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Review

Desensitization protocols improving access and outcome in transplantation

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Abstract

Sensitization to antigens of the HLA and ABO system has been the biggest barrier to access in renal transplantation and, increasingly, in transplantation of other organs. Additionally, antibody to donor antigens has been shown to result in injury to the graft ranging from catastrophic, irreversible hyperacute rejection to the slower, more insidious, chronic form of rejection. The problem of access has been recognized globally and has been the incentive for measures to overcome the disadvantage experienced by the sensitized patient. However, early attempts to reduce sensitization achieved only transient success. Newer immunosuppressive agents that affect B-cell function or viability have permitted the development of treatment protocols to eliminate and, potentially, downregulate donor-specific antibodies. The use of these protocols has achieved successful transplants that were HLA and/or ABO incompatible prior to treatment and, as such, has provided some patients with their only opportunity for transplantation.

Keywords: ABO incompatible transplantation; Desensitization; Double filtration plasmapheresis; HLA-specific antibody; Intravenous immunoglobulin (IVIg); Plasmapheresis; Sensitization; Transplantation

Abbreviations: ABO-DSA, antibody to donor ABO antigens; ABOi, ABO incompatible; AHG-CDC, antiglobulin enhanced complement-dependent cytotoxicity; AMR, antibody-mediated rejection; CDC, complement-dependent cytotoxicity; CMVIg, hyperimmune anti-CMV IVIg; CyA, cyclosporin; DFPP, double filtration plasmapheresis; DSA, donor-specific antibody; DSG, deoxyspergualin; HLA-DSA, antibody to donor HLA antigen(s); IVIg, intravenously administered, pooled human IgG; MMF, mycophenylate mofetil; OPTN, Organ Procurement and Transplantation Network; PP, plasmapheresis or plasma exchange; PRA, panel reactive antibody; RAPA, rapamycin; TAC, tacrolimus; XM, crossmatch.

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1. Introduction

The single largest barrier to access to and outcome of a renal transplant is sensitization to donor antigens of the ABO and/or HLA systems. Sensitization to antigens of these 2 systems differs in important ways. Most individuals have antibodies to nonself ABO antigens that are proposed to result from exposure to environmental substances that cross-react with those antigens. In contrast, primary sensitization to HLA antigens occurs only after contact with HLA antigens as a result of transfusion, pregnancy, or transplantation. However, the identification of epitopes on microbial cell walls that are similar to those on some HLA antigens suggests that exposure of an HLA-sensitized individual to these microorganisms may provoke an anamnestic response or a broadening of the sensitization. Antibodies to the A and B blood group antigens are usually persistent throughout a person's lifetime while HLA-specific antibodies, particularly those provoked by transfusion or pregnancy, may weaken or disappear over time. Sensitization to antigens of the ABO system renders patients incompatible with a limited portion of the population which is maximum in blood type O patients. However, the multiple, antigen-encoding loci of the HLA system, the high polymorphism of the HLA loci, and the presence of multiple antigenic epitopes on individual molecules, many of which may be shared among different HLA antigens [1,2], can result in sensitization to all but HLA identical or very closely matched donors.

Several different protocols have been used in attempts to reduce or eliminate antibodies to ABO or HLA antigens. These can be grouped into 3 categories: (1) those that remove antibody through plasma exchange or immunoadsorption (IA); (2) those that block or downregulate antibody with intravenously administered, pooled human immunoglobulin (IVIg); and (3) those that use a combination of plasmapheresis (PP) and IVIg. These protocols differ in their efficacy, applicability, and cost. Comparisons of the efficacy of different protocols are difficult because of differences in the measurement of strength and specificity of donor-specific antibody (DSA), the immunologic risks of the patients, the number and types of PP applied, the dosage and specific product of IVIg, the immunosuppression regimen followed, additional types of treatment that may have been used, and the assessment of outcome. When treatment is applied prior to transplantation for patients who do not have a live donor, efficacy is measured as a reduction in the breadth of HLA-specific antibody often gauged by the percent panel reactive antibody (PRA), which is the percent of a panel of phenotypes with which a patient's serum reacts. Efficacy is measured as reduction in the titer of antibody to a specific donor in the case of preemptive treatment of patients with a positive crossmatch (XM) to a live donor or rescue treatment during an episode of antibody-mediated rejection (AMR). Despite these confounding factors, there is a degree of consistency of efficacy within each protocol category. These protocols have been used to increase access to transplantation by applying them to patients awaiting transplantation with the goal of reducing the breadth or strength of antibody. They have also been used to rescue kidneys in patients experiencing AMR, thus improving graft outcome. With growing appreciation of the detrimental effects of HLA-DSA on transplants of organs other than the kidney, these protocols are being used, increasingly, to improve transplantation of these other organs.

The mechanism(s) of action of these different treatment protocols may be substantially different. However, as in all areas of biology, it is likely that there are multiple mechanisms

involved in all cases and they may all lead to a common, autoregulatory mechanism. Collectively, the desensitization protocols have not only overcome some of the barriers to successful transplantation but have provided unique insight into allosensitization and immune regulatory processes. However, the efficacy and risks associated must be considered for each patient. Every desensitization protocol in use adds cost to the individual transplant but when considered globally, i.e., when one takes into account the cost of dialysis, increased morbidity associated with dialysis and the cost of grafts lost to AMR, these protocols become very cost effective.

2. Extent of the problem

2.1. Sensitization and access

The impact of sensitization on transplant outcome has been recognized since the first reports of antibody-mediated, hyperacute rejection in renal transplant recipients [3,4]. Public recognition of the disadvantage conferred by sensitization was underscored in the 1984 National Organ Transplant Act, which created the Organ Procurement and Transplantation Network (OPTN). The 1984 Transplant Act specifically charged the newly created network with establishing a national system, in accordance with medical criteria, to match organs and individuals, ''especially individuals whose immune system makes it difficult for them to receive organs'' [5]. The United Network for Organ Sharing (UNOS), the agency contracted to administer the OPTN, responded by awarding some priority in renal allocation to sensitized patients. Under the policy in place since 1989, patients with high antibody levels, measured as PRA of 80% or more, are awarded an extra 4 points if they have a negative preliminary crossmatch [6].

