by donor cells. The role of donor T cells in preventing such host-versus-graft resistance presumably is a function of the ability of mature donor T cells to eliminate or suppress radioresistant host lymphocytes (143). Two groups of investigators have suggested that "partial" T cell depletion of donor marrow coupled with post-transplant immunoprophylaxis will achieve satisfactory reduction in GVHD without prohibitive failure of engraftment (117,144). However, one of these optimistic reports has already been superceded by further follow-up which demonstrates a 19% incidence of graft failure (131), and neither study has sufficiently long follow-up to permit assessment of leukemic recurrence.

Graft versus leukemia response

The graft versus leukemia (GVL) effect of allogeneic bone marrow transplantaion has now been confirmed by a variety of observations. As detailed in Figure 11, early results with BMT for acute leukemia revealed that leukemic relapse rates were lower in allogeneic marrow recipients with significant graft versus host disease (grade II-IV acute or chronic GVHD) than in syngeneic marrow recipients or allogeneic marrow recipients without severe GVHD (145). This effect has been more dramatic in ALL than in ANL, and in more recent series the development of chronic GVHD following allogeneic BMT for childhood ALL has actually been associated with improved long-term survival (see Figure 12) (146,147). The observation that T cell depletion of MHC-matched allogeneic donor marrow increases the incidence of leukemic relapse

Figure 11

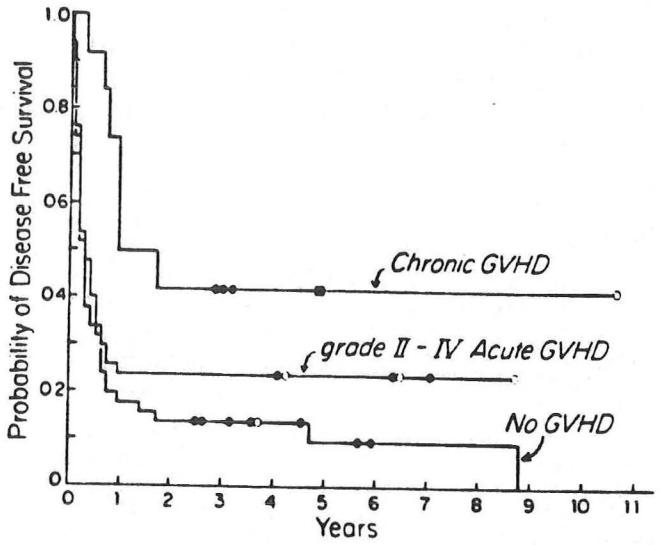


Kaplan-Meier Product Limit Estimate of the Probability (Expressed as per Cent) of Remaining in Remission from Acute Lymphoblastic or Nonlymphoblastic Leukemia as a Function of Time after Transplantation.

From Weiden PL, NEJM 300:1069

confirms that mature donor T cells are responsible for initiating the graft versus leukemia effect. NK-like tumoricidal lymphocytes capable of lysing cryopreserved host leukemic cells have been isolated from patients with GVHD and have been suggested to be the effector cells mediating this response (148). However, in animal models of syngeneic GVHD, no anti-leukemic effect can be demonstrated, and indeed, the presence on leukemic cells of GVHD inducing alloantigens appears to be a major prerequisite for the graft versus leukemia effect (149). Of note, however, exacerbation of GVHD by use of decreased post-transplant immunosuppression or reduction of GVHD by addition of cyclosporin to post-transplant GVHD prophylaxis regimens has not been associated with changes in the rate of leukemic relapse (35). Thus, whereas presence of alloantigens on host leukemic cells and transfer of mature donor T cells during BMT appear to be major prerequisites to the induction of a graft versus leukemia effect, there is some basis for hope that the GVHD and GVL-inducing capacity of the graft versus host response can be manipulated in a manner affording decreased morbidity and mortality from GVHD without sacrificing the graft versus host leukemia effect.

Figure 12



Kaplan-Meier product limit estimates of disease-free survival of children who developed chronic GVHD, acute GVHD, and no GVHD. Surviving patients transplanted in remission are represented by closed circles and surviving patients transplanted in relapse are represented by open circles.

From Sanders JE, et al, Med. Ped. Oncol. 13:170

EBV related lymphoproliferative disorders after BMT

Progressive EBV-related B-immunoblastic proliferative disorders have been described in immunosuppressed organ transplant recipients and in patients with primary immunodeficiency syndromes. However, SCID patients appear to suffer from a higher frequency of this disorder following transplantation with T cell-depleted haploidentical bone marrow (128). The Seattle group has also noted this disorder in 2 of 52 leukemic patients transplanted with T cell depleted MHC-mediated bone marrow (35). While fatal EBV-associated lymphoproliferative disorders are rare following non-T cell depleted bone marrow transplantation, in the cases where it has been reported, it has been associated frequently with use of anti-thymocyte globulin or of anti-CD3 monoclonal antibody (but not after anti-T subset monoclonal antibody) therapy of acute graft versus host disease (150,151). These observations not only emphasize the critical role of T cells in regulation of EBV induced B cell proliferation, but also tend to indicate that this syndrome is yet another obstacle to the use of T cell depletion strategies as means of preventing or controlling GVHD.

