# ABO Incompatible Kidney Transplantation -Immunological Aspect-

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**ABO incompatible kidney transplantation (ABOINCKT) has been developed in Japan because of the shortage of cadaveric donors. We have performed 76 living-donor ABOINCKT in our center. Donor blood type antibody was removed by immunoadsorption or plasmapheresis and exchange. Immunosuppression consisted of cyclosporine or tacrolimus, steroid, and cyclophosphamide or azathioprine or mycophenolate mofetil and, recently, basiliximab. Splenectomy was routinely performed during the transplantation surgery. Donor blood type antigen was strongly expressed on the vascular endothelium at all time points and in all conditions post-transplantation. Red blood cell agglutination reaction (RBAR) was positive only in renal tissues from a patient with delayed hyperacute rejection. Donor specific antibody suppression was observed in 18 ABOINCKT recipients with blood type O from a donor with blood type A1 or B. ADCC activity was detected after pre-treatment. Acute humoral rejection in ABOINCKT can result from ADCC, as well as by antigen-antibody reaction. Five year graft and patient survival rates were 75% and 64% in 37 ABOINCKT recipients from June 1989 through December 1996, however they have been 100% in 39 ABOINCKT recipients since January 1997. Accommodation has been produced in ABOINCKT with the co-existence of blood type antigen and antibody. Currently, ABOINCKT is an alternative which should be considered, particularly for blood type O patients with extended waits for cadaveric transplantation and for pediatric patients.**

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***Experimental and Clinical Transplantation (2003) 2: 112-118***



**Keywords:** *ABO Incompatible, Kidney transplantation, Accommodation*

Living related ABO incompatible kidney transplantation (ABOINCKT) was previously contraindicated because hyperacute rejection occurred during the transplant operation. However, outcomes have improved since Alexandre reported a number of successful cases [1]. Currently in Japan, ABOINCKT graft and patient survival rates from ABOINCKT do not considerably differ from those of ABO compatible kidney transplantation [2]. Pre-treatment to reduce anti-donor blood type antibody and immunosuppression are important to suppress antigen-antibody reaction and avoid hyperacute and humoral rejection. The mechanism of immunological tolerance in long-term survival of ABOINCKT cases may offer clues to improve success in transplantation of patients with positive anti-donor HLA antibodies, including those cases with a high % of panel reactive antibodies or cases of xenotransplantation.

## Materials and Methods

### Patients and pre-treatment

Seventy six recipients of ABOINCKT from June 1989 through June 2003 were enrolled in this study. Incompatible blood type matchings of blood types included blood type B donor to blood type O recipient (B to O) in 22 patients, A1 to O in 21 patients, A1B to A1 in 11 patients, A1 to B in 8 patients, A1B to B in 7 patients, B to A1 in 6 patients, and A1B to O in 1 patient. In the first series of 5 patients, the anti-donor antibodies were removed with immunoadsorption [3]. In 71 cases, the patient's plasma was replaced by 5.5% albumin solution diluted with saline 1-5 times in plasmapheresis, and plasma exchange was replaced by AB blood type fresh frozen plasma 1-2 times to reduce the titers of anti-donor blood type antibodies (IgG and M) to less than x16 before transplantation.

**Immunosuppression**
Splenectomy was performed during the operation in 74 patients. Immunosuppression consisted of triple or quadruple therapy (cyclosporine (CYA) or tacrolimus (TAC), steroid, and cyclophophamide, azathioprine or mycophenolate mofetil (MMF) with or without ALG or basiliximab). In April 2002, basiximab was introduced for induction and CYA or TAC, steroid and MMF were used for ABOINCKT. MMF was administered after the first plasmapheresis.

