Transplant International ISSN 0934-0874

REVIEW

Transplantation across previously incompatible immunological barriers

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Keywords
crossmatch, desensitization, incompatibility, rejection, transplantation.

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Received: 17 September 1995
Revision requested: 15 October 2005
Accepted: 15 November 2005

doi:10.1111/j.1432-2277.2005.00257.x

Summary

This article reviews recent advances, which allow the transplantation across or around previously incompatible immunological barriers such as a positive crossmatch or ABO blood group incompatibility.

Introduction

Transplantation across previously incompatible immunological barriers is – by definition – higher risk and not traditional standard-of-care. Nevertheless, the last 10 years have seen an explosion of interest in this area. Reasons for this increased interest are shown in Table 1. This article will concentrate on transplantation across a positive T-cell crossmatch and transplantation across or around ABO blood group incompatibilities. The focus will be on *renal* transplantation wherein most of the recent advances have occurred.

Transplantation across a positive T-cell crossmatch

In general, renal transplantation is contraindicated in the presence of a *current* positive T-cell complement-dependent cytotoxicity (CDC) crossmatch. This is because of the very high risk of hyperacute rejection, which results in irreversible destruction of the allograft. It should be noted that the type of crossmatch test and the criteria for defining a positive result vary between centers. Causes of a false-positive crossmatch such as autoantibodies must also be excluded. Therefore, close collaboration with tissue typing experts is essential in every transplant program. In most cases, a true positive T-cell crossmatch indicates the presence of noxious IgG antibodies in the serum against class I human leukocyte antigens (HLA) of the potential donor. Sensitization to HLA antigens can occur by three mechanisms: pregnancy, transfusion of blood products (usually red cells) containing fragments of leukocytes and previous transplantation. Erythropoietin has reduced the need for blood transfusion in end-stage renal disease (ESRD) patients, but as the majority of transplants are not zero-mismatched for HLA antigens, previous transplantation remains a very important cause of sensitization.

High sensitization to HLA in practice means having positive T-cell crossmatches against multiple potential donors. Traditionally, degree of sensitization was quantified as the percent of the donor pool against which the patient's serum had positive T-cell crossmatches – the panel reactive antibody (PRA) status. Definitions of 'highly sensitized' typically are a PRA persistently >50% or >80%. Advances in tissue typing technology now allow more precise characterization of the HLA against which a given patient is sensitized [1]. By knowing the gene frequencies for the relevant alleles in the donor pool, one can then estimate the percent of crossmatches that will be

Table 1. Presumed reasons why transplantation across previously incompatible barriers is becoming relatively popular.

Organ donor shortage – prolonged waiting times
General increase in, and acceptance of, living kidney donation
Powerful but relatively safe immunosuppressive regimens
Better methods of detecting and characterizing anti-HLA antibodies
Easier diagnosis and better understanding of acute antibody-mediated rejection (AMR)
Effective regimens for reversing acute AMR

Table 2. General approach to desensitization.

Remove or neutralize anti-HLA IgG
Prevent formation of new anti-HLA IgG before transplant
Transplant when crossmatch is negative
Prevent formation of new anti-HLA IgG after transplant
Rapidly diagnose and reverse acute AMR if it occurs

positive for that potential recipient. Thus, high sensitization can now be defined by methods other than PRA. These newer methods also provide useful information about which donors might be appropriate for a given sensitized patient (see section on acceptable mismatch programs below).

Until recently, patients who were highly sensitized and wanted a transplant had no option except to remain waiting for many years on the deceased donor list – some never received a transplant. Even if a living donor were available, a positive T-cell crossmatch was very common and precluded transplantation. Furthermore, attempts at overcoming the positive crossmatch (involving therapies such as cyclophosphamide, plasmapheresis, or immunoadsorption) were associated with high rates of severe rejection and infection.

Recently, two *desensitization* therapies are showing great promise in attenuating humoral alloimmune responses, overcoming positive T-cell CDC crossmatches and allowing safe transplantation: high-dose IgG and plasmapheresis – both in association with the use of the newer immunosuppressive drugs. A general approach to desensitization is shown in Table 2. Although much of the interest has focused on the high-dose IgG and plasmapheresis therapies themselves, it should be noted that these are not new therapies. Many factors are likely contributing to the recent successes in this area: the use of mycophenolate mofetil (MMF) and tacrolimus, better understanding and diagnosis of acute antibody-mediated rejection (AMR) and improved ability to reverse acute AMR. For the purposes of this article, desensitization is defined as follows:

1 In the setting of living donor transplantation: attenuating the humoral alloimmune response such that the patient becomes crossmatch negative against a specific donor.

2 In the setting of deceased donor transplantation (i.e. waiting on the list): attenuating the humoral alloimmune response (usually assessed by changes in PRA), making it *more likely* the patient will receive a deceased donor transplant.

