

## HLA-D, DR—CLINICAL SIGNIFICANCE

### A New Strategy to Improve Kidney Graft Survival: The Induction of CML Nonresponsiveness

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**A**LTHOUGH immunosuppression made renal allografting possible, the excellent results in ABO-compatible HLA-identical siblings showed that optimal matching allowed the immunosuppressive dosage to be significantly reduced. Attempts to extrapolate the sibling findings to unrelated donor-recipient pairs have until recently met with only partial success, however.

During the last half year evidence has been presented by several teams that matching for HLA-DR determinants correlates to an unprecedented level with improved renal allograft prognosis between unrelated donor-recipient pairs.

Although the effectiveness of HLA-DR matching is certainly the most exciting new data, it is equally certain that it is not the only factor influencing renal graft survival. It is impossible to give here an all encompassing and complete review. For that reason we have

selected a few topics that we think are of most interest. We will divide this review into three parts: past, present, and future.

#### THE PAST

Everybody agrees that a positive cross-match leads in most instances to graft rejection. Studies by Thomas et al. and Stiller et al. suggest that both lymphocyte-dependent antibody and cytolytic cells can precede graft rejection.<sup>1,2</sup> The point we want to stress here is that very little is known about the specificity of these antibodies and cytolytic cells. They have so far been presumed to have anti-HLA-A and B and possibly C specificity, but other systems in and outside HLA are almost certainly involved as well. This is illustrated by the following: A woman who had had three pregnancies received one blood transfusion and formed a strong anti-Cw4. She was transplanted with an HLA-A, B, and C-identical kidney. Although the crossmatch had been negative, the graft was rejected within a week. Further analysis of the pretransplant serum revealed the presence of an anti-HLA-DRw6 antibody that had not been detected in the crossmatch. The kidney donor was DRw6 positive. The findings thus imply that the DR antigens can be targets that if attacked by an antibody can lead to graft rejection. Furthermore, Conleth and Stastny found that DR antigens can also be a target in the CML test.<sup>3</sup> These examples emphasize that it is technically feasible to determine the specificity not only of the recipient's antibodies but possibly also of his lympholytic cells. It should be emphasized that one cannot rely solely on a

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crossmatch because it is well known that false-negative or so-called CYNAP reactions can occur. The situation is thus strikingly similar to that of blood transfusion.

The conclusion from this is clear: A crossmatch should always be performed. Furthermore, if the effects of preexisting immunity are to be avoided, it is essential that the specificity of antibodies formed by the patients be determined as carefully as possible. In addition, the better the match between donor and recipient for the HLA-A and B antigens, the smaller will be the chance that missed anti-HLA-A or B antibodies can cause graft rejection.

This brings us to HLA matching in cadaveric renal transplantation. It has taken us a long time, but finally those who agree that HLA-A and B matching can improve renal graft survival outnumber those who think it does not—on both sides of the Atlantic.

One could summarize the situation by saying that matching for HLA-A and B matching significantly, although not impressively, improves graft survival (10%–20% between best and poorly matched grafts). This improvement is probably due both to the importance of these antigens as targets in the homograft reaction and to the fact that they are in linkage disequilibrium with the HLA-D and DR determinants.

Next we would like to draw attention to the two-faced role of blood transfusion in transplantation. Opelz et al. were the first to present significant evidence that blood transfusion not only can cause immunization, which endangers graft survival, but can also prolong graft survival;<sup>4</sup> van Es and Balner produced experimental evidence for this in the rhesus monkey.<sup>5</sup> We want to stress in this connection two important points from our own work. The first is that we have confirmed in a prospective study our previous finding that a single transfusion improves graft survival;<sup>6</sup> 17 nontransfused patients received a single washed (i.e., buffy coat poor) blood transfusion before transplantation, and of these only three transplants failed for nonim-

munologic reasons (one with coronary occlusion and two with viral pneumonia). The second, and this was a new finding, was that 7 of the 8 patients who received blood made completely free of buffy coat cells by passing it through a cotton wool filter<sup>7</sup> rejected their kidney. These results were as poor as when no transfusions were given.<sup>6</sup> Thus for graft protection one needed the equivalent of about 50 ml of allogeneic ACD blood. Although not randomized, this was a prospective study. Since only one blood transfusion was given, it was difficult to imagine an immunogenetic system that would allow for graft protection in such a large part of the patients mediated by specific alloantibodies, and it thus seemed very unlikely that enhancing antibodies were responsible for graft protection. One alternative possibility was that it was due to the induction of a relatively nonspecific suppressor mechanism.

