Preformed cytotoxic antibodies and ABO-incompatible grafts

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Introduction

Preformed natural antibodies (PNA) against donor-incompatible blood group antigen(s) represent the major barrier against ABO-incompatible human organ transplantation. Kidney (1–6) as well as heart (7) allografting across major ABO-incompatibility leads to rapid loss of the transplant from vascular rejection in the vast majority of cases. Liver transplants tolerate ABO-incompatibility better, although crossing the ABO barrier leads to significantly shorter survival than with ABO-identical grafts (8, 9).

Preoperative elimination of these PNA with plasmapheresis in recipients of ABO-incompatible human renal allografts has allowed very successful long-term functioning of these allotransplants (10, 11). Although recipients isoantibodies return after grafting, the transplants go on functioning normally in most cases. These patients therefore represent unique cases of organ transplants that function despite the presence of complement and specific antibodies against the incompatible ABO blood group antigen present on the transplant endothelium.

An immunological phenomenon inhibits the antigen-antibody reaction between the antigenic sites on the endothelial cells and the specific antibodies present in the patient serum: this has been called "accommodation".

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Since the immunological barrier to xenografts is similarly represented by PNA of the recipient species against the donor species, we have proposed the hypothesis that adaptation could also take place in the case of xenografts. Recipient baboons were therefore plasmapherized preoperatively to get rid of the PNA and transplanted with pig kidneys. Splenectomy and bilateral nephrectomy were performed while a standard quadruple therapy combining antithymocyte globulins (ATG), cyclosporine (CSA), Imuran and steroids was instituted (12).

Although long-term success could not be achieved, a few baboons have had transplants functioning over 3 wk. For the first time, discordant renal xenografts have been able to maintain normal function postoperatively. For the first time also, a typical cellular rejection crisis could be demonstrated in a discordant xenograft, once the classical vascular rejection was overcome.

We will first deal with human ABO-incompatible renal allografts, then with xenografts.

ABO-incompatible human renal allografts

General aspects

The antigenic determinants of ABO blood groups are all of a carbohydrate nature. These blood group-active carbohydrates exist as oligosaccharides, glycoproteins and glycolipids (13). As oligosaccharides, they can be excreted in the urine; as

glycoproteins, they are found in body secretions and tissue fluids; and, as glycolipids, they are mostly found as membrane components but are also present in plasma (14).

The ABO blood groups are the product of a series of evolutionary events which span bacteria and most of the animal kingdom (14), but only the higher anthropoïd apes like chimpanzees, gibbons and orangutangs contain ABH antigens on their erythrocytes. In other primates, like baboons or vervet monkeys, the ABH antigens are expressed on the vascular endothelium but are absent from the red cells. The chimpanzee, which appears to be the closest relative to humans, has only two ABO types, namely A and O, but the latter group has only a low frequency so that one can expect no more than 200 group-O chimpanzees among all the captive chimpanzees in the United States (15).

Most populations of baboons have three ABH blood groups: A, B and AB. However, there exist a few Papio Papio baboons of type O while Group O is the only ABO type found in Geladas, monkeys living in the mountainous regions of central Ethiopia (15), a species threatened with extinction.

The full complement of four ABO groups is to be found only in humans in whom the ABH antigens are present on the erythrocytes and the vascular endothelium where they are synthesized independently of the Se and Le genes; they are also synthesized by epithelial (distal convoluted and collecting tubules) or glandular cells where expression is dependent on the Se and Le genes (16, 17).

The vascular endothelium of A_2 individuals appears to contain fewer A antigens than that of A_1 individuals (18). More recently, types A_3 and A_4 antigens have been identified in human erythrocytes (18) and kidneys (19); these have been shown to be the molecular bases behind the qualitative distinction between the subgroups A_1 and A_2 (18, 20, 21). Monoclonal antibodies may actually recognize the different types of A antigens (18, 20, 22).

The role of ABO in allotransplantation

1. In experimental models

In baboons. As long ago as 1968, van Zyl et al. (23) demonstrated a shorter graft survival in ABO-incompatible compared with ABO-compatible kidney allografts: 6 untreated ABO-incompatible allografts had an average survival time of 14.8 ± 8.4 days, with none surviving beyond 30 d, while 13 ABO-compatible grafts had an average survival time of 22.1 ± 25.6 d ($p < 0.01$) with 4 animals surviving beyond 30 d.

These results were confirmed by Murphy et al. (24) who obtained very similar results in a comparable series of 5 ABO-incompatible untreated kidney allografts (averag survival: 14.8 ± 8.4 d) and 16 ABO-compatible grafts (average survival: 21.4 ± 18.7 d ($p < 0.01$)) with less severe depression of the tubular function in the latter group.

In baboons, survival of heterotopic heart allotransplants between ABO-compatible or -incompatible nonimmunosuppressed animals does not differ significantly. However, there appears to be an approximately 30% risk of accelerated rejection in the ABO-incompatible group, with vascular rejection being a factor in destruction in almost half of the cases. In the same experimental model, immunosuppression with cyclosporine (CSA) and methylprednisolone (MP) prolonged ABO-compatible or -incompatible pairs to the same extent but there remained an approximately 30% risk of early rejection, mostly vascular (25).

In pigs. In the pig, also, the ABO system plays a role in organ transplantation: skin grafts from A-like donor pigs into O-like recipients with identical swine lymphocyte antigens (SLA) are rejected in 7 d. Skin grafts between SLA-incompatible but identical A or O blood group pigs are also rejected in 7 d but skin grafts between pigs identical for both SLA and ABH systems are only rejected after 11 d, suggesting that both systems play a role in graft rejection in this animal (26).

2. In humans

The ABH antigens are clearly a major histocompatibility barrier for transplantation. ABO-incompatible skin grafts demonstrate accelerated and/or white graft rejection in group-O recipients immunized with ABO-incompatible erythrocytes while the rejection process of ABO-compatible grafts remains unaffected by this immunization (27, 28, 29).

Hume et al., in 1955, were among the first to point out the risk of breaching the ABO-barrier in human renal transplantation after observing the early rejection by the 7th postoperative d of a group-B cadaver renal allograft into a group-O patient (1). The concept that renal allografts should not be performed across major ABO blood group compatibilities was further supported by Starzl et al. (2, 3, 4, 5) who also experienced hyperacute rejection of kidneys transplanted against the ABO barrier. These observations were also confirmed by the report of the Kidney Transplant Registry in 1967 (6). It is noteworthy that not all ABO-incompatible grafts are systematically rejected; some do function normally, and for as long as other ABO-compatible transplants. Starzl et al. reported the case of a B-to-A living donor kidney still functioning perfectly 24 years post-transplant. They also

reported 2 other cases of A kidneys transplanted to O recipients without incident (30).

