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The hyperimmunized patient: from sensitization toward transplantation

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Abstract. Hyperimmunized patients tend to accumulate on renal transplant waiting lists because their high level of sensitization leads to positive crossmatches with almost all potential organ donors. The origins of sensitization and the different efforts made to find crossmatch-negative donors for these patients are discussed. Special emphasis is given to a local strategy based on the determination of HLA-A and -B mismatches, against which the patient did not form alloantibodies, the so-called acceptable mismatches. Kidney donor selection is based on compatibility with the patients' own HLA antigens in combination with the acceptable HLA-A and -B antigens and can be operated from a central organ-sharing office.

Key words: Hyperimmunized patient - HLA - Sensitization - Acceptable mismatch - Transplantation.

The accumulation of highly sensitized patients on renal transplant waiting lists is a universal problem. Because these patients have alloantibodies against many HLA alloantigens, it is very difficult to find a crossmatch-negative graft for them. For that reason, the average waiting time is much longer for these patients than for patients with fewer or no alloantibodies [12].

Furthermore, the transplantation results in the group of highly immunized patients are generally less successful [16], resulting in a relatively high number of highly sensitized patients returning to the waiting list for retransplantation.

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In this paper we deal with three topics:

1. Why do patients become highly sensitized?
2. Which schemes are developed to increase the chance of finding a donor for highly immunized patients?
3. A special strategy developed in our center to predict and select crossmatch-negative donors for highly sensitized patients is described.

Origins of sensitization

In contrast to sensitization against the AB0 blood groups, natural antibodies do not play a role in sensitization against the HLA alloantigens. Therefore, patients with alloantibodies should have had contact with foreign HLA antigens in one way or another. The three main reasons for sensitization in patients waiting for a renal allograft are pregnancy, blood transfusion, and failed transplants [12]. It is therefore not surprising that females are predominant among the (highly) sensitized patients.

However, this higher incidence of sensitization is not only due to antibody formation against the paternal HLA antigens of the fetus during pregnancy. Several reports have suggested that multiparous women are more likely to develop broadly reactive antibodies after blood transfusions [17, 22]. Patients who are homozygous for one of the supertypic HLA antigens, i.e., Bw4 and Bw6, are also at risk of developing broadly reactive antibodies after one or a few blood transfusions, as are patients who have rejected a previous graft, especially when the rejected graft carried several HLA-A and -B mismatches [14].

Although the three risk factors (pregnancy, transfusion, and graft rejection) can easily be

determined, not every patient has the same chance of becoming sensitized after such contacts with HLA alloantigens [20]. The reason for that may be either the immunogenicity of the product used as a challenge or immune response genes in the patient that predispose to antibody formation against foreign HLA antigens. Several factors may contribute to the immunogenicity of the blood used for transfusions, such as the amount of blood given per transfusion or the number of transfusions. A very important factor is the amount of viable leukocytes in the transfusate. Systematic studies on the immunogenicity of platelets both in man [7] and in the mouse [4] have shown that the presence of viable leukocytes in the platelet suspension is a prerequisite for the induction of alloantibodies against the MHC antigens. The presence of foreign MHC class II antigens is probably necessary for the activation of T-helper cells in the recipient, which in turn will activate B-cells to develop into alloantibody-producing plasma cells. Also, others have found that the probability of sensitization in renal transplant patients increases with an increasing number of viable leukocytes in the transfused blood [15]. Of course, the number of HLA mismatches between donor and recipient plays a determining role as to whether a patient will become sensitized after an immunizing event such as blood transfusion or transplantation. However, even when challenged with several very immunogenic products, only a minority of the patients become highly sensitized.

For instance, HLA-DRw6-positive patients are more likely to reject an HLA-DR-mismatched graft [10] and to develop antibodies reactive with B-cells and monocytes after transplant rejection than are patients with other HLA-DR antigens [11]. On the other hand, the HLA-DR1 antigen has been associated with low sensitization and a high kidney transplant survival [6]. A third example is the HLA-DR2 antigen, which seems to be associated with a high level of sensitization after blood transfusion, especially in females with previous pregnancies (A. Brand, personal communication). These data suggest that genetic factors in the recipient also play a role in the level of sensitization against HLA alloantigens. As these patients have an increased or decreased reactivity against many different alloantigens, it remains to be established whether these phenomena can be explained by mechanisms similar to those involved in the function of immune response (Ir) genes that control antigen-specific immune responses.