#### THE PRESENT

An oft-repeated question has been that if HLA matching is important, why do some HLA-A and/or B-mismatched grafts do so well? An answer to this question is provided by the observation that graft survival is significantly improved by HLA-DR matching even in the face of mismatches for HLA-A and/or B.<sup>8–11</sup>

HLA-DR antigens can be recognized serologically on B cells.<sup>12–15</sup> They are probably not identical to the HLA-D determinants that stimulate in the MLC test but are very closely linked to them. Thus in Northwest Europe by typing and matching for HLA-DR one can match for HLA-D. In order to assess its importance in kidney transplantation, HLA-DR matching was performed retrospectively on peripheral blood cells of the recipients and frozen spleen cells of the kidney donors. Of the few recipients who had died after transplantation, frozen cells were available. Table 1 shows that not only HLA-DR-identical combinations but also combinations where only one HLA-DR determinant was shared showed good graft survival.<sup>11</sup> Other groups in

**Table 1. Number of Eurotransplant Patients in the Various HLA-A, B, and DR-match Classifications**

A and B Loci Combined No of Antigens Mismatched	DR Locus No of Antigens Mismatched			Totals
	0	1	2	
0	—	10/2	3/1	13/3
1	3/-	16/2	5/5	24/7
2	1/2	7/4	4/8	12/14
3	1/-	1/-	2/1	4/1
4	—	—	1/-	1/-
Totals	5/2	34/8	15/15	54/25

Slash indicates functioning/nonfunctioning grafts as of beginning of April 1978 (Adapted from Persijn et al<sup>11</sup>)

Europe have done similar studies and have published comparable data<sup>16-18</sup> (Table 2). In all series there was a striking improvement of graft survival by matching for one HLA-DR determinant in comparison to the group with two HLA-DR determinants mismatched. The improvement by matching for two HLA-DR determinants was expected, since earlier studies had shown that a low or negative MLC test between parent-child or unrelated donor-recipient pairs improved graft survival.<sup>19-21</sup> Not all HLA-D or DR-identical combinations lead to a negative or low MLC test, but many do,<sup>22</sup> and this could explain the good results in the full-house-identical group.

On the other hand, combinations that share only one HLA-DR determinant are always MLC positive. Why is it, then, that grafts mismatched for one HLA-DR antigen do so well? From the point of immunogenetics, this is, of course, heresy: a difference of an antigen between donor and recipient has always been considered to be dominant over sharing an antigen. There exists corroborating evidence

in the parent-child data. They, too, share one DR determinant and do much better than those differing by two; in fact in Holland they do as well as HLA-identical siblings (Persijn GG; Unpublished observations).

Similarly, matching for an A and B antigen combination such as A1-B8 and A3-B7, which are in strong linkage disequilibrium with a DR antigen, improved graft survival between unrelated individuals. The improvement was greater than was found if just any two HLA-A and B antigens were shared. Thus in this situation donor and recipient were matched indirectly for one DR antigen.<sup>23</sup>

From these independent lines of evidence, it is safe to conclude that matching for just one DR determinant can significantly improve graft survival. It is also clear that the limited data available are in part retrospective, and prospective trials are indicated.

It should be stressed that all donors in this study who received a kidney matched for one DR determinant had been transfused. We think that this is an important prerequisite, since not all donor-recipient combinations in this group do well.

Swedish workers found that graft survival in parent-child combinations was good only if the recipient had been transfused before transplantation.<sup>17</sup> The Dutch data tend to agree with this, although a control group of nontransfused recipients is lacking. These data, then, suggest the following:

(1) Matching for *one* HLA-DR determinant in parent-child combinations and, by inference, in the unrelated combinations leads

**Table 2 DR Matching in Europe (Functional\*/Total)**

	Zero DR Mismatches	One DR Mismatch	Two DR Mismatches
Eurotransplant <sup>11</sup>	6/7	37/42	18/30
Geneva <sup>10</sup>	0/0	22/25	12/23
Oslo <sup>9</sup>	2/2	15/24	14/31
Oxford <sup>8</sup>	4/4	32/40	27/40
Total	12/13 (92%)	106/131 (81%)	71/124 (57%)

$p = 0.60$

$p = 0.0002$

\*At 6 months after transplantation

to significant improvements, providing that the recipient had been transfused before transplantation. This conclusion is of a preliminary nature because Solheim et al. did not find a graft-protecting effect of blood transfusions in parent-child combinations.<sup>18</sup>

(2) DR serology is sufficiently accurate to select unrelated individuals who are matched for one DR determinant.

