ABSTRACT

Transplantation of hematopoietic stem cells from human leukocyte antigen (HLA)-compatible unrelated volunteer donors have become feasible for more than 70% of patients without a family match. Chronic myeloid leukemia is the most common indication for unrelated donor marrow transplantation. The 5-year survival for patients up the age of 50 years has improved to 75% with transplantation in the chronic phase within the first year from after diagnosis, the selection of a closely matched donor, and the prophylactic use of fluconazole and ganciclovir. However, graft failure, and acute and chronic graft-versus-host disease (GVHD) remain frequent causes of morbidity and death. The incidence of graft failure is correlated with multiple Class I HLA-A, B, and C mismatching in the donor. The risk of grades III-IV acute GVHD is highest with Class II HLA-DRB1 and DQB1 mismatching in the recipient. Refractoriness to glucocorticoid therapy is the dominant factor predisposing to chronic GVHD. Mismatching for a single Class I or Class II allele has no effect on survival, but mortality is increased by simultaneous mismatching for Class I and Class II alleles. Transplantation of marrow from an HLA-matched, unrelated donor is safe and effective therapy for selected patients with hematological malignancy. Since fully matched donors will not available for many patients, the challenge is developing methods for marrow transplantation that can decrease morbidity and improve survival despite genetic disparity between donor and recipient.
OVERVIEW

Transplantation of marrow stem cells from healthy individuals was initially employed for treatment of patients with marrow failure or advanced leukemia, and successful engraftment was achieved only by selecting a twin donor or a sibling donor identical for human leukocyte antigens (HLA) (1). Subsequent studies of partially HLA-matched related donors demonstrated that the degree of HLA incompatibility correlated with the incidence of graft failure and graft-versus-host disease (GVHD) (2, 3). In patients with hematologic malignancy, transplants from relatives incompatible for multiple HLA loci have been associated with lower survival than transplants from HLA-identical siblings (3). These results demonstrated that HLA is the major histocompatibility complex in man and provided the basis for testing the use of HLA compatible unrelated donors for patients without a family match. Since less than 30% of patients in developed countries have an HLA-matched sibling, the only chance of finding a compatible donor for most patients in need of an allogeneic stem cell transplant is through the identification of an HLA-compatible unrelated volunteer.

Unrelated donor transplants have become feasible and successful thanks to the identification of HLA genes and their functional products (4), the development of precise and efficient HLA typing methods using DNA technology (5), and the development of a network of registries containing more than five million HLA-typed donors worldwide (6). More than two thousands patients with acute and chronic leukemias, lymphoma, myeloma, myelodysplasia, aplastic anemia, congenital errors of metabolism and immunodeficiency syndromes are transplanted each year in the world using marrow or blood stem cells from unrelated volunteers. Many patients have achieved complete immunological tolerance and have become long-term survivors (7,8). A higher incidence of GVHD, however, was found in unrelated than in sibling transplants despite matching for HLA-A, B, and DR, indicating that the methods used initially for the assessment of histocompatibility were inadequate (8,9).

HISTOCOMPATIBILITY

HLA antigens are cell surface molecules encoded by Class I A, B, C and Class II DR, DQ, and DP genes that are located on human chromosome 6. The function of HLA molecules is to bind and present antigenic peptides to T lymphocytes, one determinant step in the initiation of the immune response. To bind the variety of ever changing environmental antigens, the HLA complex has evolved to become the most polymorphic set of known human genes. T cells
from one individual react vigorously to mismatched HLA molecules on the surface of antigen-presenting cells from another individual.

Polymorphic specificities of HLA-A, B, C, DR and DQ antigens have been routinely typed by alloantisera. A serologically defined specificity, however, does not necessarily represent a unique allele. Analysis of HLA-B27 molecules by gene sequencing, for example, has revealed that there are at least seven distinct alleles, defined B*2701-2707, each of which encode a unique primary amino acid sequence that can be distinguished by T cells (10). Hybridization of sequence specific oligonucleotide probes (SSOP) to polymerase chain reaction (PCR)-amplified DNA has proven to be a powerful method for identifying polymorphisms of Class II loci. Typing with panels of SSOPs can reveal specific alleles indistinguishable by serological typing. A single incompatibility for DRB1 or DQB1 alleles distinguished by SSOP but not by serology (for example: DR4/ DRB1*0401 versus DR4/ DRB1*0402) is associated with a significant increased risk of acute GVHD in either unrelated or related marrow transplants (8, 11). There is as yet insufficient data to assess the relevance of DP mismatching to GVHD development after unrelated donor transplants.

