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HLA DNA Typing and Transplantation

Review

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Introduction

The major histocompatibility complex (MHC) is a genetic region defined initially by the rejection of skin grafts in genetically incompatible mouse strains. In humans, the MHC, known as the human leukocyte antigen (HLA) region, comprises about 3 Mb located on the short arm of chromosome 6 (6p21.3). This region contains ~200 genes (Beck and Trowsdale, 1999; Shiina et al., 1999), many of which are involved in immune responses and some of which exhibit extensive genetic polymorphism. The genes encoding the HLA class I (A, B, and C) and the class II (DR, DQ, DP) molecules are the most polymorphic loci in the human genome, with some loci (e.g., HLA-B or DRB1) having more than 300 alleles (Marsh, 2000). Initially, genetic variation at these loci was analyzed by HLA serologic typing using reagents derived from sera of multiparous women or individuals who had received multiple blood transfusions. In addition to such serological typing methods, class II polymorphism was analyzed, in some cases, by cellular typing methods, such as the mixed lymphocyte reaction (MLR), in which antigenic differences were typed based on recognition by T cells rather than by the products (antibodies) of B cells.

The advent of recombinant DNA techniques in the mid-70s allowed the molecular cloning and characterization of the class I and class II genes, and the introduction of polymerase chain reaction (PCR) in the mid-80s made possible the analyses of the extensive allelic sequence diversity at these loci. In addition, PCR, following the determination of HLA sequence polymorphism in human populations, allowed the development of a variety of simple and rapid DNA sequence–based typing methods (Saiki et al., 1986; Erlich et al., 1991). Here, we review the application of such methods to the clinical areas of solid organ transplantation and bone marrow or stem cell transplantation.

Molecular Genetics of the HLA Region

The HLA region (6p21.3) consists of a large number of immunologically relevant genes, including the highly polymorphic HLA loci (Figure 1). These loci encode two distinct classes of highly polymorphic cell surface molecules that bind and present processed antigens in the form of peptides to T lymphocytes. The HLA class I molecules, HLA-A, -B, and -C, consist of an HLA-encoded glycoprotein chain in association with the monomorphic molecule β2-microglobulin and are found on most nucleated cells. They bind and present peptides derived primarily from endogenously synthesized proteins (e.g., viral peptides) to $CD8^+$ T cells. The other class I loci are significantly less polymorphic, and HLA class I typing for clinical transplantation is generally restricted to the HLA-A and -B loci and, in some cases, HLA-C.

The HLA class II molecules consist of HLA-encoded α and β glycoprotein chains associated as heterodimers on the cell surface of antigen-presenting cells such as B cells, macrophages, and dendritic cells. Class II molecules, HLA-DR, -DQ, and -DP, bind processed peptides derived predominantly from membrane and extracellular proteins (e.g., bacterial peptides) and present them to $CD4^+$ T cells. Both the HLA-DQ and -DP regions contain one functional gene for each of their α (DQA1 and DPA1) and β (DQB1 and DPB1) chains as well as the pseudogenes DQA2, DQB2, DPA2, and DPB2. The HLA-DR region, however, contains one functional gene for the α chain (DRA) but either one or two functional genes for the β chain, depending on the haplotype. All haplotypes encode a DRB1-encoded polymorphic polypeptide associated with the monomorphic α chain. Some haplotypes contain an additional DRB locus. The other functional class II DRB genes, DRB3, DRB4, and DRB5, encode a β chain that forms a second cell surface heterodimer with the DRA-encoded α chain on certain haplotypes (see Figure 1 legend). Many other genes with important immune functions have also been mapped within the HLA region; however, these loci are much less polymorphic than the HLA class I and class II loci and probably play a more limited role in clinical transplantation. HLA class II typing for bone marrow transplantation is usually restricted to HLA-DR and -DQ and for solid organ transplantation to DR.

HLA Allelic Diversity and Population Genetics

As noted above, the HLA class I and class II genes are the most polymorphic coding sequences in the human genome. The number of alleles at these loci as well as the number of different serological specificities (serotype) are shown in Figure 1. Virtually all of this extensive sequence diversity is localized, for the class II loci, to the second exon and, for the class I loci, to the second and third exons. The HLA class II polymorphic second exon codes for the outer domain of the α and β chains that, together, form the peptide binding groove with its characteristic β sheet floor and two α-helical walls. The first part of the second exon encodes the β sheet floor, and the second part encodes the α helix wall of the groove. For the class I molecule, the peptide binding groove is formed by a single chain; the β sheet floor

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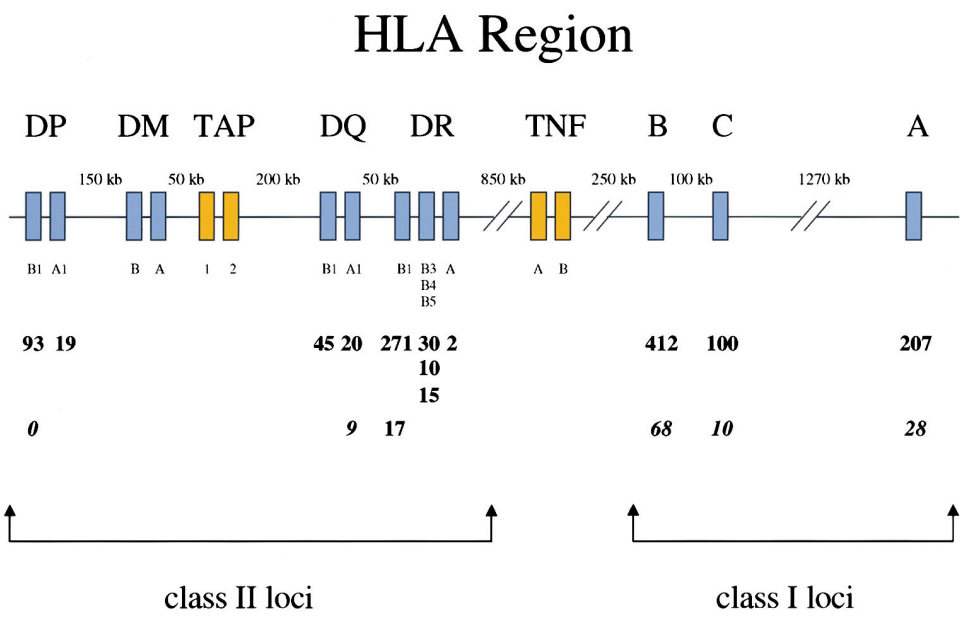

