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# Desensitization for solid organ and hematopoietic stem cell transplantation

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**Summary:** Desensitization protocols are being used worldwide to enable kidney transplantation across immunologic barriers, i.e. antibody to donor HLA or ABO antigens, which were once thought to be absolute contraindications to transplantation. Desensitization protocols are also being applied to permit transplantation of HLA mismatched hematopoietic stem cells to patients with antibody to donor HLA, to enhance the opportunity for transplantation of non-renal organs, and to treat antibody-mediated rejection. Although desensitization for organ transplantation carries an increased risk of antibody-mediated rejection, ultimately these transplants extend and enhance the quality of life for solid organ recipients, and desensitization that permits transplantation of hematopoietic stem cells is life saving for patients with limited donor options. Complex patient factors and variability in treatment protocols have made it difficult to identify, precisely, the mechanisms underlying the downregulation of donor-specific antibodies. The mechanisms underlying desensitization may differ among the various protocols in use, although there are likely to be some common features. However, it is likely that desensitization achieves a sort of immune detente by first reducing the immunologic barrier and then by creating an environment in which an autoregulatory process restricts the immune response to the allograft.

Keywords: *desensitization, donor-specific antibodies, solid organ transplantation, hematopoietic stem cell transplantation, plasmapheresis, intravenous immunoglobulin*

## Introduction

Desensitization has long been an accepted treatment for patients with asthma or allergy. In these cases, increasing doses of antigen are administered with the goal of inducing immunoglobulin G (IgG) antibodies to block IgE activation of mast cells. In transplantation, desensitization is a treatment protocol designed to eliminate antibody to donor HLA and/or ABO antigens or reduce the antibody to a level that permits successful transplantation. Desensitization protocols have been used in solid organ transplantation for two decades and today are employed worldwide to increase opportunities for transplantation. These protocols have been used most extensively in renal transplantation, and most of the data on desensitization in solid organ transplantation are from the kidney experience. However, many of the same principles

apply to transplantation of other organs and tissues. Recently, desensitization has been applied in HLA incompatible, hematopoietic stem cell transplantation (HSCT) in sensitized patients. Desensitization protocols for these patients have been modified to accommodate the necessary induction treatment. Because desensitization for stem cell transplantation is a relatively recent development with special considerations, we have dealt with that topic in a separate section.

In the section on desensitization for solid organ transplantation, we have not, in all cases for reasons of space, specified the exact immunosuppression regimens but rather have noted only the specifics related to desensitization and these have been generalized as follows. Immunoadsorption (IA) may use a staphylococcal protein A or protein G column to remove IgG, but in some cases, IA has been performed with antigen-loaded columns designed to remove anti-A or anti-B isoagglutinins specifically. High dose intravenous immunoglobulin (IVIG) is most often used at a level of 2 g IVIG per kilogram of body weight, but in some cases it is not given as a single treatment but instead divided into smaller doses, such as 400 mg/kg over 5 days. Plasmapheresis (PP) combined with low dose IVIG is most often performed as alternate day, single volume plasmapheresis or plasma exchange (PE) followed by 100 mg/kg IVIG. Minor modifications to these protocols have been employed by various programs, and this specific information can be found in the references. Finally, there is some variability in terminology used in publications. PP and PE have both been used to refer to PE, and the terms here are used interchangeably. While the term donor-specific antibody (DSA) is often used to refer to antibodies specific for donor HLA antigens, we use the terms HLA-DSA and ABO-DSA to specify antibodies to donor antigens of the HLA and ABO systems, respectively. Finally, because of the extensive literature in desensitization for solid organ transplantation, we have cited only seminal or representative articles, but for HSCT, a relatively new practice, we have cited nearly all available publications.

## The need for desensitization in solid organ transplantation

### Barriers to transplantation

The greatest barriers to both access to and success of organ transplantation remain ABO incompatibility and sensitization to HLA antigens. Among whites in the United States, the probabilities of finding ABO compatible donors are 42%, 88%, 50%, and 100% for patients of blood type O, A, B, and AB, respectively. If blood type O deceased donors must be allocated only to blood type O patients, then the probabilities of a finding an ABO compatible donor for blood types A, B, and AB are reduced to 46%, 8%, and 50%, respectively. The problem is even greater when the population is a mixture of different ethnic groups that differ in their blood type distribution. The most recent data available from the United Network for Organ Sharing, the agency that oversees organ allocation in the United States, show that the frequency of renal transplantation among patients waiting 2 years was actually less than predicted for blood types O, A, and AB which were 20%, 34%, and 45%, respectively (1). The probability of finding an ABO compatible donor increases appreciably among a patient's first degree relatives. For example, the probability that a blood type O patient will have an ABO compatible parent is 67%. However, this avenue is unavailable or severely restricted for transplants other than kidney.

The probability of finding a kidney donor to whom a patient has no or an acceptably low level of antibody to the donor's HLA phenotype is inversely proportional to the breadth of the patient's sensitization and as sensitization increases, so does the time to transplantation (2) (*Fig. 1*). This problem is worsened when there is a high degree of HLA heterogeneity in the population as occurs in the United States (3, 4) and, for very highly sensitized patients, is not improved appreciably when individuals who are related biologically to the patient are considered for donation. The frequency of sensitization differs among groups defined by race or gender with a greater proportion of sensitized patients among blacks versus whites and females versus males (5) (*Fig. 2*). Furthermore, it is likely that the frequency of sensitization is underestimated for the following reasons: (i) antibody breadth assessed as panel reactive antibody (PRA) does not take into account the collective effect of antibody to both HLA class I and class II antigens; (ii) early sensitization data were derived, at least in part, from cell-based assays, predominantly complement-dependent cytotoxicity (CDC), while the solid phase immunoassays in current use have a much higher sensitivity enabling detection of antibodies at lower levels than are detectable by CDC; (iii) PRA derived from cell-based assays reflected the HLA composition of the panel used to test the sera more than the composition of the donor population while, more recently, a calculated PRA (CPRA) used in the US and similar statistics used in other countries are determined from donor population frequencies and more accurately reflect the likelihood that an unrelated donor will be incompatible (6); and (iv) there may be undetected sensitization when the

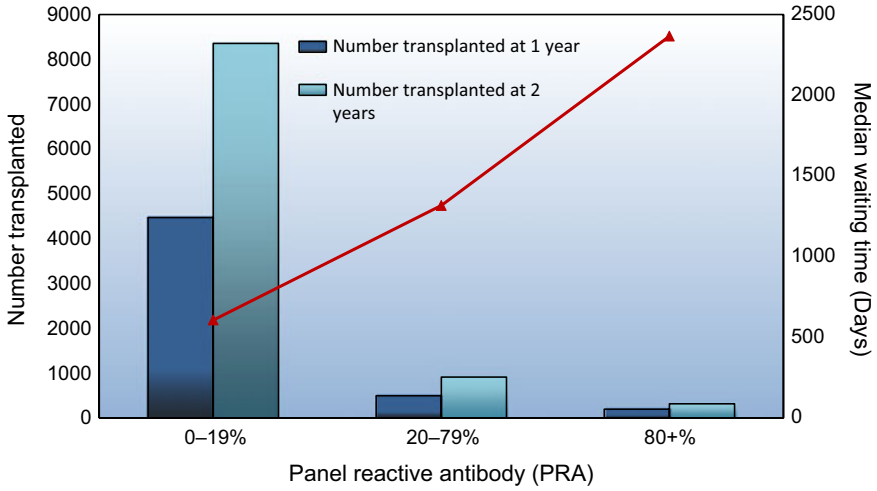


***Fig. 1.* Numbers of transplants decrease and waiting time increases with increasing breadth of sensitization.** Percent panel reactive antibody (PRA) was used until 2009 as the measure of the breadth of sensitization and reflected the number of individuals in a panel, selected to represent a wide array of HLA antigens, with whom a patient's serum gave a positive crossmatch. Patients are divided into three PRA groups: low or no sensitization (0–19), moderately sensitized (20–79), and highly sensitized (≥80). Bars represent the numbers of transplants occurring during the first and second year on a waiting list for deceased donor transplantation, and the line represents the median waiting time.

antibody is no longer present in sera available for testing. The disadvantage effected by sensitization to HLA antigens is sufficiently serious to have been addressed in the original National Organ Transplant Act (NOTA) of 1984 that specified, 'The Organ Procurement and Transplantation Network shall … establish in one location or through regional centers … a national system to match organs and individuals included in the list, especially individuals whose immune system makes it difficult for them to receive organs' (7).

The effect of ABO phenotype and/or HLA sensitization is that some patients waiting for a deceased donor renal transplant will die before a suitable donor is found or will have willing and suitable living donors who cannot donate because of an immunologic barrier. The impact is much greater on life saving transplants, such as heart, lung, and hematopoietic stem cells.

## Impact of immunologic incompatibility on graft survival

Patients sensitized to HLA antigens are known to have reduced graft survival and the extent of the effect is influenced by both breadth of sensitization and antibody strength. Mjörnstedt *et al.* (8) found that 1 year survival of renal allografts among patients with a PRA <10% was 37% greater than that of patients with PRA >50%. One year graft survival examined in a large cohort of recipients of first renal grafts from deceased donors was 79% among non-sensitized patients (n = 15 615), 78% among moderately sensitized (PRA 1–50%) patients (n = 4824), and 72% among broadly sensitized patients (n = 2615) (9). There was also an increase in delayed graft function with increasing breadth of sensitization. Süsal and Opelz (10) found that among 4136 recipients of renal grafts from deceased donors, 2 year graft survival was reduced among sensitized patients compared with non-sensitized patients and the effect of antibodies to HLA class I antigens was comparable with that of antibodies to class II antigens. However, there was a large difference in 2 year graft survival among patients sensitized only to HLA class I antigens (85%) or class II antigens (84%) compared with that of patients with antibodies to both types of HLA antigen (71%). Many early studies did not examine if antibodies found by tests of lymphocytes from a panel of individuals were donor-reactive. The impact of sensitization was found to be greater when a lymphocyte crossmatch test between donor and recipient was also found to be positive (11–14), and the risk of early graft loss and graft dysfunction was greatest when a positive crossmatch was due to antibodies to the HLA phenotype of the donor (15–17). Furthermore, the negative impact of antibodies to donor HLA antigens has been demonstrated in all organs transplanted (reviewed in 18). The association between risk to the graft and breadth of sensitization without crossmatch data is most likely due to the presence of HLA-DSA in some cases and the post-transplant development of DSA through epitope spreading (19, 20) in other cases. Today, multiplexed bead assays have replaced