The clinical impact of transplanting across an isolated positive B-cell CDC crossmatch is less well defined. In most cases, a true positive B-cell crossmatch indicates the presence of noxious IgG antibodies against class II HLA of the donor (false positives are relatively common and must be excluded). Registry data suggest that a positive B-cell crossmatch at the time of transplant is associated with inferior outcomes, including a much higher risk of early graft loss [2]. Based on the results from our center, we consider previously transplanted patients with a true positive B-cell CDC crossmatch as being high risk for early graft loss and we desensitize them against potential living donors in a manner analogous to that used for patients sensitized to class I HLA.

High-dose IgG protocol

This involves intravenous (i.v.) administration of high-dose IgG. A variety of IgG preparations are available; all are pooled from multiple donors. The mechanisms of action of IgG in this setting are not fully understood. Proposed mechanisms include: blockade of Fc receptors on mononuclear phagocytes, direct neutralization of alloantibodies (anti-idiotypic effects), inhibition of expression of CD19 on activated B-cells, inhibition of complement and inhibition of alloreactive T cells [3].

An important question is to what extent desensitization efficacy differs – if at all – between the various commercially available IgG preparations. There are certainly major differences between the preparations with regard to the blood donors used, the methods of purification and sterilization, the osmolality of the final preparation and the stabilizers used. There are no published head-to-head studies comparing the *in vivo* effects of different IgG preparations on human alloimmune responses. However, at least one group has shown significant differences between IgG preparations in their ability to reduce PRA (when added *in vitro*) [4]. Interestingly, differences were also found between different batches of the same commercial product.

Glotz *et al.* [5] reported on 15 patients who either had a PRA ≥ 50% (against class I HLA) or a positive T-cell crossmatch against a potential living donor. These patients received 2 g/kg of IgG every 4 weeks for a total of three doses. PRA and crossmatch studies were repeated 3 weeks after every dose. A negative T-cell crossmatch

against a deceased or living donor allowed transplantation to proceed. More IgG was administered post-transplant. Thirteen of the 15 patients had clinically significant falls in PRA and received a transplant (two of the 13 were from living donors). One allograft was lost from thrombosis on the first post-transplant day (note that IgG has prothrombotic effects), one was lost from severe rejection, one from polyoma virus nephropathy and two from patient death. The causes of death were post-transplant lymphoproliferative disorder (in a patient with prior leukemia) and stroke. Other allografts reportedly had good function. Jordan *et al.* [6] reported on 42 highly sensitized patients who were treated with 1–4 doses of IgG. Interestingly, they had used an *in vitro* test to select patients whom they thought would benefit most from IgG. If addition of IgG to the CDC crossmatch converted it from positive to negative, this patient was deemed a likely IgG responder and was then treated with i.v. IgG. If IgG did not reverse the positive crossmatch, i.v. IgG was not given. The 'strength' of the untreated crossmatch was not reported. Forty-two of 45 patients were *in vitro* responders and hence treated. The majority ultimately did receive living or deceased donor kidney transplants, but there were several nonkidney transplants also. The 2-year allograft survival rate was 89%. Acute rejection occurred in 31% of cases and caused three graft losses.

Jordan *et al.* [7] also reported a randomized, double-blinded controlled trial of IgG versus placebo in dialysis patients who were highly sensitized to HLA (PRA >50%). IgG was administered monthly for 4 months at 2 g/kg per dose. Follow-up was for a median 24 months. IgG therapy induced a very modest but statistically significant reduction in PRA compared with placebo and was well tolerated. It should be noted that PRA is no longer considered by some to be an ideal marker of the degree of sensitization [1] – possibly the beneficial immunomodulatory effects exceeded that measured by changes in PRA. When analyzed by adhered treatment (not by intent-to-treat), 35% of the IgG group as opposed to 17% of the placebo group underwent transplantation – mostly with deceased donor kidneys. This gives a number needed to treat (NNT) – to allow one extra transplant – of almost six. Note that the NNT will vary according to the actual availability of transplant organs in a given transplant region. This is an important number as IgG therapy is expensive. Two-year graft survival was 80% in the IgG group and 75% in the placebo group. Why the reduction in PRA was less dramatic than reported by Glotz *et al.* was unclear. Possibly, this reflected differences in patient characteristics or in the IgG preparations used.

Putting the above studies together, high-dose IgG therapy appears to be reasonably efficacious in highly sensitized patients. It should be emphasized that long-term outcomes are not yet available. The study of Glotz *et al.* does raise concerns about serious adverse effects associated with high-dose IgG protocols – note that in that study, patients also received thymoglobulin, MMF, tacrolimus and steroids. Adverse effects of high-dose IgG are summarized in Table 3. The advantages and disadvantages of high-dose IgG are summarized in Table 4.

Table 3. Adverse effects of high-dose IgG.

Adverse effect	Comment
Fever, chills	Prevent/treat by slowing infusion rate and giving acetaminophen and antihistamines
Severe headache	Prevent/treat by slowing infusion rate and giving analgesics
Anaphylaxis	Rare, can occur in those with IgA deficiency
Severe thrombosis (deep venous thrombosis, central retinal vein occlusion, transplant renal vessel thrombosis)	Reduce risk by: slow administration, using isotonic products only, ensuring patient is volume expanded, prophylactic anticoagulation (in some cases)
Nephrotoxicity	Important post-transplant; reduce risk by: slow administration, using isotonic products only, ensuring patient is volume expanded

Table 4. Advantages and disadvantages of IgG desensitization.

