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Mismatch-specific anti-HLA antibody production following aorta transplants

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Abstract In this study, we have investigated the nature and magnitude of the immunological response after implantation of human aortic segments. Five recipients of aortic segment replacement were studied for anti-HLA antibody production (specificity and Ig class), CD3, CD4, and CD8 T cell subpopulation dynamics, and aortic wall thickness. Mismatch-specific IgG antibodies to HLA class I and HLA class II antigens were first detected 1–3 months after implantation and persisted in high concentrations for at least 1 year. Computer tomography scanning showed a progressive thickness of the aortic wall. Also the absolute number of CD3, CD4, and CD8 positive lymphocytes increased progressively after implantation. In conclusion, as was observed earlier for heart valve allografts, human implanted aortic segments induce a strong anti-HLA antibody response in recipients. We speculate that these antibodies have the potential to harm the implant, for example, by having an impact on luminal narrowing.

Key words Aortic segment · Implantation · Anti-HLA antibody · Aortic wall thickness · PRA-STAT

Introduction

Clinical indications for aortic segment implantation are restricted to untreatable aorta-iliac, aorta-bifemoral, and femoropopliteal prosthesis infection. The estimated number of patients treated is small (300–500 worldwide). Despite intensive investigation of animal models [1], little or no data in humans are available on: 1) HLA immunogenicity of aortic segments (cryopreserved or stored at 4 °C), 2) the patient's immune response to implanted aortic segments, and 3) biological assessment of aortic tissues after implantation. In this study, we have evaluated anti-HLA antibody production, CD3, CD4, and CD8 T cell subpopulation dynamics, and aortic wall thickness in five patients who received aortic segment transplants.

Patients and methods

Five patients with aorta-iliac or aorta-bifemoral prosthesis infection received aortic segments from cadaver donors with an identical AB0 blood group. The aortic segments had been stored at 4 °C in modified RPMI 1640 solution for 2 days before transplantation. In all cases, recipient and donor were typed (by serological and/or molecular techniques) for HLA-A, -B, and -DR antigens. The pretransplant cross-match was negative. After aortic segment implantation, patients were treated with cyclosporine (3–5 mg/kg per day).

Anti-HLA antibody production [percentage panel reactivity (% PRA) as well as antibody specificity] was evaluated using two different techniques: standard complement-dependent cytotoxicity (CDC) and PRA-STAT (SangStat, Nantes, France). PRA-STAT is an enzyme-linked immunoabsorbent technique that allows simultaneous detection and characterization of serum IgG directed against both HLA class I and II antigens. Both methods were performed as described previously [2]. The absolute number of peripheral blood CD3, CD4, and CD8 positive lymphocytes was determined by standard flow cytometry techniques. Computer tomography scanning was performed on regular occasions after transplantation.

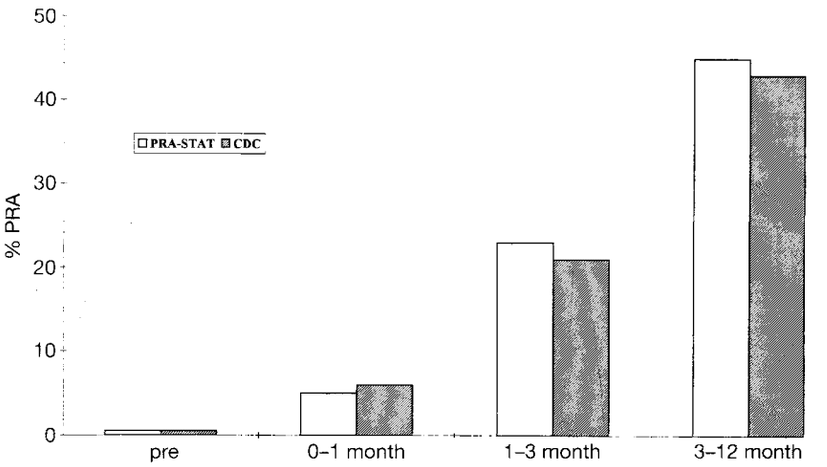

Fig. 1 Percentage panel reactivity (mean for five patients) determined by both PRA-STAT and complement-dependent cytotoxicity *(CDC)* methods at different time periods after aortic segment implantation

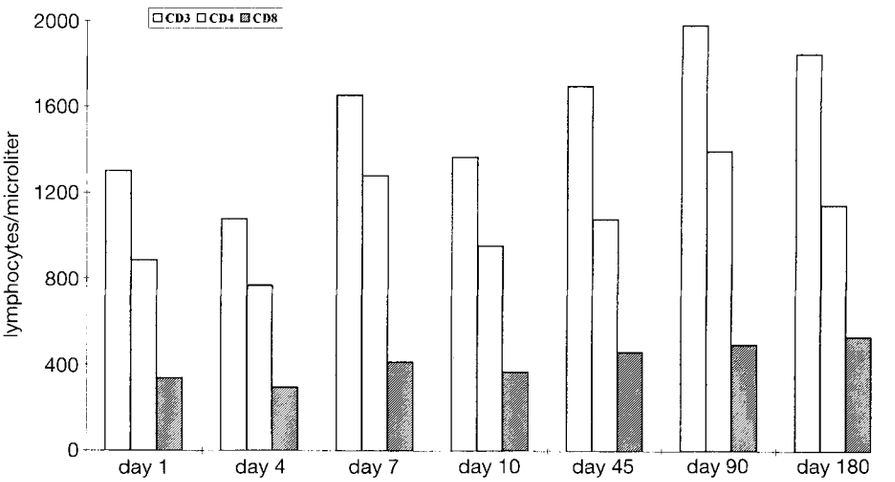

Fig. 2 Number of CD3, CD4, and CD8 T cell subpopulations (mean for five patients) at different time periods after aortic segment implantation

