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The influence of HLA-mismatches on phenotypic and functional characteristics of graft infiltrating lymphocytes after heart transplantation

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Abstract. We studied the influence of HLA mismatches on T lymphocyte cultures that were derived from endomyocardial biopsies (EMB) from 118 heart transplant recipients. From patients with DR mismatches, the majority of the EMB-derived cultures were dominated by CD4, while in patients without DR mismatches, CD8 was the predominant T cell subset. The majority (75%) of the cultures were cytotoxic against donor antigens. A significantly ($P < 0.005$) lower proportion of the cultures showed cytotoxicity (36%) against HLA-A antigens when compared to HLA-B (53%) or HLA-DR (49%). A dose effect phenomenon was detected for all HLA antigens, including HLA-A: a higher number of A, B or DR mismatches resulted in a higher number of cytotoxic cultures directed against these antigens. B and DR matching had the greatest influence on 6 month freedom from rejection. Both our experimental and clinical data indicated that HLA matching played a role in the immune response against a transplanted heart.

Key words: HLA matching – Graft infiltrating cells – Heart transplantation – Alloreactivity, Predominant phenotype

Several studies among kidney transplant recipients have shown a positive effect of HLA matching on graft outcome, especially for B and DR antigens [4, 8, 14]. The beneficial effect of DR matching has been found to be most evident in the first 5 post operative months, while the effect of matching for HLA-B antigens lasts longer [14]. In heart transplantation, the importance of HLA matching for graft survival is still debated, because in many studies numbers of patients are limited and, more importantly, numbers of well matched grafts are low, as donor hearts are randomly allocated to the recipients without reference to their HLA status. However, up to now the results indicate that HLA matching has a beneficial effect on graft survival [6, 9] or on the incidence of steroid resistant rejection [3].

The influence of HLA mismatches between donor and recipient on phenotypes and function of graft infiltrating cells has never been systematically studied. Therefore, we analyzed the effect of HLA-A, B and DR mismatches on the functional and phenotypic characteristics of these cells in a large series of endomyocardial biopsies (EMB) from 118 heart transplant recipients.

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Materials and methods

Patients. We studied 1285 biopsies from 118 heart transplant recipients transplanted between September 1984 and January 1990. All patients had received preoperative blood transfusions and all received cyclosporine and low dose prednisone as maintenance immunosuppression. The actuarial patient survival was 89% at 4 years. The mean number of HLA-mismatches between donor and recipient was 1.25, 1.62 and 1.40 for A, B, and DR, respectively. Three patients who died within 3 weeks after transplantation were excluded from this study. Endomyocardial biopsies (EMB) were taken at regular intervals. We received 4–22 biopsies from each patient (median 10).

Biopsies were cultured in interleukin 2 (IL-2) containing culture median. Phenotypes were analyzed by two-colour flow cytometry after staining with anti-Leu 4, WT31, Leu 2 and Leu 3 (Becton Dickinson, Mountain View, Calif). A more extensive phenotypic characterisation of the cultured cells is described by us in a previous study [10]. A 4-h ^{51}Cr release assay was used to measure the cytotoxic capacity against donor cells and a panel of unrelated target cells (EBV transformed B cell lines or PHA blasts) sharing one or more HLA antigens with the donor.

HLA typing. Spleen cells or peripheral blood mononuclear cells (obtained by Ficoll separation of heparinized blood) were typed for HLA class I antigens according to the standard NIH lymphocytotoxicity assay, and typed for HLA-DR by the two-colour fluorescence assay with a set of highly selected antisera [11].

Results

DR mismatches and CD4/CD8 phenotypes

In the first 180 days, the number of DR mismatches had a pronounced influence on the phenotypic composition of the EMB-derived lymphocytes (Fig. 1). Cultures from pa-

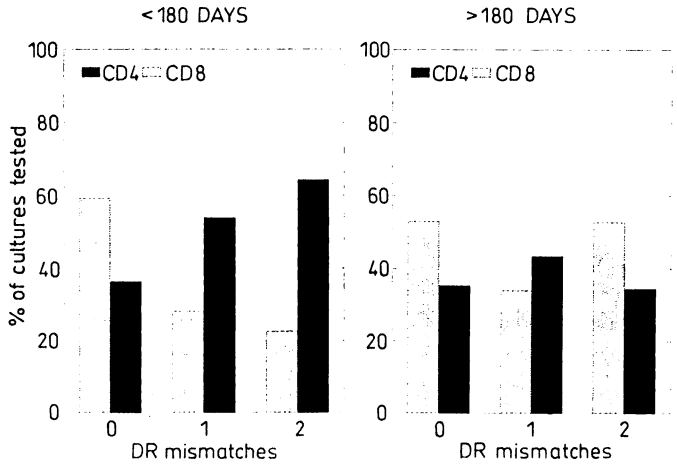

Fig. 1. Predominant phenotype of EMB-derived lymphocyte cultures in relation to the number of HLA-DR mismatches between donor and recipient

tients without DR mismatches were most often dominated by CD8, while in cultures from patients with DR mismatches CD4 was the predominant T cell subset in the majority of cultures. After these first 6 post transplant months, no significant differences were found between the groups.