Advantages
Nonimmunosuppressive
Relatively nontoxic
Easy to administer
Disadvantages
Expensive
Efficacy unpredictable
Adverse effects – see Table 3

Plasmapheresis-based protocols

Several groups have reported impressive results with plasmapheresis-based protocols [8–10]. The protocols described (and the one used in our center) are all quite similar; a general version is shown in Table 5. Briefly, plasmapheresis is used to remove anti-HLA antibodies and is immediately followed by infusion of low doses of IgG during hemodialysis. The (unproven) assumption is that even the low-dose IgG will have some beneficial immunomodulating effects. At the same time that

Table 5. Typical plasmapheresis protocol used for desensitization.

Therapy	Timing
Plasmapheresis and replacement of plasma with 5% albumin solution + isotonic saline or fresh-frozen plasma (FFP)	Three times per week
Low-dose IgG immediately after plasmapheresis	Three times per week
Tacrolimus, mycophenolate mofetil (MMF)	Twice daily
SMX-TMP, antiviral prophylaxis	Alternate days
Induction antibody	At time of transplant and several days thereafter
Rituximab (in some patients)	Day before transplant

SMX-TMP, sulfamethoxazole-trimethoprim.

plasmapheresis is started, patients begin treatment with tacrolimus, MMF ± steroids (and antimicrobial prophylaxis). Plasmapheresis is continued thrice weekly until the T-cell CDC crossmatch is negative; transplant then takes place within 24 h. Plasmapheresis and low-dose IgG are usually repeated several times during the first two post-transplant weeks to remove any rebounding antibody.

In Schweitzer's series, 11 of 15 patients were rendered crossmatch negative; of note all 11 had low pretreatment crossmatch titers. Three had rejection, but no renal allografts were lost from rejection. Gloor *et al.* [8] reported a somewhat more aggressive protocol wherein patients also underwent splenectomy and received rituximab. Pretreatment crossmatch titers varied from 1/2 to 1/16. All 14 patients treated became crossmatch negative against their living donor and received a transplant. Eleven of 14 had functioning grafts at a mean of 448 days after transplant; one graft was lost from acute AMR and one from chronic allograft nephropathy. Interestingly, acute AMR occurred mainly in those who had baseline titers ≥1/8.

Montgomery *et al.* [9] initially reported successful desensitization and transplantation of four patients. Their center has now successfully transplanted >80 sensitized patients, including some with very highly positive baseline crossmatches [11]. The Hopkins protocol is extensively discussed in Ref. [11]. It specifies the use of cytomegalovirus (CMV) hyperimmune IgG (although there are no randomized studies showing this is superior to standard IgG). Rituximab is reserved for those at highest risk of severe AMR. Risk factors for severe AMR after desensitization include previous transplants, previous early graft losses and multiple anti-HLA antibodies [11].

The plasmapheresis protocols have generally been well tolerated; adverse effects are summarized in Table 6. The advantages and disadvantages of the plasmapheresis approach are summarized in Table 7.

Table 6. Adverse effects of plasmapheresis-based protocols.

Adverse effect	Comment
Depletion of clotting factors	Follow PT and APTT closely, especially in patients getting multiple treatments and immediately before or after surgery or kidney biopsy; replace with FFP as needed
Hypocalcemia	More common if also receiving FFP; replete with PO and IV calcium
Fevers, chills	Usually these are reactions to FFP; slow infusion rate and prevent/treat with acetaminophen and antihistamines
Adverse effects of IgG	Rare as dose is small
Adverse effects of tacrolimus, MMF	Adjust doses as needed

APTT, activated partial thromboplastin time; FFP, fresh-frozen plasma; PT, prothrombin time.

Table 7. Advantages and disadvantages of plasmapheresis-based protocols.

Advantages
High efficacy
To some extent, can predict time to negative crossmatch and transplant
Also removes anti-ABO-A or anti-ABO-B antibodies, potentially allowing transplantation across two incompatible barriers
Disadvantages
Expensive
Labor intensive
Not useful if no living donor
Adverse effects – see Table 6

Other desensitization protocols

Immunoadsorption has the advantage over plasmapheresis in that it only removes IgG, not other components of the plasma such as clotting factors. Lorenz *et al.* [12] recently reported an interesting strategy in which immunoadsorption was used in highly sensitized patients with a positive CDC crossmatch against a deceased donor. If one session of immunoadsorption converted the crossmatch from positive to negative, then the transplant proceeded (one session was the limit because of the need to minimize cold ischemia time). Immunoadsorption was continued during the first post-transplant month. Nine of 14 treated patients were rendered crossmatch negative and were transplanted. Graft survival at 3 years was 78%. One disadvantage of this approach is that if the crossmatch remains positive, the subsequent recipient receives an allograft with longer cold ischemia time.