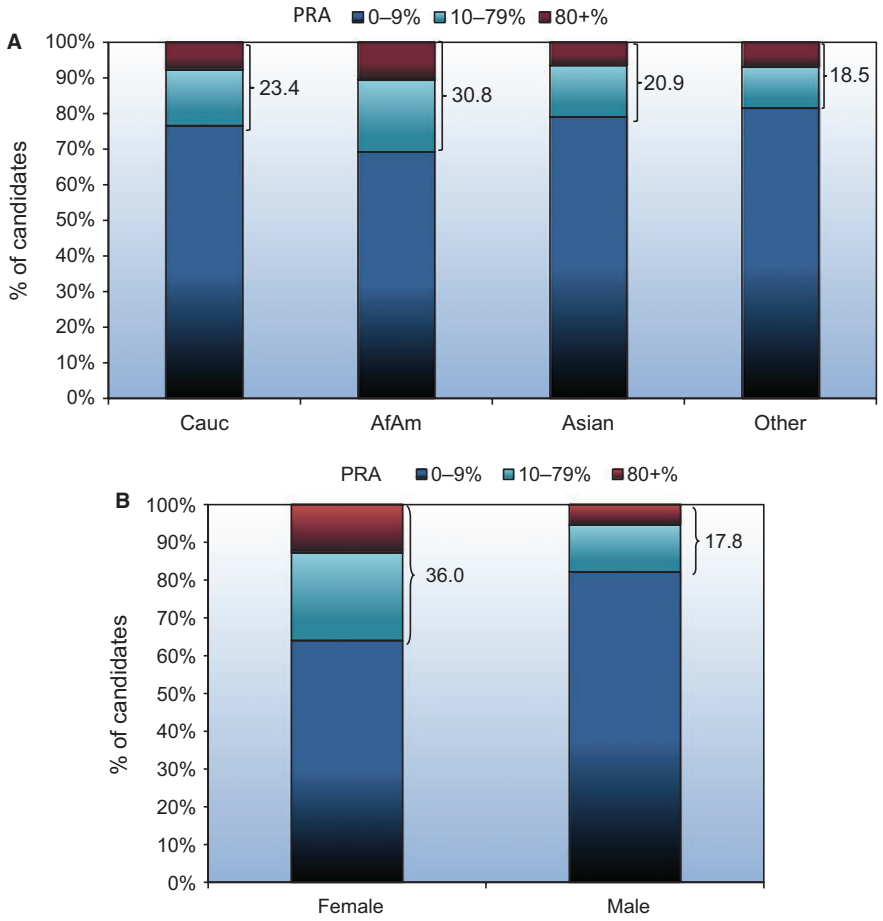


***Fig. 2.* Sensitization rates vary among patients categorized by either race or gender.** In these graphs, the panel reactive antibody (PRA) categories differ from those in *Fig. 1* with PRA 0–9 considered non-sensitized. (A) The highest frequency of sensitization (>30%) occurs in African-Americans with 10% of patients being very highly sensitized (PRA ≥80%). (B) The frequency of sensitization in females is twice that in males and the difference is even greater among the very highly sensitized.

cell-based assays for detection and characterization of HLA antibodies. These assays are more sensitive and specific and provide results in a more timely fashion than do cell-based assays, thus providing improved interpretation and clinical assessment of crossmatch results (21).

For nearly three decades, ABO incompatible organ transplantation was avoided because isohemagglutinins specific for the mismatched donor A and/or B antigen adhered to the vascular endothelium of the graft and activated complement that, in turn, led to infiltration of platelets and mononuclear cells and formation of microthrombi, ultimately resulting in vascular occlusion. The clinical outcome was hyperacute or acute humoral rejection and graft failure. An exhaustive discussion of this topic is beyond the scope of this article, but the interested reader can find an extensive review of this topic and the impact of ABO incompatibility on transplantation of various organs elsewhere (reviewed in 22).

Not only does sensitization to ABO and HLA antigens reduce the opportunity for many patients to be transplanted but may result in immediate graft loss, increased incidence of antibody-mediated rejection (AMR), graft dysfunction, and/or chronic rejection. Historically, these immunologic barriers were avoided and attempts to comply with the mandate of NOTA was to give some priority in allocation of deceased donor organs to patients highly sensitized to HLA and allocate organs from blood type O donors to only blood type O patients. These practices had limited impact, particularly before the use of erythropoietin-stimulating agents reduced sensitization via blood transfusion, because even moderately sensitized patients waited longer and often experienced a broadening of their sensitization while waiting for transplantation. The problem has been particularly egregious for patients awaiting kidney transplantation as 15.3% of those patients have been previously transplanted, comprising 95.8% of all patients waiting for retransplantation, many of

whom have been sensitized by one or more previous transplants. Furthermore, the number of patients awaiting renal transplantation is growing, increasing from 46 649 in 2001 to 104 529 at the time of this writing, with a concomitant increase in waiting time that carries the risk of broadening sensitization. Today, several options are available to deal with the ABO and HLA barriers. These include paired exchange for kidney transplantation, desensitization that can be pre-emptive to increase the likelihood of transplantation or prevent AMR or therapeutic to treat AMR and can be applied to transplants of all organs, and kidney paired donation combined with desensitization to a donor who has a reduced immunologic barrier. This review addresses desensitization.

## Desensitization protocols

Early indications that not all donor-reactive antibodies were pathologic came from successful ABO incompatible (ABOi) liver transplants (23). Subsequently an ABOi but HLA-identical kidney was transplanted successfully in a patient whose breadth of HLA sensitization required an HLA-identical donor (24). Also, successful ABOi bone marrow transplantation following removal of ABO antibodies prior to marrow infusion (25) and the success of renal transplants that had a positive historic lymphocyte crossmatch due to HLA-DSA but a negative current crossmatch (26, 27) showed that the condition of transplantation with its concomitant immunosuppression might interfere with immunologic memory. Desensitization evolved from protocols initially used to treat immunologic diseases and later found to be efficacious in treating AMR in transplantation. Three major desensitization protocols in use today are plasmapheresis with or without IA, high dose IVIG, and plasmapheresis combined with low dose IVIG. All of the protocols have been found to be more effective when immunosuppression includes a calcineurin inhibitor and agents that inhibit or eliminate T cells and/or B cells. More recently, agents that lead to apoptosis of plasma cells or inhibit complement have been included, selectively, in desensitization protocols.

### Plasmapheresis and immunoadsorption

PP or PE removes plasma from the circulation and replaces the removed plasma with plasma lacking the antibodies of interest or with a plasma substitute. In addition to antibodies, other substances in the plasma such as immune complexes, complement, and cytokines that may contribute to injury of a transplanted organ are also removed. At the onset of treatment, PP is more effective in removing IgM, which resides in the intravascular spaces, but over time, PP also reduces the amount of IgG in the circulation. Alarabi *et al.* (28) treated 23 patients, who were awaiting renal retransplantation and who had PRA ≥50%, with 12 PP treatments, cyclophosphamide, and prednisolone. Although PRA values were decreased with treatment, eight of 22 patients transplanted lost their grafts to rejection. Miura *et al.* (29) found that greater success was achieved when deoxyspergualin (DSG), a drug that inhibits interleukin-2 (IL-2)-stimulated maturation of T cells, was added to the immunosuppression regimen. Comparing AMR incidence and 5 year renal graft survival among patients treated with PP, cytoxan, antilymphocyte globulin, cyclosporine, and steroids, they found that the incidence of AMR among 10 patients treated with DSG compared with 13 patients who did not receive DSG was 49% versus 62%, respectively. Also, 5 year graft survival was better in the DSG group (83%) compared with the non-DSG group (69%). In IA, plasma extracted via PP is passed through a column, such as a staphylococcal protein A column, to remove IgG, or through a column loaded with an antigen or comparable molecule, to remove certain antibodies, such as isoagglutinins, specifically. Palmer *et al.* (30) treated 10 previously transplanted patients with protein A column IA, cyclosporine, steroids, and anti-thymocyte globulin (ATG). PRA levels were reduced in all patients but rebounded in 1 month in five of the ten patients. Of seven patients transplanted, one patient had no graft function and one other lost a graft at 1 year due to rejection mediated by a recurrence of the original DSA. IA with a protein A column reduced IgG levels in 14 patients in a study by Hakim *et al.* (31). PRA reduction was seen in 9 of the 14 patients; however, HLA antibody titers returned to baseline within 4 weeks. Thus, it seems that both PP and IA provide a transient reduction in HLA antibody levels but that a moderate level of success can be achieved when treatment includes drugs such as DSG and transplantation occurs at the HLA antibody nadir.

### High dose IVIG

IVIG is prepared from human plasma pooled from thousands of individuals and purified to be 90–99% IgG. For use in transplantation, the material should be shown to contain no ABO or HLA antibodies, as DSA in IVIG has been shown to induce acute kidney injury (32). High dose IVIG is usually a dose of 2 g IVIG per kilogram body weight. In an early study, Glotz *et al.* (33) treated five sensitized patients

and saw a reduction of many HLA antibodies in four of the patients, which lasted for more than 3 months. Later, they reported that antibody reduction occurred in 13 of 15 patients treated monthly with 2 g/kg IVIG, and 10 received renal transplants from HLA-mismatched donors (3 received transplants from matched donors). All 10 had HLA antibody prior to treatment but because of the breadth of sensitization and the limitation of the techniques available, donor-specificity could be established in only seven (34). High dose IVIG has also been used extensively by the program at Cedars-Sinai, where Tyan *et al.* (35) saw that 18 patients who had PRAs of 40–70% and who were treated with 2 g/kg IVIG had a reduction in PRA of 4–70%. Members of this same program achieved transplantation of 42 of 45 patients treated monthly with high dose IVIG (36). The pretreatment complement-dependent cytotoxicity crossmatch (CDCXM) was inhibited completely in 35 of these patients and reduced to flow cytometric crossmatch (FCXM) positive in the remaining seven. In a double-blinded, multicenter trial, it was shown that, compared with placebo, mean time to transplant for patients treated with high dose IVIG was reduced from 10.3 to 4.8 years without any reduction in graft survival (37). More recently, the Cedars-Sinai program has added rituximab, a chimeric antibody specific for CD20 and effective in eliminating peripheral B cells, to their protocol and reported the transplantation of 76 patients using this protocol. They demonstrated that most, but not all, patients showed a reduction in the strength of DSA (38). For patients awaiting deceased donor renal transplantation, using a protocol of high dose IVIG on days 1 and 30 and rituximab on day 15, the Cedars group transplanted 80 (74%) of 108 patients treated, 10 of whom received transplants with no HLA-A, HLA-B, or HLA-DR mismatches. Forty-two of these patients had a positive FCXM at the time of transplantation. Twenty-five of the 28 who had a negative FCXM had pretreatment sera tested, which were all found to be FCXM-negative, making it difficult to determine the number of transplants that resulted from the treatment (39).

In contrast to the reports above, there have been two reports indicating that high dose IVIG does not reduce sensitization or improve transplant rates. Koslowski *et al.* (40) treated five patients who had CPRAs of 56–100%. They observed a reduction in antibodies but a rebound to original levels or higher within 3 months, and none of the five patients were transplanted. Alashkar *et al.* (41) treated 27 patients who had a mean CPRA of 100 with high dose IVIG monthly. Some patients did show a transient reduction in the strength of some antibodies, but these rebounded to original levels and comparable fluctuations in antibody strength occurred in the 7 months prior to treatment. Twelve (41%) of the patients in this study were transplanted compared with only 12.8% of a matched cohort. However, all the transplanted patients had negative crossmatches with sera obtained prior to IVIG treatment and would have been transplanted without the treatment. It is likely that the inability to see an effect of IVIG in this study was because the patients all had very strong antibodies to multiple HLA antigens including the most common antigens, while the antibody reduction reported by others may have involved antibodies of lower strength (42, 43). Side effects and complications related to IVIG appear to be limited (44, 45) but possible (46, 47), and compared with patients on dialysis, treatment with IVIG is more cost effective and provides better 3 year patient survival (48). Appel *et al.* (49) treated lung transplant patients sensitized to third party HLA antigens with high dose IVIG, with or without IA, based on observations that the incidence of bronchiolitis obliterans syndrome in these patients was higher than in non-sensitized patients. They found complete elimination of HLA antibody in six of seven patients with class I antibody and one of three patients with class II antibody. IVIG has also been used, successfully, to treat antibody-mediated rejection (50–52) and to increase the opportunity for heart transplantation (36, 53).