**Staining for Donor Antigens**
We took a renal allograft biopsy to confirm acute rejection and a protocol biopsy 1 hour after reperfusion of the allograft, and 2-3 months, and 1, 3, 5, 7 and 10 years post-transplantation. Blood type (A1 or B) was stained with an indirect immunoperoxidase method, and HLA class I and DR antigens were stained with a streptoavidin-biotin (SAB) immunoperoxidase method in 35 renal tissues from 8 patients following transplantation. Seraclone anti-A and anti-B (Dainippon Pharmaceutical Co, Ltd), Clone B9.12.1 (Immunotec SA) and anti-HLA-DR (Becton Dickinson Co Ltd) were used for staining of the blood type A and B, Class I and HLA DR antigens, respectively. Fifteen specimens were obtained from 7 patients with acute rejection, including 4 patients during humoral rejection and one with delayed hyperacute rejection at nephrectomy. We classified the intensity of expression of blood type (A1 or B), HLA class I and DR antigens based on the scoring of stains (-, ±, +, ++).

**Red Blood Cell Agglutination Reaction (RBCAR)**
RBCAR is a modified Davidson's method [4] to detect the binding affinity of the anti-A1 or -B antibody (isoagglutinin) to the blood type A1 or B antigen on the tissue. Donor RBCs were diluted with saline including 10% AB serum and 0.1% Tween-80 and adjusted in 5% hematocrit. This RBC solution was incubated on the renal tissues at room temperature for 15 minutes. The slides were bathed with saline to remove non-reacted RBC. Finally, agglutination was observed microscopically. As a positive control, the same procedure was carried out after the anti-donor antibodies were pre-treated on the renal tissues.

**Measurement of Blood Type Antibodies (IgG and M)**
We serially measured the anti-donor blood type antibodies (IgG and M) both before and after plasmapheresis and exchange, and after transplantation. IgG was measured with albumin-Comb's method. IgM was initially pre-treated with Dithiothreitol (DTT) and then measured with albumin-Comb's method. In 18 stable ABOINCKT patients with blood type O received from 9 A1 and 9 B donors, we measured both anti-A1 and -B antibodies more than 6 months post-transplantation. As controls, both anti-A1 and -B antibodies were measured in 6 blood type O patients who had received renal allografts from donors with blood type O.

**Antibody Dependent Cell Mediated Cytotoxicity (ADCC)**
The mononuclear cells were extracted from the supernatant after the recipient's blood, including heparin, was centrifuged with Ficoll-Paque. They were washed with PBS and diluted in $5x10^6$/ml with RPMI 1640 including 10%FCS as the effector cells. RBCs were extracted from the donor's blood including heparin after removal of the buffy coat. RBCs were adjusted in $1x10^6$/ml with RPMI 1640 including 10%FCS as target cells, following addition of $Na_2{}^{51}CrO_4$. Recipient sera were inactivated at $56^0$C for 30 minutes and diluted in $1\sim10^{-3}$ with FCS. The target cells were reacted with these sera for 60 minutes. The effector cell/target cell ratio was adjusted to 10. The effector cells were incubated with the target cells for 5 hours. The radioisotope was measured with γ-Counter. % Cytotoxicity was defined as (experimental release–spontaneous release)/(maximum release-spontaneous release). The results are expressed as means of triplicate samples.

**Statistical Analysis**
Statistical analysis of the IgG and IgM titers was performed using Student's t-test, and $p<0.05$ was considered to be statistically significant. Patient and graft survival rates were calculated as actuarial survival rates using the Kaplan-Meier method.

## Results

**Expression of Blood Type Antigens and HLA Antigens (Table 1)**
Donor blood type antigens were strongly expressed on the vascular endothelium of the veins, and arteries, and peritubular and glomerular capillaries one hour after recirculation, and 2-3 months, and 3, 5, and 7 years post-transplantation. The intensity was stable under all conditions, including those of delayed

hyperacute, humoral or cellular rejection and stable graft function. However HLA class I and DR antigens were strongly expressed in 2 of 3 specimens of humoral rejection, 1 of 1 of hyperacute rejection, and 8 of 11 of cellular rejection.

