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NEPHROLOGY FORUM

Immunoadsorption in the sensitized transplant recipient

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Case presentation

A 36-year-old woman had developed insulin-dependent diabetes mellitus at age 7. During her first pregnancy, 19 years later, she was hypertensive and had proteinuria. This pregnancy and two further pregnancies 3 years later were unsuccessful (2 stillbirths and 1 perinatal death). Seven years later, she had a plasma creatinine of 453 μmol/liter (5.1 mg/dl) and was blind in her right eye from a retinal detachment and glaucoma.

One year later she started continuous ambulatory peritoneal dialysis (CAPD) and 2 months later she received a kidney from her sister. Prior to transplantation, she had received 5 units of blood and her percentage reactivity rose from 0% to 26%. The donor kidney was mismatched for 3 class-I HLA antigens (A1, B47, B17). Initially the graft functioned, and at 2 weeks, her plasma creatinine was 200 μmol/liter (2.3 mg/dl). She was given prednisolone and azathioprine. Despite anti-rejection therapy, she lost her kidney because of irreversible rejection 4 months after transplantation. Following graft nephrectomy, her percentage reactivity rose to 94%.

The patient returned to CAPD for the next 3 years. During that time, peritonitis and an abdominal hernia developed, prompting abandonment of CAPD. She then started hospital-based hemodialysis. Her anemia worsened and she became transfusion dependent. She tolerated hemodialysis poorly and transplantation was reconsidered.

The patient was blood group A and had been highly and persistently sensitized since the loss of her previous graft. Her percentage reactivity had remained greater than 90%, and the main HLA antibody specificities were A1, A11, and possibly Bw4. The HLA-A1 titer was 1/64. Non-HLA autoantibodies had been excluded. As a result, the chance that a suitable graft would become available was extremely remote, and the decision was made to remove her HLA antibodies by extracorporeal immunoadsorption prior to transplantation.

Following the first course of extracorporeal immunoadsorption, her HLA-A1 antibody became undetectable and her percentage reactivity fell to 32%. Despite immunosuppression with prednisolone and cyclophosphamide, however, she resynthesized the HLA antibodies and required an additional two courses of extracorporeal immunoadsorption over a 4-month period while waiting for a kidney. One month after her last course of immunoadsorption, she received a kidney carrying none of the HLA antigens against which she had previously generated antibody. The crossmatch was positive with the pretreatment sera but negative with the posttreatment sera.

After transplantation she was given prednisolone, cyclosporine, and azathioprine; she also received a 10-day course of prophylactic antithymocyte globulin. The graft functioned immediately, and at 3 months after transplantation, her plasma creatinine was 126 μmol/liter (1.4 mg/dl). At 8 months, following an upper respiratory tract infection, the plasma creatinine rose to 240 μmol/liter (2.7 mg/dl). Allograft biopsy showed acute vascular rejection. No donor-specific antibodies were detected. She was treated with high-dose steroids and a 10-day course of OKT3, following which her plasma creatinine fell to 160 μmol/liter (1.8 mg/dl). She is now well with a plasma creatinine of 175 μmol/liter (2.0 mg/dl), one year after transplantation.

Discussion

DR. DAVID TAUBE (*Consultant Nephrologist, St. Mary's Hospital, London*): This patient exemplifies the problems of highly sensitized renal transplant recipients as well as a new approach to their treatment. These unfortunate patients, in whom successful transplantation is difficult to achieve, accumulate in end-stage renal failure programs with the attendant misery and financial burdens of long-term dialysis. The scale of the problem is demonstrated by the recent estimate that 20% of the 20,000 dialysis patients awaiting transplantation in Europe and North America are sufficiently sensitized to virtually preclude transplantation [1]. I will begin by discussing the causes and consequences of sensitization and then will describe new strategies for managing these patients before and after renal transplantation. Although the term "sensitized patient" generally is used to describe the patient who has "preformed" HLA antibodies, I will briefly discuss other important, non-HLA antibodies, which include lymphocytotoxic autoantibodies, endothelial-monocyte antibodies, and blood group antibodies as well.

Documenting sensitization

The human kidney expresses class-I and, variably, class-II HLA antigens [2]. Therefore it is not surprising that the

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majority of patients with HLA antibodies damage grafts expressing the appropriate HLA antigens. This phenomenon was recognized in the early days of transplantation in 1965 and 1966, when Terasaki et al [3] and Kissmeyer-Nielsen and coworkers [4] showed that the presence of anti-graft HLA antibodies before transplantation was associated with "hyperacute" rejection [4]. Anti-graft antibodies are detected by complement-dependent lymphocytotoxicity (CDC) tests with peripheral blood lymphocytes, purified T and B cells or monocytes, and endothelial cells acting as targets. Sera from patients awaiting transplantation are screened regularly with panels of HLA-typed donor lymphocytes, selected to ensure representation of all defined class-I HLA antigens. The proportion of the panel's cells that the patient's serum kills is expressed as the percentage reactivity. The specificity of the antibody is determined from the HLA type of the donor lymphocytes. Patients with percentage reactivities of 0% to 10% are defined as being nonsensitized; with 10% to 50%, as sensitized; and with 50% to 100%, as highly sensitized. The sensitivity of these tests can be increased by the use of anti-human globulin [5, 6] and flow cytometry [7–9]. Flow cytometry can be of particular value in detecting small amounts of HLA antibodies not found by conventional CDC testing, amounts that nonetheless can lead to subsequent graft failure [8]. The overall value of these two techniques in terms of predicting graft outcome is currently uncertain, however [10, 11].

The HLA antibodies, usually IgG, are mainly directed against class-I HLA antigens [9]. In a recent study of 150 sensitized patients awaiting transplantation, 50% had class-I HLA antibodies, whereas only 10% had class-II HLA antibodies [12]. Two types of HLA antibodies are found in sensitized patients. Most patients have antibodies directed against cross-reactive groups of HLA class-I antigens similar to those detected in the sera of multiparous women [13–15]. The classic cross-reactive groups include A1, 3, 11; A2, 28, 9; B5, 15, 18, w35; B22, 7, 27, 40; and B21, 12, 13 [16]. These antibodies are directed against shared public determinants on the HLA class-I heavy chain [17, 18]. The public epitopes are shared by several HLA antigens; therefore an antibody directed against one of these antigens can cause a high percentage reactivity. Individual patients can have one to three different antibodies recognizing different groups of HLA antigens, thus causing very high percentage reactivities [17]. The degree of cross-reactivity depends on the titer of antibody. This can be shown by the use of mouse monoclonal antibodies generated against class-I HLA antigens [19] or by the examination of sera at different dilutions from highly sensitized patients [20]. We have found, in vivo, that the reduction of HLA antibody titer by plasma exchange [20] or extracorporeal immunoadsorption [21] reduces the number of cross-reactions and percentage reactivity. The presence of cross-reactive group antibodies is associated with an increased rate of early graft loss and certain cross-reactive group combinations, for example, B7, 22, 27 and B5, 18, 35, with a high rate of rejection [22]. Patients also can have high percentage reactivities because they have multiple antibodies directed against private determinants on different HLA class-I molecules. In our experience [20], approximately 60% of highly sensitized patients have cross-reactive HLA antibodies, whereas 30% have HLA antibodies directed against multiple private determinants.