Figure 1. Simplified Map of the HLA Region

The HLA class I and class II loci are shown in blue. HLA-DM is a nonpolymorphic class II molecule involved in the maturation of the antigen-presenting class II molecules DR, DQ, and DP. The number of alleles (as of 2000) is shown below each locus as is the number of serological specificities (serotypes). Kilobase distances between loci are shown above the colored boxes. For class II MHC loci, α (A) and β (B) chain descriptors are shown below the colored boxes. The number of alleles at each MHC locus is shown in bold below the α and β chain descriptors, and the number of serotypes at each locus is shown in italics. In general, the DRB3 locus is found on haplotypes where DRB1 is *03, *11, *12, *13, or *14; the DRB4 locus is found on haplotypes where DRB1 is *04, *07, and *09; and the DRB5 locus is found on haplotypes where DRB1 is 15 or 16 (*15 and *16 are subtypes of the serological type DR2). DRB1*01, *08, and *10 haplotypes typically have only the DRB1 locus.

and two α-helical walls are encoded by the second and third exons.

The patterns of allelic sequence diversity for both the class I and class II loci are highly unusual; some alleles differ in the second and third exons by as much as 15%, and the sequence variation is distributed as a patchwork of localized polymorphic sequence motifs. The extensive allelic diversity at these loci is thought to have been generated by recombinational mechanisms like gene conversion–like events or, to a lesser extent, by reciprocal recombination, which have shuffled these polymorphic sequence motifs (Erlich and Gyllensten, 1991). Of course, point mutation has also contributed to the allelic sequence diversity at the HLA loci. One consequence of this pattern of patchwork polymorphism is that, in PCR-based HLA typing, a large number of different alleles can be distinguished by using a relatively modest number of oligonucleotide primers or probes (see below) that recognize these discrete sequence motifs. Another more problematic consequence is that a given pattern of sequence motifs, detected either with probes or primers or by sequencing, may be consistent with more than a single genotype because the observed sequence motifs can be combined into more than a unique pair of alleles. This problem of "ambiguity" in DNA-based typing is discussed below.

Although a very large number of alleles (e.g., >300 for HLA-DRB1) can be found in the global human population, a much smaller number (e.g., 30–50 for HLA-DRB1) is present in most individual populations, and many populations that have gone through bottlenecks or founding events (e.g., Native Americans) show more limited allelic diversity. Different populations tend to have different frequency distributions of alleles and exhibit different patterns of linkage disequilibrium. In general, the HLA region is characterized by very strong linkage disequilibrium that may be attributed, among other factors, to selection for particular combinations of alleles. In some so-called "extended haplotypes," significant linkage disequilibrium extends over 3 Mb from HLA-A, at the telomeric end of the HLA region, to HLA-DP, at the centromeric end (i.e., the HLA-A*0101-B*0801-C*0701-DRB1*0301-DQA1*0501-DQB1*0201-DPA1*0201-DPB1*0101 haplotype among Caucasians). In fact, given the large number of alleles at the different HLA loci, it is, in part, this strong linkage disequilibrium that allows the identification of bone marrow or stem cell donors in population registries who are matched at multiple loci with a given patient/recipient (see below).

HLA DNA Typing Methods

The analysis of HLA polymorphism based on DNA sequence variation offers a number of advantages over the serological or cellular typing methods. DNA methods permit the typing of a much wider variety of samples because the viability of the cells or the expression of the relevant HLA antigen on the cell surface is not required as it is in serological typing. The initial approach to HLA DNA typing involved the use of labeled cDNA clones or genomic fragments as hybridization probes in Southern blots and the correlation of restriction fragment length polymorphism (RFLP) patterns with HLA serotypes. Although an informative method, RFLP typing required a large amount of high molecular weight

genomic DNA, and the relatively cumbersome procedure was not well suited to routine clinical typing. The most important limitation, however, was that most of the polymorphic restriction sites used for typing were not in exon 2 or exon 3, and, consequently, this method relied on linkage disequilibrium between these flanking sites and the sequence polymorphism in these exons. Thus, although RFLP analysis permitted typing at the broad serotype level and, in some cases, could subdivide these types, it failed to distinguish much of the HLA class I and class II sequence diversity.

The study of the allelic diversity at the HLA loci as well as the development of simple and rapid DNA-based typing methods was dramatically facilitated by the development of PCR amplification. In general, sequence-based typing methods allow a more accurate (fewer errors) and much more precise (more discriminating) method of typing than serological or cellular methods. For example, there are >300 alleles at the DRB1 locus but only 17 distinct serological specificities or serotypes (see Figure 1). In addition, unlike serological typing reagents, the primers, probes, and thermostable DNA polymerases used in PCR-based HLA typing can be produced as standardized reagents. PCR amplification also makes possible the HLA typing of minute samples such hair, buccal swabs, dried blood spots, or even individual cells. Finally, PCR-based typing data from which the nucleotide sequence of the amplified locus can be inferred can reveal where and how alleles differ, allowing the analysis of the role of specific polymorphic amino acid residues in peptide binding and presentation as well as in disease association and clinical transplantation outcomes (see below).

