1. Introduction

Histocompatibility in transplantation is governed by the products of the human leucocyte antigen (HLA) genes encoded within the major histocompatibility complex (MHC) on chromosome 6p21. HLA antigens define tissue type and are inherited en bloc in classical Mendelian fashion. Genotypically identical siblings inherit the same paternal and maternal chromosome 6. Haploidentical relatives share one complete chromosome 6 and the degree of HLA compatibility for the non-shared chromosome 6 is variable. Selection of unrelated donors for hematopoietic cell transplantation (HCT) is based on identity for the classical HLA genes. The precision with which an unrelated donor is “matched” with the recipient is only as rigorous as the typing technology that is used to define the alleles of class I and class II genes. New concepts in immunogenetics in transplantation include the delineation of locus-specific and allele-specific risks associated with donor-recipient disparity, the role of the chromosome 19-encoded natural killer (NK) receptor complex in modulating graft-versus-tumor effects, and the importance of the HLA haplotype as a marker of new transplantation determinants.

2. HLA Genetics

The MHC encodes over 100 loci involved in immune function including the classical and nonclassical HLA genes HLA-A, B, C, DR, DQ and DP [1]. A key feature of HLA genes is their extensive sequence diversity [2] and strong positive linkage disequilibrium (LD) across the HLA-A to HLA-DQ segment [3].

2.1. Polymorphism of HLA Genes

The extensive sequence polymorphism of HLA genes reflects their primary role in the presentation of peptides to antigen-specific T cell receptors [4]. Each HLA allele is unique and given a name by the World Health Organization Nomenclature Committee for Factors of the HLA System [2]. As of April 2006, 469 HLA-A, 794 HLA-B, 244 HLA-C, 525 HLA-DRB, 34 HLA-DQA1, 71 HLA-DQB1, 23 HLA-DPA1 and 124 HLA-DPB1 alleles are currently
recognized [Steven Marsh, personal communication]. Recently, novel HLA alleles have been discovered through DNA genotyping efforts of ethnic minority populations in volunteer bone marrow registry donors. The frequencies of HLA allele and antigen frequencies vary widely between ethnically diverse populations (www.allelefrequencies.net) [5–7]. For this reason, the likelihood of identifying suitable unrelated donors for HCT is highest when the patient and donor are of the same ethnic or racial background [8].

2.2. LD and HLA Haplotypes

LD is defined as the nonrandom association of alleles more often than would be predicted by chance alone. Within the HLA complex there is strong positive LD between the HLA-B and C loci, and between the HLA-DR and DQ loci. There is weak LD between HLA-DP and HLA-A, C, B, DR, DQ. From a clinical perspective, positive LD between HLA alleles and antigens may aid in the identification of well matched unrelated donors. For example, an HLA-A1,B8,DR3-positive recipient will have a good chance of identifying a donor with the same phenotype, because matching for HLA-A1 and B8 will often determine matching for DR3. In turn, HLA-A1, B8, DR3-positive donors and recipients have a high likelihood of being matched for HLA-DRB1’0301 and DQB1’0201 alleles. LD can exist between commonly observed alleles and antigens, as well as between less common ones [9]. When a recipient has inherited a rare combination of alleles and antigens, the probability of identifying a suitable unrelated donor is related to the size and composition of the donor registry.

The physical linkage of HLA genes on the same chromosome is called a haplotype. Haplotypes that encode highly conserved sequences thought to be derived from a common ancestor are known as “ancestral haplotypes” [The Sanger Institute; Human Chromosome 6 Project database http://www.sanger.ac.uk/HGP/Chr6/; International HapMap Project database http://www.hapmap.org/]. One of the most well-known examples of an ancestral haplotype is HLA-A1,B8,DR3 [10]. Classically, haplotypes are defined by a complete family study of the parents and children. Haploidentical related family members share one chromosome 6 and are variably matched for the non-shared HLA haplotype. A child is haploidentical to each of his/her parents. The genetic relationship between the mother and child can be described as involving “inherited maternal HLA antigens” (IMA), and that between the father and child as involving “inherited paternal HLA antigens” (IPA). HLA antigen and alleles of the non-shared haplotypes are likewise termed “non-inherited maternal antigens” (NIMA) and “non-inherited paternal antigens” (NIPA).

