Transplantation of pluripotential hematopoietic stem cells can cure some patients with otherwise deadly diseases, including leukemias, lymphomas, and other malignancies and genetic defects. The traditional source of stem cells for transplantation has been bone marrow. Increasingly, however, peripheral blood stem cells obtained byapheresis are being used, as is cord blood, albeit on an experimental basis.

Peripheral blood stem cells can be obtained without the hospitalization and anesthesia required for marrow aspiration, but the donor must receive multiple injections of a colony-stimulating factor for which the long-term effects are unknown. The immature cells in cord blood may permit greater degrees of incompatibility among human leukocyte antigens (HLA), but it may be difficult to find cells sufficient to transplant adult cells. Most stem cell transplants are still done with marrow.

**Allogenic vs Autologous Transplants**

Transplants from allogeneic donors often result in serious immunologic problems related primarily to differences in the HLA histocompatibility antigens of the donor and recipient. HLA antigens in the donor that are foreign to the recipient may result in failure of engraftment or late rejection of the graft. HLA antigens in the recipient that are foreign to the infused donor cells often stimulate graft-vs-host disease (GVHD). This condition varies from a mild skin rash to a lethal syndrome of exfoliative dermatitis, severe diarrhea, and massive liver failure.

Autologous transplants for malignant diseases, in which stem cells are withdrawn from the patient and stored frozen while intensive chemotherapy, irradiation, or both, is administered, avoid those immunologic problems, but result in greater disease recurrence. Malignant cells may be reinfused with the autologous stem cells, and the stem cell preparation lacks the graft-vs-tumor effect of an allogeneic transplant.

Allogeneic donors must be matched to the patient closely for HLA antigens. The ideal donor is a sibling who is genotypically identical to the recipient; that is, he or she has inherited the same two HLA haplotypes from his or her parents. Unfortunately, only 30% of patients find a sibling...
The human major histocompatibility complex (MHC) includes the genes for the HLA antigens. Multiple loci on the short arm of chromosome 6 produce HLA antigens of clinical importance; the most important are HLA-A, -B and -DR. The two classes of HLA antigens (Class I and Class II) differ structurally and in immunologic function. Class II antigens are the product of three genes (A, B, and C). The DR antigens of primary concern result from polymorphisms in the DRB1 gene.

Courtesy Caroline Hurley, PhD, National Marrow Donor Program, Minneapolis. Used by permission.

HUMAN MHC

HLA

CLASS II REGION

CLASS I REGION

with identical HLA genotypes. While an additional 5% may find an acceptable donor in a close relative who shares one haplotype and differs by only one HLA antigen at the other haplotype, 65% to 70% of patients who require an allogeneic stem cell transplant must rely on unrelated donors.

The cells of an unrelated donor should match those of the recipient for at least the products of the HLA-A locus, HLA-B locus, and HLA-DR locus (Fig). Because genes come in pairs (one from the mother and one from the father), finding this donor requires identity for six HLA antigens. Some transplant centers will accept a mismatch at a single HLA locus, especially if the mismatch is for cross-reacting or closely related antigens. The US National Marrow Donor Program (NMDP), Minneapolis, will release donors who match for at least five antigens.

Marrow transplants from unrelated donors have more severe immunologic problems than transplants from HLA-identical siblings. GVHD is more common and more severe, and more rejections or late failures occur (especially if the graft is depleted of T cells to reduce GVHD).

Glossary

Allele—The DNA base sequence that results in the production of a specific series of amino acids; in HLA typing, indicates that the antigen structure has been determined at the amino acid level

Allogeneic—From the same species but not of the same genotype

Bases—Purines or pyrimidines in DNA that provide the genetic code that determines the amino acid structure of proteins

Cord blood—Blood from umbilical veins and placenta after delivery of a baby; source of stem cells for transplantation

Cross-reacting antigen—An antigen that is closely related, so that two cross-reacting antigens may react with a single antibody

Genotype—The complex of genes inherited from both parents (two paired chromosomes)

Graft vs host disease (GVHD)—Immunologic attack on the recipient by immunologically competent donor cells that recognize incompatible recipient antigens

Graft vs leukemia (GVL)—Immunologic attack by donor cells on recipient malignant cells

Histocompatibility—The sharing of antigens that determine whether a graft will be rejected

HLA—Human leukocyte antigens; human antigens, usually identified on peripheral blood mononuclear cells, that play a major role in histocompatibility. Broad or public specificities are common to multiple cross-reacting alleles. Splits are specificities into which broad specificities can be divided.