Non-HLA lymphocytotoxic autoantibodies, which are usually IgM [9], cause high, broadly reactive percentage reactivities and occur in approximately 10% of our sensitized patients awaiting renal transplantation [20]. It has been recognized for many years that these antibodies do not cause hyperacute rejection [23] and that transplantation across a positive cross-match caused by these antibodies is associated with good graft survival [24, 25]. The antibodies are now readily identified by pretreating sera with dithiothreitol [26], which inactivates IgM but not IgG. Their importance lies in their ability to cause a high percentage reactivity and to "hide" significant IgG HLA antibodies [27]. They can be in found in patients with systemic lupus erythematosus [28] and can occur after viral infections, particularly with cytomegalovirus [29].

Anti-vascular endothelial cell (VEC)-monocyte antibodies are not detected in conventional CDC tests and therefore technically (that is, in in-vitro testing) are not important in sensitized patients. Increasing evidence suggests, however, that the VEC antibodies play an important role in allograft rejection. Antibodies directed against VEC antigens are found in patients with irreversible vascular rejection [30–32], particularly in recipients of HLA-identical allografts. In a multicenter, retrospective study of 35 HLA-identical, living related allograft recipients with severe rejection, 83% of the patients who lost their allograft from rejection had VEC antibodies [32]. Good evidence suggests that these antibodies cause graft loss and do not arise as a result of the rejection process [31]. They cross-react with monocytes [32] and can be difficult to detect in the presence of HLA antibodies. Antibodies directed against monocytes alone are unimportant [32]. Evidence suggests that VEC antigens are closely linked to the major histocompatibility complex (MHC) [33, 34] and that DRw6 recipients are more likely than recipients without DRw6 to develop VEC antibodies [35]. Using a donor-specific vessel cross-match technique with donor aorta and vena cava, VEC antibodies can now be detected prospectively [36]. In a preliminary study of 55 cadaver allograft recipients, 6 of 7 patients experiencing early, irreversible rejection had VEC antibodies [36].

Certain red cell antibodies are lymphocytotoxic and are therefore detected in CDC assays; hence, these too can cause "sensitization." These red cell antibodies include Lewis antibodies [37] and I and i antibodies [38]. They are not thought to be important clinically.

Causes of HLA sensitization

What prompts the formation of HLA antibodies and the consequent sensitization? These antibodies generally develop as a result of failed, mismatched transplants, blood transfusions, and multiple pregnancies [39–41]. Often a combination of these factors is involved. In a series of 2879 patients, failed transplants were thought to be the most important source of HLA antibodies, followed by pregnancy and transfusion [41]. After a failed transplant, the increase in sensitization (as judged by percentage reactivity) depends on the degree of class-I mismatching with the first transplant [42]. In a study of 449 patients who had lost a graft, the mean increase in percentage reactivity in the poorly matched group was 45%, whereas in the well-matched group, the mean increase was 34%. The number of pregnancies is important, as shown in a study of 578 parous women; only 23.6% of women with 2 pregnancies had HLA antibodies, whereas 63% of women with 6 pregnancies had

HLA antibodies [40]. Transfusions in parous women are particularly likely to induce sensitization. Opelz et al, in a study of 737 patients, found that 75% of women who had had 3 or more pregnancies and who had received 5 units of blood had percentage reactivities of at least 50% [39]. Women tend to be more sensitized than are men. In a Council of Europe study of sensitized patients, 14% of women awaiting their first transplant were highly sensitized, whereas only 5% of the male patients studied were highly sensitized [43]. This effect is partly related to the women's previous pregnancies: of these highly sensitized patients, 61% had been pregnant, but only 46% of the unsensitized women had been pregnant. With regrafts, this imbalance disappeared; 31% and 26% of female and male patients, respectively, were highly sensitized. The timing of blood transfusions also is important, as shown in the early days of HLA typing, when normal individuals were immunized with HLA antigens to produce HLA alloantisera [44]. Frequent (weekly), small-volume transfusions (50 ml) were more efficient in immunizing patients than were less frequent, larger-volume transfusions [44]; this finding might explain why donor-specific transfusion is so efficient at inducing HLA antibodies [45]. Few data are available to explain why some patients have persistently high percentage reactivities and titers of HLA antibodies, sometimes for many years. However, Deierhoi and colleagues recently showed that in many patients, persistently high percentage reactivities occur as a result of continuing blood transfusion [46]. The patient we are discussing here is a classic example of the importance of all these factors. She had several pregnancies, more than 50 transfusions, and she lost a mismatched transplant.

In 1985, Tongio and colleagues reported spontaneous HLA antibodies in 1% of normal blood donors who had not been pregnant [47]. These HLA antibodies are weak, mainly IgM; interestingly, they are often directed against HLA-B8 [47]. In 1980, Chardonnens and Jeannet found HLA antibodies against noninherited maternal antigens in newborn babies [48]. The sera and kidneys of patients after transplantation also contain HLA antibodies. Although initially the significance of these antibodies was uncertain, good evidence now suggests that the development of donor-specific antibodies is associated with rejection and a poor outcome. Ting and Morris studied sera taken at 5-day intervals for the first 2 months following initial cadaveric transplantation in nonsensitized recipients and found no correlation between the development of donor-specific antibodies and rejection or outcome [49]. Nunez et al, however, reported that the presence of class-II antibodies before and after transplantation is associated with a higher incidence of graft loss [50]. Lorden and coworkers demonstrated that antibody against a B lymphoid cell line is associated with vascular rejection and graft loss [51]. Martin et al also showed that the development of panel-reactive and donor-specific antibodies in a large group of patients is associated with a poor outcome [52]. We found donor-specific antibodies in the sera of highly sensitized patients at the time of allograft rejection [53]. Because these antibodies can be transient and present in low titer, they can be missed easily. Cytotoxic HLA antibodies can be eluted from rejected kidneys. These antibodies are not always donor specific, and the kidney can act as a nonspecific antibody "sponge" [54].

Consequences of sensitization

Highly sensitized patients wait considerably longer than do non-sensitized patients for their transplants. If these patients do receive allografts, they have a higher incidence of primary allograft nonfunction (allografts that never function) and poorer graft survival than do non-sensitized patients. Waiting times for transplantation in highly sensitized patients vary between 13 and 39 months [1]. In the Council of Europe Study, the waiting time for a highly sensitized patient, from failure of the first graft to retransplant, was a mean of 39 months, compared with 22 months in nonsensitized patients [55]. Even with the advantage of a large organ-procurement organization (for example, the Southeastern Organ Procurement Foundation [SEOPF]), less than one in 10 highly sensitized patients receives a transplant within a given year, whereas the probability of a nonsensitized or moderately sensitized patient receiving a transplant during this period is high [56]. In France, 21% of the patients in 1983 and 1984 awaiting transplantation were highly sensitized (percentage reactivity 75%). During this period, however, only 5.8% (1983) and 2.8% (1984) of the patients who received transplants were in this highly sensitized group [57].