ABH blood groups and xenotransplantation

It has been suggested that xenotransplantation would be more successful if donor and recipient had compatible blood groups (31). Between closely related species, such as the Chacma baboon and the Vervet monkey, Cooper et al. (25) showed that hyperacute rejection, surprisingly, tended to be more common in ABO-compatible than in ABO-incompatible nonimmunosuppressed animals, but there appears to be a 30% greater risk of very early graft failure for the latter group.

The results of primate-to-human organ kidney transplants performed in the 'sixties clearly demonstrated the major risk of ABO incompatibility. In 1964, Reemtsma et al. (32, 33) transplanted 8 patients with chimpanzee's kidneys, 2 of which were ABO-incompatible and failed acutely. The 6 ABO-compatible xenografts had marked initial diuresis and, although most failed within 2 months, 1 graft functioned for 9 months until the death of the patient from steroïd complications.

In 1964 also, Starzl et al. (34) transplanted a patient with a rhesus monkey kidney (most probably from group B) into a group-A patient. The transplant was removed at d 10 because of oliguria. The same author (35) also transplanted 3 group-AB and -B baboon kidneys into group-O patients and observed anuria after 10 to 25 d while 3 other ABO-compatible transplants had an average survival of 28 d with an average excretion of 44 d.

In 1967, Marchioro et al. (36) transplanted 6 patients with baboon kidneys (bilateral nephrectomy and splenectomy of the recipient were carried out concomitantly): 3 ABO-compatible grafts had an average survival time of 44 d while the 3 ABO-incompatible kidneys functioned for only 28 d with 2 kidneys developing anuria at 10 and 25 d.

More recently, Bailey et al. (31) transplanted a non-O baboon heart into a group-O baby. The failed after 20 d due to a progressive humoral response.

It should be emphasized that these results were obtained at a time when immunosuppression was far from optimal, with no attempt being made to eliminate the recipient's preformed natural antibodies against the donor ABO-incompatible antigens.

From these results, one can conclude that future clinical attempts at xenografting should respect the ABO compatibility between donor and recipient whenever possible. In this respect, the pig represents an animal of choice, since it would not be difficult to breed pigs with blood group O.

Preformed natural ABO-antibodies

There are striking differences in the blood group agglutinin titers between patients and normal controls: Cecka et al. (37) made a study of the agglutinin titers among 125 patients awaiting kidney transplantation. They found that type-O patients generally had higher titers of agglutinin antibodies against type-A than type-B erythrocytes and 17% of these patients showed no agglutinin activity against A_2. However, a small proportion of type-O patients do have quite respectable titers of anti-A_2 agglutinins.

Some believe that A_2 recipients have a minor risk of rejecting blood group-O donor kidneys. Quantitative and possibly qualitative differences have been reported in the expression of blood group-A antigens in erythrocytes of A_2 and A_1 individuals (38). It has also been reported that skin grafts from A_2 individuals will survive better in blood group-O volunteers (27, 28). This belief is based more particularly on the fact that a certain percentage of group-O patients do not carry isoagglutinins against blood group-A_2 donors; some of them, however, do carry quit respectable titers of anti-A_2 agglutinins (37).

Some association exists between the nature of the end-stage renal disease and the titer of agglutinins: there is a tendency for those patients with systemic lupus or nephrosclerosis to have lower agglutinin titers. Finally, there is no correlation between the agglutinin titers and the level of immunization against HLA antibodies, with many of the hyperimmunized patients having low titers of agglutinins.

It is a well-known phenomenon that the anti-AB PNA titer drops after ABO-incompatible transplantation due to binding of these antibodies in the graft; they reappear in the circulation after graft nephrectomy when binding in the transplant is no longer possible (39, 40, 41, 42). Indirect immunofluorescence studies clearly demonstrate binding of these PNA on endothelium of the glomeruli and peritubular capillaries of the kidney. This leads to endothelial swelling and mononuclear and polymorphonuclear leukocyte adherence to the endothelium of the glomerular and peritubular capillaries as a primary step toward intravascular coagulation and thrombosis of these vessels.

Elimination of the PNA from the recipient's serum is therefore a prerequisite for successful ABO-incompatible renal transplants. The most effective procedures to accomplish this are plasmapheresis (10) and/or immunoadsorption (43). It should be mentioned that thoracic duct drainage is an alternative technique to reduce antibodies. Indeed, surprisingly, during thoracic duct drainage,

along with the removal of T lymphocytes there is an accompanying drastic reduction, by unexplained mechanisms, of all the immunoglobulin classes (44, 45). Machleder and Paulus showed that isoagglutinins could be virtually eliminated within 3 or 4 wk of thoracic duct drainage (44).

On the other hand, no correlation could be demonstrated between early postoperative isohemagglutinin titer and initial or long-term graft acceptance (46). However, rejection episodes are connected with an increase of hemagglutinin titers over preoperative values (47).

Results of ABO-incompatible renal allografts

Cadaver transplants

Some authors believe that A_2-to-O cadaver renal transplants are a minor risk compared with A_1-to-O. The results obtained in this subgroup, however, do not confirm this hypothesis. Brynger et al. were the first to report successful A_2-to-O cadaver renal transplants (48). Their results were updated in 1987 (49): they had performed a total of 20 such transplants; no pretreatment was performed and all patients received standard immunosuppression. Eight of 20 grafts were lost within the 1st postoperative month including 1 from acute irreversible vascular rejection, another from acute irreversible cellular rejection and 2 others from arterial thrombosis in which hyperacute or accelerated rejection could not be ruled out. Twelve grafts had long-term function and, among these, 6 grafts were functioning between 4 yr and 6 months and 7 yr and 6 months after grafting. These results are much better than the 4% 1-yr graft survival of 25 cadaver donor ABO-incompatible allografts reported by Cook et al. (50).

A_2 donor kidneys have been transplanted into O recipients in a few other institutions: Jakobsen et al. (51) performed 2 A_2-to-O live donor renal transplants, both wives to husbands. One kidney developed oliguria after a few hours and was irreversibly lost from rejection while the other, which also developed oliguria after a few hours, could be partially rescued. The host, however, died on d 37 from cardiac arrest with a partially functioning graft.

Shapira et al. (52) performed 5 A_2-to-O kidney transplants, 2 cadaver grafts and 3 from live donors. Hyperacute rejection did not occur in any of the recipients and all grafts have survived between 16 and 40 months. Acute clinical rejection episodes occurred in 4 patients 6 to 10 d after transplantation. In 2 cases, the rejection crisis was mild and easily reversed while the other 2 patients suffered severe rejection crises which responded well to steroid pulse therapy and plasmapheresis. The authors found elevated isoagglutinin titers at the time of rejection, especially if the donor was a nonsecretor according to the Lewis phenotype.

