

## ABO-INCOMPATIBLE HEART TRANSPLANTATION IN INFANTS

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**ABSTRACT**

**Background** Transplantation of hearts from ABO-incompatible donors is contraindicated because of the risk of hyperacute rejection mediated by preformed antibodies in the recipient to blood-group antigens of the donor. This contraindication may not apply to newborn infants, who do not yet produce antibodies to T-cell-independent antigens, including the major blood-group antigens.

**Methods** We studied 10 infants, 4 hours to 14 months old (median, 2 months), who had congenital heart disease or cardiomyopathy and who received heart transplants from donors of incompatible blood type between 1996 and 2000. Serum isohemagglutinin titers were measured before and after transplantation. Plasma exchange was performed during cardiopulmonary bypass; no other procedures for the removal of antibodies were used. Standard immunosuppressive therapy was given, and rejection was monitored by means of endomyocardial biopsy. The results were compared with those in 10 infants who received heart transplants from ABO-compatible donors.

**Results** The overall survival rate among the 10 recipients with ABO-incompatible donors was 80 percent, with 2 early deaths due to causes presumed to be unrelated to ABO incompatibility. The duration of follow-up ranged from 11 months to 4.6 years. Two infants had serum antibodies to antigens of the donor's blood group before transplantation. No hyperacute rejection occurred; mild humoral rejection was noted at autopsy in one of the infants with antibodies. No morbidity attributable to ABO incompatibility has been observed. Despite the eventual development of antibodies to antigens of the donor's blood group in two infants, no damage to the graft has occurred. Because of the use of ABO-incompatible donors, the mortality rate among infants on the waiting list declined from 58 percent to 7 percent.

**Conclusions** ABO-incompatible heart transplantation can be performed safely during infancy before the onset of isohemagglutinin production; this technique thus contributes to a marked reduction in mortality among infants on the waiting list. (N Engl J Med 2001; 344:793-800.)

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**S**OLID-ORGAN transplantation between donors and recipients with incompatible blood groups is usually contraindicated because of the high risk of hyperacute rejection. Such rejection occurs when preexisting serum anti-A or anti-B antibodies (natural isohemagglutinins) in the recipient bind to their respective blood-group antigens on vascular endothelial cells of the donor organ, resulting

in the activation of the complement cascade and rapid thrombosis in the vasculature of the graft.<sup>1-3</sup> Although undertaken occasionally in kidney and liver transplantation,<sup>2,4-12</sup> ABO-incompatible transplantation of hearts has been reported rarely — only as an inadvertent occurrence and often with a lethal outcome.<sup>13</sup>

Newborn infants do not produce isohemagglutinins, and serum anti-A or anti-B antibody titers usually remain low until the age of 12 to 14 months.<sup>14</sup> Furthermore, the complement system is not fully competent in young infants.<sup>15</sup> Thus, the primary factors that would initiate hyperacute rejection are absent during early infancy.

Infants with lethal cardiac disease often die before transplantation because of the shortage of donor hearts,<sup>16,17</sup> with patients in blood group O encountering disproportionate competition for organs (Table 1). Furthermore, when organs become available from donors who have the less common B and AB blood types, there may be no recipients of appropriate size with compatible blood types awaiting transplantation, so these organs go unused.

We reasoned that ABO-incompatible transplantation would be safe during early infancy because of the recipients' relative immunologic immaturity. We therefore performed cardiac transplantation in 10 infants using hearts from donors of incompatible blood groups.

**METHODS****Patients**

Since January 1996, parents of fetuses and infants referred for cardiac transplantation at the Hospital for Sick Children in Toronto have been offered the heart from the first available donor of compatible size, regardless of blood type. Except for two older infants, only infants with no serum antibodies to blood-group antigens were considered. The protocol was approved by the institutional ethics committee, and all parents gave written informed consent. We compared the results in the 10 infants who received hearts from ABO-incompatible donors between January 1996 and January 2000 with those of 10 infants who received hearts from ABO-compatible donors during the same period.

**Blood Group and Antibody Status**

The blood type of recipients was determined by means of standard blood-bank procedures for all infants except those placed on the waiting list while still in utero, whose blood type was deter-

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**TABLE 1.** COMPATIBLE AND INCOMPATIBLE COMBINATIONS OF BLOOD TYPE FOR POTENTIAL ORGAN DONORS AND RECIPIENTS.

VARIABLE	COMPATIBLE BLOOD GROUPS	INCOMPATIBLE BLOOD GROUPS
Recipient's blood type		
O	O	A, B, AB
A	A, O	B, AB
B	B, O	A, AB
AB	AB, B, A, O	—
Donor's blood type		
O	O, A, B, AB	—
A	A, AB	O, B
B	B, AB	O, A
AB	AB	O, A, B

mined at birth. The blood type of donors was provided by the referring organ-procurement agency.

Serum from all infants except those placed on the waiting list antenatally was tested for the presence of anti-A and anti-B antibodies by means of standard agglutination tests with erythrocytes from blood whose type was known (reverse blood typing); 0.1 ml of the patient's serum diluted with saline in a ratio ranging from 1:1 to 1:256 was mixed with 0.1 ml of a suspension of 3 to 5 percent erythrocytes of blood groups A and B (Ortho Diagnostics), incubated at room temperature for 1 hour, and centrifuged at low speed for 60 seconds. Erythrocytes from type O blood were used as controls in testing for the presence of other alloantibodies; saline was used as a negative control. If antibodies were detected, the isotype (IgG or IgM) was not determined.