(3) The effect of matching for one DR determinant overrides the effect of incompatibility for multiple other determinants both in and outside HLA. It should, however, be emphasized that compatibility for A and B reinforces the effect on one DR match.<sup>11</sup> We cannot, of course, yet exclude the possibility that it is not DR that we should match for but another closely linked locus, e.g., HLA-D. Interracial transplants will be very useful in evaluating this.<sup>24</sup>

In an attempt to clarify the mechanism by which matching for one DR determinant overrides the effect of incompatibility of other antigens, we investigated whether or not these findings on DR matching and graft survival had an *in vitro* correlate. Both MLC and CML tests (after *in vitro* priming) were studied. Lymphocytes were taken from patients 3–18 months *after* transplantation, and these were reacted with the splenocytes, which had been stored in liquid nitrogen, from their specific kidney donor. Four patients had rejected their graft, while seven had good-functioning grafts as expected. All MLC tests

were positive. The CML findings, which can be regarded as a measure of cellular immunity *in vitro*, are shown in Figure 1. The lymphocytes of the four patients who had rejected their graft all reacted strongly in the MLC test after *in vitro* priming with the splenocytes of their specific donor (and with lymphocytes from other individuals) independently of their DR match. In contrast, the lymphocytes of four patients who had functioning grafts and who also shared one DR determinant with the donor had a negative CML test with their specific donor. This low reactivity was specific, since their lymphocytes lysed the lymphocytes of donors selected at random. Of the other three patients with functioning grafts, two differed for two DR determinants and one was DR identical to their respective donors. All three reacted about equally strongly with both the specific donors and randomly selected cells. This is, of course, a very limited set of data, but it is reassuring that we could actually show that the CML test changed from positive before transplantation to negative (as shown here) after transplantation. So far we have found CML nonreactivity only in the one DR antigen-matched group, but it is, of course, quite possible that more extensive studies will show that nonreactivity can also occur in either the two antigen-matched or mismatched group.

These preliminary findings show striking similarity to observations of Thomas et al.,<sup>25</sup> who studied CML reactivity in parent-child

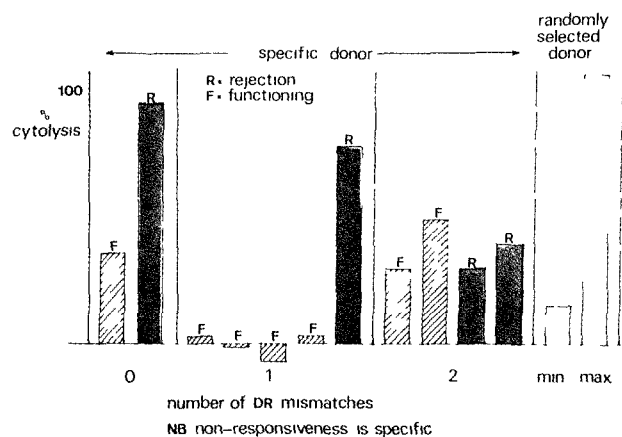


Fig. 1. CML reactivity after transplantation and DR matching.

combinations, and Woninget and Pichlmayr,<sup>26</sup> who studied cadaveric kidney transplant recipients. The new data presented here show that CML nonreactivity is found a few months and not many years after transplantation and that to improve graft survival previous blood transfusions appear to be necessary. Furthermore, under these conditions matching by DR serology in unrelated donor-recipient combinations appears to be as effective as matching by haplotype in the related parent-child combinations. The most important new finding is, of course, that for the first time CML unresponsiveness has been shown to correlate with the sharing of one DR determinant between donor and recipient. Small as the data base is, it is significant ( $p = 0.03$ , Fisher's exact test).

In summary, the current picture emerges as follows:

(1) Matching not only for two but also for one DR determinant improves graft survival to about 80% at 1 year. We should like to reiterate that our data indicate also that partial matching for HLA-A and B will improve results further to about 90%, i.e., equal to that of HLA-identical siblings.<sup>11</sup> Thus DR matching reinforces but does not replace HLA-A and B matching.

