

HLA Desensitization An Over View

Despite a marked increase in the number of living donor allografts (LDA) performed annually, many potential recipients with otherwise suitable donors are relegated to the ever expanding deceased donor waiting list secondary to either:

- Preformed Human Leukocyte Antigen (HLA) antibodies. These are acquired via pregnancy, transfusion, or prior transplant

OR

- ABO blood group incompatibility (ABOI)

If not adequately removed, the presence of such antibodies is likely to result in severe antibody mediated rejection (AMR) and early graft loss. Antibody mediated rejection or "humoral" rejection was recognized early in the history of transplantation as hyperacute rejection caused by the presence of preformed antibodies ("humors") to donor blood group ABO or HLA antigens. Introduction of pretransplant crossmatch techniques and the use of ABO compatible donors essentially eliminated hyperacute rejection.

With a wait list time of several years, and a disproportionately longer time for patients with blood group B or O, the time spent on dialysis waiting for the appropriate deceased donor allograft (DDA) to whom such antibodies do not exist in the potential allograft recipient can have a significant adverse effect on both quality and quantity of life. Preemptive transplantation (performed prior to the need for dialysis) provides

improved patient and allograft survival as compared to results in patients maintained on dialysis for an extended period of time prior to transplantation [1-3].

Approximately 14.4 percent of patients currently on the DDA waiting list are highly presensitized to HLA antigens (defined as ≥ 80 percent panel reactive antibody [PRA]) [4]. As a result, such patients are less likely to be transplanted or will spend an extended period of time on the waiting list pending availability of a suitable donor kidney. Many of these patients have potential living donors that are ruled out without further workup based upon an initial positive crossmatch with the potential recipient, indicating the presence of preformed HLA antibodies. Similarly, based upon the distribution of blood groups in the United States, approximately one-third of potential living donors are eliminated from consideration based on ABOI.

While not every potential HLA or ABO incompatible donor will prove to be a suitable candidate upon further review, the routine ability to successfully cross these barriers would significantly expand the pool of potential living donors and improve outcomes for many patients that would otherwise be relegated to the DDA waiting list.

The increased risk of hyperacute antibody mediated rejection (AMR) and subsequent allograft loss when transplanting against either preformed HLA antibodies or ABOI has engendered a general avoidance of this practice. However, reports of successful transplantation across HLA and/or ABO barriers using various desensitization protocols designed to reduce the amount of preexisting antibody to a level that allows for successful engraftment has stimulated increased interest in using

immunologically incompatible grafts [5].

A general overview of crossmatching and the techniques utilized to treat patients to overcome sensitization barriers are presented in this topic review. A discussion of the diagnosis and treatment of acute antibody mediated rejection is presented separately. ([See "C4d staining in renal allografts and treatment of antibody mediated rejection"](#)).

ABO DESENSITIZATION

ABO incompatibility — Poor outcomes combined with improvement in results using DDA limited ABOI transplantation in the United States in the past. However, an extreme lack of available deceased donor kidneys encouraged investigation into desensitization for ABOI/LDA in Japan. Data from Japan demonstrating successful long-term results combined with subsequent successful short-term results from ABOI desensitization protocols in the United States and elsewhere has renewed interest in this procedure [6-12].

ABO blood groups and antigens — The ABO blood group consists of four common categories (A, B, AB and O), with types A and O most frequently found in the US population. Antigen is expressed on red blood cells, lymphocytes, and platelets, as well as epithelial and endothelial cells. Formation of blood group antibodies occurs against those antigens not native to the host. Thus antibodies to both A and B are found in an individual with blood type O, while an individual with blood type AB has no antibodies to A or B antigens. Given the distribution of blood group antigens in the US, the waiting time on the DDA list is markedly prolonged for patients with blood group B or O [4].

Blood group A consists of two subtypes, A1 and A2. Approximately 80

percent of individuals in the US with blood group A express A1. The antigenic expression of A2 is quantitatively and qualitatively less than that of A1 and the overall immunogenic risk based on antigen expression alone is A1>B>A2 [13]. In addition, blood group O individuals tend to have higher isoagglutinin antibody titers to both the A and B antigen, as compared to the formation of anti-B by blood group A individuals or anti-A by blood group B individuals. ([See "A primer of red blood cell antigens and antibodies"](#), section on ABO (ABH) blood group system).

ABO and antigenic risk by antibody titer — Given the lower immunogenic risk of the A2 antigen, it was felt and subsequently found to be true that donor A2 kidneys could be successfully transplanted into recipients with low pretransplant anti-A titers without the use of desensitization [14-18]. Despite this categorical antigenic risk relationship, it appears that it is the initial titer of the anti A or B isoagglutinin antibody rather than the antigen targeted (A1, A2 or B) that defines the overall risk of AMR when crossing the ABOI barrier [14,19,20].

A report of ABOI kidney transplantation in 18 patients (10 A2 donors and eight non-A2 donors) further verifies the importance of the isoagglutinin titer rather than the specific antigen in determining the risk of post-transplant AMR [9,17]. Each of the eight non-A2 recipients (and one of the A2 recipients) received desensitization treatment with pretransplant plasmapheresis, intravenous immunoglobulin (IVIG) and splenectomy. An additional A2 recipient received an identical desensitization protocol, but was not splenectomized. All patients received Thymoglobulin induction therapy, with [tacrolimus](#), [mycophenolate](#) mofetil (MMF), and [prednisone](#) maintenance therapy. In this study, antibody mediated

rejection was more common in recipients of A2 kidneys than in recipients of non-A2 kidneys (4 of 10 versus 1 of 8).

Additional reported results are as follows:

- There were no episodes of hyperacute rejection reported and all episodes of AMR occurred within 12 days of transplantation. Treatment with plasmapheresis, IVIG and methylprednisolone reversed AMR in all patients except one (an A2 recipient).
- None of the recipients with a baseline total isoagglutinin titer of $\leq 1:32$ developed post-transplant AMR independent of donor blood group; however, four of eight patients with total isoagglutinin titer $\geq 1:128$ developed AMR, including three who had undergone desensitization. Four of the five episodes of AMR occurred in the A2 recipient group (two with and two without desensitization treatment).
- The only graft losses occurred in two A2 recipients with pretransplant isoagglutinin titers of 1:128 and 1:256. One patient lost their graft secondary to irreversible AMR, while the other had evidence of chronic allograft nephropathy on biopsy. Both of these patients had received pretransplant desensitization. The only non-A2 recipient with evidence of AMR had a pretransplant isoagglutinin titer of 1:512.
- While the pretransplant antibody titer was helpful in determining the likelihood of post-transplant AMR, it was not absolutely predictive of individual recipient outcome, with or without the use of pretransplant desensitization. As an example, two A2 recipients

with pretransplant antibody titers of 1:128 and 1:256, respectively, showed no evidence of AMR in the absence of desensitization treatment.

- At twelve months post-transplantation, both the total and IgM antibody titers were lower than the pretransplant titers in 15 of the 16 patients with surviving grafts. In those A2 recipients that did not undergo desensitization treatment, the IgM and total antibody titers had decreased by an average of 1.9 dilutions. In the non-A2 recipients (all had undergone pretransplant desensitization) the total antibody titer decreased an average of 3.9 dilutions and the IgM titer decreased an average of 2.3 dilutions.
- One-year graft (89 percent versus 96 percent) and patient (94 percent versus 99 percent) survival was slightly lower in the ABOI recipients as compared to ABO compatible recipients transplanted during the same interval. There was no significant difference in glomerular filtration rate (GFR) and serum creatinine (SCr) in the surviving ABOI allografts as compared to ABO compatible allografts.

The protocol in this study used a fixed number of plasmapheresis sessions and IVIG administrations pretransplant (four), that was independent of the baseline antibody titer. Post-transplantation plasmapheresis or IVIG was used only for treatment of AMR. This differs from other ABOI protocols and may have had a significant impact on the incidence of AMR reported in this analysis.

Long term results in ABOI transplantation

Long-term results of ABOI transplantation are comparable to those of ABO compatible transplantation. As examples:

- Desensitization protocols for ABOI/LDA have been used since 1989 in Japan. One report detailed the long term follow up on 441 of 494 patients that received an ABOI renal allograft during the period of 1989 through 2001 [6]. In this retrospective multicenter analysis, there was no significant difference in patient or graft survival at year one, three, five, seven or nine compared to historical data from 1055 recipients of ABO compatible living donor allografts. There was a marked improvement in graft survival in recipients less than 30 years of age at the time of transplantation (74 percent nine year graft survival versus 49 percent). This was attributed primarily to the quality of the allografts obtained in parent to child donation. The primary etiologies of graft loss were chronic allograft nephropathy, death, and acute rejection. Acute rejection within three months of transplantation was reported in 58 percent of recipients, although the authors did not differentiate between cellular and humoral processes. There was no significant difference in outcomes in recipients of an incompatible A1 versus B allograft. Protocols varied depending upon the transplant center and included immunoadsorption or plasmapheresis, deoxyspergualin, and local irradiation. Post-transplant antibody removal was not performed routinely, except in select cases. Pretransplant splenectomy was performed almost universally.
- A single center report from Japan describes marked improvement

in ABOI graft survival in 117 patients in the 2000 to 2004 era, as compared to previous same-center results following the incorporation of [tacrolimus](#) and [mycophenolate](#) in the maintenance immunosuppression regimen [21]. In this analysis, statistically superior allograft survival in the most recent period was reported at one year (94 versus 78 percent) and five years (90 versus 73 percent); acute rejection rates had also markedly improved (15 versus 48 percent).