It is well known that AMR is less common in liver transplantation, even in the presence of a positive

pretransplant crossmatch. This may reflect absorption of antidonor antibodies by the liver. At least one group has reported a small series on the use of auxiliary liver transplantation at the time of kidney transplantation in highly sensitized dialysis patients – the purpose of the segmental liver graft being only to prevent AMR of the renal allograft [13]. This is an interesting strategy, but it obviously raises a number of surgical, medical and ethical concerns.

Post-transplant immunosuppression in desensitized patients

Post-transplant immunosuppression in most series has consisted of antilymphocyte antibody induction therapy, MMF, tacrolimus and steroids. Rituximab has also been used – see below. Both thymoglobulin and IL-2 receptor blockers have been used as induction therapy. Thymoglobulin might seem preferable to IL-2 receptor blockers in that it is a more powerful immunosuppressant and is more likely to prevent acute cellular rejection. However, our center and others have found – in retrospective studies – that thymoglobulin is associated with an increase in anti-HLA antibodies after renal transplantation [14]. We therefore now use IL-2 receptor blockers in all highly sensitized patients. A prospective trial in this area would be useful.

Rituximab

Rituximab is a genetically engineered chimeric murine/human monoclonal antibody directed against the CD20 antigen on the surface of normal and malignant B cells. It is licensed for the treatment of non-Hodgkin's lymphoma but is now being tested in autoimmune diseases such as rheumatoid arthritis. An intriguing question is whether or not rituximab is a useful adjunct to the therapies described above. Note that the CD20 antigen is not expressed on plasma cells, the ultimate source of anti-HLA IgG. Nevertheless, there are at least two mechanisms by which rituximab could attenuate alloimmune responses: firstly by preventing formation of new alloantibody-producing plasma cells (by eliminating precursor B cells) and secondly by inhibiting B-cell-driven antigen presentation and costimulation of T cells.

A phase I trial of rituximab in nine highly sensitized patients on dialysis showed prolonged depletion of B cells [15]. There were modest reductions in PRA in some patients. Of note, one patient developed histoplasmosis. Currently, rituximab is being used in transplant patients – off label – in at least two situations: firstly, as adjunctive therapy to the pretransplant plasmapheresis protocols and secondly as adjunctive therapy for refractory acute AMR post-transplant [11]. The drug is expensive. A randomized-controlled trial is needed to establish its efficacy in these areas.

General comments on desensitization

The protocols described above have several limitations. Firstly, with the exception of Ref. [7] above, they have not been evaluated in randomized-controlled trials. In their defense, results are superior to historical 'controls', albeit from an era without MMF and tacrolimus. Secondly, the protocols are expensive. However, expense must be compared with that involved in remaining on dialysis. Thirdly, they often involve intense immunosuppression with the potential for high rates of polyoma virus nephropathy, systemic infections and neoplasia. Lastly, long-term outcomes are not yet available. It is conceivable that many desensitized patients will, over the long term, develop low-grade antibody-mediated rejection with deleterious effects on graft survival. On the other hand, even if graft survival is somewhat inferior to nonsensitized controls, it seems likely that quality of life and survival will still be better than remaining on dialysis.

Plasmapheresis-based protocols are usually not suitable for highly sensitized patients awaiting deceased donor transplantation because the availability of suitable organs is unpredictable and plasmapheresis is both difficult and very expensive to continue indefinitely. As soon as it is stopped, anti-HLA antibody titers rebound. A role for immunoadsorption in this setting has been proposed [12]. What about administering high-dose IgG to highly sensitized patients at the top of the waiting list? Again, there is the unpredictable availability of organs although the beneficial immunomodulatory effects probably persist longer with IgG than with plasmapheresis. This might make high-dose IgG more attractive in this setting. However, not all patients will respond to IgG and even if they do, not all will be offered a suitable organ. Therefore, the NNT will be >1 and this will have a major impact on costs.

An interesting question is to what extent alloantibody against donor or nondonor HLA antigens persists after desensitization and transplantation. Among all transplanted patients, the presence of alloantibody is associated with poorer outcomes [16]. Zachary *et al.* [17] reported elimination of antibodies against donor HLA antigens (as measured by ELISA) in patients followed for at least 2 months. Gloor *et al.* [18], however, found persistent low levels of antibody post-transplant, when measured by flow cytometry or single-antigen flow beads. These different results probably reflect differences in the sensitivity of the assays used. In fact, recent data from the Hopkins group is showing more detectable alloantibody when

highly sensitive assays are used [19]. Longer-term data are awaited with interest.

From a cost-effectiveness perspective, it seems reasonable to firstly maximize the chances for highly sensitized patients to obtain a transplant via special pathways such as acceptable mismatched programs – see below. Where such pathways prove unsuccessful or are unlikely to be successful (based on the anti-HLA antibodies of the patient), the protocols described above should be considered. These protocols should only be undertaken in programs experienced in the management of acute AMR and which have rapid access to C4d staining and sophisticated crossmatch testing. Post-transplant crossmatching can become very complicated in the presence of rituximab, thymoglobulin, or IgG.