**Table 1.** Expression of Blood Type and HLA Antigens in ABO Incompatible Renal Allografts

| | Antigens | | |
|---|---|---|---|
| | ABO Blood Type | HLA Class I | DR |
| 1 hr post-Tx(8) | 2+ | + | + |
| Protocol Bx(10) (2-3months~7 years) | 2+ | + | + |
| Acute Rejection | | | |
| Cellular(11) | 2+ | +(3),2+(8) | +(3),2+(8) |
| Hyperacute(1) | 2+ | 2+ | 2+ |
| Humoral(3) | 2+ | +1,2+(2) | 2+ |

## RBAR (Table 2)

Anti-donor antibodies were added to the renal tissues of a 1 hour biopsy together with donor red blood cells in a patient with x0 anti-donor antibodies. RBAR became positive when x64 anti-A1 IgM antibodies or x32 anti-B IgM antibody was added. However RBAR became positive only in the renal tissues from a patient with delayed hyperacute rejection. The serum titers in the patient were x64 in IgG and x64 in IgM on the day rejection began and increased to x1024 in IgG and x1024 in IgM at the time of renal allograft removal. All other specimens, including those from cases of humoral rejection, were negative in for RBAR.

**Table 2.** Red Blood Cell Agglutination Reaction
-1 Hour Biopsy- (Serum Anti-donor antibodies (IgG and IgM) titer 0)

| Titer of IgM Antibody | x32 | x64 | x128 | x256 |
|---|---|---|---|---|
| Patient's Serum | - | + or - | + | +++ |
| Anti-A IgM antibody | + | + | ++ | +++ |
| Anti-B IgM antibody | - | + | ++ | +++ |

## Pathology of Humoral Rejection

Polymorphonuclear cells infiltrated glomerular capillaries and accumulated in the peritubular capillaries. Thrombi formed in the glomerular capillaries and arteries and induced acute tubular necrosis from poor circulation. Mesangiolysis often appeared in the damaged glomeruli. Interstitial hemorrhage was observed as RBC extravasation out from the peritubular capillary. These immuno-pathological changes occurred in the region which expressed blood type antigen and were observed in 19 among 76 ABOINCKT patients.

## Blood Type Antibodies Following ABOINCKT

We reduced anti-donor antibodies (both IgG and IgM) to less than x16 by 1-5 times plasmapheresis and exchange with AB blood type plasma before transplantation. Anti-donor-antibodies rose gradually 7-14 days post-transplantation and then decreased and stabilized as low titers of IgG and IgM. The titer peaks tended to be higher, although not significantly so, in the patients with acute rejection, compared with those without acute rejection. In 9 recipients with blood type O who had a renal allograft with blood type A1 (A1 to O), the titers of anti-A1 IgG ($p< 0.002$) and IgM ($p<0.0007$) antibodies were significantly lower than those of anti-B IgG and IgM antibodies (Figure 1). These patients (A1 to O) had significantly lower titers of anti-A1 IgG ($p<0.004$) and IgM ($p<0.0001$) antibodies than the 6 O to O recipients (O to O), although anti-B antibodies did not differ between the A1 to O patients (A1 to O) and the (O to O) patients (Figure 2). In 9 recipients with blood type O who had a renal allograft with blood type B (B to O), the titers of anti-B IgG ($p<0.0001$) and IgM ($p<0.0001$) antibodies were signif-