(2) Donor-specific CML nonreactivity develops in at least some of the patients who share one DR determinant with the donor. This nonreactivity is possibly due to the induction of a suppressor cell.<sup>25</sup>

(3) Previous blood transfusions, which might induce partial aspecific tolerance and immunosuppression appear to be necessary.

#### THE FUTURE

In the first place, the implications of donor-specific CML nonreactivity will have to be studied further. On the practical level, if current trends hold up it might be possible in the immediate future to improve renal allograft survival results to 80% or 90% at 1 year. Because partial DR matching results in excellent graft survival and because of the apparent restricted polymorphism of the DR locus,

recipient pools of a few hundred persons might be sufficient to find adequately matched recipients for the majority of donor kidneys. This contrasts favorably with the pools of thousands of recipients now in use that enable one to realize only 10%–20% HLA-A and B-identical matches. This might be true not only for renal but also for other tissue transplants, including hearts.

The biomedical implications might be even more impressive. It seems that we have stumbled on a relatively simple strategy to induce donor-specific nonresponsiveness to MHC determinants and possibly other antigens as well as measured in the CML test. All that appears necessary is a single blood transfusion, a one DR antigen-matched kidney graft, and standard immunosuppression. The mechanism by which this can be done is as yet undefined but might well be the induction of suppressor cells.<sup>25</sup> The one DR-matched graft that does so well should be compared with the two DR-matched graft, which appears to do at least equally well. The central question here is whether they represent the same or different mechanisms that allow good graft survival.

The role of blood transfusion and immunosuppression needs in this context to be further analyzed. It would be of interest to investigate if there exists a link between blood transfusion, the appearance of cold B cell antibodies, and graft protection. The immunogenetic requirements, apart from a shared DR determinant, should be studied, especially the question of whether or not a dual recognition phenomenon between DR and the equivalent of the *H2-IJ* locus plays a role. Additionally, findings discussed above might perhaps be of importance in the management of autoimmune diseases (restoration of tolerance to self-antigens) and even of malignancies. It will also be of interest to determine if, and how, these findings apply also to fetal-maternal tolerance and, if so, whether or not a similar rationale can be used in the treatment of habitual abortion.

To return to the topic of this conference, it

appears to be justified to investigate whether or not pretreatment of the donor of a bone marrow graft with a blood transfusion and immunosuppression will diminish graft-versus-host reaction in MHC-identical combinations or might even make it possible to use HLA haploidentical donors. Of course, this should be first tested in animals, by preference in rhesus monkeys.

A related question also requires urgent answers: Is it really true that B cell antibodies that react primarily at  $\pm 4^{\circ}\text{C}$  are protective, while those with their optimum at  $37^{\circ}\text{C}$  can actually harm the graft?<sup>27,28</sup>

Although matching for DR will be the main topic of interest, other factors influencing graft survival should not be overlooked. There seems little doubt that monocyte antibodies, which appear to react with antigens on endothelial cells as well, can cause graft rejection.<sup>29,30</sup> Finally, the role of incompatibility for the Lewis blood group system should be evaluated by other groups in addition to the French, who noticed it first.<sup>31</sup>

We think personally that in the future the central question will be whether the recipient has been immunized or not, not only against the HLA-A, B, and C antigens but also against other antigens such as the DR antigens, the monocyte antigens, and possibly Lewis antigens. If the recipient has *not* been immunized against these antigens (and some others not yet recognized), we would not be too surprised if the specific nonresponsiveness

that can be induced by the combination of one blood transfusion, immunosuppression, and matching for one HLA-DR determinant will lead to very good graft survival. If, on the other hand, the recipient has been immunized against one or more of these determinants, matching for them as well will be obligatory.

In conclusion, we think it permissible to say that the future has never looked so bright. DR matching was developed to select DR-identical combinations. The fact that one DR-matched grafts do so well is an unexpected bonus. It provides us also with an answer to the embarrassing question of why grafts mismatched for several HLA-A and B antigens not infrequently do so well. This unpredicted finding teaches us modesty and indicates shortcomings of our experimental protocols. It is reassuring that it also shows us that one can go the right way (in this case develop DR typing) for the wrong reason (the assumption that only DR-identical matches would be good enough)

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