Approach to the Highly Sensitized Patient

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Introduction

All recipients of non-genetically identical solid organ transplants are at risk for rejection *initiated by alloantigens* and must be pharmacologically immunosuppressed to prevent rejection mediated by T cell recognition of these non-self HLA molecules or peptides as foreign. However, a potential transplant recipient may also be at risk for rejection *initiated by alloantibodies* if they were exposed pretransplant to foreign antigens that are also present on the allograft. When these preexisting alloantibodies are directed against ABO group antigens, HLA class I antigens, endothelial-monocyte antigens and perhaps HLA class II antigens that are also present on the allograft, they can initiate immediate (hyperacute) or delayed humoral immune responses against the graft [1, 2]. Patients who possess such alloantibodies prior to transplantation are considered to be "sensitized". Highly sensitized patients, especially those who have high levels of circulating anti-HLA class I antibodies, face significant difficulties in finding a compatible donor and encounter a worse prognosis for the organ after transplantation [3, 4]. According to the Organ Procurement and Transplant Network (UNOS data), graft survival rates for patients transplanted between 1997-2004 in the United States were 72.2 % at five years for those with a PRA of 0–9 % versus 65.7 % for those with a PRA of 10–80 % [5]. As such, these patients present difficult challenges to transplant physicians and to organ allocation systems. Until recent years, these patients faced hopelessly long waits for a crossmatch-negative kidney. However, in the past several years much progress has been made in managing highly sensitized patients by more optimally pairing the recipient with a compatible organ and, more recently, by suppressing the powerful humoral immune response elicited by the alloantibodies.

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Definition, Quantitation, and Monitoring of Sensitization

Sensitization is defined as the presence of preformed alloantibodies in the serum of a prospective transplant recipient [3]. In other words, it is pretransplant humoral alloimmunization. These alloantibodies are usually anti-HLA class I antibodies but may also include anti-HLA class II or non-HLA antibodies [3]. They are formed in response to prior exposure to foreign antigens encountered during events such as blood transfusions, prior transplants, and pregnancies [6]. (Of note, in addition to this humoral sensitization, there also appears to be donor-reactive T cell sensitization ("cellular sensitization"), which is measured with a delayed type hypersensitivity assay [7]. To what degree this phenomenon may be present pretransplant and manifest posttransplant is not clear.)

Anti-HLA antibodies are conventionally known as panel-reactive antibodies, or PRA, and they are quantified as the percentage of PRA that are reactive. Historically, this has been determined by testing the potential recipient's serum against a panel of lymphocytes harvested from 40 to 60 HLA-typed individuals who were chosen to represent the widest variety of HLA antigens, and an antibody-activated, complement-dependent cytotoxic (CDC) assay is used to detect antibodies against donor lymphocyte surface antigens in the recipient serum. The breadth of variety of antibodies present in the potential recipient's serum is determined by calculating the percent of donors in the panel whose cells are killed. Currently there are a variety of assays used to measure the PRA [8]. A discussion of the intricacies of the various methods is beyond the scope of this chapter and is addressed in an earlier chapter. However, in order to clarify what each test can reveal about a given recipient's level of sensitization, a discussion of some salient aspects of the methods that may be used in this setting will follow.

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The *NIH standard technique*, which has been used in much of its present form since the 1960s, essentially involves isolating each donor's peripheral T cells, placing them in individual wells of a plate, adding recipient serum, incubating, adding rabbit complement to induce killing of cells if complement-fixing antibodies directed against the donor lymphocytes are present in the recipient serum, and then distinguishing live from dead cells using a vital dye. A technologist then identifies which individual donor wells demonstrated cytotoxicity and scores the PRA as the fraction (expressed as percentage) of donors whose cells elicit a positive reaction. The sensitivity of this technique in identifying anti-donor antibodies suffers if the antibodies are not efficient enough or are not present in sufficient numbers to activate complement. The *antihuman globulin (AHG)-enhanced technique* includes the addition of antihuman immunoglobulin antibody to augment the ability of the recipient antibodies to activate complement by providing cross-linking molecules, and thereby, it increases the sensitivity of detecting these alloantibodies. Both of these cytotoxic assays (especially the more sensitive AHG-enhanced technique) will generally detect not only antibodies directed against HLA but also those directed against other non-HLA antigens that reside on lymphocytes. The latter antigens should not be present on the allograft cells and may therefore be clinically irrelevant. Also, the assays may not differentiate between immunoglobulin G (IgG) and IgM antibodies, and this distinction can be important because IgM antibodies are generally considered to be benign in this context as they are generally not directed at HLA antigens and are often drug induced. Dithiothreitol (DTT) destroys IgM and therefore is added to eliminate the effect of IgM in these PRA assays. However, it may also cause a weak IgG antibody response to disappear, thereby diminishing the sensitivity of the assay.

The *flow cytometry technique* uses soluble HLA molecules that are bound to beads, which are used in place of live donor T cells. The HLA molecules are either class I or class II and are, as with the cytotoxic assays, chosen from typed individuals with a wide spectrum of HLA antigens. Recipient serum and then antihuman antibodies labeled with a fluorochrome are then incubated with beads, and finally the antibody-labeled beads are passed through a flow cytometer that measures the intensity of light emitted from the fluorochromes and mathematically converts it into a percent PRA. Still another method uses *enzyme-linked immunoabsorption* (ELISA) methodology rather than flow cytometry to detect antibodies. The main advantage of these two molecular HLA (as opposed to live cellular) assays in the field of pretransplant PRA testing is that they specifically detect anti-HLA antibodies and not other molecules on the lymphocyte that have no significance in alloreactivity. Other advantages are that (1) the flow cytometry and ELISA techniques are considered to be more sensitive than the complement-dependent cytotoxicity assays, (2) they can distinguish between IgG and IgM antibodies, and (3) they can determine if the antibodies are directed against HLA class I or class II or both. Moreover, because ELISA reactions can be examined in a multiwell plate, determination of the specificity of HLA antibodies is possible with this technique.

