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TWO CHALLENGES IN BONE MARROW TRANSPLANTATION  
Graft versus host disease and the unrelated donor.  
(Report of the Working Party on Immunology)

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GRAFT VERSUS HOST DISEASE (GVHD)  
Immunogenetics

Goulmy has reported during the previous meeting the occurrence of cytotoxic lymphocytes (CTL's) in a patient suffering from chronic GVHD after allogeneic bone marrow transplantation for acute myeloid leukaemia (1,2). These CTL's were directed against a non-HLA determinant (HA) and were restricted by the class I antigens of the donor. They allow thus the recognition of genetic differences between HLA-identical siblings, for instance the CTL's reacted with the patient's own pretransplant lymphocytes, but not with the donor's lymphocytes. Similar CTL's have been described by Elkens et al. in a patient, who had become sensitized after multiple blood transfusions (3).

Goulmy et al. suggested that the anti-HA CTL's could and should be used to study their relevance in the occurrence of GVHD. In a collaborative effort with the bone marrow transplant group of the l'Hôpital St. Louis, in Paris (Head: Prof. E. Gluckman) and of the University Hospital Leiden (Head: Prof. Dr. J.J. Veltkamp) preliminary evidence has been collected which indicate that incompatibility for HA (or other non-HLA CTL determinants) can lead to GVHD.

Table I. Relation between mismatches for non-HLA determinants between donor and recipient and GVHD (Leiden-Paris)

|              | non-HLA mismatches |    |       |
|--------------|--------------------|----|-------|
|              | not detected       | HA | other |
| Acute GVHD   | 19                 | 0  | 0     |
| Chronic GVHD | 2                  | 3  | 4     |

Table II. CML reactivity between HLA-identical donor-recipient combinations (Leiden-Paris)

|  | CML  | chron. GVHD | acute GVHD |
|--|------|-------------|------------|
|  |      | pos.        | neg.       |
|  | pos. | 6           | 0          |
|  | neg. | 2           | 4          |