Other strategies for highly sensitized patients

Acceptable mismatch programs

Organ allocation strategies have been developed to try and safely direct more organs to highly sensitized patients. A good example of this is the Eurotransplant Acceptable Mismatch Program [20]. The goal of this program is to find acceptable HLA mismatches for a given patient – so that the patient can be offered more grafts in addition to zero-mismatched grafts. To be enrolled, patients must have a PRA >85%. On enrollment, extensive studies are performed to define those HLA against which the patient has never formed antibodies. These HLA are then considered acceptable to be offered to that recipient. Obviously, the bigger the donor pool available, the better the chances of finding compatible organs. If a presumed suitable donor organ is identified, it is mandatory to ship this to the recipient's center for final crossmatching and transplantation (if the final crossmatch is negative). The results reported by Eurotransplant have been quite impressive [20]. Fifty-seven of approximately 129 listed patients received transplants through this program over an 18-month period. Approximately, 40% of those listed have not been transplanted – such patients often have rare HLA phenotypes and react against most frequently occurring HLA. The authors have suggested reserving desensitizing therapies for such patients. Two-year allograft survival of patients transplanted through the Eurotransplant Acceptable Mismatch Program was similar to that of nonsensitized controls [20].

Living donor kidney exchange

This is also discussed below. Sensitized patients and their positive crossmatch intended donors are entered into a program with other incompatible (by crossmatch or ABO criteria) donor – recipient pairs. Computerized algorithms are then used to match compatible donors and recipients [21,22]. These algorithms can be designed to maximize the number of matches within the pool at a given time, while also minimizing the degree of HLA mismatching [23]. Donation and transplantation of two matched pairs occurs simultaneously. The Dutch program has already matched 17 pairs who had had positive crossmatches with their original intended donors [21].

The advantages of paired kidney exchanges over acceptable mismatch programs include the following: the highly sensitized patient receives a living as opposed to deceased donor transplant and two extra (living) donors are added to the total donor pool. In certain cases, however, a compatible donor cannot be found. Options then include waiting for more pairs to enter the program, desensitization against the original intended donor or pairing with a 'low-positive' crossmatch donor [24]. The more pairs enlisted in the program, the greater the chances of successful pairings. Thus, a national program has been proposed for the USA [24]. Computer simulations suggest that a national program, particularly if it used an algorithm to optimize HLA matching, could significantly increase the number of paired kidney donations/transplants; sensitized patients would benefit greatly [23]. Perceived barriers to establishing a national program include possible patient reluctance to travel long distances and the major logistical, organizational and financial burdens of administering the program.

ABO blood group incompatibility

This is a frequently encountered problem in living donor kidney transplantation. In the absence of some form of conditioning of the recipient, transplantation of an ABO-incompatible kidney results in hyperacute rejection. Several strategies are evolving to overcome or circumvent this.

Option 1: living donor kidney exchange

This is potentially an excellent solution to the problem of ABO-A and ABO-B incompatibility. Another couple with *reversed* ABO-A and ABO-B incompatibility is identified. Simultaneous donation and transplantation occur as depicted in Fig. 1. This mechanism is potentially very rewarding as both recipients receive living donor kidney transplants. It requires co-operation and trust between the transplant centers, and between the potential donors and recipients. Again, the larger the database available to search for a paired exchange, the higher the chances of finding a suitable swap. The prevalence of ABO-A or ABO-B in a given population will also affect how commonly such exchanges can occur. Successful matching

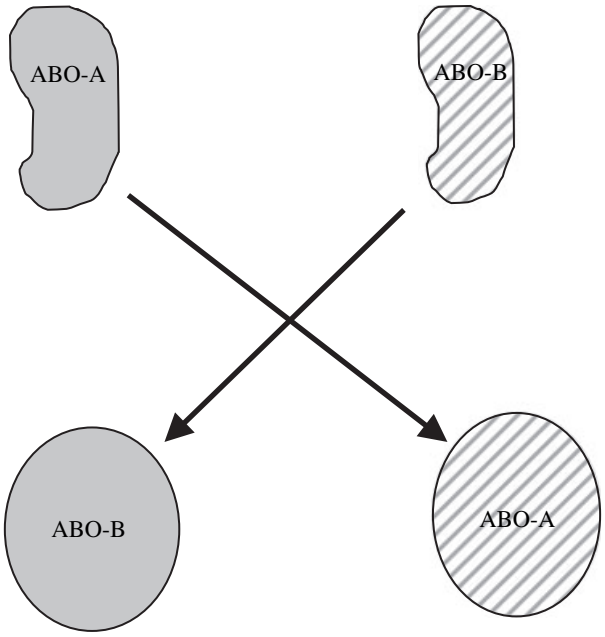

Figure 1 Living donor kidney exchange between pair 1 (gray) and pair 2 (stripes) circumvents ABO-A and ABO-B incompatibility.

and transplantation have been reported by the Dutch program and others [21,22].