When a family study is not available, haplotype frequencies can be estimated (www.allelefrequencies.net) [11]. For example, using a large unrelated donor pool from the NMDP (www.nmdpresearch.org), the estimated haplotype frequency of HLA-A1, B8, DR3 in Caucasian, Asian, African and Hispanic Americans is 0.062, 0.003, 0.012 and 0.017, respectively. Estimated haplotype frequencies have been used to determine the ideal size of unrelated donor registries for HCT [12].

2.3. Alleles and Antigens

Alleles define the genotype and antigens define the phenotype [13]. The level of resolution of HLA alleles and antigens is dictated by the specific technique or method used in the laboratory. Serologic methods were historically used to type
HLA antigens; DNA-based methods can also define the equivalent of an antigen (low resolution methods). DNA methods can further define the nucleotide sequence variation that permits the identification of a unique sequence as an allele (high resolution). For this reason, DNA-based techniques have become the gold standard in tissue typing in support of hematopoietic cell transplantation programs for the purposes of donor identification. One serologically defined antigen or phenotype (DR4) may be the product of one or more allele sequences (HLA-DRB1*0401, 0402, etc.) (Table 2-1). In this way, two individuals who share the same HLA

Table 2-1. Concepts in HLA and NK Genetics.

<table>
<thead>
<tr>
<th>Concept</th>
<th>Definition</th>
<th>Example(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HLA Mismatching</td>
<td>Antigen Donor-recipient difference between two serologically distinct proteins</td>
<td>HLA-A1 versus HLA-A2</td>
</tr>
<tr>
<td></td>
<td>Allele Donor-recipient difference between two unique HLA sequences within a serological specificity</td>
<td>HLA-A<em>0201 versus HLA-A</em>0205</td>
</tr>
<tr>
<td>HLA Vector of Incompatibility</td>
<td>HVG Donor alleles not present in the recipient</td>
<td>Donor HLA-A1,2 versus Recipient HLA-A1,24: recognition of donor A2 by recipient</td>
</tr>
<tr>
<td></td>
<td>GVH Recipient alleles not present in the donor</td>
<td>Donor HLA-A1,2 versus Recipient HLA-A1,24: recognition of recipient A24 by donor</td>
</tr>
<tr>
<td></td>
<td>Unidirectional Only one vector of incompatibility is present, either HVG or GVH (homozygous recipient with heterozygous donor, or heterozygous recipient with homozygous donor)</td>
<td>HVG: Recipient HLA-A<em>0201,0201 versus Donor HLA-A</em>0201,0205</td>
</tr>
<tr>
<td></td>
<td></td>
<td>GVH: Recipient HLA-A<em>0201,0205 versus Donor HLA-A</em>0201,0201</td>
</tr>
<tr>
<td></td>
<td>Bidirectional Both HVG and GVH vectors of incompatibility are present at the same locus (recipient and donor are both heterozygous)</td>
<td>Recipient HLA-A1,2 versus Donor HLA-A1,11; recipient HLA-A<em>0201,1101 versus Donor HLA-A</em>0205,1101</td>
</tr>
<tr>
<td>HLA Ligand</td>
<td>One or more amino acid residues of an HLA protein that binds to NK KIR receptor; known ligands include the HLA-Bw4 epitope of certain HLA-B and HLA-A antigens, and residues 77 and 80 of the HLA-C antigen</td>
<td>Bw4 ligand for 3DL1 receptor; C1 ligand for 2DL2, DL3, 2DS2 receptors; C2 ligand for 2DL1 and 2DS1 receptors</td>
</tr>
<tr>
<td>KIR Ligand Mismatch</td>
<td>Recipient lacks the HLA epitope present in the donor</td>
<td>Recipient C1, C1 homozygous (eg, HLA-Cw<em>0701, 0701) versus Donor C1, C2 heterozygous (eg, HLA-Cw</em>0701, 1602)</td>
</tr>
<tr>
<td>Missing KIR Ligand</td>
<td>Recipient is missing the HLA epitope for donor inhibitory KIR</td>
<td>Recipient C1, C1 (eg, HLA-Cw*0701, 0701); KIR2DL1 receptor for C2 ligand is not engaged, leading to release of NK inhibition</td>
</tr>
</tbody>
</table>
antigen may be mismatched for two different alleles of that antigen, and explains why unrelated donors who were historically selected based on serological identity for HLA-A, B, DR were later found to encode undetected allele mismatches when modern DNA techniques became available [14, 15]. The immunogenecity of allele mismatches and antigen mismatches is the subject of investigation in unrelated donor HCT [16–21].