Primary nonfunction and delayed graft function are associated with a high percentage reactivity. In a study of 3800 renal transplants, Sanfilippo et al found that delayed graft function was associated with both a high peak and current percentage reactivity and that graft survival was significantly lower in this group of patients [58]. In a similar, smaller multicenter study examining the causes of primary allograft nonfunction, a high current and peak percentage reactivity were major risk factors [59]. Three-quarters of these grafts were rejected [59].

Good data from the United Kingdom [60] and the United States [61] show that sensitization has a detrimental effect on allograft survival. The deleterious effect of sensitization also is found despite the use of cyclosporine in large, multicenter studies [62] as well as in smaller, single-center studies. In a study of 76 patients receiving second transplants, allograft survival was 35% at one year in the group of patients with percentage reactivities greater than 50%, and 82% in the group with percentage reactivities less than 50% [62]. More recent data suggest, however, that the use of cyclosporine may override the effect of sensitization [63]. Matas et al report a 90% one- and two-year allograft survival in a group of 33 patients with greater than 90% peak percentage reactivity [64].

Management of sensitized patients

Sensitized patients can successfully receive transplants by any of the following 3 strategies: (*1*) use of a well-matched kidney that does not express the HLA antigens against which the patient has made antibody; (*2*) use of "enlightened cross-matching"; or (*3*) removal and prevention of the resynthesis of HLA antibodies. By far the best approach to this problem, however, is the *prevention* of sensitization in potential transplant recipients.

Recent advances in transplantation immunosuppression have improved allograft survival and hopefully will minimize the most important cause of sensitization, namely, allograft failure. Because there is little we can do about sensitization arising as a result of previous pregnancies, I intend to concentrate here on the role of blood transfusion. Patients with renal failure gener-

ally are transfused because of hemorrhage, anemia, and because blood transfusions improve the probability of graft survival. Although transfusion in patients who are actively bleeding is essential, there is now little reason to transfuse patients who have symptomatic anemia. Recombinant human erythropoietin (rHuEPO) is widely available and highly effective, although a small proportion of patients receiving this agent become hypertensive, thrombose dialysis fistulae and grafts, or have cerebrovascular accidents [65]. With increased experience in the use of rHuEPO, the incidence of these side effects may be reduced. As yet, however, little evidence suggests that the elimination of regular transfusion by the use of rHuEPO substantially reduces percentage reactivity in previously sensitized patients [66]. A small number of sensitized pediatric patients at Guy's Hospital who were treated with rHuEPO showed a steady decline in HLA antibody titer and, to a lesser extent, in percentage reactivity (Dr. S. Rigden, personal communication). One certainly would hope that the use of rHuEPO will have a major impact on the sensitization induced by regular blood transfusions [46]. In contrast, the depletion of leukocytes from blood by cotton wool filtration and red cell washing does not prevent sensitization [67], particularly as red cells can express class-I antigens [68].

After Opelz et al reported the beneficial effects of blood transfusion on renal transplant survival in 1973 [69], most transplant units started to transfuse (and also sensitize) their patients routinely prior to transplantation [70]. It has become clear, however, that over the past 3 years or so, the "transfusion effect" has become negligible in terms of graft survival, as confirmed in several large multicenter studies [70, 71] as well as in single centers [72]. This seeming disappearance of the "transfusion effect" has been attributed to the overriding benefits of cyclosporine therapy [70–72]. At present, with the use of cyclosporine, I do not believe that patients should receive pretransplant transfusions for the sole purpose of enhancing the probability of graft survival. In particular, patients who are already sensitized or at risk of further sensitization—for example, parous women—should not be transfused unless it is clinically indicated.

Clearly the simplest approach to transplantation in a highly sensitized recipient is finding a *well-matched organ* that does not carry the HLA antigens against which the recipient has generated antibody. Living related donors are the best source but are available for only a small minority of our patients. Consequently, special schemes have been developed for finding well-matched cadaver donors for highly sensitized patients; 11 special programs were started in Europe between 1976 and 1986 [73]. In 1984, the United Kingdom Transplant Service's "Save Our Sensitised" (SOS) scheme was started. The initial results, however, have been disappointing, with a 6-month graft survival of only 56.6% [74]. Scandia Transplant, which has an organ-sharing program for highly sensitized patients, had comparably poor results. They reported a one-year graft survival of 48.6% in 158 patients (most of whom received cyclosporine) [75]. Slightly better results were reported by SEOPF in 3766 sensitized patients; their one-year graft survival was 63% (with historically positive cross-match) to 69% (with historically negative cross match) [76]. The search for an appropriate donor can be facilitated by predetermining which donor HLA specificities will give a negative cross-match. Recently Claas et al described a computer-aided method of predicting allowable donor HLA antigens by screening recipient sera [77]. Using this technique, they successfully transplanted organs into 40 patients (one-year graft survival, 80%), some of whom had been waiting more than 10 years. The accurate prediction of the acceptable HLA antigens was greatly aided by the knowledge of the patient's mother's HLA type; this parameter of screening was based on the assumption that the patient would have a negative cross-match with noninherited maternal antigens (because of tolerance acquired in utero). In a parallel study, 15 of 26 patients had negative cross-matches with noninherited maternal antigens [78]. This finding indicates that a mother may be able to induce partial tolerance in her fetus.

Because of the danger of hyperacute rejection when a kidney is transplanted across a positive cross-match, a negative cross-match had been an absolute prerequisite before any transplant operation. Over the past 5 years, this former absolute rule has lost its grip on us. I use the term "*enlightened cross-matching*" to describe the developments in cross-matching that have led to the successful transplantation of organs in sensitized patients even in the presence of a positive cross-match or previous mis-match. Examples include the recognition of the benign nature of IgM autoantibodies, which I already mentioned, of the benign nature of positive cross-matching with historic sera but negative cross-matching with current sera, and of the acceptability of mis-matched HLA antigens on previously failed grafts. In 1983, Cardella and colleagues described successful transplantation in a group of patients who had positive cross-matches with their donors when historic sera were used but who had negative cross-matches with current sera [79]. This concept is of particular value in the management of sensitized patients, because at least 50% of them show a decline in percentage reactivity with time, particularly if further transfusion is avoided [46]. Several studies now have confirmed the efficacy of Cardella's approach [76, 80, 81]. Extending this concept, we recently assessed whether a previous mis-match is important in subsequent retransplantation [82]. Provided that grafts carrying HLA antigens against which the recipient is known to have made cytotoxic antibody are avoided, and prophylactic antithymocyte or antilymphocyte globulin is used, retransplantation after previous mis-matches has been successful in our hands, with a one-year graft survival of 79%.

The third branch of our management triad is *removal and prevention of the resynthesis of HLA antibodies*. In 1983, an 18-year-old patient of ours was in serious trouble. She had had two unsuccessful living related transplants, she was unable to tolerate CAPD, and she had virtually no further access sites for hemodialysis. She was transfusion-dependent, with a percentage reactivity persistently greater than 90%; she had had positive cross-matches with at least 40 potential donors. We established that her high percentage reactivity was due to a high titer (1/64) HLA-A2 antibody, which cross-reacted with multiple HLA antigens. We decided to remove her HLA antibody by plasma exchange and to prevent its resynthesis using cyclophosphamide and steroids. Following such treatment, her antibody titer fell to less than 1/10, and her percentage reactivity to 43%; she was given a non-HLA-A2 cadaveric kidney. The cross-match with her pretreatment sera was positive, but negative with the posttreatment sera. Her immunosuppresion regimen post transplant consisted of prednisone and azathioprine.

