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Review Article

Humoral immunity in renal transplantation: epitopes, Cw and DP, and complement-activating capability – an update

Filippone EJ, Farber JL. Humoral immunity in renal transplantation: epitopes, Cw and DP, and complement-activating capability – an update.

Abstract: Humoral immune responses can destroy a renal allograft. In January 2013, Consensus Guidelines were published regarding testing and management concerns with respect to antibodies in transplantation. New studies have been reported over the past two yr and controversies remain. We review here the new data in light of the Consensus Guidelines and the relevant prior research with emphasis on antibody characteristics and potential for pathogenicity. The heart of immune recognition, epitopes, is stressed, including the realization that DQ (and probably DP) epitopes may be determined not only by eplets within a given α- or β-chain, but also by specific α- and β-chain pairings. The significance of Cw and DP loci are discussed. To better understand which donor-specific antibodies are pathogenic, IgG subclass determination has been studied, and in *in vitro* complement fixation assays, such as the C4d and C1q assays, have been evaluated.

Edward J. Filippone[a] and John L. Farber[b]

[a]Division of Nephrology, Department of Medicine and [b]Department of Pathology, Thomas Jefferson University Hospital, Philadelphia, PA, USA

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Corresponding author: Edward J. Filippone, MD, 2228 South Broad Street, Philadelphia, PA 19145, USA.
Tel.: +1 215 467 8955; fax: +1 215 467 8956; e-mail: kidneys@comcast.net

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The detrimental effect of a humoral immune response on renal transplants is well described. In January 2013, Consensus Guidelines were published regarding testing and management concerns with respect to antibodies in transplantation (1). Over the past two yr, new studies were reported and controversies remain. We review here the new data in light of the Consensus Guidelines and the relevant prior research. The heart of immune recognition, epitopes, will be stressed, including the realization that DQ (and probably DP) epitopes may be determined by specific α- and β-chain pairings. The significance of Cw and DP loci, IgG subclass determination, and in *in vitro* complement fixation are evaluated.

Epitopes

The Consensus Guidelines extensively review the technical aspects of anti-HLA antibody detection, including the use of cell-based and solid-phase assays (SPA). The difficulty and complexity in interpreting the epitope specificity of these antibodies, however, is noted but not discussed in detail. Anti-HLA antibodies react with specific regions within HLA antigens known as epitopes (2). Structural epitopes contain 15–25 contact amino acid residues in an area of about 700–900 $Å^2$ that interact with the six complementarity determining regions (CDR) of the variable heavy (V_H) and light (V_L) chains of a given HLA antibody (3). Within this structural epitope is a functional epitope, or eplet, a small polymorphic residue (1–5 amino acids within a 3 Å radius) that is expressed on the surface of the HLA antigen and that binds the centrally located third CDR of V_H (CDR-H3). These functional epitopes may be determined by a computer algorithm (accessible at http://www.HLA-Matchmaker.net) that compares polymorphic residues between specific alleles. The antigenicity of

these eplets can be verified by specific monoclonal antibody binding, as described by El-Awar and Terasaki (often referred to as TerEps), as well as by others (4, 5). As of November 2013, a total of 95 antibody-verified class I epitopes (eplets) have been recorded in the International Registry of Antibody-defined HLA Epitopes (http://www.epregistry.com.br) (6). A given eplet may be sufficient to be immunogenic; hence, all serologically defined antigens containing this eplet will be reactive. In about a third of cases, however, the eplet may have to be paired with one or more additional, non-contiguous, superficial polymorphic residues (eplets) within a radius of 16 Å (corresponding to the 900 $Å^2$ area of the antibody paratope) (6, 7). Occasionally, the paired eplet will not be superficial, but will affect the tertiary structure of the functional eplet. To be recognized by B cells and result in antibody production, a functional eplet is typically non-self, but not always (8). Current understanding holds that when an additional eplet is required for recognition, it should be identical (i.e., self), or nearly so, between the immunizer and responder, the so-called non-self/self-hypothesis (7, 9). Otherwise, the responder B cells will not be stimulated by the non-self functional eplet. Hence, not all alleles expressing the eplet are necessarily reactive. A given functional epitope may be restricted to one or a few HLA alleles or may be more broadly expressed across many alleles, previously designated as cross-reactive groups.