Although various regulatory bodies (UNOS, HCFA, etc.) may dictate which tests to use and how often they should be done, a PRA should generally be measured at least every 3 months on all patients on the cadaveric waiting list. Because kidney transplant candidates with high PRAs statistically face longer wait list times for compatible organs, they are given priority in the cadaveric renal allograft allocation system for potentially compatible organs. As discussed later, PRA levels are instrumental in guiding cost- and (cold ischemia) time-efficient crossmatching immediately prior to deceased donor renal transplantation, and they impact greatly on posttransplant allograft outcome.

Various terms and definitions have been used to grade the degree of sensitization. Although there appears to be a graded or stepwise effect on outcomes [9], the term "highly sensitized" or "broadly sensitized" is applied to those who have a PRA of at least 30–50 %. A designation of "unsensitized" has been applied to patients with a PRA of 0 % up to 10 %. Some authors hold that the peak pretransplant PRA is more predictive of graft outcome than the PRA level at the time of surgery [8, 10] and that therefore the sensitization status a given patient may best be determined by the highest pretransplant PRA. Other investigators have argued that only those antibodies present in the serum at the time of transplantation (current crossmatch) are relevant to outcome [11]. The peak PRA may be less pertinent if histocompatibility laboratories have a conscientious sampling and screening program that excludes recipients from receiving allografts that harbor mismatched antigens against which the recipient has ever developed specific antibodies [12].

Importance of Sensitization

UNOS data indicate that approximately 20 % of patients on the transplant wait list have a PRA of greater than 20 % and in France more than 50 % of patients have reactive PRAs [48]. Although hyperacute rejection (HAR) with immediate and universal graft loss historically was a common occurrence in these patients, this problem has been largely eliminated using sensitive crossmatch techniques. Still, sensitized patients wait longer for compatible allograft [3] and are at increased risk for early acute humoral rejection [13–17] and have worse short-term and long-term outcomes [4].

The presence of anti-HLA antibodies present posttransplant is associated with acute and chronic rejection as well as decreased graft survival in various organs transplanted,

including kidneys [18]. Renal transplant patients with posttransplant HLA alloantibodies were 5–6 times more likely to develop chronic rejection [19, 20]. Animal and in vitro human models suggest that a repair response to donor-specific antibodies may result in arterial thickening associated with chronic rejection [21]. To what extent patients with pretransplant sensitization may be at risk for this phenomenon of posttransplant alloantibody-induced graft pathology and to what extent posttransplant humorally mediated rejection involves a de novo versus an amnestic response is not yet clear. Highly sensitized patients are clearly at increased risk for antibody-mediated (humoral) rejection, and a thorough discussion of this is beyond the scope of this chapter.

Causes of Sensitization

There are three primary sources of sensitization of kidney transplant patients: pregnancy, blood transfusions, and prior transplants [8, 22]. All three of these situations may present the potential recipient's immune system with "a look at" foreign antigens, including HLA molecules. If the potential recipient is not immunosuppressed, he or she will appropriately produce antibodies against these alloantigens. These sensitizing events appear to have a cumulative and interacting impact on the PRA.

Blood transfusions: Early in the history of solid organ transplantation, the "transfusion effect" was observed by Opelz et al. [23] and others [24] when they demonstrated a benefit on graft outcome if preoperative blood transfusions were given in combination with immunosuppressive drugs or X-radiation [25, 26]. The mechanism of this beneficial effect has still to this day not been elucidated. It has been theorized that (a) it may allow for preselection of a population with a high response (that later will presumably fail crossmatch when reexposed to antigens), (b) it may induce clonal deletion or activate suppressor mechanisms of alloreactive T cells, or (c) it may block allo- or anti-idiotypic antibodies [27]. Whatever the case, over the years, it has also become clear that blood transfusions may also induce sensitization, and by the late 1990s, the previously noted beneficial "transfusion effect" had given way to a deleterious effect, with worsening graft survival associated with the greater numbers of transfusions in sensitized and nonsensitized patients [28].

Prior transplants and pregnancies: The rate of sensitization seems greater in regraft as compared to initial graft recipients. The sensitizing effect of pregnancy appears to be more important in initial transplants than in retransplants. Increasing numbers of transfusions are associated with increasing PRAs, and the effect seems to be modulated by sex and pregnancies [28]. It has been hypothesized that pregnant women may be sensitized at the time of delivery with exposure to parental HLA antigens expressed by fetal cells [29].

Other as yet unidentified factors: Among patients receiving their first kidney transplant with no known history of blood transfusions, approximately 20 % of nulliparous women and 13 % of men were sensitized (PRA > 10 %) [27]. It is not clear how such a large proportion of patients lacking risk factors became sensitized. As this observation was noted in patients who actually received a kidney and as the prevalence of sensitization is overall higher in wait-listed patients, it is likely that a higher percent of such patients on the transplant wait list may be sensitized. Certainly, underreporting of these sensitizing events may have occurred. Additional factors in women may have included unrecognized pregnancies, alloantigenic stimulation from sperm, or an augmenting estrogen effect [30].