The relevance of Class I allele mismatching to clinical marrow transplantation has first been suggested by the occurrence of marrow graft rejection in a case where an unrelated donor was mismatched only for HLA-B*4402 versus B*4403 two alleles that differ by a single amino acid residue but are serologically indistinguishable. Anti B*4403-specific CTL (cytotoxic T lymphocytes) were found in the patient's blood following rejection of the B*4403-positive donor graft (12). Initial data using sequencing of PCR-amplified DNA have indicated that mismatching for Class I HLA-A, B and C alleles is a risk factor for marrow graft failure and death (13,14). These observations have demonstrated that serological typing methods are not adequate for identifying all HLA antigens relevant to marrow transplantation. At this time, HLA Class I gene typing is becoming a routine technique in many tissue typing laboratories.

Theoretical considerations have suggested that pairs of unrelated individuals are more likely to have disparity for non-HLA minor histocompatibility genes than occurs between related individuals (15). Unfortunately, only two non-HLA minor histocompatibility genes has been well characterized in humans: the male-associated gene H-Y, and HA-1 (16,17). Functional assays may test reactivity of donor T cells against non-HLA minor histocompatibility determinants of the recipients, and vice versa (18). Both functional and genetic assays may help to select more closely matched donors, but they have not yet been widely implemented for donor selection.
REGISTRIES OF HLA TYPED VOLUNTEER DONORS

Because of the enormous polymorphism of HLA-A, B and DR genes, the probability of matching two random individuals for both alleles at all three loci is small. The success of matching is higher than expected, however, because of non-random association between the individual alleles of one HLA locus with alleles of the other loci on the same chromosome, a phenomenon termed linkage disequilibrium. As for racial traits, HLA types are associated with the ethnic background of the individual person, and populations living in different geographic areas demonstrate variable degrees of HLA polymorphism that correlate to some extent with the ethnic heterogeneity of the population. Therefore, the probability of matching a patient with a donor in a pool of unrelated volunteers depends on the number of donors in the pool, the ethnic heterogeneity of the population and the donor pool, the relationship of the patient with the ethnic composition of the donor pool, and the HLA diversity of the ethnic groups. The problem of finding a suitable donor becomes especially complex when intergroup marriages have occurred.

The United States of America represent an example of great complexity. In 1986 it was predicted that a marrow donor registry containing at least 100,000 volunteers was needed to find an HLA-A, B and DR match for at least 50% of patients (19). The United States National Marrow Donor Program (NMDP) began search operations in September 1987. Initially the donor registry contained HLA data for approximately 18,000 volunteers. By 1998, the NMDP registry contained more than 3.4 million donors typed for HLA-A and B and more than 1.5 million also typed for DR. The NMDP network includes 105 donor centers, 111 marrow collection centers and 76 transplant centers located in 40 different states and 10 countries. In addition, NMDP maintains cooperative donor search agreements with other national networks in Australia, Austria, Canada, United Kingdom, France and Switzerland which combine more than 500,000 additional donors. This international donor search agreement expands the pool of HLA typed unrelated volunteers that can be accessed through the NMDP to more than 4 million. By 1998 more than 7,000 patients have been transplanted through NMDP.

DONOR SEARCH

The probability of finding an HLA-A, B, DR match at the initial search has increased from 10-15% in 1987 to 80% in 1998 and has crossed the 50% mark, just when the number of HLA-A, B, and DR-typed volunteers was 100,000. Some patients also find a match through DR typing of HLA-A and B matched donors.
By molecular typing, approximately 2/3 of HLA-A, B, DR matched donors are also identical for the HLA-DRB1 allele (8). All but 3% of patients find at least one HLA-A and B matched donor. Since 80% of the donors in the registry are Caucasian, the probability of finding a match is highest for Caucasians and lower for other racial groups which are represented in North America and the NMDP as minorities.

The time interval from the initiation of the search to transplant varies according to the patient's HLA type and diagnosis but currently averages 3-4 months. This long search time is a concern especially for patients with marrow failure or acute leukemia. A substantial amount of this time is necessary for contacting donors to provide blood samples for additional typing. With recent advances in molecular HLA typing technology and with the establishment of a repository containing donor DNA it may no longer be necessary to call in specific donors for complete typing. These changes might decrease the duration of the donor search.

