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ORIGINAL ARTICLE

The mechanism responsible for accommodation after living-related kidney transplantations across the blood barrier

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Summary

The mechanism responsible for accommodation in renal transplantations across the blood barrier remains unclear. We recently encountered two patients with accommodated status after living-related kidney transplantations across the blood barrier. Both developed elevations of anti-blood-group antibodies to titers over 128× after transplantation, despite excellent renal function. We investigated the serum samples after the establishment of accommodation bound to the erythrocyte membrane of the donors or the third party with the same blood group. After the establishment of accommodation, the serum samples from both accommodated patients demonstrated a significant decrease in binding to the donors' erythrocyte membrane, but did not show any decrease in binding to the erythrocyte membrane of the third party. By contrast, serum samples from patients with graft loss after unsuccessful accommodation showed high anti-blood-type antibody activity directed towards both the donor's and the third party's erythrocytes. The result of this study suggests the difference of quality in antibodies produced by accommodated and nonaccommodated recipients.

More than 200 renal transplantations across the blood barrier have been performed in our institution [1,2]. ABO-incompatibility is the most important risk factor affecting long-term graft survival time in ABO-incompatible renal transplantations. Natural antibodies induce hyperacute rejection of grafts, but if they are removed from the recipient prior to transplantation, one of three types of immune response may occur [3]. (i) Most patients seem to become immunotolerant to the incompatible blood antigens because they do not reject the incompatible graft and do not produce anti-A or anti-B antibodies against it; (ii) 2–5% of patients produce antibodies to the incompatible blood antigens that mediate rejection of the graft, and they ultimately lose the grafts; (iii) some patients display accommodation state of the allograft, which is characterized by antibody production, but the graft is not rejected. The apparent resistance of a vascularized graft to humoral rejection despite the presence of antibodies in the recipient's body is 'accommodation' [4]. The phenomenon, in which temporary depletion of anti-blood group antibodies allows the permanent engraftment of an ABO-incompatible organ, was referred to as accommodation. The concept of accommodation and the success of ABO-incompatible renal transplantations provide important information toward the clinical application of xenograft. The clinical utility of xenotransplantation would be significantly increased by achieving accommodation, because it would allow the withdrawal of treatments aimed at depleting natural antibodies and complements from the recipients. Not only does the occurrence of accommodation eliminate some of the need for continuing immunomodulation, but it may also provide clues to manipulate the donor or recipient that might prevent humoral rejection or the development

Table 1. Patient characteristics in this study.

	Patient 1	Patient 2	Patient 3	Patient 4
Age (years)	20	32	34	36
Gender	F	M	F	F
Day of rejection	–	–	31	30
Cr*	1	1.1	HD	HD
Maximal Ab†	×128	×256	×256	×128
ABO incompatibility donor	AB/B‡	A/B	B/O	A/O
Age (years)	45	35	40	60
Relation	Mother	Sibling	Sibling	Mother

*Serum creatinine level at the time of discharge.
†Maximal anti-blood-type antibody titer by saline agglutination assay.
‡Donor blood type/recipient blood type.

of chronic rejection. Thus, the study for accommodation may provide a new insight in various medical fields, not just in the transplantation field.

We encountered two recipients with excellent renal function whose anti-blood-type titers rose to more than 128 times after the operation (patients 1 and 2 in Table 1). One patient (patient 1) was blood-type B and received a blood-type AB renal graft from her mother. The other patient (patient 2) was blood-type B and received a blood-type A renal allograft from his sibling. The anti-blood-type antibody titers of these accommodated patients showed similar changes, namely a rapid decrease to less than four times immediately after surgery followed by a gradual increase to 128 times within 1 month of transplantation. The serum creatinine levels were very stable, 1.0 and 1.1 mg/dl, throughout this period.

Patients who lost their renal grafts and had high elevations of anti-blood-type antibody titers were also selected as subjects of this study (patients 3 and 4 in Table 1). One of them (patient 3) developed oliguria and a rapid increase in serum creatinine 3 weeks after renal transplantation, and the pathological findings showed severe antibody-mediated vascular rejection. The other patient (patient 4) who lost the graft experienced deteriorating renal function associated with antibody elevation to over 128 times 1 month after transplantation. No pathological confirmation was obtained because of the high risk of puncturing the graft which was swollen by the vigorous humoral response. Unfortunately, patients 3 and 4 were discharged from the hospital without achieving diuresis.