According to the International Registry of Antibody-defined HLA Epitopes (accessed on August 1, 2014), 54 of 151 identified HLA-DR epitopes have been confirmed by antibodies. Similarly, 18 of 83 DQ-α-β and six of 58 DP-α-β epitopes have been confirmed. In the case of all class I HLA antigens, the epitopes reside only in the alpha chain. By contrast with DR antigens, all epitopes are within the beta-chain. With DP and DQ antigens, both the α- and β-chains are polymorphic, and antibodies against DQ (and probably DP) may be produced to epitopes restricted to one chain only or are determined by the specific combinations of α- and β-chains, not just either in isolation. Using sera from 74 renal transplant patients and 35 specific mouse DQβ1 momoclonal antibodies, Terasaki and colleagues defined three DQα1 and 15 DQβ1 epitopes. Twenty-one patients produced antibodies against one of the DQα1 epitopes and 27 against one of the DQβ1 epitopes (10). Of note, reaction to a DQβ1 epitope was not restricted to pairing with a specific DQα1 allele and vice versa. This group did not have the ability to define inter-chain epitopes determined by the sequences of both chains combined.

Others have described the dependence of specific DQα and DQβ pairings (11–14). In a study of 104 wait-listed patients with known anti-DQ antibodies (11), 71% of the 104 serum samples contained antibodies reactive with beads containing the patient's own DQα- or DQβ-chains that were paired with non-self chains. Of note, 15 such patients had moderate to strong flow cross-match (FCXM) positivity, because the functional epitope was determined by the specific α/β combination and not by either chain alone. Patients with certain DQ serologic specificities (of the seven known DQ serotypes) more commonly have antibodies against self-DQβ (e.g., 46% of patients in this study with serologic DQ7, owing to multiple documented pairings with DQα). Overall, 90% of serum samples had antibodies recognizing epitopes contributed by sequences of the β-chain, 13% by sequences of the α-chain, and 33% had antibodies recognizing epitopes contributed by both chains.

Tambur et al. retrospectively evaluated 2037 assays performed in 2011 (12). Of these, 1173 (58% of patients) were sensitized: 9% against class II alone and 48% against both class I and II (overall 57% against class II). Fifty-six percentage of the class II antibodies were anti-DQ (31% DQ only, 25% DR and DQ, 44% DR, DP, and DQ). FCXM assays were performed against 259 potential donors during this time, 112 of which were positive, due at least in part to donor-specific DQ antibodies. In another 35 cases, patients had antibodies with donor-specific serologic DQ specificity, but they were negative when considering specific α/β DQ combinations between donor and recipient, and had, as predicted, negative FCXM. Hence, of the 147 patients with serologically specific anti-DQ donor-specific antibodies (DSA), 24% did not have antibodies against the actual donor α/β combination. Furthermore, they had negative FCXM and would otherwise have been denied the opportunity (if typed just serologically) for these possibly compatible kidneys.

Tambur et al. (13) studied the effect of allele-specific typing of both DQα- and DQβ-chains on the calculated panel reactive antibodies (cPRA) and compared the results to the current UNOS algorithm that uses only the serologic nomenclature for the DQ locus. The distribution of α/β alleles within the seven DQ serogroups varied, as patients usually did not react with all α/β allele combinations found within a serogroup otherwise defined as unacceptable for that particular patient, thereby making serologic calculations of cPRA problematic. For example, of 904 patients tested for class II antibodies by high-resolution typing, the use of allele-specific frequencies to determine

cPRA affected over one-third of those with moderate or strong serologic DQ reactivity. Fully 36% of patients had reductions of their cPRA, with 27% having 11–40% reductions due to the lack of reactions to specific α/β combinations in the particular serogroup. Thus, many patients will be assigned higher cPRA points than are actually warranted, whereas some may be denied the chance for possibly compatible kidneys. Furthermore, the authors note the imperfect linkage disequilibrium between DRβ1, DQα1, and DQβ1. They confirmed 3182 DRβ1–DQα1–DQβ1 haplotypes from 2182 individuals and found 113 (approximately 4%) that were unique (observed less than three times), similarly supporting their view that both DQα- and DQβ-typing are required for cPRA determination.