Pathogenesis of Sensitization

Essentially, sensitization develops when a non-immunosuppressed patient is exposed to foreign human cells that have HLA molecules and other surface antigens that are recognized as nonself. A humoral response ensues, and it appears to be initiated via the T cell-dependent, Th2 cytokine-driven humoral response (as opposed to T cell-independent B cell activation). Recent studies have demonstrated that a Bcl-6-expressing T cell subset found with B cell follicles (i.e., T follicular helper cells (Tfh)) is important in the development of germinal center B cells. However, because B cells and plasma cells have short life spans, it is not clear how a patient may sustain an anti-HLA antibody response and thus a high PRA without ongoing antigenic stimulation. Possible explanations include (a) the persistence of residual donor protein antigens in long-lived follicular dendritic cells, (b) the presence of cross-reactive environmental antigens, or (c) the development of chimerism (the presence of donor stem cells in the host) or microchimerism (small numbers of such cells) [31]. In support of theory of a chimeric mechanism, one group has identified non-genomic DNA in sensitized patients (Y chromosome material in sensitized females) [30], and another group demonstrated more than two HLA-DR antigens in a higher percentage of sensitized as compared to nonsensitized individuals [32]. In addition, it has been described that a small proportion of plasma cells become established as long-term antibody factories, producing IgG, in the bone marrow, or within a number of limited niches (i.e., allografts, inflamed tissues), and antibodies are the critical mediators of humoral immunity [33]. Tertiary lymphoid organs have also been observed in human renal allografts suggesting possible direct B cell activation in the graft [34]. B cells produce VEGF-A and lymphotoxin-β, which drive

lymphoid organ formation and lymphangiogenesis, pointing to a role in how they develop within allografts [33].

Immunobiology of Rejection in Sensitized Recipients

In 1966, it was reported that sensitized recipients of renal allografts may undergo fulminant rejection within minutes or hours of implantation [35], and it became clear that the process, known as HAR, was the result of the preexisting antibodies that bind to donor allograft endothelium and activate complement cascade with subsequent rapid immune and ischemic destruction of the graft. With the implementation and refinement of pretransplant crossmatching techniques, this disaster has become exceedingly rare. Still, a highly sensitized patient who does eventually receive a kidney transplant faces a more hostile posttransplant immunologic environment with a higher risk of rejection, including the accelerated humoral form (ACCR). Although HAR and ACCR are probably on the same continuum in terms of pathophysiology (both induced by preformed alloantibodies), the onset and tempo differ. It may be that the level of preexisting antibodies at the time of engraftment may determine whether HAR (if levels are high) or ACCR develop. If anti-donor alloantibody levels are low (perhaps too low to be identified with a cytotoxic crossmatch), then ACCR may develop as titers rise to a level sufficient to activate complement following reexposure to the antigen on the allograft [3]. Some investigators define HAR as rejection within 24 h and ACCR as rejection within the first few days. In both forms of early rejection, microthrombi with aggregates of fibrin, red blood cells, and platelets occlude small arteries and glomeruli; polymorphonuclear neutrophils (PMNs) accumulate within glomeruli, juxtaglomerular arterioles, and intertubular capillaries. Meanwhile, medium and large vessels are spared. Typically, complement C4d is observed with immunofluorescence staining in the peritubular capillaries (PTCs) and circulating anti-donor antibodies can be detected on crossmatching [26]. (Of note, important diagnostic criteria of acute humoral rejection include PTC C4d deposition, granulocytes in PTC, and severe renal allograft dysfunction [36].) If the process is not reversed, intense vasospasm and intravascular microthrombi causes ischemic necrosis of the parenchyma and permanent loss of graft function.

The time course of events during early allograft rejection in heart allografts transplanted into highly sensitized patients has been elucidated, and it probably parallels that which occurs in renal allografts in this setting. Early in the course of the process, the graft endothelial cells are coated with dense deposits of IgM, IgG, and complement C3, with only trace amounts of fibrin. Then, fibrin deposition on capillary and small vessel endothelium increases progressively such that by 18–24 h, dense fibrin deposits occlude essentially all capillaries and venules. Graft cellularity rapidly increases, with the appearance of polymorphonuclear leukocytes (PMNs) within the first hour after engraftment. By 3–6 h, cell adhesion molecules such as E-selectin and ICAM-1 appear, and this is followed by deposition of the extracellular matrix proteins laminin and fibronectin and then the massive influx of PMNs and mononuclear cells (>75 % macrophages, 10–20 % T cells/natural killer cells, <1 % B cells). Cytokines clearly are involved in this rejection process as well, with expression of tumor necrosis factor (TNF)-α, interleukin (IL)-12, IL-1β, IL-6, IL-2, and interferon (INF)-γ [26].

Although the humoral arm of the immune system has received most of the attention in discussions of the mechanism of early rejection in these highly sensitized patients, it has become clear that cellular immunity is also involved. Although preformed antibodies initiate the process, current theories posit an interdependence of the two pathways with key roles for CD4 T cells and macrophages along with their cytokine mediators in the initiation and control of B cell differentiation and immunoglobulin production. In fact, both Th1 and Th2 cytokine elaboration profiles are observed. Contrary to what would be expected in a largely humorally mediated rejection, the Th1 subsets (which include IL-2 and INF-γ and which promote cell-mediated immunity and tend to suppress the humoral response) seem to dominate the Th2 subsets (which typically promote antibody production by B cells). These Th1 cytokines as well as IL-12 seem to effect an isotype switch of IgG to the IgG2b subclass, which appears to deposit at intragraft epithelial cells and seems to be very effective in inducing complement activation [26].

Strategies to Optimize Outcomes in Sensitized Patients

Limit alloimmunizing events: Avoiding transfusions and pregnancies in patients who may eventually need kidney transplantations may lessen exposure to alloantigens. Two studies [37, 38] have suggested cyclosporine administration with pretransplant transfusions ("under cyclosporine cover") may prevent the development of alloimmunization and furthermore may improve posttransplant graft survival and increase anti-idiotypic antibody activity. In the second study cited above [35], cyclosporine was started 4 days prior to transfusion and continued until 1 month after. The feasibility and safety of this strategy in patients with advanced chronic kidney disease or hyperkalemia who are not yet on dialysis obviously may be problematic, and it does not appear to have achieved widespread use. Although to our knowledge no studies have shown convincing benefits of leukoreduction (to remove HLA-bearing leukocytes) of transfused products on

sensitization rates in this population, leukocyte filtering of blood products is routinely done [39].