### Plasmapheresis and low dose IVIG

Plasmapheresis followed by low dose (100 mg/Kg) IVIG is a widely used protocol for desensitization to HLA antigens. This protocol is applied pre-emptively for living donor renal transplantation and immediately after deceased donor transplantation when DSA is present. It is also used to treat AMR in renal, heart, and lung transplantation. Although there are numerous minor variations among centers, the protocol pioneered at the Johns Hopkins Comprehensive Transplant Center is alternate day, single volume plasmapheresis followed by 100 mg/kg pooled, hyperimmune anti-CMV immunoglobulin (CMVIG). Tacrolimus and MMF are administered at the start of treatment, and steroids and induction agents such as daclizumab or anti-thymocyte globulin are given at the time of transplant (reviewed in 54). After using this protocol to achieve successful treatment of AMR (55, 56), we next applied it to preemptive treatment of DSA, which was initiated prior to living donor transplantation or immediately after deceased donor

transplantation. In an initial cohort of 49 patients with DSA that ranged in strength from FCXM+ to a CDCXM titer of 4096, 63% had eliminated DSA by the end of treatment, while third party antibody was eliminated in only 27% at the same point. However, we found that among 38 patients who had sera tested for 2–90 (mean 13) months after the end of treatment, 34 (89%) no longer had DSA but that the percentage of patients with third party antibodies had increased to 81% due to recurrence of those antibodies (57). Antibody testing in this group of patients had been performed using cell-based techniques and enzyme-linked immunosorbent assay (ELISA) with soluble HLA molecule targets. We later tested sera from 67 desensitized patients using the more sensitive, multiplexed bead assays and found that the incidence of persistent antibody was higher than what had been detected by ELISA and that persistence of DSA was most affected by titer and specificity. Among patients whose pretreatment crossmatch was positive by CDC, 67.9% had persistent DSA, while only 30.8% of those with a positive FCXM had persistent DSA. When categorized by specificity for HLA-A or HLA-B, HLA-DRB1 or HLA-DQ, and HLA-DRB3-5, we found that DSA persisted for 24%, 40%, and 80% of those antibodies, respectively (58). Gloor *et al.* (59) treated 14 renal patients, who had DSA to living donors, with a combination of plasmapheresis, low dose (100 mg/kg) IVIG, rituximab, and splenectomy. Pre-treatment CDCXM titers ranged from 2 to 16, and all 14 achieved negative crossmatches prior to transplantation. They later examined DSA persistence in 12 of 33 patients treated with this protocol (60). All 12 patients had positive CDCXM with living donors prior to treatment, and all 12 had negative CDCXM at the time of transplantation but eight of the 12 were positive by FCXM. Four months after transplantation, 6 patients remained $FCXM^+$, and nine of 11 tested in the multiplexed bead assay were positive for DSA. As with high dose IVIG, PP combined with low dose IVIG has also been used to treat AMR successfully (55, 56, 61–64).

There have been few comparisons of the efficacy of high dose IVIG and PP/IVIG in reducing HLA-specific antibodies. With both protocols, antibody reduction is transient and will eventually rebound if transplantation does not occur. The duration of antibody reduction can be several months with high dose IVIG, while rebound after cessation of PP/IVIG can be immediate making high dose IVIG better suited for patients awaiting deceased donor transplantation. However, we and others (40–42, 65) have found that PP/IVIG is more effective with strong antibodies than is high dose IVIG. In Addition, 98% of patients entered into the desensitization protocol at the Johns Hopkins Comprehensive Transplant Center have been transplanted.

### Desensitization for ABO incompatibility

Once considered an insurmountable barrier and an absolute contraindication to transplantation, successful renal transplantation with an ABOi donor has been achieved using protocols that incorporate plasmapheresis combined, in some cases, with IA, and/or B-cell depletion (66–68). It was initially believed that splenectomy was necessary to ensure graft survival. However, this practice was replaced with use of a B-cell-depleting agent, and ultimately it was found that the standard PP/IVIG protocol without B-cell depletion was equally effective (68) in achieving successful ABOi transplantation. It is not necessary to achieve a negative red cell crossmatch and there is usually some increase in ABO-DSA titer after transplantation. Almost incredibly, successful transplantation has been achieved in the presence of antibodies to both ABO and HLA antigens of the donor (69–71).

### New agents

Recently, the protocols described above have been augmented with new therapeutic agents. Two of these, in wide use, are bortezomib, a proteasome inhibitor that leads to apoptosis of plasma cells, and eculizumab, a humanized antibody specific for the C5 component of complement that prevents formation of the membrane attack complex (MAC). While removal of HLA-DSA and depletion of B cells have been effective in reducing antibody levels, plasma cells are unaffected by these treatments and can lead to sustained low levels of DSA or a strong rebound following cessation of desensitization. Bortezomib effectively depletes plasma cells (72); however, mixed results for HLA-DSA reduction have been reported (73–76). Given its demonstrated effect on plasma cells, it may be that desensitization protocols in use do not optimize the effectiveness of bortezomib. There are insufficient data to draw conclusions about the utility of eculizumab in desensitization (77), and it will be important to determine its effect on infection risk.

### Dealing with unrecognized sensitization

Sensitized patients may stop making antibody, and if the only samples available are those without antibody, sensitization may go undetected. Although this may be true of any patient, those at greatest risk for unrecognized sensitization are previously transplanted patients and parous females. We

developed an assay for detecting and quantifying HLA-specific B cells by staining with HLA tetramers and showed that patients with low levels of B cells specific for a particular HLA antigen did not make antibody to that antigen following transplantation, even when the antigen was a mismatched donor antigen. We anticipated that the trauma of surgery, a very pro-inflammatory event, could lead to non-specific activation of memory B cells. We did see that patients who had increased levels of B cells to an HLA antigen did make antibody to that antigen in the immediate post-transplant period, whether or not they were mismatched for that antigen (78, 79). We further investigated if B-cell depletion at the time of transplantation would prevent an anamnestic response in patients who had elevated B cells specific for an HLA antigen but did not have antibody to that antigen. Among patients with elevated frequencies of B cells but no antibody to specific HLA antigens, 12 of 14 (85.7%) patients who were not treated with rituximab made antibody to those antigens following transplantation. In contrast, none of 10 such patients who were treated with rituximab made antibody to the specific HLA antigens after transplantation ($P = 0.00004$) (80). We also examined the effect of rituximab on the levels of 256 HLA antibodies still present after transplantation in 50 desensitized patients. Among rituximab-treated patients, the levels increased in 7% and 33% of antibodies to donor HLA and non-donor HLA, respectively, while greater increases of these antibodies (32% and 55%, respectively) occurred among patients not treated with rituximab (Jackson AM, *et al*. Presented at the Cutting Edge of Transplantation Meeting, February 14, 2013). Data showing that B-cell depletion at the time of transplantation both prevents an anamnestic response and reduces the amount of rebound of existing antibodies following transplantation support the clinical utility of this practice.

## Outcomes of transplantation after desensitization

### Desensitization for HLA antibody

Desensitization protocols have enabled transplantation of many sensitized patients. In turn, this has proven both to increase opportunities for living donor transplantation and to extend life. However, patients transplanted after desensitization to HLA have a higher incidence of AMR and reduced graft survival, compared with non-sensitized patients. Data on AMR incidence and graft survival vary due to the variability, among centers and among patients, of several factors: (i) criteria for patient eligibility for desensitization; (ii) treatment protocols; (iii) treatment endpoints; (iv) antibody evaluation; (v) crossmatch procedures; (vi) assessment of graft function; (vii) donor type (living or deceased); (viii) DSA strength pretreatment and at transplant; (ix) length of follow-up; and (x) additional risk factors such as repeated mismatches. *Tables 1 and 2* give AMR rates and graft survival data, respectively, for selected studies representing variations of the high dose IVIG and PP/low dose IVIG protocols (35, 39, 40, 65, 71, 75, 81–86). Because of the variability noted above, these data cannot be taken as a meaningful comparison of the different protocols but, rather, show that despite the increased risk of AMR, good graft and patient survival can be achieved. One of the greatest risks for AMR is the strength of antibody at the time of transplantation or before desensitization. Reinsmoen *et al*. (87) examined antibody levels at transplantation in 16 patients who had undergone desensitization with high dose IVIG and rituximab and given alemtuzumab at the time of transplant. In the first 6 months post-transplantation, no AMR occurred in 11 patients who had either a negative FCXM or a positive FCXM with a mean channel shift (MCS) less than 200. However, three of five patients with MCS >200 did experience AMR in the same time period. Similarly, Gloor *et al*. (88) found that risk of AMR correlated with the strength of DSA prior to desensitization. They divided patients with DSA into three groups: (i) positive CDCXM; (ii) negative CDCXM, positive FCXM with MCS >300; and (iii) FCXM with MCS <300. Groups one and two were desensitized with PP/low dose IVIG and group three received no preconditioning. The AMR rates were 50, 38,

***Table 1.* Incidence of antibody-mediated rejection among patients desensitized for HLA antibody**

| Reference | Treatment | *N* | AMR, % |
|---|---|---|---|
| (65) | IVIG high dose | 13 | 80 |
| (39) | IVIG high dose + rituximab | 76 | 37 |
| (40) | IVIG high dose + rituximab | 70 | 42* |
| (71) | IVIG high dose ± PP | 124 | 4 |
| (75) | PP/Low dose IVIG | 51 | 41.2 |
| (75) | PP/Low dose IVIG + eculizumab | 26 | 7.7 |
| (65) | PP/Low dose IVIG + rituximab | 32 | 37 |
| (81) | PP/Low dose IVIG + rituximab | 6 | 0 |
| (82) | PP/Low dose IVIG + rituximab | 20 | 55 |
| (83) | PP/Low dose CMVIG ± rituximab | 100 | 31† |
| (84) | 3 protocols: PP/low dose IVIg/ splenectomy (16); PP/high dose IVIg (48); IVIg high dose (21), no treatment (17) | 102 | 37.2 |
| (89) | IA or PP + rituximab | 23 | 22 |

*N*, number of patients; AMR, antibody-mediated rejection; IVIG, intravenous immunoglobulin; PP, plasmapheresis; IA, immunoadsorption.
*Excluding patients who received zero mismatched grafts.
†Current data from nearly 300 patients show a 22% AMR incidence.

***Table* 2. Graft and patient survival in renal transplantation after desensitization**

| Reference | Treatment | *N* | Term | Graft survival, % | Patient survival, % |
|---|---|---|---|---|---|
| (84) | 3 protocols: PP/low dose IVIG; PP/high dose IVIg; IVIG high dose | 102 | 5 years | 70.7 | 92.5 |
| (46) | IVIG high dose | 79 | 3 years | 87.1 | 97.5 |
| (71) | IVIG high dose ± PP | 124 | 2 years | 96.0 | 98.0 |
| (39) | IVIG high dose + rituximab | 76 | 2 years | 84.0 | 95.0 |
| (35) | IVIG high dose | 15 | 1 year | 81.8 | 100.0 |
| (81) | PP/Low dose IVIG + rituximab | 6 | 33 months | 100.0 | 100.0 |
| (75) | PP/Low dose IVIG + eculizumab | 16 | 1 year | 100.0 | 100.0 |
| (85) | PP/low dose IVIG | 51 | 2 years | 81.0 | 91.0 |
| (86) | PP/low dose IVIG ± rituximab | 211 | 1 year | >90 | 90.6 |
| (83) | 3 protocols: PP/low dose IVIG/splenectomy (16); PP/high dose IVIG (48); IVIG high dose (21), no treatment (17) | 102 | 5 years | 70.7 | 83.5 |
| (89) | IA or PP + rituximab | 23 | 2 years | 100.0 | 100.0 |

PP, plasmapheresis; IVIG, intravenous immunoglobulin; IA, immunoadsorption.

and 30% in groups 1, 2, and 3, respectively. Klein *et al.* (89) desensitized 23 patients with a combination of IA or plasmapheresis and rituximab. They had 100% graft survival at 2 years. However, 2 of 11 patients who had +CDCXM lost their grafts in the third post-transplant year. Hirai *et al.* (90) examined the need for desensitization among patients with low level DSA detectable in bead assays. Among 24 patients who were not desensitized, the rate of AMR was 33% while among 54 patients with low level DSA who were plasmapheresed and received rituximab, the AMR rate was only 4.7%. However, six of the 24 patients in the untreated group had positive FCXM making it difficult to assess the DSA strength associated with risk.