The magnitude of sensitization among transplant candidates is not small. Sensitized patients, i.e., those with PRAs of 10% or greater, currently comprise 33% of the wait list [7]. (We note that a better definition of sensitization is the production of HLA-specific antibody, regardless of PRA, but these data are not available in The United Network for Organ Sharing database.) However, the rates of sensitization are not equal among different groups of transplant candidates. This is illustrated in Fig. 1a, where it can be seen that the proportion of sensitized women (PRA $\geqslant$ 10) is twice that of men. There is also disparity in rates of sensitization among different racial groups (Fig. 1b). The highest percentage of highly sensitized patients, those with PRA $\geqslant$ 80, occurs among African Americans (10.6%), compared to 7.7% and 6.5% of Caucasians and Asians, respectively (OPTN/SRTR 2004 Report). When lower levels of sensitization are included (PRA $\geqslant$ 10), African Americans are even more disadvantaged, with 30.8% being sensitized compared to 23.4% of Caucasians and 20.9% of Asians.

It is clear that any level of sensitization significantly prolongs waiting time. Factors influencing waiting times were analyzed in a report from the Scientific Registry of Transplant Recipients, and those that increased waiting times are listed in Table 1 [8]. The factor with the greatest effect on wait time was a high level of sensitization (PRA $\geqslant$ 80), followed by more moderate degrees of sensitization (PRA from 41% to 79%). Lower levels of sensitization (PRA from 10% to 40%) also had a substantial impact on the rate of transplantation. There are also significant differences in waiting times for some racial/ethnic groups, particularly for

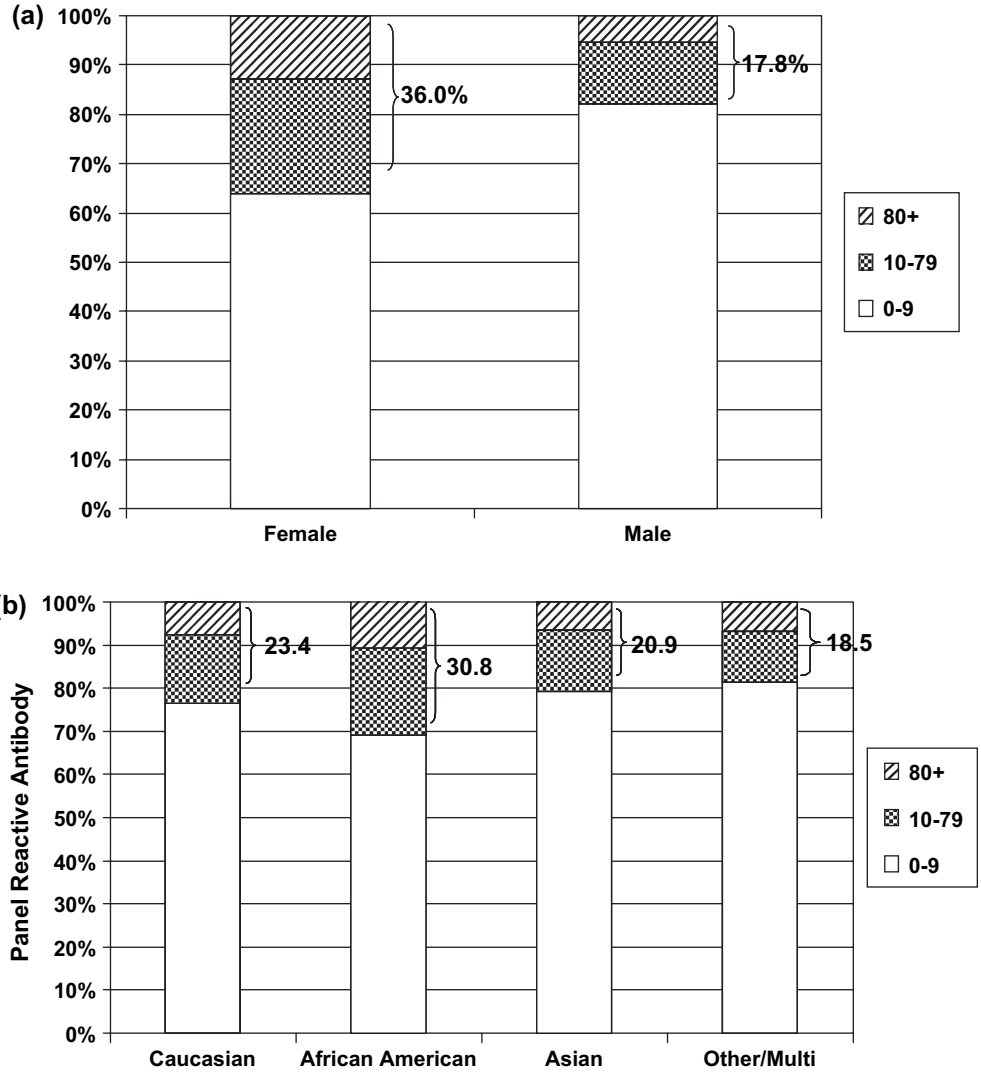

Fig. 1. (a) Proportions of sensitized wait list patients by gender. Data from 22 086 registrants with peak PRA listed in 2003. From 2004 OPTN/SRTR Annual Report. (b) Proportions of sensitized wait list patients by race. Data from 22 083 registrants with peak PRA listed in 2003. From 2004 OPTN/SRTR Annual Report.

African Americans who experience the longest median waiting times. However, higher rates of sensitization occurring in this group, result in a higher frequency of positive pretransplant crossmatches, which accounts for much of this racial disparity [9]. Increased sensitivity of newer antibody detection methods may further reduce access to transplantation.

2.2. Sensitization and graft function

There is a significant association between pretransplant sensitization and AMR of renal allografts [10]. However, sensitization increases the risk of renal graft loss due to any kind

Table 1
Recipient factors that increase waiting times

Factor	Relative rate of transplantation	Reference group
Age ⩾ 65	0.912	Age 35–49
Blood type B	0.850	Type O
African American	0.766	Caucasian American
Hispanic/Latino	0.904	Non-Hispanic/Latino
Peak PRA 10–40%	0.707	PRA 0–9%
Peak PRA 41–79%	0.483	PRA 0–9%
Peak PRA ⩾ 80%	0.413	PRA 0–9%
Previous transplant	0.559	No previous transplant

Adapted from reference [8].

of rejection, humoral or cellular, during the first year posttransplant. In an analysis of the primary causes of graft loss categorized by PRA level, 47.2% of cases were attributable to rejection in patients with PRA ⩾ 80, compared to 30.1% in patients with PRAs from 0 to 19 (OPTN data as of February 18, 2005). Over the long term, high levels of sensitization correlate with an 8–9% decrease in renal graft survival from that observed with non- or minimally sensitized patients (Fig. 2). In addition to correlating with increased rejection, HLA-specific antibodies show a significant correlation with the development of transplant-related coronary artery disease [11].