- The long term outcomes of 60 consecutive ABOI kidney transplantations after the use of a protocol that incorporated antigen-specific immunoadsorption, [rituximab](#), and IVIG were compared to that of 276 ABO compatible live donor transplant recipients in three centers in Sweden [22]. At follow-up of up to 61 months, allograft survival was 97 percent for the ABOI group versus 95 percent for the ABO compatible recipients. Patient survival was identical for both (98 percent). At this time, isoagglutinin immunoadsorption filters are not approved for use in the United States; however, the outstanding initial results from this study are very encouraging and of extreme interest given the antigen specificity of this procedure.

Is splenectomy necessary? — Although controversial, splenectomy has been commonly used in desensitization protocol for ABOI transplantation to reduce the risk of AMR. This is particularly true in Japan where the majority of the experience and long-term data are available. Early reports describing hyperacute irreversible vascular rejection in recipients of an ABOI allograft not undergoing pretransplant

splenectomy as compared to favorable outcomes in recipients that had been splenectomized promoted this practice [23,24]; however, the combination of an additional surgical risk and an increased risk of serious infection, especially in the setting of chronic immunosuppressive therapy, served to curtail enthusiasm for ABOI transplantation in the United States.

The routine use of pretransplant splenectomy in ABOI transplantation has been called into question. One report described that the suppression of ABO antibodies after splenectomy is not significantly different than in ABOI recipients that are not splenectomized [25].

Newly described ABOI desensitization protocols with attention to pretransplant antibody titers using standard pre- and post-transplant antibody reduction with either plasmapheresis or immunoadsorption and [rituximab](#) (to perform a partial pharmacologic splenectomy) may allow for the avoidance of a surgical splenectomy [10,11,26].

[Rituximab](#) is a humanized mouse monoclonal antibody that targets CD20, which is expressed on the majority of B cells. Rituximab has been reported to allow successful ABOI transplantation when added to a standard protocol in patients otherwise resistant to other desensitization procedures [27]. Despite an absence of detectable splenic B cells after rituximab administration, plasma cells remain, as the majority of plasma cells lack CD20 receptors [27,28]. While the intact plasma cells are able to produce isoagglutinin antibodies, the deletion of plasma cell precursors may decrease the risk of AMR if used in conjunction with other antibody depleting measures.

A report on six ABOI renal allograft recipients (from A1, A2 and B

donors) describes successful transplantation and graft survival without any evidence of AMR through a median follow-up of one year despite the avoidance of splenectomy [10]. The desensitization protocol included plasmapheresis, IVIG and [rituximab](#) and used [daclizumab](#) induction therapy with [tacrolimus](#), MMF and [prednisone](#) maintenance therapy. Rituximab was also given as a one-time pretransplant dose of 375 mg/m² and anti-cytomegalovirus antibody immune globulin (CMVIVIG) was administered at a dose of 100 mg/kg after each plasmapheresis session. Although any additional clinical benefit is uncertain, this form of IVIG is supplied by a stable pool of donors and has less chance for batch-to-batch immunologic variability than other pooled IVIG.

As most AMR in the setting of ABOI transplantation has been reported to occur in the first several months post-transplantation, the authors of this study felt that the use of [rituximab](#) to provide several months of a partial pharmacologic splenectomy would prevent AMR and avoid the long term risks of a surgical splenectomy. The number of plasmapheresis sessions and IVIG administrations was based on the isoagglutinin titer, and transplantation was not performed until the titer was $\leq 1:16$. The highest pretransplant isoagglutinin titers present were in three patients with titers of 1:128. The median number of pretransplant plasmapheresis sessions was five and sessions were routinely continued on post-transplant day one, three and five.

The patients ranged in age from 32 to 73 years (mean 58) and the mean follow up serum creatinine concentration was 1.3 ± 0.1 mg/dL.

Isoagglutinin titers remained lower than pretransplant baseline. Protocol biopsies demonstrated one episode of cellular rejection and no histologic evidence of AMR. Of note, positive peritubular capillary (PTC) C4d

staining was present in 10 of 15 cases. This approximates the percentage of C4d+ biopsies present in recipients of ABOI renal allografts at that institution when splenectomy has been employed rather than [rituximab](#).

Similar findings were noted in a study of 11 recipients of ABOI renal allografts that included A1 and B donors and a non-splenectomy protocol employing [rituximab](#) and immunoadsorption [11].

Although long term follow-up is needed, these reports suggest that attention to the isoagglutinin titer at the time of transplantation and routine post-transplant antibody reduction with either plasmapheresis or immunoadsorption may significantly reduce the risk of AMR and allow for the elimination of splenectomy from the ABOI desensitization protocol.

Further support for this approach was provided by a study in which therapy consisting of plasmapheresis and low dose CMVIVIG (but without splenectomy or anti-CD20 therapy) was successful in four ABO incompatible renal transplant recipients [29]. All patients had antihuman globulin phase titers of 64 or higher. There were no cases of acute rejection and the mean serum creatinine concentration was approximately 1.1 mg/dL (97 µmol/L).

Given these results, combined with continued experience, most transplant centers in the United States and Europe avoid routine splenectomy prior to ABOI transplantation. However, splenectomy may remain an effective treatment for refractory antibody mediated rejection [30]. The utility of routine [rituximab](#) administration remains uncertain.

A question of accommodation — The presence of detectable isoagglutinin titers, AB endothelial antigen expression and positive PTC C4d staining despite the absence of any histologic evidence of AMR has been thought to represent ABOI immunologic accommodation [31]. The presence of positive PTC C4d staining in ABOI transplantation has been described by others [9,32-34], and appears to have a different connotation than when seen in the setting of AMR secondary to HLA incompatibility.

Based upon the findings from one study [10], the authors stated that they no longer recommend initiation of therapy targeting AMR based upon an isolated finding of positive PTC C4d staining in ABOI transplantation. When graft dysfunction secondary to AMR is present in the setting of ABOI transplantation, PTC C4d is likely to be positive, but histologic manifestations associated with AMR are also likely to be present [9].

Another analysis found that C4d positive staining was present in 80 percent of protocol ABOI allograft biopsies and in 58 percent of ABOI biopsies performed for graft dysfunction [33]. There was no correlation between this finding and histologic evidence of antibody mediated rejection or graft injury. Although unable to ascertain with certainty given the small sample size in this analysis, positive C3d staining in ABOI allografts may correlate with antibody mediated graft injury. Whether the early presence of PTC C4d in these protocols impacts on long term graft function remains to be determined.

Isoagglutinin antibody titers — As previously discussed, higher baseline isoagglutinin antibody titers generally predispose to an increased risk of antibody mediated rejection. What is not clear is whether a significantly elevated baseline antibody titer predisposes to a poor long term outcome, even in the absence of initial success through desensitization.

There is a marked difference in the reporting of antibody titers among institutions. The classic tube dilution method is most commonly used to report an IgM isoagglutinin titer and a total isoagglutinin titer. These have commonly been reported in the literature as an IgM and IgG titer, but actually represent IgM and total titer. In addition, given observer variation, the reported result should be considered an approximation. Thus, a titer reported at 1:128 may represent a titer of 1:64 to 1:256.

Improved assays, such as ELISA based technology, should assist in helping to define and standardize acceptable IgM and IgG isoagglutinin titers at baseline and in the peritransplant period. Although center specific protocols vary, the total isoagglutinin antibody titer is generally reduced to at least 1:32 (and often to $\leq 1:8$) before proceeding to transplantation.

Most centers also use immunoadsorption or plasmapheresis to maintain an isoagglutinin titer $\leq 1:16$ during the critical two week period following ABOI transplantation. If fresh frozen plasma (FFP) replacement is required as a result of plasmapheresis treatment, DONOR BLOOD TYPE or AB donor FFP should be given in order to avoid the administration of anti-allograft isoagglutinin antibodies.

Summary — At present, it is unclear which strategy, plasmapheresis, immunoadsorption, IVIG infusion, rabbit antithymocyte globulin, anti-IL-2 receptor antibody, splenectomy, anti-CD20 or a combination of therapies is more effective or cost effective than any of the others described in this section. Only a limited number of institutions have published their results, which are principally short term. While a variety of protocols seem to be effective in allowing successful transplantation across the blood group barrier, much work remains to determine optimal protocols, acceptable baseline and peri-transplant antibody titers, and to define the mechanisms responsible for this perceived process of accommodation.

HLA SENSITIZATION

Types and importance of anti-HLA antibodies — Patients with end stage renal disease that are broadly sensitized to HLA antigens via previously administered blood products, pregnancy, or prior transplantation often possess antibodies directed against deceased donor and potential living donor kidneys. Assays that define sensitization detect antibodies against the broad spectrum of potential donors. By comparison, the crossmatch identifies antibodies against a specific donor.