Based on the available database of class I and class II allelic sequence diversity, a variety of PCR-based typing methods have been developed and applied to clinical HLA typing.

Most typing methods involve the design of primer pairs that are capable of amplifying all alleles at the target locus with the polymorphic sequence motifs localized between the primer sites. The sequences between the primers are subsequently characterized by a variety of analytic approaches, including hybridization with oligonucleotide probes, digestion with restriction enzymes, and chain termination sequencing reactions, or are inferred from the conformation-based mobility of the PCR products using gel electrophoresis. The other main approach to HLA typing uses the PCR itself as a method of distinguishing polymorphisms by exploiting the specificity of oligonucleotide primer extension and places the 3′ end of the primer at the polymorphic site.

Typing by Hybridization to Sequence-Specific Oligonucleotide Probes

The first PCR-based approaches to HLA typing utilized labeled sequence-specific oligonucleotide (SSO) probes to hybridize to PCR products amplified from the sample and immobilized on a nylon or nitrocellulose filter—the "dot blot" method (Saiki et al., 1986). Under appropriate hybridization and wash conditions, the labeled SSO probes would bind only to the complementary sequence in the amplified DNA immobilized on the filter and were able to distinguish single nucleotide differences. Using a panel of probes specific for informative sequence motifs, the HLA alleles in the sample could be inferred from the pattern of probe reactivity (Erlich et al., 1991). The probes, labeled initially with P^{32}, are now typically labeled either directly with an enzyme (e.g., HRP) or with biotin. The biotin-labeled probes can be detected with streptavidin conjugated to HRP (or AP) and can be used with chromogenic or chemoluminescent subtrates for sensitive, simple, and robust detection. More recently, digoxigenin-labeled probes and antibody to digoxigenin detection methods have been introduced.

Currently, computer programs are routinely used to infer the genotype from the probe reactivity pattern. The genotype interpretation programs have to be updated periodically to incorporate newly identified alleles. In some cases, the identification of new alleles and their introduction into the genotyping software may require the addition of probes to the typing system. In most cases, however, the new allele represents a new combination of known polymorphic sequence motifs recognized by existing probes and, thus, does not create the need for additional probes. In this case, however, the incorporation of the new allele into the typing system and software may create additional ambiguities, arising when a given probe reactivity pattern is consistent with more than one genotype. Given enough primers and probes, the PCR/SSO method is, in principle, capable of distinguishing all of the alleles at a given HLA locus. To achieve allele-level typing, however, it is usually necessary to amplify separately the alleles of a heterozygote (see below) and analyze the probe reactivity pattern for each individual allele.

In the SSO probe typing approach, the sequence of the primers and the amplification reaction conditions (thermal profile, Mg^{2+} concentration, primer concentrations, etc.) are designed so that all alleles at the target locus are amplified in a single PCR, and, whenever possible, the amplification is locus specific. In some cases, such as generic DRB typing, the same primer pairs amplify the DRB1, DRB3, DRB4, and DRB5 second exons. This approach allows the typing of all these loci from a single PCR. On the other hand, amplification of more than one locus can complicate the interpretation of probe reactivity if the complementary sequence motif is found in more than one locus. In the case of DRB1, it is difficult to find a single primer pair that is locus specific and that also amplifies all alleles of DRB1.

Following the initial SSO probe typing system for DQA1, PCR/SSO dot blot typing systems for DRB1, DQB1, DPB1, and DPA1 have been developed as well as, more recently, for the HLA-A, B and C loci. In dot blot typing, as the number of probes in the typing system and, hence, the number of separate hybridizations increases, the procedure can be somewhat cumbersome, particularly for the typing of small numbers of samples. Reverse hybridization approaches using immobilized SSO probe arrays and labeled PCR products, developed to simplify SSO typing, are described below.

Typing Based on Differential Primer Extension and Sequence-Specific Amplification

Another PCR-based approach, based on the specificity of primer extension rather than that of probe hybridiza-

tion, has also been applied to HLA typing. This method is known variously as allele-specific amplification (ASA) (Wu et al., 1989), sequence-specific priming (SSP) (Newton et al., 1989), and the amplification refractory mutation system (ARMS) (Olerup and Zetterquist, 1992). Here, a specific primer pair is designed for each polymorphic sequence motif or pair of motifs and is based on the differential ability to extend a primer that is matched or mismatched with the template at the 3′ end. The presence of the two targeted polymorphic sequences in a sample is detected as a positive PCR product, typically identified as a band on a gel. In SSP/ARMS typing, if the PCR is negative and no product is detected, the sample is assumed to lack one or both of the specific motifs. Since inhibition of the PCR or absence of template also yields a negative result, each reaction should include a positive control, that is, PCR primers for an unrelated monomorphic target sequence that produces a fragment that can be distinguished (e.g., resolved by differential gel mobility) from the HLA PCR product. One virtue of SSP/ARMS typing is that it can "set phase," in that the two polymorphic sequence motifs detected by the primers must be linked on the same allele. SSP/ARMS typing methods have been developed for both the class I and class II loci, and the introduction of robotics has, in some cases, simplified this procedure. Detection methods that are not based on visualizing a band in a gel and can therefore eliminate the gel electrophoresis step have been developed recently. Although informative and relatively fast for small numbers of samples, the SSP approach requires many separate PCR's to achieve intermediate or high level typing and, in its current format, is not well suited to rapid throughput of a large sample of numbers. As noted above, allele-specific amplification can be used in conjunction with SSO probe typing for high-resolution typing by allowing the separate amplification of the two alleles in a heterozygote.

