

# Human Leukocyte Antigen Class II Antibodies and Transplant Outcome

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During recent years, increasing evidence has accumulated that preformed antidonor human leukocyte antigen (HLA) class II antibodies represent significant risk factors for transplant dysfunction and failure (1–6). Also, the posttransplant development of anticlass II antibodies is associated with a higher incidence of acute and chronic rejection (7–14).

In this issue, the report by Issa et al. (15) on “Transplant Glomerulopathy: Risk and Prognosis Relate to Anti-HLA Class II Antibody Levels” describes a pretransplant serum analysis of anticlass II antibodies with solid-phase assays using single HLA antigen-coated flow beads. Their patients had been previously transplanted solely on the basis of a negative T-cell antiglobulin-augmented, complement-dependent cytotoxicity crossmatch, a standard approach during that era. This retrospective study showed that 28% of these patients had pretransplant anticlass II antibodies and approximately one-half of them were donor specific. This group had a higher incidence of transplant glomerulopathy and subsequent graft failure than patients without anticlass II antibodies pretransplant. Higher anticlass II antibody levels were also associated with the presence of CD4 in transplant biopsies. These findings provide further support of the concept that anticlass II antibodies have a detrimental effect on kidney transplant outcome.

This analysis did not consider crossmatches with B-cells that express class II antigens presumably because they were not used for this cohort of transplant recipients. Although many reports show that positive B-cell crossmatches represent risk factors for early graft loss and lower graft survivals, there has been widespread debate about the value of the B-cell crossmatch (16, 17). The test seems technically difficult and false-positive result can often occur with non-HLA-specific antibodies. Therefore, it is difficult to say

whether a positive B-cell crossmatch could have predicted a greater risk for transplant glomerulopathy, a long-term complication.

In the study by Issa et al. (15), almost 50% of the patients with relatively strong anticlass II antibody reactivity and approximately 25% of these patients with weaker antibody reactivity developed transplant glomerulopathy during a 4-year follow-up period. One might raise the question whether any particular antibody specificity patterns could be implicated. Although Issa et al. indicated that their sensitized patients had similar incidence of anti-DR and anti-DQ antibodies, it seems worthwhile to differentiate between antibodies reacting with each class II gene product including DRB1, DRB3/4/5, DQB, DQA, and even DPB and DPA. Solid-phase assays with single DRB, DQ, and DP alleles together with high-resolution typing for class II loci of donor and recipient are now routinely performed in many tissue typing laboratories. Such detailed information about donor-specific class II antibody specificity may reveal why some patients develop transplant glomerulopathy and others do not. In addition, the determination of glomerular expression of different class II genes may provide a better understanding of the pathogenesis of class II antibody-associated transplant glomerulopathy.

In the management of sensitized patients considered for transplantation, any HLA antibody specificity analysis should consider the fact that HLA antigens have multiple epitopes that now can be readily identified from molecular structural modeling and amino acid sequence differences between HLA antigens. Figure 1 shows the polymorphic amino acid residues on the surface of stereochemical models of crystallized DR and DQ molecules. The structural polymorphisms of DR are restricted to the  $\beta$  chains. The DR  $\alpha$  chain is largely monomorphic. Many polymorphic DRB residues are on the top of the molecule adjacent to the bound peptide and are often in contiguous sequences. Polymorphic residues on the side of the molecule generally comprise distinct clusters in both  $\beta 1$  and  $\beta 2$  domains. Some polymorphic residues reside at the bottom part of the molecule that is nearby the cell membrane. Both  $\alpha$  and  $\beta$  chains of the DQ-heterodimer have polymorphic positions.

The structural polymorphism of HLA is obviously complex, and the determination of the epitope repertoires cannot be solely based on single polymorphic residues. Recent studies based on stereochemical modeling of crystallized complexes of antibodies with different protein

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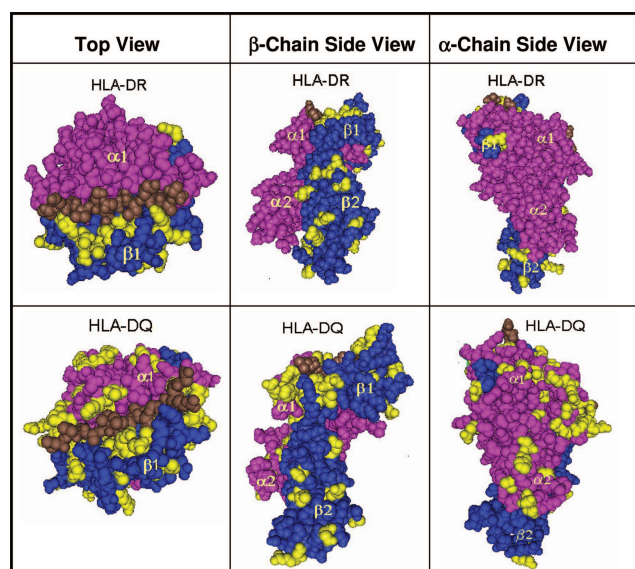
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**FIGURE 1.** Visualization of polymorphic amino residue positions (in yellow) on HLA-DRB and HLA-DQ molecules; the peptides are in green. The crystallographic structural models of DRA1\*0101, DRB1\*0101 (PDB #1KG0) and DQA1\*0301, DQB1\*0302 (PDB #1JK8) were retrieved on the NCBI Web site.

antigens have led to the concept that functional epitopes consist of patches of amino acid residues that are approximately 3 Å apart from each other and at least one of them is non-self and exposed on the molecular surface (18, 19). This concept has been applied to defining epitopes on HLA antigens and the term “eplet” is used to list the polymorphic residues in a patch with a radius of 3.0 Å. A computer algorithm called HLA Matchmaker considers the notion that the recipient’s own HLA antigens represent the repertoire of self-eplets to which no antibodies can be made, and structurally based compatibility determines for each mismatched HLA antigen, which eplets in corresponding sequence positions are different. Eplet-based compatibility testing and serum analysis programs can be downloaded from the Web site <http://www.HLAMatchmaker.net>.

A recent study on kidney transplant patients has demonstrated the identification of antibodies specific for eplets on DRB1, DRB3/4/5, DQB, DQA, DPB, and DPA antigens (20). Interestingly, for each class II locus the donor-specific antibody response seems to be dominated by a subset of presumably immunogenic eplets. An epitope-based approach may permit a fine-tuning of the HLA antibody specificity patterns and their clinical relevance in relation to mismatch acceptability and transplant outcome.

It has become apparent that HLA compatibility should be defined at the epitope rather than the antigen level. Several studies have shown that the epitope load of a mismatched antigen correlates with the HLA antibody response (21–24). Conventional donor selection criteria consider a DR antigen as a match or a mismatch, but this approach is an inadequate reflection of histocompatibility.

Each DR antigen should be viewed as a package of DR+DQ+DP antigens, and its class II epitope load depends on the patient’s DR, DQ, and DP type representing the repertoire of self-epitopes. Some DR antigen matches have considerable epitope loads because of incompatibility at DRB3/4/5, DQ, and/or DP. Conversely, certain DR mismatches have low epitope loads whereas others have many mismatched epitopes; it all depends on the DR, DQ, and DP type of the patient. Information about epitope load and epitope immunogenicity may permit strategies for HLA-mismatch permissibility to prevent alloimmunization.

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