2.4. Vector of Incompatibility

The vector of incompatibility refers to the direction of allorecognition between the donor and recipient (Table 2-1). When a donor and recipient are incompatible in the host-versus-graft (HVG) vector, there are donor antigens or alleles not shared by the recipient. HVG vector mismatches correlate closely with graft failure [22]. When a donor and recipient are incompatible in the graft-versus-host (GVH) vector there are recipient antigens or alleles not shared by the donor; GVH vector disparity closely correlates with the risk of acute and chronic GVHD [22]. Unidirectional mismatches refer to disparity in only the HVG or the GVH vector at a given locus. Unidirectional mismatching occurs when the donor is homozygous (donor HLA-A1,1 versus recipient HLA-A1,2 produces a GVH vector mismatch only) or when the recipient is homozygous (donor HLA-A1,2 versus recipient HLA-A2,2 produces an HVG vector mismatch only). Bidirectional mismatching refers to the presence of both HVG and GVH mismatching at the same locus, and occurs when the donor and recipient are each heterozygous for two different alleles or antigens. For example, when a donor is HLA-A1,2 and the recipient is HLA-A1,11, the mismatch between the A2 and the A11 is bidirectional.

3. NK Genetics

The NK family of killer immunoglobulin-like receptor (KIR) genes is encoded within a 150 kb region of chromosome 19q13.4 and segregates independently of HLA. The concept that certain HLA class I gene products may interact with NK cells to distinguish self from non-self has been one of the most important advances in human immunogenetics [23]. The role of NK-mediated alloreactivity provides a unique therapeutic avenue for graft-versus-tumor effects, and the selection of potential donors has recently included consideration of the HLA ligands of NK receptors.

3.1. Ligand-Receptor Diversity

HLA-B, HLA-C and some HLA-A antigens serve as the ligands for inhibitory KIRs [24]. Although the polymorphisms that distinguish HLA-B and C alleles are distributed across three exons, the epitopes that define specificity of KIR receptor binding are governed by residues 77 and 80 of HLA-C and by HLA-Bw4 present on some HLA-B and HLA-A molecules. HLA-C molecules that encode Asn at position 77 and Lys at position 80 define the group 2 (C2) ligands; this polymorphism is present in HLA-Cw2, Cw’0307, Cw’0315, Cw4, Cw5, Cw6, Cw’0707, Cw’0709, Cw’1205, Cw’12041/2, Cw15 (except Cw’1507), Cw’1602, Cw17 and Cw18. Group C2 HLA ligands are recognized by the KIR2DL1 and 2DS1 receptors. HLA-C molecules that encode Ser77 and Asn80 (HLA-Cw1, Cw3 [except Cw’0307, 0310, 0315], Cw7 [except
Cw’0707, 0709], Cw8, Cw12 [except Cw’1205,1204/2], Cw13, Cw14
[except Cw’1404], Cw’1507 and Cw16 [except Cw’1602]) are collectively
referred to as the group 1 or C1 ligands; they are recognized by the 2DL2,
2DL3 and 2DS2 KIR receptors. The HLA-Bw4 epitope present in B5, B13,
B17, B27, B37, B38, B44, B47, B49, B51, B52,B 53, B58, B59, B63, B77,
B’1513, B’1516, B’1517, B’1523 and B’1524 serves as a ligand for the inhibi-
tory KIR3DL1 receptor. The KIR receptor genes display allelic and haplo-
typic polymorphism [24–31]. The clinical importance of KIR receptor diversity in
transplantation is unknown.

3.2. Mechanisms of HLA Ligand- KIR Receptor Recognition in
Allogeneic HCT

When HLA-B and/or C ligands are engaged with their inhibitory KIR receptor,
the NK cell is inhibited from killing the target cell [32]. Killing of host cells,
including residual host leukemia or tumor cells, occurs when the inhibitory
KIR of the donor-derived NK cell does not recognize the patient’s HLA class
I allele, either because the allele is different or because it is not present (Table
2-1). In this situation, eliminating the host leukemic cells leads to lowered
post-transplant relapse; the elimination of host antigen-presenting cells leads
to lowered GVHD [32, 33]. Among HLA-B and/or C mismatched haploi-
dentical siblings, if the recipient is homozygous for the HLA ligand, or if the
recipient is missing an HLA ligand for which the donor NK cells express the
inhibitory KIR receptor, then this may lead to release of inhibition of the donor
NK cells and result in killing of the target (host) cell. Note that C1, C2 and/or
Bw4-homozygous recipient cells may also be the target of donor NK-medi-
ated killing, even when the recipient is HLA matched with his or her donor
(Table 2-1).