Depending upon the assay used to detect sensitization or perform a crossmatch, different types of antibodies can be identified. The clinical

relevance of the antibodies detected may vary, and in some cases is uncertain.

- IgG versus IgM — IgG antibodies detected by sensitization or crossmatch assays are generally considered to reflect true sensitization against HLA antigens, while IgM antibodies are not considered typical of a true anti-HLA response.
- T cell versus B cell — Antibodies detected against T cells have been generally considered to represent true anti-HLA sensitization against class I antigens. By comparison, T cell negative, B cell positive crossmatch results have been considered to represent HLA class II antibodies of less significance clinically. However, the realization that B cells also express HLA class I antigen at a level quantitatively greater than on T cells meant that low titers of preexistent HLA class I antibody, rather than only HLA class II antibody, could produce this result. Thus, T cell negative/B cell positive reactions may be secondary to either class I or class II antibodies, while a T cell positive/B cell negative reaction most likely results from a non-HLA antibody, as class I antigen is expressed on both T and B cells.
- Complement fixing versus noncomplement fixing — Antibodies that must fix complement to produce a positive assay are considered cytotoxic and likely to be of clinical importance. Antibodies detected by means that are independent of complement fixation, such as flow cytometric assays, may detect other antibodies that may not be cytotoxic and for which the clinical significance is unclear. While data do suggest that a positive flow crossmatch is

associated with an increased risk of rejection and decreased graft survival, the positive predictive value of this assay is likely dependent upon whether or not donor specific antibodies are present [35] and, if so, whether the antibody is able to fix complement [36].

Most assays detect the presence of antibody but not the precise HLA specificity. Assays to identify the specific HLA antigens targeted require additional time to perform, and can only be undertaken in settings such as LDA, when time constraints are not an issue.

Transplantation should not proceed if there is evidence of a positive crossmatch secondary to a cytotoxic IgG anti-HLA antibody.

Determination of sensitization — Several different assays are available to help determine how sensitized a potential transplant recipient is to HLA antigens. Depending upon the institutional preference, at least one of these tests is performed at the time of the initial transplant evaluation. Patient sensitization is classically reported as the percent Panel Reactive Antibody (PRA) and is an estimate of the likelihood of a positive crossmatch to a pool of potential donors.

The PRA is reported as historical (the highest value recorded on previous testing) and as current PRA. When using cytotoxicity assays, the percent PRA may vary from one testing date to another in an individual patient secondary to either a change in antibody levels in the potential recipient, or a change in the composition of HLA antigens in the assay utilized. Enzyme linked immunoadsorption assays (ELISA) and flow cytometry/flow bead technology can also be used to estimate PRA using a fixed composition of HLA antigens.

In 2005, data from the United States reported that 20 percent of patients on the kidney waiting list had a PRA of 10 to 79 percent and 14.4 percent were highly sensitized (as defined by a PRA \geq 80 percent) [37].

The different assays that can be used to determine the PRA include the following:

- The CDC assay estimates PRA by adding potential recipient serum to microtiter plates that contain a pool of lymphocytes with defined HLA antigens. Rabbit complement is added and the plates are viewed after addition of a vital stain. The PRA can then be determined based upon the number of cytotoxic reactions that are observed.
- The ELISA assay uses microtest trays containing known HLA antigens to which potential recipient serum is added. This test is more rapid than the CDC assay and the HLA antigens used for screening can be adjusted as necessary to reflect the presumed potential donor pool.
- The PRA can also be estimated using flow cytometry, using defined HLA "flow beads." Flow cytometry measures the fluorescence after patient serum has been added to a defined set of HLA antigen flow beads and a positive test is determined by the mean channel shift in intensity. One center has used "flow beads" to help identify specific HLA antigens to which the patient is sensitized [38]. Highly sensitized patients are not included in a match run if the donor possesses such unacceptable antigens, thereby increasing the probability of a negative final crossmatch.

Other multiplexed particle-based flow cytometric (Luminex) and ELISA based assays are increasingly being used to detect the presence of HLA antibodies and donor specific antibodies.

Algorithms employing such a "virtual crossmatch" can improve efficiency by decreasing the risk of a subsequent positive final crossmatch. The use of very sensitive assays to determine the presence of HLA antibodies may also improve outcomes by avoiding transplantation against the defined antigens, should the final crossmatch be negative. However, this is likely a patient and antigen-specific event, and antibodies detectable only by flow beads or other sensitive assays prior to transplantation may not always denote poor graft outcome and could result in an unnecessary bias against some potential recipients. The degree of ischemic injury and subsequent antigen expression may also impact the risk of acute antibody mediated rejection in this setting. At present, the long term effects on graft function and the risk of acute or chronic antibody mediated rejection in the setting of a "positive virtual crossmatch only" transplant is unknown and much work remains to be done in this area.

Crossmatch — The PRA has no bearing on crossmatch outcome for the recipient with a specific donor. When a potential donor is identified, a crossmatch is performed prior to transplantation to evaluate for any evidence of preformed antibodies with specificity for the potential donor that could lead to hyperacute or acute antibody mediated rejection. The crossmatch assays available differ in the degree of sensitivity.

Transplant centers may adopt a conservative crossmatch policy wherein all testing is done using a very sensitive assay, such as flow cytometry. This may help to significantly reduce the chance of post-transplant hyperacute or acute rejection.

However, this approach may also be too sensitive in that clinically irrelevant, non-HLA antibodies are detected and potentially viable transplant opportunities are bypassed. Thus, many centers use an approach that matches the crossmatch assay to the clinical concern given the potential for sensitization in the potential recipient. With this strategy, flow cytometry is used only in specific cases. This remains a controversial area.

The pretransplant crossmatch may be performed using three different sets of sera from the same potential recipient against the donor lymphocytes:

- Serum from the potential recipient at the time of the highest historical PRA
- Serum from the potential recipient that was most recently stored
- Serum that is obtained when the patient is called in for a potential transplant (final crossmatch)

The final crossmatch (from fresh serum) is done in all cases and must be negative to proceed with transplantation. The scenario of current sera negative, historical sera positive may indicate the presence of preformed antibodies that have waned in titer, but also the presence of a memory cell line that could rapidly expand and produce rejection if rechallenged

with donor antigen. Although this is not an absolute contraindication to proceeding with transplantation, it should be evaluated in the clinical context of presumed patient risk and with close post-transplant monitoring.

The following is an overview of the different types of crossmatch tests and the significance of their results:

Complement dependent cytotoxicity (CDC or standard NIH-CDC)

The complement dependent cytotoxicity (CDC or standard NIH-CDC) is similar to the CDC test used to define PRA. It is performed using wells containing donor cells rather than a pool of lymphocytes. Potential recipient serum is added along with rabbit complement and cytotoxicity is determined after administration of a vital dye. The test is done in dilutions to rule out prozone effect and to help estimate the magnitude of the immunologic reaction.

Modifications of this test include:

- A wash step to help eliminate clinically irrelevant antibodies (Amos-modified CDC)
- The addition of antihuman globulin (AHG-modified CDC) to increase the sensitivity by inducing cross linking of any antibody present and thus increasing the likelihood of visualizing cytotoxicity.

If the crossmatch is positive by CDC, the process is repeated with the addition of Dithiothreitol (DTT). This step reduces the disulfide bonds

present when the antibody is IgM. A test that is CDC positive/DTT negative (presence of an IgM antibody only) should not preclude transplantation. By comparison, the presence of a CDC positive/DTT positive test is an indication of IgG anti-donor antibody and is a contraindication to transplantation without the use of a desensitization procedure, especially if a donor-specific antibody has been defined. ([See "Summary" below](#)[See "Summary" below](#)[See "Summary" below](#)).

Flow Cytometry — Flow cytometry can be used as a crossmatch test and is routinely performed in some institutions but only selectively in others. As an acute screening test for DDA, it can define a positive T or B cell reaction and whether the antibody present is an IgG or an IgM; however, this may potentially result from non-HLA antibody. In the setting of LDA, when acute results are not necessary, flow beads or other sensitive single antigen antibody detection methods can be used to determine if an IgG HLA antibody is responsible for the registered shift in fluorescent intensity.

ELISA test — The ELISA test can be used post-transplant to rapidly determine the presence of donor specific antibodies (DSA). However, it cannot be used as a pretransplant crossmatch test.

Other assays — A number of highly sensitive screening techniques, particularly the single antigen bead assay, have been introduced [\[39-41\]](#). With this technique, microspheres coated with single HLA antigens are incubated with patient sera, resulting in the detection of both complement and non-complement activating antibodies.

These assays are helpful in determining the etiology of a positive crossmatch test, but the clinical relevance in the setting of a negative

crossmatch is unclear. The presumed strength of an antibody compared to a control and prior antigen exposure history should be considered prior to transplantation in the setting of detectable DSA but negative crossmatch. If performed, close monitoring should ensue post-transplant, given a presumed increased risk of antibody mediated rejection. As with transplantation after HLA desensitization, long-term results of this practice are unknown.