She experienced no hyperacute rejection and, although she had two severe rejection episodes, she remains well 7 years later, with a current plasma creatinine of 105 μmol/liter (1.2 mg/dl). No increase in HLA-A2 antibody titer occurred after transplantation, even during her rejection episodes. We were encouraged to make similar attempts in other patients and, although one patient died from immunosuppression-induced sepsis, we successfully transplanted kidneys into 4 additional patients using this regimen [20]. We then began to use cyclosporine, and, because of the high incidence of early rejection, we added a 10-day prophylactic course of antithymocyte globulin, starting at the time of transplantation.

Extracorporeal immunoadsorption with staphylococcal protein A is a new technique by which circulating IgG can be efficiently removed [84]. We therefore decided to use immunoadsorption rather than plasma exchange [85] and recently published our results with 10 patients [21]. In our hands, this technique is an effective method of removing HLA antibodies. We recently reviewed the outcome of 19 patients whose HLA antibodies were removed by either plasma exchange or immunoadsorption and who have had a successful allograft for a minimum of one year [86]. Only one graft failed as a result of rejection. Two patients died (one from sepsis and one from a myocardial infarction 3 years post transplant). The one-year patient and graft survival were 94% and 88%, respectively. These results compare favorably with those obtained using the conventional "wait for a good match strategy." We select our patients carefully. Their HLA antibodies are fully characterized and in general are directed against one or two cross-reactive groups. We also ensure that the patients are fit enough to tolerate the immunosuppression. Our approach using both plasma exchange and immunoadsorption has been used successfully by several other groups [87–89]. Limited data suggest that the prevention of HLA antibody resynthesis depends on the use of cyclophosphamide. Hillebrand et al were unable to prevent the resynthesis of HLA antibody using azathioprine (in 3 patients) or cyclosporine (in 2 patients) [90]. We used cyclosporine in 4 patients [91]. In the 2 patients with relatively low titers of HLA antibody, we were able to suppress HLA antibody resynthesis, but in 2 other patients with higher titers, we were unsuccessful. Most of the deaths in the patients treated with this technique resulted from infection. Before this technique can be used more widely, a less toxic method of preventing antibody resynthesis must be developed. At present, therefore, I believe that HLA antibody removal should be reserved for the fit patient with well-characterized antibodies who needs urgent transplantation.

How does one manage the sensitized patient after transplantation? As I said earlier, sensitized patients have a higher incidence of rejection and impaired graft survival when compared with nonsensitized patients. We routinely add a 10-day course of antithymocyte globulin to our standard regimen of prednisolone and cyclosporine for our sensitized patients. With this regimen, we achieve a one- and two-year patient survival rate of 96% and a one- and two-year graft survival rate of 84% and 79%, respectively [92]. Similar results with sensitized patients also have been reported with other regimens using prophylactic antithymocyte or antilymphocyte globulin. DeMasi and coworkers recently reported one-year patient and allograft survival rates of 97.0% and 82.5%, respectively, using a quadruple immunosuppression regimen [93]. Sommer et al used antilymphocyte globulin, cyclosporine, and prednisone sequentially, and obtained a two-year patient and graft survival of 91% and 72%, respectively [94]. These results are better than those described earlier [59, 60, 64]; the prophylactic use of antithymocyte and antilymphocyte globulin possibly improves graft survival in sensitized patients without significantly increasing the risk of infection or neoplasia. No formal proof confirms this hypothesis, however, because no randomized, controlled trial has been performed.

In conclusion, the sensitized patient presents several problems, none of which is insurmountable, as demonstrated by the patient discussed today. Improvements in graft survival, the use of rHuEPO, and the cessation of routine pretransplant transfusions should soon make sensitization much less problematic than at present.

Questions and answers

DR. JOHN T. HARRINGTON (*Chief of Medicine, Newton-Wellesley Hospital, Newton, Massachusetts*): I always have thought that patients with any degree of percentage reactivity are, in fact, sensitized. You have given us certain ranges of percentage reactivity representing different degrees of sensitization. What do these ranges of percentage reactivity indicate, and what is the reproducibility and accuracy of the test?

DR. TAUBE: Your first comment is entirely relevant. The ranges of percentage reactivity that I have discussed are arbitrary. The validity of the percentage reactivity depends on the size of the panel and the technical excellence and expertise of the tissue typing laboratory. Small panels can be particularly misleading. Blood transfusions and viral infections can cause marked, often transient, fluctuations in an individual patient's percentage reactivity. It is therefore essential that we measure a patient's percentage reactivity several times over a period of time before deciding on the degree of sensitization.

DR. M. MCGEOWN (*Professorial Fellow, Queen's University, Belfast, Northern Ireland*): Although the current results of the United Kingdom Transplant Service's SOS program matching scheme are disappointing, we hope to improve them by increasing the degree of matching between donor and recipient.

DR. TAUBE: The avoidance of the HLA antigens against which the recipient has made antibody ensures a certain degree of matching. Gerhardt Opelz has data showing that good matching in highly sensitized patients greatly improves graft survival (personal communication; presented at 1989 EDTA/ERA Congress in Göteborg, Sweden). But finding a good match entails a mean wait for a transplant of 4 years.

DR. C. M. LOCKWOOD (*Lecturer in Medicine, University of Cambridge, Cambridge, England*): How long should one use prophylactic antithymocyte or antilymphocyte globulin after transplantation in these highly sensitized patients? Do patients with higher titers of HLA antibodies require larger doses and extended courses of antithymocyte or antilymphocyte globulin?

DR. TAUBE: We generally give a 10-day course of antithymocyte or antilymphocyte globulin to these patients after transplantation. Little evidence suggests that a 5- or 7-day course is less effective than a 10-day course, however. We have no evidence suggesting that the more highly sensitized patients require more prophylactic antithymocyte or antilymphocyte globulin.

DR. A. REES (*Consultant Nephrologist, Royal Post Graduate Medical School, Hammersmith Hospital, London, England*): You made some tantalizing comments about monocyte-endothelial antibodies. One of them was that monocyte antibodies do not cause rejection; another was that this system is MHC linked.

DR. TAUBE: This group of antibodies recognizes epitopes both on endothelial cells and on monocytes. There is good evidence showing that endothelial cell antibodies cause rejection and that monocyte antibodies are harmless [32]. Some evidence suggests that this system is linked to the major histocompatibility complex [33]. Recently Brasile et al found that patients who are DRw6+ are more likely to develop endothelial cell antibodies [35].

DR. G. NEILD (*Senior Lecturer, Institute of Urology, London*): You came out rather strongly against blood transfusions. One of the pieces of information you cited was that the benefit in the cyclosporine era is only 5%. Would you like to qualify this? My understanding from the literature is that if you use well-matched grafts, there is no benefit. I think the Scandinavian group showed this. I thought, however, that transfusion had a beneficial effect on the survival of poorly matched grafts.

DR. TAUBE: You are correct; the Scandinavian group found no beneficial effect of matching or pretransplant transfusion on graft or patient survival in cyclosporine-treated patients [95]. Also, DR matching was associated with a lower incidence of rejection [95]. I do not think that there was any evidence that transfusion improved graft survival in the poorly matched group.