RESULTS OF UNRELATED DONOR TRANSPLANTS

Engraftment

One of the mechanisms for failure of unrelated donor grafts is immunological rejection of donor hematopoietic cells by recipient T cells that recognize incompatible HLA determinants (12). It has also been proposed that recipient natural killer cells might mediate rejection of marrow grafts by destroying donor hematopoietic cells incompatible for HLA Class I determinants (20,21). Consistent with this hypothesis, we found that the risk of graft failure is increased by HLA-A, B and C mismatching of the donor (13,14).

A matched case-control study was designed in Seattle to evaluate the role of HLA-C disparity assessed by DNA sequencing methods in 21 patients who experienced graft failure (cases) following transplantation with unmanipulated marrow (13). The donor was unrelated, either HLA-A, B serologically matched, DRB1 matched (n = 14) or single locus mismatched (n = 7) with the recipients. Graft failure was more frequent in patients with CML than patients with any other diagnosis. For each case, two patients who successfully engrafted were selected as controls based on similarity for factors known or suspected to influence engraftment. The estimated odds ratio (OR) of graft failure for an HLA-C mismatch relative to match was 5.2 (95% CI: 1.4, 19; p=0.01). Serologically undetectable HLA-A or HLA-B allele disparity was also associated with graft failure. The association between HLA-C disparity and graft failure remained significant even after accounting for the contribution of HLA-A and B allele disparity (OR 4.0; 95% CI: 1.1, 15; p=0.03).
A subsequent study utilized DNA amplification and sequencing to identify the HLA-A, B, and C alleles of 300 patients with CML and their donors (14). Graft failure occurred in 3/146 (2%) HLA-A, B, C, DRB1 and DQB1 compatible transplants, and in no cases of transplants incompatible only at DRB1 or DQB1. The incidence of graft failure was increased with multiple Class I A, B, or C mismatching (9/31 cases, 29% incidence; OR 10.5, 95% CI: 2.2 to 49.8; p=0.003) or Class I A, B or C combined with Class II DRB1 or DQB1 mismatching (4/34 cases, 12% incidence; OR 10, 95% CI: 1.7 to 58.4; p=0.01). These results show that mismatch for HLA-A, B and C alleles that are not appreciated by serological typing is biologically important. Furthermore, data indicate that mismatching for a single Class I HLA allele does not increase the risk of graft failure in patients receiving conditioning with cyclophosphamide plus whole body irradiation. However, mismatching for multiple Class I HLA alleles or mismatching for Class I and Class II HLA alleles combined significantly increases the risk of graft failure.

Additional factors associated with an increased incidence of graft failure are sensitization of the recipient against donor antigens, a less intense conditioning regimen before transplantation, a less intense post transplant immunosuppression, lower marrow cell dose, and by depletion of donor T lymphocytes from the marrow inoculum. The use of hematopoietic growth factors can accelerate engraftment, but their utility in decreasing the risk of graft failure and improving outcome of unrelated transplants remains unproven (22). Conversely, the use of blood stem cells is expected to improve the probability of engraftment, especially in HLA incompatible transplants that are depleted of T cells (23).

Acute GVHD

Clinical GVHD results from an immune reaction of mature donor T lymphocytes contained in the marrow inoculum against histocompatibility determinants of the recipient. This reaction is directed towards normal tissues such as skin, gastrointestinal mucosa and hepatic biliary tract, and also against normal as well as malignant lymphohematopoietic cells. Human clinical trials evaluating T cell-depleted marrow transplants have found a reduction in incidence of GVHD but also an increase in the incidence of graft failure and relapse of malignancy (24). Current studies are evaluating whether less complete removal of donor T cells or T cell subset depletion can be sufficient to prevent GVHD without increasing the risk of graft failure and leukemia relapse. Initial data from the Milwaukee transplant team reported favorable results when a low degree (1.5 log) of T cell depletion was achieved by in vitro treatment of marrow cells with the anti-T cell receptor antibody T10B9 and complement in recipients of unrelated donor grafts (25,26).
The alternative approach for GVHD prevention is to deliver post-transplant immunosuppression. The first large series of successful unrelated transplants was reported after the introduction of combination therapy with cyclosporine and methotrexate used in 1985. The incidence of moderate to severe acute GVHD was significantly higher in HLA matched unrelated transplants (79%) than in HLA matched sibling transplants (35%) (9). In a subsequent study of patients less than 36 years of age, the probability of moderate to severe acute GVHD was 95% in 42 transplants mismatched for one A, B or D/DRB1 locus compared to 70% in 70 HLA-A, B, D/DRB1 matched unrelated donor transplants (p=0.05) (27). Since post transplant immunosuppression with cyclosporine and methotrexate is insufficient to control GVHD in most unrelated transplants, alternative modalities for GVHD prevention are being explored.