Sensitive single antigen assays are also useful for listed patients with persistently positive crossmatch results against deceased donors. Evidence for a lack of DSA on recent recipient screening tests can allow transplantation to proceed when positive crossmatch results occur as a result of autoantibodies or other non-HLA antibodies (negative virtual crossmatch).

The advent of these assays has been extremely useful, but work remains to determine the significance of positive DSA results in an individual setting and the expected outcome in a desensitization protocol. Further endeavors will include comparing in-vitro results to in-vivo events, as not every antibody detected is necessarily expressed or accessible in-vivo and may be irrelevant. Likewise, complement activation is not required for a positive test result, and the significance of non-complement activating antibodies in-vivo is unclear.

What we test — We routinely perform an NIH-CDC T and B-cell AHG crossmatch on all allograft recipients. For living donor recipients, we also perform a monocyte crossmatch, which may help to detect anti-endothelial antibodies. In addition, we perform a flow crossmatch on potential recipients with a second transplant (who are presumed to be highly sensitized) or with a history of a positive PRA as detected by

either ELISA or standard CDC assays. A flow crossmatch is also performed in the setting of any high risk case for presensitization (child to mother or husband to wife donation).

We currently use luminex testing to assess for the presence or absence of HLA antibodies and potential donor specific antibodies in potential DDA recipients and to assess donor specific antibodies in potential LDA recipients. Any potential LDA recipient with evidence of donor specific antibodies undergoes a flow crossmatch in addition to the cytotoxicity crossmatch. All of this information is used to help define the presumed risk of antibody mediated rejection and plan for desensitization, if appropriate, or to recommend against transplantation with a particular donor.

Summary and risk assessment — Regardless of the test used, highly sensitized patients are often crossmatch positive to multiple potential donors and require a zero antigen mismatch allograft to increase success. These patients are therefore relegated to the deceased donor waiting list and have a very low rate of eventual transplantation. For this reason, desensitization against preformed HLA antibodies is being used for both DDA and LDA recipients.

The following is a summary of the contraindications for transplantation and a proposal for risk assessment for acute antibody mediated rejection based on crossmatch results, as determined by the Profiling Work Group for AMR [\[42,43\]](#).

- A current positive CDC or AHG-CDC is associated with a high risk for AMR.

- A current positive CDC is a contraindication to transplantation, unless DSA can be reduced with desensitization protocols.
- A current positive flow crossmatch or a remote (historic) positive CDC or AHG-CDC crossmatch is associated with an intermediate risk for AMR. In this setting, augmented immunosuppression may be required.
- A low risk is noted in patients with negative current and remote flow or AHG-CDC crossmatch. Such patients can undergo conventional immunosuppressive therapy.

The work group also stated that current and remote negative crossmatches, if obtained only by CDC, are not necessarily associated with a low risk for AMR given the highly variable sensitivity of the CDC technique.

DESENSITIZATION PROTOCOLS

At present, variations of two protocols are principally used in an attempt to desensitize potential recipients with preformed HLA antibodies and allow a successful transplant across this barrier [44].

Desensitization can be used for a potential recipient of a LDA with defined HLA/DSA against the donor in an attempt to reduce the level of those specific antibodies. It can also be used for an individual on the DDA waiting list broadly sensitized to HLA antigens (PRA \geq approximately 30 percent) with repeated positive crossmatch results.

One method uses high dose IVIG and the other uses low dose IVIG and plasmapheresis in a protocol essentially identical to that previously

described for ABOI transplantation.

Monitoring for AMR post-transplantation consists of:

- Laboratory evaluation
- Monitoring DSA titers
- Protocol biopsies

Use of IVIG — Pooled immune globulins likely have multiple mechanisms of action that are of relevance in modulating the immune response [45,46]. These include the presence of anti-idiotypic antibodies, a reduction in antibody formation, inhibition of complement-dependent injury and other immunomodulatory actions [47-50]. Several reports describe successful use of IVIG in HLA desensitization protocols [51-56]. Intravenous immunoglobulins have also been used in the treatment of biopsy proven AMR independent of desensitization and in cases of steroid and antilymphocyte globulin resistant rejection [51,57-59].

The optimal dosing and frequency of administration of IVIG in either the high or low dose protocol is unclear and the optimal regimens remain to be defined.

High dose IVIG — The use of high dose IVIG for desensitization was initially reported in the early 1990s [60,61]. Subsequently, other reports of successful use of this protocol have been published [55,62].

In the first of these reports [62], 15 patients with either a panel reactive antibody (PRA) of >50 percent or with a positive crossmatch to their potential living donor were given 2 g/kg of IVIG monthly for three months. Thirteen of the 15 showed evidence of desensitization (reduction of PRA by at least 50 percent or a repeat negative crossmatch to the living

donor) and underwent renal transplantation (11 DDA and 2 LDA). The mean decrease in PRA for recipients of a DDA was 80 percent and a post-IVIG administration NIH cytotoxicity crossmatch was negative prior to transplantation. The IVIG was repeated at the same dose on post-transplant day zero and one. Thymoglobulin was used for induction and maintenance immunosuppression consisted of MMF, [corticosteroids](#) and [tacrolimus](#). The IVIG was again repeated at post-transplant day 20 or 21 and 40 or 41.

The following results were reported:

- One graft was lost secondary to thrombosis and one graft was lost secondary to rejection.
- No other episodes of rejection were reported in the remaining allografts during follow-up of over one year.
- The success rate for desensitization was higher than that reported previously by the same group (87 percent versus 50 percent) and was attributed to a lower dose of IVIG administered in the earlier trial.

In the other reported analysis of desensitization using high dose IVIG [\[55\]](#), 45 patients highly sensitized to HLA antibody (15 DDA, 28 LDA, and 2 cardiac transplant patients) underwent a similar protocol. The 15 potential DDA recipients all had exhibited a positive crossmatch to multiple potential donors and had been on dialysis for greater than five years. The 28 potential LDA recipients all had a positive crossmatch with their respective donor. The two potential cardiac transplant recipients

were also highly HLA sensitized.

The potential LDA recipients underwent an initial in vitro cytotoxicity test to evaluate the inhibition or reduction of DSA. If the in vitro test demonstrated inhibition, the recipient was given IVIG at a dose of 2 g/kg (maximum dose of 140 g). If the repeat crossmatch test was negative or the reaction significantly decreased, the transplantation was performed within 24 to 72 hours. An additional dose of IVIG at 2 g/kg was administered one month after transplantation. 26 of the 28 potential LDA recipients exhibited an acceptable crossmatch after only one dose of IVIG. The remaining two patients required three additional monthly doses to achieve an acceptable crossmatch.

For potential deceased donor kidney or cardiac recipients, the PRA test was repeated in the presence of IVIG. If the percent PRA was significantly decreased, the patients received 2 g/kg of IVIG monthly for four months. Sixteen of the 17 patients in this category underwent transplantation (12 kidneys, one cardiac-liver, one cardiac-kidney, one liver-kidney, and one cardiac). Ten of these had a negative cytotoxicity crossmatch and negative flow cytometry to the potential donor at the time of transplantation and six of these had a negative cytotoxicity crossmatch, but a positive flow cytometry at the time of transplantation. Intravenous immunoglobulin at a dose of 2 g/kg was initiated prior to the surgery and continued intraoperatively. Potential LDA recipients with a positive in vitro crossmatch despite IVIG or non-LDA recipients not exhibiting a reduction in PRA were not considered for transplantation. All recipients in this protocol received induction with [daclizumab](#) and were maintained on [corticosteroids](#), [tacrolimus](#) and MMF.

The results were as follows:

- Thirteen of the 42 recipients (31 percent) had an episode of acute rejection (all within two months post-transplant) and three grafts were lost to rejection. Recurrent rejection was unusual.
- The mean serum creatinine at two years was 1.4 mg/d (124 $\mu\text{mol/L}$) with a patient survival of 98 percent and a graft survival of 89 percent.

A large placebo controlled trial among highly sensitized patients awaiting kidney transplantation also found that high dose IVIG therapy successfully reduced anti-HLA antibody levels, thereby improving transplantation rates [63]. In this multicenter study, 101 patients with PRAs ≥ 50 percent were randomly assigned to IVIG (2 g/kg monthly for four months) or placebo. Active therapy significantly reduced PRA levels and increased the transplantation rate (35 versus 17 percent for placebo). Although rejection episodes were more common among the IVIG group, allograft survival rates at a median period of two years post-surgery were similar in both groups (80 and 75 percent for IVIG and placebo, respectively).

The effectiveness of high dose IVIG therapy appears to be the same with induction therapy with thymoglobulin versus that associated with [daclizumab](#). A retrospective study found similar allograft survival rates as well as incidence of antibody mediated rejection [64].

- **Summary** — Advantages of the high dose IVIG method include the ability to desensitize patients on the DDA waiting list as well as potential LDA recipients. This protocol is much less

resource intense and less expensive than low dose IVIG protocols. Patients on hemodialysis can also receive monthly IVIG infusions while undergoing dialysis treatment.

