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Extending options for highly sensitized patients to receive a suitable kidney graft

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Highly sensitized patients (anti-HLA) on the kidney waiting list wait longer for a suitable crossmatch negative organ. At the moment there are two strategies to enhance transplantation of these patients. One approach is the determination of acceptable HLA mismatches and application of this knowledge for the selection of crossmatch negative donors, and the second is the desensitization of patients with intravenous immunoglobulin-based protocols to enable transplantation of an organ from a donor towards which antibodies were originally present. Both approaches have advantages and disadvantages and are only successful in a proportion of the patients. The optimal solution is an integrated strategy whereby desensitization is used for those patients for whom the acceptable mismatch approach is not successful.

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Introduction

Donor-specific antibodies against the human leukocyte antigens (HLAs) play an important role in the outcome of solid organ transplantation. Kissmeyer-Nielsen *et al.* [1], and Patel and Terasaki [2] were the first to observe that pre-existing anti-donor HLA antibodies lead to hyperacute or accelerated rejection of the transplanted kidney. Introduction of pre-transplantation crossmatching reduced the incidence of hyperacute rejection to less than 0.5%. The goal of organ-exchange organizations is to offer a crossmatch-negative organ [3•] to the patients on the waiting list. Sensitization caused by HLA alloantibodies is found in patients who received blood transfusions, transplants or, in case of females, delivered a child.

Sensitization reduces the chance of receiving a crossmatch-negative organ, especially if the patient has an odd or rare HLA phenotype in relation to the organ donor population [4,5,6••,7]. Highly sensitized patients (HSPs) with a panel reactive antibody (PRA) value of 85% or more (i.e. HLA alloantibodies in their serum react with 85% or more of unselected donors in the crossmatch) accumulate on the waiting list of the organ exchange organizations [3•]. The obvious solution is to find an HLA-identical donor, but because of the enormous polymorphism of the HLA system, this is virtually impossible for the majority of HSPs. In general, there are two other options to realize transplantation of these patients: removal of the antibodies causing the positive crossmatch or definition of the holes in their antibody repertoire to enhance selection of crossmatch-negative HLA mismatched donors.

Removal or reduction of donor-specific HLA antibodies can be obtained by extracorporeal immunoadsorption columns [8,9,10••], or by *in vivo* application of intravenous immunoglobulin preparations (IvIgs) [11–18,19••,20,21,22••,23••,24,25]. *In vivo* absorption of detrimental donor-specific antibodies by simultaneous liver/kidney transplantation is suggested by Olausson *et al.* [26] and Gutierrez *et al.* [27] as an alternative option for transplant patients with donor-specific antibodies. Alternatively, one can accept that a patient is highly sensitized and look for a crossmatch-negative donor organ via special programs, such as the acceptable mismatch (AM) program organized by Eurotransplant (ET), or a priority system such as that used in the UK [28].

Desensitization protocols

Immunoabsorption

Several desensitization protocols have been used to remove the detrimental donor-specific alloantibodies from the circulation of the potential recipient. Application of extracorporeal immunoabsorption [8] was reported in 1989, with successful short-term results for six out of seven HSPs; however, a rebound effect, resulting in reappearance of the antibodies, is observed in many patients [9]. Recently [10••], it was demonstrated that, after peritransplant immunoabsorption in 40 HSPs crossmatch negative and crossmatch positive patients (current mean PRA value of 77%; 38 re-transplants) no significant difference in three years graft survival (71% versus 78%) or serum creatinine 1.57 mg/dL versus 1.23 mg/dL) was observed.

Intravenous immunoglobulin preparations

An alternative approach to removing donor-specific antibodies is by treatment with IvIg. Historically, IvIg was used to treat immune haematological diseases and infections [29]. In solid organ transplantation IvIg is used not only for desensitization of the patient but also for treatment of acute rejections [30–32]. Application of IvIg is not restricted to renal transplantation, but is also adapted to other organs as heart or pancreas [33,34]. Desensitization is not restricted to HLA alloantibodies, but can also be used to facilitate transplantation of ABO blood group incompatible donor organs combined either with splenectomy [21,35–37] or without splenectomy [38]. Even desensitization of donor HLA specific antibodies combined with a major blood group incompatibility has led to successful transplantations. During the past few years, application of IvIg for removal of HLA alloantibodies has become a very popular approach, especially in the USA.