Most recently, Tambur et al. (14) studied by HLA Matchmaker analysis with Cn3D software (accessible at http://www.ncbi.nlm.nih.gov/Structure) 40 previously unsensitized recipients with graft loss (GL) due to rejection in the presence of de novo donor-specific DQ antibodies to highlight the exact position of the culprit eplet. They identified 10 DQα-eplets or eplet combinations and 13 DQβ-eplets or combinations. In all but one, the structural epitope footprint involved both the α- and β-chains. They discuss in detail four situations in which: (i) the eplet may reside in the DQβ-chain but the antibody paratope covers both α- and β-chains; (ii) the eplet resides in the DQβ-chain and the paratope covers both chains, but only the β-chain residues are critical; (iii) the eplet is located on the α-chain with the paratope covering both chains; and (iv) the eplet and paratope are both restricted to the α-chain. These results lend further support to the concept that DQ antigens are not necessarily composed of two independent components and should be considered in light of the specific DQα/DQβ combination when evaluating acceptable mismatches and cPRA calculations.

At this time, it remains unclear if pre-transplantation epitope analysis should be routinely incorporated into HLA-matching algorithms. Randomized controlled trials are needed to determine whether the increased time and cost are outweighed by definitive benefits, such as more equitable organ allocation, better graft survival, and/or reduced future sensitization. In the meantime, we agree with Tambur et al. that specific α/β-chain analysis should replace serologic DQ typing currently in use. Furthermore, it remains to be proven that epitope analysis in the post-translantion setting can better guide intensity of immunosuppression, monitoring for donor-specific HLA antibody development, and/or affect treatment if such antibodies are detected.

Antibodies against Cw and DP

The role of a specific alloantibody response against the HLA-C locus is not discussed in detail in the Consensus Guidelines. Due to its low tissue expression, HLA-C is considered weakly immunogenic (15). Earlier studies showed a lack of effect of HLA-C mismatches on transplant outcome. They were based solely on serologic typing, however, and were also hampered by the strong linkage disequilibrium of HLA-C with HLA-B (16). More recently, Duquesnoy and Marrari evaluated after graft nephrectomy sera from 45 HLA-C mismatched, failed primary transplants that had 56 such mismatches (17). Twenty-seven (60%) recipients had HLA-C DSA. Using the HLA Matchmaker algorithm, 56 HLA-C eplets were detected in their Luminex panels, 17 unique for only one or more HLA-C alleles, and 39 shared with HLA-A and/or HLA-B alleles. At least 19 of these have been verified by specific antibodies (e.g., TerEps). In total, 352 mismatched HLA-C donor/recipient eplets were identified, with 84 (24%) resulting in antibodies against either a particular eplet (69 instances) or eplet pair (15 instances). The latter typically included the donor eplet and a self (recipient)-eplet. Whether any of these particular antibodies were directly responsible for GL could not be determined. In a study of 2260 deceased donor renal transplants (DDRTs), however, all matched for HLA-B and typed be PCR-SSP, HLA-C mismatching was significantly associated with decreased graft survival (GS) (18). This effect was seen only in pre-sensitized recipients, but by multivariable analysis was similar in magnitude to DR mismatching. With monoclonal antibody determined HLA-C epitopes, mismatching three of eight epitopes was associated with significantly reduced GS.

In another study of 251 sensitized wait-listed patients, the frequency and median mean fluorescence intensity (MFI) values of anti-Cw antibodies (56%, 4955) were significantly lower than both anti-HLA-A (79%, 10194) and anti-HLA-B (86%, 11 235) antibodies (19). Eight patients with isolated anti-HLA-Cw received transplants with no rejection and 100% GS at six months. By contrast, a retrospective study compared the outcome of 22 recipients with isolated anti-Cw DSA with 88 sensitized (but DSA negative) case–controls (20). It was found the incidence of antibody-mediated rejection (AMR; 27%) was significantly higher than the controls (9.1%) and was predicted by the day 0 anti-Cw MFI. Isolated case reports also highlight the potential deleterious effects of HLA-C DSA (21–23).