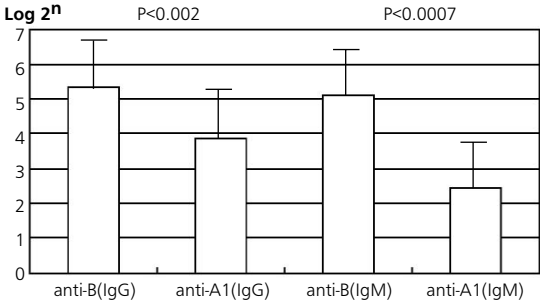


**Figure 1.** Donor Specific Antibody Suppression in ABO Incompatible Kidney Transplantation (A1 to O;N=9)

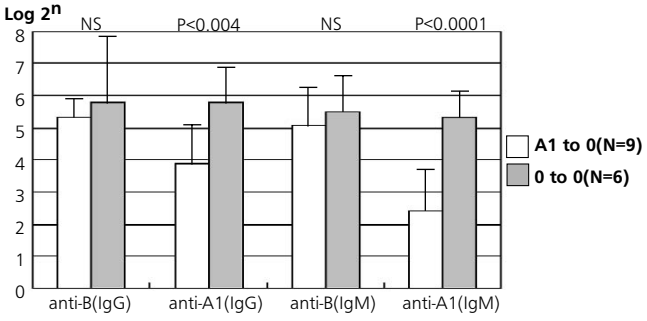


**Figure 2.** Blood Type Antibody Status (A1 to O compared with O to O)

icantly lower than those of anti-A1 IgG and IgM antibodies (Figure 3). These patients (B to O) had significantly lower titers of anti-B IgG ($p<0.0009$) and IgM ($p<0.0001$) antibodies than the 6 (O to O) recipients, although anti-A1 antibodies did not differ between these patient groups (Figure 4). In one patient (A1 to O) with delayed hyperacute rejection, high titers of anti-A1 IgG and IgM antibodies ($x2^{10}$-$x2^{12}$) persisted after removal of the renal allograft, even after the patient had a second cadaveric transplantation (O to O) under immunosuppression. However, 3 patients, who lost graft function because of chronic allograft nephropathy without removal of renal allografts, maintained low titers of anti-donor IgG and IgM antibodies ($x2^{0}$-$x2^{4}$).

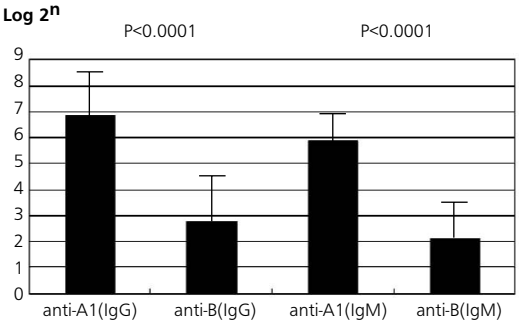


**Figure 3.** Donor Specific Antibody Suppression in ABO Incompatible Kidney Transplantation (B to O;N=9)

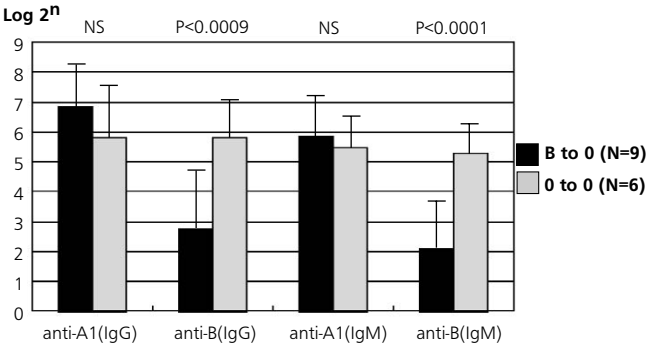


**Figure 4.** Blood Type Antibody Status (B to O compared with O to O)

## ADCC (Figure 5)

We studied ADCC in 2 patients before and after immunoadsorption to remove the anti-donor blood type antibodies. ADCC activity was detected in 1/10 – 1/100 of patients' serum. One of the patients had ADCC activity even after removal of antibodies with immunoadsorption. Anti-donor blood type antibody (IgG) was mediated by the ADCC activity.

