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Decrease of blood type antigenicity over the long-term after ABO-incompatible kidney transplantation

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ABSTRACT

Background: Few studies have investigated the changes in the antigenicities of the transplanted organs after transplantation.
Methods: We examined, by immunohistochemical assay, the changes in expression of the blood-type antigens on the transplanted kidneys over the long-term after ABO-incompatible kidney transplantation with A- or B-antigen incompatibility (blood type A to B and B to A). The subjects were six patients, including one case with graft loss, who had received ABO-incompatible kidney allografts more than ten years previously. As normal controls, four cases of ABO-compatible transplantation during the same period, including two recipient/donor pairs each with blood group A1 and blood group B were enrolled.
Results: Expression of the blood-type A or B antigens decreased gradually to 91.8% during the first three months after transplantation, 85.8% during the first five years, 64.1% during the first ten years, and 57.6% over ten years after transplantation on average in ABO-incompatible transplant recipients. In one patient with graft loss due to severe antibody-mediated rejection, the donor's type B blood-type antigens on the transplanted graft had changed but partially to the recipient's blood-type A antigen by 2582 days after the transplantation, suggestive of the occurrence of blood-type chimerism on the endothelium. In ABO-compatible transplant recipients, such changes in expression were not observed. The average percentage of blood-type antigen-positive vessels at more than ten years after the renal transplantation was 99.8%.
Conclusions: Decrease in the expression of the donor's blood-type antigen on the endothelium of the graft has been considered as one of the mechanisms underlying the accommodation occurring over the long-term after ABO-incompatible kidney transplantation. On the other hand, establishment of antigenic chimerism on the graft endothelium could be one of the hallmarks of the immunological reaction associated with antibody-mediated rejection.

1. Introduction

We have performed more than 432 ABO-incompatible transplantations at our center since 1989 [1]. More than 20 years have elapsed since the introduction of ABO-incompatible transplantation in Japan because of the serious problem for organ shortage [2]. Studies of transplantation across the blood group barrier provided the first definitive evidence that hyperacute rejection is caused by anti-donor antibodies. Such surgery therefore became a testing ground for various methods of preventing hyperacute rejection. Numerous strategies based on the removal of anti-blood type antibodies have been reported for successful transplantation across the blood group barrier. However, some studies have reported that acute antibody-mediated rejection (AAMR) still occurs in up to 33% of cases undergoing ABO incompatible kidney transplantation [1,3]. Among cases in which AAMR is triggered in the early postoperative period, the prevalence of transplant glomerulopathy (TGP) at one year post transplantation is reported to be 22% [1]. Aita et al. [4] suggested that thickening of the peritubular capillary basement membrane can be a novel diagnostic marker of chronic rejection. These findings associated with chronic antibody-mediated rejection (CAAMR) that is related to the damage of endothelial cells. Endothelial cells that are found at the interface between the graft and the immune system of the recipient play a major role in the immunological processes occurring after organ transplantation.

In general, complement binding to the incompatible grafts, as shown by C4d deposition on the peritubular capillary basement membrane, has been clearly demonstrated to occur to some or the other degree in all recipients of ABO-incompatible transplantation [5,6]. However, patients have been encountered who show negative conversion of C4d

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deposition over the long-term after ABO-incompatible transplantation, after detection of positive C4d staining immediately following the transplant surgery. These findings suggest the possible occurrence of some qualitative changes on the graft endothelium that might lead to the C4d-negative conversion observed over the long-term after kidney transplantation.

2. Objective

In this study, we investigated the changes in the blood-type antigens on the transplanted grafts by immunohistochemical assay on serial graft biopsies obtained for more than ten years after kidney transplantation.

3. Material and methods

All study procedures were approved by the Ethics Committee of Tokyo Women's Medical University. Written informed consent was obtained from all of the patients in the renal transplant program at our institution who were enrolled in this study. Clinical and laboratory information was extracted from the electronic databases and patient medical records.

3.1. Patients

Recipients or donors with blood group O were excluded from this study, since the H epitope is not expressed on renal endothelial cells. From October 1989 to December 2001, we performed six consecutive living-donor kidney transplant recipients with blood group B who had received renal allografts from donors with blood group A1, and thirteen living donor kidney recipients with blood group A1 who had received renal allografts from donors with blood group B at our department at the Tokyo Women's Medical University. Nine of the 19 allografts were removed from this study because of graft failure at short period after kidney transplantation. Among the remaining 10 patients who still had functioning allografts, serial biopsies, at least three times, could not be obtained in four patients, because the patient refused to provide consent. Among all nineteen patients, six cases were enrolled in this study, because they had been followed up for a long duration of time (at least 10 years) and they had undergone three or more transplant biopsies, at least one of which was more than 5 years post-transplantation. As normal controls, cases of ABO-compatible transplantation during the same period, including two recipient/donor pairs each with blood group A1 and blood group B were enrolled.