All four patients in this study underwent three sessions of plasmapheresis prior to surgery to decrease their anti-blood-type antibody titer to less than 16 times, and splenectomy was routinely performed during the transplantation procedure [2]. An immunosuppressive regimen consisting of tacrolimus (FK506), methylprednisolone and mycophenolate mofetil was used in the induction phase.

We investigated the antibody specificity in these patients' serum by enzyme-linked immunosorbent assay (ELISA). ELISA is more sensitive than the classical agglutination assay and was used to detect humoral changes in IgM or IgG classes [5–7]. Briefly for performing ELISA, red cell membranes were obtained by hypotonic shock lysis and repeatedly washed until the ghosts are white. Suspensions of red cell ghosts were brought to a concentration of 2 mg/ml in carbonate buffer (pH 9.5), and dried overnight as 50 μl aliquots per well in ELISA plate (Falcon 3912; Falcon, Franklin Lakes, NJ, USA). The dried membranes adhere to the ELISA wells and are blocked with 1% phosphate bicarbonate saline (PBS)–bovine serum albumin (BSA). Serum samples from patients were administered on the plates at serial twofold dilutions in PBS–BSA. After 2 h incubation at room temperature, the plates were washed with PBS containing 0.05% Tween, then alkaline phosphate coupled rabbit anti-human antibodies IgM or IgG (Sigma, diluted 1:1000) was plated in 50 μl aliquots for 1 h, as secondary antibody. p-nitrophosphate (Sigma) was added for color reaction and absorbance was measured at 405 nm. It should be stressed that this assay cannot be performed with peroxidase coupled secondary antibody, because the residual peroxidase activity in the red cell ghosts generates a very high background for the assay. The experiments were carried out in triplicate with plates coated on different days. Statistical comparisons were made by the unpaired Student's *t*-test. A value of <0.01 was regarded as significant.

Patients' serum that contained maximal antibody titer was used as samples in this study. Erythrocytes were harvested from each of the donor, and from healthy volunteers with various blood types as controls. The erythrocyte membranes of each blood type used in this experiment were mixtures from at least five volunteers, because there are individual differences in blood antigen expression on erythrocyte membranes.

Figure 1a and b show anti-blood-type antibody activity in patient 1 who was in the accommodated state, before and after renal transplantation. As expected, before transplantation, the serum from patient 1 with an anti-blood type A antibody titer over 128× showed strong activity, stronger than 3.0 OD against blood group A antigen (Fig. 1a). However, after the establishment of accommodation, binding of the anti-blood type A antibody of patient 1 to the donor's membrane was clearly suppressed, although it easily bound to the erythrocytes from the third party with the same blood group (mean antibody binding suppression at each serum dilution: 65%) (Figs 1 and 2). This hypo-responsiveness was also observed in the other accommodated patient (Fig. 2a and b). Furthermore, we investigated hypo-responsiveness in the patients without