Detrimental effects of HLA-DSA have been demonstrated for every type of solid transplant performed. In addition to kidneys, HLA-specific antibodies have been associated with increased frequencies of rejection and decreased graft survival with heart, lung, liver, and corneal transplants [12]. The effects range from hyperacute rejection leading to immediate and irreversible graft failure to the slow, ongoing deterioration of graft function that results

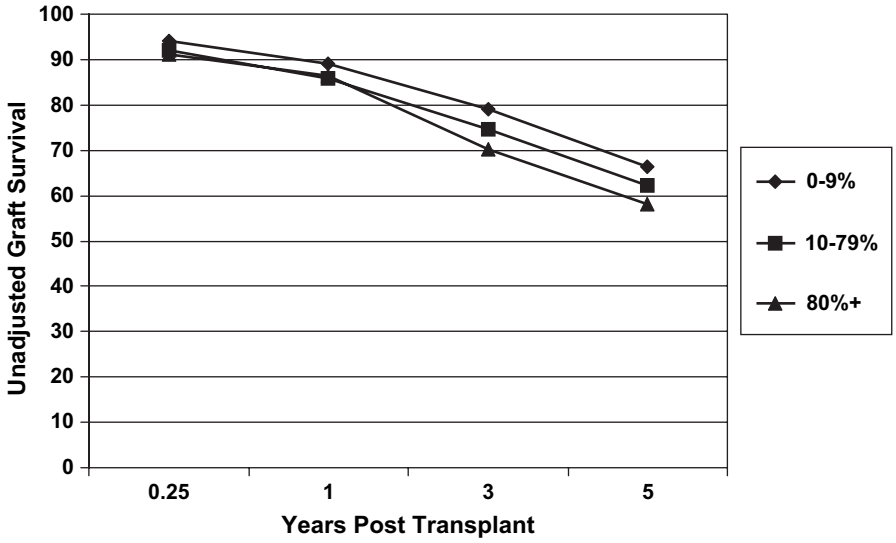

Fig. 2. Impact of sensitization on graft survival.

from chronic rejection. The effect varies with the type of organ, the strength and specificity of the antibody, the nature of the target antigen, differences among individuals in antigen expression and immune responsiveness, and the physiologic condition of the transplanted organ. The subject is too extensive to be covered here, and the reader is directed to any of a number of reviews on the topic, only a few of which are identified here [13–19].

The traditionally accepted routes of sensitization have included pregnancy, transfusion, as well as prior transplantation, but it has also been recognized that there are other stimuli, including left-ventricular assist devices, cryopreserved tissue grafts, and even nonspecific inflammatory events, that can provoke or expand anti-HLA sensitization [20–22]. Moreover, new technologies using solubilized and recombinant HLA molecules in solid-phase immunoassays have dramatically improved detection and characterization of HLA-specific antibodies in recent years. In our experience, introduction of an enzyme-linked immunosorbent assay–based assay for detection of HLA-specific antibodies doubled the incidence of sensitization that was detected among renal transplant candidates [23,24], and subsequent use of an multianalyte assay using microspheres coated with soluble HLA molecules (Luminex xMAP® technology) provided a further 2-fold increase [25–27]. It is indeed likely that the current rates of sensitization among transplant candidates are underestimated. Therefore, overcoming the effects of presensitization will remain a critical issue in clinical transplantation.

3. Treatment protocols: lymphocyte-reactive antibody

3.1. Comment

Early studies of histocompatibility, other than ABO compatibility, identified incompatibility using lymphocytotoxicity assays, commonly referred to by the acronym CDC (for complement-dependent cytotoxicity). The inadequate sensitivity and specificity of these assays often made it difficult to determine the basis of a positive crossmatch. Thus interpreting the results obtained in early studies—engraftment in the face of an apparent humoral incompatibility or graft failure in the apparent absence of such—must take the test shortcomings into account [reviewed in reference 14]. The addition of an antiglobulin reagent results in the highest level of sensitivity among cytotoxicity assays, and cell-based flow cytometry permits detection of very low levels of antibody including antibodies that do not activate complement effectively. However, neither procedure improves test specificity. It was the development of solid-phase antibody assays using soluble HLA molecules as targets that provided the sensitivity and specificity needed to detect and define low levels of antibody specific for donor HLA. Recommendations of a working group regarding antibody identification, risk assessment, and AMR determination have been published [28] and provide important guidelines for increasing consistency in the reporting of results.

4. Renal transplantation

4.1. Plasma exchange

Effective removal of antibody from the circulation can be achieved through plasmapheresis (PP). Antibodies of the IgM class, located predominantly in the intravascular spaces,

are removed more effectively, initially, than is IgG, which is distributed throughout the interstitium. In the absence of immunosuppression, immunoglobulin levels rapidly return to normal. An early attempt to remove antibody to HLA-class I antigens augmented plasma exchange with IA [29]. IA has, for the most part, found greater use in protocols designed to overcome ABO incompatibility, discussed below. However, in a recent study, Hickstein et al. [30] treated 6 patients with protein A column IA and were able to reduce PRA from a mean value of 65% to a posttreatment mean of 15%. They did see some antibody rebound but 5 of 6 patients transplanted had no vascular rejection. Alarabi et al. [31] were able to achieve a significant ($P < 0.001$) reduction in PRA, from 70% to 30% in 23 patients treated with plasmapheresis, cyclophosphamide, and prednisolone. However, grafts were lost to irreversible rejection in 8 of 22 patients transplanted following this treatment, and 1-year graft survival was only 50%. Studies in rats [32] and humans [33–35] have shown that deoxyspergualin (DSG), rapamycin, and mycophenylate mofetil (MMF) effectively inhibit antibody production when combined with plasmapheresis. Miura et al. [36] treated 23 patients with double filtration plasmapheresis (DFPP) and cyclophosphamide to abrogate a positive complement-dependent cytotoxicity (CDC) crossmatch with a live, renal donor. All patients also received cyclosporin, antilymphocyte globulin, and steroids. They found that freedom from rejection occurred in a higher percentage of patients ($n = 10$) who received DSG than among those who did not (60% vs. 38%) and that the DSG-treated patients were also free of severe accelerated rejection while the patients who did not receive DSG were not. Three- and 5-year graft survival among DSG-treated patients was also better (83% vs. 77% and 83% vs. 69%).