Typing Based on the Gel Mobility of the PCR Products

Other PCR-based methods involve the use of multiple restriction endonucleases and gel electrophoresis (PCR-RFLP) to characterize the polymorphisms in the PCR product; these approaches have been developed for class I and class II typing. Conformation-based gel mobility analyses, such as PCR-SSCP (single strand conformation polymorphism) and PCR-DHA (directed heteroduplex analysis), have also been developed for class II typing, but these approaches, unlike SSO or SSP methods, are not widely used in clinical settings. A recent modification of the directed heteroduplex approach, termed double-stranded conformation analysis (DSCA), utilizes a fluorescent single-stranded probe specific for a particular HLA allele (i.e., HLA-A*0101) to hybridize to the PCR products (e.g., the two HLA-A alleles) amplified from the sample, thereby generating labeled heteroduplex molecules. The mobility of these labeled molecules can be systematically compared to a standard set of markers for each allele (Arguello, 1998).

Reverse Hybridization with Immobilized SSO Probe Arrays

Reverse hybridization approaches to SSO probe analysis (Saiki et al., 1989; Bugawan et al., 1994; Begovich and Erlich, 1995) of HLA polymorphism have greatly facilitated PCR-based typing with multiple probes. The conventional dot blot involved an immobilized PCR product that is hybridized to each of many labeled SSO probes. The "reverse blot" (or immobilized probe) method is based on the hybridization of PCR product, labeled with biotinylated primers during the amplification, to an array of immobilized probes on a membrane. A variety of immobilization methods can now be used. The presence of the PCR product bound to a specific probe at a specific location is detected using streptavidin-HRP and a chromogenic substrate. This procedure requires only a single PCR and a single hybridization reaction to obtain information from the entire SSO probe panel; all of the probe reactivity information is contained on a single membrane, making it amenable to automating the hybridization, wash, and color development as well as the data capture and interpretation. The critical challenge in this approach is to design a large number of SSO probes that will hybridize specifically under a single set of conditions. In addition, because the PCR product is denatured and hybridized in solution to the immobilized complementary probe, secondary structure in the labeled target single strand that would prevent binding to the probe must be minimized.

This approach has been applied to a variety of genetic typing systems and was the basis for the first commercial PCR test, the AmpliType HLA-DQ-α Forensic test. In order to accommodate more probes on the membrane, the probes are now immobilized as lines rather than as dots (Figure 2). A commercially available HLA-DRB test, the DYNAL/Reli HLA-DRB test, uses 36 probes and provides intermediate-level typing for alleles at the DRB1, DRB3, DRB4, and DRB5 loci. Immobilized probe tests using the same PCR thermal profile and hybridization conditions have been developed for DQB1 and the HLA class I loci, A, B, and C and are also commercially available. Our current higher resolution research B locus typing system uses 83 probes for exons 2 and 3, which are coamplified with two primer pairs, and our research HLA-A typing system uses 57 immobilized probes. Two instruments to automate the hybridization, wash, and color development of these immobilized probe strips are commercially available (SLT Profiblot and the DYNAL Autoreli48) as is software to read and interpret the probe reactivity patterns. The immobilized probe approach is well suited to the rapid typing of a few samples as well as to high-throughput typing of large sample numbers. As noted above, high-resolution typing often requires the separate amplification of the two alleles in a heterozygote. For this purpose, primers designed to polymorphic regions with many sequence variants (i.e., codons 9–12 of DRB1, a region with eight different sequence motifs) are valuable to carry out allele-specific amplification. Figure 2 shows the probe reactivity pattern, defined by the blue lines on the nylon strip, for the HLA-A locus for a panel of homozygous and heterozygous cells.

Sequence-Based Typing

The application of semiautomated chain termination sequencing using either fluorescent primers or fluorescent dideoxy terminators, with the development of appropriate sequence analysis software, has become a powerful approach to allele-level HLA typing. Several com-