In summary, HLA-C matching is relevant. Mismatched HLA-C epitopes, whether restricted to the C locus or shared by other class I loci, can result in DSA, acute or chronic allograft injury, and reduced GS.

The role of a specific alloantibody response against the HLA-DP locus is also not discussed in detail in the Consensus Guidelines. HLA-DP is expressed constitutively at low levels on endothelial cells (24). Reactivity to DP is less common than to DR or DQ. Anti-DP antibodies are detectable in 5–40% (19, 25) of wait-listed patients, and up to 60% or more in those with failed transplants (25, 26). Sensitized waiting list patients less commonly reacted to DP antigens (35%) than DR (66%) and DQ (69%). They did so with less intensity (mean MFI 2945 vs. 7866 and 8283, respectively). There are six known hypervariable regions of DPB1 (27), but only two dimorphisms of DPB1 define immunodominant serologic epitopes (28). One mutually exclusive dimorphism is at position 56 (E vs. A), the other at positions 85–87 (EAV vs. GPM). Together, these result in four possible epitopes that are immunodominant for the DP locus. These epitopes may be restricted to DP antigens or may be expressed as well on HLA-DR antigens. Non-immunodominant epitopes exist. Only when a patient carries all of these dominant ones as self-antigens would they be predisposed to form antibodies against the non-dominant ones (29).

Early studies suggested that HLA-DP mismatch did not adversely affect GS (30), except possibly in retransplants or in highly sensitized patients (31, 32). As most patients with DP antibodies also have anti-DR and or DQ antibodies, the role of DP specifically has been hard to assess (25). High-resolution typing and epitope analysis by HLA Matchmaker have allowed identification of patients with isolated anti-DP antibodies that have suffered AMR and GL (29, 33–38). A highly sensitized retransplant mismatched only at DPA1 and DPB1 with low-level HLA-DP DSA was reported (38). Despite a negative B-cell FCXM, the graft rapidly developed AMR that was shown to be caused by intra-allele epitope spreading and inter-allele epitope sharing with post-transplant sera reacting to previously unreactive eplets.

Thus, similar to HLA-C, antibodies against DP eplets can be pathogenic. Interestingly, donor–recipient pairs with compatibility at HLA-A, B, C, DR, and DQ still have a reasonably high chance of a DP mismatch (39). High-resolution typing of donor and recipient DPA and DPB may be needed in highly sensitized recipients or in those with unexplained positive B-cell cross-matches, especially with a prior failed transplant.

HLA antibody subclass determination and *in vitro* complement binding assays

Pre-transplant donor-specific complement-dependent cytotoxicity (CDC) precludes successful renal transplantation (40). CDC lacks sensitivity and specificity, however, and requires rabbit complement. FCXM have been extensively used over the past 25 yr and has increased sensitivity compared to CDC, although positive reactions are not specific for complement binding antibodies. Currently, SPA using ELISA or bead-based technology with single antigen (SAB) or multiple antigen beads (pooled or single-celled phenotype) analyzed on a Luminex platform markedly enhance sensitivity with specificity down to the allele level. Whereas antibodies detectable only by these more sensitive SPA represent a risk for AMR and reduced GS (41), they are not always pathogenic. Many patients have no detectable adverse consequences. Technical aspects and potential pitfalls of interpreting these more sensitive assays are discussed in detail in the Consensus Guidelines and have recently been reviewed (42). Whereas HLA antibodies can cause allograft damage by direct activation of graft endothelial cells (43) and can directly recruit effector cells (e.g., NK cells) (44), a major way they cause damage is by complement activation. Hence, to better define the potential detrimental effects of IgG antibodies detectable by SPA, IgG subtyping can be performed to determine complement-activating capability (IgG1 and three being strong complement activators, IgG2 and IgG4 weak or not at all). We will discuss in detail here studies addressing IgG subclass analysis in ascertaining the potential pathogenicity of anti-HLA antibodies, a topic not discussed in the Consensus Guidelines. Alternatively, the ability to fix complement *in vitro* can be determined by directly detecting C4d or C1q on SABs by flow or Luminex technology, as noted in the Consensus Guidelines. We will review the data evaluating these two *in vitro* assays, including studies published over the past two yr.

