# Anti-B–Mediated Rejection of an ABO-Incompatible Cardiac Allograft Despite Aggressive Plasma Exchange Transfusion

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THIS REPORT describes the use of plasma exchange with replacement by fresh frozen plasma (FFP) in an ABO-incompatible cardiac transplant recipient.

## METHODS

### *Case Report*

A 47-year-old white man had a myocardial infarction in 1984 and later refused coronary artery bypass surgery. He developed severe ischemic cardiomyopathy and was eventually referred for cardiac transplantation. The patient had blood group A but he received a group B cardiac allograft. This was discovered as he came off cardiopulmonary bypass. Plasma exchange was used in an attempt to reduce the recipient's anti-B titer. The patient mounted a secondary anti-B response beginning on day 5. Associated with the rise in anti-B titer, the patient's cardiac function deteriorated, and on the 13th posttransplant day, he received a compatible group A heart. The compatible heart functioned well initially but ultimately was rejected. Death occurred 51 days after the initial transplantation.

### *Laboratory Procedures*

Serum IgM and IgG anti-B were measured using a modified dithiothreitol (DTT) technique.[1] Direct antiglobulin (Coombs') testing was performed on all negative samples using rabbit anti-human IgG. Indirect peroxidase-antiperoxidase staining of cardiac tissue was performed using goat anti-rabbit IgG $F(ab')_2$ (Cappel, Cooper Biomedical, Inc, Malvern, PA) *v* rabbit anti-human IgG and IgM (Dako Corporation, Santa Barbara, CA).[2]

Single plasma volume exchanges were carried out using an IBM 2997 portable blood separator (Cobe Laboratories, Inc, Lakewood, CO). The volume exchange per procedure was approximately 3 L. The time per procedure varied from 2 to 2½ hours, and the replacement fluid was group AB FFP.

Representative left ventricular samples of both allografts and a control heart were analyzed for the presence of tissue-bound anti-B antibodies.[3] One gram of quick-frozen heart muscle was finely minced, washed three times in 0.3 mol/L buffered sucrose, centrifuged (14,000 × g, 15 minutes), washed two more times in buffered 0.175 mol/L potassium chloride, and then homogenized in 9 vol of buffered potassium chloride. After centrifugation (1,500 × g, 15 minutes) the supernatant was separated and designated as the first homogenate supernatant. The homogenization procedure was repeated and a second homogenate supernatant was obtained. The pellet was then eluted in 5 vol of 0.2 mol/L glycine, pH 2.8, centrifuged (12,000 × g) for two minutes and the supernatant was adjusted to pH 7.4. This was designated the glycine eluate. Both homogenate supernatants and the glycine eluate were then dialyzed *v* phosphate buffered saline and titrated *v* washed group B red cells.

## RESULTS AND DISCUSSION

As soon as the allograft incompatibility was discovered, the blood bank was immediately consulted and the recipient's pretransplant anti-B titer was determined to be 1:32. The options available to minimize allograft damage mediated by the recipient's anti-B antibodies were considered to be: (1) wait and see, relying on routine immunosuppressive drugs to control the recipient's anti-B response; (2) adsorption of anti-B antibody by filtering the recipient's plasma over a column of B coated beads (this, however, needed to be done immediately and no such columns were available to us); and (3) plasma exchange to remove circulating anti-B antibodies. The dilemma associated with plasma exchange concerned the ABO group of the FFP that could be used for replacement (Table 1). With group 0 FFP,

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**Table 1. FFP Blood Group Dilemma**

| Recipient's RBC Group A | Allograft Group B | | |
|---|---|---|---|
| | Advantages | Disadvantages | Acceptability |
| Group O FFP | None | Attacks patient's RBC<br>Attacks allograft | No |
| Group B FFP | Not attack allograft | Attacks patient's RBC<br>B-substance may ↑ anti-B | No |
| Group A FFP | Not attack patient's RBC | Attacks allograft | No |
| Group AB FFP | Not attack allograft<br>Not attack patient's RBC | B-substance may ↑ anti-B | Probably yes |

anti-A antibody could attack the patient's red blood cells and the anti-B could attack the allograft. The group B FFP would not affect the allograft but it would attack the patient's red blood cells; its soluble B substance could possibly increase the recipient's anti-B titer. Group A FFP would not affect the patient's red blood cells but the anti-B antibody would attack the allograft. The last choice was group AB FFP, which would neither attack the allograft nor the patient's red blood cells. However, the B substance present in the AB FFP might stimulate the recipient's anti-B titer. Under the circumstances, it was decided to use group AB FFP.

Figure 1 shows the recipient's anti-B titers and the volumes of the plasma exchanges performed until he received a second allograft on day 13. Included also are the dosage levels of immunosuppressant drugs administered. The secondary rise in anti-B titer could have been stimulated either by the B antigen intrinsic to the allograft, the soluble B substance in the AB FFP used for replacement, or both.

Figure 2 shows IgM and IgG anti-B titers determined using more specific and sensitive techniques (modified DTT). After the initial plasma exchange the anti-B titer dropped to a low level and remained there until approximately day 5, when both IgM and IgG anti-B titers rose despite daily plasma exchange. The reduction in the anti-B titer from 1:32 to 1:2 following the first plasma exchange is greater than would be expected from the exchange alone, which should have reduced the titer by approximately 70% (1:32 to 1:8).[4] The additional reduction in titer almost certainly reflects neutralization of circulating anti-B by soluble group B substance present in the group AB FFP used for replacement.