Two main protocols are used: a so-called high-dose IvIg treatment proposed by the Cedars-Sinai (2 g/kg IvIg; [17]) for patients awaiting either a deceased or live donor, and a low-dose IvIg protocol used at the John Hopkins (100 mg/kg; [21]) in combination with plasmapheresis, with or without treatment with anti-CD20, applied for live donors only. Desensitization was monitored by *in vitro* testing and was successful for the majority of the patients (40–60% depending of the study and protocol) [25,39], although other reports do not confirm these positive findings [40].

The biological function of IvIg is still unclear. In addition to its anti-inflammatory effect, a decreased Fc-receptor-mediated immune phagocytosis, complement consumption [41], and regulatory effects on B and T cells have been suggested. Part of the regulatory function has been attributed to anti-idiotypic antibodies present in the IvIg preparations. [25]. The immunomodulatory effects of IvIg were also attributed to modulation of surface molecule expression and induction of apoptosis in B cells [42]. Irrespective of the mechanism, it is clear that IvIg is able to modulate the immune response to alloantigens [43]. In combination with rapamycin, additional effects on cell proliferation are observed [44]. Reported side-effects of IvIg include serious headaches [19^{••}], nephrotoxicity, a negative interaction with other treatment modalities (such as ATG) [45], and the induction of isometric tubular epithelial vacuolization post-transplantation [46]. Batch differences and the stabilizing agent also play a significant role in the efficacy of the treatment. The results in term of graft survival are similar in all groups using IvIg following either the high or the low dose protocol. An average of 80–85% two years graft survival has been reported [19^{••},22^{••}]. In some patients, the persistence of low levels of donor-specific alloantibodies is observed [47[•]], but has not been confirmed by others [39]. Both treatment modalities (high or low dose) are associated with

increased additional costs of 35 000 to 45 000 USD per patient (see Table 1).

Special programs for the selection of crossmatch-negative donors

The AM program of ET is a multinational and multicenter program with the aim of defining HLA antigens towards which the patient never formed antibodies [6^{••}], thereby predicting negative crossmatches with specific HLA mismatches. This can be achieved by ‘trial and error’ by performing crossmatches with randomly selected ABO-compatible donors. A more sophisticated approach is to perform crossmatches with blood donors with a single HLA-A, or -B mismatch to the patient [4,7] or by using alternative methods, such as cell lines expressing single HLA antigens [48] or single antigen beads [49]. In addition, a recently developed computer algorithm ‘HLAMatchmaker’ [50,51^{••}] can be used to identify acceptable mismatches. The concept of HLAMatchmaker is that the HLA type of the patient represents six strings of self-triplets (antibody epitopes consisting of three consecutive amino acids on the HLA-A, -B, -C antigens). Patients will not form antibodies against these triplets because they represent self-structures. Each mismatched donor HLA antigen represents a string of triplets. Compatibility is assessed by lining-up donor triplet strings with patient triplet strings to determine differences between patient and donor (mismatches). This concept was validated and has been adopted in the analysis of acceptable mismatches for HSP candidates in the AM Program [51^{••}]. The HLA type of the patients and their acceptable mismatch are entered in the AM allocation program and, upon availability of an organ donor, recipients are selected on the basis of compatibility of the donor with the patient’s blood group and HLA-A, -B, and -DR antigens in combination with the AM. Compatible organs are immediately dispatched to the transplantation centre of the recipient where the definitive crossmatch is performed. From the establishment of this program more than 450 HSPs have profited from the AM program. Currently, 45 HSPs per year receive a suitable crossmatch-negative transplant, from a pool of ca. 3 100 organs from deceased donors of the ET pool. Every patient entering the AM program has a 43% chance of receiving a transplant within 12 months or 58% within 21 months [6^{••}]. Graft survival at two years is 87% identical to the non-sensitized patients transplanted in the same period.

In vivo absorption of alloantibodies by combined transplantations

It is generally accepted that donor-specific HLA alloantibodies, which are detrimental in kidney transplantation, have no negative effect in liver transplantation. The liver, or soluble HLA antigens derived from the liver, seem to be able to absorb these antibodies, preventing antibody-mediated effector mechanisms. As a consequence, this combined liver–kidney transplantation was reported to be

Table 1

Comparison between the two main modalities for highly sensitized patients.