Locus—The area on the chromosome occupied by a specific gene

Lymphocytes—Small, white blood cells with a single nucleus that play a major role in immune responses, with 85% as T lymphocytes, which provide cellular immunity and help B cells, and 15% as B lymphocytes, which produce antibodies

Oligonucleotide—A short section of a DNA strand used as a probe or primer in the polymerase chain reaction

Peripheral blood mononuclear cells—White blood cells, primarily lymphocytes and monocytes, excluding granulocytes

Pluripotential hematopoietic stem cells—Primitive stem cells capable of self-renewal or differentiation into stem cells that are committed to specific blood cell lines

Polymerase chain reaction—A method that results in logarithmic increase (amplification) of a segment of DNA

Polymorphism—Variation in structure

Primer—An oligonucleotide that determines the section of a DNA strand that will be amplified by the polymerase chain reaction

Probe—An oligonucleotide that binds to and identifies a specific section of DNA

Stem cells—Primitive cells that have the capability of differentiating into functional cells

T-cell depletion—Removal of T lymphocytes from a source of stem cells with the goal of limiting graft-vs-host disease
These increased problems are related to the fact that HLA antigens that appear identical between two unrelated individuals may, in fact, have some differences. Moreover, while identity for HLA-A, -B, and -DR between siblings almost always ensures that other genes within the same general region are also identical, this is less likely to be true with unrelated donors.

Although transplants from an unrelated donor have more immunologic problems than those from HLA-identical siblings, malignant diseases are less likely to recur with the former. The histocompatibility differences presumably contribute to a graft-vs-leukemia effect. The cumulative effect of these opposing influences is that disease-free survival increasingly is approaching that achieved with HLA-identical sibling donors, provided that the donor and recipient are matched accurately.²

HLA Typing for Unrelated Stem Cell Transplants
HLA genes are extremely polymorphic,³ and a large file of typed volunteers is required to give most patients a chance to find an HLA-compatible donor. The NMDP lists more than 2 million donors by identification number and HLA type. Volunteer donors primarily are white (Table 1), although efforts to correct the imbalance are intense. The likelihood of finding an HLA-compatible donor in the NMDP registry varies with the ethnic origin of the patient (Table 2), but the chances are much better for all groups if a five-out-of-six antigen match is accepted.⁴

The initial typing of unrelated volunteer donors for stem cell registries commonly is restricted to HLA-A and HLA-B. The first donors listed with the NMDP were donors of platelets at blood banks who needed to be typed for only HLA-A and -B. Typing for HLA-DR is more complex and expensive. Patients who find an HLA-A,B match in the file are expected to pay for the DR typing of that donor. Funds from the US Naval Medical Research and Development Command in Bethesda, Md, have made it possible to type other donors prospectively for HLA-DR, with emphasis on DR typing of minority donors. More than 35% of donors in the NMDP registry have been DR typed.

HLA Typing Methods
Since the 1960s, the standard method for HLA typing has used lymphocytes as a target, with rabbit complement to lyse the lymphocytes to which antibody attached. Although typing for HLA-A and -B uses all peripheral blood mononuclear cells, the usual approaches to HLA-DR typing require isolation of B lymphocytes, because the majority of lymphocytes (T cells) do not manifest DR in a resting state. The antisera used are human sera containing antibodies stimulated by pregnancy, blood transfusion, or organ transplant. Most sera containing lymphocytotoxic antibodies react with broad or multiple specificities, do not define the narrower specificities, and are not useful for typing.

The definition of antigens required the discovery of appropriate human alloantisera. As the years went by, many antigens previously accepted were split by newly discovered sera. Accepted HLA specificities have been defined by a nomenclature committee under the auspices of the World Health Organization.⁴ Sera to define more newly recognized specificities often are in short

### Table 1. National Marrow Donor Program Volunteer Donors by Race*

<table>
<thead>
<tr>
<th>Race</th>
<th>NMDP Donors</th>
</tr>
</thead>
<tbody>
<tr>
<td>White</td>
<td>1,279,027</td>
</tr>
<tr>
<td>Black</td>
<td>153,740</td>
</tr>
<tr>
<td>Asian/Pacific Islander</td>
<td>101,084</td>
</tr>
<tr>
<td>Hispanic</td>
<td>140,554</td>
</tr>
<tr>
<td>Native American</td>
<td>26,436</td>
</tr>
<tr>
<td>Other minorities</td>
<td>10,788</td>
</tr>
<tr>
<td>Multiple race</td>
<td>929</td>
</tr>
<tr>
<td>Unknown</td>
<td>445,916</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>2,158,474</td>
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</tbody>
</table>

* As of May 1, 1996.