Testing for serum antibodies was repeated periodically, and it was performed immediately when a potential donor was identified. The tests were also conducted during the operation before the release of the aortic cross-clamp and every six hours for four days after transplantation. In recipients without antibody production before transplantation, subsequent testing was repeated daily for two weeks, weekly for two months, then monthly. In the older recipients, testing was repeated more frequently.

**Preparations of Blood Products for Cardiopulmonary Bypass**

For recipients with type O blood, the bypass circuit was primed with plasma from blood of either the donor's blood type or type AB, which therefore carried no antibodies to the donor's blood group. In the one recipient with type A blood who received a heart from a donor with type B blood, plasma from type AB blood was given to avoid introducing antibodies directed against the blood types of both the donor and the recipient. All cellular products (except platelets) administered intraoperatively and postoperatively were from group O blood (or the recipient's blood group) and therefore expressed no exogenous A or B antigens. All plasma products were from group AB blood (or the donor's blood type when the recipient had blood group O) and therefore carried few or no exogenous anti-A or anti-B antibodies (Table 2).

Although platelets express low levels of A or B antigens, platelets from group O blood were not used with recipients with blood type O, because substantial quantities of anti-A and anti-B antibodies would be expected to appear in preparations of platelets from group O blood. These antibodies could be more detrimental in initiating hyperacute rejection of ABO-incompatible grafts than additional A or B blood-group antigens expressed in preparations of platelets from blood of the donor's blood group. Thus, platelets from group AB were used (or from blood of the donor's blood type,

**TABLE 2.** BLOOD GROUPS REQUIRED FOR BLOOD PRODUCTS ADMINISTERED DURING ABO-INCOMPATIBLE HEART TRANSPLANTATION.

DONOR'S BLOOD GROUP	RECIPIENT'S BLOOD GROUP	ANTIBODIES TO AVOID	INDICATED BLOOD GROUP		
			PLASMA	RED CELLS	PLATELETS
AB	O	Anti-A (vs. graft) Anti-B (vs. graft)	AB	O	AB
B	O	Anti-B (vs. graft)	AB or B	O	AB or B
A	O	Anti-A (vs. graft)	AB or A	O	AB or A
AB	B	Anti-A (vs. graft) Anti-B (vs. graft and recipient)	AB	O or B	AB
A	B	Anti-A (vs. graft) Anti-B (vs. recipient)	AB	O or B	AB
AB	A	Anti-B (vs. graft) Anti-A (vs. graft and recipient)	AB	O or A	AB
B	A	Anti-B (vs. graft) Anti-A (vs. recipient)	AB	O or A	AB

in the case of recipients with type O blood). Because sufficient platelets from type AB blood were not available, saline-washed platelets from type A blood were also given, with no apparent adverse effect.

**Intraoperative Procedures**

Orthotopic heart transplantation was performed by standard surgical techniques. During cardiopulmonary bypass, approximately three times the total body volume of plasma was exchanged. Blood equal in volume to the estimated circulating blood volume was removed from the infant during the initiation of bypass through the line for venous return to the bypass circuit and was replaced with an equal volume of blood from a blood bank and plasma from type AB blood. The infant's blood was separated into plasma and red-cell fractions by the hospital's blood bank or by means of a commercial blood-salvage system (Sequestra 1000, Medtronic). The plasma was discarded and the red-cell fraction was returned through the bypass circuit. This procedure was repeated up to three times during bypass to reduce to undetectable levels the concentrations of circulating antibodies to blood-group antigens; the reduction was confirmed before the aortic cross-clamp was released.

**Immunosuppressive Treatment**

All infants received 30 mg of methylprednisolone per kilogram of body weight intravenously during the operation, and they received induction immunosuppression with the use of rabbit polyclonal antithymocyte antibody immediately after the operation and until renal function normalized (median duration, 6 days [range, 3 to 14]; dose, 0.15 ml per kilogram per day intravenously, adjusted to yield a lymphocyte count of 200 to 400 per cubic millimeter). Primary immunosuppression of the first two recipients of hearts from ABO-incompatible donors and all recipients of hearts from ABO-compatible donors consisted of cyclosporine (Neoral, Novartis Canada; initial oral dose, 8 to 10 mg per kilogram per day; target plasma concentration, 250 to 325 ng per milliliter, according to enzyme-multiplied immunoassay), azathioprine (2 mg per kilogram per day orally), and prednisone (3 mg per kilogram per day orally, decreasing to 0.25 mg per kilogram per day during the first 10 days after transplantation). Subsequent recipients of hearts from

ABO-incompatible donors received primary immunosuppression with newly available drugs that may have a greater effect than the combination of cyclosporine and azathioprine on B-lymphocytes and thus on antibody production.<sup>18,19</sup> These patients were given tacrolimus (Prograf, Fujisawa Canada; initial oral dose, 0.1 to 0.3 mg per kilogram per day; target plasma concentration, 8 to 12 ng per milliliter) with mycophenolate mofetil (Cellcept, Hoffmann-LaRoche Canada; initial oral dose, 20 to 40 mg per kilogram per day) and prednisone (in the doses described above).