4. Donor Selection

Donor selection begins with a full evaluation of available family members of
the transplant recipient (Fig. 2-1). A family study includes tissue typing of the
father, mother, full siblings and additional relatives, where indicated. The fam-
ily study confirms the recipient’s genotype and haplotypes, and provides infor-
mation on the immediate availability of genotypically matched siblings and
haploidentical family members. Further consideration of related donors may
include the number of HLA disparities on the mismatched maternal or paternal
haplotype, and the presence or absence of disparity and of homozygosity for
HLA KIR ligands. For unrelated donors, the number of HLA mismatches and
the specific loci mismatched are important elements in the selection of donors
(Table 2-2).

4.1. Related Donors

Segregation of chromosome 6 in Mendelian fashion gives rise to a 25 percent
probability that two siblings will be genotypically identical, a 50 percent
probability that two siblings will be haploidentical (share one paternal chro-
mosome 6 or one maternal chromosome 6) and a 25 percent chance that they
inherited different parental chromosome 6s. Clinical outcome after haploiden-
tical transplantation differs depending on whether NIMA or NIPA is involved
Fig. 2-1. Algorithm for Donor Identification

**Patient is a Candidate for Allogeneic HCT**
Perform Family HLA Study
1. Define recipient HLA genotype
2. Confirm recipient HLA haplotypes
3. Identify suitable related donors

**Related Donor - Available**

- HLA Genotypically – Identical Sibling

Assess recipient class I genotype for missing KIR ligand for risk-assessment purposes

- Haploidentical Mismatched – Related Donor

- Define
  1. Number of HLA mismatches on non-shared haplotype
  2. NIMA/MPA relationship
  3. Mismatched KIR ligands
  4. Missing recipient KIR ligands

Attempt 5 – locus allele matching if possible; weigh risks of 9/10 donor

Unrelated Cord Blood
Consider single versus double unit (refer to Chapter 14)

Consider CMV, gender, ABO when multiple equally matched donors are available

Ideal time frame for HCT: within 1 month. Identify and prioritize first available donors (availability of high-resolution typing will accelerate selection); consider CB haploidentical related donor if available and search does not yield URDs

Unrelated Cord Blood
Consider single versus double unit (refer to Chapter 14)

Ideal time frame for HCT: within 3 months. Consider 10/10, 9/10, 8/10. Weigh risks and benefits of prolonged search if suitable donors are not available, and disease progression; consider CB haploidentical related donor if available and search does not yield URDs

Unrelated Cord Blood

Consider single versus double unit (refer to Chapter 14)

Assess Disease Risk
1. Low (CML-CP1; Acute leukemia in remission; early MDS)
2. Intermediate
3. High (refractory or active disease; short remission duration)

**Related Donor – Not available**

Ideal time frame for HCT: within 1 month. Identify and prioritize first available donors (availability of high-resolution typing will accelerate selection); consider CB haploidentical related donor if available and search does not yield URDs

Unrelated Cord Blood

Consider single versus double unit (refer to Chapter 14)
(described below) and, hence, NIMA and NIPA are used to further select among potential family donors (Table 2-2). Haploidentical siblings may be variably mismatched for the non-shared haplotype if the maternal and paternal HLA haplotypes fortuitously encode the same HLA antigen(s) or allele(s).

In addition to maternal and paternal sharing of HLA antigens, selecting haploidentical family members can also include considering the presence of mismatched HLA KIR ligands encoded by HLA-B, HLA-C and certain HLA-A alleles. For both related and unrelated donors, the availability of high resolution typing of HLA class I alleles of the recipient provides the necessary information to determine whether a patient is Bw4-positive or negative, and whether the recipient is C1/C2 heterozygous, C1/C1 homozygous or C2/C2 homozygous.