Summary and Future Prospects

During the greater than three decades of its use as a therapy for human disease, allogeneic bone marrow transplantation has been limited by a rather constant set of obstacles which include GVHD, host resistance to engraftment, opportunistic infections, and relapse of the malignancies for which this therapy is most commonly utilized. By the late 1970's regimens of radiation and chemotherapy had been devised which achieved nearly uniform donor marrow engraftment and an acceptable incidence of leukemic relapse when applied early in the disease course to patients in remission from leukemia. Modified versions of these regimens were proven to be even more efficacious when applied to nonmalignant diseases such as aplastic anemia. However, the morbidity and mortality posed by GVHD has largely prevented use of this therapy in patients over age 40 who lack MHC-matched sibling donors. Based on mechanisms of GVHD induction defined in a variety of animal models, and with the development of more specific anti-human T cell reagents, a number of protocols for prevention of GVHD by T cell depletion of donor marrow have been devised and applied clinically. Whereas such protocols have been impressively successful in decreasing the incidence and severity of GVHD, as summarized in Table XV, such benefits have been largely counterbalanced by an increased frequency of the other previously recognized obstacles to BMT.

TABLE XV
EFFECTS OF T CELL DEPLETION OF DONOR BONE MARROW
ON CLINICAL OUTCOME OF ALLOGENEIC BMT

Benefits	Adverse Effects
Decreased GVHD (50,51)	Increased graft failure and/or mixed chimerism (50,51,123-125,128,152)
	Increased leukemic relapse (50,51)
	Increased EBV related B lymphoproliferative disorders(35,128)

The difficulties in achieving donor engraftment with T cell depleted human allogeneic donor marrow appear to be related largely to host resistance factors. Mechanisms of marrow graft resistance in animal models have been defined. Based on these observations, a variety of approaches to overcoming host resistance have been devised. The approaches summarized in Table XVI have all been shown to be of value in permitting enhanced engraftment of allogeneic T cell depleted marrow in animal and/or early human trials.

TABLE XVI
RECIPIENT CONDITIONING REGIMENS DEVISED TO ENHANCE ENGRAFTMENT
OF T CELL-DEPLETED ALLOGENEIC BONE MARROW

In vivo anti-T cell monoclonal antibody therapy (142,153,154)*
In vivo anti-NK cell monoclonal antibody therapy (139)*
In vivo anti-LFA1 monoclonal antibody therapy (155)+
Intensified total body irradiation (132,156)*+
Total lymphoid irradiation (65,157,158)*+
Intensified chemotherapy (156)*

*Demonstrated to be efficacious in animal models of resistant allogeneic marrow engraftment; +Positive effects on engraftment of T cell depleted allogeneic bone marrow in preliminary human trials

Use of intensified chemoradiotherapy regimens has been the standard recipient preparative approach which, when coupled with depletion of donor marrow, has permitted GVHD free establishment of allogeneic chimeras with relative ease in rodent models. Humans and large animals have a much lower tolerance for total body irradiation than do rodents (159), and current preparative regimens are already associated with substantial morbidity including increased risk of interstitial lung disease (98-100), hepatic veno-occlusive disease (160,161), cardiac toxicity (28,162, and late malignancy (163). Thus, it is unclear whether increased total body irradiation or intensified chemotherapy will be of overall benefit even if decreases in graft failure rates are noted. Addition of total lymphoid irradiation or of in vivo monoclonal antibody therapy to more moderate chemoradiotherapy preparative regimens would appear to hold greater promise for improved outcome of allogeneic bone marrow transplantation. This would especially appear to be the case in non-malignant diseases where the opposing demands of GVHD prevention and enhancement of engraftment are the major obstacles to be overcome. Even in this setting, all such regimens are likely to result in an even more immunocompromised patient in the immediate post-transplant period, and prevention of EBV-related B cell proliferative disorders or other infectious processes will continue to be a major challenge.

The barriers to improved outcome of allogeneic BMT as therapy for leukemia are far more substantial. The occurrence of leukemia relapse continues to be the major problem limiting life expectancy in this setting. Major hope for improved outcome rests in the possibility that intensified chemoradiotherpy preparative regimens will simultaneously decrease leukemic relapse and lower host resistance to engraftment of T cell depleted allogeneic bone marrow, or that donor T cell responses can be manipulated in a way that achieves satisfactory elimination of host resistance factors (presumably host T and NK cells) and adequate graft versus leukemia effects while not producing severe GVHD. Whereas substantial obstacles still remain to the therapeutic use of allogeneic BMT and at present limit its application largely to diseases with high short-term mortality rates, the success of this procedure has and is continuing to steadily improve. Of note, it is now appreciated that human bone marrow transplantation achieves not only long-term reconstitution of hematopoietic cells, but also provides the host with a new immune system which has been reconstituted in a unique fashion. Bone marrow transplantation is the only organ transplant model in which development of specific transplantation tolerance is the rule rather than the exception. Thus, as is detailed in Table XVII, the list of dieases thought to be future candidates for allogeneic BMT therapy includes not only additional "deficiency" disease but also all diseases correctable by solid organ transplantation as well as HLA-linked autoimmune diseases.

TABLE XVII
SOME POTENTIAL FUTURE APPLICATIONS OF ALLOGENEIC
BONE MARROW TRANSPLANT THERAPY

Use for current disease indications, in patients >40 years of age or without HLA-matched sibling donors
Treatment of sickle cell anemia and other lympho-hematopoietic genetic diseases not presently routinely treated by BMT (164)
Induction of specific transplantation tolerance in solid organ allograft recipients (165)
Correction of HLA-linked autoimmune diseases (166)

Clearly, widespread adoption of allogeneic BMT for use in the disease categories listed in the above table is absolutely dependent upon significant additional improvement in survival and quality of life after BMT. However, whereas application of allogeneic BMT to some of the diseases listed in Table XVII may appear to be wildly speculative, such suggestions are probably no more speculative than were thoughts in the mid-1970's of widespread application of allogeneic BMT therapy in leukemia.

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