In all infants, the doses of adjunct immunosuppressive medications (prednisone or either azathioprine or mycophenolate mofetil) were gradually decreased, and the drugs were discontinued if no rejection occurred. The doses of calcineurin-inhibitor drugs were reduced over time (target plasma concentration one year after transplantation, 150 to 200 ng per milliliter for cyclosporine and 4 to 6 ng per milliliter for tacrolimus), so that total immunosuppression was maintained at the lowest level required to prevent rejection. Infants who received cyclosporine-based therapy and who had recurrent rejection or unacceptable side effects or in whom it was impossible to reduce the drugs to low doses or discontinue glucocorticoid therapy were switched to tacrolimus-based maintenance therapy. Patients 1 and 7 were switched from tacrolimus to cyclosporine because of gastrointestinal disturbance and anemia (in one patient each). Acute cellular rejection was treated with methylprednisolone (given in intravenous doses of 10 to 15 mg per kilogram per day for three days). If humoral rejection had occurred, plasmapheresis would have been performed, combined if necessary with the administration of cyclophosphamide (1 to 2 mg per kilogram per day).

#### Surveillance for Rejection

All infants underwent endomyocardial biopsy for the detection of acute cellular and humoral rejection at intervals of approximately three to four weeks, or earlier if possible rejection was indicated by changes in clinical status (fever, hypotension, lethargy, irritability) or by the results of electrocardiographic studies (decreased QRS voltages or new rhythm abnormalities) or echocardiographic studies (decreased ejection fraction or increased wall thickness). The biopsy specimens were stained with hematoxylin and eosin and were examined by pathologists with expertise in cardiac-transplant biopsies, who assessed them according to the criteria of the International Society for Heart and Lung Transplantation.<sup>20</sup> Histopathological features of antibody-mediated rejection included interstitial edema or hemorrhage and swelling of the endothelial cells. Immunohistochemical analysis was performed with the use of fluorescent tagged antibodies to IgG, IgM, IgA, C3, and fibrinogen. Coronary angiography was performed within six months after transplantation, then annually, to identify chronic rejection (accelerated coronary artery disease in the graft), a process that may be associated with antibody-mediated damage.<sup>21</sup>

## RESULTS

The median age at the time of transplantation in the recipients of grafts from ABO-incompatible donors (Patients 1 through 10) was two months (Table 3). A retransplantation (with a graft from an ABO-compatible donor) was performed urgently in Patient 2 after a traumatic injury to the initial graft during transport. Data on 10 recipients of grafts from ABO-compatible donors (Patients 11 through 20) who underwent transplantation at a median age of 5.5 months were used for comparison (Table 3).

#### Serum Antibody Titers before Transplantation

Serum anti-A or anti-B antibodies were detected before transplantation in four recipients of hearts from

ABO-incompatible donors (Table 4). Patients 2 and 10 had low titers of isohemagglutinins (1:2 or lower), presumably maternal in origin, given the infants' ages (six months or younger). Patients 4 and 9 had serum antibody titers consistent with normal isohemagglutinin production for their age (14 months).

#### Mortality

Patients 3 and 4 died of causes that were presumed to be unrelated to ABO incompatibility. In Patient 3 (donor's blood type, A; recipient's blood type, O), progressive cardiac hypertrophy developed two weeks after transplantation, and the patient died on day 29. Post-transplantation endomyocardial biopsy and post-mortem examination of the transplanted heart revealed no cellular or humoral rejection. No serum anti-A or anti-B antibodies were detected, and there was no deposition of immunoglobulin or complement within the graft. Possible factors contributing to the patient's lethal cardiac hypertrophy were the use of multiple inotropic drugs in high doses<sup>22</sup> and neonatal myocardial sensitivity to glucocorticoids<sup>23</sup> or tacrolimus<sup>24</sup> (which is unlikely at the low doses that were administered).

Patient 4 (donor's blood type, B; recipient's blood type, O), who had detectable serum anti-B antibodies before transplantation that were eliminated during transplantation, died of aspiration pneumonia on day 24 after transplantation. Serum anti-B antibodies were detected the day before death (titer, 1:4), but graft function was normal on echocardiography; a biopsy revealed mild cellular rejection and no humoral rejection. Postmortem examination demonstrated mild cellular and humoral rejection — specifically, edema and vasculitis, with deposition within the graft of immunoglobulin and fibrinogen but not complement. The importance of these findings relative to the clinical outcome is unclear.

#### Rejection and Modulation of Immunosuppression

No infant had hyperacute rejection. In the recipients with ABO-incompatible donors, acute cellular rejection occurred infrequently: no infant had more than one episode, and the episodes that did occur resolved easily with short-term, high-dose glucocorticoid therapy (Table 5). Among the recipients with ABO-compatible donors, there was a slightly higher incidence of rejection episodes overall (Table 5), perhaps because they were not given the combination of tacrolimus and mycophenolate mofetil used for immunosuppression in most recipients with ABO-incompatible donors or because they were older at the time of transplantation (median age, 5.5 vs. 2 months).<sup>25</sup> There were no findings consistent with humoral rejection in the recipients with ABO-incompatible donors, except for the autopsy findings, described above, in Patient 4. Chronic rejection (coronary vasculopathy in the graft), as diagnosed by coronary angiography, has not oc-

**TABLE 3.** CHARACTERISTICS OF PATIENTS WHO RECEIVED HEART TRANSPLANTS FROM ABO-INCOMPATIBLE OR ABO-COMPATIBLE DONORS.\*