The use of PP, with or without drugs known to inhibit antibody production, has shown moderate success in the treatment of rejection. In one study, 22 of 28 (78.6%) patients with grade 3 rejection showed increased graft function when treated with PP, cyclosporin, azathioprine, and steroids [37] although in another study, 5 of 11 patients treated with DFPP lost grafts to rejection [38]. Comparable results were obtained in 2 small studies that used MMF with PP. This treatment achieved rescue of 3 of 4 (75%) renal transplants in patients experiencing acute AMR of renal transplants [39] in 1 study and of 14 of 18 (77.8%) renal transplants in patients with accelerated AMR in another [40]. Comparable results in treating AMR were obtained with the use of PP in combination with thymoglobulin, with rescue of transplants in 6 of 7 (85.8%) patients [41], or with DSG, which achieved recovery of graft function in 4 of 5 patients [42]. Ravichandran et al. [43] added splenic radiation to a regimen that included DFPP and MMF to treat 16 positive crossmatch patients. One graft was lost to hyperacute rejection, 3 other patients experienced reversible AMR, and 12 patients remained rejection-free at 3 months to 1 year posttransplant.

4.2. Intravenous immunoglobulin

Although first used for the treatment of hypogammaglobulinemia, several reports demonstrated beneficial effects of IVIg in patients with immune disorders [47,48, reviewed in reference 49]. Further, it had been shown that IVIg was able to reduce the incidence of and death due to acute graft-versus-host disease in recipients of bone marrow transplants [50]. These findings led to investigations of the use of IVIg in reducing sensitization in renal

transplantation. Glotz et al. [51] treated 5 patients, who had PRA ⩾ 25%, determined by CDC, with 400 mg/kg of IVIg administered in 4 consecutive dialysis procedures. They achieved a 40–80% decrease in the PRAs of 4 of the 5 patients, which lasted throughout the 3 month follow-up. They also tested 19 sera from these patients, in vitro, and saw a decrease in PRA in 18 of the 19 and a decrease in titer in all the sera. Tyan et al. [52] also achieved in vitro blocking of sera with HLA-specific antibodies detectable by CDC. The effect varied among sera, both in PRA reduction (4–70%) and degree of inhibition (4–100%), and this variability was not related to either the antibody titer or specificity. Treatment of a renal transplant candidate led to a substantial reduction in PRA and successful transplantation. Peraldi et al. [53] used IVIg treatment to prevent AMR in 21 of 41 renal regraft patients. They found that the IVIg group had a shorter time to graft function (3.4 vs. 9.9 days) and better 5-year graft survival (68% vs. 50%) that was comparable to patients receiving primary grafts at their center. These early successes led investigators to treat larger numbers of patients as a means to improving access to transplantation among sensitized patients.

More recently, Glotz et al. [54] reported that 13 of 15 patients treated with 2 g/kg of IVIg monthly for 4 months were desensitized and transplanted. However, only 10 of these had historic antibodies to the donor (7 with known specificity for donor HLA) and as such, had transplantation enabled by the treatment. Patients also received MMF, steroids, thymoglobulin, and tacrolimus (TAC). Two of the 13 transplants failed, 1 graft thrombosed the day of transplant, and 1 was lost later to rejection. The remaining 11 had uneventful courses during the first year but 3 patients were negative historic crossmatches and of the other 8, only 5 had clearly identified HLA-DSA. Thus, while this study was able to demonstrate elimination of HLA-DSA in only a minority of the patients treated, it did substantiate the ability of IVIg treatment to eliminate such antibody. It also illustrated the need for improved methods for identifying and characterizing HLA-specific antibodies. Jordan et al. [55] reported on 45 patients awaiting transplantation (43 kidney and 2 heart) who were treated with 2 g/kg IVIg monthly. Forty-two patients achieved negative crossmatches and were transplanted, 35 had negative flow cytometric crossmatches, and 7 had crossmatches that were negative by CDC but positive by flow cytometry. Following transplantation, all patients received Zenapax, MMF, TAC, steroids, and 1 additional IVIg treatment 1 month posttransplant. AMR occurred in 31% of the patients, all within the first 2 posttransplant months. At 24 months, graft survival was 89.1%, and the mean serum creatinine was 1.3 mg/dl.

These results differ substantially from those reported by Mahmoud et al. [56] who treated 11 patients who had a positive CDC XM with a live donor and who had PRA ⩾ 20%. They saw a decrease in PRA in only 4 of the 11 patients and achieved a negative crossmatch in none. This disparity from the results obtained by others may have been due to differences in treatment protocol (6 doses each of 500 mg/kg vs. 4 monthly treatments of 2 g/kg) and to use of the least sensitive CDC test which would be positive only in patients with high antibody levels. Another variation in treatment was followed by Golconda et al. [57] who treated patients with a hyperimmune anti-CMV immunoglobulin (CMVIg). Patients had a negative basic CDC and positive antiglobulin-enhanced complement-dependent cytotoxicity XM with a live donor and received 500 mg/kg CMVIg. Patients who achieved a negative antiglobulin-enhanced complement-dependent cytotoxicity XM were transplanted and were

treated with MMF, steroids, thymoglobulin, TAC, and one more dose of CMVIg. Of 7 transplants, 1 graft was lost to thrombosis on day 5 posttransplant and 5 patients experienced cell-mediated rejection and/or AMR. In yet another variation of treatment protocols, Akalin and Bromberg [58] treated patients with low-dose (100 mg/kg) IVIg and thymoglobulin for 5 days. They treated 17 patients with positive crossmatches—10 were positive by CDC and 7 by flow cytometry only. Eleven of the patients were shown to have HLA-DSA by CDC or by solid-phase immunoassay. At a median follow-up of 28 months, there were no incidents of acute cellular rejection but 3 patients, all with HLA-DSA, had acute AMR, 1 of whom lost the graft. They also demonstrated that 2 of 8 class I-specific antibodies and 4 of 8 class II-specific antibodies were lost after transplantation.

In addition to the efforts of individual programs, a randomized, double blind, NIH-sponsored, multicenter clinical trial enrolled 101 adult patients who had PRA $\geqslant$ 50% [59]. Patients were treated with 2 g/kg of either IVIg or a placebo monthly for 4 months with treatment repeated at 12 and 24 months and posttransplant. The results for the IVIg group vs. the placebo group were as follows: (1) a significantly greater decrease in PRA; (2) increased rate of transplantation (35% vs. 17%); (3) higher incidence of rejection (52.9% vs. 10%); and (4) comparable 2-year graft survival (80% vs. 75%) but with poorer serum creatinine values (1.68 vs. 1.28).