Pretransplantation Crossmatching

The pretransplant crossmatch tests for the presence of donor-directed antibodies in the sera of a potential transplant recipient may doom the allograft to early humoral rejection and more rapid graft failure. It is used primarily to prevent hyperacute rejection, an immediate and irreversible HLA class I antibody-mediated rejection of the allograft. While universal use of basic crossmatching techniques has relegated hyperacute rejection to the history books, more sophisticated techniques of crossmatching have extended their use to also identifying the presence of less potent alloantibodies that do not produce hyperacute rejection but may predict less fulminant adverse immunologic outcomes [7].

While nonsensitized patients are extremely unlikely to have positive crossmatches, the highly sensitized patient is much more likely to be eliminated from consideration for transplantation from a given deceased donor or living donor by a positive pretransplant crossmatch. So as not to deprive a highly sensitized patient any realistic hope of ever receiving an allograft, organ allocation systems and individual clinicians may have to accept less than optimal highly sensitive crossmatch results and the attendant increased immunologic risk. An understanding of the implications of the results of the various crossmatching techniques is critical in optimizing the matching of these highly sensitized recipients with an organ.

The basic procedure of the CDC crossmatch techniques is essentially the same as the PRA tests that were discussed above, with the important difference being that the recipient's serum is tested against the specific donor candidate's lymphocytes rather than against a panel of anonymous individuals' lymphocytes. The NIH standard technique is the least sensitive but the most specific crossmatch assay. If recipient's serum produces a complement-mediated cytotoxic reaction with this test, then it strongly suggests the presence of anti-HLA antibodies and is highly predictive of hyperacute rejection. A positive result has been considered an absolute contraindication to transplant of renal, pancreas, and heart transplants (although aggressive treatments may be capable of converting a positive to a negative result in certain settings). However, the test may fail to detect small amounts of HLA class I or II antibodies such that a negative test cannot exclude the possibility of poor immunologic outcome. In the late 1960s, when this test was first used clinically, sensitized patients demonstrated an 80 % immediate graft failure rate if the pretransplant NIH standard crossmatch was positive. However, a negative test in a sensitized patient was still associated with a 15 % immediate graft failure rate (i.e., many grafts would still be squandered if allocated to sensitized patients with a negative assay) [1].

The AHG-enhanced technique (as discussed previously) increases the sensitivity of the NIH assay by adding AHG to crosslink antibodies that may not have been numerous or efficient enough to activate complement by themselves. When the AHG-enhanced assay is positive after the standard NIH test was negative, the antibodies that were insufficient to cause lysis of cells in the unenhanced test are likewise probably not capable of causing hyperacute rejection. Nevertheless, the presence of these antibodies in the recipient seems to adversely impact graft survival to the extent that it is widely considered prudent to forgo transplantation of the recipient–donor pair.

The above crossmatch assays generally use live donor T cells as targets for recipient alloantibodies, but in certain circumstances, B cells may be substituted for T cells. The latter is known as the *B cell crossmatch*, and it is used to detect anti-HLA II antibodies. Resting T cells express only HLA class I and not class II antigens on their surface. Class I HLA molecules are present on the endothelial and interstitial cells of the transplanted organ, and these are felt to be the primary targets of the alloimmune response. Class II molecules have a much more restricted tissue distribution than class I molecules, but can be induced to be expressed on injured endothelium and interstitial cells of renal allografts. Although class I antigens appear to be responsible for most episodes of HAR, anti-HLA class II antibodies appear to also adversely impact graft survival, with a more delayed rather than immediate effect (although there have been rare reports of HLA class II antibody-induced hyperacute rejection). Substituting B cells, which express class II antigens, for T cells in the CDC assay allows for the detection of anti-donor class II antibodies. Among recipients with a negative standard T cell crossmatch, those with a negative B cell crossmatch demonstrated improved 2-year survival as compared to those with a positive result. The test seems to be most discriminating in retransplant patients [40–42].

Flow cytometry technology may be used in place of the CDC assay to identify the presence of donor-directed antibodies in recipient serum. When using the flow cytometry crossmatch (FCXM), recipient serum is mixed with lymphocytes from the potential donor, and then this mixture is incubated with fluorochrome-labeled, xeno (mouse, goat, or other species) antihuman immunoglobulin antibodies. When recipient antibodies bind to antigens on the donor lymphocytes, fluorochrome-labeled AHG antibodies will conjugate with the recipient antibodies. The lymphocytes (rather than the HLA-coated beads used to determine the PRA, as discussed above) are then passed through the flow cytometer. If the lymphocytes have alloantibody-AHG complexes attached, the fluorochrome will be activated by the flow cytometer laser beam and emit photons that will be measured