Modality	Desensitization (Ivlg)		Special program
Factor	High dose ¹	Low dose ²	Acceptable mismatch ³
Donor type	Deceased	Live	Deceased
Additional costs	35 000 \$	45 000 \$	4,100 \$
Transplantation rate at end of treatment (max 24 months) ⁴	40%	63%	58%
Panel reactive antibodies ⁵	>30%	>0%	≥85%
Two year graft survival	80%	85%	87%

The transplantation rate in the standard allocation or the use of placebo in the controlled study was 11%⁶. ¹Jordan *et al.* [16,19**]. ²Montgomery and Zachary [21,22**]. ³Claas *et al.* [5,6**]. ⁴Patients in the high dose protocol and the AM program still have the chance to be transplanted after the 24-month period. ⁵In the Ivlg studies all sensitized patients can be included. In the AM only HSPs are accepted [6**]. ⁶HSPs in ET [3*] have a 11% chance per year for a crossmatch-negative organ via the allocation system.

an alternative solution for *in vivo* absorption of HLA antibodies enabling transplantation of HSP in the presence of a positive donor-specific crossmatch. Olausson *et al.* [26], and later Gutierrez *et al.* [27], reported good graft and patient survival with positive crossmatches turning negative after transplantation. In these studies with low numbers of patients (five in total) no hyperacute or accelerated acute rejections were observed. Earlier results, however, do not confirm these results [52]. It remains to be established in larger groups of patients if this surgical approach can be substantiated and more widely accepted.

Discussion and future perspectives

Highly sensitized patients are very difficult to transplant and the best solution would be to avoid sensitization of the patients. This is not always possible as females have a chance of about 25% to become sensitized post-partum. The introduction of recombinant erythropoietin has led to a reduction of the incidence of sensitization of patients to 28% [53]. The re-transplant candidates remain problematic. These patients become sensitized against the mismatched HLA antigens of the rejected organ. The sensitization incidence after a failed transplant depends on the number of HLA mismatches of the donor, and can vary between 20% (0, 1 mismatches) and 46–52% (5,6 mismatches). Reduction of the HLA mismatches in previous transplants will reduce the incidence of sensitization.

The aim of all programs presented here is to provide a crossmatch-negative donor organ with the prospect of a long graft survival to (highly) sensitized patients. The philosophy of the two main modalities differs significantly. The acceptable mismatch program aims at a clear definition of acceptable HLA mismatches based on extensive laboratory studies. This knowledge is used for donor selection, and the allocation is performed with the highest priority (mandatory). The program can be successfully implemented in regions where a substantial number of organs are available (ca. 3000 per year), which is the case for large organ-exchange organizations in Europe and the USA. That such exchange is feasible is

also shown with the 6-antigen match promoted by United Network for Organ Sharing (UNOS). Desensitization of the patients by Ivlg treatment is performed locally with the aim of directly helping patients on a local basis. The driving force for using Ivlg is not the degree of sensitization but rather the desensitization of the patient to overcome a positive crossmatch. The long-term effects of Ivlg treatment (in combination with the additional immunosuppression therapy needed and splenectomy) are not yet known.

The main characteristics of the two main modalities are shown in Table 1, where the Ivlg group is split into the high- and the low-dose protocol. The low-dose protocol is restricted to live donors, whereas the high-dose protocol also involves the use of deceased donors. The rate of transplantation is high in the low-dose protocol, but one should realize that the degree of sensitization in the patients transplanted so far is also low. The results of the transplants performed with these different approaches are good: two years graft survival is 80% for the high dose Ivlg, 85% for the low dose Ivlg and 87% for the AM program. An important aspect is the difference in costs of the approaches. These costs are significantly higher (almost 10-fold) for the Ivlg treatment.

A problem is that all of these modalities are successful in only a proportion of the patients. The optimal situation would be a combined protocol starting with the most cost-effective approach. Therefore, we propose that, for countries with a large donor-organ pool with a well-functioning organ exchange organization, the prioritization of HSPs, similar to the Eurotransplant acceptable mismatch program, should be established. The allocation should be mandatory and prioritized. Definition of AM is currently simple and reliable with the modern screening techniques combined with the HLAMatchmaker algorithm. In this way at least 60% of the patients will be transplanted with a suitable graft within two years, leading to excellent graft survival and acceptable costs. The remaining patients, with mostly rare HLA phenotypes and antigen combinations in comparison to the donor population, should be considered

for desensitization protocols. Finally, if desensitization before transplantation is not successful, alternative approaches such as combined kidney–liver transplantation could be considered.

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