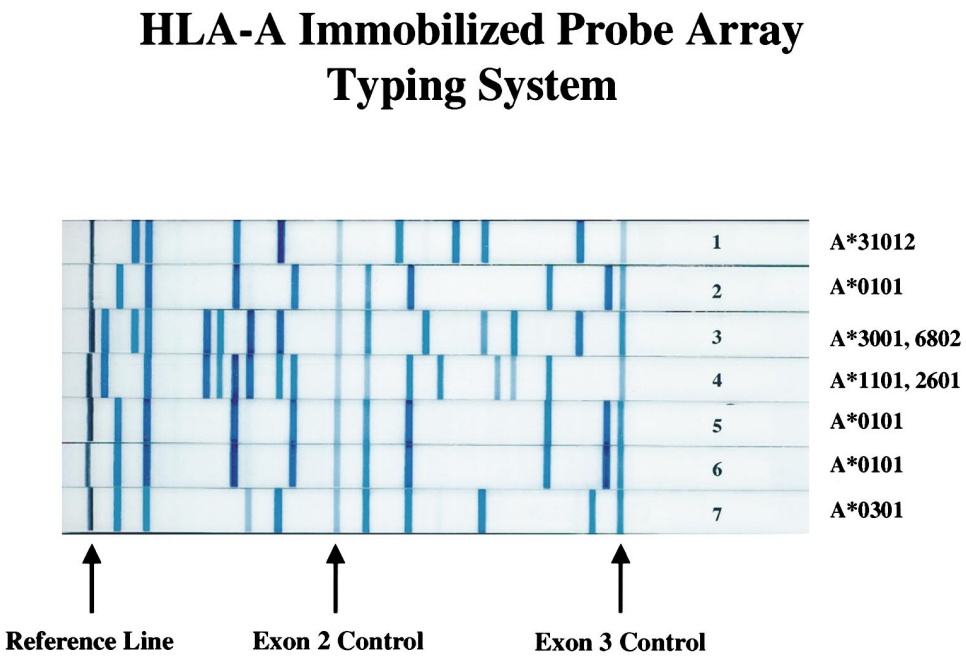

Figure 2. HLA-A-Immobilized SSO Probe Line Array Strips

Seven HLA-A line array strips (labeled 1–7) are shown with the probe reactivity patterns detected using Streptavidin-HRP and TMB, a chromogenic substrate. The inferred heterozygous and homozygous HLA-A genotypes are shown on the right. The black reference line and control probe lines for HLA-A exon 2 and exon 3 are indicated along the bottom of the figure. Positively reacting probes appear as blue lines. Control probes (that hybridize to all alleles) are titered to be lower in signal intensity than all other positive probes, permitting quantitative discrimination between truly positive probes and any cross-hybridizing probe.

mercial approaches to automating the gel electrophoresis step are now available, and, recently, the introduction of modified thermostable DNA polymerases capable of efficiently incorporating fluorescent dideoxy nucleotides has made sequencing a more robust typing method. The recent introduction of capillary-based systems has increased the throughput and decreased the cost of HLA sequence-based typing. High-resolution typing by sequencing, which requires the separate amplification and analysis of the two alleles, is sometimes carried out for the final matching of bone marrow donor and recipient. However, in its current format, SBT is still a somewhat expensive procedure and not ideally suited to large-scale typing or routine clinical typing.

HLA Typing Requirements

In general, the desired resolution and properties of a PCR-based HLA typing method will depend on the application. Bone marrow donor registry screening and recruitment requires a method that provides intermediate-level resolution typing and is low cost and high throughput. The typing data should facilitate the cost-effective storage and search of the donor typings for A, B, and DRB1 in the donor registry for possible matches with the patient/recipient. The final HLA typing for matching potential bone marrow donors and recipients should be done with a method that allows high-resolution typing and should include the DRB1, DQB1, and the A, B, and C loci. For solid organ transplantation, the typing methods should be relatively fast and provide an intermediate level of resolution for the A, B, and DRB1 loci.

Mechanisms of Alloreactivity

Graft rejection by the host as well as graft versus host disease, the attack of recipient tissue by the bone marrow stem cell graft, is mediated by alloreactive T cells (Dennert et al., 1985; Hansen et al., 1997). HLA molecules have a major effect in transplantation, due to the fundamental role they play in T cell activation and the alloresponse. A significant proportion of circulating T cells are alloreactive, that is, they recognize "foreign" or "nonself" HLA. The structural differences encoded by sequence polymorphisms in the class I and class II HLA genes and the minor histocompatibility antigens (mHA) presented by class I and class II molecules induce T cell activation and immune reactions mediated by alloreactive T cells and B cells producing anti-HLA antibodies. The polymorphic residues that define each unique HLA molecule are located within or very near the peptide binding groove (Parham, 1992). These differences may alter the size, shape, and charge of the contact sites for peptide binding and thereby profoundly change the repertoire of peptides that are bound (Rammensee, 1995). The genetic differences recognized by alloreactive T cells reflect the sequence polymorphisms in HLA molecules and the mHA HLA-bound peptides encoded by biallelic genes that distinguish donor and recipient. Allorecognition can be classified as the "direct" or the "indirect" pathway. Direct alloreactivity refers to the direct recognition of foreign HLA-peptide complexes, whereas indirect recognition refers to foreign HLA molecules and mHA that are processed by host APC and presented by host HLA. If we consider the clinically critical graft versus host disease in a bone marrow trans-