### 4.2. Unrelated Donors

Early in the unrelated HCT clinical experience, matching volunteer donors included consideration of HLA-A, B and DR antigens. As laboratory technology for discriminating HLA alleles became available, a transition from serologically based phenotyping to DNA-based genotyping for DRB1 and DQB1 was made in the early 1990s; application of DNA-based genotyping to class I HLA-A, B and C genes followed soon thereafter.

As robust DNA methods were applied to address research questions on the importance of genetic variation in transplant outcome, new information became available demonstrating the importance of HLA-C [16–18] and HLA-DQB1
and criteria for donor evaluation and selection of volunteer donors became more complex. Today, consideration of 5 loci HLA-A, B, C, DRB1 and DQB1 is considered standard, and gives rise to the 10 possible alleles (two at each loci) for matching (“10/10” allele-matched donor). Although outcome after transplantation from a 10/10 allele-matched donor is outstanding and, in some diseases, approaches the results for HLA identical sibling transplantation, 20 to 80 percent of patients who initiate a donor search are able to find a suitable donor. As described below, a major research impetus has been underway to define specific allele and antigen mismatch combinations that do not increase post-transplant risks. In this way, the use of mismatched donors with “permissible” mismatches may open the possibility that all patients in need of a transplant will have a suitable donor. Overall outcome after unrelated HCT is influenced by the stage of disease at the time of transplantation, and disease activity remains one of the most challenging aspects of searching volunteer donors in a timely manner, particularly for patients with less common genotypes and haplotypes [36]. When more than one equally matched unrelated donor is available, additional criteria include CMV serostatus, donor age, donor gender or ABO [37].

5. Importance of Donor Matching in Allogeneic HCT

HLA genotypically identical siblings are the preferred donor for allogeneic transplantation because they are genetically identical for the entire chromosome 6, including all non-HLA genetic variation encoded on the MHC haplotype. When a matched sibling is not available, a haploidentical relative, cord blood (CB) unit(s) or a volunteer donor are considered (Fig. 2-1). Choice of these alternative donors is shaped by the urgency of the transplant as well as outcome using specific conditioning regimens and GVHD prophylaxis. When the timing of a transplant is urgent, as in the case of high risk patients whose disease tempo precludes time for a donor search, an available haploidentical donor or CB unit provides a highly attractive strategy. The recent elucidation of the NK KIR receptor complex and its role in donor-antihost alloreactivity after allogeneic transplantation provides a novel approach for optimizing overall results of HCT, especially for patients at high risk for disease recurrence.

5.1. HLA Effects on Transplant Outcome

5.1.1. Haploidentical Mismatched Related Donor Transplantation

A decisive summary of the haploidentical transplant experience is provided elsewhere in this textbook (Chapter 15). The specific risks associated with HLA disparity in this patient population include increased risk of graft failure, GVHD and delayed immune reconstitution [22, 38–43]. The risks of GVHD and graft failure increase as the number of HLA mismatches of the non-shared HLA haplotype [44–47] increase, and are especially high in the presence of mismatching for two or more antigens from a T replete grafting source [44, 46]. Use of alternative donors with limited numbers of HLA mismatches may approach the favorable results observed after HLA-identical related HCT [48–51]. Higher risks of acute GVHD are observed with donors mismatched for one or two antigens, compared to phenotypically identical donors [48]. Additive effects of HLA mismatches are measurable in patients transplanted
from unrelated donors with two or more class I mismatches, or from 6/10 (or lower) haploidentical related donors, compared to matched or single antigen mismatched unrelated donors or 7/10, 8/10 or 9/10 HLA-A, B, C, DR, DQ haploidentical related donors [50]. Compared to matched unrelated donors, use of mismatched unrelated or related donors is associated with higher TRM after transplantation [49], and in patients transplanted from partially matched related donors, increased non-relapse mortality has been a limitation [51].

When multiple haploidentical family members are available to serve as donors, additional HLA criteria may be helpful for prioritizing the optimal donor. Donor-specific suppression of T cell responses against the non-inherited maternal HLA antigens provides a basis for the use of NIMA-mismatched haploidentical donors and may be an effective strategy for patients whose primary risk is TRM [52–54]. Lower risks of acute and chronic GVHD and TRM are observed after T-replete bone marrow transplantation between mother and offspring, compared to father and offspring [52]. These data support an immunological basis for the NIMA effect in which in utero exposure to NIMA is tolerizing, whereas exposure to IPA is immunizing. The NIMA effects have been observed after conditioning with myeloablative [53, 54] and reduced intensity [55, 56] regimens, in which overall survival at five years post-transplant is significantly higher and TRM lower among recipients of maternal grafts compared to paternal grafts.