Despite the increased risk of AMR, transplantation after desensitization provides increased patient survival compared to dialysis. Jordan *et al.* (40) reported that at 3 years, patient survival among those desensitized and transplanted was 97% compared with 78% for a set of matched patients on dialysis. Montgomery *et al.* (86) compared patient survival at 1, 3, 5, and 8 years for patients transplanted after desensitization with PP/low dose IVIG to that of a matched set of patients on hemodialysis. Patient survival rates at these time points were 90.6%, 85.7%, 80.6%, and 80.6% for the transplanted group and 90.1%, 67.2%, 51.5%, and 30.5% for the group on dialysis so that at 8 years, the transplanted patients were more than two and a half times as likely to be alive than if they had remained on dialysis. Importantly, survival rates among the transplanted patients were inversely proportional to DSA strength with the largest decrement in survival occurring among patients with a positive CDCXM.

We and others have observed that DSA may persist post-transplant after completion of desensitization which may be related to the increased incidence of AMR in desensitized patients. Gloor *et al.* (60) followed 12 patients desensitized with PP/low dose IVIG and rituximab for 4 months after transplantation. They saw persistent antibody at the level of a positive FCXM or lower in 11 of the 12 patients with four patients experiencing AMR. We evaluated the level of persistent antibody among 67 patients and the relationship of antibody strength to AMR (58). All the persistent antibodies were below the level of a CDCXM and were categorized as ELISA+, which in our hands is equivalent to a positive FCXM, ELISA- but positive in a multianalyte bead assay, or negative in the bead assay, which we considered as evidence of no DSA. As shown in *Fig.* 3, there was only a slight

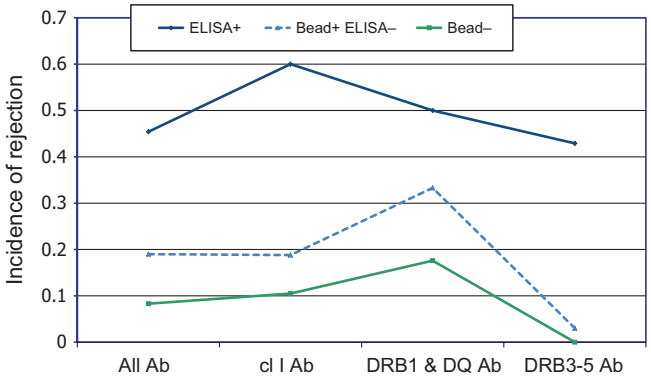


***Fig.* 3. The incidence of antibody-mediated rejection (AMR) among patients with donor-specific antibody (DSA) that persists after transplantation is affected by both strength and specificity of the antibody.** The incidence of AMR among patients with low levels of antibody (ELISA−/Bead+) is only slightly higher than among patients with no persistent DSA. However, there is a substantially increased incidence of AMR among patients with DSA strong enough to be positive in an ELISA. The highest risk of AMR is for DSA to HLA class I antigens among patients with ELISA+ DSA and for DSA to HLA-DRB1 and/or -DQ for lower levels of antibody. Antibodies to antigens encoded by HLA-DRB3-5 carried the lowest risk of AMR at either level of antibody.

increase in AMR incidence among those with any DSA positive in the bead assay only (19%) compared to those with no antibody (8%). However, there was a significantly higher incidence of those with DSA at the level of ELISA (45%) with the highest incidence occurring in patients with DSA specific for HLA class I antigens (60%).

There have been a limited number of studies that have examined subclinical rejection in patients desensitized for HLA antibody. Yamanaga *et al.* (91) examined the incidence of subclinical chronic (CAMR) detectable in protocol biopsies from 26 patients desensitized with double filtration plasmapheresis and rituximab. They found that subclinical CAMR was present in 36% of these patients. Interestingly, they found that CAMR was not associated with the class of HLA antibody but that by 1 year, most class I DSA (94%) had been cleared while three quarters of class II DSA persisted. For those with persistent class II DSA, DR antibody was associated with CAMR but DQ antibody was not. Kraus *et al.* (92) examined protocol biopsies taken in the first year from 50 patients desensitized for HLA antibody with PP/CMVIG. They found that 20–30% of the biopsies, taken at any time, had diffuse C4d staining, while biopsies from 29% of 17 patients with biopsies taken quarterly for the whole year remained C4d positive at all time points. In all cases with positive C4d staining, there was subclinical AMR. DSA was detected in the serum whenever a biopsy was positive for C4d, but only 48% of biopsies were C4d positive when DSA was present. Occurrence of subclinical AMR was associated with a positive CDCXM pretreatment and with two mismatches of antigens encoded by the DRB3, DRB4, or DRB5 loci, antibodies to which were shown, previously, to be persistent in 80% of patients (58).

### Desensitization for ABO antibody

Following cessation of treatment, isoagglutinins persist and may rebound slightly in strength among patients desensitized and transplanted with ABOi kidneys. However, as shown in *Table* 3, excellent patient and graft survival have been achieved by multiple groups (93–96). Initially, splenectomy was believed necessary to avoid irreversible AMR; however, this practice has been replaced in many centers with rituximab treatment (reviewed in 97). Gloor *et al.* (98) found that using rituximab, patient and graft survival was comparable to those for splenectomy, and with a lower risk of infection. Montgomery *et al.* (68) achieved comparable results in ABOi kidney transplantation following desensitization with PP/IVIG and with either splenectomy or rituximab or with neither splenectomy nor rituximab. Not surprisingly, as with patients with HLA-DSA, AMR is more frequent in ABOi transplants than in ABO compatible transplants and ranges from 4% to 33% (68, 96, 99, 100). Also as with HLA incompatible transplants, the risk of AMR is proportional to the titer of the ABO-DSA. Gloor *et al.* (98) found that ABO-DSA titers greater than 256 were associated with a high risk of AMR in both splenectomized and rituximab-treated patients. Tobian *et al.* (101) showed that the median ABO-DSA titers for those with and without AMR were 64 and 16, respectively. However, there was great overlap with the titers ranging from 4 to 512 among patients with AMR and 2–256 among patients without AMR. Furthermore, although titer >64 represented an increased risk of AMR in the first post-transplant year, it had a low positive predictive value. Desensitization for ABOi transplants in other organs is limited to post-transplant treatment with the exception of partial lung and liver transplants from living donors. Sanada *et al.* (102) successfully performed ABOi, living donor liver transplantation in 11 pediatric patients following PE or DFPP and rituximab pretransplantation and splenectomy post-transplantation. Song *et al.* (103) performed ABOi living donor liver transplants in 10 adults following PE and rituximab but without splenectomy. Graft and patient survival at 2 years was 90%. An excellent review of the current status of ABOi liver transplantation can be found in Raut and Uemoto (104). Lori West has shown that ABOi heart transplants can be performed in infants without the need for antibody depletion (105). In older children and adults, ABOi heart transplantation can be performed successfully using the various desensitization protocols applied to ABOi kidney transplantation (reviewed in 106).

***Table* 3. Outcomes of ABO incompatible renal transplants**

| Reference | *N* | One year survival | | Three year survival | | Five year survival | |
|---|---|---|---|---|---|---|---|
| | | Patient, % | Graft, % | Patient, % | Graft, % | Patient, % | Graft, % |
| (93) | 50 | NG | NG | 98 | 97 | NG | NG |
| (94) | 738 | 94 | 94 | 88 | 90 | 74 | 73 |
| (95) | 1878 | 97 | 93 | 95 | 89 | 93 | 84 |
| (68) | 60 | 96 | 98 | 96 | 93 | 89 | 89 |
| (96) | 50 | 100 | 100 | 100 | 100 | 100 | 100 |

NG, not given.

## Mechanisms: desensitization for HLA antibody

Most likely there is no single mechanism, even within one type of protocol, underlying the success of desensitization for HLA incompatible transplants. Furthermore, the same

variables noted above, that make it difficult to compare rates of graft survival and AMR, probably affect the specific manner in which desensitization is achieved in any given patient. With both the high dose IVIG and PP/IVIG protocols, the effect of desensitization appears to be specific and long lasting but requires transplantation or else rebound of antibody occurs. Of course, transplantation is accompanied by the use of potent immunosuppressive agents, and it is possible that by lowering DSA levels sufficiently to prevent immediate damage to the graft, the immunosuppressive agents, particularly when they include the use of B-cell-inhibitory or -depleting agents, are sufficient to prevent rebound. However, rebound does occur as does the *de novo* production of antibody in the presence of strong immunosuppression, suggesting that suppression of DSA is not due to immunosuppression therapy alone. There are data to suggest that antibody reduction may provide immediate protection from antibody-mediated injury and provide an environment that supports an active modulation of alloreactivity.

High doses of IVIG have been shown to be effective therapy in a wide variety of autoimmune disorders including idiopathic thrombocytic purpura, Kawasaki disease, systemic lupus erythematosus, myasthenia gravis, multiple sclerosis, and many others (reviewed in 107). Many mechanisms have been proposed for the effect of IVIG, predominantly through *in vitro* studies (reviewed in 108, 109). These mechanisms include anti-idiotypic antibodies, inhibition of a variety of cells including T cells and dendritic cells, anti-complementary activity (inhibition, depletion, interference with MAC formation), neutralization of BAFF and APRIL that interferes with activation of B cells and their transformation to plasma cells, and inhibition of cytokine genes and cytokine activity, among others. Two studies (34, 36) have implicated anti-idiotypic antibodies as one mechanism for IVIG suppression of HLA antibodies. It is unlikely that this is the major mechanism of IVIG, because the effect would be transient, i.e., only when the IVIG is in the circulation, not long lasting. One would expect that reduction in HLA antibodies would be consistent for certain antibody specificities, and this has not been observed. One of the most interesting potential mechanisms in both treatment of autoimmune disease and transplantation desensitization is through binding of Fc receptors (FcRs). The various ways in which FcR binding may be immunomodulatory or protective include: induction of FcγRIIB (an inhibitory receptor on B cells), cross-linking of FcγRIIB on plasma cells to induce apoptosis, induction of immunosuppressive activity in dendritic cells via binding of FcγRIII, protection from endothelial cell injury via induction of FcRn on endothelial cells, and competition with DSA for binding to FcRn with subsequent reduction of the half-life of DSA (45, 108).