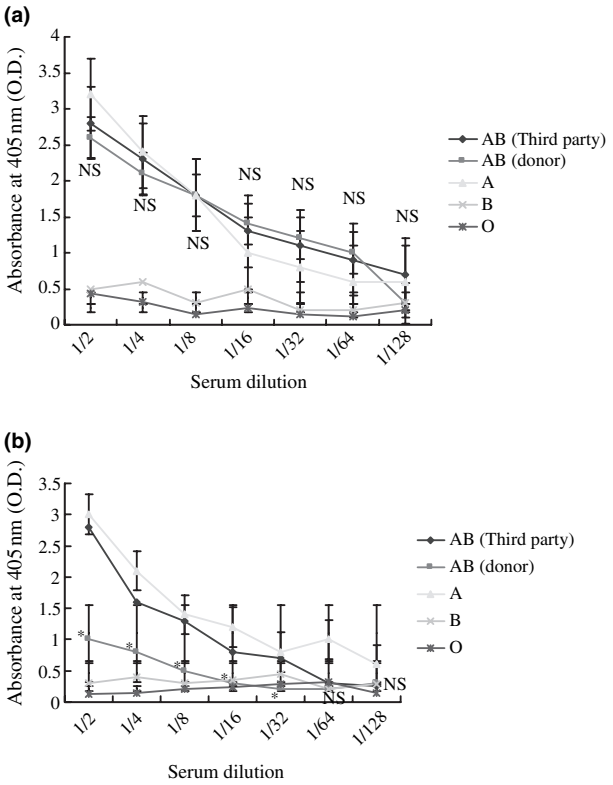

Figure 1 (a) ELISA of serum from patient 1 (anti-blood-type A before plasmapheresis). Patient 1 (blood type B) had a very high anti-blood type A titer, ×128, on admission (before plasmapheresis to remove antibody). As expected, the serum with high anti-A titer attached to blood-type AB (third party and donor) and blood type-A, but not to blood type-B or -O. Points represent the means of triplicate measurements, bars represent SEM. Statistical comparisons were made using the unpaired Student's *t*-test between antibody-binding to donor and third party erythrocytes. A value of <0.01 was regarded as significant (*). (b) ELISA of serum from patient 1 (anti-blood-type A: 2 weeks after transplantation). Patient 1 had high anti-blood-type A titer, ×128, postoperatively despite having excellent renal function. The serum at this time did not attach to the donor's erythrocyte membranes, but it attached strongly to other A antigens. Points represent the means of triplicate measurements, bars represent SEM. Statistical comparisons were made using the unpaired Student's *t*-test between antibody-binding to donor and third party erythrocytes. A value of <0.01 was regarded as significant (*).

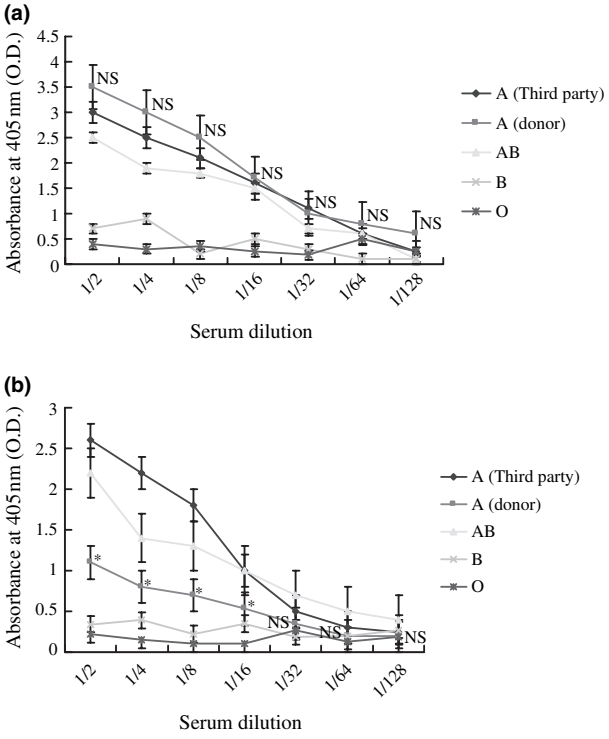

Figure 2 (a) ELISA of serum from patient 2 (anti-blood-type A before plasmapheresis). Patient 2 (blood type B) had also high anti-blood-type A titer, ×256, on admission (before plasmapheresis). As expected, the serum with a high anti-A titer attached to blood-type A (third party and donor) and blood-type AB erythrocyte membranes, but not to blood-type B or -O. Points represent the means of triplicate measurements, bars represent SEM. Statistical comparisons were made using the unpaired Student's *t*-test between antibody-binding to donor and third party erythrocytes. A value of <0.01 was regarded as significant (*). (b) ELISA of serum from patient 2 (anti-blood-type A: 2 weeks after transplantation). Patient 2 had a high anti-blood type A titer of 128 × postoperatively, despite having excellent renal function. The serum at this time did not attach to the donor's blood-type A erythrocyte membranes, although it attached strongly to other A antigens. Points represent the means of triplicate measurements, bars represent SEM. Statistical comparisons were made using the unpaired Student's *t*-test between antibody-binding to donor and third party erythrocytes. A value of <0.01 was regarded as significant (*).

accommodation after transplantation. The results (Fig. 3a and b) showed that the anti-blood-type antibody in patients without accommodation attached to all relevant erythrocyte membranes, suggesting that, in these non-accommodated recipients, there was no hypo-responsiveness of antibody against donor's erythrocyte membranes as clearly seen in accommodated patients 1 and 2.