PATIENT No.	DIAGNOSIS	AGE AT TRANSPLANTATION	AGE AT LAST FOLLOW-UP	INTERVAL FROM TRANSPLANTATION TO LAST FOLLOW-UP	DONOR'S BLOOD GROUP	RECIPIENT'S BLOOD GROUP
<b>ABO-incompatible</b>						
1	Hypoplastic left heart syndrome	25 days	4.7 yr	4.6 yr	AB	O
2†	Dilated cardiomyopathy	6 mo	4.7 yr	4.2 yr	B, O	O
3‡	Endocardial fibroelastosis, ventricular dysfunction	4 hr	30 days	29 days	A	O
4‡	Dilated cardiomyopathy	14 mo	15 mo	24 days	B	O
5	Hypoplastic left heart syndrome	2 mo	3.1 yr	2.9 yr	B	A
6	Single ventricle, right atrial isomerism	2 mo	2.9 yr	2.7 yr	A	O
7	Hypoplastic left heart syndrome	2 days	22 mo	22 mo	B	O
8	Hypoplastic left heart syndrome	7 wk	21 mo	19 mo	B	O
9	Hypoplastic left heart syndrome, after unsuccessful surgical palliation	14 mo	2.5 yr	17 mo	A	O
10	Hypoplastic left heart syndrome	10 wk	13 mo	11 mo	A	O
<b>ABO-compatible</b>						
11‡	Single ventricle, right atrial isomerism, pulmonary atresia	10 wk	10.5 wk	4 days	A	A
12	Hypoplastic left heart syndrome	2 mo	3.4 yr	3.2 yr	A	A
13	Hypoplastic left heart syndrome, after unsuccessful surgical palliation	7 mo	3.4 yr	2.8 yr	A	A
14	Dilated cardiomyopathy	12 mo	3.4 yr	2.4 yr	O	O
15	Dilated cardiomyopathy	11 mo	2.5 yr	1.6 yr	A	A
16	Restrictive cardiomyopathy	7 mo	2.1 yr	1.5 yr	O	A
17	Hypoplastic left heart syndrome, coarctation	5 mo	19 mo	14 mo	O	O
18	Single ventricle, atrioventricular valve regurgitation	9 wk	16 mo	14 mo	B	B
19	Dilated cardiomyopathy	6 mo	19 mo	13 mo	A	A
20	Hypoplastic left heart syndrome, double-outlet right ventricle	5 wk	12 mo	11 mo	O	O

\*Among the recipients with ABO-incompatible donors, the median age at transplantation was 2 months (range, 4 hours to 14 months); the median age at last follow-up for the eight surviving patients was 2.7 years (range, 13 months to 4.7 years), and the median interval between transplantation and last follow-up was 2.25 years (range, 11 months to 4.6 years). Among the recipients with ABO-compatible donors, the median age at transplantation was 5.5 months (range, 5 weeks to 12 months); the median age at follow-up of the nine surviving patients was 2.1 years (range, 12 months to 3.4 years), and the median interval between transplantation and last follow-up was 1.3 years (range, 16 months to 3.2 years).

†Patient 2 underwent urgent retransplantation (of a heart from a donor with blood group O) after a traumatic injury to the initial graft.

‡The patient died.

curred as of this writing in any recipient with an ABO-incompatible donor.

Adjunct immunosuppressive therapy has been discontinued in most recipients with ABO-incompatible donors. Five of the eight surviving patients now receive calcineurin-inhibitor monotherapy, and weaning from additional immunosuppressive therapy continues in two patients who underwent transplantation recently. Because of preexisting serum isohemagglutinins at the time of transplantation, Patient 9 has continued to receive low-dose tacrolimus and mycophenolate mofetil.

**Morbidity**

The recipients with ABO-incompatible donors have had no more chronic problems after transplantation than the recipients with ABO-compatible donors, and

increased immunosuppression has not been necessary to prevent or treat rejection. Specifically, no cases of post-transplantation lymphoproliferative disease, severe opportunistic infections, or diabetes mellitus have developed in recipients with ABO-incompatible donors. The frequency of anemia, leukopenia, hypertension, renal dysfunction, serum lipid abnormalities, and feeding difficulties has been similar in the two groups.

**Development of Isohemagglutinins after Transplantation**

Of the seven surviving recipients with ABO-incompatible donors who still have their original grafts, six were not producing isohemagglutinins before transplantation. According to the most recent follow-up data, production of antibodies to the A or B blood-group antigens in the ABO-incompatible graft has been detected in only two infants (Patients 1 and 6);

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**TABLE 4.** PRETRANSPLANTATION SERUM ISOHEMAGGLUTININ TITERS IN RECIPIENTS OF HEART TRANSPLANTS FROM ABO-INCOMPATIBLE DONORS.

PATIENT NO.	AGE AT TRANSPLANTATION	RECIPIENT'S BLOOD GROUP	DONOR'S BLOOD GROUP	SERUM ANTI-A TITER	SERUM ANTI-B TITER
1	25 days	O	AB	0	0
2*	6 mo	O	B, O	1:2	0
3	4 hr	O	A	0	0
4	14 mo	O	B	1:256	1:8
5	2 mo	A	B	0	0
6	2 mo	O	A	0	0
7	2 days	O	B	0	0
8	7 wk	O	B	0	0
9	14 mo	O	A	1:128	1:16
10	10 wk	O	A	1:1	1:1

\*Patient 2 underwent urgent retransplantation (of a heart from a donor with blood group O) after the traumatic injury of the initial graft.

in these cases, the onset of antibody production was delayed, and titers have remained low (Fig. 1). Despite the production of antibody to the antigens in these grafts, repeated biopsies revealed no evidence of humoral rejection or deposition of immunoglobulin, fibrinogen, or complement within the graft.