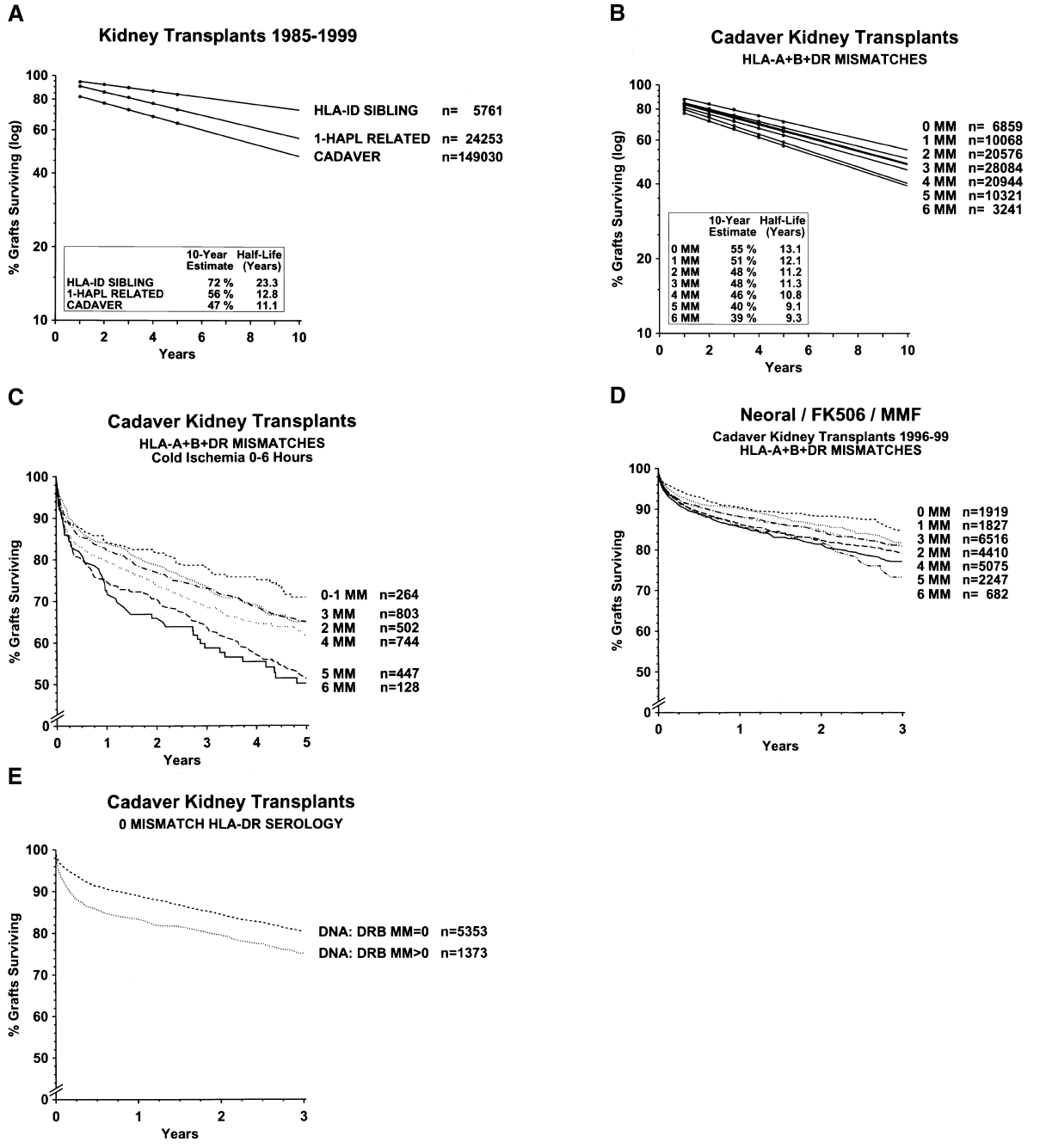

Figure 3. Kidney Graft Survival and HLA Matching

P values for correlations of HLA matches with graft survival were based on a "weighted regression analysis." It considers the number of patients studied in each group and "expects" a stepwise decline in success from zero to six mismatches (Dunn and Clark, 1974). With the large patient numbers analyzed, p values were usually less than 0.0001 (Collaborative Transplant Study, University of Heidelberg, Germany). The international reference source for organ transplant survival data is http://www.ctstransplant.org. (A) Comparison of kidney graft survival with related and cadaveric donors. (B) Survival of cadaveric kidney transplants and HLA-A, -B, and -DR matching. (C) Survival of cadaveric kidney transplants with limited cold ischemia. (D) Survival of cadaveric kidney transplants with most recent immune suppression protocols. All patients who received any of the three drugs Neoral, FK506, and/or MMF (in any combination) were included. (E) Cadaveric kidney transplant survival and DNA matching at HLA-DRB among serogically matched donors. The two curves were compared using the log rank test ($p < 0.0001$).

plant, in the direct pathway, alloreactive donor T cells specific for the recipient HLA molecule can recognize and attack recipient cells that bear this foreign molecule. In the indirect pathway, donor T cells can also recognize donor cells with self HLA that have bound a recipient (foreign) peptide. Direct alloreactivity is thought to contribute primarily to acute rejection, whereas indirect allorecognition may be responsible for sustaining chronic rejection.

T cells and B cells may not be the only mediators of

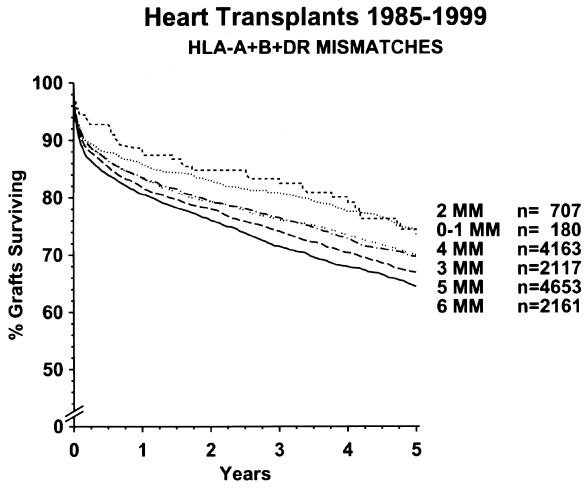

Figure 4. Heart Transplant Survival and HLA-A, -B, and -DR Matching

alloimmunity, especially in marrow transplantation. The recent discovery of a family of genes encoding natural killer cell (NK-KIR) receptors, known to interact with specific polymorphic epitopes on HLA class I molecules, has suggested the participation of NK cells in graft rejection and possibly GVHD (Colonna et al., 1992; Litwin et al., 1994; Valiante and Parham, 1997; Winter et al., 1998).

The consequences of mismatching at different HLA loci may be different for these different pathways. In general, precise HLA class I and class II matching appears to be more critical for bone marrow or stem cell transplantation than for solid organ transplantation because, among other factors, the graft is immunologically competent and, thus, the immune recognition is bidirectional, involving the response of the graft to the host as well as that of the host to the graft (Hansen et al., 1997).