During the process of affinity maturation, IgG subclass switching proceeds irreversibly from subclass 3 to 1 to 2 and eventually to 4. Only IgG3 and IgG1 can fix complement by the classical pathway, although the other mechanisms of injury mentioned above are still possible for the non-complement fixing antibodies (NCF) IgG2 and IgG4. In 94 explanted failed kidney grafts, pan IgG was eluted from 58, which in 16 were NCF. Interestingly, seven of these 16 (54%) were donor specific (45). Another series presented three cases with positive B-cell FCXM but negative by CDC. More

than 50% of the IgG DSA were NCF with favorable short-term outcome, a result suggesting less pathogenicity if NCF subclasses predominate (46). Honger et al. (47) retrospectively evaluated the IgG subclass distribution of 141 HLA DSA detected pre-transplant by SAB in 71 renal transplant patients, 38 of whom had AMR in the first six months. The majority of antibodies were either strong activators of complement (IgG1 and/or IgG3) or mixtures of strong and NCF subclasses (IgG2 and/or IgG4): 21 patients with 48 strong antibodies alone and 46 with 67 mixtures, respectively. Only four patients with nine antibodies total had only IgG2 and/or IgG4 alone. No difference in AMR, GS, or death-censored GS (DCGS) was seen in those with just strong activators versus those with mixtures of strong and NCF antibodies. The group with just weak/non-activators was too small to analyze, but only one had AMR and none lost their graft.

Previously, Arnold and colleagues analyzed re-transplant candidates for NCF antibodies (IgG2/4 and IgA) and found them in 25–40%, mostly against mismatches from the prior failed transplant (48, 49). Recently, Arnold et al. (50) evaluated the effect on GS of NCF anti-HLA antibodies detected post-transplantation in 274 patients. They found that 50 (18.2%) developed NCF, 16 with IgG2/4, 31 with IgGA, and three with both. In 17 of the 50 patients, the antibodies were donor specific. Of the 17, 16 also had strong complement activators (IgG1/3). The presence of a NCF anti-HLA response had no effect on GS, whether DSA alone, non-DSA HLA alone, or all patients combined were considered. In another study, 51 highly sensitized patients with 148 anti-HLA specificities were analyzed by pan-IgG Luminex for subclass determination. As in previous reports, IgG1 was the predominant subclass (approximately 90%), and only one of 138 definable specificities lacked a strong complement fixing subtype (IgG1 or IgG3) (51). Among 284 primary renal transplants screened for *de novo* HLA antibodies, 54 had anti-DQ specificity, including 20 with other specificities as well. Approximately 85% (46) reacted with IgG subclass-specific antibodies. Of these 46, 100% had IgG1, and an 24 additionally had IgG3. The presence of both IgG1 + IgG3 was more likely to be associated with AR than if both IgG2 + IgG4 were present, but only in the subgroup of 34 patients with just anti-DQ (52). By univariate analysis, IgG subclass distribution was not significantly associated with GS, with the exception of the presence of IgG3.

Most recently, 2665 pan-IgG-positive SABs of 8535 total SABs were tested against the sera of 73 sensitized pre-transplant patients. Similar to prior studies, 86% of these pan-IgG-positive beads reacted with subclass-specific antibodies. IgG1 and IgG3 accounted for a median of 99% of the total IgG, and only 4% of subclass-positive beads reacted with IgG2 and/or IgG4 only (53). Hence, the vast majority of patients with anti-HLA antibodies pre- or post-transplant have strong activators of complement (IgG1 and/or IgG3) with or without NCF. Currently, there appears to be no benefit in subclass determination to assess potential pathogenicity in the setting of renal transplantation, although there may be a role with other solid organ transplants. For example, the presence of an IgG3 subclass DSA predicted increased AR and reduced GS in liver transplants (54). Nevertheless, it remains unclear whether the very small percent of renal transplant patients with just NCF antibodies will have a different outcome.