3.2. Immunosuppressive regimens

Before 1996, cyclosporine (CsA), azathioprine (AZ), methylprednisolone (MP), deoxyspergualine (DSG), and antialymphocyte globulin (ALG) were used in the induction phase, as described previously [2]. Since 1996, Tacrolimus (FK) has been used as the calcineurin inhibitor in place of CsA and since 2000, MMF has been used as the antimetabolite immunosuppressive drug in place of AZ. In all the ABO-incompatible recipients, three or four sessions of double-filtration plasmapheresis and/or some sessions of plasma exchange were undertaken before the surgery and splenectomy was performed simultaneously at the time of the transplantation.

3.3. Biopsies

At our center, 0-hr protocol renal allograft biopsy has been performed in all patients for more than two decades. Subsequent protocol biopsies are performed between one and three months after the transplantation. Episode biopsy is performed in cases with suspected rejections. Histologic evaluation was performed in our cases by two independent observers on formalin-fixed paraffin sections stained with hematoxylin and eosin, periodic acid-Schiff stain, Masson trichrome stain, and periodic acid methenamine silver stain. The renal histopathology was then characterized according to the Banff classification 2007 [7] and light-microscopic photographs were obtained (AX-80: Olympus, Tokyo, Japan).

3.4. Immunohistochemistry

Formalin-fixed, paraffin-embedded blocks were sectioned at 3 μm, then deparaffinized and rehydrated. Endogenous peroxidase activity was quenched with hydrogen peroxide. The primary antibodies, anti-blood-group A (clone HE-193; Abcam, Tokyo, Japan) and anti-blood group B (clone HEB-29; Abcam, Tokyo, Japan) antibodies, diluted 1:40, were applied, followed by incubation at room temperature for 60 min in phosphate-buffered saline/1% bovine serum albumin and then with the Histofine Simple Stain MAX-PO (Nichirei, Tokyo, Japan) for 30 min. Mouse anti-CD34 (clone QBEnd/10, Novocastra, UK), diluted 1:50, was also used to identify the endothelial cells. Immunohistochemical reactivity is detected by incubation with diaminobenzidine tetrahydrochloride (DAB) for 5 min to produce a brown color, followed by counterstaining of the sections with hematoxylin. For immunohistochemical double staining, sections were first reacted with anti-blood-group A antibody and then with Histofine Simple Stain AP, reactivity is detected by PermaBlue/AP (Japan Tanner Co, Japan). Next, the sections were reacted with anti-blood-group B antibody as described above, and coloring with PermaRed/AP.

C4d staining is routinely performed in all renal allografts by indirect immunofluorescence on cryostat sections, using a mouse monoclonal anti-human C4d antibody (1:40; Quidel, San Diego, CA) and fluorescein isothiocyanate-conjugated goat anti-mouse IgG.

3.5. Quantification of immunostaining

To assess the blood-type antigenicities, we calculated the percentage of blood-type antigen-positive vessels and CD34-positive vessels. Immunohistochemical staining for CD34 is known as an endothelial cell marker in kidney tissue [8]. The count of vessels positive for blood-type A and B antigens was established by counting the number of positive capillaries, venules and arterioles per defined surface area at a magnification of ×400 in 20 fields under a light microscope, excluding the glomerular area. In serial sections, CD34-positive vessels were also counted the same way. The results were expressed as percentages of capillaries positive for blood type A or B antigens as compared with the total number of capillaries defined by the density of capillaries positive for CD34.

4. Results

4.1. Patients

Table 1 shows the clinical characteristics of the ABO-incompatible recipients, including the age, gender, primary disease, HLA-mismatch, initial serum anti-A or B antibody titers, and the induction immunosuppression therapy employed. The patients consisted of three males and three females, with a mean age of 38 years (range 25–52). The primary renal disease was chronic glomerulonephritis or IgA nephropathy in all the patients. The initial anti blood-type IgG titers ranged between 1:2 and 1:128. Half of all the patients were treated with an immunosuppressive drug regimen based on CSA and TAC-based regimen that was used in the other three patients. Splenectomy at the time of the renal transplant was undertaken in all the patients.

Four patients, including three males and one female (mean age, 36.8 years; range, 23–48 years), who underwent ABO-compatible living kidney transplantation during the same period were enrolled as the control group. The primary renal diseases in this group were chronic glomerulonephritis (#8, #10), membranoproliferative glomerulonephritis (#7), and polycystic kidney disease (#9). Three patients received a TAC-based immunosuppressant regimen, while the remaining one patient received a CSA-based regimen (data not shown).

4.2. Allograft function

The findings on renal biopsy and the allograft function are shown in Table 2. The mean follow-up period was 4620 days (range 3088–6886). Allograft function was