Welsh et al. (53) performed 16 A_2 cadaver grafts into 1 B and 15 O recipients. Rejection played a major causal role for all 7 of the lost grafts. The authors insist that the recipient anti-A_2 IgM titer (with A_2 cells) is a critical factor of long-term success in these A_2-to-O cadaver grafts. Taking 1/64 as an arbitrary level for the IgM anti-A_2 titer preoperatively, 3 of 4 patients with higher titers lost their grafts early between the 9th and 17th d and the 4th required ATG and five courses of high-dose steroid therapy, while 7 of 9 grafts survived for more than 1 yr when the preoperative IgM anti-A_2 was 1/64 or less.

Slapak et al. (54) performed 16 renal allografts across the ABO barrier. In 14 patients a cadaveric donor and in 2 patients a living-related donor was used. With the exception of the first 2 cadaveric graft recipients, the remaining 14 patients received one or two pretransplant plasma exchange. Usually, isoaglutinin titres of 1:8 or less were obtained immediately prior to transplantation. Concomitant splenectomy was performed in 1 living donor graft and in 4 of the cadaver graft recipients. One living donor graft is well-functioning after more than 7 yr; The other LRD graft was lost after 18 months due to ureteric obstruction. The 1-yr graft survival of the CD group was 78%; 3 patients had functioning grafts after more than 5 yr.

Others have now confirmed the feasibility of renal transplantation across the major ABO barrier. Bannett et al. in a series of 6 patients also depleted recipient ABO blood group antibody before transplantation. However they did so by using immunoadsorption over synthetic antigen instead of plasmapheresis (55). The efficacy of this procedure in eliminating recipient isoagglutinins might be questioned since, in 3 other patients in whom the immunoadsorption columns were applied, the isoagglutinins were refractory to immunoadsorption. For this reason, the authors chose not to transplant these patients. In a 4th patient, immunoadsorption could only reduce the titer of Ig anti-A to 1/32; further reduction of this titer to 1/4 necessitated plasmapheresis and donor-type specific plasma transfusions. Five out of 6 grafts functioned; 1 was acutely and irreversibly rejected on d 7 although donor and recipient were HLA-identical. It should be mentioned that all recipient-donor pairs in Bannett's series were either two-haplotype identical or were not mismatched for HLA. All recipients were splenectomized but immunosuppression was rela-

tively light, consisting of azathioprine and steroids in 5 out of the 6 cases.

Takahashi et al. (56) also reported good results in a series of 14 ABO-incompatible living donor renal transplants. Preparation of the recipients and the immunosuppressive therapy differed from ours since they used one session of double filtration plasmapheresis and three to four sessions of immunoadsorption prior to transplantation to remove the recipient's isoagglutinins. They also used a quintuple immunosuppressive regimen: methylprednisolone, CyA, ALG (14 d) and deoxyspergualin (5 d) with splenectomy. One patient died at 8 months from a B-cell lymphoma, another at 2 months from a cerebral hemorrhage. All the other patients retained a functioning graft but only 1 functioned over 1 yr. Interestingly, 5 out of the 14 patients presented no rejection crisis: these are also the cases who had low preoperative anti-A and/or anti-B antibody titers prior to plasmapheresis. They are also the cases with the best HLA match.

Haberal et al. (57) have also performed ABO-incompatible living donor kidney transplants. To prepare the recipient, these authors transplant a donor-specific skin graft 3 wk prior to kidney transplantation to decide whether or not the ABO-incompatible kidney graft should be performed. Out of a series of 28 patients submitted to the procedure, 12 patients were eliminated because of complete skin graft rejection. Fifteen patients who showed less than complete rejection of the specific donor skin graft received the ABO-incompatible kidney and, of these, 3 grafts were lost – which amounts to 80% graft survival. The authors, however, do not specify the period of time after which this rate of success was obtained. Splenectomy at the time of skin grafting was performed in the first 9 cases.

The experience of ABO-incompatible kidney transplants in our center

Thirty-seven patients received 38 ABO-incompatible renal allografts (1 patient received successively 2 ABO-incompatible kidney transplants). All patients received living donor kidneys, except the 1st who was inadvertently transplanted with an ABO-incompatible cadaver graft. The source of the transplant was the mother in 19 cases, the father in 8 cases, the spouse in 7 cases, the sister in 2 cases and an uncle in 1 case.

The donor-recipient ABO incompatibilities were A_1–to–O in 16 cases including the cadaver transplant, A_2–to–O in 4 cases, A_1–to–B in 4 cases, B–to–O in 9 cases, A_1B–to–B in 1 case, A_3B–to–B in 1 case, A_1B–to–A_1 in 1 case, A_1B–to–O in 1 case and B–to–A_1 in 1 case.

Thirty-four patients received primary transplants; 1 patient who received an ABO-incompatible transplant from her husband had been transplanted before with an ABO-compatible graft from her father which she rejected after 6 months. Another patient had previously rejected 2 cadaver kidneys before receiving an ABO-incompatible transplant from his mother. One patient received a secondary ABO-incompatible kidney from his uncle after having rejected a first ABO-incompatible transplant from his mother in a period of 27 months.

Plasmaphereses were performed in all the patients on d −5, −2 and +1 pretransplant to eliminate the ABO-isoagglutinins. The replacement therapy used during plasma exchange consists of 60% stable plasma protein solution, 20% albumin solution and 20% Hartmann's solution. One to 2 g of calcium bicarbonate is added to each liter of replacement solution. When the ABO-antibody titer remains superior to 1/4 after the third plasmapheresis, a supplementary plasmapheresis is performed to lower the titer of isoagglutinins below 1/4, at which time 5 to 10 ml substance A or/and B (Centre National de Transfusion Sanguine, Paris, France) diluted in 150 ml saline are administered during a period of 30 to 60 min. Hydrocortisone at a dose of 250 mg is given to the patient prior to the administration of substance A or B to avoid muscular and/or abdominal cramps which may occur during infusion of the product. One hour after administration of substance A or B, the isoagglutinin titer is again determined; if it is not equal to or lower than 1/2, another infusion of 5 ml substance A and/or B is given immediately prior to the transplantation procedure.

Piracetam (Nootropil, Union Chimique Belge, Belgium) at a dose of 9 g (200 mg/kg) is given intravenously during the donor nephrectomy procedure and the same dose (3 g given i.v. every 8 h) is added to the recipient therapy as a platelet antiaggregant during the first 2 postoperative wk.