Disadvantages of the high dose IVIG protocol include a current lack of significant experience in either ABOI or in patients with known high titer HLA DSA. Crossmatch monitoring and monitoring of post-transplant DSA for approximately three weeks after high dose IVIG administration is limited to the less sensitive NIH cytotoxicity assay secondary to interference of high dose IVIG with other more sensitive assays.

With the requirement to use the less sensitive NIH cytotoxicity assay for crossmatch screening rather than the more sensitive anti-human globulin (AHG) cytotoxicity assay, one could infer that the recipients in the high dose IVIG protocols are likely to be more sensitized (false negative crossmatch) and thus at higher risk of rejection than those in the low dose IVIG protocols, which uses the AHG assay.

Infusion of high dose IVIG may result in side effects such as headache, fever, muscle pain, shortness of breath, and chest discomfort [65].

Complications can be minimized or eliminated by using products with an osmolality that approximates that of plasma [45]. Administering the necessary volume of an iso-osmolar IVIG solution while the patient is on dialysis allows for simultaneous correction of volume issues. When administering high dose IVIG to a patient not receiving simultaneous hemodialysis, individual product guidelines for rate of administration should be followed to decrease the risk of complications related to a high osmotic load. These complications can generally be avoided by using a non-sucrose based, iso-osmolar product.

Low dose IVIG — Desensitization using a low dose IVIG protocol in combination with antibody reduction via plasmapheresis in renal transplantation was initially described by two groups [51,53]. The low dose IVIG protocol for HLA desensitization is essentially identical to that described previously for ABOI. It has been used to simultaneously cross both barriers [66].

Alternate day plasmapheresis is used pretransplantation with 100 mg/kg of IVIG (or CMVIG depending on the institutional protocol) administered after each session. The number of pretransplant treatments is estimated based on the baseline anti-HLA antibody titer. Once the antibody is no longer detectable and the pretransplant AHG cytotoxicity crossmatch is negative, transplantation proceeds.

Some centers elect to proceed with transplantation despite the persistence of a low cytotoxicity antibody titer, generally $\leq 1:4$. The advisability of transplantation in this setting remains to be determined, as the risk of rejection and early graft loss may be substantial [67]. The allowable risk, as determined by the titer of a persistent DSA, is currently a case-specific and transplant center dependent decision.

Albumin is generally used for replacement in plasmapheresis in most desensitization protocols. This avoids the potential for HLA sensitization when using fresh frozen plasma (FFP). Two to three units of FFP may be substituted at the end of the treatment in lieu of albumin in the setting of a significantly increased PT or PTT, decreased fibrinogen level, or within 24 hours of a renal biopsy or other invasive procedure. ([See "Acute renal allograft rejection: Diagnosis"](#)).

In one protocol using CMVIVIG, [daclizumab](#) was used for induction and

maintenance therapy consisting of [tacrolimus](#), MMF, and [prednisone](#) [51]. Plasmapheresis and CMVIVIG are continued postoperatively on an alternate day regimen with the duration dependent on the pretransplant titer or as indicated by evidence of AMR. A follow-up report by the same group on 49 patients undergoing the low dose IVIG protocol described the absence of detectable DSA in 89 percent of recipients [68].

Another study described 14 patients with a positive AHG cytotoxicity crossmatch to a potential living donor [54]. Patients underwent plasmapheresis on days four, three, and one pretransplant, on the day of transplantation, and on day one and three post-transplantation.

Intravenous immunoglobulin 100 mg/kg (non-CMVIVIG) was administered after each plasmapheresis session. [Rituximab](#) at a dose of 375 mg/m² was given on post-transplant day four. Splenectomy was performed at the time of transplantation in those with an intact spleen (two had previously been splenectomized). Thymoglobulin was used for induction and [tacrolimus](#), MMF and [corticosteroids](#) were used for maintenance therapy. The following were reported:

- Patient survival at a mean follow-up of 448 days was 86 percent. Two grafts were lost secondary to chronic allograft nephropathy following AMR and one functioning graft was lost secondary to patient death.
- The mean serum creatinine concentration was 1.4 mg/dL (124 µmol/L) and all creatinine concentrations are ≤2.0 mg/d (.177 µmol/L).
- Histologic evidence of AMR occurred in 6 of 14 patients (43

percent). The risk of AMR was related to the baseline anti-HLA antibody titer. Ten of the 14 recipients had baseline anti-HLA titers $\leq 1:4$ and subclinical rejection was found in only two of these on protocol biopsy. The remaining four episodes of AMR (two subclinical) were present in recipients with baseline titers $\geq 1:8$.

- Both clinical and subclinical AMR was treated with pulse steroids, plasmapheresis, and IVIG. All four subclinical episodes responded to treatment and follow-up protocol biopsies showed no histologic evidence of rejection. Both episodes of rejection defined as clinically significant AMR demonstrated evidence of chronic allograft nephropathy on subsequent biopsies.

In a separate publication, none of the recipients had a positive AHG crossmatch at four months post-transplant [69]. However, 9 of 11 recipients tested using a sensitive single antigen flow bead assay did demonstrate persistent low levels of DSA.

European studies using immunoadsorption combined with Thymoglobulin induction along with [cyclosporine](#), [corticosteroids](#), and MMF as maintenance therapy has also been used for desensitization in sensitized DDA recipients [70,71]. In one analysis of 20 DDA recipients using immunoadsorption immediately prior to and in the post-transplant period for a median of 11 sessions, the 12-month patient survival was 95 percent and the graft survival was 80 percent [70]. Immunoadsorption is not yet routinely available in the United States.

- **Summary** — Advantages of the low dose IVIG protocol include its documented use in patients with high titer anti-HLA

antibodies with desensitization and successful transplantation in patients with DSA titers of up to 1:256 [68]. Although patients with high titer DSA may respond to this protocol, numerous pretransplant sessions may be required to obtain an adequately low titer that will allow successful transplantation across the HLA barrier as well as numerous post-transplant sessions to limit the rise of DSA titers and prevent AMR. As with the ABOI protocol, the number of pretransplant plasmapheresis sessions required can usually be estimated based on the initial antibody titer.

When using low dose IVIG, the more sensitive AHG crossmatch can be used prior to transplantation, which may help detect the presence of preformed antibodies missed using the NIH cytotoxicity assay. After transplantation, it is possible to follow crossmatch and DSA by several assays, rather than only by the least sensitive NIH cytotoxicity assay.

Interference of these tests by adjunct agents, such as Thymoglobulin or [rituximab](#), requires special steps to remove the administered antibody from the assay to avoid false-positives. Accidental removal of anti-HLA antibody when attempting to remove Thymoglobulin or rituximab from the crossmatch assay could therefore result in a false negative.

Disadvantages include the high financial cost and resource requirements to perform this desensitization protocol. As the timing of the actual transplant procedure must be adjusted to allow for an acceptable antibody titer, this protocol is only suitable for potential sensitized recipients of a LDA.

Comparative studies — Only one study has directly compared high dose IVIG with plasmapheresis/low dose IVIG protocols. The

plasmapheresis regimens provided significantly better outcomes, although the study has significant limitations. In this report from the Mayo Clinic, the following three protocols were employed sequentially in renal transplant recipients with high DSA levels [67]:

- Plasmapheresis plus low dose IVIG plus anti-CD20 antibody (32 patients), referred to as the plasmapheresis group. 19 of the 32 patients in this group also underwent splenectomy; post-transplant plasmapheresis and low dose IVIG were continued on post-surgery days one to three for a total of two to three sessions.
- High single dose IVIG (13 patients), which is the high dose IVIG group
- Plasmapheresis plus low dose IVIG plus anti-CD20 antibody plus pretransplant Thymoglobulin combined with post-transplant DSA monitoring (16 patients), which is the plasmapheresis/monitoring group. Post-operative plasmapheresis and low dose IVIG were continued on post-transplant days one through three.

Achieving a negative crossmatch was significantly more likely with both plasmapheresis protocols versus high dose IVIG (84, 88, and 38 percent for the plasmapheresis, plasmapheresis/monitoring, and high dose IVIG groups, respectively).

However, the significantly lower response rate in this report with high dose IVIG may have resulted from the lack of in vitro testing of patients in the high dose IVIG group to identify potential responders prior to systemic administration of IVIG. Patients with low baseline antibody titers ($\leq 1:4$), were successfully desensitized by any of the three methods. By

comparison, only two of ten patients with a baseline titer $\geq 1:8$ were successfully desensitized with the high single dose protocol versus twelve of nineteen in the plasmapheresis/low dose IVIG groups. Three of the eight patients not responding to the high dose IVIG regimen did respond when switched to the plasmapheresis/low dose IVIG protocol. Only one of ten patients with a baseline titer $\geq 1:32$ responded to any of the three methods. Significantly lower humoral rejection rates were also reported with the plasmapheresis protocols (37, 29, and 80 percent, respectively), although none of the patients in the high single dose IVIG group received [rituximab](#) or post-transplant administration of IVIG.