as a quantitation of the amount of anti-donor antibody present in the serum. Donor lymphocytes may include T cells, B cells, or both, depending on whether the clinician hopes to identify HLA class I or HLA class I and II antibodies. As opposed to the CDC crossmatch techniques, live donor lymphocytes are not needed for the flow cytometry crossmatching and previously frozen donor cells may be used. This may be a critical advantage in certain situations when live donor lymphocytes are not available or if posttransplant testing for donor-specific alloreactivity is needed (e.g., to assist in the diagnosis of humoral rejection). This technique is able to detect very low levels of circulating antibodies and is therefore very sensitive and may be positive when CDC crossmatches are negative. The FCXM, however, lacks specificity, and a positive result does not necessarily doom the graft to immediate rejection. A review of UNOS Scientific Transplant Registry data in the late 1990s [43] indicated that a positive FCXM (T cell or B cell) was associated with suboptimal allograft function as evidenced by an increased need for posttransplant dialysis, higher incidence of primary nonfunction, longer hospital stays, and a higher incidence of rejection. The impact of graft survival was greatest among retransplanted patients (60 % 3-year graft survival with a positive FCXM versus 79 % with a negative FCXM, $p=0.003$) although significant differences were also noted in primary transplants (76 % 3-year graft survival with positive FCXM versus 81 % with negative FCXM, $p<0.001$). Although a subsequent single-center retrospective study suggested that a positive FCXM (of course with a negative AHG-enhanced CDC crossmatch) did not have a negative impact on graft survival or rejection frequency [44], a more recent prospective single-center study indicated that low-level preformed alloantibodies detected by FCXM represents a risk for rejection even in those patients with no additional immunologic risk factors and that the risk seemed to be due to donor-specific memory rather than to a direct effect of the antibodies [45].

Properly using these tests is critical. It is important not to waste precious organs by failing to identify an incompatible match, and it is likewise important to not deny the highly sensitized patient a rare opportunity to get an acceptable allograft because of a false positive crossmatch. The University of Maryland has adopted a policy of selective application of the FCXM to optimize allograft selection, cost, and cold ischemia time. Because of the higher pretest probability of HLA alloimmunization in highly sensitized patients and thus the lower risk for false positives (i.e., a higher positive predictive value), the FCXM is performed in potential recipients with a PRA over 40 %. Additionally, all potential recipients who had a prior transplant receive the FCXM because a positive test seems to be a more strongly negative prognostic factor in this population. The FCXM is performed simultaneously with the AHG crossmatch if sequential AHG-then-flow testing would increase cold ischemic time. The combination of negative AHG crossmatch and positive flow crossmatch is not considered an absolute contraindication as the anti-donor antibody level is relatively low (below the detection level for the less sensitive AHG assay) in this situation such that hyperacute rejection is unlikely. Depending on the situation, the transplant surgeon decides whether to bypass the potential recipient or to proceed with the transplant under cover of antilymphocyte antibody ± rituximab induction and plasmapheresis (to prevent accelerated humoral rejection).

Immunosuppression: Aspects of immunosuppression of highly sensitized kidney transplant patients include desensitization therapy prior to transplantation, induction therapy with transplantation, maintenance therapy after transplantation, and rescue therapy in the event of acute humoral rejection. In addition to the standard immunosuppressive agents used, the following modalities have been found to be useful in controlling the antibody-mediated response in certain highly sensitized patients in the desensitization, induction, and rescue phases of therapy.

Intravenous immunoglobulins (IVIg): IVIgs are commercially prepared mixtures of IgG derived from pooled human plasma from at least 50,000 to 100,000 screened donors, and they probably contain the entire complement of antibodies that are found in normal human serum. While the composition is >90 % intact IgG, some dimers or aggregates, a few F(ab′)2 fragments, and traces of IgM and IgA are also present. Multiple theories have been proposed to explain the immunomodulatory effects of IVIg, and the mechanism may vary depending on the setting and the indication of use. Its mechanism of action in the setting of the sensitized transplant patient may involve reduction or neutralization of alloantibodies via anti-idiotypic binding, inhibition of inflammatory cytokine generation, inhibitory binding of complement components with inhibition of complement-mediated injury, and inhibition of antibody production. It is now recognized that IVIg can also modify antigen-presenting cell and B cell activity. It causes a number of inhibitory effects on B cells through upregulation of the inhibitory receptor FcγIIB, leading to crosslinking and then apoptosis of plasma cells. IVIg can also inhibit growth factors for B cell development and maturation [46]. There are data demonstrating inhibitor effects of IVIg on T cell proliferation in culture, which was associated with significant reduction in co-stimulatory and adhesion molecules. It has also recently been shown in vitro and in vivo (in humans with autoimmune disease) to enhance the expansion and effector function of Tregs [46]. IVIg has shown success in treating steroid and antilymphocyte–antibody-resistant rejection episodes, suggesting that IVIg exerts its antirejection effects via different

mechanisms than that of standard anti-T cell therapies [47]. Whatever the exact mechanism, the beneficial effects of IVIg on the alloimmune response appear to persist long after the half-life of the IgG, suggesting that it has the ability to produce long-term suppression of antibody production.

At least five trials have used IVIg in the treatment of highly sensitized patients as part of a desensitizing protocol:

1. Leffell et al. at Johns Hopkins [48] provided plasmapheresis and IVIg until crossmatch was negative and they gave methylprednisolone 500 mg per day for three doses to four patients before or during this course. Maintenance immunosuppression consisted of triple therapy with tacrolimus, mycophenolate mofetil (MMF), and prednisone. All four patients were transplanted and subsequently developed antibody-mediated rejection that was treated successfully with IVIg/plasmapheresis with no graft loss and serum creatinines ranging from 0.8 to 1.2 mg/dL at follow-up periods ranging from 4.4 to 17 months.
2. Schweitzer et al. at the University of Maryland [49] utilized a protocol in 15 AHG-crossmatch positive live donor kidney transplant recipients that included preconditioning with plasmapheresis three times weekly for a maximum of six treatments along with IVIg, tacrolimus, MMF, and prednisone. The 11 patients who were successfully desensitized were given 10 days of OKT3 following living donor transplantation. Three patients developed antibody-mediated rejection, and each case was successfully rescued with additional plasmapheresis, OKT3, or antithymocyte globulin (ATG), and IVIg. A fourth patient developed mild acute cellular rejection that was treated successfully with pulse steroids. All 11 patients were dialysis free and the group had a mean serum creatinine of 1.6 ± 0.2 (range 1.1–2.4) mg/dL at mean follow-up period of 13.3 ± 2.4 months. The protocol was well tolerated without excessive infectious complications. Of note, two patients received cadaveric pancreas transplants simultaneously with the kidney transplantation. One was lost to accelerated chronic rejection, and the other to early thrombosis which may have been caused by antibody-mediated rejection.
3. Glotz et al. from Hospital Europeén Georges Pompidou [50] utilized a regimen of IVIg given as three monthly courses of 2 g/kg body weight. Thirteen of 15 patients with a PRA of at least 50 % were successfully desensitized (defined as at least 50 % decrease in PRA) and underwent immediate transplantation, 11 of whom received the first available ABO-matched, IgG T cell crossmatch-negative cadaveric kidney and two of whom received a living donor kidney against which pretreatment crossmatch was positive. Posttransplant immunosuppression consisted of Thymoglobulin®, tacrolimus, MMF, and steroids. One graft was lost from thrombosis and one from rejection. All other patients had uneventful courses, without any episodes of rejection at a mean follow-up of more than 1 year.
4. The NIH sponsored a randomized, double-blind, placebo-controlled clinical trial (the IGO2 study) to compare IVIg versus placebo in highly sensitized patients awaiting kidney transplantation. Between 1997 and 2000, 101 patients with ESRD who were highly sensitized to HLA antigens (PRA $\geq$ 50 %) received either 2 g/kg body weight of IVIg monthly for 4 months or placebo infusions. If transplanted, patients were given additional monthly infusions for 4 months. IVIg significantly reduced PRA levels in study subjects compared with placebo. Sixteen IVIG patients (35 %) and eight placebo patients (17 %) were transplanted. Seven graft failures occurred (four IVIG, three placebo) among adherent patients, with similar 2-year graft survival rates (80 % IVIG, 75 % placebo). Viable transplants functioned normally with a mean ± SEM serum creatinine of 1.68 ± 0.28 for IVIG versus 1.28 ± 0.13 mg/dl for placebo after a median follow-up of 2 years posttransplant. They concluded that IVIg was effective in reducing anti-HLA antibody levels and improving transplantation rates in highly sensitized patients with ESRD. In addition, IVIg desensitizes highly sensitized patients and offers them significant transplantation opportunities without excessive graft loss [51].
5. Montogmery et al. from Johns Hopkins Hospital enrolled 211 HLA-sensitized patients who underwent living donor kidney transplantation (215 patients were desensitized, 4 did not undergo transplantation). They compared rates of death between the group undergoing desensitization and two matched control groups from the UNOS kidney transplant waiting list who either continued with dialysis or who underwent transplantation. Patients received plasmapheresis and then IVIg at a dose of 100 mg/kg after each pheresis session. Numbers of treatments varied depending on the level of donor-specific anti-HLA antibody at baseline, with the goal of conversion to a negative crossmatch before transplantation. MMF and tacrolimus were given with PP before transplantation and induction therapy consisted of daclizumab or Thymoglobulin with intraop and post-op steroids. The average calculated PRA was 82 and 32 % of patients had PRA of 98 % or more. During the overall study periods, desensitization was associated with a significant increase in the rate of patient survival, as compared with the rates in the dialysis-only group and the dialysis organ transplantation group. The survival rates in the treatment group were similar up to 12 months with the dialysis-only group and up to 18 months with the dialysis-or-transplant group, but were higher in the treatment group compared to both control groups, thereafter. The survival benefit was preserved in all three subgroups based on the level of donor specific anti-HLA antibiody [52].

As can be seen, various regimens that employ IVIg in combination with other modalities such as plasmapheresis and immunosuppressive drugs resulted in the successful transplantation of patients who would otherwise have been considered non-transplantable.

The main side effects associated with IVIg therapy have been infusion-related reactions that can usually be controlled with slowing the rate of infusion or treating with anti-inflammatory drugs, very rare anaphylactic transfusion reactions, acute renal failure which appears to be secondary to tubular toxicity related to osmolarity of sucrose-containing preparations and which can be prevented by avoiding such preparations, and acute thrombotic events such as myocardial infarction, deep vein thrombosis, central retinal vein occlusion, stroke, and pulmonary embolism. Various IVIg preparations are available, and these vary in terms of diluent, sodium content, sugar moiety, and content and osmolarity. The side effect profiles differ significantly among the preparations, with higher concentration sucrose mixtures having a higher incidence of acute tubular necrosis and lower concentration products causing more problems with volume overload. Difficulties obtaining Medicare and private insurance coverage for this expensive drug have limited its usefulness in the highly sensitized potential renal transplant [43].

Protein A immunoabsorption and plasma exchange have been used in combinations with other modalities and are very effective in rapidly removing alloantibodies from serum. These modalities have shown success in the desensitization regimens as well as rescue/treatment regimens in patients with humoral rejection.

Rituximab is a chimeric murine human monoclonal antibody directed against the B cell surface molecule CD20. CD20 is not found on pro-B cells or mature plasma cells, so rituximab attacks peripheral B cells and does not prevent the regeneration of B cells from precursors or directly affect immunoglobulin levels. Several groups have reported reduction in DSA titers, and there is a fair amount of published data on its use in combination with IVIg and/or plasma exchange for desensitization and treatment of ABMR. For example, Vo et al. showed that high-dose IVIg in conjunction with anti-CD20 antibody, rituximab, improved transplantation rates of highly allosensitized living and deceased donor kidney transplant candidates. Twenty patients received 2 g/kg body weight IVIg and two infusions of rituximab. PRA levels decreased significantly following treatment, and 16 patients were transplanted within 4 months of treatment, though 13 of 16 recipients had persistent positive crossmatches at the time of transplantation. The AMR rate after transplantation was 31 % but the 1-year allograft survival was 94 % [53]. Addition of rituximab to the IVIg (NIH protocol) improved transplant rates for deceased donors to 73% [54]. Thielke et al. reported that a negative crossmatch was successfully achieved in 51 of 57 positive crossmatch patients treated with antithymocyte and anti-CD20 antibody induction therapy in addition to plasma exchange and low-dose IVIg. The rate of allograft survival was 93 % at 1 year and 81 % at 2 years [55]. Stanley Jordan's group from Cedars-Sinai Medical Center is currently conducting a placebo-controlled, multicenter, randomized clinical trial of high-dose IVIg ± rituximab for desensitization to help determine if rituximab is superior to IVIg alone in improving rates of transplantation for highly sensitized deceased donor candidates on the UNOS waiting list (NCT01178216). A preconditioning regimen consisting of rituximab infusions and a splenectomy was also found to be effective in allowing transplantation and preventing early rejection (which would be expected to be humorally mediated) in a recipient of an ABO-incompatible kidney transplantation after conventional preconditioning regimen with plasmapheresis had failed [56].