IVIg has also been used to treat AMR. Jordan et al. [60] treated 10 patients, who were experiencing severe AMR, with IVIg. The rejection episodes were resolved within 2–5 days in all patients accompanied by a decrease in DSA. Nine of the patients remained rejection-free throughout the follow-up period. In a randomized study of 30 patients with steroid-resistant rejection, a 7-day course of 500 mg/kg/day of IVIg was as effective as a 14-day course of 5 mg/day of OKT3 in reversing rejection [61]. Further the long-term outcome was the same in both groups with fewer side effects in the IVIg group. The needs of infected patients experiencing rejection are dichotomous: increased immunosuppression to treat the rejection and decreased immunosuppression to overcome the infection. IVIg may represent the ideal compromise because it both immunomodulates and reconstitutes immune responsiveness. Moger et al. [62] successfully reversed steroid-resistant rejection episodes in 2 such patients using IVIg.

Thus, the use of high-dose IVIg, which is usually a dose of 2 g/kg given monthly for several months or 400–500 mg/kg given daily for several consecutive days, has been demonstrated to increase access to transplantation by reducing sensitization. Patients who receive transplants as a result of desensitization have graft survival comparable to patients who have negative crossmatches without desensitization although they have a significantly higher frequency of AMR episodes. There is variability in the ability of IVIg to eliminate or reduce HLA-specific antibodies prior to transplantation, which is not related to specificity or titer, and there is a population of patients who are IVIg ''nonresponders.'' The effect in vivo can be predicted by in vitro testing, although in vitro testing is complicated by the presence of the large amount of IgG present. Tyan [52] has developed a modified CDC assay that overcomes this problem, and this assay is described in detail in a review by Jordan et al. [65]. Interestingly, the consistent success of IVIg in treating AMR is in contradiction to the variability seen with preemptive treatment. One interpretation of these findings is that the presence of target antigen enhances desensitization. However, because most of the

studies were done using CDC assays to assess antibody elimination, there exists the possibility of the persistence of antibody at levels below that detectable by CDC. Thus, apparent differences in antibody elimination seen in preemptive vs. rescue treatment need further evaluation with newer, more sensitive assays.

4.3. Plasmapheresis with IVIg

The Johns Hopkins Comprehensive Transplant Center began desensitization using a combination of plasmapheresis and low-dose IVIg to treat AMR in the mid-1990s [66]. In 1998 this same protocol was adapted to desensitize patients with a positive crossmatch or ABO incompatibility with a live donor in preparation for renal transplantation [67]. The protocol consisted of alternate day plasmapheresis followed by low-dose (100 mg/kg) CMVIg and quadruple, sequential immunosuppression comprised of Zenapax, MMF, TAC, and steroids. In an initial report of 4 patients treated preemptively and 3 patients treated for AMR, there were no immediate graft losses, and good renal function was achieved [67]. The patients treated preemptively had positive crossmatches by CDC in 1 case and by flow cytometry in 3 other cases. Further, all patients had 1 or more additional risk factors for AMR such as repeat mismatch, previous transplant, or husband to wife or child to mother transplant. Subsequently, we reported on an additional 18 patients with positive pretransplant crossmatches (8 by CDC and 10 by flow cytometry) who were treated preemptively [68]. HLA-DSA was identified in 16 of the 18 patients. There was only 1 graft loss among this group, which occurred as a result of noncompliance. Five patients experienced 1 or more episodes of AMR, all of which were treated successfully with additional courses of PP/CMVIg. At a mean follow-up of 17.3 months, the mean serum creatinine was 1.1 mg/dl. Schweitzer et al. [69] treated 15 patients, all of whom had a positive CDC crossmatch with a potential live donor, with alternate day PP, daily doses of 70 mg/kg IVIg for 7 days, and OKT3 for 10 days. Eleven patients converted to a negative crossmatch and were transplanted. Four patients had an episode of AMR and at a mean follow-up time of more than 13 months, serum creatinines ranged from 1.1 to 2.4. The group at the Mayo Clinic [70] reported on 14 patients who had positive CDC crossmatches and who were transplanted under a protocol consisting of 7 single-volume plasmapheresis exchanges (from transplant days -4 to $+3$) followed by 100 mg/kg IVIg. Patients also received anti-CD20, thymoglobulin, TAC, MMF, and steroids and were splenectomized on day of transplant. All patients had a negative CDC XM at time of transplant. Specificity to donor HLA was identified in 5 patients and to antigens crossreactive with donor HLA in 4 patients. No DSA could be identified in 2 patients, and antibody specificity could not be determined in the remaining 3. Two patients lost their grafts in the 11th posttransplant month, both of whom had reverted to a positive CDC XM at follow-up, and 6 of the 14 patients had an episode of AMR. Thielke et al. [71] achieved excellent results in patients whose baseline DSA levels were low. They treated 16 patients who had positive flow cytometric crossmatches with alternate day PP and low-dose (100 mg/kg) IVIg. Six patients who converted to a negative XM within 3 treatments were transplanted and were immunosuppressed with thymoglobulin, TAC, MMF, and steroids. Their 1-year graft survival was 100%, incidence of AMR was 25%, and mean 6-month serum creatinine value was 1.4.

Several groups have reported successful treatment AMR with the combination of PP and IVIg. Pascual et al. [35] successfully treated 5 patients, who had AMR within the first post-transplant month, with 4–7 daily PP treatments followed by one 400 mg/kg dose of IVIg. A similar protocol, but with a high dose of IVIg (2 g/kg) was used at Duke University [72]. They found that 15 patients who were treated immediately after the diagnosis of AMR, all responded positively while 1 patient whose treatment was delayed for 1 week, lost the graft to rejection. They showed here and in a later report of a slightly larger cohort [73] that graft survival in the AMR group was not significantly different from that of patients experiencing cellular rejection. A third group using the protocol of alternate day PP followed by a single dose of IVIg [74] was able to rescue 8 of 9 patients with AMR. The 8 successes were all patients who were not presensitized while the graft was lost in single presensitized patient. Unfortunately, this report did not include information on the characterization of DSA. Comparable success was not achieved by the Cedars Sinai group who treated C4d+ acute rejection episodes with a combination of steroids, IVIg, PP, and thymoglobulin [75]. They reported a 25% graft loss (5 of 20 patients) treated in this protocol.