### Table 2. Patients Registered in the US National Marrow Donor Program for Whom an A, B, DR, or Identical Match Was Found*

<table>
<thead>
<tr>
<th></th>
<th>11/1/93–10/31/94 (12 mo)</th>
<th>11/1/94–10/31/95 (12 mo)</th>
<th>11/1/95–4/30/96 (6 mo)</th>
</tr>
</thead>
<tbody>
<tr>
<td>White</td>
<td>67% (2,040)</td>
<td>72% (2,762)</td>
<td>76% (1,523)</td>
</tr>
<tr>
<td>Black</td>
<td>30% (76)</td>
<td>31% (124)</td>
<td>48% (107)</td>
</tr>
<tr>
<td>Asian/Pacific Islander</td>
<td>50% (72)</td>
<td>51% (79)</td>
<td>60% (47)</td>
</tr>
<tr>
<td>Hispanic</td>
<td>49% (140)</td>
<td>64% (213)</td>
<td>68% (127)</td>
</tr>
<tr>
<td>Native American</td>
<td>90% (9)</td>
<td>77% (20)</td>
<td>33% (4)</td>
</tr>
<tr>
<td>All patients</td>
<td>60% (2,495)</td>
<td>67% (3,531)</td>
<td>72% (1,994)</td>
</tr>
</tbody>
</table>

*The figures in parentheses are the numbers of patients in each category.
supply, and laboratories may differ in their ability to discriminate newer splits or to detect newly defined antigens.

Requirements of the unrelated stem cell donor registry dictated that large numbers of samples be typed at the lowest possible prices. This resulted in a high proportion of initial typings (48.3%) on which laboratories disagreed in regard to at least one antigen. Discrepant typing results were more common among cells from minority donors, because most available typing sera have resulted from pregnancies in white women. Although the disagreements commonly related to the distinction between two closely related HLA antigens (splits), and NMDP’s search strategy takes the possible inaccuracies of initial typings into account so that compatible donors are less likely to be missed, initial misidentification of an HLA antigen may result in rejection of a donor previously believed to be acceptable or in failure to recognize a compatible donor. Although true compatibility is ensured by complete and accurate typing of the recipient and the selected donor at the transplant center, incorrect preliminary assignment of HLA types results in delays that may prove fatal to the recipient.

Newly developed technology in which DNA techniques define HLA specificities may solve the problem of discrepancies among laboratories. These new techniques identify specific base sequences within genes. These base sequences determine the critical amino acid sequences of the HLA antigens. The techniques use the polymerase chain reaction to amplify HLA gene sequences. Specificity is ensured by using either oligonucleotide probes that bind only to the base sequences of specific alleles or specific primers that amplify only certain alleles. These approaches can distinguish HLA antigens that differ by only a single amino acid.

Unlike serology, DNA typing does not require living cells. DNA can be extracted from whole blood frozen without a cryoprotectant. Reagents for HLA DNA typing should not be in short supply, because both primers and probes are synthesized. The remarkable accuracy of the approach is shown by NMDP studies in which more than 99% of the types were assigned correctly. The technique was developed first for Class II HLA antigens (HLA-DR, -DQ, and -DP), because these are less polymorphic than Class I genes (HLA-A, -B, and -C). Results were so successful that all Class II typing for NMDP now must be done by DNA techniques. Initial typings can use primers and probes that distinguish HLA antigens at a level comparable with serology or somewhat better. Further tests using appropriately selected primers or probes then can identify specific alleles.

DNA Class I typing is not yet routine. NMDP trials in progress are so successful, however, that DNA Class I typing should begin within 6 months and be routine for all within 2 years.

Stem cell donors and recipients must be matched at the allelic level. Evidence exists that a single amino acid difference can be important, and the team at the Fred Hutchinson Cancer Research Center in Seattle has shown that matching at the DR allelic level provides better results. A requirement to match at the allelic level will make identification of a compatible donor more difficult for most patients, however. The question remains regarding how much of a mismatch can be tolerated and whether certain mismatches can be ignored. The NMDP is attempting to answer these questions through ongoing studies in which cells of transplant donors and recipients were saved and are being retyped at the allelic level. Also, researchers are trying to determine whether matching for HLA-C and HLA-DP, routinely ignored, can improve graft results.

Conclusion
Compared to those who receive stem cells from siblings with identical HLA, patients who receive stem cells from unrelated donors experience greater immunologic problems, including graft rejection and GVHD, but are less likely to see the recurrence of malignant disease.

HLA matching by traditional serologic techniques is inexact and subject to a high degree of error. Newer DNA typing techniques have a high degree of accuracy and can distinguish among HLA alleles that differ by a single amino acid. Matching for stem cell transplants at the allelic level improves results.

References

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