In contrast, the production of antibodies to the A or B blood-group antigens not expressed in either donor or recipient (for example, production of anti-B antibody in a recipient with blood type O and a graft from a donor with blood type A) has proceeded in a fashion similar to that in the recipients with ABO-compatible donors, suggesting that deficient production of antibody to the antigens in the ABO-incompatible graft is not due to immunosuppression therapy.

Before transplantation, both of the older infants we studied had serum antibodies to the antigens in the ABO-incompatible graft. In Patient 4 (donor's blood type, B; recipient's blood type, O), serum anti-B antibodies reaccumulated within three weeks after transplantation, although the titer remained low at death (1:4), whereas antibody to nonexpressed blood-group A antigen had reaccumulated to a titer of 1:64. Pa-

**TABLE 5.** IMMUNOSUPPRESSIVE THERAPY AND REJECTION PATTERNS IN RECIPIENTS OF HEART TRANSPLANTS FROM ABO-INCOMPATIBLE AND ABO-COMPATIBLE DONORS.\*

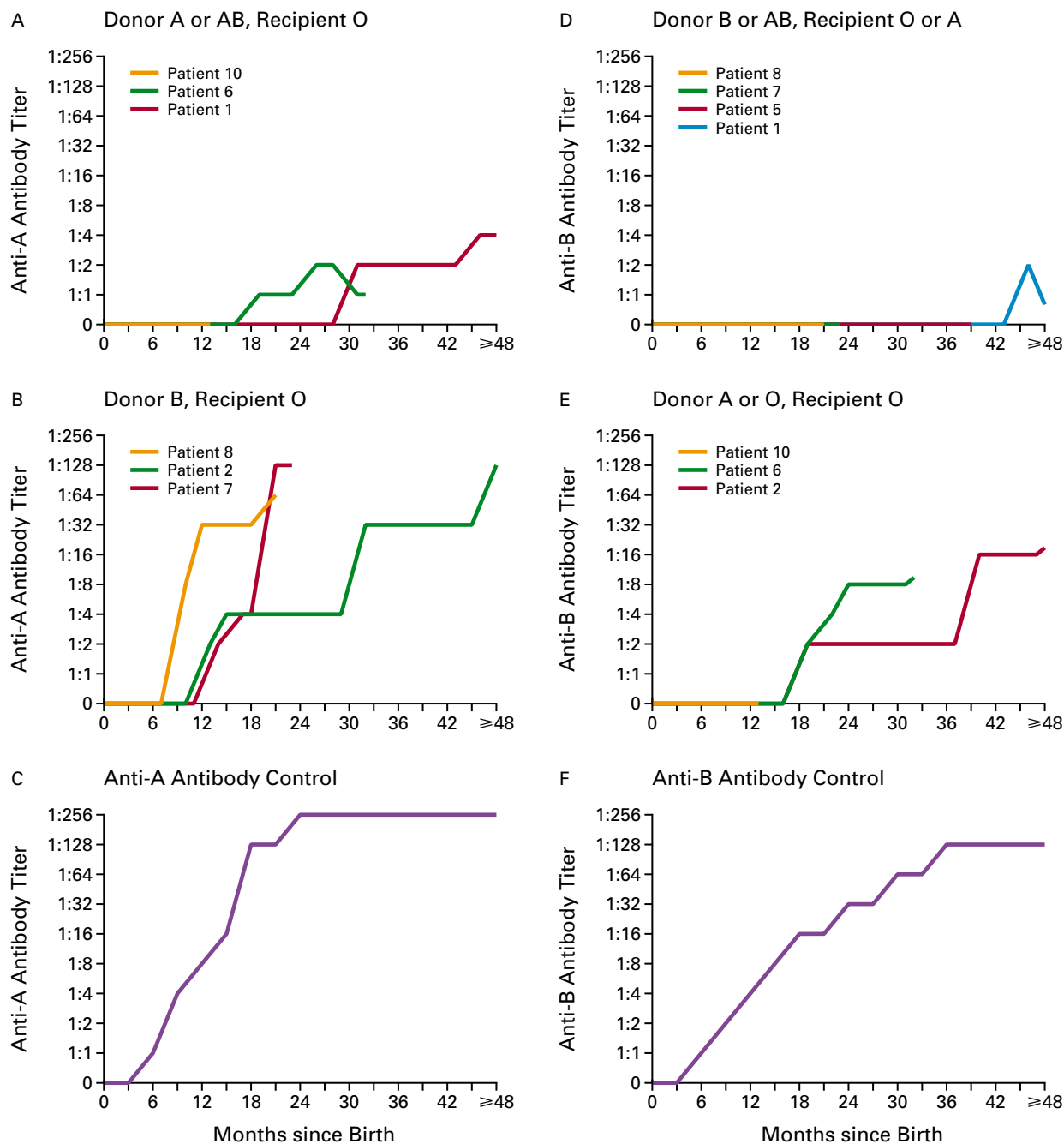
PATIENT NO.	DONOR'S BLOOD GROUP	RECIPIENT'S BLOOD GROUP	AGE AT TRANSPLANTATION	DAYS OF RABBIT POLYCLONAL ANTITHYMOCYTE ANTIBODY	IMMUNOSUPPRESSIVE DRUGS			No. OF REJECTION EPISODES†	
					AT	12 MO AFTER	CURRENT	≤6 MO AFTER	>6 MO AFTER
					TRANSPLANTATION	TRANSPLANTATION	(MO OF FOLLOW-UP)	TRANSPLANTATION	TRANSPLANTATION
<b>ABO-incompatible</b>									
1	AB	O	25 days	6	C, MP, A	T	C (55)	1	0
2‡	B, O	O	6 mo	14	C, MP, A	T, P, A	T (50)	1	0
3§	A	O	4 hr	10	T, MP	—	—	—	—
4§	B	O	14 mo	6	T, MP, A	—	—	1	—
5	B	A	2 mo	3	T, MP, MM	T, MM	T (35)	1	0
6	A	O	2 mo	5	T, MP, MM	T, MM	T (32)	0	0
7	B	O	2 days	7	T, MP, A	C, MM	C, MM (22)	0	0
8	B	O	7 wk	6	T, MP, MM	T, P, MM	T, MM (19)	1	0
9	A	O	14 mo	6	T, MP, MM	T, MM	T, MM (17)	0	0
10	A	O	10 wk	5	T, MP, A	NA	T, MM (10.5)	1	0
<b>ABO-compatible</b>									
11§	A	A	10 wk	4	C, MP, A	—	—	—	—
12	A	A	2 mo	0	C, MP, A	T, MM	T (39)	1	1
13	A	A	7 mo	6	C, MP, A	T, P, A	T (33)	1	1
14	O	O	12 mo	4	C, MP, A	T, P, MM	T, P, MM (29)	2	1
15	A	A	11 mo	5	C, MP, A	T, P, A	T, P, MM (19)	3	1
16	O	A	7 mo	5	C, MP, A	C, MM	C, MM (18)	1	0
17	O	O	5 mo	7	C, MP, A	T, P, A	T, P, A (14)	0	1
18	B	B	9 wk	5	C, MP, A	C, P, A	C, P, A (14)	2	1
19	A	A	6 mo	5	C, MP, A	C, P	C, P (13)	0	1
20	O	O	5 wk	6	C, MP, A	NA	T, MM (11)	2	1

