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ORIGINAL ARTICLE

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In vitro assessment of a new ABO immunosorbent with synthetic carbohydrates attached to sepharose

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Abstract Transplantation across the ABO barrier is sometimes done in cases of emergency, such as acute liver failure, but is also carried out in elective cases, e.g. kidneys from living donors. Reducing the recipient anti-A/B antibody titres is often necessary in ABO-incompatible kidney transplantation. This is usually done by the use of techniques such as plasmapheresis and protein A- or sepharose-linked anti-human Ig immunoadsorption. A new ABO immunosorbent with synthetic A- or B-trisaccharide carbohydrate epitopes linked to a sepharose matrix has been tested. Columns made of this material have been tested in vitro with plasma from A- and B-individuals, assessed for antibody reduction capacity, flow characteristics, biocompatibility, and unspecific protein adsorption. The columns have a high capacity for ABO antibody removal, reducing titres by three to seven steps in one passage. We noted a high biocompatibility, with no unspecific protein adsorption, no activation of coagulation factors, and a low activation of complement, no immune complex formation and no cytotoxicity towards cultured mammalian L929 cells.

Keywords Immunosorbent · ABO antibodies · Biocompatibility · ABO incompatible transplantation

Introduction

Removal of ABO antibodies may be necessary in certain situations such as bone marrow transplantation across the ABO barriers [1, 2]. In solid-organ transplantation, the ABO barrier is usually not crossed. The shortage of organs and increasing waiting time for transplant surgery justifies an increased use of ABO-incompatible grafts [3]. Several hundred ABO-incompatible liver and kidney transplantations have been performed during the past decades [4], with a 1-year graft survival rate of approximately 50% to 60% [5]. For kidney recipients that have high anti-A/B antibody titres, removal of those prior to ABO-incompatible grafting is necessary [6]. Some years ago a commercial ABO immunosorbent, based on synthetic A- and B-carbohydrates linked to silica particles, was available [7], but this material is now off the market. Thus, for the removal of ABO antibodies, unspecific methods, such as plasmapheresis [8] or immunoadsorption, using protein A [9] or anti-human Ig [10] columns, have been used during the past decade.

These techniques can have disadvantages such as loss of essential plasma components, e.g., fibrinogen and antithrombin 3 can cause coagulation disorders and general loss of immunoglobulins. Recently, a new immunosorbent material, with synthetic A- or B-trisaccharide epitopes linked via a six-hydrocarbon spacer to sepharose, has been developed. The advantage of the use of columns based on this material is that they specifically remove the proteins (A/B antibodies) responsible for hyper-acute rejection in ABO-incompatible solid-organ transplantation. We carried out an in vitro experimental study to estimate the efficacy of these columns to remove anti-A and anti-B antibodies from large volumes of human plasma as well as their biocompatibility.

Materials and methods

Columns

Immuno-adsorbents, Glycosorb-ABO, A-columns and B-columns, were obtained from Glycorex Transplantation AB (Lund, Sweden). Blood group A-trisaccharides [GalNAcα1–3(Fucα1–2)Gal, Glycosorb-ABO, A-column] and blood group B-trisaccharides [Galα1–3(Fucα1–2)Gal, Glycosorb-ABO, B-column] were covalently bound via a six-hydrocarbon spacer to sepharose particles and packed in columns made of polycarbonate plastic and steam sterilized (fluid path). The A- and B-trisaccharides were synthesised by Glycorex, using in vitro chemo-enzymatic procedures. Both products are EC-certified as medical devices (CE-0413).

Plasma samples

Our study was approved by the human ethics committee at Sahlgrenska University Hospital. Plasma used for absorption experiments were from two different sources.

For antibody removal and biocompatibility tests, we collected plasma by plasmapheresis from healthy blood donors of the same ABO blood type. The plasma from two donors was mixed in one bag, and sodium citrate solution (4%) was added at a volume of 1:8. The total volume of plasma was approximately 1.2 l and was used within 1 h of collection. The blood group A columns were tested with three plasma batches of blood group O and one of blood group B, while all five B-columns were tested by O plasma. To test the maximum antibody binding capacity, we collected a large volume of plasma (approximately 5 l) from either patients that were being treated by plasmapheresis due to neurological diseases or from stored blood bank plasma pooled from a number of blood donors.