In addition to these novel treatments, antilymphocyte–antibody induction is probably necessary to prevent reformation of removed antibodies and to suppress cell-mediated rejection in these high-risk patients. In a randomized, controlled clinical trial, ATG showed benefit when added to cyclosporine, steroids, and azathioprine in sensitized kidney recipients: the ATG group demonstrated lower incidence of biopsy-proven rejection (38 % in ATG versus 64 % in controls), 1-year graft survival (89 % in ATG versus 76 % in controls), and 1-year inulin clearances (49 ± 18 in ATG versus 37 ± 15 in controls) at the expense of higher incidence of leukopenia and thrombocytopenia with ATG administration [57]. Nevertheless, OKT3 is favored by other experts because of its documented superiority in the setting of vascular rejection (which may be humorally mediated) and its success as part of the University of Maryland regimen that successfully allowed successful transplantation in high PRA patients with a positive crossmatch. Potent triple-drug maintenance immunosuppression therapy with a calcineurin inhibitor, MMF, and steroids is probably indicated to prevent later rejection in this immunologically high-risk population.

University of Maryland experience with sensitized patient: In a retrospective comparative cohort study, we examined the long-term results of living donor kidney transplant recipients who had undergone desensitization therapy for positive FC crossmatch. We compared results to transplant outcomes in a group of matched recipients with negative crossmatch who were chosen in a blind fashion by 1:1 matching for age, gender, race, year of transplantation, and retransplantation status from a pool of 946 LDKT patients transplanted at our center. A total of 41 live donor kidney recipients with preoperative desensitization regimen for removal of donor-specific alloantibody, as detailed in

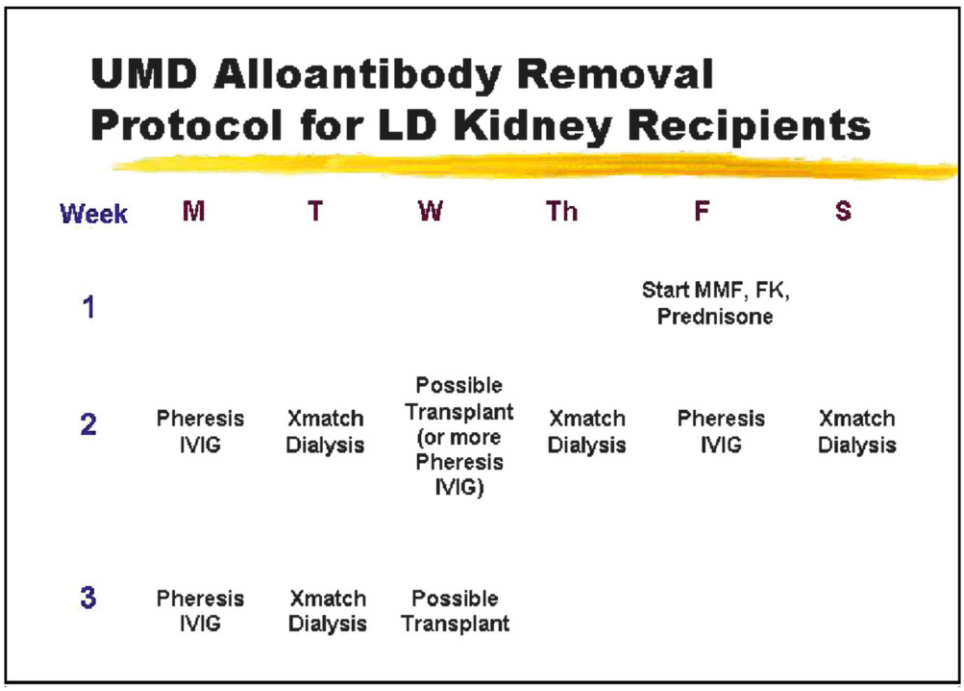

Fig. 5.1 University of Maryland alloantibody removal protocol for live donor kidney recipients who have a positive crossmatch with their donor