Throughout the desensitization program, the Hopkins group has monitored the course of HLA-DSA using solid-phase immunoassays. Initial antibody evaluations were done by enzyme-linked immunosorbent assay screens against purified HLA targets and by flow cytometric and CDC crossmatches. In our report of 49 patients with confirmed HLA-DSA there were 43 patients with HLA-DSA prior to transplantation and 6 patients who developed DSA posttransplant [78]. Crossmatches were positive by CDC in 14 patients, with titers ranging from 1 to 4096, and by flow cytometry in 35 others. Immunosuppression consisted of MMF, daclizumab, TAC, and steroids. Patients with good graft function had PP/CMVIg treatment terminated, whether or not HLA-DSA had been completely eliminated. At the end of treatment, 63% of patients had eliminated DSA while only 27% had eliminated antibody to third-party HLA. Antibody studies were able to be continued for a minimum of 2 months in 38 patients with a mean follow-up of 13 months. At follow-up, 89% of patients had lost DSA while third-party antibody was no longer present in only 19% of patients who had third-party antibody at the start of treatment. We also examined the effect of treatment on antibody to viral antigens and found no effect of treatment on these antibodies. In contrast to treatment using high-dose IVIg, treatment with PP/CMVIg seemed, with one exception, to be effective in eliminating DSA specific for any HLA antigen. The exception was for antibodies specific for antigens encoded by the HLA-DRB3 and -DRB4 loci, HLA-DR52 and -DR53, respectively. Three patients whose DSA was reduced to low levels but not completely eliminated all had pretreatment positive CDC crossmatches with titers of 64, 256, and 4096. The antibody specificities were to DR52 in 1 patient and to DR53 in 2 others. There were other patients with antibodies to these antigens who did eliminate DSA, none of whose crossmatches were positive by CDC.

In contrast, Gloor et al. [79] found that 6 of 12 patients with DSA specific for donor class I antigens and who were CDC XM negative at the time of transplant, were positive by flow cytometric XM 4 months posttransplant. Further, 9 of 11 patients tested by the very sensitive flow cytometry assay using beads coated each with a single type of HLA antigen were found to have HLA-DSA. Since our initial report on antibody persistence, we have added Luminex-based antibody tests to our testing repertoire and reexamined antibody persistence in

patients treated in the PP/CMVIg protocol. Not surprisingly, given the high degree of sensitivity of this assay, we saw a higher percentage (46.3%) of patients with persistent HLA-DSA than we had seen initially [80]. However, as we had seen earlier, this varied according to specificity with elimination of HLA-class I, -class II (DRB1 and DQ antigens), and -DRB3-5 antigens occurring in 75.6%, 60.0%, and 20.0% of patients, respectively. The elimination of HLA-DSA was higher for class I-specific antibody than that seen by Gloor et al. [79], which occurred in only 50% of patients when measured by flow cytometric crossmatch and in only 18.2% of patients when measured by flow cytometric bead technology. Possible explanations of these differences are differences in product, immunosuppression, or baseline antibody levels. We found that baseline DSA strength did affect antibody elimination with only 57.9% of CDC XM–positive patients eliminating DSA specific for HLA-class I. The only other factor that affected antibody elimination was whether the patient had antibodies to only 1 member of a crossreactive group of antigens, potentially to a private epitope, or to several members of a crossreactive group, a condition that might reflect antibody to a public epitope [1,2]. In the former case, antibody elimination, overall was only 29.2% while 73.5% of antibodies specific for multiple members of a crossreactive group were eliminated. This difference was consistent for antibodies specific for either class I or class II.

Thus, treatment with a combination of plasmapheresis and IVIg permits successful transplantation of patients with a positive crossmatch and rescue of transplants undergoing AMR. These protocols often use substantially lower doses of IVIg than protocols that use IVIg alone and this, in turn, has permitted the use of very sensitive methods of antibody detection during and after treatment. These sensitive assays are being applied, increasingly, to patients being treated with high-dose IVIg. Publication of these data will reveal if that protocol results in the complete elimination of HLA-DSA or simply reduces it to very low levels. The clinical relevance of such low levels of HLA-DSA also remains to be determined. Gloor et al. [79] found no evidence, by either renal function or histology, of antibody-mediated damage among patients with persistent antibody. However, Hourmant et al. [81] examined a large cohort (1229) of renal transplant recipients and found that patients who developed HLA-specific antibodies after transplantation, whether specific for donor or third-party HLA, had significantly lower long-term graft survival and poorer graft function than patients who did not. The level of antibody may be relevant because the Mayo Clinic study used the highly sensitive bead assays while the Hourmant study did not. Further, it cannot be determined whether the presence of HLA-specific antibody was responsible for the reduced graft function or simply a marker of an inflammatory process related to graft impairment.

4.4. Heart and lung transplantation

4.4.1. Plasma exchange

There are only a few reports on the use of plasmapheresis in nonrenal transplantation. Among the earliest was a report of rescue of a heart transplant from rejection [44]. A more recent report [45] describes the rescue of a lung transplant from an HLA-DSA–mediated hyperacute rejection using PP, antithymocyte globulin, and cyclophosphamide. Grauhan et al.

[46] compared treatment of AMR in heart transplant recipients using cyclosporin, steroids, and cytolytic antibodies to a protocol that added plasmapheresis. Five of 7 patients in the first group died while all 6 patients treated with plasmapheresis survived. Thus, early indications are that plasmapheresis may be a useful adjunct for treating rejection of organs other than the kidney.

4.4.2. Intravenous immunoglobulin

The use of IVIg to reduce HLA-specific antibodies in patients awaiting transplants of organs other than the kidney has been limited. The implantation of left-ventricular assist devices (LVAD) is accompanied by transfusions that often sensitize patients awaiting heart transplantation. As noted above, 2 heart patients were included in the study of positive crossmatch patients reported by Jordan et al. [55]. In LVAD patients sensitized to HLA-class I antigens, John et al. [63] used either high-dose (2 g/kg) IVIg or plasmapheresis to reduce the antibodies. IVIg-treated patients experienced a reduction in PRA comparable to that occurring in the patients being treated with plasmapheresis (33%). However, plasmapheresis treatment took longer to eliminate antibodies and resulted in a 50% infection rate. Appel et al. [64] treated lung transplant recipients who had antibody to third-party HLA antigens (i.e., HLA antigens not present in the donor) with high-dose (2 g/kg) IVIg and IA at the time of and following transplantation. Antibody studies of high sensitivity and specificity, using flow cytometric crossmatches and solid-phase immunoassays, were performed on 12 patients who had third-party HLA-specific antibodies and who survived more than 90 days. They found that 6 of 7 patients eliminated class I-specific antibody, one of 3 patients lost class II-specific antibody, and 3 patients had lost their antibody prior to treatment. They saw less obliterative bronchiolitis among the treated patients compared to patients not receiving this treatment, although the difference was not statistically significant. However, there was a significantly lower mean number of acute rejection episodes among treated patients. They also treated transplant patients who had newly formed antibody to donor HLA ($n = 5$) or to third-party HLA ($n = 3$) using IVIg. Among these patients, the frequency of antibody elimination was lower than in the previous group with 1 of 1 patient clearing class I-specific antibody and 3 of 7 eliminating class II-specific antibody. Improvement in graft function did not correlate with antibody elimination and antibodies often returned.