Column absorption procedure

The perfusion set-up consisted of a pump, a BEH10 Gambro (Gambro BCT, Lund, Sweden), a plasma heater (Sthiler Electronics, Germany) and tubing from Hosp-Med (BHL/460). The columns were pre-washed with approximately 1,000 ml isotonic NaCl, and the plasma perfusion was started. The plasma was pumped from the bag to the plasma heater (38°C) and through the immuno-adsorption column and then discarded. Sampling ports were placed in the tube line directly before and after the column, and serum samples were collected from the inflow of the column at 5, 10 and 20 min, and from the outflow at 5, 10, 20, 30, and 40 min. The plasma flow was 30–35 ml/min. Serum samples were kept frozen at −80°C if not analysed immediately.

The temperature gradient from the heater to the outflow from the column was 38°C to 31°C. After the perfusion, the columns were washed with 500 ml NaCl, and bound ABO antibodies were eluted with glycine buffer (0.1 mol/l, pH 2.8).

Antibody titre analysis

Titrations were done by standard blood banking techniques. One hundred microlitres of serum in two-step serial dilution was put into a 96-well microtitre plate, and 30 µl of a 2% solution of A_1 and B erythrocytes, suspended in isotonic saline solution, was added. The mixture was incubated at room temperature for 30 min. After the plates had been shaken, haemagglutination (HA) was read macroscopically.

Immune antibodies (IATs) were titrated as follows: 40 µl of the erythrocyte–serum mixture in the microtitre plates was transferred to gel cards containing indirect antiglobulin serum (DiaMed AG, Cressier s/Morat, Switzerland). The gel cards were incubated at 37°C for 15 min, and, after being spun, they were read macroscopically.

Plasma protein determination

Albumin, IgG, IgM and IgA were determined by nephelometry. C3, C3d, C4 were determined by radial immunodiffusion. Immune complex formation was determined by enzyme immunoassay [11]. Circulating immune complex was precipitated by polyethylene glycol. After being washing, the complexes were adsorbed onto anti-C3-coated micro-plates and visualised with enzyme-linked anti-human IgG.

C3a and sC5b-9 (terminal cascade complex) were determined by a commercially available enzyme immunoassay (Quidel, San Diego, Calif., USA) [12]. FVIII:C was determined by nephelometry, and prothrombin

fractions 1+2 by an immuno-enzymatic method. All analyses were done by the Department of Clinical Chemistry and Clinical Immunology at Sahlgrenska University Hospital except for C3a and C5b-9, which were done at a research laboratory [12].

Cell cytotoxicity was tested in vitro on cultured mammalian L929 cells (mouse fibroblasts), in accordance with ISO standard 10993-5, to exclude release of cytotoxic material from the columns. The columns were filled with 0.9% NaCl and incubated for 24 h at 37°C. The eluate was diluted to 10% and 2.5% v/v with fresh culture medium and added to the cells. Cell cultures were inspected with an inverted phase microscope.

Anti-A/B antibody characterisation

The antibodies bound to the columns and eluted with glycine buffer (0.1 mol/l, pH 2.8) were dialysed against 1% PBS at 8°C for 2 days, lyophilised, and dissolved in 8 ml PBS for each antibody column batch. The antibody batches were tested for protein content by electrophoresis and their blood group A and B red blood cell (RBC) agglutination titres. The antigen-binding epitope was specificity tested against several structurally different blood group A and B glycolipid antigens in a chromatogen binding assay [13]. In this assay, total neutral glycolipid fractions, isolated from various human tissues (Fig. 1), were applied to high-performance thin-layer plates (HPTLC, Merck, Darmstadt, Germany) and eluted by chloroform:methanol:water (60:35:8 by volume). These glycolipid fractions contained a large variety of structurally different blood group A and B components based on different core-saccharide chains [14]. The plates were incubated with BSA followed by patient serum in a dilution of 1:20. After washing, the ^{125}I-labelled secondary antibody (rabbit anti-human IgM, Dakopatts, Copenhagen, Denmark; no. A090) was applied for 2 h, and the plates were dried and autoradiographed. The reference plates were stained with the chemical reagent anisaldehyde [14].