Various observations suggest immunomodulatory mechanisms for PP/IVIG. Data suggest that antigen presentation may be important. Elimination of DSA is inversely proportional to antigen expression. That is, DSA to class I, DR and DQ, and DR51-53 antigens are eliminated with decreasing effectiveness. Although DR antigens are expressed at levels comparable with that of class I, their constitutive expression is limited to professional antigen presenting cells. The HLA-DR52 and -53 antigens and, quite possibly -DR51, are expressed at 14–20% the level of those encoded by the DRB1 locus. In addition, antibody was eliminated more effectively in patients whose antibody was specific for public HLA epitopes versus private epitopes, the former of which would be present in greater numbers compared with the latter (58). Differential effects of induction with either daclizumab, an anti-IL2 receptor antibody, or an ATG suggest a possible role for regulatory T cells. DSA is eliminated more rapidly and there is less rebound when daclizumab rather than ATG is used for induction and may imply that ATG eliminates Tregs (57, 110). It has been shown that pro-inflammatory events, such as infection, may cause a rebound and even an expansion of DSA (111), and the use of CMVIG may prevent viral activation helping to maintain reduced levels of DSA.

As noted above, many patients maintain low levels of DSA following transplantation. Several groups have shown that low levels of antibody to HLA class I antigens can induce a state of accommodation rendering endothelial cells (ECs) resistant to complement-mediated lysis (112–114). Exposure of ECs to sub-saturating doses of HLA class I antibody results in decreased expression of ICAM-1 and VCAM-1, an increased expression of certain anti-apoptotic genes, and an induction of the PI3/Akt pathway, conferring resistance of these cells to complement-mediated lysis. Reed's group (115, 116) has shown that ligation of HLA class I molecules can result either in accommodation or in the induction of cell survival and proliferation leading to the transplant vasculopathy seen in chronic rejection and that the outcome is dependent on the antibody level. This is supported by the data shown in *Fig.* 3, where persistent class I DSA at the level detectable only in bead assays resulted in very little increase in AMR, while at higher levels, there is a significant increase in AMR. Although persistent class I DSA may be beneficial or harmful, it is unlikely that HLA-DSA

levels remain constant but may increase with infection or trauma or may decrease through normal immunoregulatory processes. Therefore, it is likely that persistent HLA-DSA to class I antigens will result in chronic AMR negatively impacting graft survival. Conflicting data exist on the constitutive expression of HLA class II on the vascular endothelium. This is further confounded by the fact that alteration in gene promoters results in a reduced expression of antigens encoded by the DRB3 and DRB4 loci. This may explain why antibodies to the DRB3-5 encoded antigens show no increase in AMR when present at low levels and at higher levels, result in less AMR than do antibodies to class I, HLA-DRB1, or HLA-DQ (*Fig. 3*). We have followed the levels of HLA-specific B cells following desensitization and transplantation and found that, in the absence of B-cell-reducing therapy, there is little change in the frequencies of these B cells (authors' unpublished data). Thus, even when DSA is completely eliminated, desensitized patients remain at risk for an anamnestic response.

There are data indicating that desensitization treatments impact cellular immunity; however, the studies on the effect of treatments on cell-mediated immunity have been performed predominantly in the setting of autoimmune disease. Identifying the effect on cellular immunity of high dose IVIG, PP/low dose IVIG, or PP/IA in transplantation is confounded by several factors including: the number of HLA-specific T cells, which far outnumber autoreactive T cells; the occurrence of transplantation followed soon thereafter by cessation of the desensitization treatment; and the wide and varied array of immunosuppressive agents used in transplantation. Nonetheless, a number of *in vitro* experiments and observations of the *in vivo* effect of desensitization suggest that there is a downregulation of cellular responsiveness. *In vitro* experiments performed by Tha-In *et al.* (117) showed that IVIG activated regulatory T cells (Treg) and increased their ability to suppress proliferation of allogeneic T cells. Sharma *et al.* (118) examined the effects of *in vitro* treatment of cells with IVIG and/or mycophenolic acid (MPA), a common immunosuppressive agent in desensitization protocols, on cell proliferation in a two-way mixed lymphocyte reaction. They found that both drugs, individually, suppressed cell proliferation and when used together, acted synergistically to amplify the effect. They also showed that IVIG, but not MPA, induced apoptosis. In studies of autoimmune disease and immunodeficiencies, Tjon *et al.* (119) demonstrated that high dose IVIG increased the activation status of Tregs, while not changing the numbers of those cells.

In the treatment of myasthenia gravis with single volume, double filtration plasmapheresis, Chien *et al.* (120) observed a significant decrease in the percentage of $CD3^+$ T cells and a concomitant decrease in the T-helper/T-suppressor ratio, but there was a significant increase in the percentage of NK cells. Interestingly, after three treatments, all the T-cell subsets had returned to base line levels perhaps due to homeostatic proliferation. Kiprov *et al.* (121) has also shown that plasmapheresis decreased the ratio of T-helper/T-suppressor cells. Sadeghi *et al.* (122) observed a decrease in some T-cell subsets following plasmapheresis along with a modulation of T-cell activation. As noted earlier, our data suggest that the effectiveness of our PP/IVIG protocol may be dependent on antigen presentation and the possible involvement of Tregs, as elimination or reduction in DSA is less effective when ATG is used as an induction agent (110). This is in contrast to the findings of Krystufkova *et al.* (123), who found an increase in Tregs by post-transplant day 14 among patients treated with ATG. The difference in these two findings may be due to an effect of plasmapheresis and the timing of Treg development.

### Desensitization for ABO antibodies

Accommodation is a well-recognized phenomenon in ABOi transplantation. The precise mechanism underlying this process has not been elucidated. However, there are data suggesting several possible mechanisms. Mohiuddin *et al.* (124) have shown in a mouse model that mice that accommodated to blood group incompatible heart grafts showed a shift in IgG subclass to one that activates complement poorly. Takahashi (125) suggests that following transplantation, there is a decrease in the levels of circulating glycosyltransferase, which in turn, leads to decreased ABO antigen production and reduced susceptibility to antibody-mediated injury. This is supported by data showing an ongoing decrease in the expression of A or B antigens on ABO incompatible renal transplants (126). Accommodation most likely accounts for the success of ABOi liver transplants. Two other groups have suggested that B cells specific for the ABO incompatible antigens are either suppressed, rendered tolerant, or eliminated (127, 128). West has shown that ABOi heart transplantation in infants generates a B-cell tolerance and an absence of antibody to the mismatched antigen (128).

It is likely that the mechanisms permitting successful transplantation in HLAi versus ABOi transplants differ. HLA antigens are proteins while the ABO antigens are carbohydrates. Furthermore, graft failures in ABOi transplants occur

in the early post-transplant period (94), suggesting that the mechanisms leading to chronic rejection due to HLA-DSA may not exist in ABOi transplantation.

The studies cited above have shown a benefit to the use of rituximab or splenectomy in desensitization. Splenectomy together with plasmapheresis results in a rapid and drastic drop of DSA (129), most likely due to a debulking of plasma cells. Rituximab can prevent an anamnestic response (80) and reduce antibody rebound following transplantation. However, there may be a more long-lasting benefit to the use of B-cell-depleting therapies. Several studies have indicated that long-term graft survival and transplantation tolerance is associated with the presence of regulatory B cells and furthermore, that regulatory B cells arise following B-cell depletion (reviewed in 130).

In the last 10 years, there has been a growing recognition of the importance in transplantation of antibodies to antigens other than HLA and ABO (reviewed in 131, 132). However, little information is available on the effect of desensitization on these antibodies. Jackson *et al.* (133) reported a case of hyperacute rejection in a desensitized patient who had a positive crossmatch with precursor endothelial cells but lacked antibody to donor HLA. Desensitization consisted of PP/IVIG and rituximab. The graft became anuric within 24 h, and efforts to rescue the graft, which included daily PP/IVIG, rituximab, eculizumab, and at 3 days post-transplant, splenectomy, were unsuccessful. Similarly, Eng *et al.* (134) showed that desensitization with PP/IVIG reduced levels of AT1R antibody, but that three of four patients with very high baseline levels of this antibody (>40 units/ml) showed a rebound within 3–6 months after transplantation. The levels in two patients returned to the high baseline level. Both of these patients experienced AMR not due to HLA-DSA and one graft was lost to rejection.

## The need for desensitization in HSCT

### Increasing use of HLA-mismatched donors

HSCT is used as curative treatment for certain hematological malignancies and as cellular therapy for some congenital immunodeficiencies (reviewed in 135, 136). More recently, HSCT has been applied in treatment of autoimmune disorders, including systemic lupus erythematosus and multiple sclerosis, and may be applied in solid organ transplantation as clinical trials have been initiated combining donor bone marrow with a solid organ allograft in efforts to achieve a state of tolerance toward the donor organ (136). The impact of HLA incompatibility in HSCT differs from that of solid organ transplantation in that the allograft is comprised of immunologically competent cells conferring the potential of bidirectional alloreactive immune responses, both host versus graft (HVG) rejection and graft versus host disease (GVHD). The degree of HLA matching is, therefore, more critical in HSCT than in solid organ transplantation and the preferred donor choice for HSCT has been a bone marrow graft from an HLA-identical sibling, as such transplants were matched for the major HLA loci, with the possible exception of HLA-DP, as well as, on average half of minor histocompatibility antigens. However, it is well recognized that the availability of HLA-identical sibling donors is limited to, at best, 30% of HSCT candidates and current practice in allogeneic HSCT increasingly includes the use of alternative donor stem cell sources that are partially HLA mismatched. These sources may include bone marrow or mobilized peripheral blood stem cells from unrelated donors or HLA-haploidentical family members, as well as stem cells from umbilical cord blood (UCB) (135, 137–139).

With these alternative donor sources the degree of HLA mismatch can range from one or more HLA alleles to a full HLA haplotype. Data from the National Marrow Donor Program retrospective studies indicate that less than 50% of candidates can be matched with unrelated donors at the allele level for HLA-A, B, C, and DRB1 and that mismatches for HLA-DP alleles may be greater than 80% when donors and recipients are matched at the HLA-A, B, C, DRB1, DQA1, and DQB1 loci (140, 141). Therefore, unrelated registry donors often convey one or more HLA allele and/or antigen level mismatches. Stem cells from umbilical cord blood generally have higher degrees of HLA mismatching than encountered with mobilized peripheral blood or marrow from registry donors as attempts to match are based generally on HLA-A, B, and DRB1. Despite the increased incompatibility, UCB grafts do not confer a greater incidence of GVHD, presumably due to the immunologic immaturity of the transplanted cells. UCB units also can be obtained and transplanted in far less time than the average of 3–4 months required for an unrelated registry search (142). HLA haplotype identical (haplo-ID), related transplants, often performed under reduced intensity or non-myeloablative conditioning, offer candidates additional donor options and afford several advantages that have led to growth in this practice (139, 143). Haplo-ID HSCT greatly increases the number of potential donors, with over 95% of patients having at least one donor among their parents, children, and siblings with whom they share one HLA haplotype. Haplo-ID donors are also readily available, which

may be critical for patients with aggressive disease for whom the time required for an unrelated registry search may be prohibitive. The use of reduced intensity and non-myeloablative conditioning prior to transplant in haplo-ID regimens permits transplantation of older patients for whom co-morbidities prevent full myeloablation. In general, unless the stem cell grafts are depleted of T lymphocytes, increasing degrees of HLA mismatch have been associated with increased rates of severe acute and chronic GVHD. However, the non-myeloablative haplo-identical protocol with post-transplantation cyclophosphamide has been shown to provide acceptable rates of severe acute or chronic GVHD (143, 144). In a retrospective study of 185 recipients of haploidentical transplants, Kasamon *et al.* (145) found no significant difference in event-free survival or the risk of acute grade II-IV for recipients mismatched for 3–4 HLA antigens versus those with fewer mismatches. The higher degree of HLA mismatching in haploidentical transplants provides another reason for the growing use of haploidentical donors, i.e., an associated 'graft versus leukemia' benefit. This effect, manifested by reduced rates of disease relapse, may be mediated by alloreactive donor T cells and/or KIR (killer cell immunoglobulin-like receptors) mismatched NK cells (146, 147).