HLA and Transplantation

HLA-matched sibling bone marrow or stem cell donors are, with the exception of very rare recombination events, genotypically identical at all HLA loci with the patient. Most patients, however, do not have an HLA-matched sibling. Registries of HLA-typed unrelated bone marrow donors have been developed and can be searched to find phenotypically matched potential donors. Given the extensive polymorphism at the HLA loci, the probability of finding a serologically matched donor at three loci (i.e., A, B, and DRB1) is very low, requiring registries consisting of millions of potential bone marrow donors to provide a matched donor for most patients (www.bmdw.org). Given the allelic sequence diversity within a serotype, the probability of finding a DNA-matched donor is even lower, making the concept of "permissible" mismatches (see below) a clinically relevant issue. The bone marrow transplant HLA data discussed here are based on typing at high (allele level) resolution. The large data set for solid organ transplantation discussed here relies primarily on serologic typing. Thus, the power of DNA typing to distinguish allelic variants within a serological group (i.e., any of the >30 DRB1*04 alleles within the DR4 serotype) and the effect on graft survival and acute graft versus host disease is restricted to the studies of bone marrow transplantation (see below). As a result of the different levels of HLA typing, the meaning of a "mismatch" differs in these two studies; in the solid organ transplant, mismatch means a serotype difference (e.g., DR4 versus DR3), while, in the bone marrow studies of chronic myeloid leukemia (CML) recipients, mismatch refers to an allele difference (i.e., DRB1*0401 versus DRB1*0404) between individuals with the same serotype (DR4). As a consequence of the different ways that donors are identified or recruited for bone marrow and solid organ transplants, bone marrow donors are more likely to be serologically matched with the recipient than are solid organ donors. Although these solid organ transplants involve typing only at the serologic level of resolution, these studies have much larger numbers and, hence, greater statistical power than do the bone marrow transplant studies.

Table 1. Bone Marrow Graft Failure According to Donor HLA Disparity

Donor Disparity	Graft Failures (%)
Allele-matched for HLA-A, -B, and -C	3 (2%)
Single allele mismatch for HLA-A, -B, or -C	1 (2%)
Multiple alleles mismatch for HLA-A, -B, and/or -C	9 (29%)
Single allele mismatch for HLA-DR or -DQ	0
Multiple allele mismatches for HLA-DR and/or -DQ	0
Class I and class II mismatches	4 (12%)

Graft failure among 300 patients with chronic myeloid leukemia that were transplanted with marrow from an unrelated donor (Petersdorf et al., 1998).

Solid Organ Transplantation

The analysis of the role of HLA matching for kidney graft survival over the period 1985–1999 is, as noted above, primarily based on serologic typing data. The comparison of DNA typing and serology that indicates that DNA-matched grafts survive better than do serologically matched grafts (see below) probably reflects differences in accuracy (fewer errors for DNA typing) rather than greater discrimination, because, in these comparisons, the DNA typing was carried out at a low to intermediate level of resolution (does not distinguish all alleles).

For kidney transplants, the significance of HLA matching for graft survival is illustrated in Figure 3A by comparing related and cadaveric donors. The half-life survival for a graft from an HLA-identical sibling is 23.4 years compared to a one haplotype–related donor with a half-life of 12.8 years and to a cadaveric donor with a half-life of 11.1 years. The effect of serologic mismatches at either the HLA-A, -B, or -DR loci for the survival of cadaveric kidney transplants is shown in Figure 3B. Contrary to some claims, HLA matching has a statistically significant effect on kidney graft survival even under conditions of very short ischemia (0–6 hr under optimal local transport conditions) (Figure 3C). The effect of HLA matching is, as expected, enhanced with presensitized recipients (panel reactive antibody [PRA] >50%; data

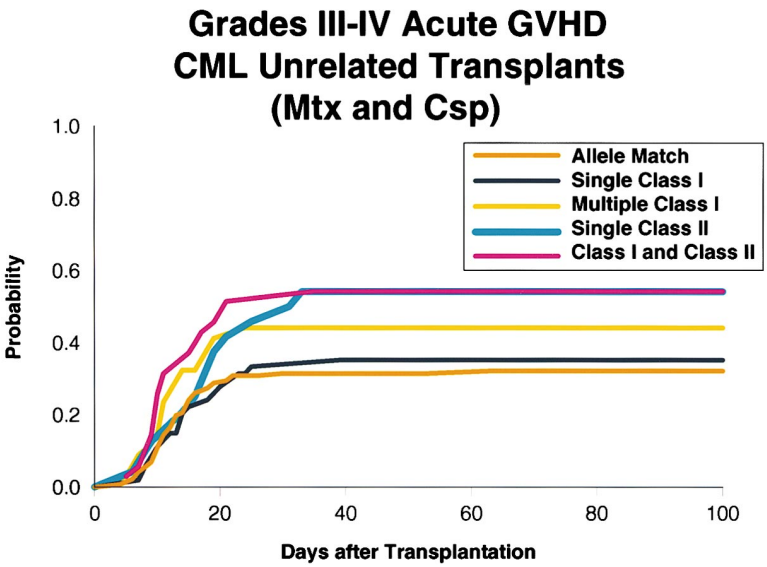

Figure 5. Probability of Grades III–IV Acute GVHD in Patients with Chronic Myeloid Leukemia Transplanted with Marrow Cells from an Unrelated Donor According to HLA Allele Match

All recipients were treated with methotrexate and cyclosporin A (Csp).

not shown) and is also statistically significant among kidney grafts from unrelated living donors (data not shown). HLA-A, -B, and -DR matching remains signficant for cadaveric kidney tranplants, even with the most recent forms of immunosuppression (Figure 3D). The effect of HLA matching is relatively weak over the first 2 years and emerges over time, suggesting that the new drugs are more potent in avoiding early acute rejections. However, as the drug dosages are tapered over time, the HLA-matching effect becomes significant. DNA typing for HLA-DRB1, albeit at an intermediate level of resolution, provides better cadaveric kidney graft survival than does serological typing (Figure 3E). This effect is probably due, given the serological level of the DNA-based HLA typing, to enhanced accuracy rather than to increased discrimination (better allelic definition). Recent studies, however, have indicated that the DNA typing of loci that cannot be typed serologically (i.e., HLA-DP) (Mytilineos et al., 1997) as well as DNA typing that distinguishes alleles within a serotype can have clinical relevance in cadaveric kidney transplants. In both of these studies, the enhanced graft survival was observed only in presensitized (retransplant) recipients. The effect of HLA-A, -B, and -DR matching on the survival of heart transplants is also statistically significant (Figure 4) but not for liver transplants (data not shown).