Results

Antibody titre reduction

The elimination of anti-A/anti-B antibodies was tested in two different experimental set-ups. First, the antibody titre reduction in 1.2 l fresh plasma obtained by plasmapheresis was determined after passage through the column. Second, the total antibody binding capacity was tested using large batches of human plasma.

The mean titre reduction (HA and IAT titre steps) of anti-A and anti-B antibodies in the 1.2 l fresh plasma batches are listed in Table 1. The data for the anti-A reduction are based on four column adsorption experiments, while the anti-B titre reduction is based on five column adsorptions. The mean titre reduction of the

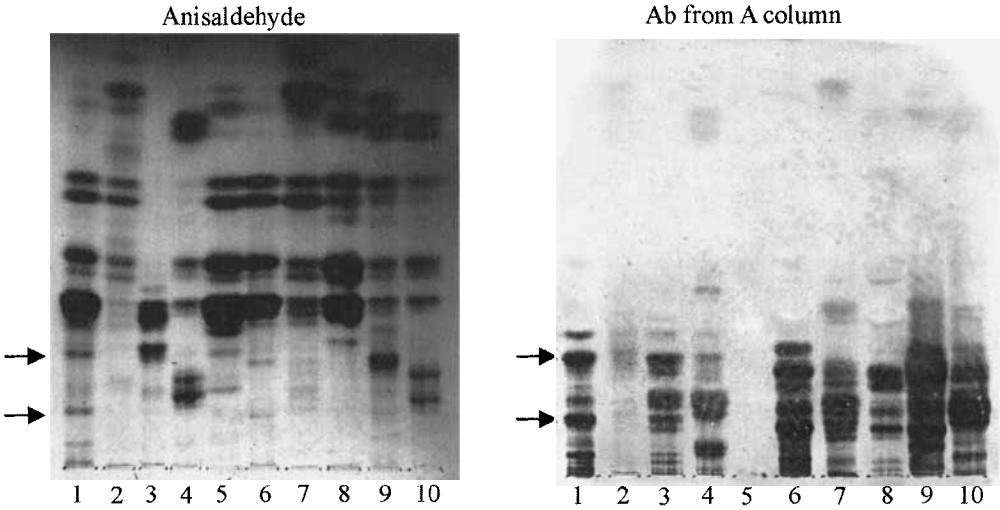

Fig. 1 Chromatogram binding assay of antibodies eluted from a blood group A immuno-adsorption column that had bound antibodies from 1.2 l blood group O plasma. The human tissue glycolipid fractions tested contain a large structural variation in the blood group A and B compounds, and glycolipids from human blood group O erythrocytes (*lane 5*) were used as negative control. The glycolipid fractions applied were neutral glycolipids from A_1Le(a−b+) erythrocytes (*lane 1*), A_1Le(a−b+) plasma (*lane 2*), blood group A kidneys (*lane 3*), blood group A_1Le(a−b+) small intestine (*lane 4*), blood group OLe(a−b+) erythrocytes (*lane 5*), blood group BLe(a−b+) erythrocytes (*lane 6*), blood group BLe(a−b+) plasma (*lane 7*), blood group BLe(a−b+) kidney (*lane 8*), blood group BLe(a−b+) pancreas (*lane 9*) and blood group BLe(a−b+) small intestine (*lane 10*). *To the left* is the thin-layer plate stained with a chemical reagent (anisaldehyde) and *to the right* is the immunostained plate. *The arrows* indicates the mobility of blood group A compounds with six and eight sugar residues in blood group A erythrocytes (*lane 1*).

Table 1 Haemagglutinating (*HA*) and immune (*IAT*) antibody titres of serum samples collected before and after passage through blood group A or B trisaccharide immuno-adsorbent columns

Column	HA			IAT		
	Before	After	Titre step reduction	Before	After	Titre step reduction
A	8	0	4	64	0	7
A	16	0	5	256	16	4
A	16	0	5	256	1	8
A	8	1	3	8	1	3
B	4	0	3	64	0	7
B	4	0	3	128	0	8
B	16	0	5	128	1	7
B	4	0	3	32	0	6
B	16	0	5	32	0	6

haemagglutinating antibodies was by approximately four titre steps for both columns (titre range 4–16 before and 0–1 after), and the mean titre reduction of immune antibodies was by more than five titre steps for the A-columns and seven for the B-columns (titre range 32–512 before and 0–16 after).