What about the situation where the intended recipient is ABO-O and the potential donor is ABO-A or ABO-B? Doing a paired living donor exchange is more difficult than the scenario described in the above paragraph, as usually an ABO-O potential donor will be able to donate to their non-ABO-O partner (ABO-O being the universal blood donor) and therefore would be unlikely to enter the exchange program. One situation where direct donation might be contraindicated is a positive T-cell crossmatch – see Fig. 2. However, for this swap to proceed, there would need to be a negative (or much weaker) crossmatch with the ABO-O recipient – perhaps unlikely if the ABO-O patient is highly sensitized. Nevertheless, some ABO-O patients have been transplanted through these programs [21].

Option 2: list donor kidney exchange

This strategy is more controversial. It is currently being used by Region 1 of UNOS in the USA *if a living donor kidney exchange for a given pair is not achievable in the region*. It involves the ABO-incompatible or positive crossmatch living donor donating a kidney onto the deceased donor list. This kidney is allocated according to usual criteria. This donation then allows the partner of the donor to ascend to the top of the list and obtain a deceased donor ABO-compatible, crossmatch negative kidney relatively quickly. In practice, this usually involves an ABO-A or ABO-B-living donor kidney being donated to the list and then an ABO-O patient going to the top of the ABO-O list and receiving a deceased donor kidney. The potential advantages

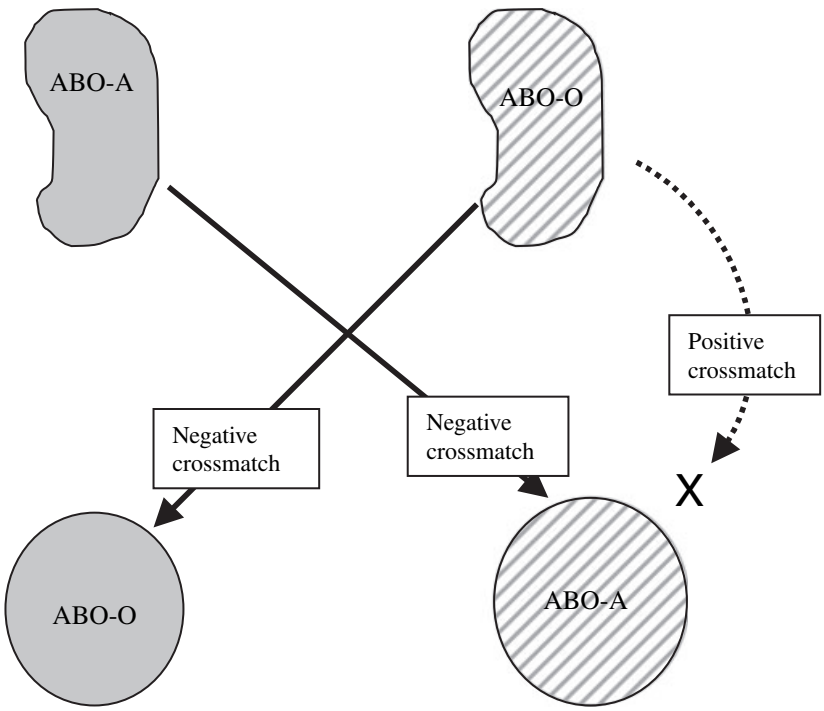

Figure 2 Living donor kidney exchange between pair 1 (gray) and pair 2 (stripes) circumvents problem of positive crossmatch that exists within pair 2.

Table 8. Advantages and disadvantages of donor exchange with the list.

Advantages	Comment
Allows the ABO-incompatible donor the opportunity to help their partner	Small paired living donor exchange programs may not be able to achieve this result – especially for ABO-O patients
An extra (living donor) kidney transplant is created	Opponents would argue that living donation might still occur if there was a very large living donor exchange program
Disadvantages	
Loved one receives a deceased donor rather than living donor transplant	Informed consent is essential
Timing of the deceased donor transplant unpredictable	In practice, usually happens within a few weeks
Other ABO-O waitlisted patients wait longer	This is the most controversial issue
Not suitable for an ABO-O patient highly sensitized to HLA antigens	Sensitized patients might never receive a transplant from the list

and disadvantages of this approach are summarized in Table 8.

The major controversy surrounds the impact on already-waitlisted ABO-O patients [25]. These patients already wait the longest of any ABO group and must wait longer every time an ABO-O list donor exchange is performed. The counter-argument is that waiting time is only extended a few weeks to months and that *extra* ABO-O patients are not actually being added to the list; the early transplant of the donor's partner means that they leave the list and several years later someone else receives a transplant more quickly [26].

Option 3: ABO-incompatible living donor kidney transplantation

This has become relatively common in Japan over the last 20 years because of the very limited number of deceased donors there. Reported outcomes from Japan have actually been almost as good as in ABO-compatible controls [27]. These reports and the factors listed in Table 1 have stimulated interest in Europe and the USA in transplanting across the ABO barrier.

Current protocols to allow this typically involve the following [28–30]:

1 Pretransplant plasmapheresis or immunoadsorption to remove IgG and IgM antibodies against the ABO group of the potential recipient – such that the serum concentration falls below a clinically significant titer (typically an IgG titer ≤8). Immunoadsorption with special columns has the advantage over plasmapheresis of depleting only anti-A or anti-B antibodies [31,32].