Immunodepression in the first cases was achieved with a triple-drug therapy consisting of antilymphocyte serum (ALG, Behringwerke, Germany), Imuran and steroids. Thereafter, some patients received a triple therapy consisting of ALG, cyclosporine and steroids. In the last series, a quadruple-drug therapy was used consisting of antithymocyte serum (ATG, Fresenius, Germany), cyclosporine, Imuran and steroids (see Tables 1, 2, 3, and 4). ATG is administered at a dose of 3 mg/kg from d −3 up to the 10th postoperative d; cyclosporine is also started at d −3: the dose is adjusted according to the whole blood trough level;

Table 1. Related ABO-incompatible living donor kidney transplants: from mother (with splenectomy)

No.	Transpl. date	Donor	Don. ABO	Rec. ABO	Prophylactic immunodepressive therapy	Serum creatinine and follow-up (April 1991)
1.	06/30/82	Mother	A_1	0	ALG-Im-Ster.	1.35 mg%
2.	11/11/82	Mother	A_1	0	idem	1.00 mg%
3.	11/17/82	Mother	A_1	0	idem	1.26 mg%
4.	12/08/82	Mother	B	0	idem	R.D. 09/26/89
5.**	11/16/83	Mother	B	0	CSA-Ster	3rd graft: 1.48 mg%
6.	05/09/84	Mother	A_1B	0	ALG-CSA-Ster	1.70 mg%
7.	07/04/84	Mother	A_1	B	idem	Chronic rejection; (2nd graft ABO-inc. (see no. 4, table II))
8.	07/18/84	Mother	A_2	0	idem	2.06 mg%
9.	12/05/84	Mother	A_1	0	ALG-Im-CSA-Ster	Acute irrevers. rejection (12/14/84)
10.	07/03/85	Mother	A_1	0	idem	2 mg%
11.	10/16/85	Mother	A_1B	A_1	idem	R.D. 12/31/90
12.	07/16/86	Mother	B	0	idem	1.37 mg%
13.*	05/13/87	Mother	A_2	0	idem	0.8 mg%
14.	07/08/87	Mother	A_1	0	ATG-Im-CSA-Ster	1.06 mg%
15.	12/23/87	Mother	B	0	idem	1.36 mg%
16.	05/18/88	Mother	A_2	0	idem	1.05 mg%
17.	06/03/88	Mother	B	A	idem	1.09 mg%
18.	06/16/88	Mother	B	0	idem	1.51 mg%

RD = Return in dialysis; Im = Imuran; Ster = steroids; * = secondary graft; ** = tertiary graft.

Imuran is started at d −2 at a dose of 2 mg/kg up to the 2nd postoperative d, at which time the dose is reduced to 1 mg/kg/d. Hydrocortisone at a dose of 250 mg is given peroperatively, methylprednisolone at a dose of 250 mg is administered 6 h postoperatively and, from the 2nd postoperative d, a dose of 25 mg of prednisolone is given daily in the adult during the 1st month and is tapered slowly thereafter to reach a dose of 0.2 mg/kg/d at the end of the 9th postop month.

The overall results are better in the series of related living donor transplants (11, 58), the best results being obtained in the 18 cases of mother-to-child kidneys. In this latter series, only 1 graft was rejected in the first 2 yr. The first patients of the series are now surviving at more than 8 yr with a functioning graft.

In all cases, the recipient's isoantibodies return after grafting. Upon a rejection crisis the isoantibody titer may increase sharply and diminish again, although more slowly when the rejection crisis is being controlled. When the increase is too sharp, the transplant may be violently and irreversibly rejected.

All long-term functioning ABO-incompatible transplants retain their function notwithstanding the presence of specific isoantibodies directed at the ABO antigens of the graft endothelium. The isoantibody postoperative titer may even remain higher than the preoperative one.

Discordant renal xenografts from pig to baboon

As already pointed out, the major obstacle to xenografting between discordant animal species such as pig to baboon or pig to human is also represented by natural preformed antibodies (PNA) of the recipient species against the donor species producing acute vascular irreversible rejection of the transplanted organ. There is therefore a similarity be-

Table 2. Related ABO-incompatible living donor kidney transplants: from other than mother (with splenectomy)

No.	Transpl. date	Donor	Don ABO	Rec ABO	Prophylactic immunodepressive therapy	Serum creatinine and follow-up (April 1991)
1.	11/30/83	Sister	B	0	ALG-CSA-Ster.	2.2 mg%
2.	09/26/84	Father	A_1	0	ALG-CSA-Ster.	1.6 mg%
3.	03/20/85	Father	A_1	0	ALG-Im-CSA-Ster.	2.07 mg%
4.	10/15/86	Uncle	A_1	B	Idem	0.9 mg% (2nd graft ABO inc.)
5.	10/22/86	Father	B	0	Idem	2.8 mg%
6.	05/20/87	Father	A_1	0	Idem	Acute irreversible rejection (06/03/87)
7.	06/24/87	Father	A_3B	B	ATG-Im-CSA-Ster.	1.78 mg%
8.	11/04/87	Father	B	0	ALG-Im-CSA-Ster.	1.5 mg%
9.	02/08/89	Sister	A_1B	B	ATG-Im-CSA-Ster.	Hyperacute irreversible rejection (TX: 02/22/89)
10.	11/10/89	Father	A_1	0	Idem	Hyperacute irreversible rejection (TX: 11/29/89)

Im = Imuran; Ster. = Steroids.

Table 3. Unrelated ABO-incompatible living donor kidney transplants (with splenectomy)

No.	Transpl. date	Donor	Don ABO	Rec ABO	Prophylactic immunodepressive therapy	Serum creatinine and follow-up (April 91)
1.*	02/02/83	Spouse	A_1	0	ALG-CSA-Ster.	2nd graft (creat.: 1.37%)
2.	09/09/83	Spouse	A_1	B	Idem	RD: 03/27/84
3.	01/23/85	Spouse	A_1	0	ALG-Im-CSA-Ster.	1.22 mg%
4.	06/05/85	Spouse	A_1	0	Idem	RD: 05/24/89.
5.	12/04/85	Spouse	A_1	0	Idem	Acute irreversible rejection (12/23/85)
6.	04/16/86	Spouse	A_2	0	Idem	Death from CMV infection (06/24/86)
7.	05/11/88	Spouse	A_{1B}	0	ATG-Im-CSA-Ster.	Death from pneumocystis carinii infection (08/26/88)

RD = Return in dialysis; Im = Imuran; Ster. = Steroids; * = Secondary graft.

tween ABO-incompatible renal transplantation in the human and renal discordant xenotransplantation since PNA are responsible of the violent vascular rejection phenomenon observed in both models. Incidentally, the titer of baboon or human agglutinins against pig red cells is in the range of the isoagglutinin titers which can be found in the recipients of ABO-incompatible kidneys in the human.

Similarly to the situation existing in human ABO-incompatible renal transplantation, manipulation of these natural antibodies in such a manner as to avoid the triggering of the xenograft rejection phenomenon is a prerequisite for successful long-term organ xenografting.