In comparison with IgG subclass determination, the ability of antibodies to fix complement *in vitro* can also be determined. This was first described using flow cytometry (FC) to detect C4d on cell-derived phenotypic beads (multiple HLA antigens per bead) after exposure to recipient serum using normal human serum as a source of complement (55). The methodology was then modified to SAB technology (56, 57). Alternatively, C1q was detected on SAB by adding human C1q without the need for normal human serum (58). About half of sera positive by SAB technology, that is, pan IgG positive, are C1q positive (C1q+), and about one-fourth of these (10–15% overall) will be CDC positive.

Upon evaluating pre-renal transplant PRA by CDC, pan-IgG FCXM, and C4d+ FCXM methodology, decreased function and GS was demonstrated with complement fixation (CDC+ PRA or C4d+ FC PRA) (55). Subsequently, using SAB technology on FCXM IgG+ patients pre-transplant, both C4d+ DSA and C4d+ non-DSA HLA had significantly increased graft dysfunction associated with biopsy staining for C4d, as compared to patients with C4d-HLA. Within the group of DSA+ patients, however, C4d+DSA did not influence renal function or GS, as compared to C4d-DSA. Many in the C4d+DSA+ group were known to be at higher risk requiring peri-transplant immunoabsorption (56). This same group retrospectively evaluated 64 patients with low-level DSA (only SAB positive) and found 11 (17%) C4d+ by SAB using FC. There was no difference in AMR (55% vs. 53%) or C4d peritubular capillary staining (55% vs. 40%) on biopsy (59). In heart transplants, significantly decreased survival was shown with C4d+ DSA pre-transplant, although nearly

half of the C4d+ group also had a positive CDC cross-match (57). A retrospective evaluation of 52 renal transplant patients with DSA only detectable on SAB with FC found that 10 were C4d+. Biopsy-proven AMR was significantly higher in the C4Dd+ group (70% vs. 19%) (60). In general, however, insufficient evidence is available to recommend this C4d+ assay to determine pathogenic potential in questionable cases.

An alternative HLA *in vitro* complement-activating assay involves detection of C1q on SABs after addition of purified human C1q (58, 61). As noted above, it is five times more sensitive than CDC (58). About half of IgG HLA antibodies will give a positive reaction, although as low as 4% was found in one study (62). This assay has been applied both pre- and post-transplant to predict outcomes. The pre-transplant serum of 837 renal transplant patients was retrospectively studied, and IgG DSA was found in 35% (62). Only 30 (4%) were C1q+ with no difference in GS, as compared to the C1q− group. Similarly, the pre-transplant sera of 355 kidney transplants performed across a negative CDC were retrospectively analyzed using SABs (63). Of 28 patients with DSA+ >2000 MFI, 15 were C1q+ and 13 C1q−. No difference was found in AR, graft function, or GS upon comparing both DSA+ groups. In a small series of pediatric heart transplants, five of 18 patients developed early AMR, and two of the five had a positive pre-transplant C1q+ virtual cross-match (64). Interestingly, the remaining three patients were C1q− pre-transplant, but turned C1q+ post-transplant prior to the AR. At the present time, the pre-transplant characterization of HLA or DSA antibodies in terms of C1q positivity is not justified by the available data.