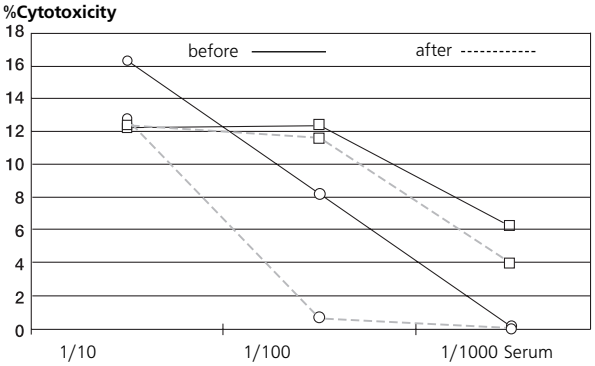


**Figure 5.** ADCC against Donor Red Blood Cells before and after immunoadsorption

## Outcome of ABOINCKT

To date, we have performed 76 ABOINCKT since June 1989. Only one patient suffered delayed hyperacute rejection 4 days post-transplantation and lost graft function despite of administration of OKT3. Thirty eight among 76 recipients had 51 clinical acute rejection episodes including 19 acute humoral rejection episodes in 19 recipients. Acute rejection, irrespective of cellular and humoral rejection, was treated with steroid pulse therapy (methylprednisolone 250-500mg daily for 3 days) with or without deoxysperguline 4mg/kg daily for 5-7 days. OKT3 was administered only in 3 patients with steroid resistant rejection including delayed hyperacute rejection. In the introduction period of ABOINCKT, from June 1989 through December 1996, patient/graft survival rates (n=37) were 89/86% at 1 year, 86/81% at 3 years, 75/64% at 5 years, 75/61% at 7 years and 71/44% at 10 years. However, the patient/graft survival rates (n=39) are 100/100% at 5 years from January 1997 up to the present.

## Discussion

Pre-treatment is critically important to reduce anti-donor antibodies for successful ABOINCKT. Plasmapheresis can easily remove IgM antibody, however IgG antibody is more difficult because of its low molecular weight. We usually used plasma exchange to remove IgG antibody, replacing with AB blood type plasma one day before transplantation. In plasmapheresis, plasma is replaced with only albumin and saline. However plasma exchange can supply γ-globulin, fibrinogen and coagulation factors which are beneficial for hemostasis during surgery. In addition,

AB blood type plasma contains soluble blood type A and B antigens which can neutralize anti-A and -B antibodies. In contrast, AB blood type plasma contains no blood type antibodies whatsoever. Plasma exchange possesses more advantages than plasmapheresis for ABOINCKT, though there is a risk of allergy and infection. To avoid such transfusion risks we utilized specific immunoadsorption using a Biosynsorb column as pre-treatment [3]. However, because of the prohibitive expense, we reverted to plasmapheresis and exchange. Tyden discussed the benefits of immunoadsorption using a carbohydrated column with A or B blood type antigen linked to a Sepharose matrix [5].

Immunosuppression in ABOINCKT is similar to that of ABO compatible kidney transplantation (ABOCKT). In ABOINCKT, however, antibody production must be suppressed by MMF, cyclophophamide or azathioprine, and steroid to avoid hyperacute or acute humoral rejection. Acute humoral rejection usually occurs within one month post-transplantation, although cellular rejection can occur at any time after transplantation. Therefore, MMF or azathioprine must be reduced from 2 to 1-1.5g daily or from 2 to 1mg/kg daily, respectively, 1 month post-transplantation to avoid excessive immunosuppression. We formerly used ALG in the induction period, however, the incidence of infection was higher, though the incidence of rejection was similar. Triple therapy without ALG was sufficient for ABOINCKT [5]. Recently, we introduced a new regimen consisting of basiliximab in the induction period, MMF, TAC or CYA and steroid. The dose of methylprednisolone was rapidly tapered from 20mg/day and maintained at 4-8mg/day 2-3 months post-transplantation.

Alexandre's first series of 3 patients without splenectomy failed with ABO incompatible grafts due to delayed hyperacute rejection. As a result, he concluded that splenectomy was essential for ABOINCKT [1]. However, Glore reported that splenectomy was not necessary for a non-A2 donor if anti-CD20 antibody (rituximab) and intravenous immunoglobulin (IVIG) were included in the immunosuppressive regimen [6]. Tyden also reported that ABOINCKT without splenectomy was successful in one patient with A1 to O incompatible matching [5]; however, the titers of IgG and IgM before pretreatment were low (less than x128). We did not perform splenectomy in 2 (B to O, A1 to O) of the 76 patients. Their titers of anti-B and -A1 were low (IgG x64, x32 and IgM x64, x64, respectively) before pre-treatment. At this writing, both of them remain well without acute rejection 5 years 1 month and 11 months post-transplantation. However, the splenectomy remains standard for ABOINCKT, particularly in patients with high titers or rebound phenomenon after immunoadsorption or plasmapheresis. Blood type antigens were strongly expressed on the vascular endothelium of peritubular and glomerular capillaries, veins and arteries [7]. Blood type antigens persisted in the renal tissue long after transplantation, without any change in pattern or intensity [8]. Expression of blood type antigens also did not change with any alteration of immunological status, including acute rejection [8]. However, HLA Class I and DR antigens were strongly expressed in acute humoral, as well as in cellular rejection. It was suggested that antigen-antibody reaction and T cell mediated cellular immunity did not affect expression of blood type antigens on the renal tissue. In contrast expression of HLA Class I and DR antigens was augmented by either cellular or humoral rejection.