We thought that if "the same causes produce the same effects", then treating a baboon recipient of a pig kidney by the same regimen as a human recipient of an ABO-incompatible renal allograft might allow a breach of the barrier presented by the PNA in the field of xenotransplantation.

In October 1984, the first pig (minipig)-to-baboon renal xenograft was thus performed in our laboratory. Preoperative plasmaphereses were performed under general anesthesia with ketamine (10 mg/kg) on d −2, −1 and on the day of transplantation to eliminate antipig xenoagglutinins; replacement therapy was achieved with a stable solution of human plasma proteins (SSPP). Soluble blood group substance A and B (Behasil Corporation, Miami) were administered to the recipient at the end of the last plasmapheresis in order to further eliminate remaining xenoagglutinins. A splenectomy with bilateral nephrectomy was performed and the abdominal cavity was closed in two layers.

Recipient immunosuppression was started on d −2 with rabbit antihuman antithymocyte serum (Fresenius, West Germany) 18 mg/kg, given intravenously for 10 d. Imuran was also administered i.v. daily, at a dose of 1 mg/kg while cyclosporine was administered intramuscularly at a dose of 10 mg/kg. Methylprednisolone (10 mg/kg) was administered i.v. during the operation and given intramuscularly daily thereafter at decreasing doses.

This renal xenograft succeeded in maintaining a satisfactory function for 13 d (12) whereas control grafts last a maximum of 35 min to a few hours. The combination of preoperative plasmapheresis with splenectomy and a quadruple immunosuppressive therapy thus demonstrated its effectiveness in prolonging a discordant renal xenograft far beyond the survival time that could be so far obtained in this type of model.

In the series we have performed to date, the follow-up of 3 other baboons is worth a more detailed description since they survived, respectively, for 10, 22 and 23 d. The baboon which lived for 10 d died of a pulmonary infection; the renal function was normal (1 mg%) at the time of death and histology of the xenograft was remarkably normal. There was no edema, practically no infiltrate; most glomeruli as well as the large vessels looked normal. Only in a few glomeruli could some very small focal vascular thromboses be identified.

Another baboon lived for 22 d. Renal function

Table 4. ABO-incompatible living donor kidney transplants (no splenectomy)

No.	Transpl. date	Donor	Don ABO	Rec ABO	Prophylactic immunodepressive therapy	Serum creatinine and follow-up (April 1991)
1.	04/27/83	Father	A_1	B	ATG-Im-Ster.	Hyperacute rejection transplantectomy 10th day
2.	06/29/83	Spouse	B	0	ALG-CSA-Ster.	Hyperacute rejection transplantectomy 7th day
3.	07/20/83	Mother	A_1	0	ALG-Im-Ster.	Hyperacute rejection transplantectomy 7th day

Im = Imuran; Ster. = Steroids.

remained normal during the 5 first postoperative d at which time serum creatinine increased. A biopsy showed acute vascular rejection. At the same time cyclosporine serum trough level was more than 500 ng/ml, demonstrating cyclosporine cytotoxicity. Rejection was treated by administration of methylprednisolone at a dose of 250 mg/d for 3 d; cyclosporine was stopped temporarily and renal function returned to normal up to the 17th postoperative d at which time progressive impairment of the renal function occurred. On the 22nd d, the animal became anuric and was sacrificed. Autopsy revealed clear-cut, irreversible vascular rejection.

The longest survivor of the series lived for 23 d. At d 3, while serum creatinine was 3.3 mg%, a biopsy of the transplant was taken which showed tubular necrosis without cellular infiltrate. A swelling of the small arteries' endothelial cells could be noted. Serum creatinine decreased to 1.9 mg% at d 5 but increased suddenly at d 6 at which time a second biopsy was taken which demonstrated a moderate mononuclear cell infiltrate similar to a first set allograft rejection. This rejection crisis was treated with rabbit antihuman ATG; renal function became normal (creatinine: 1.3 mg%) until d 15 at which time renal function decreased progressively with irreversible rejection and sacrifice of the animal at d 23.

Discussion

There is no doubt that preformed anti-ABO isoagglutinins represent the major factor involved in the mechanism of acute vascular rejection of ABO-incompatible human renal transplants. Breaching the barrier of major ABO compatibility without immunological manipulation of the recipient to eliminate antidonor isoantibodies almost invariably leads to hyperacute rejection of the kidney, followed by its rapid destruction (50, 59, 60). Slapak et al. were the first to demonstrate the reversibility of an acute vascular rejection by the use of plasmapheresis in a case of an inadvertent major ABO-mismatched cadaver transplant. Until then, there had been no report of graft survival after clinical manifestation of intravascular coagulation.

Hyperacute vascular rejection in cases of major ABO-incompatibility is, however, not at all inevitable. Starzl et al. (30) have reported long-term normal function of several kidney transplants across the major ABO barrier. Transplantation of A_2 kidneys to O recipients has also been successful without requiring any pretreatment of the patients (48, 61).

A beneficial effect of plasmapheresis is known to occur in a series of antibody-mediated syndromes. Pinching et al. demonstrated such an effect already in 1976 in the treatment of myasthenia gravis by the reduction of acethylcholine antibodies (62) but plasmapheresis is now applied in a series of diseases such as feto-maternal rhesus incompatibilities, polyarthritis, systemic lupus, Raynaud's disease, dermatomyositis, panarteritis and so on.

In the field of ABO-incompatible human kidney transplantation, preoperative elimination of these PNA has allowed very successful long-term functioning renal allotransplants with survival rates similar to those obtained with ABO-compatible grafts. However, isoantibodies return after grafting. In some cases, upon the occurrence of a rejection crisis, these antibodies increase sharply. Two possibilities then exist: either the crisis is controlled with the antirejection therapy or the crisis is uncontrolled and the transplant undergoes a violent vascular (humoral) irreversible rejection.

In the first case, the isoantibody titer, after control of the rejection crisis, decreases to a lower level and stabilizes. Our studies have shown that the isoagglutinins that return after grafting are still directed at the recipient's ABO antigens and that these persist on the graft's endothelium. An equilibrium at this stage is established between the incompatible ABO antigens of the graft endothelium, the most accessible site for immune injury (63, 64), and the circulating isoantibodies. F. H. Bach proposes the term "accommodation" to refer to this phenomenon of equilibrium.

Pratt et al. (65) propose several explanations for this phenomenon of accommodation:
- a change in the susceptibility of the graft endothelial cells to injury from the isoagglutinins. The gradual (how much gradual remains to be settled) postoperative return and increase of the isoagglutinins might elicit a progressive resistance to these antibodies. As an example of this possibility, Pratt et al. refer to the work of Hamilton and colleagues (66) who showed that assembly of the membrane attack complex (MAC) on endothelial cell surfaces causes loss of membrane vesicles that bind the components of the activated prothrombinase complex but spare thrombomodulin, an important inhibitor of coagulation;
- a change in affinity and/or specificity from the natural antibodies present before grafting:
- a modulation of the epitopes normally expressed by the graft endothelial cells.