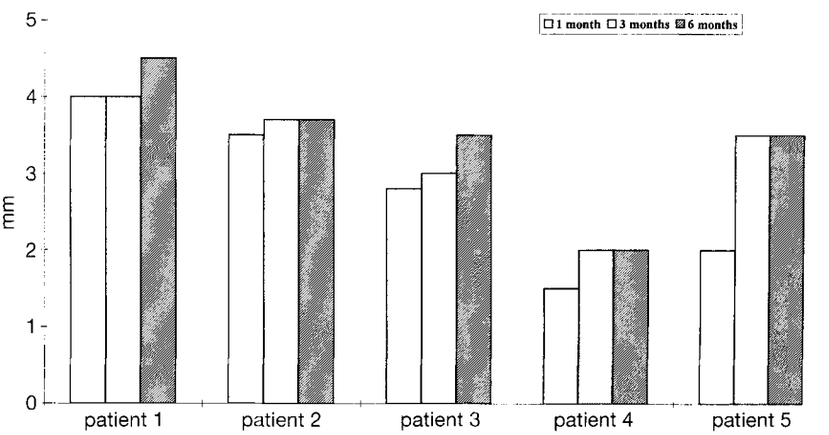

Fig. 3 Evolution of aortic wall thickness after implantation

Table 1 PRA-STAT evaluation (percentage panel reactivity and antibody specificity) for each patient

Patient	PRA-STAT detected anti-HLA specificity	HLA mismatch
1	B5(51), B13	A29, A30, B5(52), B18, DR6(13), DQ1(6)
2	**A10,** B15, B22(54), DR1, DR2, **DQ1(5)**	A3, **A10,** B35, DR6(13), **DQ1(5),** DQ1(6)
3	**A1,** A9(24), A11, **B8, DR3(17),** DR7, **DQ2**	**A1, B8, DR3(17), DQ2**
4	A2, A32, A33, B5, **DR2, DR4, DQ1(6), DQ3(8)**	A30, B14, **DR2, DR4, DQ1(6), DQ3(8)**
5	**A2,** DQ3(7)	A1, **A2,** B5, DR2(16), DQ1(5)

Results

On the day of surgery, before segment implantation, antibodies could not be detected (0% PRA) in any of the patients. As early as 1 month after transplantation, a significant increase in % PRA was observed in all patients by both CDC and PRA-STAT screening methods (Fig. 1). For four of the five patients, PRA-STAT data showed that the sera tested contained complex mixtures of anti-HLA class I and class II antibodies. Of importance, these antibodies were directed against specificities of the mismatch (Table 1). For the fifth patient, only anti-HLA class I antibodies were detected against a specificity (B51) that cross-reacts with the mismatch (B52) (Table 1). All recipients produced antibodies of the IgG class (the conjugate in the PRA-STAT assay is specific for human IgG and, as a consequence, no IgM antibodies can be detected. The absolute number of peripheral blood CD3, CD4, and CD8 positive lymphocytes increased progressively after aortic segment implantation, probably in relation to the ongoing anti-HLA immune response (Fig. 2). The CD4/CD8 ratio, on the contrary, did not change (results not shown). Computer tomography scanning showed a progressive thickness of the aortic wall (Fig. 3).

Discussion

In this study, we showed that human implanted aortic segments induce a strong anti-HLA antibody response. PRA-STAT data demonstrate that mismatch-specific IgG antibodies for HLA class I as well as class II antigens are produced. The antibody response became detectable during the 1st month after surgery and persisted for at least 6–12 months in all five patients studied. The fact that the antibody response was of the IgG class and was still marked 1 year after implantation would require a sustained nature of the antigenic stimulus. Most likely, viable dendritic and/or endothelial cells, capable of synthesizing and presenting donor HLA class I and II antigens to recipient T cells, remain present in the implanted aortic segment. Alternatively, donor HLA antigens could be released into the circulation. These data confirm similar observations in human heart valve allografts, where donor-specific cellular as well as humoral immune responses have been reported [3–7].

The posttransplant immunological activation we observed supports the existence of the antigen allorecognition mechanisms recently hypothesized by Callow [8]. In these patients, despite the use of cyclosporine A, the immune system appears active; the risk of infection due to immunosuppressive treatment is, therefore, limited. A potential cause of antibody production is perioperative blood transfusions. However, no correlation between blood transfusion and antibody formation was observed in our study. In combination with the fact that the antibodies are specific for the mismatch antigens of the aorta donor, we feel confident that the antibodies are not evoked by blood transfusions. The rat arterial model described by Plissonnier [9] suggests that sequential early cellular and late humoral injury takes place on different targets (allogeneic endothelial cells and medial smooth muscle cells, respectively). The mechanisms operating in our patients could be very similar. The clinical significance of this response requires further investigation. We speculate that these antibodies could have deleterious effects on the implant. For example, they could play a role in the progressive luminal narrowing, that was observed in all 5 patients. Because aortic segments are not considered to be immunogenic, implantation is performed without HLA matching and no immunosuppressive therapy is given. Our data show that, at least for high-risk patients, strategies aimed at reducing the immune response should be taken into consideration: prospective matching, low-dose immunosuppression, and preoperative manipulation of the segment to reduce its antigenicity. In conclusion, our data suggest that aortic tissues continue to maintain biological activity for a considerable period after implantation. They should, therefore, be regarded as a biologically active real vascular transplant rather than a mechanical tissue implant.

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