RBAR demonstrated tissue agglutination activity. We observed agglutination in renal tissue following addition of more than x32 anti-A IgM antibody or x64 anti-B IgM antibodies. However agglutination was never observed, even in renal tissues of recipients with high serum titers (>x128 IgM), except for those in delayed hyperacute rejection. The amount and the affinity of tissue-bound antibody appeared to be much greater than those of serum antibody. Agglutination appeared to be caused by an antigen-antibody reaction. Activated compliments, cytokines and chemokines must appear together with the anti-donor antibody in the renal tissue with hyperacute rejection.

Characteristically, humoral rejection occurred in the vascular endothelium of peritubular and glomerular capillaries, arterioles and veins. When the endothelium was injured or a thrombus formed in the glomerular and peritubular capillaries and arteries, poor circulation resulted in acute tubular necrosis and renal dysfunction [9]. Glomerular changes were also observed more frequently in ABOINCKT than in ABOCKT [10]. Damaged mesangial cells often succumbed to mesangiolysis [9].

We found that anti-donor blood type antibodies were specifically suppressed in long-term ABOINCKT

recipients with blood type O. The recipients with blood type O can produce both anti-A and -B antibodies. In fact, the titers of anti-A antibodies did not differ from those of anti-B antibodies in a blood type O recipient from a blood type O donor (O to O). However only anti-donor antibodies were significantly suppressed compared with other antibodies in ABOINCKT recipients with blood type O (A1 to O and B to O). Anti-donor antibodies must not be absorbed in ABOINCKT renal allograft in the recipients with stable renal function long-term post-transplantation. Because antibodies were not discovered in any protocol biopsy specimen, except those from cases of hyperacute rejection, we believe production of anti-donor antibodies is specifically suppressed due to immunological tolerance. Interestingly, the patient who underwent allograft removal because of hyperacute rejection maintained a high titer of anti-donor antibodies even after re-transplantation from a donor with blood type O under immunosuppression. By contrast, in those patients who retained allografts and were reintroduced to dialysis due to chronic allograft nephropathy, titers of anti-donor antibodies were low. These findings suggest that donor's allograft antigens are necessary for suppression of anti-donor antibodies.

Co-existence of blood type antigen and low titers of anti-donor antibody in long-term ABOINCKT appears to be accommodation. Park et al suggested that accommodation may be caused by disruption of normal signal transduction, attenuation of cellular adhesion and the prevention of apoptosis [11].

ADCC presents antibody mediated cellular immunity. We found K cells could act on donor RBCs through anti-donor blood type IgG antibody. ADCC activity in 2 patients remained higher than 10% cytotoxic in 1/10 of patient's serum after immunoadsorption. Anti-donor blood type IgG antibody may injure the renal vascular endothelium, not only by humoral rejection through antigen-antibody reaction, but by cellular immunity through ADCC.

Recent outcomes with ABOINCKT have been excellent. In fact 5 year patient and graft survival rates (n=39) have been 100% since January 1997 (Figure 6). Shishido et al. reported that patient and graft survival rates in ABOINCKT for pediatric patients were also good (100% and 85% at 5 years) [12]. Adoption of proper immunosuppression and improved management of patients including infection control can result in good ABOINCKT outcomes.

ABOINCKT is a beneficial option for patients, particularly in blood type O patients with extended waits for cadaveric transplantation and in pediatric patients.