The maximum capacity of the columns to remove anti-A/B antibodies was tested in two experiments, and the titre reduction is listed in Table 2. Blood group O plasma, 4.7 l from a patient with Guillain–Barré disease, was passed through an A-column. Haemagglutinating titres were reduced from 8 to 2 (two-titre step reduction) and remained at this level during the experiment, which lasted 105 min. The corresponding IAT titre was reduced from 512 to 16 (five-titre step reduction) 10 min after the start of column absorption and was 32 at the end of the experiment.

Five litres of plasma, collected from blood group A blood donors, was passed through a B-column. The HA and IAT titres decreased from 4 to 0 (three-titre step reduction) after passage and increased to 1 at the end of the experiment.

Characterisation of antibodies eluted from the columns

The eluted antibodies were theoretically concentrated by a factor of 150, compared with serum-based antibodies, on the assumption that all anti-A/B antibodies in 1,200 ml serum were bound to the column and that all were eluted. The antibodies eluted from the A- and B-columns agglutinated A and B erythrocytes, respectively (Table 3). The eluates also cross-reacted with other blood group erythrocytes, e.g., eluate from A-columns agglutinated blood group B erythrocytes. This cross-reactivity showed a slightly lower titre of approximately two titre steps. When compared with the original serum the eluates had a higher titre for the B-column (three to five titre steps), whereas for the A eluates one had a similar titre (16 compared to 32) and the other was two titre steps lower (Table 3).

The eluted antibodies were tested for their ability to react with structurally different blood group A and B glycolipid antigens in a chromatogram binding assay. The results showed that there was a similar binding pattern for the antibodies eluted from both the A and the B immunosorbents (Fig. 1). The antibodies reacted with all different A and B glycolipids, and no reaction was seen with glycolipids from blood group O red blood cells, used as a negative control. We found only slight difference in binding strength but no qualitative differences in the binding pattern.

Biocompatibility

The concentration of albumin, IgG, IgA and IgM determined before and 20/30 min after column adsorption to A- and B-columns, respectively, is shown in Table 4. No significant effect on the total amount of these parameters was noted. The antibody eluates were also tested by protein electrophoresis, and only immunoglobulins were found.

The concentration of C3, C4, and C3d before and 20/30 min after column adsorption, to A- and B-columns respectively, is shown in Table 5. Neither a reduction in the levels of C3 and C4 nor any significant increase in C3d levels could be seen.

The C3a and sC5b-9 values before and after column adsorption, to A- and B-columns respectively, is shown in Table 5. An increased formation of C3a and sC5b-9 occurred in the effluent samples collected at 20, 30 and 40 min. The P-FVIII:C, P-APTT, P-PK and P-prothrombin fraction 1+2 values were not affected by

Table 2 Test of the maximum capacity of the blood group A and B columns to remove anti-A/anti-B antibodies. The anti-A and anti-B titres in the samples collected before and after the passage through the column are given for certain time points

Anti-A (A column)					Anti-B (B column)			
Time (min)	HA before	HA after	IAT before	IAT after	HA before	HA after	IAT before	IAT after
10	8	2	512	16	8	1	16	0
20		2		32		1		0
40	4	2	512	32	8	1	16	0
60		2		32		0		0
80	8	2	512	32	8	0	16	0
105		2		32		0		1
120					8	0	16	1

Table 3 Anti-A and anti-B antibody titres in the glycine buffer eluates from two blood group A and B columns, respectively. The antibody titre of the serum is also listed. The HA titres were tested against human A and B erythrocytes

Column	HA titre B RBCs	HA titre A RBCs	HA titre of starting plasma	
			A RBCs	B RBCs
A	8	32		4
A	32	64		4
B	128	32	16	
B	32	4	256	

column adsorption, as shown in Table 6. No immune complex formation or cytotoxicity to cultured cells was seen in any of the experiments.