Another possible explanation for the lower likelihood of a negative crossmatch result with the high dose IVIG protocol in this analysis as compared to previous high dose IVIG desensitization reports may have been the use of the AHG cytotoxicity assay. The increased sensitivity of the AHG-CDC assay as compared to the standard CDC assay used in previous high dose IVIG reports may have produced a greater number of persistent positive crossmatch results.

Additional limitations with this study include nonrandomization and low numbers of patients. While this report suggests that plasmapheresis-based desensitization protocols may provide superior desensitization outcomes and lower rates of acute antibody mediated rejection as compared to a high single dose IVIG regimen, the avoidance of pretransplant [rituximab](#) and absence of post-transplant administration of IVIG in the single dose group may have biased the results.

Patients with low baseline antibody titers responding to high dose IVIG may do equally as well with further optimization of therapy. However, whether or not the administration of [rituximab](#) or the routine post-

transplant administration of IVIG would be of benefit in reducing the incidence of acute rejection in a high dose IVIG protocol is unclear at this time.

The utility of routine post-transplant plasmapheresis and low dose IVIG administration in response to rising DSA titers is unclear. In this analysis, DSA monitoring with reinitiation of plasmapheresis/IVIG delayed the onset of, but did not reduce the incidence of antibody mediated rejection. Routine post-transplant plasmapheresis and IVIG administration for one to three sessions, as described in this study, in the absence of graft dysfunction or evidence of rejection, is of uncertain benefit.

Given the high risk of acute rejection, however, most clinicians would favor this approach until more data are available. Subsequent treatments are then based upon results of protocol or indication biopsies. However, a rising DSA titer in the early post-transplant setting should prompt an allograft biopsy and suggests the need for intensification of therapy, in the absence of graft dysfunction; reinitiation of plasmapheresis/IVIG or other treatment should be based on biopsy results.

The need for a completely negative crossmatch versus reduction to a low titer antibody response prior to attempting transplantation has been debated. The authors of this study describe their experience transplanting across a low titer positive crossmatch following desensitization, in which seven of ten patients had acute antibody mediated rejection and five of ten had early graft loss.

Summary — Given the results of the previous comparative study and the lack of long-term data or late protocol biopsy information for HLA desensitization, the following generalized recommendations can

be made pending further investigations:

- A negative crossmatch is desirable in desensitization protocols. Transplantation in the setting of even a low titer positive crossmatch may significantly increase the risk of early graft loss and require prolonged, intense post-transplant therapy with uncertain long-term results.
- Desensitization attempts in the patient with higher baseline titer HLA antibodies ($\geq 1:32$) may be very resource intense with a low likelihood of producing a completely negative crossmatch. Transplantation attempts following a desensitization protocol in these patients likely carries with it an increased risk of repeated episodes of antibody mediated rejection and early graft loss.
- Single, high-dose IVIG desensitization protocols may be useful in patients with low baseline antibody titers ($\leq 1:4$) and optimization of therapy may allow for a reduced incidence of acute antibody mediated rejection.
- Plasmapheresis/low dose IVIG is more likely to produce a negative crossmatch in patients with mid-level titers (1:8 to 1:16) of HLA antibodies than is single high dose IVIG. Optimal post-transplant monitoring and timing of reinitiation of therapy remains to be determined.
- A high risk of acute rejection and increased risk of early graft loss along with uncertain long-term outcome is present with any HLA desensitization protocol and this information should be understood

by both donor and recipient prior to proceeding.

Diagnosis and treatment of AMR

All patients who have undergone transplantation using HLA desensitization should be considered at high risk for antibody mediated rejection. As a result, there is a low threshold for biopsy ([show figure 1](#)) and protocol biopsies ([show figure 2](#)) are performed on these allografts, independent of evidence of clinical allograft dysfunction.

Therapy is based in part on the time of AMR:

- In the early post-transplant setting in these patients, treatment including IVIG and/or plasmapheresis is commonly initiated based on positive C4d staining or evidence of DSA alone, independent of histologic findings. Unlike the case with C4d staining in ABOI transplantation, positive C4d staining in the setting of HLA desensitization is much more likely to be associated with antibody mediated rejection [33]. Treatment for apparent AMR is essentially the same as for desensitization, although the duration of plasmapheresis and IVIG is dependent upon an improvement in renal function and a decrease in the titer of DSA. Other rescue therapies, such as [rituximab](#) or splenectomy are sometimes employed [30].

- In the late post-transplant course, C4d and/or DSA may be present in the absence of graft dysfunction. The implications of these findings are not clear. In this setting, hypervigilance with potential modification of maintenance immunosuppression is the norm, rather than initiating intensive therapy using IVIG and/or plasmapheresis. Subclinical rejection (as defined by positive C4d staining associated with histologic evidence of antibody mediated rejection) on protocol biopsies may be associated with future clinical antibody mediated rejection and/or subsequent evidence of chronic allograft injury [72]. Whether or not treatment of subclinical rejection in this setting has a benefit on long-term graft survival is unknown.

In ABO incompatible transplantation, it is not unusual for C4d to be positive on protocol biopsies, and this probably has a different connotation than when seen in HLA incompatible transplantation. Thus, in the early post-transplant setting in ABO incompatible transplantation, reinitiation of plasmapheresis and IVIG is used with graft dysfunction or a rising isoagglutinin antibody titer, but not with evidence of positive C4d staining alone.

Long term results

The long term results for graft and patient outcome using either of these protocols of HLA antibody desensitization are unknown. Early results with both protocols have been encouraging and they have allowed patients to successfully undergo a transplantation procedure that would

have otherwise been unavailable to them. Accelerated chronic rejection and early graft loss might be the penalty for attempting to cross the HLA barrier, or conversely, these protocols may help induce a state of accommodation or tolerance with excellent long term graft function as has been reported in ABOI transplantation [31].

Results from long term follow up and protocol biopsies will be necessary to judge the utility of HLA desensitization. One report described the findings of one year protocol biopsies for 61 patients that had undergone a desensitization protocol for either HLA (37 patients) or blood group incompatibility (24 patients) and 198 conventional controls [73].

Transplant glomerulopathy was significantly more common in those patients undergoing HLA incompatible (22 percent) versus blood group incompatible (13 percent) or standard (8 percent) renal transplantation. Prior antibody mediated rejection was associated with glomerulopathy, while no difference was noted among the groups in the absence of this event.

Although glomerulopathy in any group was associated with increased proteinuria, there was no association between glomerulopathy and renal function at one year and there was no significant overall difference between groups in serum creatinine or estimated renal function. The pretransplant antibody titer was predictive of subsequent antibody mediated rejection, with a 21 percent rate in those with titers $\leq 1:64$ and an 80 percent rate in those with titers $> 1:64$. The use of various assays to measure HLA antibody titers prevented a definite predictive association for antibody mediated rejection in this group.

In the absence of HR, no difference in histologic changes was seen between groups, although all three groups had a demonstrable mild

increase in interstitial fibrosis from biopsies performed at the time of transplant.

Thus, although HR is associated with an increase in TG, in its absence allograft histology is similar in +XM, ABOI and conventional allografts one year post-transplant.

FUTURE OF ABOI AND HLA DESENSITIZATION PROTOCOLS

Excellent long term results (with an acceptance of a high rate of AMR in HLA incompatible transplantation and an awareness that early graft loss will be likely be greater than is currently experienced in nonsensitized recipients) are needed to justify the financial cost and resource utilization, particularly with the low dose IVIG protocols. If long term allograft function is routinely maintained using either of these protocols, an overall financial benefit in terms of health care dollars will be realized [45].

Additional comparative studies between the high and low dose IVIG protocols are needed. Further information regarding what level of antibody titer may be amenable to transplantation with or without desensitization is also needed, as is information on the appropriate adjunctive desensitization treatments. For patients with the option of continuing hemodialysis or peritoneal dialysis, any long term survival benefit from transplantation using an intensive desensitization protocol with uncertain results as compared to continued dialysis remains to be determined.

DONOR EXCHANGE — An alternative to either ABOI or HLA desensitization is the use of a paired exchange program. Although fraught with ethical and potentially legal concerns, willing participants can choose to allow either an ABO or HLA incompatible donor in one case to donate their kidney to an alternate recipient also with an ABO or HLA incompatible donor that is agreeable to donating to their kidney to the remaining recipient [74,75].

This process avoids the need for desensitization and provides a living donor allograft to each recipient. Obviously, both parties must be in full agreement and have an understanding of the potential post-transplantation ramifications as well as making a decision on whether or not the parties should remain anonymous to one another.

Paired exchange programs can help provide allografts to a select number of individuals. In a single center study, for example, marked success was reported among 22 patients who received ten paired donations including two triple exchanges [76]. However, this approach is unlikely to have a significant impact on reducing the wait list time when the exchange program is confined to an individual transplant center.

Although fraught with logistical problems, increasing the exchange programs to a regional or national level would potentially expand the number of suitable donor pairs considerably. This was shown in a modeling study in which the success of such programs was analyzed using increasing population sizes [77]. In the United States, a donor kidney exchange program has been implemented in Region 1 of the United Network for Organ Sharing system, resulting in limited early success [31]. A national kidney paired donation is being considered in

the United States.