DR. N. MALLICK (*Consultant Nephrologist, Royal Infirmary, Manchester, England*): To prevent subsequent sensitization, do you think we ought to forbid transplantation across certain HLA mis-matches?

DR. TAUBE: You raise an important point. I think we should avoid mis-matches with common antigens such as HLA-A2. If a patient undergoes unsuccessful transplantation with a graft mis-matched at HLA-A2, and subsequently generates A2 antibody, that patient will be able to receive only 50% of the donor kidneys, as approximately 50% of our donor population is A2+.

DR. M. BOULTON-JONES (*Consultant Nephrologist, Royal Infirmary, Glasgow, Scotland*): Does long-term immunosuppressive treatment without antibody removal alter the nature of the HLA antibodies? Does any evidence suggest that these antibodies are beneficial (that is, anti-idiotypic) rather than cytotoxic?

DR. TAUBE: I know of no data or evidence showing that immunosuppressive therapy alone reduces HLA antibody titers or induces the formation of anti-idiotypic antibodies.

DR. M. VENNING (*Lecturer in Medicine, University of Newcastle upon Tyne, Newcastle, England*): You suggest that we should only use rHuEPO and avoid transfusing anemic patients. Occasionally we have to transfuse our patients. Do you think we ought to use filters to remove leukocytes and platelets to prevent sensitization on these occasions?

DR. TAUBE: As I mentioned briefly, unfortunately removal of leukocytes from whole blood by filters, cotton wool, and red cell washing does not completely remove them and sensitization still results [68]. Red cells also express class-I MHC antigens [68]. In these situations, it might be worthwhile using filters to reduce sensitization, but I have no solid evidence to prove this.

DR. C. PUSEY (*Senior Lecturer in Medicine, Royal Post Graduate Medical School, Hammersmith Hospital*): You presented data showing that approximately one-half of your patients had a more rapid rebound in HLA antibody titer after antibody removal than the others. Is there any way of predicting which patients will have a less rapid rebound so that we can identify the patients who will require less treatment? Does this group have less rejection after transplantation?

DR. TAUBE: Patients with HLA antibody titers of 1:8 or less prior to plasma exchange or extracorporeal immunoadsorption are easy to treat, in that they require less plasma exchange or immunoadsorption and have a minimal antibody rebound. After transplantation there is no correlation between the degree of sensitization and the incidence or severity of rejection.

DR. PUSEY: Is immunoadsorption more effective than plasma exchange in the removal of HLA antibodies?

DR. TAUBE: There are no formal, controlled data showing that immunoadsorption is more effective than plasma exchange. We were unable to remove the HLA antibody from two of our very heavily sensitized patients by plasma exchange. We could, however, successfully remove their antibody later with immunoadsorption.

DR. J. S. CAMERON (*Professor of Nephrology, Guy's Hospital, London*): Have you tried using cyclosporine or other less toxic substances than cyclophosphamide to prevent antibody resynthesis, particularly 3 to 4 months post immunoadsorption? This might avoid the long-term toxicity of cyclophosphamide.

DR. TAUBE: That is a good suggestion. We have not tried switching to cyclosporine and steroids 3 to 4 months post immunoadsorption. Stopping the cyclophosphamide at that time usually results in antibody rebound even if the patient is neutropenic. We have tried using cyclosporine and steroids at the time of immunoadsorption to prevent antibody resynthesis, but we have had only limited success [91].

DR. M. JONES (*Lecturer, Department of Medicine, University of Aberdeen, Aberdeen, Scotland*): In transplantation with living related donors, azathioprine reduces the incidence of sensitization following donor-specific transfusion by approximately 50%. Also, some data suggest that cyclosporine reduces the incidence of sensitization by random third-party transfusion in cadaveric transplantation [96]. We also have preliminary, unpublished data suggesting that cyclosporine and third-party transfusion might stimulate the formation of anti-idiotypic antibodies. Do you think that we ought to give cyclosporine with blood transfusions to prevent sensitization?

DR. TAUBE: I agree that recipients of living related transplants who receive donor-specific transfusions should receive azathioprine to prevent the development of cytotoxic antibodies. I am aware of your group's work with third-party transfusions and cyclosporine. As far as I know, you have only treated small numbers of patients, several of whom have yet to receive transplants. My view is that if we persist in transfusing patients before transplantation, any relatively harmless maneuver that might reduce the incidence of sensitization is of value.

DR. A. WING (*Consultant Physician, St. Thomas' Hospital, London*): Forgive this question, but I would like to discuss the cost of HLA antibody removal by immunoadsorption. You mentioned that it was expensive. Do you have any details?

DR. TAUBE: A pair of immunoadsorption protein A columns currently costs approximately £3600 ($5735). The buffers, lines, and plasma filters cost another £1500 ($2390). The equivalent amount of plasma exchange costs slightly less. However, the same pair of protein A columns can be used for an individual patient for as long as one year. Therefore immunoadsorption will be cheaper than plasma exchange if multiple courses of treatment are needed.

DR. HARRINGTON: You mentioned that the scheme of HLA antibody removal and prevention of its resynthesis could be applied to approximately two-thirds of your highly sensitized patients. What about the other one-third?

DR. TAUBE: The group of patients you refer to presents a difficult problem because they have antibodies against multiple HLA antigens. Their high percentage reactivities are not caused by antibody directed against cross-reactive groups. Therefore, HLA antibody removal does not work in this group of patients. Occasionally, however, we can remove all the antibody directed against a particular antigen. We did this in one patient who had antibody against multiple HLA antigens, including A2 [21]. We were able to remove all his A2 antibody and prevent its resynthesis with cyclophosphamide and steroids. One year later, he successfully received an A2+ kidney, which is functioning well 18 months post transplant. This maneuver might be a good approach in patients with multiple HLA antibodies against multiple HLA antigens.

DR. J. DONOHOE (*Consultant in Nephrology, Beaumont Hospital, Dublin, Ireland*): Is living-related-donor transplantation a good option for highly sensitized patients?

DR. TAUBE: Yes, particularly if the donor and recipient are well matched. Unfortunately, such a scenario is uncommon.

DR. HARRINGTON: What are the results of other groups doing comparable work on HLA antibody removal?

DR. TAUBE: Several other groups have used plasma exchange to remove HLA antibody. In general they have done well [87, 88], particularly when the results are compared with those obtained by the organ matching programs [74, 75]. Immuno sorption is a relatively new technique, and as yet there are few data other than our own.

DR. R. GABRIEL (*Consultant Physician, St. Mary's Hospital, London*): You implied that transplantation is the only treatment of chronic renal failure. There must be situations in which chronic dialysis is the better choice.

DR. TAUBE: Transplantation provides the cheapest and most effective form of treatment for patients with end-stage renal failure. Certain patients—for example, diabetics with extensive vascular and ischemic heart disease—do badly after transplantation and probably are best left on dialysis. However, highly sensitized patients often are young and fit and are otherwise ideal candidates for transplantation. Because transplantation has become so successful, the real problem with it is the shortage of donor kidneys.