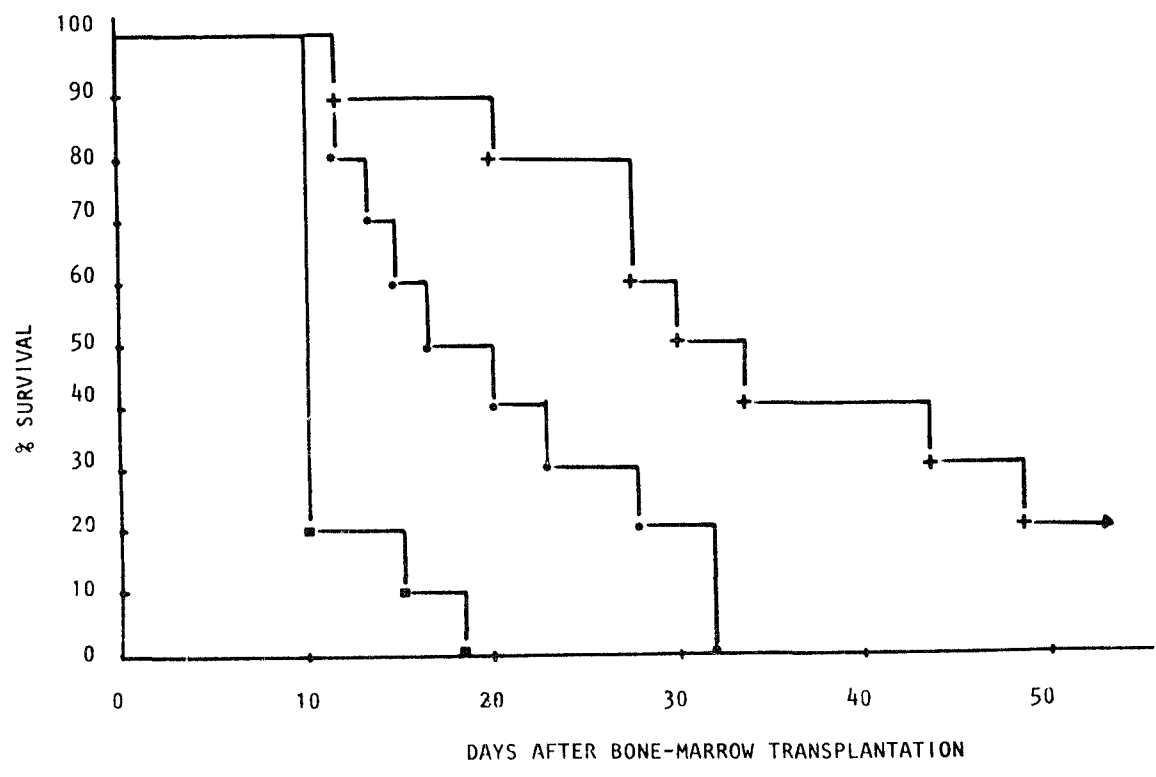
p = 0.02

Table I shows that in chronic GVHD but not in acute GVHD differences for HA (or its allele) could be found. Likewise a positive CML was found between post- and pretransplant lymphocytes from patients suffering from chronic GVHD but not from such patients suffering from acute GVHD (Table II). Our preliminary conclusion is that such CTL's can recognize non-HLA but MHC restricted determinants and that incompatibility for these determinants could lead to GVHD.

Immune modulation

We have discussed in previous reports the possibility that the finding that pretransplant blood transfusion can facilitate renal allograft survival might also be relevant in bone marrow transplantation. Recently it has been shown that in primates and mice not only whole blood but also pure platelets can induce this so-called pretransplant blood transfusion effect (4,5). In mice it was shown that heat pretreated leucocytes behaved as platelets as far as the graft protecting effect was concerned. On the basis of these findings it was investigated in a mouse model whether platelets obtained from the future recipient and given to the bone marrow donor would mitigate GVHD. Figure 1 shows that this is indeed the case. Note that if the donor receives recipient's leucocytes GVHD is more severe, while survival is improved (and GVHD is lessened) if pure platelets are given.

Figure 1. Survival of (B10.T(6R) x B10.A(2R))F1 mice following a bone marrow transplantation with B10.AQR spleen cells. Donor pretreatment: ■ = pretreated with recipient leucocytes; ● = not pretreated; + = pretreatment with heat-treated leucocytes of the recipient. (Claas et al. 1982)



Of course these data cannot be extrapolated to man without extensive preclinical testing (dogs, monkeys). Even if such experiments would corroborate the findings in the mouse, ethical considerations would

probably exclude their application in the clinical situation. However if we would understand the mechanism of the pretransplant blood transfusion effect we might be able to "treat" the bone marrow in vitro and induce in that manner the "blood transfusion effect" without harming the bone marrow donor.

THE UNRELATED DONOR

Haploidentical bone marrow donors (e.g. the parents) seem to give good results especially in younger leukaemic patients. For older especially aplastic anaemia patients unrelated well matched donors seem at the moment the best choice. Very little is known how "well matched" should be interpreted. Is complete identity necessary or can partially mismatched bone marrow be used as well? Especially relevant in this respect are the experiments by Wagemaker et al. who could show that stem cell preparation infused in decontaminated monkeys would not lead to GVHD if donor and recipient were matched for class I antigens, while matching for class II antigens seemed to be less important (6). Termijtelen et al. has described the difficulties of finding an HLA-A-B-C, -DR MLC negative donor (7). We understand now better than before why HLA-DR identical unrelated individuals are so often MLC positive. This is certainly due to incompatibility for MLC stimulating determinants other than HLA-DR. Two of these have been identified; products of the SB (PL3) locus to the left of HLA-DR and the LB-Q locus to the right. Mismatches for these determinants seem not to be a deterrent in renal transplantation; in bone marrow transplantation their relevance remains to be studied.