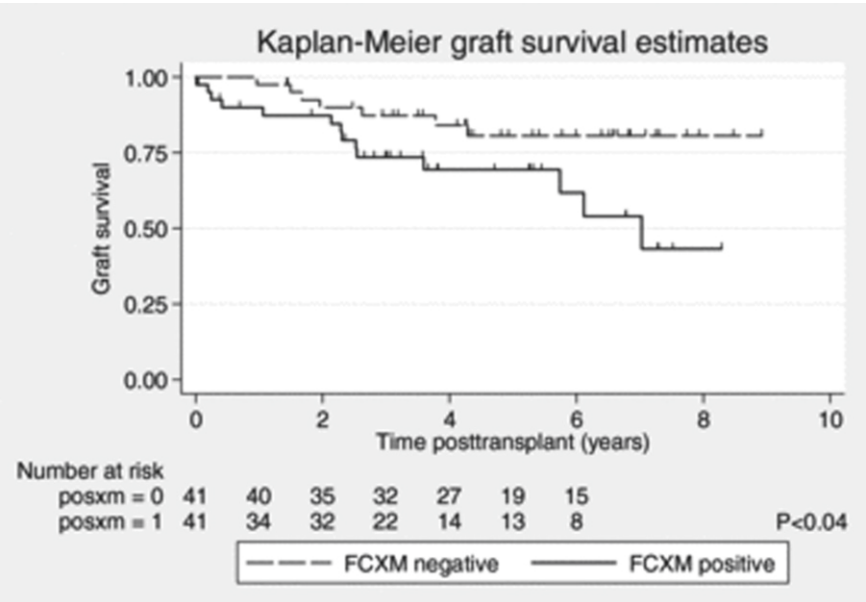

Fig. 5.2 Graft survival in positive crossmatch cases compared with controls

Fig. 5.1. Prior to desensitization, 33 patients were positive for T-flow crossmatch, 35 patients were positive for B cell crossmatch, and 27 patients were both T and B cell FC crossmatch positive. After desensitization and prior to operation, 18 patients remained T-FCXM positive and 17 patients remained B-FCXM positive and 15 had both tests positive, though with reduced median channel value (MCV). Patients were followed for up to 9 years. Serum creatinine at 1, 3, and 5 years posttransplant was not statistically different between the positive crossmatch group and controls. As shown in Fig. 5.2, positive crossmatch recipients had significantly worse graft survival than matched controls ($p = 0.04$). Graft survival at 1 year was 89.9 % for the presensitized patient and 97.6 % for the controls. At 5 years, it

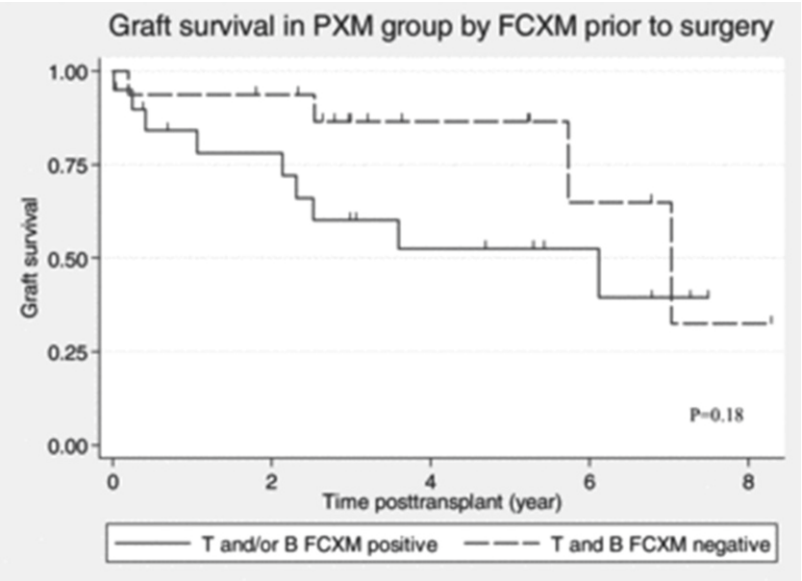

Fig. 5.3 Graft survival in positive crossmatch group in those who achieved negative crossmatch versus those with persistently positive flow crossmatch at the time of transplant following course of desensitization

was 69.4 % and 80.6 %, respectively. After adjusting for acute cellular rejection, presensitization was associated with inferior graft survival during the observation period (HR for graft loss: 2.6, $p=0.04$, 95 % CI 1.03–6.4). Acute antibody-mediated rejection was a strong predictor for worse graft survival in the positive crossmatch group (HR: 9.1, $p<0.001$, 95 % CI 2.9–28.8). One- and 5-year graft survival rates for positive T cell FC crossmatch patients were 90.6 % and 69.2 % and 87.5 % and 72.9 %, respectively, for patients with positive B cell FC crossmatch. Of the factors examined as possible predictors of poor outcome within the positive crossmatch group (i.e., age, sex, race, BMI, pretransplant diabetes), negative crossmatch and PP/IVIg prior to transplant surgery were associated with improved graft survival when compared with positivity in either both T and B cell crossmatch, though it was not statistically significant (HR: 0.45, $p=0.2$, 95 % CI 0.13–1.6). Previous transplantation was significantly associated with graft loss (HR 3.4, $p<0.05$, 95 % CI 1.0–11.8). In patients with DSA and those without identifiable DSA, there was no difference in graft survival rates. Patients who had a negative T and B cell crossmatch before surgery had a nonstatistically significant trend toward better graft survival rates than those with low-grade positivity, as shown in Fig. 5.3. We noted shortcomings of this study included limited power to detect true differences (given the small number of cases) and the lack of protocol biopsies and posttransplant DSA monitoring which could adversely affect outcomes. Overall, the study suggests that desensitization of positive FC crossmatch can provide fair short-term results but suboptimal medium to long-term outcomes that are inferior to LDKT. The 5-year survival rates seem comparable to non-ECD and perhaps better than ECD DDKT [58].

Future Directions

If regimen of administering IVIG to patients on the transplant wait list (such as those protocols of monthly infusions with dialysis described above) is found to be effective, logistically feasible, cost-effective in clinical practice, then perhaps this could be an option for highly sensitized kidney transplant candidates who are unable to find a living donor. Also, given the evidence that chimerism or microchimerism may be involved in the genesis of sustained sensitization and given the inability to produce long-term suppression of sensitization with our current regimens, some authors have suggested that actively eliminating chimerism in sensitized patients may provide an alternative strategy to control alloimmunization [31]. Immunopotentiating agents such as interferon or administration of antibodies with specificity of chimeric HLA are two strategies that could be employed in the near future.

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