HLA Matching and Marrow or Hematopoietic Stem Cell Transplantation

The analysis of the role of HLA matching for HSC transplantation has largely depended on the development of high-resolution typing for completely defining the polymorphism of HLA-A, -B, -C, DRB1, and DQB1 alleles in patients and donors. HLA compatibility affects not only the ability to achieve sustained engraftment of donor HSC but also the risk of developing acute and chronic

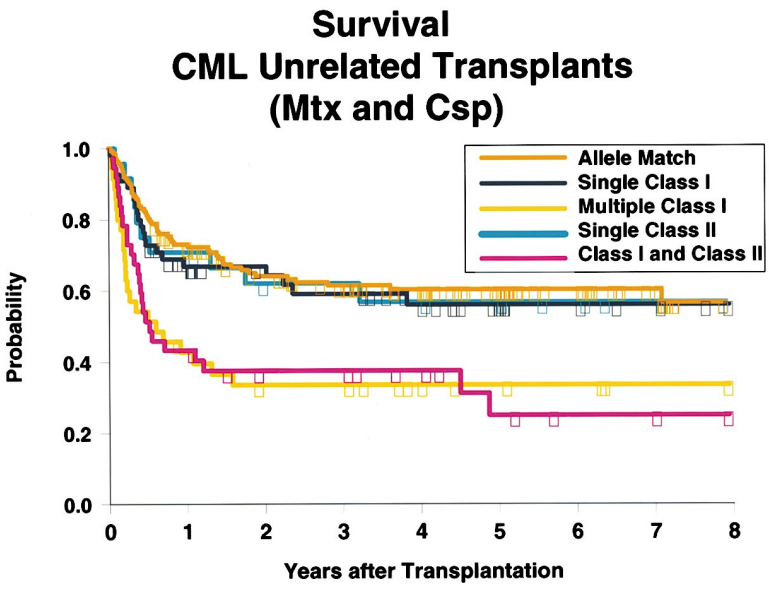

Figure 6. Probability of Survival in Patients with Chronic Myeloid Leukemia Transplanted with Marrow Cells from an Unrelated Donor According to HLA Allele Match

GVHD (Beatty et al., 1985; Anasetti et al., 1989). Availability of these methods has led to the finding that risk of graft failure is affected primarily by donor disparity for class I alleles including HLA-C, suggesting that HLA-C can also function as a classical transplantation antigen (Petersdorf et al., 1998). The incidence of rejection correlated with the number of donor-incompatible alleles. Among 300 cases analyzed, the incidence of graft rejection was 0.7% for zero, 8% for a single, and 19% for multiple class I allele donor incompatibilities (Table 1). Donor disparity for class II did not increase the risk of rejection.

Analysis of HLA and GVHD focuses on mismatches in the host recognized by donor T cells. Generally, host disparity for class II has a greater risk of GVHD than disparity for class I. Patients with a single class II mismatch at DR or DQ have a hazard ratio for acute GVHD of ~1.8 compared to matches (Figure 5) (Petersdorf et al., 1998). Single class I mismatches are well tolerated with respect to GVHD, but combined mismatching at class I and II confers a hazard ratio of ~2.0. Allele mismatching for HLA-A, -B, -C, DRB1, and DQB1 is also a significant risk factor for survival (Figure 6). Patients mismatched for more than one class I allele and those mismatched at both class I and class II have a significantly lower survival than patients and donors fully matched for the HLA-A, -B, -C, DRB1, and DQB1 alleles. A single class I mismatch or single class II mismatch does not appear to affect survival.

Conclusions and Summary

PCR-based methods of HLA class I and class II typing have been developed that are simple, rapid, highly informative, and automatable and can be carried out at either intermediate or high levels of allelic resolution in a clinical diagnostic setting as well as for research studies. For solid organ transplants, the data indicate that the degree of HLA matching, in terms of the number of mismatched A, B, and DR antigens, significantly affects the survival of kidney grafts, for both cadaveric and living unrelated donors, even with the most recent protocols of immunosuppresion. The degree of HLA matching also significantly affects the survival of heart transplants but not of liver transplants. For bone marrow transplants, where the HLA typing was carried out at the allele level, the comparison of DNA-matched with DNA-mismatched among serologically matched donor and recipient pairs indicates that both graft rejection and graft versus host disease occur more frequently in allele-mismatched transplants. Acute graft versus host disease is increased with recipient disparity for one DR or DQ allele or for two or more class I alleles. Bone marrow graft rejection was associated with donor disparity for two or more class I alleles. It is likely that the effect of mismatching on these various clinical outcomes will depend on the specific HLA alleles that differ between the donor and recipient. The increased use of DNA HLA typing in transplantation should help define the "rules" that govern the clinical outcomes of mismatched transplants and allow the identification of "permissible" (relatively well-tolerated) mismatches.

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