Discussion

The shortage of donor organs in solid-organ transplantation has renewed the practice of crossing the ABO barrier and using ABO-incompatible grafts. In Japan more than 300 living-donor kidney transplantations were performed between 1989 and 1998 [15]. One prerequisite is the pre-transplantation removal of anti-A/anti-B antibodies in the recipient. Columns for specific removal of ABO antibodies from plasma have not been available commercially for a number of years. Specific anti-A/B immunosorbents, with synthetic saccharides covalently coupled to macroporous glass beads via polyacrylamide [16], have been produced, but this product is not yet on the market.

A new specific immunosorbent, based on synthetic A- and B-trisaccharides linked to a sepharose matrix, has been tested for its capacity to remove anti-A/B antibodies and for its biocompatibility. The columns have shown high capacity to remove anti-A/B antibodies in one session, with a capacity for removal of more than 90%.

The capacity to remove anti-A/B antibodies in large volumes of plasma has also been tested. The titres were reduced by one passage, leaving remaining titres of haemagglutinating and immune antibodies at low levels,

Table 4 Mean values and range of total serum albumin (*Alb*), IgG, IgA and IgM in plasmapheresis plasma before and after adsorption on synthetic A- or B-trisaccharides linked to sepharose columns

Parameter	A-column ($n=4$)		B-column ($n=5$)	
	Before	After 20/30 min	Before	After 20/30 min
Alb (g/l)	34 (30–37)	34 (30–36)	34 (31–36)	35 (33–36)
IgG (g/l)	9.1 (7.4–10)	8.9 (7.5–9.8)	8.8 (7.3–11)	8.6 (7.1–10)
IgA (g/l)	1.7 (1.3–2.2)	1.7 (1.2–2.2)	1.4 (1.2–1.8)	1.4 (1.1–1.8)
IgM (g/l)	0.76 (0.73–0.78)	0.75 (0.72–0.79)	0.65 (0.41–0.77)	0.64 (0.42–0.75)

Table 5 Mean values and range of total serum C3, C4, C3a, C3d and C5b-9 in plasmapheresis plasma before and after column adsorption on synthetic A- or B-trisaccharides linked to sepharose columns

Parameter	A-column ($n=4$)		B-column ($n=5$)	
	Before	After	Before	After
C3 (g/l)	1.31 (1.08–1.64)	1.32 (1.03–1.61)	1.37 (1.30–1.40)	1.39 (1.29–1.42)
C4 (g/l)	0.25 (0.20–0.34)	0.24 (0.20–0.31)	0.23 (0.19–0.27)	0.23 (0.18–0.28)
C3d (mg/l)	8.0 (5.3–12.5)	8.5 (5.6–13.4)	8.1 (6.6–10.4)	7.6 (6.6–8.8)
C3a (ng/ml)	2,252 (870–4,891) ($n=2$)	2,267 (798–3,275) ($n=2$)	1,075 (754–1,590) ($n=3$)	1,872 (935–4,891) ($n=3$)
sC5b-9 (TCC) (ng/ml)	907 (115–2,272) ($n=2$)	598 (73–1,106) ($n=2$)	395 (135–795) ($n=3$)	1,872 (73–1,660) ($n=3$)

Table 6 Mean values and range of P-FVIII:C (IU/ml), APTT, PK and prothrombin fractions 1+2 in plasmapheresis plasma before and after column adsorption on synthetic A- or B-trisaccharides linked to sepharose columns (*INR* international normalised ratio)

Parameter	A-column ($n=2$)		B-column ($n=4$)	
	Before	After	Before	After
FVIII:C IU/ml	0.98 (0.75–1.44) ($n=4$)	1.04 (0.79–1.56) ($n=4$)	0.93 (0.80–1.09) ($n=5$)	0.88 (0.80–1.14) ($n=5$)
P-APTT (s)	37 (36–38) ($n=2$)	38 (36–39) ($n=2$)	35 (34–37) ($n=4$)	35 (34–37) ($n=4$)
P-PK (INR)	1.1 (1.1–1.1) ($n=2$)	1.1 (1.0–1.1) ($n=2$)	1.0 (1.0–1.0) ($n=4$)	0.95 (0.9–1.0) ($n=4$)
P-thrombin fraction 1+2 (nmol/l)			1.4 (1.4–1.4) ($n=2$)	1.4 (1.3–1.4) ($n=2$)

and with no obvious sign of saturation of the filter. The remaining antibodies that were not removed by the immunosorbent column may require carbohydrate epitopes with four or more sugars, including part of the core chain structure, to be adsorbed [17].