Leaving matching for the class II antigens aside, we have analysed the feasibility of finding class I identical bone marrow donors in a file of 10.000. For 28 patients who had no HLA identical sibling donor, we would have been able to provide for 18 of them 5 to over a 100 unrelated class I identical donors. Although the logistics offer thus for about 2/3 of our patients no unsurmountable problems the implementation of such an operation will take considerable time. Such protocols should be reviewed by the medical ethics committee, informed consent should be obtained as well as adequate reimbursement of the costs.

The question remains whether HLA identity is really necessary. The haploidentical grafts which often function so well, testify that this is not always the case. Recent data in renal allograft studies have provided us with data which are relevant in this respect.

They showed that some AB and DR mismatched renal grafts did extremely well. It was assumed that this was due to a low responder characteristic of the recipient, at least as far as HLA allo-antigens were concerned. The first significant evidence that immune response genes might be involved in developing immunity against non-HLA antigens was unearthed by Baldwin et al. when they showed that only HLA-DRw6 positive individuals formed antibodies against antigens present on some endothelial cells and monocytes (8). HLA-DRw6 appeared to act as an immune response (Ir) gene for these EM antigens.

This study was undermore criticized because it is notoriously difficult to type for HLA-DRw6. Recently however the definition of these class II antigens has been much improved (9) and it seemed to be a good moment to assess the importance of HLA-DRw6 as Ir gene for HLA alloantigens.

Figure 2 shows that overall renal graft survival is poorer in HLA-DRw6 positive than in negative ones. (10) A finding which in the meantime has been confirmed by others (P.J. Morris personal communication). In contrast matching for HLA-DR seems to be very effective in DRw6 positive and only of borderline importance in DRw6 negative recipients. That this was not a red herring is shown in figure 3, which shows the influence of matching for HLA-DR on renal graft survival in Eurotransplant (G.F.J. Hendriks personal communication).

Figure 2.

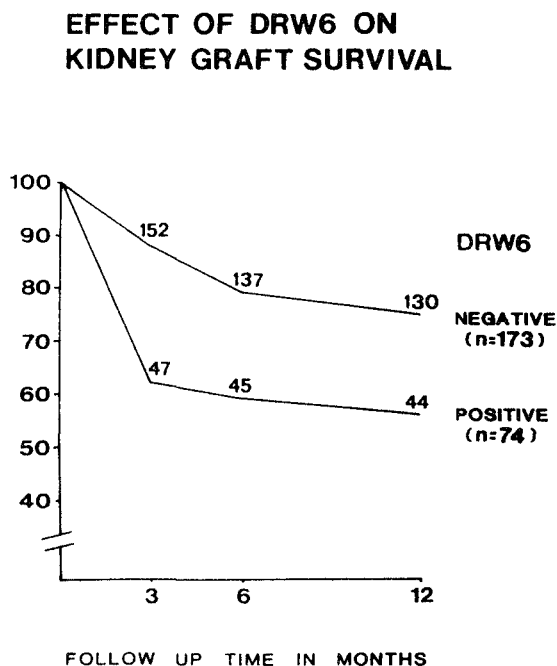
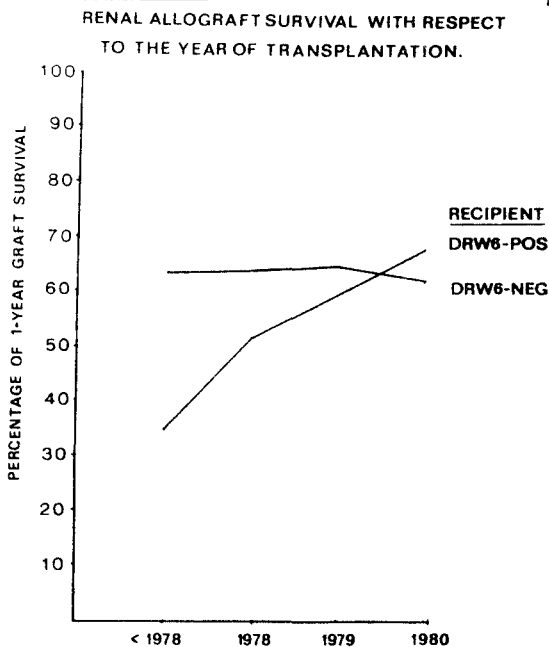


Figure 3.