### Incidence of HLA-specific antibodies among HSCT candidates

While the use of partially HLA-mismatched donors makes HSCT a possibility for more candidates, the presence of HLA-DSA can present a substantial barrier to HSCT. Other factors, including the underlying disease, the degree of HLA mismatch, the stem cell source, the timing of the transplant, the pre-transplant conditioning regimen, and post-transplant immunosuppression (135, 140, 142, 148–150), all significantly affect HSCT outcomes and HLA-DSA may increase significantly the risk of adverse outcomes. The role of HLA-specific antibodies in HSCT is of much current interest and has been the topic of other recent, excellent reviews (151–153).

The major causes of humoral sensitization to HLA antigens include transfusion, previous transplantation, and pregnancy. Candidates for HSCT often receive transfusion support and many are multiparous women; therefore, it is not surprising that many candidates have HLA-specific antibodies prior to transplantation. Several studies utilizing current, sensitive solid phase immunoassays have shown that the incidence of HLA sensitization ranges from 20 to >40% for the presence of any HLA antibodies and from 3 to 24% for HLA-DSA, with differences varying with the type of donor transplant and the degree of HLA mismatch. Some of the reported incidence rates for different types of HLA-mismatched HSCT are given in *Table* 4. Two recent studies have evaluated rates of HLA sensitization among recipients of mismatched unrelated HSCT. In a retrospective, case–control study of 115 patients, Spellman *et al.* (154) observed an incidence of 37% of any HLA-specific antibody and 8.7% for DSA. Ciurea *et al.* (155) found that 20% of 592 unrelated donor recipients had HLA-DP specific antibodies and among these, 3.4% were donor specific. The presence of HLA-specific antibodies among UCB candidates has been the subject of several recent reports, as the degree of HLA mismatching is usually higher with UCB transplants than those with unrelated donors and because the cell dose of stem cells in UCB may be less than optimal and, consequently, more susceptible to antibody-mediated rejection (156–160). The antibody incidence is often higher for candidates receiving double cord blood units compared to those receiv-

***Table* 4. Incidence and impact of HLA-specific antibodies in HSCT**

| Reference | HSCT type | *N* | HLA-Ab *N* (%) | DSA *N* (%) | Significant impact of DSA and comments |
|---|---|---|---|---|---|
| (154) | Unrelated | 115 | NG | 10 (8.7) | Associated with graft failure |
| (155) | Unrelated | 592 | 116 (19.6) | 8 (1.4) | Associated with graft failure; All DSA were anti-HLA-DP |
| (156) | sUCB | 386 | 89 (23.1) | 20 (5.2) | Associated with graft failure, reduced OS and EFS |
| (157) | dUCB | 126 | 50 (39.7) | 18 (14.3) | No difference in engraftment with and without DSA |
| (158) | dUCB | 73 | NG | 18 (24.7) | Associated with graft failure, excess 100 day mortality or relapse |
| (160) | d,sUCB | 293 | 62 (21.2) | 14 (4.8) | Associated with graft failure and OS |
| (159) | sUCB | 70 | 31 (44.3) | 12 (17.1) | Both DSA and any HLA-Ab associated with reduced engraftment; DSA associated with reduced OS |
| (165) | Haplo-ID | 24 | NG | 5 (20.8) | Associated with high rate of graft failure |
| (161) | Haplo-ID | 79 | 16 (20.3) | 11 (13.9) | Associated with graft failure |
| (162) | Haplo-ID | 296 | 68 (23) | 43 (14.5) | None observed; DSA was avoided or reduced by treatment |

The incidence and impact of HLA-specific antibodies on outcomes of HSCT are given from recent studies that used current sensitive and specific solid phase immunoassays for detection and characterization of donor HLA-specific antibodies. HLA-Ab, the presence of any HLA-specific antibody; DSA, donor HLA-specific antibody; HLA-ID, HLA-identical donor; OS, overall survival; EFS, event-free survival; sUCB, single umbilical cord blood unit; dUCB, double UCB units; Haplo-ID, HLA-haploidentical donor.

ing single units, as each unit may have different mismatches with potential recipients. For example, Takanasi *et al.* (156) found a 23% prevalence of any HLA antibodies and a 5.2% incidence of DSA among 386 single UCB cases, while Brunstein *et al.* (157), reported respective incidences for any antibody and DSA of 40% and 14.3% among double UCB cases. Among haplo-ID candidates, two studies reported similar results to each other for both the presence of any HLA-specific antibody, and DSA. Yoshihara *et al.* (161), in a study of 79 cases, found respective incidences of 20% and 13.9% for any HLA antibodies and DSA, while, in a larger study of 296 cases conducted at the Johns Hopkins Sidney Kimmel Cancer Center, we reported 23% and 14.5%, respectively (162). As a major cause of HLA sensitization is via pregnancy, it is not surprising that the prevalence of HLA antibodies is greatest among multiparous women. We observed that 43% of parous females had DSA compared with 12.5% of nulliparous females and 4.9% of males (162). Similar findings have been reported by Ciurea *et al.* (155).

### The impact of HLA antibodies

The potential impact of HLA antibodies on the outcome of HSCT has been recognized since the late 1980s, as both engraftment failure (163) and reduced overall survival (164) were observed among patients with antibody levels high enough to result in positive pre-transplant CDCXM. However, until recently, most research centered on the T-cell-mediated alloreactivity as the cause of both GVHD and allograft rejection. Studies in the last few years using HLA-specific and highly sensitive solid phase immunoassays indicate that much lower levels of antibody than can be detected in CDCXM tests confer increased risk in HSCT for engraftment failure, event-free survival, disease relapse, and overall survival. The majority of clinical studies employing solid phase immunoassays to date have demonstrated significant associations of DSA with engraftment failure, regardless of the degree of HLA mismatch or stem cell source. Several of these recent reports evaluating the impact of HLA-specific antibodies on engraftment and overall survival in HSCT are summarized in *Table* 4.

Spellman *et al.* (154) used archived pre-transplant sera for a case-controlled analysis of the relationship of HLA antibodies to graft failure among 115 recipients of unrelated HSCT. Their study groups included 37 recipients with failed grafts and 78 matched controls. The presence of DSA was significantly associated with graft failure ($P < 0.001$), with a 24% incidence of graft failure among DSA+ patients compared with 1% among the controls. The antibodies were directed to both HLA class I and II mismatched alleles, including HLA-A, -B, and -DP, and although single DSA antibodies to HLA class I or II alleles conferred increased risk, the presence of antibodies to both HLA classes was most significant. Ciurea *et al.* (155) confirmed that antibodies to HLA-DP antigens, which they demonstrated to be expressed at lower levels on the cell surface of both peripheral blood lymphocytes and $CD34^+$ stem cells than were HLA-A, -B, and -DR antigens, were also associated with engraftment failure. Among 8 cases with DSA to HLA-DP antigens, graft failure occurred in 3 (37.5%). In their multivariate analysis, the DSAs were the only factor highly associated with graft failure ($P = 0.0001$).

Among UCB transplants, Cutler *et al.* (158) found significant associations with DSA for increased engraftment failure and reduced event-free survival among recipients of double UCB. Graft failure rates were 5.5% versus 18.2% versus 57.1% for cases with no DSA, DSA to a single UCB unit, and DSA to both UCB units, respectively ($P = 0.0001$). These authors also observed an impact of increased 100 day mortality or disease relapse with DSA to one unit (36.4%) and DSA to both units (71.4%) compared with no DSA (23.6%). Two other reports in UCB transplants have shown an impact of DSA on overall patient survival, as well as on engraftment failure. Ruggeri *et al.* (160) reported decreased overall survival of 29% among 14 UCB recipients with DSA compared with 42% among those with no DSA ($P = 0.07$). Takanashi *et al.* (156) found significantly decreased neutrophil and platelet recovery ($P < 0.0001$), as well as decreased event-free survival and overall survival ($P = 0.0001$, $P = 0.03$, respectively) for 20 DSA+ patients compared with non-donor antibody positive and antibody negative cases. These authors also reported no impact of HLA antibodies, whether donor specific or not, on grades II-IV acute GVHD, disease relapse, or transplant related mortality.

The incidences of DSA-associated engraftment failures in two reports of haplo-ID HSCT range from 27% to 75%. Ciurea *et al.* (165) reported a significant rate of graft failure ($P = 0.008$) among 3 of 4 haplo-ID recipients with DSA compared to 1 of 20 with no DSA. Yoshihara *et al.* (161) in a prospective study observed DSA among 11 patients receiving haplo-ID HSCT. Ten of these patients were treated before transplant to reduce their DSA levels (discussed further below). The cumulative incidence of neutrophil recovery among the pre-transplant and post-treatment DSA+ patients was significantly lower compared with DSA−

patients (61.9 versus 94.4%, P = 0.026). Engraftment failure occurred in three of five patients with high levels of DSA. Development of DSA arising after HSCT has been further reported from a multi-center study conducted as a component of the 15th International Histocompatibility and Immunogenetics Workshops (166). Not surprisingly, given the greater degree of incompatibility conferred by a full HLA haplotype mismatched donor, 15.7% of haplo-ID recipients (N = 51) developed DSA post-transplantation, while no DSA was observed among 89 recipients of 1–2 allele mismatched, unrelated donor or partially mismatched, UCB transplants. Unfortunately, long term follow-up was not available in this study, so the clinical relevance of DSA developing post-HSCT remains open to investigation.

The potential mechanisms for antibody-mediated rejection of allogeneic stem cells may include complement-mediated lysis, antibody-dependent cell-mediated cytotoxicity (ADCC), or phagocytosis by macrophages or other FcR-bearing cells. The end result of any of these mechanisms is the prevention of the homing of stem cells to niches in the recipient's marrow. Taylor *et al.* (167) demonstrated in a murine model that the major effect of alloantibody was a rapid elimination of donor stem cells through ADCC by $FcR^+$ macrophages and NK cells. While the effect of alloantibody on stem cell rejection does not rule out concomitant T-lymphocyte effector functions, passive transfer of DSA in animal models has shown that alloantibody alone is sufficient to prevent donor stem cell engraftment (168). The impact of HLA antibodies also may depend on the level of expression of the corresponding antigens on donor stem cells. Ciurea *et al.* (155) examined HLA antigen expression by flow cytometry on $CD34^+$ stem cells from eight normal donor bone marrow and peripheral blood samples. The expression of HLA-DP antigens was significantly lower than that of HLA class I antigens (HLA-A, -B, and -C) with median values of 36.1% versus 99.9%, respectively (P < 0.001). However, as noted above, these same authors also found a significant association of antibody to DP antigens with graft failure. As the expression of both HLA class I and II molecules can be upregulated by inflammatory cytokines stimulated by complement activation, it is not unreasonable that even minimal antibody binding to some mismatched donor HLA antigens on transplanted stem cells could result in an inflammatory amplification of HLA expression and subsequently, increased destruction of the allograft cells.