\*C denotes cyclosporine, MP methylprednisolone, P prednisone, A azathioprine, T tacrolimus, MM mycophenolate mofetil, and NA not applicable.

†Data are for rejection episodes of grade 2 or higher.

‡Patient 2 underwent urgent retransplantation (of a heart from a donor with blood group O) after the traumatic injury of the initial graft.

§The patient died.



**Figure 1.** Serum Antibody Development in Surviving Infants with Grafts from ABO-Incompatible Donors Transplanted before the Onset of Isohemagglutinin Production.

Panels A and D represent the production of antibodies to the blood-group A or B antigen in the incompatible graft; Panels B and E represent the production of antibodies to non-expressed blood-group A or B antigen; and Panels C and F represent normal antibody development (extrapolated from Fong et al.).<sup>14</sup>

tient 9 (donor's blood type, A; recipient's blood type, O), who had serum anti-A and anti-B antibody titers of 1:128 and 1:16, respectively, before transplantation, had not had a reaccumulation of antibody to the blood-group A antigen in the ABO-incompatible graft 17 months after transplantation, despite having a reaccumulation of antibody to nonexpressed blood-group B antigen 2 months after transplantation.

#### Effect on Waiting-List Mortality

Before the initiation of this protocol, there were 7 deaths among 12 infants (58 percent) younger than six months old who had been placed on the waiting list for transplants since 1990, with only 2 infants receiving transplants and 3 infants receiving (unsuccessful) surgical palliation. Between January 1996, when our study began, and October 2000, 29 infants younger than six months old were listed as potential recipients of organs from a donor of any blood group, of whom 2 (7 percent) died after waiting six and nine weeks, respectively. Twenty-two infants received transplants (9 from ABO-incompatible donors and 13 from ABO-compatible donors), 3 were treated with other surgery, and 2 were removed from the list after two days of waiting (1 had a lethal genetic syndrome and 1 had an intracerebral hemorrhage).

### DISCUSSION

Heart transplantation is a successful therapy for infants with potentially lethal cardiac disease, and results are improving steadily; five-year survival rates are approaching 80 percent in centers with extensive experience.<sup>25,26</sup> However, efficient allocation of the rarely available organs is hampered by the presumed need for a recipient who has a blood type that is compatible with that of the donor.

Some aspects of antibody production are not yet developed in infants. In particular, stimulation by T-cell-independent polysaccharide antigens, such as the capsular components of bacteria (e.g., pneumococci), does not elicit a serum antibody response early in life.<sup>27,28</sup> Similarly, the production of antibodies to the carbohydrate blood-group antigens begins at the age of six to eight months, in infants of susceptible genotypes, as a cross-reactive immune response after the colonization of the gut with polysaccharide-bearing *Escherichia coli*.<sup>14,29</sup>

Blood-group antigens and isohemagglutinins that develop in persons who lack antigen expression are a major immunologic barrier to organ transplantation.<sup>1-3</sup> Nonetheless, the scarcity of donors has spurred efforts to use ABO-incompatible organs, with some success in kidney<sup>2,4-10</sup> and liver<sup>1,11,12,30</sup> transplantation. The simple removal of antibodies by techniques such as plasmapheresis,<sup>4,6,12</sup> immunoabsorption,<sup>5,31</sup> or administration of competitive soluble carbohydrate antigen<sup>32</sup> is sometimes successful, but reaccumulation of antibodies usually occurs.