5.1.2. Unrelated Donor Transplantation

There are currently over 10.3 million volunteer donors worldwide (National Marrow Donor Program [NMDP], www.marrow.org; Bone Marrow Donors worldwide [BMDW], www.bmdw.leidenuniv.nl; World Marrow Donor Association [WMDA], www.worldmarrow.org). Good-risk patients who do not have an HLA matched sibling to serve as donor may have superior disease-free survival (DFS) with transplantation from well matched unrelated donors [49, 50, 57–62], and the safety of reduced intensity and non-myeloablative regimens has further broadened the application of unrelated HCT to patients who, due to advanced age or medical infirmities, would not otherwise be considered ideal candidates for traditional myeloablative transplantation procedures [63, 64].

The introduction of polymerase chain reaction (PCR) technology for tissue typing has dramatically changed how unrelated donors are selected. Methods define low (equivalent to serologically defined antigen), intermediate and high (unique allele sequence) resolution of the polymorphic exons that encode HLA molecules. A preliminary search of unrelated donors begins with screening of registry donors typed either at low or high resolution for at least HLA-A, B and DR, and a listing of the HLA types of potentially matched donors. From these potential donors, individual donors who most closely meet the criteria for the planned transplantation procedure are selected for the next phase of “confirmatory typing” (CT) of HLA-A, B, C, DRB1 and DQB1 alleles.

Currently, 20 percent to 80 percent of patients who initiate a search ultimately identify a suitable unrelated donor. The likelihood of identifying a matched unrelated donor depends on the patient’s genotype, his or her racial background, and the composition and size of the donor registries [8]. If the HLA criteria for donor selection is too stringent, then few patients will find suitable donors. The need to broaden availability of unrelated HCT for patients who lack a matched donor has provided the rationale to define permissible HLA mismatches.
The optimal set of HLA criteria that allows patients the opportunity for cure while avoiding risks associated with disparity is the subject of ongoing research efforts worldwide.

Retrospective analysis of clinical outcome related to donor HLA match status has played an important role in refining criteria for prospective donor selection. DNA-based methods are now considered the gold standard, as they are reliable, cost-effective and can be applied to samples with low cell concentration and with archived DNA. DNA methods are the chief means through which novel or undetected sequence variation is detected [14, 15, 65] and, therefore, are instrumental in evaluating genotypes of patients and donors representing diverse ethnic and racial backgrounds.

Donors and recipients who share the same serologically defined antigen may differ based on the allele variant of the antigen. HLA class I and class II allele disparities are functional [16–20, 63, 66], and, furthermore, the risks of graft failure and GVHD increase with the increasing degree of donor-recipient incompatibility [16–20, 66–68]. Therefore, comprehensive evaluation of each of the five classical loci at the allele level (i.e., 10 alleles in total) is performed to ascertain not only the match status at each locus, but also the total number of disparities (a single mismatch is referred to as a “9/10” match; two mismatches constitute a “8/10” match, etc).

Current understanding is that some HLA mismatches may be better tolerated than others. Active research questions include whether all six HLA-A, B, C, DR, DQ and DP loci are important for donor matching, whether class I and class II mismatches each contribute to graft failure or GVHD, whether allele mismatches are less detrimental than antigen mismatches and how HLA effects interact with nongenetic factors that affect clinical outcome [16–19, 21, 34, 58, 66, 68–73]. Allele mismatches might be less immunogenic than antigen mismatches [19–20]; however, the differences may be difficult to measure when disease stage is considered [21].

In a recent analysis by the NMDP and CIBMTR, the risks of HLA disparity and the relative importance of recipient and donor factors in clinical outcome were measured retrospectively in a large study population of patients who received T-replete myeloablative transplantation for leukemia and MDS [74]. The average overall survival rate was 7 to 8 percent lower with each additional HLA mismatch, compared to 10/10 allele matching, and 12 to 15 percent lower if the disease progressed from early to intermediate to advanced stage. Among single mismatches, disparity at the HLA-A locus was associated with a statistically significant risk of mortality and lower DFS. HLA-A, B and C were each associated with increased risk of acute GVHD; there were no HLA associations with chronic GVHD.