In conclusion, the main risk factors for a patient's becoming highly sensitized are pregnancy, blood tranfusion, and graft rejection. However, both the immunogenicity of these allogeneic factors and genetic factors in the recipient will determine whether a certain patient will actually become a highly immunized patient, for whom it is very difficult to find a suitable donor.

Schemes to increase the chance of finding a kidney donor for highly immunized patients

Highly immunized patients have antibodies against almost all foreign HLA antigens and are very difficult to transplant because the crossmatches with almost all donors are positive. When these patients are treated like all other patients in terms of donor selection and urgency, they accumulate on the renal transplant waiting lists. Therefore, several strategies have been developed to increase the chance of finding a crossmatch-negative donor for them. One possibility is to select HLA-A and -B identical or compatible donors, but the chance of finding such a donor is often very low due to the enormous polymorphism of the HLA system. Other schemes involve the distribution of the sera from these patients to many tissue-typing laboratories. Each potential donor is tested against these sera, and by trial and error donor-recipient combinations with negative crossmatches are identified [1, 21]. HLA matching is hardly or not at all involved in these schemes, although several reports have suggested that matching the donor and recipient for the HLA-DR antigens will significantly improve graft survival [12, 13]. These schemes have contributed to a shorter waiting time for highly immunized patients, although transplantation results could be further improved with DR matching.

Other approaches have also been suggested to solve the problem of highly sensitized patients, who have so many different antibodies in their serum that all crossmatches with HLA-mismatched donors are positive. For instance, the removal of preformed alloantibodies by cyclophosphamide treatment in combination with plasma exchange has been reported to be helpful in some highly sensitized patients [24]. However, this treatment is rather aggressive, often resulting in infectious complications and even death in some patients [9].

A new approach has recently been described for the removal of alloantibodies from the serum of highly immunized patients by the extracorporal immunoadsorption of the anti-HLA antibodies by staphylococcus protein A columns [18]. Although up to now only two patients have been successfully transplanted, with a positive crossmatch with serum

before immunoadsorption and a negative crossmatch postadsorption, these preliminary results suggest that this method may be helpful for at least some highly immunized patients.

Also, the statistical evidence that crossmatch-positive historical sera have not been predictive of the outcome of the transplantation [2, 8] may contribute to a shorter waiting time for some highly sensitized patients. This approach may be especially helpful for patients with a declining panel reactivity, although the role of memory cells or undetectable antibody levels in (hyperacute) rejection in individual patients remains to be established.

In this context, the detection of anti-idiotypic antibodies directed against previously formed HLA alloantibodies may be helpful [23]. Graft survival in donor-recipient combinations with positive crossmatches using historical sera and negative crossmatches with serum taken just before transplantation has been reported to be excellent when the latter can block the positive reaction of the historical sera [19]. In contrast, when the current serum did not contain such blocking factors, graft survival was found to be very poor. If confirmed, these data may be very useful in establishing the importance of the positive crossmatch with historical sera. Until now, we have preferred not to transplant when the historical crossmatch was positive due to specific alloantibodies directed against the HLA class I antigens of the donors, especially when IgG antibodies were involved [3].

In conclusion, several schemes have been developed to increase the chance of finding a donor for highly immunized patients, with variable success rates. However, some of these schemes can only be applied in subgroups of the highly sensitized patients, whereas others include a serious risk to the health of the patient. Nevertheless, the introduction of specific approaches for highly sensitized patients will contribute to shorter waiting times for these patients.

Donor selection for highly immunized patients based on acceptable HLA-A and -B mismatches

We have developed a special strategy to help highly immunized patients, which does not need the distribution of patient sera and can be operated from a laboratory adjunct to a central organ-sharing office. Our protocol is based on data obtained from Eurotransplant showing that, in the case of a negative crossmatch with both current and historical sera, HLA-A and -B mismatches are less important for graft survival in highly sensitized patients, whereas matching for HLA-DR has a significant influence especially on retransplantation [12].

The aim of the scheme is an exact definition of those HLA-A and -B incompatibilities that will not result in a positive crossmatch. Therefore, the sera from highly immunized patients are screened against a panel of lymphocyte donors, who are selected on the basis of having only one HLA-A or -B mismatch with the specific patient (Table 1). Using this approach, HLA-A and/or -B antigens against which the patient did not develop antibodies can be detected. Kidney donor selection takes place by entering the HLA-A, -B, and -DR antigens of every potential donor for each patient so analyzed in the central computer, which will select crossmatch-negative recipients on the basis of their own HLA-A, -B, and -DR antigens in combination with the acceptable HLA-A and -B mismatches [5]. In this way the donor is always matched for HLA-DR (either identical or compatible) but may have several HLA-A and -B mismatches.