In cardiac transplantation, the particularly devastating effects of antibody-mediated rejection, both hyperacute and delayed, have dictated the avoidance of situations in which antibody-mediated rejection may occur. Thus, although HLA compatibility is not the principal determinant of outcome in cardiac transplantation, sensitized patients who have antibodies to the donor's HLA antigens before transplantation are at high risk for graft loss due to humoral rejection.<sup>33</sup> Similar considerations have prevented the intentional use of organs from ABO-incompatible donors. In an international survey,<sup>13</sup> Cooper found only eight cases of ABO-incompatible heart transplantation, all in adults and all occurring inadvertently because of errors in determining the blood type of the donor. Despite urgent removal of antibodies or retransplantation, only two patients survived.

In this study, we found that heart transplantation between donors and recipients of incompatible blood groups can be performed safely during early infancy. In the absence of preformed antibodies against blood-group antigens, hyperacute rejection did not occur, in keeping with predictions based on experimental evidence in animals and limited clinical experience with the transplantation of other organs. Other than plasma exchange during cardiopulmonary bypass, invasive preparatory procedures to remove antibodies were not required, nor was splenectomy performed to diminish antibody production.

For patients placed on the waiting list antenatally, fetal blood typing was unnecessary. After transplantation, there was no rapid production of antibodies to the incompatible blood-group antigens, in contrast to the clinical course in most adult patients in whom ABO-incompatible transplantation has been attempted. This lack of antibody production is probably due to the patients' immature immune responsiveness to stimulation by T-cell-independent antigens. Thus, in infants who underwent transplantation before the initial development of isohemagglutinins, the clinical course was similar to that of infant recipients with ABO-compatible donors.

Antibodies to the blood-group antigens in the ABO-incompatible graft have thus far developed in only two infants who underwent transplantation before antibody production had begun, and no graft damage has become apparent on histologic examination. Early experience suggested that if ABO-incompatible transplantation could be performed in persons or animals with minimal serum titers of preformed antibodies, graft damage would be avoided when antibodies reaccumulated — a process called accommodation.<sup>4-6,32,34-36</sup>

Patients 4 and 9 represent a different phase of immunologic maturation. Isohemagglutinin production was under way in these older infants, with relatively high serum antibody titers, which predict a high risk of hyperacute rejection. Nonetheless, hyperacute re-

jection was avoided with the use of intraoperative plasma exchange as the sole method for the removal of antibodies.

The absence of antibody production or its delay beyond the time expected because of early immunosuppression may be evidence of partial B-cell tolerance induced by exposure to donor antigens during the maturation of the immune system.<sup>37-39</sup> This is particularly obvious in Patients 6 and 9 (both recipients with type O blood whose donors had type A blood), who have had increasing production of anti-B antibody but abnormally low production of anti-A antibody.

The generally good results in this series reflect the fundamental immunologic malleability of young infants. Thus, caution must be exercised to avoid excessive immunosuppression in such infants solely because of ABO incompatibility.

It should be noted that these infants were desperately ill at the time of transplantation, most with severe cardiac malformations. Their highest risk of mortality derived from the insufficient availability of ABO-compatible donors. The maximal age at which this protocol may be used safely remains to be determined, but the appropriate population could include all infants in whom serum isohemagglutinins have not yet developed.

*We are indebted to the clinical heart transplant team and the cardiovascular perfusionists for their expert assistance in the care of these patients; to the technical staff of the Hospital for Sick Children blood bank; and to Elmer Cruz for assistance with the manuscript.*