The extent to which unrecognized mismatching for alleles that encode DR1-18 contribute to the increased risk of acute GVHD and overall survival was initially investigated in patients receiving transplants from HLA-A, B, DR serologically matched donors (11). DRB1 alleles were typed by SSOP hybridization methods and selected alleles were confirmed by DNA sequencing. Of the 365 pairs, 306 were matched and 59 were mismatched for DRB1. The probability of moderate to severe acute GVHD was 47% for the matched and 70% for the mismatched patients. Compared to mismatched patients, the estimated relative risk (RR) of GVHD for matched patients was 0.58 (95% CI: 0.40, 0.84). DRB1 matching decreased the risk of transplant-related mortality (RR 0.65; CI: 0.44, 0.96) and was associated with decreased overall mortality (RR 0.7; CI: 0.5, 0.99). Acute GVHD and survival after unrelated marrow transplantation are significantly improved by matching DRB1 alleles of the donor and recipient.

A further study to address the relevance of DQB1 matching was conducted in 449 HLA-A, B, and DR serologically matched transplants (28). Molecular typing of HLA-DRB1 and DQB1 revealed 335 DRB1 and DQB1 matched pairs; 41 DRB1 matched and DQB1 mismatched pairs; 48 DRB1 mismatched and DQB1 matched pairs; and 25 DRB1 and DQB1 mismatched pairs. The conditional probabilities of grades III-IV acute GVHD were 42%, 61%, 55%, and 71%, respectively. The relative risk associated with a single locus DQB1 mismatch was 1.8 (CI: 1.1, 2.7; p=0.01), and the risk associated with any HLA-DQB1 and/or DRB1 mismatch was 1.6 (CI: 1.2, 2.2; p=0.003). The dominant role of incompatibility for HLA-DRB1 and DQB1 in the development of acute GVHD has remained even after accounting for mismatching at HLA-A, B, and C alleles (14). These results provide evidence that matching donors and recipients for DRB1 and DQB1 can further decrease the incidence of GVHD. Therefore,
prospective matching of patients and donors for DRB1 and DQB1 alleles is warranted.

**Chronic GVHD**

Beyond 100 days from transplantation, GVHD may involve skin, oral mucosa, eyes, liver and lungs and assume features resembling scleroderma, biliary cirrhosis and obliterative bronchiolitis. Approximately 35% of patients transplanted with unmodified marrow grafts show manifestations of chronic GVHD on day 100. These patients and an other 35% who develop chronic GVHD after day 100 require continued immunosuppressive treatment. The incidence of chronic GVHD is increased in recipients of female grafts, and in patients with acute GVHD who fail to achieve a complete and sustained response to glucocorticoids (29). Approximately 30% of all patients do not develop chronic GVHD, and in this situation, immunosuppression can be terminated by 5-6 months after transplantation. The median time for successful withdrawal of immunosuppressive therapy is 18 months, but 3% of patients continue to require some degree of immunosuppression four years or more after transplant (8). The duration of therapy for chronic GVHD is increased in patients older than 20 year old, in recipients of HLA-incompatible grafts, and in male patients transplanted from a female donor (29). Twenty-five to 30% of all patients die from complications of chronic GVHD while receiving immunosuppressive therapy. Mortality is increased in patients with history of glucocorticoid-resistant acute GVHD, and in those patients with serum bilirubin greater than 2 mg/dl or platelet count less than 100,000/ L at the onset of chronic GVHD (29). By one year after transplantation 75% of surviving patients have recovered a good performance status and by four years 95% have done so. Permanent disability may be caused by side effects of glucocorticoid therapy, cataract formation, osteoporosis, and avascular bone necrosis or by complications of chronic GVHD such as scleroderma and chronic obstructive pulmonary disease (29).