From our clinical experience, it appears that a rapid postoperative increase of the antibody titer generally leads to irreversible graft rejection, while those patients maintaining a low profile of these antibodies generally continue to have normally functioning transplants.

Therefore, a primary condition to obtain accom-

modation must be the control of the postoperative production of the natural antibodies during the initial postoperative period. In the series of patients herein reported, we never observed any humoral crisis of rejection after the 3rd postoperative w, which indicates that there exists a critical period during which the ABO-incompatible transplant is at risk of acute vascular (humoral) rejection but after which the transplant behaves in a conventional manner.

The immunosuppressive regimen administered to the recipient might be of particular importance in this special category of transplants. Breimer et al. (21) noticed a reduced success in CyA-treated patients in comparison to azathioprine-treated recipients. None of 14 grafts treated with azathioprine-prednisone was lost of hyperacute rejection and 10 grafts had long-term function, while none of their last 6 transplants treated with CyA had long-term function. Although no definite conclusion could be drawn because some of these 6 grafts were lost from causes unrelated to rejection, it is nevertheless a factor to bear in mind. In our own experience, all our first recipients were treated with azathioprine-steroids and prophylactic ALG.

Azathioprine could be more important than CyA in ABO-incompatible organ transplantation since CyA selectively suppresses T-cell function and does not affect B cells, in contrast to azathioprine (67). We should not forget that, 30 years ago, Hitchings and Elion showed that azathioprine was a very potent drug in inhibiting the antibody response to sheep red blood cells in mice. According to some authors, the anti-B cell action of azathioprine could also be of importance in suppressing the occurrence of autoantibodies in blood group A, B and AB patients receiving O kidney grafts (68, 69).

Another observation is that, in any particular patient, we have never observed two successive vascular rejections, so that accommodation is either produced progressively when the patient does not present any rejection crisis, or the accommodation is produced at the outset of a controlled rejection crisis. Since the majority of patients receiving an ABO-incompatible kidney transplant present once vascular (humoral) rejection crisis, one may believe that accommodation is brought about in the majority of cases when the patient is coming out of a successfully treated rejection crisis.

The long-term success of major ABO-mismatch in renal transplantation is an interesting issue to address, especially in the light of a possible accomodation phenomenon. Stock et al. (73) noted a significant decrease in the long-term success rate observed in ABO-mismatched human renal allografts but this study concentrates on minor ABO mismatching such as O–to–A, O–to–B and O–to–AB.

In major ABO-incompatible grafts, we cannot confirm this finding, but our series are much smaller. Comparing 19 ABO-incompatible with 40 ABO-compatible living donor renal transplants performed at our center during the same period of time, we have shown that the quality of surviving grafts seems to be as good in the ABO-incompatible as in the ABO-compatible group, as witnessed by an equivalent serum creatinine level throughout the follow-up period (46).

However, in the ABO-incompatible transplants there is a higher risk of losing the graft from irreversible vascular rejection in the early postoperative period. Once "accommodation" is obtained, it is not at all clear whether ABO-incompatible transplants carry a higher risk at long term. There could be, on the contrary, some protective effect afforded by accommodation which awaits further assessment.

The favorable role of splenectomy remains more difficult to explain. Quantitatively, it is by no means a big reservoir of B lymphocytes – the producers of the natural antibodies. Qualitatively, Rowley (70) in 1955 has shown that splenectomized patients fail practically to respond to sheep erythrocytes while normal controls respond with a significant rise in antibody titer in the 2nd postinjection wk. Quantitatively, in the rat, splenectomy alone leads to an early antibody rebound effect after isovolemic plasma exchange (71). In hepatic xenografts from hamster to rat, splenectomy was shown to decrease antibody formation but had no effect on graft survival, while a significant suppression of antibody formation could be demonstrated when splenectomy was combined with CyA administration (72).

Owing to the success obtained in our series of ABO-incompatible renal transplants, we chose to explore the possibility of achieving pig-to-baboon renal xenotransplantation. We choose the pig because it could be a good candidate for a donor species to human subjects. It is ubiquitous, easily bred, even under germ-free conditions; it is a prolific species whose physiology resembles that of human physiology. The baboon was choosen as being the closest species to the human which could be used without too much difficulty for mimicking a possible future clinical application of pig organ transplantation to humans.

Although distinct major immunological differences exist between ABO-incompatible human renal allografts and minipig-to-baboon discordant xenografts, it is noteworthy that the immunological preparation and the immunosuppressive therapy applied in the human model are also effective in

the xenograft model. In both models, recipients possess PNA against the donor which produce vascular rejection when present at the time of transplantation.

ABO-incompatible human renal allografts and minipig-to-baboon discordant renal xenografts thus resemble to each other in the way they can be treated pre-, per- and postoperatively. ABO-incompatible human organ allografting could therefore be considered to a certain extent as being a model for evaluating new procedures of xenografting.

In the field of experimental organ xenotransplantation, the pig-to-baboon model appears very appropriate. In other models, such as pig-to-dog or hamster-to-rat, the xenografts behave differently and it is not at all certain that the observations made in these models might be extrapolated to the human situation.

In our model there are, however, clear practical difficulties such as the need for a general anesthesia every time blood is to be drawn, and frequent general anesthesia might also influence the recipient immunological reaction to the xenograft, although some sophisticated devices could help in solving these problems.

In conclusion, we may hope that the similarities observed between ABO-incompatible human renal allografts and minipig-to-baboon renal xenografts are suggestive of a similar immunological phenomenon in both models. However, a better comprehension of the conditions needed to obtain accommodation are necessary before any clinical xenografting can be initiated. Obviously, efficient means of dealing with the natural antibodies will be the primary tools. Although plasmapheresis and/or immunoadsorption are effective ways of dealing with these antibodies, they are incapable of completely eliminating the PNA during a relatively long period of time which, most probably, constitutes one of the prerequisites to obtain accommodation. Still, the possibility that "accommodation" exists also in xenografts opens a new frontier and gives hope for future clinical applicability of pig organ xenografts in human patients.