2 Splenectomy shortly before or at the time of the transplant.

3 Tacrolimus, MMF and steroids, sometimes started at the time of the first plasmapheresis session.

4 Induction antibody therapy with thymoglobulin or IL-2 receptor blockers.

5 Very close monitoring of antibody titers in the first 2–4 weeks with plasmapheresis if titers are ≥16 [30].

Splenectomy has traditionally been a component of these protocols, based on the idea that it contributed to elimination of cells involved in the production of anti-A and -B antibodies; furthermore, early published work suggested that those who were not splenectomized had a much higher risk of severe AMR and graft loss. The need for splenectomy – with its associated risk of bacterial infections – has recently been called into question as short-term results suggest substitution of rituximab for splenectomy may be sufficient [29,31]. Rituximab has been discussed above. Acute AMR after ABO-incompatible transplantation is diagnosed on the basis of allograft dysfunction, neutrophil capillaritis, positive C4d staining and usually rising anti-A/B titers. In the setting of ABO-incompatible transplantation, it appears that the presence of C4d alone does not imply rejection [29]. Treatment of acute AMR includes pulse steroids and plasmapheresis.

Interestingly, about 20% of Caucasians with ABO-A have the ABO-A2 subtype. Donor kidneys with this subtype (i.e. ABO-A2 or ABO-A2B) appear less likely to elicit severe AMR in their ABO-incompatible recipient; less intensive protocols have been used with success in recipients with low baseline anti-A titers [33]. Nevertheless, acute AMR can still occur [28].

Short-term results from Europe and the USA with protocols as described above have been impressive [28,29,31]. For example, in the series of Tyden *et al.* (using an anti-A or -B adsorption column and rituximab but no splenectomy), 11 patients with baseline anti-A or -B IgG titers of two to 128 were successfully transplanted. There were no episodes of acute rejection and plasma creatinine at follow-up was 22–168 μmol/l.

An extraordinary feature of ABO-incompatible renal transplantation is that over the long term, 'toxic' levels of anti-A/B antibody are still detectable in the recipient's serum (albeit at lower concentrations than pretransplant) – yet acute AMR does not occur [34]. This peaceful co-existence of antigen and antibody has been termed accommodation. A better understanding of the

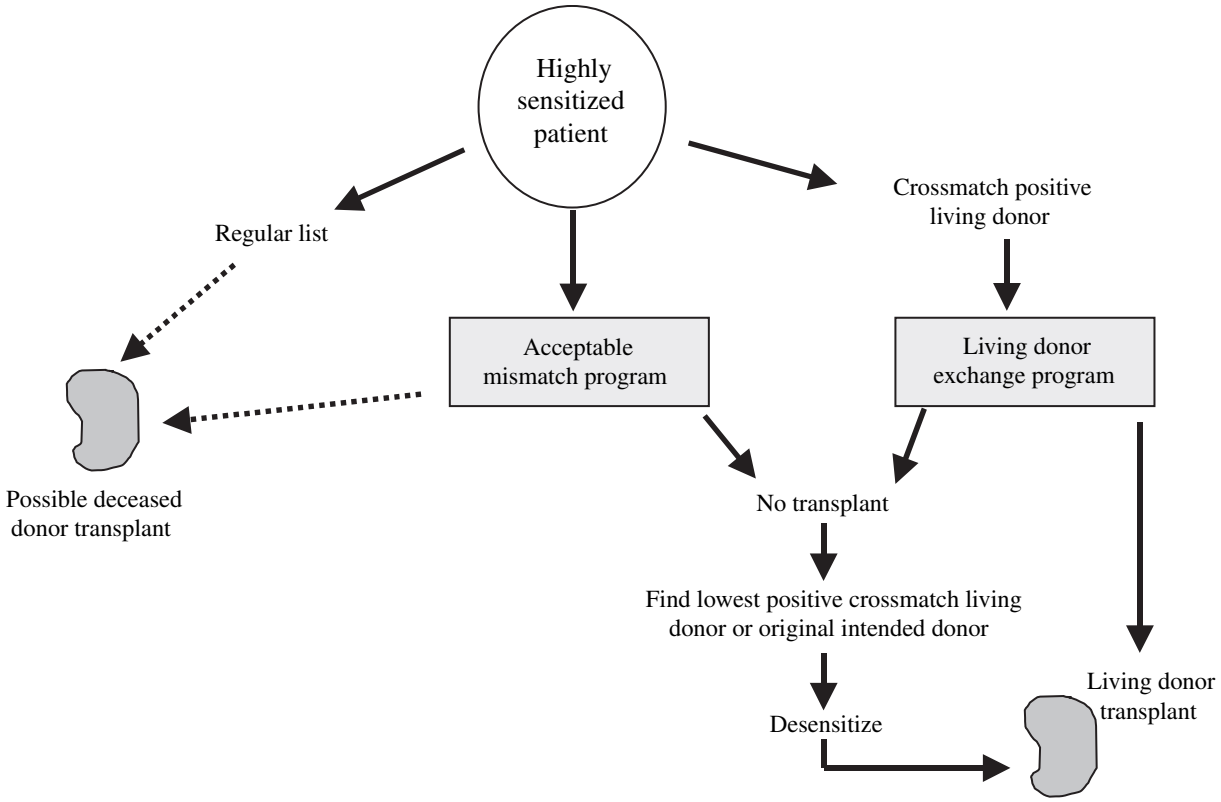

Figure 3 Proposed approach for optimizing transplantation of a highly sensitized patient with a crossmatch-positive living donor.