4.4.3. Plasmapheresis with IVIg

The use of a PP/IVIg protocol in nonrenal transplantation has been limited to heart transplantation although Thielke et al. [71] did include 1 combined renal–pancreas transplant in their renal group. Pisani et al. [76] treated 16 patients who had PRA $> 10\%$ with plasmapheresis and 20 g of IVIg immediately prior to heart transplantation. They found that sensitized patients so treated did as well as nonsensitized patients with respect to length of hospital stay, rejection, and mortality. However, it is unknown what portion, if any, of this patient group had HLA-DSA prior to treatment. Another group treated 4 heart transplant patients, postoperatively, who had a positive pretransplant CDC XM with PP/IVIg, basiliximab, steroids, cyclosporin, OKT3, and antithymocyte globulin [77]. Three patients experienced rejection episodes but all 4 had good graft function at follow-up ranging from 17 to 57 months.

5. Treatment protocols: ABO incompatible transplantation

Alexandre et al. [82] provided dramatic evidence that plasmapheresis and standard immunosuppression were not sufficient to transplant across the ABO barrier when 3 patients who were not splenectomized, hyperacutely rejected their renal transplants. However, once splenectomy was added to the pretransplant conditioning, reasonable levels of success could be achieved. The largest single center cohort of ABO incompatible (ABOi) transplants has been performed in Tokyo. This group has used DFPP with or without IA, graft irradiation, and splenectomy to perform transplants across all ABO incompatibilities. They found that the outcome was not related to the ABO-DSA titer before the start of treatment [83] but that significant improvement in graft survival was achieved when the titer of this antibody was reduced to 16 or less before transplant [84]. They were also able to show that when titers could not be reduced sufficiently with DFPP, treatment with repeated doses of anti-CD20 followed by splenectomy permitted successful transplantation [85]. In a review of 141 patients, they showed that graft survival was significantly lower than in ABO compatible transplants, at 1, 5, and 10 years posttransplant. However, newer immunosuppressive agents have reduced acute rejection episodes and have brought current survival rates to the level of ABO compatible transplants [86,87]. This group has also performed ABOi transplants in pediatric patients with even better outcomes [88].

At the Mayo Clinic, the PP/low-dose IVIg protocol used to transplant patients with HLA-DSA was adapted for ABOi transplants by the addition of splenectomy. They have achieved an 85% graft survival at a mean follow-up of 400 days with no hyperacute rejections [70,89]. There was a high frequency (46%) of AMR but it was reversible with additional plasmapheresis in the vast majority (83%) of patients. The acceptance of ABOi transplantation by patients and referring doctors has been limited by the need for splenectomy and the resultant lifetime susceptibility to bacteremia. We [90] and others [91] hypothesized that the temporary ''biologic splenectomy'' that could be achieved using anti-CD20 would provide protection against AMR during the first 3 months when the risk of graft loss is highest and then allow the B-cell compartment to reconstitute with the spleen in place. Tyden et al. [90] reported good success without splenectomy using a protocol of IA on sepharose columns, 500 mg/kg IVIg, anti-CD20, tacrolimus, MMF, and steroids. They transplanted 4 patients with A1, A2, or B incompatibilities and pretreatment, IgG isoagglutinin titers of 16–64. At follow-up ranging from 3 months to 2 years, all patients had good renal function and antibody to donor ABO antigens titers ranging from 1 to 8. In our series, successful ABOi transplants have been achieved without splenectomy using our PP/CMVIg protocol with anti-CD20 [91]. Six patients that included A1, A2, and B incompatibilities and starting ABO-DSA titers of 8–128 were transplanted under this protocol. At follow-up ranging from 4 to 14 months, there were no graft losses and mean serum creatinine was 1.3 mg/dl. These last 2 studies demonstrate the ability to achieve successful ABOi renal transplantation without the long-term risks associated with splenectomy. We have recently reported 4 patients with A1 (2 patients), A2 (1 patient), and B (1 patient) who underwent successful transplantation across an ABOi barrier with PP/CMVIg alone without splenectomy or anti-CD20 [92]. We have also performed 3 transplants in patients who had positive crossmatches with their donors due to HLA-DSA and were ABOi [93]. All 3 patients underwent splenectomy at the time of transplant and 1 of the patients

received anti-CD20. Two patients were CDC XM–positive at start of treatment and 1 was positive by flow cytometric crossmatch. The incompatibilities were A2, A1, and B. At follow-up of 11, 9, and 9 months, serum creatinines of the patients were 1.2, 1.2, and 1.1, respectively. ABOi protocols currently being used in the United States, Europe, and Japan are providing 1- and 3-year graft survival rates equivalent to those of blood type compatible live donor transplants.

6. High-dose IVIg vs. plasmapheresis with low-dose IVIg

High-dose IVIg and plasmapheresis combined with low-dose IVIg are the 2 most common protocols used for overcoming the HLA-DSA barrier. A meaningful comparison of the 2 protocols is difficult because, as noted earlier, there have been numerous protocol variations used and there have been no randomized trials. Further, there are several different IVIg products that vary in the size of the donor pool and in several aspects of their composition. While this subject is too extensive to discuss here, the interested reader may refer to several review articles that cover this subject [49,94–97]. Considering the caveats given above, it is interesting to make some comparisons on efficacy and applicability, adverse effects, and possible mechanisms of these 2 types of protocol.

6.1. Efficacy and applicability

Efficacy may be measured by the percentage of treated patients who are transplanted, the graft survival and function, and by the effect on HLA-DSA. In the studies of high-dose IVIg carried out by Glotz and Jordan groups and in the NIH-sponsored IG02 study, high-dose IVIg was given to patients who did not have a designated donor and were on a deceased donor waiting list. While the majority of patients treated by Glotz and by Jordan were transplanted, it is difficult to determine the extent to which treatment facilitated transplant. For example, in 1 study, although 13 of 15 patients treated were transplanted, only 10 had historical antibody to the donor, and HLA-DSA could be identified in only 7 [54]. Jordan et al. [55] transplanted 42 of 45 patients treated with high-dose IVIg. Among these were 28 live donor transplants that were converted from positive to negative CDC crossmatch by the treatment. In the multicenter trial that treated patients with PRA $\geqslant$ 50, only 35% of those treated with IVIg were transplanted. However, this is twice the transplantation rate of those treated with placebo. In these studies, transplantation occurred only when the CDC XM was negative. Comparable transplantation rates were achieved by Schweitzer et al. [69] using PP/IVIg and they were able to transplant 11 of 15 patients who had converted to a negative crossmatch. In our program, we have treated more than 110 patients with HLA-DSA (95 preemptively and 15 rescue) and 31 with antibody to donor ABO antigens and transplanted all but 4 of the 126 patients who were treated preemptively (97% transplantation rate). However, we have transplanted several patients who had positive CDC XM at the time of transplant and have shown that these crossmatches become negative with further treatment. Despite wide variability in immunosuppression regimens, it appears that both types of protocol yield comparable graft outcomes with 1- and 2-year graft survivals in the high 80 percentages. There is also an appreciable rate of AMR (35–50%) seen with both protocols and, at least for the PP/IVIg protocol, is related to persistence of HLA-DSA. Both types of protocols are efficacious in treating AMR.