We investigated the time course changes in anti-blood-type antibody titers during follow up by ELISA (Fig. 4a and b). Hypo-responsiveness persisted in patients 1 and 2 for at least 1 month. Exposure of B cells capable of producing anti-blood-type antibodies for more than 1 month to corresponding incompatible blood group antigens may result in induction of tolerance in which no antibodies can be produced.

As a possible explanation of this mechanism of accommodation in this study, we have proposed one hypothesis about the presence of non-blood-group antigens and non-blood-group antibodies that varies between individuals. In the publishing data regarding the mechanism for accommodation in α-gal K/O mouse model [6,7], Tanemura *et al.* attributed the difference in accommodation

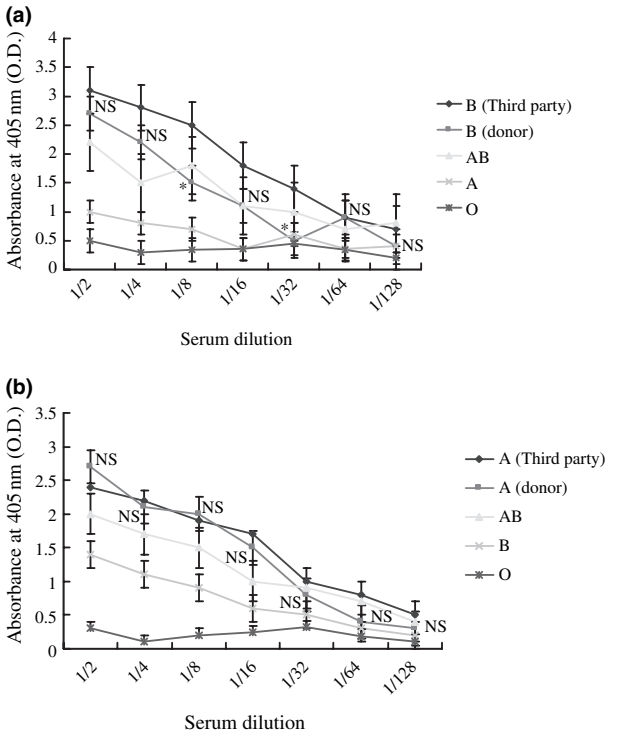

Figure 3 (a) ELISA of serum from patient 3 (anti-blood-type B: 2 weeks after transplantation). Patient 3 lost the graft approximately 1 month postoperatively. The serum sample at the time the graft was lost did not show hypo-responsiveness of patient 3's antibody to donor's erythrocyte membrane, unlike patients 1 and 2. Points represent the means of triplicate measurements, bars represent SEM. Statistical comparisons were made using the unpaired Student's *t*-test between antibody-binding to donor and third party erythrocytes. A value of <0.01 was regarded as significant (*). (b) ELISA of serum from patient 4 (anti-blood-type A: 2 weeks after transplantation). Patient 4 also lost the graft approximately 1 month postoperatively. The serum sample did not show hypo-responsiveness of patient 4's antibody against the donor's blood antigen. Points represent the means of triplicate measurements, bars represent SEM. Statistical comparisons were made using the unpaired Student's *t*-test between antibody-binding to donor and third party erythrocytes. A value of <0.01 was regarded as significant (*).