References

1. HUME DM. In: PEER LA, ed. Transplantation of tissues. Baltimore: Williams & Wilkins, 1959 pp 2, 484,
2. STARZL TE, MACHIORO TL, HERMANN GG, et al. Renal homografts in patients with major donor-recipient blood group incompatiblities. Surg Forum 1963: 14: 214.
3. STARZL TE. Experience in renal transplantation. Philadelphia: Saunders, 1964, p. 37.
4. STARZL TE, MARCHIORO TL, HOLMES JH; et al. Renal homografts in patients with major donor-recipient blood group incompatibilities. Surgery 1964: 55: 195.
5. WADDELL WR. The incidence, cause and significance of immediate and delayed oliguria or anuria after human renal transplantation. Surg Gynecol Obstet 1964: 118: 819.
6. GLEASON RE, MURRAY JE. Report from kidney transplant registry: analysis of variables in the function of human kidney transplants. I. Blood group compatibility and splenectomy. Transplantation 1967: 5: 343.
7. COOPER DKC. Clinical Survey of heart transplantation between ABO blood group-incompatible recipients and donors. J Heart Transplant 1990: 9: 376.
8. GORDON RD, IWATSUKI S, ESQUIVEL CO, et al. Experience with primary liver transplantation across ABO blood groups. Transplant Proc 1987: 19: 4575.
9. REDING R, VEYCKEMANS F, DE VILLE DE GOYET J, et al. ABO-incompatible orthotopic liver allografting in urgent indications. Surg Gynecol Obstet 1991 (in press).
10. ALEXANDRE GPJ, DE BRUYÈRE M, SQUIFFLET JP, et al. Human ABO-incompatible living donor renal homografts. Neth J Med 1985: 28: 231.
11. ALEXANDRE GPJ, SQUIFFLET JP, DE BRUYÈRE M, et al. Present experience in a series of 26 ABO-incompatible living donor renal allografts. Transplant Proc 1987: 19: 4538–4542.
12. ALEXANDRE GPJ, GIANELLO P, LATINNE D, et al. In: HARDY A, ed. Xenograft 25. Elsevier Science publishers (Biomedical Divison) 1989. 259–266.
13. SAMUELSSON BE, BREIMER ME. ABH Antigens: some basic aspects. In: BANNETT A, ed. ABO incompatiblity and transplantation. Grune & Stratton 1987.
14. RUFFIÉ J, SOCHA WW. Les groupes sanguins érythrocytaires des primates non-hominiens. Nouv Rev Fr Hématol 1980: 22– 147.
15. SOCHA WW, MARBOE CC, MICHLER RE, et al. Primate animal model for the study of ABO incompatibility in organ transplantation. In: BANNETT AA, ed. ABO incompatibility and transplantation, Grune & Stratton 1987.
16. SZULMAN AE. The histological distribution of blood group substances A and B in man. J Exp Med 1960: 111: 785.
17. BARIÉTY J, ORIOL R, HINGLAIS N, et al. Distribution of blood group antigen A in normal and pathologic human kidneys. Kidney Int 1980: 17: 820.
18. CLAUSEN H, LEVERY SB, NUDELMAN E, et al. Repetitive A epitope (type 3 chain A) defined by blood group A_1-specific monoclonal antibody TH-1: chemical basis of qualitative A_1 and A_2 distinction. Proc Natl Acad Sci USA 1985: 82: 1199.
19. BREIMER ME, JOVALL PA. Structural characterization of a blood group A heptaglycosylceramide with globo-series structure. FEBS Lett 1985: 179: 165.
20. BREIMER ME, SAMUELSSON BE. The specific distribution of glycolipid-based blood group A antigens in human kidney related to A_1/A_2, Lewis and secretor status of single individuals. Transplantation 1986: 42: 88.
21. BREIMER ME, BRYNGER H, LE PENDU J, et al. Blood group ABO-incompatible kidney transplantation. Biochemical and Immunochemical studies of blood group A glycolipids antigens in human kidney and characterization of the antibody response (antigen specificity and antibody class) in O recipients receiving A2 grafts. Transplant Proc 1987: 19: 226.
22. CLAUSEN H, LEVERY SB, NUDELMAN E, et al. Further characterization of type 2 and type 3 chain blood group A glycospingolipids from human erythrocyte membranes. Biochemistry 1986: 25: 7075.
23. VAN ZYL JA, MURPHY GP, WEBER HW, et al. Kidney allotransplantation in the baboon. Analysis of 26 control transplants: S Afr Med J 1968: 42: 6 (suppl).
24. MURPHY GP, WEBER HW, BREDE HD, et al. The significance of human ABO blood groups in the survival of untreated baboon renal allotransplants. Am Surg 1969: 35: 292.