The use of donor exchange programs to perform wait list exchange (wherein the incompatible donor donates to an individual on the waiting list and the incompatible recipient moves to the front of the wait list) as compared to paired exchange is somewhat controversial.

This process may result in even longer delays for those patients already with the longest wait times, in particular blood group O recipients. Such a strategy may not be acceptable to some patients, particularly those with blood group O [78].

Acceptable mismatch program — In Europe, an acceptable mismatch program is in place that assists in providing DDA to highly sensitized individuals through an enhanced distribution and immunologic screening protocol [79,80]. Using this protocol, there is no statistically significant difference in graft survival in the highly sensitized recipients versus that of non-HLA sensitized recipients.

Summary — Despite success with these programs in increasing the likelihood of an acceptable DDA being offered to a potential recipient highly sensitized to HLA antigens, some authors note that a pool of patients remain essentially non-transplantable secondary to broad immunogenicity. For this reason, further investigation of desensitization protocols continues.

REFERENCES

Meier-Kriesche, HU, Kaplan, B. Waiting time on dialysis as the strongest modifiable risk factor for renal transplant outcomes: a paired donor kidney analysis. *Transplantation* 2002; 74:1377.

Kasiske, BL, Snyder, JJ, Matas, AJ, et al. Preemptive kidney transplantation: the advantage and the advantaged. *J Am Soc Nephrol* 2002; 13:1358.

Mange, KC, Joffe, MM, Feldman, HI. Effect of the use or nonuse of long-term dialysis on the subsequent survival of renal transplants from living donors. *N Engl J Med* 2001; 344:726.

2006 OPTN/SRTR Annual Report: Transplant Data 1996 - 2007. www.ustransplant.org (Accessed 9/07).

Glantz, D, Antoine, C, Duboust, A. Antidonor antibodies and transplantation: How to deal with them before and after transplantation. *Transplantation* 2005; 79:S30.

Takahashi, K, Saito, K, Takahara, S, et al. Excellent long-term

outcome of ABO-incompatible living donor kidney transplantation in Japan. *Am J Transplant* 2004; 4:1089.

Tanabe, K, Takahashi, K, Agishi, T, et al. Removal of anti-A/B antibodies for successful kidney transplantation between ABO blood type incompatible couples. *Transfus Sci* 1996; 17:455.

Tanabe, K, Takahashi, K, Sonda, K, et al. Long-term results of ABO-incompatible living kidney transplantation: a single-center experience. *Transplantation* 1998; 65:224.

Gloor, JM, Lager, DJ, Moore, SB, et al. ABO-incompatible kidney transplantation using both A2 and non-A2 living donors. *Transplantation* 2003; 75:971.

0. Sonnenday, CJ, Warren, DS, Cooper, M, et al. Plasmapheresis, CMV hyperimmune globulin, and anti-CD20 allow ABO-incompatible renal transplantation without splenectomy. *Am J Transplant* 2004; 4:1315.

1. Tyden, G, Kumlien, G, Genberg, H, et al. ABO incompatible kidney transplantations without splenectomy, using antigen-specific immunoabsorption and rituximab. *Am J Transplant* 2005; 5:145.

2. Tanabe, K. Japanese experience of ABO-incompatible living kidney transplantation. *Transplantation* 2007; 84:S4.

3. Breimer, ME, Samuelsson, BE. The specific distribution of glycolipid-based blood group A antigens in human kidney related to A1/A2, Lewis, and secretor status of single individuals. A possible molecular explanation for the successful transplantation of A2 kidneys into O recipients. *Transplantation* 1986; 42:88.

4. Norman, DJ, Prather, JC, Alkhunaizi, AM, et al. Use of A(2) kidneys for B and O kidney transplant recipients: report of a series of patients transplanted at a single center spanning a decade. *Transplant Proc* 2001; 33:3327.

5. Nelson, PW, Landreneau, MD, Luger, AM, et al. Ten-year experience in transplantation of A2 kidneys into B and O recipients. *Transplantation* 1998; 65:256.

6. Sorensen, JB, Grant, WJ, Belnap, LP, et al. Transplantation of ABO group A2 kidneys from living donors into group O and B recipients. *Am J Transplant* 2001; 1:296.

7. Stegall, MD, Dean, PG, Gloor, JM. ABO-incompatible kidney transplantation. *Transplantation* 2004; 78:635.

8. Bryan, CF, Winklhofer, FT, Murillo, D, et al. Improving access to kidney transplantation without decreasing graft survival: Long-term

outcomes of blood group A2/A2B deceased donor kidneys in B recipients. *Transplantation* 2005; 80:75.

9. Alkhunaizi, AM, de Mattos, AM, Barry, JM, et al. Renal transplantation across the ABO barrier using A2 kidneys. *Transplantation* 1999; 67:1319.
0. Shimmura, H, Tanabe, K, Ishikawa, N, et al. Role of anti-A/B antibody titers in results of ABO-incompatible kidney transplantation. *Transplantation* 2000; 70:1331.
1. Ishida, H, Miyamoto, N, Shirakawa, H, et al. Evaluation of immunosuppressive regimens in ABO-incompatible living kidney transplantation--single center analysis. *Am J Transplant* 2007; 7:825.
2. Tyden, G, Donauer, J, Wadstrom, J, et al. Implementation of a Protocol for ABO-incompatible kidney transplantation--a three-center experience with 60 consecutive transplantations. *Transplantation* 2007; 83:1153.
3. Alexandre, GP, De Bruyere, M, Squifflet, JP, et al. Human ABO-incompatible living donor renal homografts. *Neth J Med* 1985; 28:231.
4. Alexandre, GP, Squifflet, JP, De Bruyere, M, et al. Present experiences in a series of 26 ABO-incompatible living donor renal allografts. *Transplant Proc* 1987; 19:4538.
5. Ishida, H, Koyama, I, Sawada, T, et al. Anti-AB titer changes in patients with ABO incompatibility after living related kidney transplantations: survey of 101 cases to determine whether splenectomies are necessary for successful transplantation. *Transplantation* 2000; 70:681.
6. Gloor, JM, Lager, DJ, Fidler, ME, et al. A Comparison of splenectomy versus intensive posttransplant antidonor blood group antibody monitoring without splenectomy in ABO-incompatible kidney transplantation. *Transplantation* 2005; 80:1572.
7. Sawada, T, Fuchinoue, S, Kawase, T, et al. Preconditioning regimen consisting of anti-CD20 monoclonal antibody infusions, splenectomy and DFPP-enabled non-responders to undergo ABO-incompatible kidney transplantation. *Clin Transplant* 2004; 18:254.
8. Sawada, T, Fuchinoue, S, Teraoka, S. Successful A1-to-O ABO-incompatible kidney transplantation after a preconditioning regimen consisting of anti-CD20 monoclonal antibody infusions, splenectomy, and double-filtration plasmapheresis. *Transplantation* 2002; 74:1207.
9. Segev, DL, Simpkins, CE, Warren, DS, et al. ABO incompatible high-titer renal transplantation without splenectomy or anti-CD20

treatment. *Am J Transplant* 2005; 5:2570.

0. Locke, JE, Zachary, AA, Haas, M, et al. The utility of splenectomy as rescue treatment for severe acute antibody mediated rejection. *Am J Transplant* 2007; 7:842.
1. Park, WD, Grande, JP, Ninova, D, et al. Accommodation in ABO-incompatible kidney allografts, a novel mechanism of self-protection against antibody-mediated injury. *Am J Transplant* 2003; 3:952.
2. Onitsuka, S, Yamaguchi, Y, Tanabe, K, et al. Peritubular capillary deposition of C4d complement fragment in ABO-incompatible renal transplantation with humoral rejection. *Clin Transplant* 1999; 13 Suppl 1:33.
3. Haas, M, Rahman, MH, Racusen, LC, et al. C4d and C3d staining in biopsies of ABO- and HLA-incompatible renal allografts: Correlation with histologic findings. *Am J Transplant* 2006; 6:1829.
4. Setoguchi, K, Ishida, H, Shimmura, H, et al. Analysis of renal transplant protocol biopsies in ABO-incompatible kidney transplantation. *Am J Transplant* 2008; 8:86.
5. Pollinger, HS, Stegall, MD, Gloor, JM, et al. Kidney transplantation in patients with antibodies against donor HLA class II. *Am J Transplant* 2007; 7:857.
6. Bartel, G, Wahrman, M, Exner, M, et al. Determinants of the complement-fixing ability of recipient presensitization against HLA antigens. *Transplantation* 2007; 83:727.
7. 2006 OPTN/SRTR Annual Report: Transplant Data 1996 - 2005. www.ustransplant.org (Accessed 9/07).
8. Bray, RA, Nolen, JD, Larsen, C, et al. Transplanting the highly sensitized patient: The Emory algorithm. *Am J Transplant* 2006; 6:2307.
9. Pei, R, Lee, JH, Shih, NJ, et al. Single human leukocyte antigen flow cytometry beads for accurate identification of human leukocyte antigen antibody specificities. *Transplantation* 2003; 75:43.
0. El-Awar, N, Lee, J, Terasaki, PI. HLA antibody identification with single antigen beads compared to conventional methods. *Hum Immunol* 2005; 66:989.
1. van den, Berg-Loonen EM, Billen, EV, Voorter, CE, et al. Clinical Relevance of Pretransplant Donor-Directed Antibodies Detected by Single Antigen Beads in Highly Sensitized Renal Transplant Patients. *Transplantation* 2008; 85:1086.
2. Takemoto, SK, Zeevi, A, Feng, S, et al. National conference to assess antibody-mediated rejection in solid organ transplantation. *Am J*

Transplant 2004; 4:1033.