Cytotoxicity

As described by us in a previous study [10], the majority (75%) of the cultures tested (n = 324) were cytotoxic against donor antigens. In the first 6 months after transplantation a high proportion of the cultures was found to be cytotoxic against HLA-B and DR mismatches between donor and recipient, and significantly fewer were cytotoxic against HLA-A antigens (Table 1). After 6 months these differences were no longer detectable, because of a decline in the incidence of HLA-B and DR directed cytotoxicity.

HLA-mismatches and acute rejection

In the DR matched patient group 56% of patients remained free from rejection of 6 months, compared with 29% of patients with one DR mismatch and 22% with two DR mismatches. For the combination of HLA-B and DR antigens a significant effect on freedom from rejection was found: 37% in patients with two or less B and DR mismatches and 24% in patients with three or four B and DR mismatches at 6 months (P = 0.05, log rank test). No significant relationship between the number of acute rejection episodes in the 1st year and the number of mismatches on the individual A, B or DR locus was observed.

Discussion

Direct CML assays of biopsy-derived lymphocytes revealed that in the first 6 months after transplantation, HLA-B and DR antigens were important epitopes for cytotoxic lymphocytes. Cytotoxicity against B and/or DR mismatches was found significantly more often than against HLA-A antigens. This may account for the significantly lower freedom from rejection rates in the patient group with more than two B and DR mismatches. This association between the number of B and DR mismatches and freedom from rejection has also been described by others [7]. Studies on the effect of matching for HLA antigens in renal [4, 8, 14] and heart [6, 9] transplantation have shown that matching for HLA-B and DR has a significant influence on graft survival.

Table 1. CML specificity of EMB-derived cultures against panel cells sharing mismatched HLA A, B or DR antigens with the donor. Relation with time after transplantation. In the first 6 months, the incidence of HLA-A directed cytotoxicity was significantly lower than that against B or DR antigens ($P < 0.001$)

	Numbers of reactive cultures		
CML specificity	< 180 days n (%[1])	> 180 days n (%)	P value[2]
HLA-A	79 (38)	28 (33)	n.s.
HLA-B	127 (57)	38 (40)	< 0.01
HLA-DR	120 (55)	34 (37)	< 0.01

[1] Percentage reactive cultures
[2] X^2 test

In the one and two DR mismatched heart allograft recipients, significantly more CD4-dominated cultures were derived from the biopsies than in the DR-matched group. $CD4^+$ cells are known to be of crucial importance in initiating rejection [1, 5]. Interaction of these cells with donor class II MHC antigens, expressed on the graft tissue and an passenger leucocytes of donor origin, results in activation of $CD8^+$ cells which recognize MHC class I antigens. Both $CD4^+$ and $CD8^+$ cells play a role in the rejection of grossly mismatched graft. Rejection of class I disparate grafts appears to be most dependent on $CD8^+$ cells, although $CD4^+$ cells can be activated as well via presentation of donor MHC class I antigens on recipient antigen presenting cells in the context of HLA class II molecules. Our data were compatible with this theory. A decline in the number of the CD4 dominated cultures was found after 180 days post transplant. Also the the incidence of DR directed cytotoxicity showed a significant decline in this period. This may be due to a lower expression of donor type class II antigens on graft tissue, and the replacement of donor dendritic cells by the patient's antigen presenting cells after such a long period following transplantation [12, 13]. As a consequence, fewer class II specific $CD4^+$ lymphocytes may be attracted to the graft.

In conclusion, we showed that in the first 6 months after transplantation, the number of DR mismatches between donor and recipient had a pronounced influence on CD4 predominance in EMB-derived cultures. Furthermore, in this period a significantly higher percentage of cytotoxic cultures was directed against HLA-B and DR mismatches than against HLA-A. This was in keeping with results of graft survival studies in renal and heart transplantation. A higher incidence of freedom from rejection was associated with a lower number of HLA-B and DR mismatches. This study showed that HLA matching between donor and recipient played a role in the immune response against a transplanted heart.

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