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References

1. KEOWN PA: The highly sensitized patient: Aetiology, impact and management. *Transplant Proc* 19:74–78, 1987
2. FUGGLE SV, ERRASTI P, DAAR AS, FABRE JW, TING A, MORRIS PJ: Localisation of MHC (HLA-ABC and DR) antigens in 46 kidneys. Differences in HLA-DR staining of tubules among kidneys. *Transplantation* 35:385–390, 1983
3. TERASAKI PI, MARCHIORO TL, STARZL TE: Sero-typing of human leukocyte antigens: preliminary trials on long-term kidney homograft survivors, in *Histocompatibility Testing*, edited by RUSSELL PS, WINN HJ, AMOS DB, National Academy of Sciences, Washington, DC, 1965, p 83
4. KISSMEYER-NIELSEN F, OLSEN F, PETERSON VP, FJELDBORG O: Hyperacute rejection of kidney allografts associated with pre-existing humoral antibodies against donor cells. *Lancet* 1:662–665, 1966
5. JOHNSON AH, ROSSEN RD, BUTLER WT: Detection of alloantibodies using a sensitive antiglobulin microtoxicity test: identification of low levels of preformed antibodies in accelerated allograft rejection. *Tissue Antigens* 2:215–219, 1972
6. FULLER TC, PHELAN D, GEBEL HM, RODEY GE: Antigenic specificity of antibody reactive in the antiglobulin-augmented lymphocytotoxicity test. *Transplantation* 34:25–29, 1982
7. GAROVOY MR, RHEINSCHMIDT MA, BIGOS M, PERKINS H, COLOMBE B, FEDUSKA N, SALVATIERRA O: Flow cytometry analysis: a high technology crossmatch technique facilitating transplantation. *Transplant Proc* 15:1939–1944, 1983
8. CHAPMAN JR, DEIERHOI MH, CARTER NP, TING A, MORRIS PJ: Analysis of flow cytometry and cytotoxicity crossmatches in renal transplantation. *Transplant Proc* 17:2480–2481, 1985
9. CHAPMAN JR, TAYLOR CJ, TING A, MORRIS PJ: Immunoglobulin class and specificity of antibodies causing positive T cell crossmatches. *Transplantation* 42:608–613, 1986
10. COOK DJ, TERASAKI PI, IWAKI G, ET AL: Donor factors that influence flow cytometry crossmatching. *Transplant Proc* 20:81–83, 1988
11. TALBOT D, GIVAN AL, SHENTON BK, PROUD G, TAYLOR RMR: The nature of donor-specific IgG isotypes identified by flow cytometry in the preoperative crossmatch. *Transplant Proc* 20:84–85, 1988
12. BRASILE L, CLARKE J, CERILI J: Frequency of DR antibody in patients awaiting a renal allograft. *Transplant Proc* 21:706–707, 1989
13. SVEJGAARD A, KISSMEYER-NIELSEN F: Cross-reactive human HL-A iso-antibodies. *Nature* (Lond) 219:868–869, 1968
14. MUELLER-ECKHARDT C, HEINRICH D, ROTHENBERG V: Studies on cross reactive HL-A antibodies by elution with platelets. *Tissue Antigens* 2:436–446, 1972
15. COLOMBANI J, COLOMBANI M, DAUSSET J: Cross reaction in the HL-A system with special reference to the Da6 cross reacting group. Description of Da22, 23, 24, HL-A antigens defined by platelet complement fixation, in *Histocompatibility Testing*, Copenhagen, Munksgaard, 1970, pp 72–92
16. SNELL GD, DAUSSET J, NATHENSON S: *Histocompability*. New York, Academic, 1976, p 196
17. OLDFATHER J, MORA A, PHELAN D, KEHL L, FULLER T, ANDERSON C, SICARO G, ETHEREDGE E, RODEY G: The occurrence of cross-reactive "public" antibodies in the sera of highly sensitized dialysis patients. *Transplant Proc* 15:1212–1214, 1983
18. OLDFATHER JW, ANDERSON CB, PHELAN DL, CROSS DE, LUGER AM, RODEY EG: Prediction of crossmatch outcome in highly sensitized dialysis patients based on the identification of serum HLA antibodies. *Transplantation* 42:267–270, 1986
19. SENSI A, BARICORDI OR, MELCHIORRI L, BALBONI A, PLETSCHETTE M, GRAPPA MT, MATTIUZ PL: A monoclonal antibody (GF 22.1) detecting different patterns of HLA class I cross-reactivities at different dilutions. *Tissue Antigens* 26:204–209, 1985
20. TAUBE D, WILLIAMS DG, CAMERON JS, SEWICK M, OGG CSA, RUDGE CJ, WELSH KE, KENNEDY LA, THICK MG: Renal transplantation after removal and prevention of resynthesis of HLA antibodies. *Lancet* 1:824–826, 1984
21. PALMER A, TAUBE D, WELSH K, BEWICK M, GJOSTRUP P, THICK M: Removal of anti-HLA antibodies by extracorporeal immunoadsorption to enable renal transplantation. *Lancet* 1:10–12, 1989
22. GAROVOY MR, CHO S, CARPENTER CB: Cross-reactive antibodies