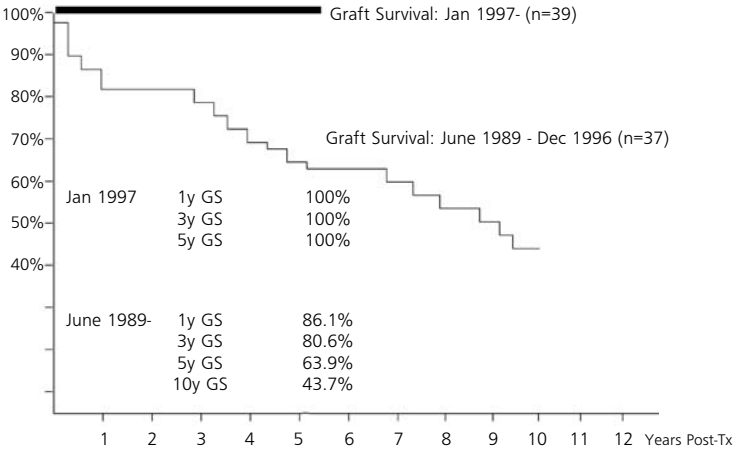


**Figure 6.** Graft Survival in ABO Incompatible Kidney Transplantation
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## References


1. Alexandre GP, Squifflet JP, De Bruyere M, Latinne D, Reding R, Gianello P et al. Present experiences in a series of 26 ABO-incompatible living donor renal allografts.Transplant Proc 1987;19:4538-42
2. Takahashi K. Current status of ABO-incompatible kidney transplantation in Japan, 1999: Results of a questionnaire-based survey. In:Takahashi K, eds. ABO incompatible kidney transplantation. Amsterdam. Elsevier Science BV, 2001, p73-99
3. Takahashi K. Blood adsorption and immunoadsorption. Pretransplant extracorporeal immunomodulation. Immunosuppressive therapy. In:Takahashi K, eds. ABO incompatible kidney transplantation. Amsterdam, Elsevier Sciences BV, 2001, p37-42
4. Davidsohn I. Early immunologic diagnosis and prognosis of carcinoma, Philip Levine Award Address. Am J Clin Pathol 1972;57: 715-30
5. Tyden G, Kumlien G, Fehrman I. Successful ABO-incompatible kidney transplantation without splenectomy using antigen-specifi immunoadsorption. Transplantation 2003;76:730-1.
6. Gloor JM, Lager DJ, Moore SB, Pineda AA, Fidler ME, Larson TS et al. ABO-incompatible kidney transplantation using both A2 and non-A2 living donors. Transplantation 2003;75:971-7
7. Aikawa A, Ohara T, Hasegawa A, Yamashita M, Hadano T, Shindo M et al. ABO incompatible kidney transplantation on triple therapy-compared with quadruple therapy. Transplant Proc 1998;30:1337-8
8. Aikawa A, Hadano T, Ohara T, Hasegawa A, Yamashita M, Shindo M et al. Relation between ABO blood type antigen and antibody, acute vascular rejection in ABO incompatible kidney transplantation. Transplant Proc 1998;30:3507-9
9. Hasegawa A, Ohara T, Hirayama N, Aikawa A, Arai K, Tajima E et al. Reversible anuria associated with glomerular fibrin thrombi in ABO-incompatible renal transplants. Transplant Proc 1995;27:1024-7
10. Aikawa A, Miyagi M, Hasegawa A, Ohara T, Hadano T, Nakano H et al. Glomerular change in a 1-year protocol biopsy as a useful pr dictive indicator in ABO incompatible kidney transplantation. Transplant Proc 2000;32:301-5
11. Park WD, Grande JP, Ninova D, Nath KA, Platt JL, Gloor JM et al. Accommodation in ABO-incompatible kidney allografts, a novel mechanism of self protection against antibody-mediated injury. Am

J Transplant 2003;3:952-60
12. Shishido S, Asanuma H, Tajima E, Hoshinaga K, Ogawa O, Hasegawa A et al. ABO-incompatible living–donor kidney transplantation in children. Transplantation 2001;72:1037-42