Histologic examination of the incompatible allograft demonstrated diffuse destruction of the microvasculature and endothelial necrosis in larger vessels, associated with acute (neutrophilic) inflammation of vessels beginning on day 5. Immunoperoxidase staining demonstrated deposition of IgM and IgG immuno-

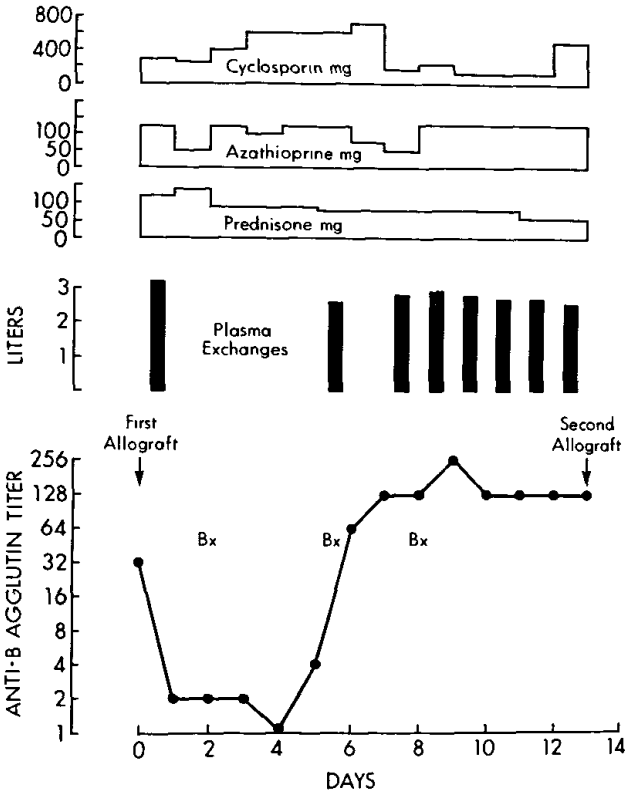


**Fig 1. Anti-B titers during clinical course.**

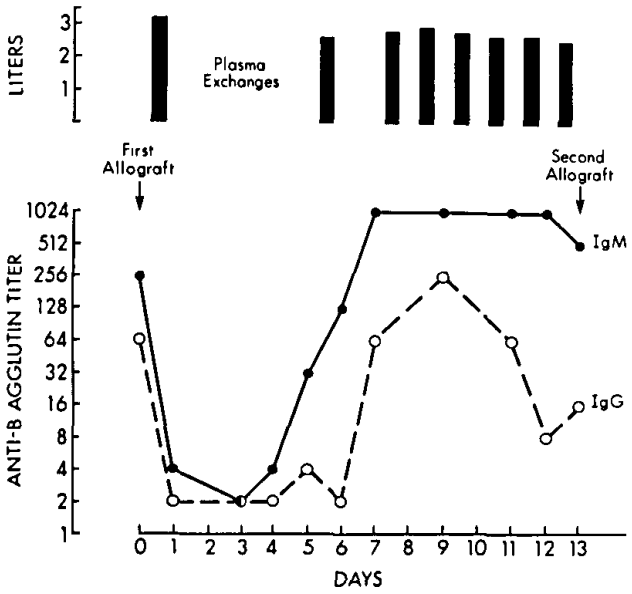


**Fig 2. IgM and IgG anti-B titers.**

globulin in the coronary vasculature. These histologic findings are indicative of antibody-mediated rejection. By contrast, the compatible allograft showed marked perivascular and interstitial inflammation composed primarily of lymphocytes with scattered monocytes and only rare polymorphonuclear cells, a picture typical of cell-mediated allograft rejection.

Table 2 is a comparison of the IgM and IgG anti-B antibody titers in extracts of the incompatible allograft, the compatible allograft and a group A control heart, along with measurements of serum antibody titers. Abundant IgG and IgM antibody was detected in all three extracts of the incompatible heart, primarily in the first homogenate, whereas anti-B was not detected in extracts of the compatible or control hearts.

## SUMMARY

A group A recipient received a group B cardiac allograft. Aggressive plasma exchange with replacement by group AB FFP initially reduced the recipient's anti-B titer to a low level. Once a secondary anti-B response was mounted, plasma exchange was ineffective and IgM and IgG anti-B titers rose to high levels. Associated with the increased anti-B titers, cardiac function deteriorated and on day 13 the group B heart was replaced by a group A allograft. The compatible allograft functioned well initially but was eventually rejected, and the patient died 51 days after the initial transplantation. Histologic examination of the first allograft revealed a delayed form of typical antibody-mediated rejection with destruction of the microvasculature associated with antibody deposition and acute inflammation. By contrast, the histopathology of the second compatible allograft was typical of cell-mediated allograft rejection. Extracts of myocardium from the incompatible heart contained IgM and IgG anti-B, while no anti-B alloantibody was demonstrable in the extracts of the ABO-compatible allograft and a control heart. The utility of plasma exchange with group AB FFP replacement in such a circumstance requires further study.

**Table 2. Demonstration of Anti-B in Heart Tissue Extracts**

| | Titers Against Group B RBC | | | | | |
|---|---|---|---|---|---|---|
| | Incompatible Group B Heart | | Compatible Group A Heart | | Control Heart | |
| | IgM* | IgG* | IgM | IgG | IgM | IgG |
| First homogenate supernatant | 8 | 16 | 0 | 0 | 0 | 0 |
| Second homogenate supernatant | 8 | 0 | 0 | 0 | 0 | 0 |
| Glycine-HCl eluate | 8 | 0 | 0 | 0 | 0 | 0 |
| Serum† | 512 | 16 | 1024 | 32 | 128 | 32 |

*Results negative with group A RBC.

†On day heart removed.

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