While there is clear evidence of the detrimental impact of HLA antibodies on engraftment, it should be noted that patients with DSA are also likely to have increased numbers of alloreactive memory T cells which also can mediate rejection of stem cell grafts. Evidence of the role of T-cell memory in rejection of bone marrow grafts was shown by Levesque *et al.* (169) in a murine model. Rejection of bone marrow grafts in allo-presensitized mice was reduced among B-cell-deficient mice, while no reduction in rejection was observed among wildtype mice whose alloantibody had cleared or decreased to minimal levels. These results indicate that B-cell-dependent, memory T-cell effectors can mediate stem cell graft rejection independently of alloantibody.

As with solid organ transplantation, an open question is what level of DSA is detrimental to HSCT outcomes. While the majority of studies to date indicate that even low levels of DSA detected by current, sensitive solid phase immunoassays confer some increased risk of engraftment failure, the available data are limited to relatively small numbers of cases and not all reports have included antibody levels associated with either engraftment or graft failure. Most recent studies have utilized single HLA antigen assays on the Luminex® platform which confounds comparison between different reports, as these assays are not quantitative and are inherently variable due to their high sensitivity (21). There are also considerable differences in the concentrations of HLA antigens on the microbeads, resulting in varying levels of reactivity for antibodies of comparable strength. For example, the level of antibodies to HLA-DQ antigens that yield a positive crossmatch can be twice that of anti-DR antibodies. Such variations in antigen reactivity likely are a factor in the ranges of antibody levels that have been associated with either engraftment failure or success. Furthermore, the thresholds for antibody levels that are considered positive range from 500 to 5000 MFI in different reports. What appears to be clear is that high antibody levels, consistent with positive cell-based crossmatch tests, carry a significantly increased risk of graft failure. Yoshihara *et al.* (161) found that antibodies with MFI values >10 000 were significantly associated with graft failure. Similarly, Cutler *et al.* (158) observed engraftment failure associated with antibodies with a median value of 17 650 MFI. Low level DSA in reports by Ciurea *et al.* (165) and Cutler *et al.* (158) were found to be permissive for engraftment. We provided correlation of the MFI ranges of reactivity with cell-based crossmatch tests, and the engraftment permissive levels in our desensitized patients (discussed below) were all well below the levels that would be positive in a FCXM (162). From other studies, however, the antibody levels associated with increased risk for graft failure range from as low as 1000 to >10 000 MFI. Ishiyama *et al.* (170) achieved successful engraftment

in a single case of a patient with CDCXM+ DSA with MFI values >10 000 on the day of transplant. This patient was treated with IVIG (400 mg/kg) from day −2 through day +2, and the IVIG likely facilitated engraftment by interfering with $FcR^+$ cell clearance of DSA-coated stem cells (discussed further below). In a report of 18 double cord blood recipients with DSA to at least one of the two UCB units, Brunstein *et al.* (157) found no difference in the cumulative incidence of engraftment between patients with and without DSA (83% versus 78%) with the DSA levels ranging from 536 to 12, 951 MFI. Among their engrafted patients, however, in 9 of 13 cases there was either no DSA detected to the long-term dominant UCB unit or the level was <1000 MFI. Despite differences in the reported levels of DSA associated with engraftment failure, the risk imposed by DSA is sufficient to have led to recommendations that screening for HLA-specific antibodies should be routine practice for any HLA-mismatched HSCT (152, 171). Additional and larger studies will be required to determine if there is a safe threshold of DSA for HSCT. Correlations will need to consider differences in threshold levels and reactivity to different HLA antigens, as well as accounting for test variability. In the interim, in addition to avoidance of the presence of any DSA toward a potential donor, a practical approach would be for transplant centers to correlate their antibody assays with actual crossmatch assays to establish acceptable levels of HLA-specific antibodies.

### Possible impact of non-HLA antibodies

While most studies to date have implicated a significant impact of donor HLA-specific antibodies on HSCT outcomes, there are two reports suggesting that non-HLA-specific antibodies may also be deleterious. Ansari *et al.* (159) observed an association of antibodies to the major histocompatibility class I-related chain A antigens (MICA) with reduced platelet recovery after HSCT, while Norlander *et al.* (172) found that donor-specific antibodies to $CD34^+$ $VEGFR\text{-}2^+$ cells appeared to contribute to graft failure.

## ABOi HSCT

ABO blood group incompatibility is not generally considered as a major contraindication for HSCT, although ABO incompatibility between donors and recipient can occur in 30–40% of patients receiving HSCT. Recipients of ABOi HSCT are at increased risk for immune-mediated hematological complications including immediate and delayed hemolysis, delayed red blood cell (RBC) engraftment, and pure red cell aplasia (reviewed in 173–175). Crossing an ABO incompatibility has not been associated with a significant impact on overall survival or transplant related mortality, although the reports as to whether there is an associated increased incidence of GVHD have been equivocal (150, 174–176) and Remberger *et al.* (177) have observed an increased risk for graft failure among recipients of a major ABOi transplant. ABOi HSCT is classified in three categories: (i) a major ABOi (HVG) occurs when the recipient has preformed anti-donor isohemagglutinins, e.g. an ABO-A donor to an -O recipient; (ii) a minor ABOi (GVH) may occur when passenger donor B lymphocytes and plasma cells produce anti-recipient isoagglutinins, e.g. an ABO-O donor to an -A or -B recipient; and (iii) a bidirectional incompatibility results when there is a combination of both major and minor ABOi, e.g. an ABO-A donor to a -B recipient (173, 175, 178). A major ABOi can result in immediate and severe hemolysis depending on the level of recipient isoagglutinins and the quantity of RBC in the stem cell infusion component. The risk of immediate hemolysis and delayed RBC recovery has been reported to vary with the type of graft, occurring mainly after bone marrow transplants but not after peripheral blood or cord blood grafts (178). Major ABOi HSCT may also result in pure red cell aplasia. Minor ABOi grafts confer a risk for delayed hemolysis, while bidirectional grafts carry risks of both immediate and delayed hemolysis, delayed RBC engraftment, and red cell aplasia (173–175).

In contrast to desensitization for incompatible solid organ transplants, management of ABOi HSCT is aimed primarily at reducing the risk of the hematological complications rather than prevention of graft rejection. Initially, all donors and recipients must be typed for ABO and Rh blood groups, and antibody screens on both donors and recipients should be performed for non-ABO red cell antibodies that could also result in hemolysis (174). There are two general approaches for management of major ABOi HSCT (175): removal of the anti-donor isoagglutinins, and depletion of RBCs from the stem cell product. Techniques for removal of isoagglutinins include PE, column IA, and *in vivo* adsorption with ABOi RBC or fresh frozen plasma. Depletion of RBC from the stem cell infusion component can be achieved by gravity sedimentation, centrifugation, Ficoll-Hypaque gradients, or continuous flow blood cell separation. While RBC depletion currently is used in many centers, it can result in a loss of 50–60% of nucleated cells from the infusion component (174). The recipient's isoagglutinin level can be used to guide the need for treatment. Lower titer antibodies ($\leq$1:16) are thought to be safely infused without interven-

tion, while RBC depletion of the component should be considered with higher titers, particularly for bone marrow grafts due to their higher RBC concentrations. For patients with high IgG isoagglutinin titers (>1:256), PE or IA in addition to red cell depletion of the stem cell component has been recommended (173). The primary approach for minor ABOi HSCT is to reduce high titer donor isoagglutins by removing plasma from the component simply by centrifugation (173, 174). For bidirectional HSCT, all of the above considerations may be applicable. While the above techniques can minimize immunohematological complications, the best practice is to avoid ABOi HSCT whenever possible.

### Desensitization protocols in HSCT

Because allogeneic HSCT offers the only curative option for many patients, interest is growing in desensitization for candidates with options limited to donors to which there is donor-specific antibody. The protocols reported to date are summarized in *Table* 5. Most desensitization methods have involved pre-transplant reduction in antibody levels through PE, adsorption via irradiated donor lymphocytes, donor or surrogate platelets, or a staphylococcal protein A column. While the total experience is limited to less than 30 patients, in the majority of cases in which DSA was reduced (21/23), reduction of DSA to low MFI values or crossmatch negative levels was associated with post-transplant engraftment. PE has been tried most often but was not effective in two attempts when it was the only treatment employed to reduce antibodies that were present at crossmatch positive levels (179, 180). As noted above in the section of solid organ transplantation, antibody reduction after PE is known to be transient without other immunosuppression or immunomodulation; therefore, most of the PE trials have been coupled with IVIG or rituximab in addition to standard post-HSCT immunosuppression. Results combining PE with the anti-CD20 monoclonal antibody, rituximab, have been mixed. Ciurea *et al.* (165) achieved engraftment in two patients for whom the DSA levels were reduced to MFI values <500 after treatment, while two other patients with higher MFI had graft failures. Yoshihara *et al.* (161) observed DSA reduction for one patient with PE and rituximab, but not with another whose post-treatment MFI remained high at 12 736 MFI, but both of these patients engrafted. There has been one successful report by Braun *et al.* (180) using adsorption of antibodies via a staphylococcal protein A column. The donor-specific antibody was reduced from a level that was positive in a FCXM to a negative crossmatch prior to transplantation.

PE in combination with IVIG has been an effective treatment for HLA-specific antibodies in the largest number of cases, with 11/13 patients engrafting among the trials shown in *Table* 5. In single cases, both Pollack *et al.* (181) and Costa *et al.* (182) combined PE with high dose IVIG (2 and 1 g/kg, respectively) and achieved both DSA reduction to low levels and successful engraftment. Norlander *et al.* (172) employed a combination of PE, rituximab, and IVIG (250 mg/kg) for treatment of HLA-DSA with engraftment in one patient but not in another. Interestingly, these authors also treated two patients with donor-reactive, anti-VEGFR-2 antibodies. Prior to treatment, the VEGFR-2 antibodies tested positive in a CDCXM against $CD34^+$ $VEGFR\text{-}2^+$ cells isolated from donor bone marrow or mobilized peripheral blood by positive selection with magnetic beads coated with anti-CD34 and anti-VEGFR-2 monoclonal antibodies. Following the desensitization treatment, the anti-VEGFR-2 reactivity was CDCXM- and the patient engrafted, but the other patient whose anti-VEGFR-2 reactivity remained positive had graft failure. The report (162) from The Johns Hopkins center of nine patients is the largest series to date of desensitization of HLA-DSA for haplo-ID transplantation. Our protocol is a modification of the desensitization protocol comprised of alternate day PE with low dose IVIG at 100 mg/kg developed for renal transplantation (56). Varying numbers of treatments were planned prior to the initiation of the conditioning regimen based on the pre-treatment DSA levels. Treatment was discontinued during conditioning with one additional treatment on pre-transplant day −1. Three patients received an additional PE-IVIG treatment on post-transplant day +1 due to some antibody rebound during the conditioning interval. A semi-quantitative evaluation of DSA reduction was provided on the basis of normalized DSA levels, and a mean DSA reduction of 68.1% was achieved by the end of treatment. Engraftment was successful in all eight patients who were transplanted. The DSA for the one patient who was not transplanted was only reduced from a CDCXM+ level to a FCXM level, and as the patient was to receive full myeloablation, the risk of engraftment failure was considered too high to proceed. In the other eight cases, the DSA were reduced to levels well below that consistent with positive FCXMs with full donor hematopoietic engraftment by day +60. Since the publication of our first series of desensitized patients, three more patients with donor DSA have been successfully treated with