There is much more information available about persistence of HLA-DSA in the studies using PP/IVIg treatment. It appears that a substantial percentage of these patients maintain low levels of antibody, detectable only by the most sensitive techniques. Because HLA-class II antigens are not expressed constitutively on vascular endothelium, it is likely that antibodies specific for donor class II antigens will be problematic only at times when antigen expression is induced. Low levels of antibody to donor class I have been reported to induce accommodation [98–100], enhancing graft survival. However, in the presence of proinflammatory cytokines, such antibodies promote transduction of proliferative signals which, in turn, may result in graft vascular changes associated with chronic rejection [101]. Thus, the long-term consequences of persistent HLA-DSA remain to be seen and necessitate accurate and consistent evaluation of antibody status.

Tyan et al. [52] have shown that it is possible to predict the efficacy of high-dose IVIg treatment through in vitro testing. The opportunity for predictive in vitro testing and being able to treat patients with monthly injections make the high-dose IVIg protocol much more suitable than PP/IVIg for patients on deceased donor transplant waiting lists. Further, high-dose IVIg treatment does not need the specialized equipment and training necessary for performing plasmapheresis. However, high-dose IVIg is variable in its effect on different HLA-specific antibodies. Despite persistence of very low antibody levels, the PP/IVIg protocol allows successful transplantation regardless of antibody specificity. Therefore, it may be better suited, than high-dose IVIg, for live donor transplantation.

6.2. Adverse effects

A variety of side effects have been reported for IVIg. The presence of IgG aggregates may produce headaches, fever, and flushing [reviewed in reference 49]. Reported thrombotic events may be related to the product's sodium content and osmolarity [reviewed in reference 96]. Certainly, for use in renal transplantation, the most disconcerting effects are hypotension, renal dysfunction, and even acute renal failure [102–106]. Kidney-related problems appear to be related to the sucrose content, which is variable among the different IVIg products and, therefore, should be avoidable.

These sorts of problems are not seen with IVIg and CMVIg given at low doses. Haas et al. [107] showed that vacuolization of tubular epithelial cells and osmotic nephrosis seen with high-dose IVIg can also be seen in biopsies of patients treated with low-dose CMVIg. However, it does not appear to have clinical consequences, and we have not seen any cases of acute renal failure with low-dose CMVIg. Repeated plasmapheresis is associated with increased risk of infection [63]. Further, choice of material for volume replacement should take into account the need for restoring adequate levels of clotting factors at the time of surgery or other invasive procedures.

6.3. Mechanisms

Because of its use in a variety of other conditions, there is a fair amount known about the actions of IVIg. Basta et al. [108] demonstrated the ability of IVIg to suppress binding of complement to IgG-sensitized red blood cells. Several investigators have reported that the

binding of IVIg to Fc receptor may result in a blockade of this receptor, preventing salvage of endogenous IgG and leading to B-cell apoptosis [109,110]. Others have demonstrated an effect on antigen presenting cells (APCs). Blasczyk [111] found that IVIg preparations contain soluble CD4 that may block APCs. Bayry et al. [112] demonstrated that IVIg inhibits the maturation of dendritic cells and modulates the secretion of cytokines by those cells. All of these properties would support a suppression of the humoral response in the transplant setting but do not explain the in vitro effects. This is unlikely to be due to interference with complement because the IVIg does not block antibody reactions in the CDC assay uniformly. Tyan et al. [52] and Glotz et al. [51] have suggested that the major mode of action, both in vivo and in vitro, is via anti-idiotypic antibodies in the IVIg. They propose that these antibodies block in vitro and, initially, bind the HLA-specific antibodies in vivo and that this binding promotes development or expansion of autologous anti-idiotypic antibodies. This may well be the main mode of action of high-dose IVIg and would account for the dose dependency of the effect. However, biology is never simple and there are probably several mechanisms involved.

Much less is known about immunomodulatory properties of CMVIg. Sivasai et al. [113] showed that Cytogam® could block the mixed lymphocyte reaction when added to the assay in vitro but sera from patients receiving 1 dose of 50 mg/kg did not block the reaction. However, these sera did inhibit the cytotoxic T lymphocyte assay. Perhaps the mode of action is not different than that of high-dose IVIg but that the dose requirement is reduced in the face of plasmapheresis. Certainly plasmapheresis has been shown to reduce antibody levels and most likely reduces a number of other serum factors with the effect of minimizing inflammation and HLA expression. Although it is likely that CMVIg also contains anti-idiotypic antibodies, we have not achieved in vitro blocking and have found anti-idiotypic antibodies in patients treated in our protocol, only sporadically (unpublished data).

7. Summary

Sensitization to HLA antigens has been a significant barrier to access to transplantation. The use of recombinant erythropoetin and concomitant reduction in transfusions has mitigated this problem somewhat. However, sensitization remains a significant barrier, which affects African Americans and women disproportionately. These antibodies have long been recognized for their ability to cause hyperacute rejection but, more recently, it is being appreciated that they most likely are involved in all types of rejection. Therefore, the ability to suppress production of these antibodies would be expected to increase access to transplantation, improve graft function, and prolong graft survival. Antibodies to antigens of the ABO system, with its much more limited polymorphism, are not as restrictive as are HLA-specific antibodies. However, the shortage of organ donors has made the ability to cross the ABO barrier more of a necessity than a desire. This is particularly true for patients whose only opportunity for a live donor transplant is with an ABOi donor.

Protocols have been developed that have crossed these immunologic barriers and achieved excellent results. This has been possible because of the availability of newer immunosuppressive agents that affect B-cell activity and survival and the development of

technologies that permit sensitive detection and accurate characterization of HLA-specific antibodies. The use of these protocols has also provided fertile ground for investigation into allorecognition, alloreactivity, and peripheral tolerance. Further, despite the initial increase in cost for a transplant, the ability to reduce the waiting time for patients makes the use of the desensitization protocols highly cost effective [reviewed in references 94 and 114]. However, these treatment protocols are labor intensive and require expertise and support in a variety of areas and should only be undertaken after careful consideration of the needs of such a program.

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