and decreased renal graft survival. *Transplant Proc* 11:370–373, 1979
23. Cross DE, Greiner R, Whittier FC: Importance of the autocontrol crossmatch in human renal transplantation. *Transplantation* 21:307–311, 1976
24. Ting A, Morris PJ: Crossmatching in clinical transplantation, in *Progress in Transplantation* (vol 2), edited by Morris PJ, Tilney NL, Edinburgh, Churchill Livingstone, 1985, pp 44–68
25. Alarif L, Snyder T, Light JA: Transplantation of highly sensitized patients based on crossmatches using DTT-treated sera. *Transplant Proc* 21:742–744, 1989
26. Rudy T, Opelz G: Dithiothreitol treatment of crossmatch sera in highly immunized transplant recipients. *Transplant Proc* 19:800–802, 1987
27. Shroyer TW, Diethelm AG, Deierhoi MH, Barber WH, Barger BO: Lymphocytic IgM antibody in highly sensitized (>50% PRA) autologous negative renal allograft candidates. *Transplant Proc* 21:739–741, 1989
28. Terasaki PI, Mottironi VD, Barnett EV: Cytotoxins in disease: autocytotoxins in lupus. *N Engl J Med* 283:784, 1970
29. MacLeod AM, Kurtz J, Chapman JR, Ting A, Morris PJ: Autolymphocytotoxins and virus infection in renal transplantation. *Transplant Proc* 19:901, 1987
30. Paul LC, van Es LA, van Rood JJ, van Leeuwen A, de la Riviere GB, de Graeff J: Antibodies directed against antigens on the endothelium of peritubular capillaries in patients with rejecting renal allografts. *Transplantation* 27:175–179, 1979
31. Paul LC, Frans MD, Class HS, van Es LA, Kalff MW, de Graeff J: Accelerated rejection of a renal allograft associated with pretransplantation antibodies directed against donor antigens on endothelium and monocytes. *N Engl J Med* 300:1258–1260, 1979
32. Cerilli J, Brasile L, Galouzis T, Lempert N, Clarke J: The vascular endothelial cell antigen system. *Transplantation* 39:286–289, 1985
33. Brasile L, Clarke J, Cerilli J: VEC antigens are closely linked to the MHC. *Transplant Proc* 19:894–895, 1987
34. Kalil J, Guilherme L, Neumann J, Rosales C, Marin M, Saldanha L, Chocair PR, Ianhez LE, Sabbaga E: Humoral rejection in 2 HLA identical living related donor kidney transplants. *Transplant Proc* 21:711–713, 1989
35. Brasile J, Clarke J, Abrams A, Cerilli J: Risk factors in the development of antibodies to vascular endothelial cells. *Transplant Proc* 21:716–717, 1989
36. Cerilli J, Clarke J, Doolin T, Cerilli G, Brasile J: The significance of a donor specific vessel crossmatch in renal transplantation. *Transplantation* 46:358–361, 1988
37. Park MS, Oriol R, Nakata S, Terasaki PI, Ford R, Bernoco D: ABH and Lewis antigens on lymphocytes: screening of pregnant women's sera with the B-cell cytotoxicity test. *Transplant Proc* 11:1947–1949, 1979
38. Shumak KH, Rachewich RA, Crookston MC, Crookston JH: Antigens of the Ii system on lymphocytes. *Nature* 231:148–149, 1971
39. Opelz G, Graver B, Mickey MR, Terasaki PI: Lymphocytotoxic antibody responses to transfusions in potential kidney recipients. *Transplantation* 32:177–183, 1981
40. Terasaki PI, Michey MR, Yamazaki JN, Vredevoe D: Incidence of HLA antibodies and possible associations with congenital abnormalities. *Transplantation* 1:538–543, 1970
41. Sanfilippo F, Vaughn WK, Bollinger RR, Spees EK: Comparative effects of pregnancy, transfusion, and prior graft rejection on sensitization and renal transplant results. *Transplantation* 34: 360–366, 1982
42. Sanfilippo F, Goeken N, Niblack G, Scornik J, Vaughn WK: The effect of first cadaver renal transplant HLA-A, B match on sensitization levels and retransplant rates following graft failure. *Transplantation* 43:240–244, 1987
43. Gore SM, Bradley BA: *Renal Transplantation: Sense and Sensitization*. Amsterdam, Kluwer Academic Publishers, 1988, p 95
44. Curtoni ES, Scudeller G, Mattuiz PL, Savi M, Ceppellini R: Anti-HL-A antibody evolution in recipients of planned transfusions. *Tissue Antigens* 2:415–426, 1972
45. Salvatierra A, Melzer J, Potter D, Garovoy M, Vincenti F, Amend WJC, Husing R, Hopper S, Feduska NJ: A seven-year experience with donor-specific blood transfusions. *Transplantation* 40:654–659, 1985
46. Deierhoi MH, Shroyer TW, Hudson SL, Barger BO, Barber WH, Curtis JJ, Julian BA, Diethelm AG: Sustained high panel reactive antibody levels in highly sensitised patients: significance of continued transfusions. *Transplant Proc* 21:771–772, 1989
47. Tongio MM, Falkenrodt A, Mitsuishi Y, Urlacher A, Berger JP, North ML, Mayer S: Natural HLA antibodies. *Tissue Antigens* 26:271–285, 1985
48. Chardonnens X, Jeannet M: Immunobiology of pregnancy: Evidence for a fetal immune response against the mother. *Tissue Antigens* 15:401–406, 1980
49. Ting A, Morris PJ: Development of donor-specific B lymphocyte antibodies after renal transplantation. *Transplantation* 28:13–17, 1979
50. Nunez G, McPhaul JJ, Stastny P: Characterisation of B cell antibodies in kidney transplant recipients. *Transplantation* 33: 446–452, 1983
51. Lorden RE, Stelzer GT, Ferrone S: Early post transplant prognosis of acute renal allograft rejection as determined by detection of cytotoxic antibodies to lymphoid B cell lines. *Transplantation* 38:493–497, 1984
52. Martin S, Dyer PA, Mallick NP, Gokal R, Harris R, Johnson RWG: Posttransplant antidonor lymphocytotoxic antibody production in relation to graft outcome. *Transplantation* 44: 50–53, 1987
53. Palmer A, Bewick M, Kennedy L, Welsh K, Taube D: Anti-HLA antibodies following the transplantation of highly sensitized renal allograft recipients. *Transplant Proc* 21:766–767, 1989
54. Vogeler U, Doxiadis I, Bucholz B, Eigler FW, Lison AE, Niebel W, Zastrow F, Grosse-Wilde H: Cytotoxicity of antibodies eluted from rejected kidney allografts. *Transplant Proc* 17:2630–2634, 1985
55. Gore SM, Bradley BA: *Renal Transplantation: Sense and Sensitization*, Amsterdam, Kluwer Academic Publishers, 1988, p 168
56. Pfaff WW, Vaughn WK, Sanfilippo F: Effect of Class I HLA matching on graft survival in sensitised patients. *Transplant Proc* 19:716–719, 1987
57. Raffoux C, Busson M, Bouteiller AM, Hors J: Some contributions brought by the histocompatibility laboratories in favour of hyperimmunized patients waiting for a kidney transplant, in *Transplantation and Clinical Immunology* XVII, edited by Touraine JL et al, Amsterdam, Elsevier, 1985, pp 131–138
58. Sanfilippo F, Vaughn WK, Spees EK, Lucas BA: The detrimental effect of delayed graft function in cadaver donor renal transplantation. *Transplantation* 38:643–648, 1984
59. Halloran P, Aprile M, Farewell V: Factors influencing early renal function in cadaver kidney transplants. *Transplantation* 45: 122–127, 1988
60. Gilks WR, Bradley BA, Gore SM, Selwood NH: Immunogenetic and clinical factors affecting renal transplantation. *Transplantation* 42:39–45, 1986
61. Sanfilippo F, Vaughn KW, LeFor WM, Spees EK: Multivariate analysis of risk factors in cadaver donor kidney transplantation. *Transplantation* 42:28–34, 1986
62. Opelz G: Kidney transplantation in highly sensitized patients. *Transplant Proc* 19:3737–3741, 1987
63. Stratta RJ, Oh CS, Sollinger HW, Pirsch JD, Kalayoglu M, Belzer FO: Kidney retransplantation in the cyclosporin era. *Transplantation* 45:40–45, 1988
64. Matas AJ, Tellis VA, Quinn TA, Glicklich D, Soberman R, Veith FJ: Successful transplantation of highly sensitized patients without regard to HLA matching. *Transplantation* 45:338–342, 1988
65. Eschbach JW, Adamson JW: Recombinant human erythropoietin: implications for nephrology. *Am J Kidney Dis* 11:203–209, 1988
66. Winearls CG: Erythropoietin and the anaemia of renal failure, in *Transplantation Reviews* (vol 3), edited by Tilney N, Morris PJ, in press
67. Scornik JC, Ireland JE, Howard RJ, Fennell RS, Pfaff WW: Role of regular and leukocyte-free blood transfusions in the generation of broad sensitisation. *Transplantation* 38:594–598, 1984