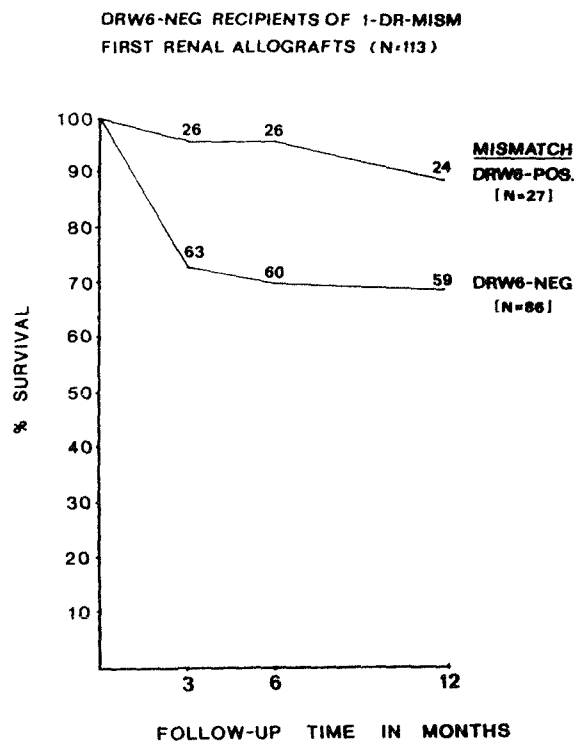


In the years before 1978 typing for HLA-DR was possible in Leiden only, thus donors were typed but not matched with recipients for HLA-DR. In 1980 most centers had implemented HLA-DR typing and matching became feasible. Over these years graft survival at one year post-transplant in the HLA-DRw6 negative group remained unchanged but in the HLA-DRw6 positive group rose from about 35% to over 70%. This is thus a confirmation on an independent set of data of the findings shown in figure 2. Finally it could be shown that in HLA-DRw6 negative individuals pretransplant blood transfusion improved graft survival quite substantially while in DRw6 positive individuals it appeared to have no effect. These are thus 3 different observations which all suggest that DRw6 is a strong Ir gene for incompatible HLA antigens, and that matching for DR (but not pretransplant blood transfusion) turns the DRw6 positive recipient from a high in a low responder. The situation appears to be inverted if DRw6 is present in the donor. In that situation it appears to act as an activator of suppressor cells. In figure 4 it is shown that if a HLA-DRw6 negative recipient is transplanted with kidney from a donor which is mismatched for one HLA-DR antigen, graft survival is excellent (>90%), if that antigen is DRw6 but only average ( $\pm$  70%) if it is any other DR antigen (p =

0.01). Similar findings were observed when the outcome of grafts which were mismatched for two DR antigens were analysed (Hendriks et al. to be submitted).

In conclusion, it appears that DRw6 in the recipient of a renal allograft acts as a strong Ir gene both for non-HLA and HLA antigens, but when it is present in the donor it acts as an activator of suppressor cells. To which extent these data can be extrapolated to the situation in bone marrow transplantation remains to be seen. The partly mismatched family transplants might be better material to study and this the working party on immunology plans to do.

Figure 4



Even if such studies would not reveal a relation between HLA-DRw6 and the prognosis of partly mismatched bone marrow grafts, the results in renal transplantation remain relevant for haematologists. This is so because they provide for the first time an indication that it is possible to activate the suppressor circuit in man. This is obviously not only important for the control of the homograft reaction, but also of the graft versus host reaction and possibly even of autoimmune disease.

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