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Review

Histocompatibility testing after fifty years of transplantation

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ABSTRACT

Histocompatibility testing has been used in support of solid organ and hematopoietic stem cell transplantation for over fifty years and transplantation has clearly served as a major stimulus for interest in the human major histocompatibility complex, the HLA system. Until the 1990s, typing and definition of antibodies to HLA antigens was performed primarily by serologic techniques using cell-based assays. Two major technological advances have greatly increased knowledge of HLA alleles and HLA-specific antibodies, namely the introduction of DNA based molecular typing and solid phase immunoassays using purified HLA antigens as targets. By virtue of these advances, the number of recognized HLA alleles has increased from a few hundred to greater than 6000 and definition of the specificities of antibodies to HLA antigens is now possible at the level of individual epitopes. The technological advancements have also raised new challenges. The vast and ever increasing number of HLA alleles has resulted in ambiguities in allele assignments which confound matching for hematopoietic stem cell transplants. Similarly, the ability to detect extremely low levels of HLA-specific antibodies has raised questions about whether such low levels are clinically relevant. Next generation DNA sequencing methods likely will offer the solution to many of the HLA typing ambiguities and future studies on the nature of both HLA and non-HLA-specific antibodies will clarify their impact on transplant outcomes.

Contents

Abbreviations: AMR, antibody mediated rejection; CDC, complement dependent cytotoxicity; CFSE, carboxyfluorescein succinimidyl ester; CPRA, calculated panel reactive antibody; CREG, cross-reactive antigen group; DSA, donor specific antibody; DHSA, donor HLA-specific antibody; FCXM, flow cytometric crossmatch; GVHD, graft-versus-host-disease; HLA, human leukocyte antigen; HSCT, hematopoietic stem cell transplant; HTC, homozygous typing cell; IHIWS, International Histocompatibility and Immunogenetics Workshops; IMGT, international ImMunoGeneTics project; MESF, molecules of equivalent soluble fluorochrome; MFI, mean fluorescence intensity; mHags, minor histocompatibility antigens; MHC, major histocompatibility complex; MIC, MHC class I related chain molecule; MLC, mixed lymphocyte culture; NK, natural killer cell; NMDP, National Marrow Donor Program; OPTN, Organ Procurement and Transplantation Network; PLT, primed lymphocyte typing; PRA, panel reactive antibody; RFLP, restriction fragment length polymorphism; SBT, sequencing based typing; SNP, single nucleotide polymorphic substitution; SPI, solid phase immunoassay; SSOPH, sequence specific oligonucleotide probe hybridization; SSP, sequence specific primer amplification; UNOS, United Network for Organ Sharing; WHO, World Health Organization.

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1. Introduction and background

Solid organ transplantation became a reality in 1954 when the first successful renal transplantation between identical twins was performed by Drs. Murray, Merrill and Harrison at the Peter Bent Brigham Hospital in Boston (Merrill et al., 1956). The extension of transplantation to genetically disparate individuals required both definition of the human histocompatibility complex (MHC) and development of immunosuppressive regimens. With the co-evolution of surgical techniques, immunopharmacology, and histocompatibility assays, success was achieved with allogeneic hematopoietic stem cell and solid organ transplants, including kidney, pancreas, liver, lung, heart, and small bowel. More recently, composite tissue grafts such as hand and face transplants have been accomplished (Siemionow et al., 2009). Transplantation has been a major driving force for both the definition of the human MHC and the development of assays to assess histocompatibility.

Recognition of genes encoding transplantation or histocompatibility antigens was first appreciated in murine models. In 1909, scientists working in cancer research described the phenomenon of the acceptance of transplanted tumors in inbred mice but their rejection in genetically disparate mice (Little and Tyzzer, 1916). Peter Gorer identified a blood group locus in mice in 1936 and showed that the gene product, *antigen II*, was associated with tumor graft rejection (Gorer, 1936, 1937). At the same time, George Snell at the Jackson Laboratory in Bar Harbor, Maine, independently mapped the gene that determined the rejection of tumor grafts and named it the *Histocompatibility* (H) locus (Snell, 1937). Gorer and Snell later showed that the *antigen II* and the *H* locus were actually one and the same. Their collaboration led to the recognition of the first major histocompatibility gene in rodents, "*H2*", a combination of "*H*" and "*II*" (Gorer et al., 1948). However, discovery of *H2* in mice did not elicit an immediate search (Klein, 1971; Thorsby, 1971) for a counterpart in man. Growing interest in transplantation and immunogenetic disease associations stimulated investigations of the human MHC, commonly known as the HLA, or human leukocyte antigen system. The HLA system was studied with human anti-sera reactive with human leukocytes, whereas the H2 system was studied by serological testing of murine erythrocytes. The fact that the *HLA complex* was homologous to *H2* in mice was not recognized until 1971 (Klein, 1971; Thorsby, 1971).

In 1952, Jean Dausset in France observed an unexpected aggregation of leukocytes when mixing a serum from a patient who had received multiple blood transfusions with another donor's leukocytes. The patient's serum did not react with autologous leukocytes suggesting the agglutinins were alloreactive. In 1958, Dausset described the first HLA antigen, which he named "MAC", currently known as HLA-A2 (Dausset, 1958, 1962). Dausset's discovery stimulated an intense interest in studying leukocyte antigens. This led to the definition of additional HLA antigens by other scientists using their own reagents. Van Rood in Holland identified the 4a and 4b antigens, now recognized as the Bw4 and Bw6 epitopes, while Rose Payne and Walter Bodmer described the LA antigen that was later found to be the MAC antigen defined by Dausset (van Rood and van Leeuwen, 1963; Payne, 1962).

As the number of new HLA antigens increased, it was appreciated that the exchange of typing reagents between laboratories was crucial for accelerating the identification of new antigens and for standardizing their definition. In fact, trading of sera worldwide was the only way that laboratories could type for antigens as they became recognized. The International Histocompatibility Workshop (IHWS) was established to create opportunities for collaboration, exchange of precious sera, comparison of assays, and sharing of unpublished data. The workshops brought together scientists from different disciplines and expedited understanding of the genetics and functions of the MHC gene products. The first IHWS was held at Duke University in Durham, North Carolina in June 1964, organized by Dr. Bernard Amos (Amos, 1965). Important data generated during this workshop shaped the direction for future research and also accelerated the establishment of histocompatibility testing in clinical transplantation, since the first studies of the influence of the HLA compatibility on renal and skin graft acceptability were also discussed (Terasaki et al., 1965). The micro-droplet lymphocytotoxicity assay developed by Paul Terasaki was introduced and was compared to other assay methods such as agglutination, cytotoxicity, leukocyte and platelet complement fixation, and mixed hemabsorption. Following its success, workshops have been held every 2–5 years since to discuss contemporary issues related to histocompatibility testing and immunogenetics. In the more recent workshops, now known as the International Histocompatibility and Immunogenetics Workshops (IHIWS), the scope of studies has been expanded to the genes encoding minor histocompatibility antigens, the class I related MICA and MICB molecules, KIR receptors, and clinical applications in drug hypersensitivity and disease susceptibility.

Through family studies conducted at the 2nd and 3rd IHWS, the leukocyte antigens MAC, 4a, and 4b were shown to