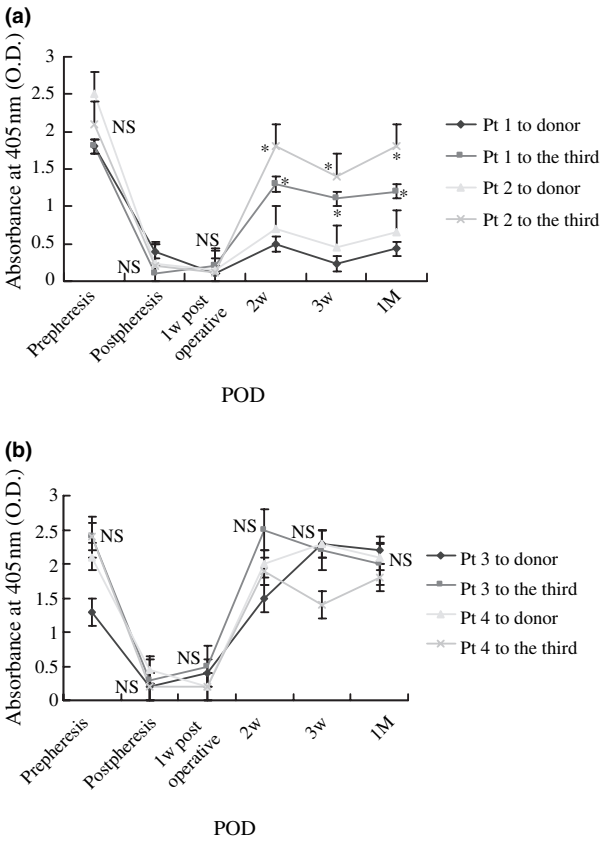

Figure 4 (a) Time course of ELISA in patients 1 and 2. ELISA was performed using 1:8 dilutions of samples from patients 1 and 2. Donor specific hypo-responsiveness was observed during the follow-up period. Points represent the means of triplicate measurements, bars represent SEM. Statistical comparisons were made using the unpaired Student's *t*-test between antibody-binding to donor and third party erythrocytes. A value of <0.01 was regarded as significant (*). (b) Time course of ELISA in patients 3 and 4. ELISA was performed using 1:8 dilutions of samples from patients 3 and 4. In contrast to patients 1 and 2, no donor-specific hypo-responsiveness was observed during the follow-up period. Points represent the means of triplicate measurements, bars represent SEM. Statistical comparisons were made using the unpaired Student's *t*-test between antibody-binding to donor and third party erythrocytes. A value of <0.01 was regarded as significant (*).

establishment between xenotransplantation and ABO-incompatible transplantation to the differences of peptide of alloglycoprotein and xenoglycoprotein linked to carbohydrate, not the differences of carbohydrate epitope itself. We can also believe that the difference in human individual peptides linked to carbohydrate blood antigens may yield the difference of antibody binding to antigens. When blood-group antibodies or non-blood-group antibodies in circulating plasma have been sufficiently removed preoperatively and T cells have been completely inhibited with immunosuppressive agents, peptides linked to incompatible carbohydrate antigens stimulate the BCR of B cells directly without the help of T cells, and as a result, B cells exposed to peptides linked to incompatible carbohydrate for a certain period start to produce non-cytolytic antibody that does not react with the incompatible carbohydrate antigen and peptides, i.e. accommodated antibody [7]. This new natural antibody production has been demonstrated by research at the gene level, and it appeared that junctional diversity and somatic mutations may play a major part in the process of antibody formation during human evolution [8]. As the serum of patients 1 and 2 contained this special accommodated antibody, a phenomenon is postulated in which it does not bind to donor

membranes but usually binds to the membrane of third parties with no recognition memory [7]. By contrast, when blood-group antibodies or non-blood-group antibodies in circulating plasma are not adequately removed and T-cell inhibition by immunosuppressive agents is inadequate, peptides linked to incompatible carbohydrate antigens indirectly stimulates B cells through T-cell help. When that happens, the IgG antibody produced with T-cell help is cytolytic antibody, i.e. non-accommodated antibody, and injures the graft. The antibody contained in the serum of patients 3 and 4 appears to have been this type of antibody.

The results of this study supported a decrease in antigen–antibody interaction and the influence of non-blood-group antigens/non-blood-group antibodies. The results also suggest that functional or structural changes in anti-blood-type antibodies greatly contributed to successful accommodation after ABO incompatible organ transplantation.

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