25. Cooper DKC, Human PA, Rose AG. Is ABO compatibility essential in xenografting between closely related species. Transplant Proc 1987: XIX: 4437–4440.
26. Leight GS, Kirkman R, Benjamin A, et al. Transplantation in miniature swine. III: Effects of MSLA and A-O Blood groups matching on skin allograft survival. Tissue antigens 1978: 12: 65.
27. Dausset J, Rapaport FT. Role of ABO erythrocyte groups in human histocompatibility reactions. Nature 1966: 209: 209.
28. Ceppellini R, Bigliani S, Curtoni ES, et al. Experimental allotransplantation in man. II: The role of A_1, A_2 and B antigens. III: Enhancement by circulating antibody. Transplant Proc 1969: 1: 390.
29. Rapaport FT, Dausset J, Legrand L, et al. Erythrocytes in human transplantation: effects of pretreatment with ABO group-specific antigens. J. Clin Invest 1968: 47: 2206.
30. Starzl TE, Tzakis A, Makowka L, et al. The definition of ABO factors in transplantation: relation to other humoral antibody states. In: Bannett A. ed. ABO incompatiblity and transplantation. Grune & Stratton 1987: p96.
31. Bailey LL, Nehlsen-Cannarella SL, Concepcion W, et al. Baboon-to-human cardiac xenotransplantation in a neonate. JAMA 1985: 254: 3321.
32. Reemtsma K, McCracken BH, Schlegel JU, et al. Renal heterotransplantation in man. Ann Surg 1964: 160: 384.
33. Reemtsma K, McCracken BH, Schlegel JU, et al. Heterotransplantation of the kidney: two clinical experiences. Science 1964: 143: 700.
34. Starzl TE, Hitchcock TE. Renal heterotransplantation from baboon to man: experience with 6 cases. Transplantation 1964: 2: 752.
35. Starzl TE. Experience in renal transplantation. Philadelphia: Saunders, 1964, p262.
36. Marchioro TL, Hitchcock CR, Porter KA, et al. Renal heterotransplantation from baboon to man: experience of six cases. In: Vagtborg H, ed. The baboon in medical research. 1967. p.849.
37. Cecka JM, Breidenthal SE, Terasaki PI. Low anti-A and anti-B titers in some type O patients may permit renal transplantation across the ABO barrier. In: Bannett A, ed. ABO incompatibility and transplantation. Grune & Stratton, 1987. p.111.
38. Watkins WM. Genetic and biochemistry of some human blood groups. Proc R Soc Lond (Biol) 1978: 202: 31.
39. Hume DM, Magee JH, Prout Jr, GR, et al. Studies of renal homotransplantation in man. Ann NY Acad Sci 1964: 120: 578.
40. Sheil AGR, Stewart JH, Tiller DJ, et al. ABO blood group incompatibility in renal transplantation. Transplantation 1969: 8: 299.
41. Wilbrandt R, Tung KSK, Deodhar SD, et al. ABO blood group incompatibility in human renal homotransplantation. Am J Clin Pathol 1969: 51: 15.
42. Paul LC, van Es LA, Brutel de la Riviere G, et al. Blood group B antigen on renal endothelium as the target for rejection in an ABO-incompatible recipient. Transplantation 1978: 26: 268.
43. Raja R, McAlack R, Mendez P, et al. Technical aspects of antibody immunoadsorption prior to ABO-incompatible renal transplant. Transplant Proc 1987: 19: 4525.
44. Machleder HI, Paulus H. Clinical and immunological alterations observed in patients undergoing long-term thoracic duct drainage. Surgery 1978: 84: 157.
45. Starzl TE, Weil R III, Koep LJ, et al. Thoracic duct fistula and renal transplantation. Ann Surg 1979: 190: 474.
46. Reding R, Squifflet JP, Pirson Y, et al. Living-related and unrelated donor kidney transplantation: comparison between ABO-compatible and incompatible grafts. Transplant Proc 1987: 19: 1511–1513.
47. Reding R, Squifflet JP, Latinne D, et al. Early postoperative monitoring of natural anti-A and anti-B isoantibodies in ABO-incompatible living donor renal allografts. Transplant Proc 1987: 19: 1989–1990.
48. Brynger H, Rydberg L, Samuelsson B, et al. Renal transplantation across a blood group barrier – "A_2" kidneys to "O" recipients. Proc EDTA 1982: 19: 427.
49. Rydberg L, Breimer ME, Samuelsson BE, Brynger H. Blood group ABO-incompatible (A2 to O) kidney transplantation in human sujects: a clinical, serologic and biochemical approach. In: Bannett A, ed. ABO incompatibility and transplantation Grune & Stratton, 1987. p.132.
50. Cook DJ, Graver B, Terasaki P. ABO incompatibility in cadaver donor kidney allografts. In: Bannett A, ed. ABO incompatibility and transplantation Grune & Stratton 1987: p.153.
51. Jakobsen A, Solheim BG, Albrechtsen D, et al. Accelerated acute rejection in blood group O recipients of renal grafts from blood group A2 incompatible living donors. In: Bannett A, ed. ABO incompatibility and transplantation. Grune & Stratton, 1987, p.165.
52. Shapira Z, Yussim A, Shmueli D, et al. Experience with blood group A2 renal grafts in ABO-incompatible recipients. In: Bannett A, ed. ABO incompatibility and transplantation. Grune & Stratton, 1987, p.166.
53. Welsh KI, van Dam M, Koffman CG, et al. Transplantation of blood A2 kidneys into O or B recipients: the effect of pretransplant anti-A titers on graft survival. In: Bannett A, ed. ABO incompatibility and transplantation. Grune & Stratton, 1987, p.169.
54. Slapak M, Digard N, Ahmed M, et al. Renal transplantation across the ABO barrier-A 9 year experience. Transplant Proc 1990: 22: 1425.
55. Bannett AD, McAlack RF, Raja R, et al. Experiences with known ABO-mismatched renal transplants. In: Bannett A, ed. ABO incompatibility and transplantation. Grune & Stratton, 1987, p.147.
56. Takahashi K, Tanabe K, Ooba S, et al. Prophylactic use of a new immunosuppressive agent, deoxyspergualin, in patients with kidney transplantation from ABO-incompatible or preformed antibody-positive donors. Transplant Proc 1991: 23: 1078.
57. Haberal M, Gülay H, Sert S, et al. ABO-incompatible living related donor criteria. Transplant Proc 1990: 22: 345.
58. Reding R, Squifflet JP, Pirson Y, et al. Unrelated living donor kidney transplantation: a comparison with well-matched cadaver grafts. Clin Transplantation 1987: 1: 278–280.
59. Hume DM, Merrill JP, Miller BF, et al. Experiences with renal homotransplantation in the human: report of nine cases. J Clin Invest 1955: 34: 327.
60. Hamburger J, Crosnier J, Dormont J. Experience with 45 renal homotransplantations in man. Lancet 1965: 1: 985.
61. Breimer ME, Brynger H, Rydberg L, et al. Transplantation of blood group A2 kidneys to O recipients. Biochemical and Immunological studies of blood group A antigens in human kidneys. Transplant Proc 1985: 17: 2640.
62. Pinching AJ, Peters DK, Davies JN. Plasma exchange in myasthenia gravis. Lancet 1977: 1: 428.
63. Paul LC, Baldwin WM III, Van Es La. Vascular endothelial alloantigens in renal transplantation. Transplantation 1985: 40: 117.
64. Baldwin WM III, Paul LC, Claas FHJ, et al. In: Morris PJ, Tilney NL, eds. Progress in transplantation, Vol. 3. Churchill Livingstone, 1986, p.85.
65. Pratt LP, Vercelotti GM, Dalmasso AP, et al. Transplantation of discordant xenografts: a review of progress. Immunology Today 1990: 11: 450–456.

66. HAMILTON KK; HATTORI R, ESMON CH.T, et al. Complement proteins C5b-9 induce vesiculation of the endothelial plasma membrane and expose catalytic surface for assembly of the prothrombinase enzyme complex. J Biol Chem 1990: 265: 3809.
67. BOREL JF, FEURER C, GUBLER HU, et al. Biological effects of cyclosporin A: a new antilymphocytic agent. Agents Actions 1976: 6: 468.
68. MANGAL AK, GROWE GH, SINCLAIR M, et al. Acquired hemolytic anemia due to "auto"-anti-A or "auto" anti-B induced by group O homograft in renal transplant recipients. Transfusion 1984: 24: 201.
69. MINAKUCHI J, TOMA H, TAKAHASHI K, et al. Autoanti-A and -B antibody induced by ABO unmatched blood group kidney allograft. Transplant Proc 1985: 17: 2297.
70. ROWLEY DA. The formation of circulating antibody in the splenectomized human being following intravenous injection of heterologous erythrocytes. J Immunol 1950: 65: 515.
71. REDING R, WHITE DJG, BRONS G, et al. Effect of splenectomy on early rebound after plasma exchange in the rat. Eur Surg Res 1987: 19: 79–80.
72. VALDIVIA LA, MONDEN M, GOTOH M, et al. Hepatic xenografts from hamster to rat. Transplant Proc 1987: 19: y1158.
73. STOCK P, SUTHERLAND DER, FRYD DS, et al. ABO-compatible mismatching decreases 5-year actuarial graft survival after renal transplantation. Transplant Proc 1987: 19: y711.