In the past 2 years, the sera from 73 highly sensitized patients were tested for the absence of specific alloantibodies against HLA-A and -B antigens using such patient-specific panels. All these patients showed antibody reactivity against more than 85% of the panel donors in complement-de-

Table 1. Principle of detection of acceptable HLA-A and/or -B mismatches in highly sensitized patients

HLA type of the patient: A1 A2 B7 B8	
Selected panel donors	Crossmatch with patient sera
A1 A2 B7 *B44*	+
A1 A2 B2 *B35*	−
A1 A2 B7 *BW60*	+
A1 *A3* B7 B8	−
Acceptable mismatches: A3 and B35	

Table 2. Consequences of acceptable mismatches for kidney donor selection

Patient: A1 A2 B7 B8 DR2 DR3 (100% panel reactivity)

Acceptable mismatches: A3 B35

DR-compatible donors, who will be selected on the basis of their acceptable mismatches

A1 A3 B7 B8	A2 A2 B35 B35	A3 A3 B35 B35
A2 A3 B7 B8	A2 A3 B7 B35	A3 A3 B7 B7
A3 A3 B7 B8	A2 A3 B8 B35	A3 A3 B8 B8
A1 A2 B7 B35	A1 A3 B7 B35	A1 A3 B35 B35
A1 A2 B8 B35	A1 A3 B8 B35	A1 A3 B7 B7
A1 A2 B35 B35	A3 A3 B7 B35	A1 A3 B8 B8
A1 A1 B35 B35	A3 A3 B8 B35	A2 A3 B35 B35
A2 A3 B8 B8	A2 A3 B7 B7	

pendent lymphocytotoxicity. This antibody reactivity was due to multispecific alloantibodies to HLA class I antigens; autoantibodies were excluded. In patients with less than 100% panel reactivity, a first indication concerning these acceptable HLA-A and -B mismatches could be deduced from the HLA antigens of the negative panel donors in the screening.

Nevertheless, patients with 100% panel reactivity were also found to have acceptable mismatches, which, due to the composition of the panel, were not detectable in the standard screening. A panel of lymphocyte donors who have only one HLA-A or -B mismatch with the specific patient, however, will reveal these "holes" in the antibody repertoire of the highly immunized patient. In 80% of the patients it was possible to define such acceptable mismatches using these patient-specific panels.

Even HLA antigens with a high phenotype frequency, such as HLA-A1 and HLA-A3, were found to be acceptable in some patients. Addition of the acceptable HLA-A and -B antigens to the patients' own HLA-A, -B, and -DR antigens in kidney donor selection will significantly increase the chance of finding a crossmatch-negative donor (Table 2). In the meantime, 34 of these patients have been transplanted according to this scheme, with a 1-year graft survival of over 80%. Some of these patients waited for more than 10 years to receive an HLA-compatible graft.

We conclude that the determination of acceptable HLA-A and -B mismatches may contribute to a significant increase in the potential donor pool for highly immunized patients. Based on laboratory work that is carried out beforehand in the recipient center, a central organ-sharing office can select all HLA-DR-compatible kidney donors who have a negative crossmatch with all sera from a certain highly immunized patient. This approach has several advantages compared to other schemes for selecting crossmatch-negative donors for highly sensitized patients:

1. There is no need for distribution of patient sera to other tissue-typing centers.

2. Instead of crossmatches being carried out on all donors, most of whom will be positive, selection is based on a predictable, negative crossmatch.

3. Selection of potential donors is based on data from the recipient center, which has all the information concerning the immunological background (e.g., transfusions, specific alloantibodies, autoantibodies) of the patient, and not on a negative crossmatch done in another tissue-typing center.

4. Selection is based on HLA-DR compatibility between the donor and recipient, which is, in our analyses and those of others [11, 13], important for an optimal prognosis of the graft survival in highly immunized patients.

A disadvantage of the scheme might be that the amount of work is considerable. The determination of acceptable mismatches in a highly sensitized patient takes at least 2-3 weeks, especially in those cases in which the screening results do not give a clue toward finding such antigens. Therefore, extensive panel studies are necessary before the acceptable mismatches can be detected. However, our very recent finding that in about 50% of the highly immunized patients the acceptable mismatches include HLA antigens of the mother of the patient, which were not inherited by the patient, may significantly facilitate the search for such acceptable HLA-A and -B antigens (manuscript in preparation). Moreover, these observations suggest the existence of neonatal tolerance in man, which will be a topic for future research in our laboratory.

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