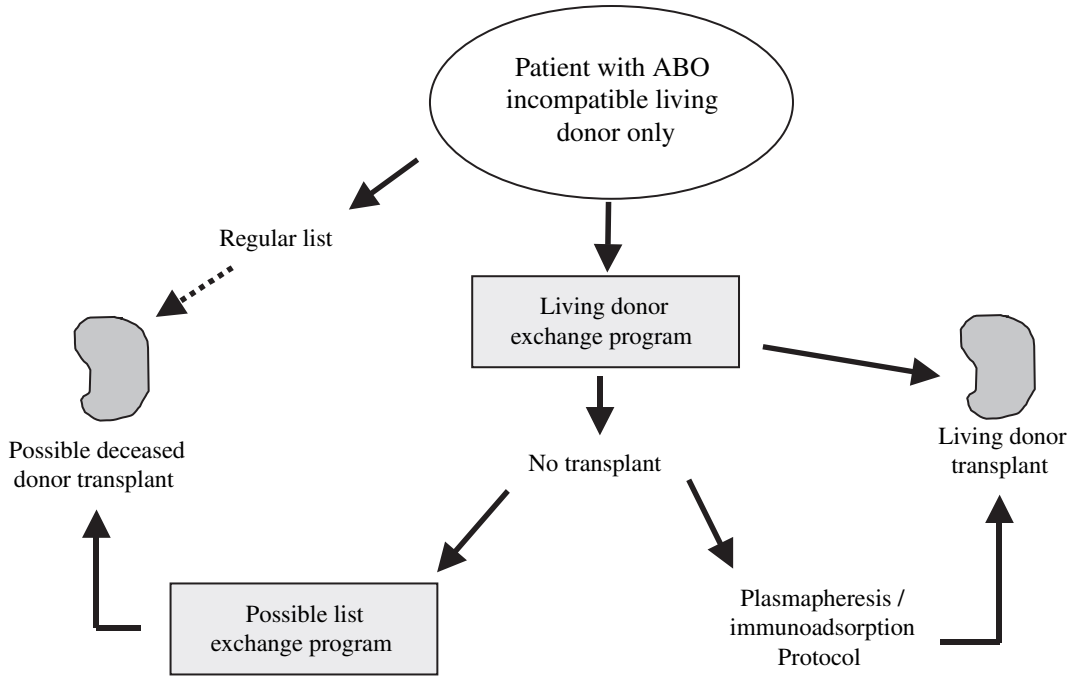

Figure 4 Proposed approach for optimizing transplantation of a patient with an ABO-incompatible living donor only.

mechanisms of accommodation [34] might facilitate progress in xenotransplantation, where AMR remains problematic.

Transplantation across ABO-incompatibility and a positive crossmatch

The plasmapheresis-based protocols for positive crossmatch or ABO-incompatibility are quite similar. What about using these protocols to overcome a positive crossmatch *and* ABO-incompatibility in the same recipient – living donor combination? Warren *et al.* [35] reported three cases in which plasmapheresis + IgG + splenectomy + MMF + tacrolimus + steroids + daclizumab ± rituximab were used. Short-term outcomes in the three ABO-O recipients were excellent. Our case involved a woman of ABO-O blood group who had a potential ABO-A-living donor. Her baseline anti-A titers were 128 and baseline CDC T-cell crossmatch was 128. She was conditioned with plasmapheresis + low-dose IgG + MMF + tacrolimus; she then underwent simultaneous splenectomy and renal transplantation and received basiliximab + rituximab. Allograft function has been excellent with no evidence of acute AMR on the single biopsy performed. Although these 'dual barrier' transplants are highly complex, they will likely find a role for a small number of patients, predominantly those who are ABO-O and highly sensitized.

Conclusion

The outlook for patients who are highly sensitized to HLA antigens or have ABO-incompatible living donors is improving. Although protocols involving plasmapheresis or high-dose IgG are yielding exciting results, the safest and most cost-effective approach is probably to first maximize their chances of receiving crossmatch negative, ABO-compatible allografts via acceptable mismatch programs and living donor exchange programs. A lot of work is now needed to build such programs at the national or even international level. If these programs do not provide a suitable allograft for a given patient after a reasonable waiting period, transplantation across the barriers of a positive CDC crossmatch or ABO-incompatibility should be strongly considered. Suggested approaches are illustrated in Figs 3 and 4.

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