Several different strategies have been used to identify permissible HLA mismatches. Analyzing specific class I epitopes involved in T cell recognition has provided one avenue for identifying GVHD risk determinants [75]. Functional assays have been employed to identify tolerable HLA-DP [73] and class I mismatches [76]. Since one underlying mechanism for permissiveness may be related to the specific allele and antigen mismatch combinations, which reflect the ethnic and racial background of the donor and recipient, another approach has been to examine allele mismatches within common antigen groups shared between ethnically diverse transplant populations [77, 78]. These data suggest that HLA and other genetic factors may explain differences in outcomes [79].
5.1.2.1. Non-HLA Factors of Importance in Unrelated HCT
Non-HLA factors that impact transplant outcome include disease stage at the time of transplantation (intermediate versus early; late versus early), CMV-seropositive recipient and recipient age older than 20 years [37, 74]. Younger patients tolerate higher degrees of HLA mismatching [50, 59–61]. Patients transplanted from single allele mismatched donors for low risk CML are at an increased risk of post-transplant complications, compared to patients transplanted from fully matched donors for the same stage of their disease. However, the outcome for patients with higher risk disease following transplantation from mismatched donors is similar to that of matched recipients [21]. These data suggest that the stage of disease is a powerful indicator of transplant outcome and that the detrimental effects of HLA disparity may be obscured by the negative effects of disease stage.

5.1.2.2. HLA Disparity and GVL after Unrelated HCT
Most of the published HLA studies have focused on quantifying risks associated with HLA mismatching and graft failure, GVHD and mortality. One potential benefit of HLA disparity is lower disease recurrence arising from graft-versus-leukemia in patients with clinical GVHD [80]. HLA-DP mismatching has a lower risk of disease recurrence, particularly in patients with lymphoid malignancies [72]. GVL effects may also be contributed by non-HLA loci; lower post-transplant relapse has been observed after non-myeloablative unrelated, compared to related donor HCT, and likely reflect the impact of undetected genetic disparity among unrelated individuals who are HLA matched [64].

5.2. KIR Effects
Demonstrating the powerful effect of HLA KIR ligand on outcome after haploidentical mismatched related HCT is a novel approach for selecting alternative donors and the integrating post-transplant immunotherapy into the treatment plan [32, 33, 81, 82]. Originally described in the setting of megadose \textit{ex vivo} T cell depleted transplantation from NK-alloreactive donors [33], durable engraftment, low risk of GVHD, low post-transplant disease recurrence (particularly in patients with AML) and superior survival have been confirmed in several series [32, 83, 84]. The beneficial effects of donor NK-mediated alloreactivity may not be entirely uniform under different conditioning intensities and GVHD preventive measures, and remains an important research question [85–90].

Since HLA and KIR genes segregate independently, the beneficial effects of donor-mediated GVL may be the result of HLA ligand incompatibility and/or recipient lack of ligand when the donor has a complete repertoire of inhibitory KIR receptors. In the latter scenario, population genetic analysis demonstrates that most individuals encode KIR2DL2, 2DL3, 2DL1 and 3DL1 KIR receptors; however, the distribution of HLA-C and B alleles that define the group C1, group C2 and HLA-Bw4 ligands varies substantially. Even when the donor and recipient are HLA matched, the recipient may lack the ligand for which the donor is the receptor [91].

A test of the potential effect of ligand disparity and missing ligand demonstrates a complex interaction between HLA and KIR-driven alloreactivity [89]. Lowered post-transplant relapse was observed in HLA-mismatched recipients homozygous for HLA-Bw6 and group C1 or C2 (missing KIR ligand) after T-replete marrow, or PBSC after myeloablative conditioning. However, the beneficial effect of lowered relapse was not observed among HLA-matched transplants. These data suggest that KIR ligand absence in the recipient may
be a useful pre-transplant indicator for lowered disease recurrence after T-replete myeloablative conditioning, and could provide clinicians with a strategy to plan specific transplant treatment for patients at highest risk of relapse. Since recipients are now routinely assessed pre-transplant using high resolution HLA typing methods, patients may be readily assessed as to whether they lack C1, C2 or Bw4 ligands to assist in assessing risk.

Research is ongoing to assess the importance of donor KIR receptor polymorphism in donor selection and transplant outcome [90, 92, 93]. Future studies on the clinical impact of ligand/receptor genotype with different conditioning and GVHD preventive regimens are needed to adequately assess the role for pre-transplant receptor genotyping in donor selection.