3. Fuggle, SV, Martin, S. Toward performing transplantation in highly sensitized patients. *Transplantation* 2004; 78:186.
4. Jordan, SC, Pescovitz, MD. Presensitization: The problem and its management. *Clin J Am Soc Nephrol* 2006; 1:421.
5. Jordan S, Cunningham-Rundles, C, McEwan, R. Utility of intravenous immune globulin in kidney transplantation: efficacy, safety, and cost implications. *Am J Transplant* 2003; 3:653.
6. Jordan, SC, Tyan, D, Czer, L, Toyoda, M. Immunomodulatory actions of intravenous immunoglobulin (IVIG): potential applications in solid organ transplant recipients. *Pediatr Transplant* 1998; 2:92.
7. Kazatchkine, MD, Kaveri, SV. Immunomodulation of autoimmune and inflammatory diseases with intravenous immune globulin. *N Engl J Med* 2001; 345:747.
8. Toyoda, M, Pao, A, Petrosian, A, Jordan, SC. Pooled human gammaglobulin modulates surface molecule expression and induces apoptosis in human B cells. *Am J Transplant* 2003; 3:156.
9. Toyoda, M, Zhang, XM, Petrosian, A, et al. Inhibition of allospecific responses in the mixed lymphocyte reaction by pooled human gamma-globulin. *Transpl Immunol* 1994; 2:337.
0. Toyoda, M, Zhang, X, Petrosian, A, et al. Modulation of immunoglobulin production and cytokine mRNA expression in peripheral blood mononuclear cells by intravenous immunoglobulin. *J Clin Immunol* 1994; 14:178.
1. Montgomery, RA, Zachary, AA, Racusen, LC, et al. Plasmapheresis and intravenous immune globulin provides effective rescue therapy for refractory humoral rejection and allows kidneys to be successfully transplanted into cross-match-positive recipients. *Transplantation* 2000; 70:887.
2. Sonnenday, CJ, Ratner, LE, Zachary, AA, et al. Preemptive therapy with plasmapheresis/intravenous immunoglobulin allows successful live donor renal transplantation in patients with a positive cross-match. *Transplant Proc* 2002; 34:1614.
3. Schweitzer, EJ, Wilson, JS, Fernandez-Vina, M, et al. A high panel-reactive antibody rescue protocol for cross-match-positive live donor kidney transplants. *Transplantation* 2000; 70:1531.
4. Gloor, JM, DeGoey, SR, Pineda, AA, et al. Overcoming a positive crossmatch in living-donor kidney transplantation. *Am J Transplant* 2003; 3:1017.

5. Jordan, SC, Vo, A, Bunnapradist, S, et al. Intravenous immune globulin treatment inhibits crossmatch positivity and allows for successful transplantation of incompatible organs in living-donor and cadaver recipients. *Transplantation* 2003; 76:631.
6. Jordan, SC, Vo, AA, Peng, A, et al. Intravenous gammaglobulin (IVIg): A novel approach to improve transplant rates and outcomes in highly HLA-sensitized patients. *Am J Transplant* 2006; 6:459.
7. Casadei, DH, del CRM, Opelz, G, et al. A randomized and prospective study comparing treatment with high-dose intravenous immunoglobulin with monoclonal antibodies for rescue of kidney grafts with steroid-resistant rejection. *Transplantation* 2001; 71:53.
8. Luke, PP, Scantlebury, VP, Jordan, ML, et al. Reversal of steroid- and anti-lymphocyte antibody-resistant rejection using intravenous immunoglobulin (IVIg) in renal transplant recipients. *Transplantation* 2001; 72:419.
9. Rocha, PN, Butterly, DW, Greenberg, A, et al. Beneficial effect of plasmapheresis and intravenous immunoglobulin on renal allograft survival of patients with acute humoral rejection. *Transplantation* 2003; 75:1490.
0. Glotz, D, Haymann, JP, Sansonetti, N, et al. Suppression of HLA-specific alloantibodies by high-dose intravenous immunoglobulins (IVIg). A potential tool for transplantation of immunized patients. *Transplantation* 1993; 56:335.
1. Tyan DB, Li, VA, Czer, L, et al. Intravenous immunoglobulin suppression of HLA alloantibody in highly sensitized transplant candidates and transplantation with a histoincompatible organ. *Transplantation* 1994; 57:553.
2. Glotz, D, Antoine, C, Julia, P, et al. Desensitization and subsequent kidney transplantation of patients using intravenous immunoglobulins (IVIg). *Am J Transplant* 2002; 2:758.
3. Jordan, SC, Tyan, D, Stablein, D, et al. Evaluation of intravenous immunoglobulin as an agent to lower allosensitization and improve transplantation in highly sensitized adult patients with end-stage renal disease: Report of the NIH IG02 trial. *J Am Soc Nephrol* 2004; 15:3256.
4. Vo, AA, Toyoda, M, Peng, A, et al. Effect of induction therapy protocols on transplant outcomes in crossmatch positive renal allograft recipients desensitized with IVIg. *Am J Transplant* 2006; 6:2384.
5. Schiff, RI, Williams, LW, Nelson, RP, et al. Multicenter crossover comparison of the safety and efficacy of Intraglobin-F with Gamimune-N,

Sandoglobulin, and Gammagard in patients with primary immunodeficiency diseases. *J Clin Immunol* 1997; 17:21.

6. Warren, DS, Zachary, AA, Sonnenday, CJ, et al. Successful renal transplantation across simultaneous ABO incompatible and positive crossmatch barriers. *Am J Transplant* 2004; 4:561.
7. Stegall, MD, Gloor, J, Winters, JL, et al. A comparison of plasmapheresis versus high-dose IVIG desensitization in renal allograft recipients with high levels of donor specific alloantibody. *Am J Transplant* 2006; 6:346.
8. Zachary, AA, Montgomery, RA, Ratner, LE, et al. Specific and durable elimination of antibody to donor HLA antigens in renal-transplant patients. *Transplantation* 2003; 76:1519.
9. Gloor, JM, DeGoey, S, Ploeger, N, et al. Persistence of low levels of alloantibody after desensitization in crossmatch-positive living-donor kidney transplantation. *Transplantation* 2004; 78:221.
0. Haas, M, Bohmig, GA, Leko-Mohr, Z, et al. Peri-operative immunoadsorption in sensitized renal transplant recipients. *Nephrol Dial Transplant* 2002; 17:1503.
1. Lorenz, M, Regele, H, Schillinger, M, et al. Peritransplant immunoadsorption: a strategy enabling transplantation in highly sensitized crossmatch-positive cadaveric kidney allograft recipients. *Transplantation* 2005; 79:696.
2. Haas, M, Montgomery, RA, Segev, DL, et al. Subclinical acute antibody-mediated rejection in positive crossmatch renal allografts. *Am J Transplant* 2007; 7:576.
3. Gloor, JM, Cosio, FG, Rea, DJ, et al. Histologic findings one year after positive crossmatch or ABO blood group incompatible living donor kidney transplantation. *Am J Transplant* 2006; 6:1841.
4. Delmonico, FL, Morrissey, PE, Lipkowitz, GS, et al. Donor kidney exchanges. *Am J Transplant* 2004; 4:1628.
5. de Klerk, M, Keizer, KM, Claas, FH, et al. The Dutch national living donor kidney exchange program. *Am J Transplant* 2005; 5:2302.
6. Montgomery, RA, Zachary, AA, Ratner, LE, et al. Clinical results from transplanting incompatible live kidney donor/recipient pairs using kidney paired donation. *JAMA* 2005; 294:1655.
7. Gentry, SE, Segev, DL, Montgomery, RA. A comparison of populations served by kidney paired donation and list paired donation. *Am J Transplant* 2005; 5:1914.
8. Ackerman, PD, Thistlethwaite, JR Jr, Ross, LF. Attitudes of

minority patients with end-stage renal disease regarding ABO-incompatible list-paired exchanges. Am J Transplant 2006; 6:83.

9. Doxiadis, II, Smits, JM, Persijn, GG, et al. It takes six to boogie: allocating cadaver kidneys in Eurotransplant. Transplantation 2004; 77:615.
0. Claas, FH, Witvliet, MD, Duquesnoy, RJ, et al. The acceptable mismatch program as a fast tool for highly sensitized patients awaiting a cadaveric kidney transplantation: short waiting time and excellent graft outcome. Transplantation 2004; 78:190.