## REFERENCES

1. Starzl TE, Ishikawa M, Putnam CW, et al. Progress in and deterrents to orthotopic liver transplantation, with special reference to survival, resistance to hyperacute rejection, and biliary duct reconstruction. *Transplant Proc* 1974;6:Suppl 1:129-39.
2. Paul LC, Baldwin WM III. Humoral rejection mechanisms and ABO incompatibility in renal transplantation. *Transplant Proc* 1987;19:4463-7.
3. Stock P, Sutherland DE, Fryd DS, et al. Detrimental effect of ABO mismatching in renal transplantation. *Transplant Proc* 1987;19:711-2.
4. Slapak M, Naik RB, Lee HA. Renal transplant in a patient with major donor-recipient blood group incompatibility: reversal of acute rejection by the use of modified plasmapheresis. *Transplantation* 1981;31:4-7.
5. Bennett AD, Bensinger WI, Raja R, Baquero A, McAlack RE. Immunosorption and renal transplant in two patients with a major ABO incompatibility. *Transplantation* 1987;43:909-11.
6. Alexandre GPJ, Squifflet JP, De Bruyere M, et al. Present experiences in a series of 26 ABO-incompatible living donor renal allografts. *Transplant Proc* 1987;19:4538-42.
7. Sutherland DE, Fryd DS, So SK, et al. Long-term effect of splenectomy versus no splenectomy in renal transplant patients: reanalysis of a randomized prospective study. *Transplantation* 1984;38:619-24.
8. Takahashi K, Yagisawa T, Sonda K, et al. ABO-incompatible kidney transplantation in a single-center trial. *Transplant Proc* 1993;25:271-3.
9. Tanabe K, Takahashi K, Sonda K, et al. ABO-incompatible living kidney donor transplantation: results and immunological aspects. *Transplant Proc* 1995;27:1020-3.
10. Osorio AV, Sullivan EK, Alexander SR, Bryan CF, Shield CF, Warady BA. ABO-mismatched renal transplantation in children: a report of the North American Pediatric Renal Transplant Cooperative Study (NAPRTCS) and the Midwest Organ Bank (MOB). *Pediatr Transplant* 1998;2:26-9.
11. Gugenheim J, Samuel D, Reynes M, Bismuth H. Liver transplantation across ABO blood group barriers. *Lancet* 1990;336:519-23.
12. Eid A, Zamir G, Yaron I, et al. Liver transplantation across the ABO barrier: the role of plasmapheresis. *Transplant Proc* 1998;30:701-3.
13. Cooper DKC. Clinical survey of heart transplantation between ABO blood group-incompatible recipients and donors. *J Heart Transplant* 1990;9:376-81.
14. Fong SW, Qaquadah BY, Taylor WF. Developmental patterns of ABO isoagglutinins in normal children correlated with the effects of age, sex, and maternal isoagglutinins. *Transfusion* 1974;14:551-9.
15. Ferriani VP, Barbosa JE, de Carvalho IF. Serum haemolytic classical and alternative pathways of complement in infancy: age-related changes. *Acta Paediatr Scand* 1990;79:322-7.
16. Boucek MM, Faro A, Novick RJ, et al. The Registry of the International Society of Heart and Lung Transplantation: third official pediatric report-1999. *J Heart Lung Transplant* 1999;18:1151-72.
17. Morrow WR, Naftel D, Chinnock R, et al. Outcome of listing for heart transplantation in infants younger than six months: predictors of death and interval to transplantation. *J Heart Lung Transplant* 1997;16:1255-66.
18. Becker BN. Mycophenolate mofetil. *Transplant Proc* 1999;31:2777-8.
19. Seebacher G, Weigel G, Griesmacher A, et al. One and a half years of experience with mycophenolate mofetil (Cellcept) in cardiac transplantation: a prospective, randomized study. *Transplant Proc* 1999;31:3291-3.
20. Billingham ME, Cary NR, Hammond ME, et al. A working formulation for the standardization of nomenclature in the diagnosis of heart and lung rejection. *J Heart Transplant* 1990;9:587-93.
21. Fredrich R, Toyoda M, Czer LS, et al. The clinical significance of antibodies to human vascular endothelial cells after cardiac transplantation. *Transplantation* 1999;67:385-91.
22. Larson DF, Copeland JG, Russell DH. Catecholamine-induced cardiac hypertrophy in a denervated, hemodynamically non-stressed heart transplant. *Life Sci* 1985;36:2477-89.
23. Werner JC, Sicard RE, Hansen TW, Solomon E, Cowett RM, Oh W. Hypertrophic cardiomyopathy associated with dexamethasone therapy for bronchopulmonary dysplasia. *J Pediatr* 1992;120:286-91.
24. Atkison P, Joubert G, Barron A, et al. Hypertrophic cardiomyopathy associated with tacrolimus in paediatric transplant patients. *Lancet* 1995;345:894-6.
25. Bailey LL, Gundry SR, Razzouk AJ, Wang N, Sciolaro CM, Chiavarelli M. Bless the babies: one hundred fifteen late survivors of heart transplantation during the first year of life. *J Thorac Cardiovasc Surg* 1993;105:805-15.
26. Dapper F, Bauer J, Kroll J, et al. Clinical experience with heart transplantation in infants. *Eur J Cardiothorac Surg* 1998;14:1-5.
27. Cadoz M. Potential and limitations of polysaccharide vaccines in infancy. *Vaccine* 1998;16:1391-5.
28. Ahmad H, Chapnick EK. Conjugated polysaccharide vaccines. *Infect Dis Clin North Am* 1999;13:113-33.
29. Springer GF, Horton RE. Blood group isoantibody stimulation in man by feeding blood group-active bacteria. *J Clin Invest* 1969;48:1280-91.
30. Yandza T, Lambert T, Alvarez F, et al. Outcome of ABO-incompatible liver transplantation in children with no specific alloantibodies at the time of transplantation. *Transplantation* 1994;58:46-50.
31. Rieben R, Korchagina EY, von Allmen E, et al. In vitro evaluation of the efficacy and biocompatibility of new, synthetic ABO immunoabsorbents. *Transplantation* 1995;60:425-30.
32. Cooper DKC, Ye Y, Mickrasz M, et al. Specific intravenous carbohydrate therapy: a new concept in inhibiting antibody-mediated rejection — experience with ABO-incompatible cardiac allografting in the baboon. *Transplantation* 1993;56:769-77.
33. Thompson JS, Thacker LR II, Takemoto S. The influence of conventional and cross-reactive group HLA matching on cardiac transplant outcome: an analysis from the United Network of Organ Sharing Scientific Registry. *Transplantation* 2000;69:2178-86.
34. Platt J. A perspective on xenograft rejection and accommodation. *Immunol Rev* 1994;141:127-49.
35. Dalmaso AP, Platt JL. Prevention of complement-mediated activation of xenogeneic endothelial cells in an in vitro model of xenograft hyperacute rejection by C1 inhibitor. *Transplantation* 1993;56:1171-6.
36. Bach FH, Ferran C, Hechenleitner P, et al. Accommodation of vascularized xenografts: expression of "protective genes" by donor endothelial cells in a host Th2 cytokine environment. *Nat Med* 1997;3:196-204.
37. Rieben R, Tucci A, Nydegger UE, Zubler RH. Self tolerance to human A and B histo-blood group antigens exists at the B cell level and cannot be broken by potent polyclonal B cell activation in vitro. *Eur J Immunol* 1992;22:2713-7.
38. Klinman NR. The "clonal selection hypothesis" and current concepts of B cell tolerance. *Immunity* 1996;5:189-95.
39. Dintzis RZ, Middleton MH, Dintzis HM. Studies on the immunogenicity and tolerogenicity of T-independent antigens. *J Immunol* 1983;131:2196-203.

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