68. EVERETT ET, KAO KJ, SCORNIK JC: Class I HLA molecules on human erythrocytes. *Transplantation* 44:123–129, 1987
69. OPELZ G, SENGER DPS, MICKEY MR, TERASAKI PI: Effect of blood transfusions on subsequent kidney transplants. *Transplant Proc* 5:253–259, 1973
70. OPELZ G: Improved kidney graft survival in nontransfused recipients. *Transplant Proc* 19:149–152, 1987
71. GROTH CG: There is no need to give blood transfusions as pretreatment for renal transplantation in the cyclosporin era. *Transplant Proc* 19:153–154, 1987
72. KERMAN RH, VAN BUREN CT, LEWIS RM, KAHAN BD: Successful transplantation of 100 untransfused cyclosporin-treated primary recipients of cadaveric renal allografts. *Transplantation* 45:37–40, 1988
73. GORE SM, BRADLEY BA: *Renal Transplantation: Sense and Sensitization*, Amsterdam, Kluwer Academic Publishers, 1988, pp 269–281
74. KLOUDA PT, RAY TC, GORE SM, BRADLEY BA: Organ sharing for highly sensitized patients. *Transplant Proc* 19:731–732, 1987
75. JACOBSEN SEH, FJELDBORG O, MADSEN M, LAMM LU: Kidney graft survival in sensitized cyclosporin A treated recipients in Scandia Transplant. *Transplant Proc* 20:365–366, 1988
76. BOLLINGER RR, VAUGHN W, SANFILIPPO F, LUCAS B, LEFOR W, PETERS T, BIAS W: Results of the SEOPF strategy for transplanting sensitized patients. *Transplant Proc* 19:1964–1966, 1987
77. CLAAS FHJ, GIJBELS Y, VEEN AV, DE WAAL LP, D'AMARO J, PERSIJN GG, VAN ROOD JJ: Selection of crossmatch negative HLA-A and/or B mismatched donors for highly sensitised patients. *Transplant Proc* 21:665–666, 1989
78. CLAAS FHJ, GIJBELS Y, VAN DER VELDER DE MUNCK J, VAN ROOD JJ: Induction of B cell unresponsiveness to noninherited maternal HLA antigens during fetal life. *Science* 241:1815–1817, 1988
79. CARDELLA CJ, FALK JA, NICHOLSON MJ, HARDING M, COOK GT: Successful transplantation in patients with T cell reactivity to donor. *Lancet* 2:1240–1243, 1982
80. SANFILIPPO F, VAUGHN WK, SPEES EK, BOLLINGER RR: Cadaver renal transplantation ignoring peak-reactive sera in patients with markedly decreasing pretransplant sensitization. *Transplantation* 38:119–124, 1984
81. NORMAN DJ, BARRY JM, WETZSTEON PJ: Successful kidney transplantation in patients highly sensitised by blood transfusions: unimportance of the most reactive serum in the pretransplant crossmatch. *Transplantation* 39:253–255, 1985
82. WELSH KI, VAN DAM M, BEWICK M, KOFFMAN GK, TAUBE DH, RAFTERY MJ: Successful transplantation of kidneys bearing previously mismatched HLA A and B locus antigens. *Transplant Int* 1:190–195, 1988
83. TAUBE DH, WELSH KI, KENNEDY LA, THICK MG, BEWICK M, CAMERON JS, OGG CS, RUDGE CJ, WILLIAMS DG: Successful removal and prevention of resynthesis of anti-HLA antibody. *Transplantation* 37:254–255, 1984
84. NILSSON IM, JONSSON S, SUNDQVIST SB, AHLBERG A, BERGENTZ SE: A procedure for removing high titre antibodies by extracorporeal protein-A-sepharose adsorption in haemophilia: substitution therapy and surgery in a patient with Haemophilia B and antibodies. *Blood* 58:34–44, 1981
85. PALMER A, TAUBE D, WELSH K, BRYNGER H, DELIN K, GJOSTRUP P, KONAR J, SODERSTROM T: Extracorporeal immunoadsorption of anti-HLA antibodies: preliminary clinical experience. *Transplant Proc* 19:3750–3751, 1987
86. TAUBE D, PALMER A, WELSH K, BEWICK M, SNOWDEN S, THICK M: Removal of anti-HLA antibodies prior to transplantation: an effective and successful strategy for highly sensitized renal allograft recipients. *Transplant Proc* 21:694–695, 1989
87. FAUCHALD P, LEIVESTAD T, BRATLIE A, ALBRECHTSEN D, TALSETH T, FLATMARK A: Plasma exchange and immunosuppressive therapy before renal transplantation in allosensitised patients. *Transplant Proc* 19:3748–3749, 1987
88. BACKMAN U, FELLSTROM B, FRODIN L, SJOBERG O, TUFVESON G, WIKSTROM B: Successful transplantation in highly sensitised patients. *Transplant Proc* 21:762–763, 1989
89. BROCARD JF, FARAHMAND H, FASSI S, PLAISANT B, FRIES E, CANTAROVICH M, BISMUTH A, LAMBERT T, HIESSE C, LANTZ O, FRIES D, CHARPENTIER B: Attempt at depletion of anti-HLA antibodies in sensitized patients awaiting transplantation using extracorporeal immunoadsorption, polyclonal IgG and immunosuppressive drugs. *Transplant Proc* 21:733–734, 1989
90. HILLEBRAND G, CASTRO LA, SAMTLEBEN W, ALBERT E, SCHOLZ S, ILLNER WD, LAND W, GURLAND HJ: Removal of preformed cytotoxic antibodies in highly sensitized patients using plasma exchange and immunosuppressive therapy, azathioprine, or cyclosporine prior to renal transplantation. *Transplant Proc* 17:2501–2504, 1985
91. TAUBE D, WELSH K, VAN DAM M, KENNEDY L, BRIGGS D, BIGNARDI L, THICK M: Cyclophosphamide, prednisolone and cyclosporin in the removal and prevention of the resynthesis of anti-HLA antibodies, in *Transplantation and Clinical Immunology* 17:179–185, 1985
92. PALMER A, BEWICK M, WELSH K, SNOWDEN S, PARSONS V, TAUBE D: Low-dose cyclosporin and antithymocyte globulin improve renal allograft survival in highly sensitised patients. *Transplant Proc* 20:198–200, 1988
93. DEMASI R, THOMAS F, CUNNINGHAM P, SASH C, CLARK G, THOMAS J: Improved immunosuppression for secondary renal transplants in high risk patients using quadruple immunosuppression with cyclosporin and rabbit anti-thymocyte globulin. *Transplant Proc* 21:1729–1731, 1989
94. SOMMER BG, HENRY M, FERGUSON RM: Sequential antilymphoblast globulin and cyclosporin for renal transplantation. *Transplantation* 43:85–90, 1987
95. LUNDGREN G, GROTH CG, ALBRECTSEN D, BRYNGER H, FLATMARK A, FRODIN L, GABEL H, HUSBERG B, KLINTMALM G, MAURER W, PERSSON H, THORSBY E: HLA matching and pretransplant blood transfusions in cadaveric renal transplantation—a changing picture with cyclosporin. *Lancet* 2:66–69, 1986
96. RAFTERY MJ, LANG CJ, SHWARZ A, O'SHEA JM, VARGHESE Z, SWENY P, FERNANDO ON, MOORHEAD JF: Prevention of sensitization resulting from third-party transfusion. *Transplant Proc* 17: 2499–2500, 1985