**Table 5. Protocols for desensitization in HSCT**

| Reference | Desensitization method* | *N* | Ab test† | HSCT type‡ | Stem cell source§ | DSA reduction at EOT¶ | F/U Ab test‖ | Engraftment? |
|---|---|---|---|---|---|---|---|---|
| (A) Antibodies defined by cell-based tests | | | | | | | | |
| (180) | Staph protein A adsorption | 1 | FCXM+ | Haplo-ID | PBSC | Yes | FCXM- | Yes |
| (179) | PE | 1 | CDC+ | Haplo-ID | BM | ND | ND | No |
| (183) | PE + irradiated DLI | 1 | AHG-CDC+ | MM-sibling | BM | Yes | AHG-CDC- | Yes |
| (181) | PE | 1 | FCXM+ | MM-sibling | PBSC | No | FCXM+ | No |
| | PE + IVIG (2 g/kg) | | FCXM+ | Re-Tx, same donor | PBSC | Yes | FCXM- | Yes |
| (184) | Donor platelets + rituximab | 1 | AHG-CDC+ | MM-sibling | PBSC | Yes | AHG-CDC- | Yes |
| (B) Antibodies defined by solid phase immunoassays | | | | | | | | |
| (165) | PE + rituximab | 2 | Luminex-SAB | Haplo-ID | PBSC | Yes | 2 – MFI <500 | Yes |
| | | 2 | | | | 1 – no; 1 – yes | MFI 1500–3000; MFI 500–1500 | No |
| (182) | PE + IVIG (1000 mg/kg) | 1 | Luminex-SAB | MM-unrelated | BM, PBSC | Yes | MFI <1000 | Yes |
| (172) | PE + rituximab + IVIG (250 mg/kg) | 2 | 2 – Phenotype panels | MM-unrelated | BM, PBSC | 1 – yes; 1 – no | PRA <5 PRA >80 | 1 – yes; 1 – no |
| | | 2 | 2 – CDC+ for anti-VEGFR-2 | MM-unrelated | UCB | 1 – yes; 1 – no | CDCXM- CDCXM+ | 1 – yes; 1 – no |
| (161) | PE + rituximab | 2 | Luminex-SAB | Haplo-ID | BM/PBSC | 1 – yes; 1 – no | MFI = 3036; 12 736 | Yes |
| | Platelet transfusion | 2 | | | | 2 – yes | MFI <1000 | Yes |
| | Bortezomib | 1 | | | | Moderate | MFI: 13 334 to 9289 | Yes |
| (170) | High dose IVIG (400 mg/kg) | 1 | Luminex-SAB; CDC XM+ | Haplo-ID | BM | Moderate MFI >10 000 | MFI <500 | Yes |
| (162) | PE + IVIG (100 mg/kg) | 9 | Phenotype panels; SAB | Haplo-ID | BM | Mean decrease = 68.1% | Last F/U mean decrease = 94.9% | 8 – yes; 1 – not tx'd |

Results are summarized of available reports of trials to reduce donor-reactive antibodies prior to HSCT according to whether the antibodies were detected by cell-based methods (A) earlier reports or by current, sensitive solid phase assays (B).

*Desensitization methods include: PE, plasma exchange; DLI, donor lymphocyte infusion; IVIG, intravenous immunoglobulin.

†Test methods for antibody (Ab) detection include: FCXM, flow cytometric crossmatch; CDC, complement-dependent cytotoxicity crossmatch; AHG, anti-human immunoglobulin; and SAB, single antigen bead assay.

‡Types of HSCT include: Haplo-ID, HLA-haploidentical donor and MM, mismatched unrelated donor.

§Stem cell sources were PBSC, peripheral blood stem cells and BM, bone marrow.

¶Reduction in DSA at the end of treatment (EOT) is indicated as designated by each report as simply yes or no; as moderate with median fluorescence intensity (MFI) in one report, and as a percentage reduction for one report.

‖Levels of DSA reactivity are given at the time of last follow-up (F/U) testing as reported by crossmatch test results, by panel reactive antibody (PRA), by MFI values, or as a percentage of DSA reduction.

this regimen and have fully engrafted after haplo-ID transplants (authors' unpublished data).

Among the other regimens used to reduce DSA levels, combinations using donor or surrogate cell adsorption also appear to be effective. Maruta *et al.* (183) coupled PE with adsorption via irradiated donor lymphocytes in one patient and reduced antiglobulin−CDCXM+ antibodies to a negative level with subsequent engraftment. Similar antibody levels were successfully treated by Narimatsu *et al.* (184) using a combination of donor platelet adsorption and rituximab. Yoshihara *et al.* (161) found that platelet adsorption was highly effective using surrogate donors matched for the HLA class I antigens corresponding to the DSA. The DSA was reduced to low levels (MFI <1000) with both of the two treated patients fully engrafting. However, as noted by these investigators, platelet adsorption would only be effective for patients with antibody to HLA class I antigens, as platelets do not express HLA class II molecules.

There are two reports of attempts to reduce DSA levels without either PE or other adsorption. Yoshihara *et al.* (161) treated one patient with DSA to HLA class II antigens with the proteasome inhibitor bortezomib. Only moderate antibody reduction resulted with the DSA remaining at a relatively high level (MFI = 9289); however, the patient engrafted. Ishiyama *et al.* (170) tried IVIG at a dose of 400 mg/kg for one patient with HLA class II antibodies. This group also only achieved moderate DSA reduction, but the patient engrafted. However, as was noted previously, the engraftment in this patient probably was facilitated by the IVIG. Although the available data are limited to the attempts shown in *Table* 5, taken together, the numbers of successful engraftment are encouraging. Future studies may be able to address the levels of DSA that should be treated or avoided, as well as defining optimal protocols for desensitization. However, a large, prospective and blinded study is not likely to occur as such trial could be considered unethical due to the risk of graft failure imposed by HLA antibodies for randomized, non-treated patients.

### Possible mechanisms for effective desensitization for HSCT

The mechanisms underlying the success of desensitization in HSCT likely differ from those operational in desensitization for solid organ transplantation. Importantly, the establishment of long-term chimerism may induce B-cell as well as T-cell tolerance, resulting in a continued decline in donor HLA-specific antibodies. Sykes and colleagues (185–188) have demonstrated clearly in a series of studies in murine models that B-cell tolerance can be induced by mixed chimerism after allogenic bone marrow transplantation. These authors achieved tolerance of B cells producing antibodies to the Gal α1,3 Galβ1,4G1cNAc-R epitope (Gal) in α1, 3-galactosyltransferase knockout mice through bone marrow transplantation from $GalT^{+/+}$ wildtype mice (185, 186). The Gal epitope is widely expressed on glycoproteins and glycolipids of most mammalian species, and natural occurring antibodies to the Gal epitope present a major barrier to the potential of xenotransplantation, as they result in hyperacute rejection of xeno-allografts. Following a non-myeloablative regimen comprised of $CD4^+$ and $CD8^+$ T-cell depletion combined with non-lethal whole body and thymic irradiation, long-lasting multilineage $H\text{-}2^{bxd}$ $GalT^{+/+}$ + $H\text{-}2^{d}$ $GalT^{-/-}$ mixed chimerism was achieved and the levels of anti-Gal antibodies were substantially reduced by 2 weeks and completely undetectable at later time points (185, 186). Although a higher marrow dose was required, long-lasting chimerism could also be produced in animals with high levels of anti-Gal antibodies induced by prior immunization with rabbit red blood cells (187). Through *in vitro* and adoptive transfer studies, the mechanisms underlying the B-cell tolerance in the studies by Sykes and her colleagues appear to involve an antigen-dependent anergy initially, followed by long-term unresponsiveness through clonal deletion and/or receptor editing (188).

Both the pre-transplant conditioning and the post-transplant immunosuppression given to control GVHD and graft rejection may also contribute to the efficacy of desensitization protocols in HSCT. In the trials that used IVIG, interference or blocking of the Fc receptors on macrophages and NK cells may have prevented ADCC of the donor cells as suggested by the studies of Taylor *et al.* (167). However, as previously discussed for solid organ desensitization, IVIG is known to have multiple immunosuppressive effects; therefore, other mechanisms may have contributed to its effects in HSCT desensitization. In our series of eight patients treated and transplanted with haploidentical donors, the post-transplant immunosuppression included two high doses of cyclophosphamide (50 mg/kg) on days +3 and +4, along with mycophenolate mofetil on days +5 through +35, and tacrolimus on days +5 through +180 (162). Cyclophosphamide appears to induce apoptosis of proliferating, alloreactive T lymphocytes while sparing donor stem cells, effectively reducing both GVHD and rejection (137). While the cyclophosphamide is thought to act principally against activated, alloreactive T lymphocytes, there is some evidence that it may have similar effect against alloreactive B lymphocytes resulting in a gradual decline in

antibody over time. Brodsky *et al.* (189) observed significant reduction or complete elimination of HLA antibodies in four of five aplastic anemia patients treated with high dose cyclophosphamide without HSCT. In both our study and that of Ishiyama *et al.* (170), DSA reduction continued after HSCT, gradually declining until the DSA levels became extremely weak or negative. Interestingly, in our series, the antibody reduction was significantly greater for DSA than for third party antibodies with a mean reduction of 94.9% of DSA compared with 37% for third party antibodies (162). This observation could indicate that the post-HSCT cyclophosphamide affected donor-specific alloreactive B lymphocytes more than non-donor directed B cells. It is also possible that the engrafting donor cells adsorbed the circulating DSA and, as the cell numbers increased, the DSA gradually declined. However, a similar differential effect on DSA and third party antibody was observed in desensitization of renal transplant recipients using the PE, low dose IVIG protocol, minus the post -transplant cyclophosphamide, suggesting that there may be some antigen-specific immunoregulation resulting in the DSA decline (57). Clearly, as indicated by the work of Sykes and colleagues (185–188) and as suggested by Luznik *et al.* (137) for the desensitization regimen using post-transplantation cyclophosphamide, there may be multiple mechanisms facilitating the downregulation of donor-specific antibodies including a balance of effector and regulatory T cells maintained by post-transplant immunosuppression until long-term tolerance can be established.

### Considerations for HSCT desensitization

Although the trials to date indicate that desensitization can be effective in reducing DSA to levels that permit successful HSCT, we have noted that it should be reserved for patients with no other or limited donor options (162). This would include patients with very broad HLA sensitization that eliminates most, if not all, potential donors and patients whose disease status requires rapid transplantation, ruling out the possibility of unrelated donor searches. Importantly, desensitization requires thorough and on-going characterization and monitoring of DSA levels prior to desensitization, during desensitization, and immediately post-HSCT to determine if additional treatment is needed. Close communication between the transplant physicians and the histocompatibility laboratory is essential. More experience is needed to determine what levels of DSA can be successfully reduced, and until such knowledge is available, alternative treatment options may be advisable for candidates with very high DSA levels. Nonetheless, these preliminary trials indicate that DSA does not need to be an absolute barrier for HLA-mismatched HSCT.

### Future directions

Undoubtedly desensitization will continue to afford sensitized patients the opportunity for successful transplantation, but it also offers an excellent model for immunologic research. Basic science studies in the mechanisms of antibody-mediated graft injury, B-cell biology, and gene expression are providing insight into how desensitization protocols can be modified and what immuno-modifying therapies can be used to optimize outcomes. However, the environment and the immune system's response to the environment are not static. Evolution has endowed the immune system with many ways to circumvent or overcome agents designed to suppress the immune response. Therefore, what factors are needed are improved methods and markers for monitoring changes in the immune system and the graft after desensitization and transplantation.

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