6. Identifying Novel Transplantation Determinants

6.1. Microsatellites as a Mapping Tool

Although complete and precise donor-recipient HLA matching can optimize the results of unrelated donor HCT, clinical experience demonstrates that GVHD remains a severe, potentially life-threatening complication. Given the extreme genetic diversity of the MHC, new hypotheses about the potential role of undefined non-HLA MHC-resident genetic variation may be developed. Laboratory approaches to characterizing sequence diversity between the classical HLA loci include direct sequencing and other high throughput platforms for the MHC. Microsatellite (Msat) markers have been used for disease mapping. Although indirect, Msat markers provide information over a great genetic distance of the MHC because Msat alleles are in LD with HLA alleles and haplotypes [94–97]. In this way, Msats have been an effective tool for estimating optimal size and composition of donor registries [98, 99], and for donor selection [100].

Mapping novel transplantation determinants may be developed using either donor-recipient Msat disparity or recipient and/or donor Msat genotype as the marker. Two recent studies have explored the MHC region with such an approach [97, 101]. Although no statistically significant associations between donor-recipient Msat disparity and risks of acute or chronic GVHD, graft failure, relapse or survival were observed in one study, Msat identity for the tumor necrosis factor [TNF] locus was associated with lower survival, compared to matching at this locus among patients developing clinical GVHD [97], suggesting the class III region to be of interest. A second analysis of a large international dataset of unrelated donor transplants uncovered an increased risk of death among patients mismatched for class III and class I Msat markers [101]. Taken together, these studies indicate that undetected genetic variation within the MHC may be functional, and in-depth analysis of the class I, II and III region is warranted.

6.2. Single Nucleotide Polymorphisms (SNPs)

Msats provide an indirect measurement of linked variation. Direct examination of sequence diversity can be attained by directly assaying single nucleotide polymorphisms (SNPs) [1, 102–105]. A catalogue of MHC region SNPs is now available from The MHC Haplotype Project (http://sanger.ac.uk/HGP/Chr6?MHC). This work showcases the extreme diversity of the HLA region, and the organization of SNPs into haplotypes. Unique HLA alleles are also defined as part and parcel of these SNP haplotypes [1], and multi-block haplotypes
may define antigens and alleles encoded at HLA-A, B, C and DR loci [106, 107]. These data demonstrate that SNP haplotypes could serve as a surrogate marker for certain HLA alleles, and may provide an alternative strategy for mapping HLA-associated SNPs that may have functional significance.

6.3. Haplotype-Based Approaches for Mapping Genes Important in Transplantation

Donor-recipient matching for blocks of conserved regions within the MHC (“haplotype blocks”) has been shown to correlate with improved clinical outcome after transplantation [108, 109]. The HLA haplotype may be an informative approach for understanding the significance of MHC variation. A family study to confirm the haplotypes of an unrelated donor is not feasible, and although statistical methods can be used to infer haplotypes [11, 110], their accuracy in predicting an individual’s haplotypes may be limited by the frequency of the donor’s HLA alleles [111].

The great genetic distance between HLA loci is a technological challenge. Recently, a novel approach for isolating high quality 2 Mb-long genomic DNA fragments specific for only one of the two HLA haplotypes in heterozygous samples has been developed [112]. The phasing method has been applied to test the hypothesis that haplotype mismatching may occur among HLA identical unrelated donor-recipient pairs, and that haplotype mismatching is a marker for higher risk of GVHD [113]. These data suggest that long-range phasing methods may provide a strategy for mapping MHC-resident variation important in transplantation.

7. Summary and Conclusions

HLA and NK participate in the transplantation barrier. Optimizing transplant outcome includes donor matching for the highly polymorphic HLA loci. When HLA disparity cannot be avoided, judiciously selecting a donor with the fewest HLA mismatches and avoiding certain loci may provide patients with the opportunity for life-saving transplantation. Disease stage remains a strong predictor of overall transplant outcome, and expediency in timing of transplantation for patients with high risk disease is paramount. Haploidentical mismatched related donors and cord blood units provide attractive alternatives for patients whose disease status cannot wait for a prolonged donor search. New research avenues include identifying novel MHC resident genetic variation that may contribute to risks of GVHD and TRM.

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