**Opportunistic Infections**

Immune reconstitution is severely impaired by acute and chronic GVHD and by prolonged immunosuppressive treatment. Repopulation by mature T cells and recovery of immunoglobulin production is extremely slow after unrelated donor transplantation. Immune deficiency and glucocorticoid therapy predispose patients to opportunistic infections, predominantly with aspergillus and cytomegalovirus (CMV). Disseminated aspergillus has an incidence of approximately 15% and is associated with more than 90% mortality in unrelated transplants. No improvement has been achieved in the prevention or treatment of aspergillus infection over the last several years. CMV seropositive patients have an increased incidence of CMV disease and CMV-associated mortality after transplantation compared to CMV seronegative patients (7, 27). The risk is also slightly increased in seronegative patients transplanted from seropositive
donors. Controlled clinical trials have shown that ganciclovir can prevent cytomegalovirus disease in CMV seropositive recipients of allogeneic marrow transplants (30). Post transplant prophylaxis or preemptive therapy with ganciclovir at the first evidence of CMV reactivation can significantly decrease CMV morbidity and mortality in seropositive recipients of marrow transplants from unrelated donors (31). Prophylaxis with fluconazole has also improved survival by decreasing the risk of disseminated Candida Albicans infection and the use of amphotericin B (31,32).

Recurrent Malignancy after Transplantation

The probability of relapse after transplantation depends predominantly on the diagnosis, stage of disease, and tumor load at the time of transplant (33,34). There is a trend however, for a lower probability of relapse in patients transplanted from an unrelated donor than patients transplanted from a HLA-matched sibling (9). This trend is particularly apparent in patients transplanted for CML in chronic phase. The data from Seattle indicate a 5% probability of relapse in unrelated transplants compared to 18% in HLA matched sibling transplants. Data from Milwaukee indicates a 5% relapse probability in unrelated transplants compared to approximately 50% in HLA matched siblings treated with the same T cell depletion regimen (35). There is also a further reduction in the probability of relapse after transplantation by using an HLA incompatible as opposed to a HLA compatible unrelated donor (34).

Survival

The probability of survival after transplantation depends predominantly on the diagnosis and stage of the disease at the time of transplant. Chronic myelogenous leukemia is the most common indication for unrelated donor transplantation and therefore provides the largest homogeneous group of patients for analysis. In a study of CML patients transplanted in Seattle from 1985 through 1994, the probability of disease-free survival at 5 years was 56% for 196 patients transplanted in first chronic phase, 45% for 17 patients in second chronic phase, 42% for 71 patients in accelerated phase and 6% for 35 patients in blast phase (8). Survival was 74% at 5 years in patients transplanted in chronic phase within the first year of diagnosis (31). These data, therefore demonstrate better survival for patients transplanted early in the course of the disease. Over the last 10 years, survival has improved thanks to the use of antiviral and antifungal prophylaxis, and the selection of better matched donors (14,31,32,36). Multiple Class I mismatching (hazard ratio [HR] 3.5, 95% CI: 2.1 to 5.9; p<0.001) or Class I combined with Class II mismatching between donor and recipient (HR 3.3, 95% CI: 2.0 to 5.5; p<0.001) correlated with increased patient mortality. Thus, molecular-based methods for pretransplant assessment of Class I and Class II compatibility should be utilized for the selection of unrelated marrow donors.
Results for patients with acute leukemia are less favorable, because until now most patients treated with unrelated transplantation have had advanced disease. Survival has exceeded 50% in patients with high risk AML or ALL transplanted in the first remission (34,37). Risk factors for an increased risk of transplant-associated mortality are older patient age, positive patient CMV serology in absence of ganciclovir prophylaxis, low marrow cell dose, and lack of compliance with post-transplant immunosuppression (7, 8, 34). HLA mismatch has been associated with worse survival in one study (7) but not in another (34). This apparent discrepancy likely reflects different levels of precision in HLA typing and different criteria for matching among transplant centers (7, 34).

CONCLUSION

Use of an HLA-compatible unrelated donor has become standard practice for patients who need an allogeneic marrow transplant and lack a HLA-compatible family member. Current efforts are directed towards improving the probability of finding a donor by expanding the size and the genetic heterogeneity of donor registries, decreasing the search time by implementing more efficient strategies for donor typing, and improving outcome by selecting better matches. Clinical studies are investigating new approaches for GVHD prevention and treatment. Improved treatment protocols could make unrelated marrow transplantation safer and simpler, thereby allowing this therapeutic approach to be exported from few selected highly specialized centers to a larger number of marrow transplant units worldwide.

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Literature Cited