In the post-transplant setting, however, the results are different. Among 193 pediatric renal transplant patients, 19% were found to have developed *de novo* DSA, 43% of which were C1q+ (65). The C1q+ group was significantly more likely to have AR, C4d+ allograft staining, and GL (47% vs. 15%). A small, retrospective pilot study in adults analyzed 31 patients that had a renal biopsy (13 of which were C4d+ and 18 C4d−) with both pre-transplant and concurrent serum available (66). Of the 31 patients, 17 had IgG DSA, 13 of which were *de novo,* and 10 of these 13 were C1q+. There was no difference in DCGS in the C1q+ group, although their absence had a strong negative predictive value for transplant glomerulopathy and graft function. More impressively, 1016 renal transplant patients were followed for the impact of C1q+ DSA detectable in the first year after transplantation (67). The 77 C1q+ DSA patients had significantly worse five-yr GS (54%), as compared to the 239 with C1q− DSA (93%) and the 700 without any DSA (94%), with a multivariable adjusted HR for GL of nearly 5. C1q+ DSA also were associated with AMR, C4d deposition, and more microvascular inflammation. In the study of *de novo* DQ antibodies noted above, both groups of DQ-DSA-positive patients (34 with DQ only and 20 with DQ plus other DSA) had significantly greater AR rate if C1q+ than if C1q−, although C1q+ did not significantly affect GS (52). In the study of Schaub et al. noted above on 2665 pan IgG+ SABs, about 25% were C1q+ (53). Sensitivity was enhanced by an anti-human globulin modification, with about 50% now C1q positive by that assay. As noted above, however, strong complement fixing IgG subclasses (IgG1/3) were present on 99% of beads. Hence, a negative C1q reaction did not preclude strong complement binding subclasses (53).

As either IgG1 and/or IgG3 are nearly universally present, the ability to fix complement *in vitro* may simply be a surrogate for the intensity of the humoral response, that is, the pan-IgG MFI. Some studies note a significant relationship (53, 62, 63), whereas others do not (58, 66). For example, in the Schaub study cited above, IgG pan MFI alone showed a strong correlation with C1q+ ($r^2 = 0.72$) (53). Whereas in their original description of the C1q assay (58), a correlation was not found. Lachmann et al. compared standard CDC with pan IgG by Luminex SAB, C1q Luminex SAB, and a modified C4d Luminex SAB assays in 45 highly immunized patients (pan-IgG Luminex virtual PRAs of ~98%, CDC PRAs of 56%) (68). They found excellent concordance between C4d and C1q assays, with both detecting about three-fold more specificities than CDC. Of note, pan-IgG luminex MFI often did not predict C1q and C4d positivity, with some low MFI pan IgG assays having high MFI complement activation (usually against relevant epitopes from prior failed transplants) and vice versa. This same study confirmed that average MFIs for the C1q assay are 10-fold higher than found with C4d (68).

Possible explanations can be adduced for why the MFI of a given HLA antibody may not correlate with a positive C1q assay. A previous study demonstrated that combinations of antibodies directed against different epitopes on an HLA antigen may result in complement activation, even if each is not capable individually (69). It has been hypothesized that recognition of the functional eplet may be sufficient to bind the antibody (pan IgG positive). To fix C1q, however, a higher degree of interaction between structural epitope and anti-

body paratope is required (i.e., more CDR interactions are involved) and even more interaction may be required to proceed to further complement activation, that is to reach C4d (70). Another possibility is the prozone effect, whereby a false-negative relationship occurs with neat (undiluted) serum owing to a very high concentration of antibodies that becomes positive with dilution. In addition, pan-IgG detection on bead assays can be inhibited by complement components, an observation that may also help to explain the prozone phenomenon A recent study using both SAB and multiple antigen bead assays showed that complement activation proceeded through C3 activation, including alternate pathway participation (factor B), with resulting covalent binding of C4d and C3d (71). The result was inhibition of pan-IgG detection as well as interference with C4d and C1q detection.

In summary, it remains to be established whether IgG subclass determination or detection of *in vitro* complement activation can direct therapy in the individual patient.

Conclusions

The evaluation of humoral immunity in renal transplantation continues to evolve. Epitope analysis is a potential way to better define the antigenicity and immunogenicity of HLA mismatches. Such analysis has the potential to improve organ allocation, allow tailoring of immunosuppression, and affect post-transplantation events such as graft survival and future sensitization. Randomized controlled trials are needed to test the validity of this hypothesis and to compare any benefit to the increased cost, time, and labor required. Antibodies against the Cw and DP loci can mediate graft injury and should be considered when mismatches are present, especially when antibodies against HLA-A, B, and DR are not detected. At the present time, the utility of IgG subclass determination of HLA antibodies is not defined and requires more study. It also remains to be determined whether *in vitro* complement fixing capability can guide therapy in the individual patient.

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