The flow rate of 30–35 ml/min seems ideal; an increase up to 50 ml/min increased the pressure. However, an increase in the pump rate did not influence the efficacy of the antibody removal.

The eluted antibodies from the B-columns showed high titres against B-erythrocytes but also titres, although lower, against A-erythrocytes. Likewise, eluted antibodies from A-columns showed high titres against A-erythrocytes, but also lower titres against B-erythrocytes. This means that a large proportion of the bound antibodies have the specificity for anti-A/B antibody, recognising a common core carbohydrate structure in A- and B-antigens. This specificity is common in blood group O individuals [18], especially after they have been immunised with A or B blood-group-like substances [19]. This can also be seen in the thin-layer immunostaining assay, where eluted antibodies bind to both A-active and B-active glycolipids but not to glycolipids prepared from blood group O individuals.

No specific adsorption of proteins, as measured by total serum albumin, IgG, IgA and IgM, could be seen. The columns showed high biocompatibility, as complement factors were unaffected, FVIII:C did not decrease significantly and P-APTT and P-PK values were unaffected. No formation of immune complexes could be noted in any of the experiments.

During the passage of plasma through the column an activation of the complement cascade occurred, as shown by the increase in concentrations of C3a and sC5b-9. It is well known that activation of complement occurs in most cases of exposure of human blood/plasma to artificial surfaces such as haemodialysis filters or plastic tubing [20]. The most sensitive parameter of complement activation is the formation of C3a and sC5b-9. The values obtained are in the same range as in other extracorporeal perfusion treatments [21, 22]. Thus, these new columns are efficient, they specifically remove only anti-A/B antibodies and they are biocompatible.

The columns have been used, so far, for pretreatment of three blood group O recipients in our centre with a satisfactory reduction in haemagglutinin and IAT anti-A/B titres. No side effects of the column treatments were recorded, and the kidneys were grafted successfully. Although the columns have not been tested to a great extent in patients, the capacity to remove anti-A/B antibodies indicates that three sessions will be sufficient to reduce titres to levels of 8 or below (i.e. levels at which it has been shown possible to perform successful transplantations across the ABO barrier) [23]. This new antibody removal may help to alleviate the situation for a number of patients on the waiting list for kidney transplantation who are fortunate to have a living donor but who happen to be ABO incompatible.

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References

1. Berkman EM, Caplan S, Kim CS. ABO-incompatible bone marrow transplantation: preparation by plasma exchange and in vivo antibody adsorption. Transfusion 1978; 18:504.
2. Osterwalder B, Gratwohl A, Nissen C, Speck B. Immunoadsorption for removal of anti-A and anti-B blood group antibodies in ABO-incompatible bone marrow transplantation. Blut 1986; 53:379.
3. Alexandre GPJ, de Bruyere M, Latinne D, et al. ABO-incompatible related and unrelated living donor renal allografts. Transplant Proc 1986; 18:452.
4. Rydberg L. ABO-incompatible solid organ transplantation. Transfus Med 2001; 11:325.
5. Wu A, Bühler LH, Cooper DKC. ABO-incompatible organ and bone marrow transplantation: current status. Transpl Int 2003; 16:201.
6. Tanabe K, Takahashi K, Sonda K, et al. Long-term results of ABO-incompatible living kidney transplantation. Transplantation 1998; 65:224.
7. Bensinger WI, Baker DA, Buckner CD, Clift RA, Thomas ED. In vitro and in vivo removal of anti-A erythrocytic antibody by adsorption to a synthetic immunoadsorbent. Transfusion 1981; 21:335.
8. Agishi T, Takahashi K, Ota K, Yagisawa T. Comparative evaluation of immunoadsorption and double filtration plasmapheresis for removal of anti-A and anti-B antibody in ABO-incompatible kidney transplantation. Transplant Proc 1992; 24:557.
9. Gjörstrup P, Watt RM. Therapeutic protein A immunoadsorption. A review. Transfus Sci 1990; 11:281.
10. Leventhal JR, John R, Fryer JP, et al. Removal of baboon and human anti-porcine IgG and IgM natural antibodies by immunoadsorption. Results of in vitro and in vivo studies. Transplantation 1995; 59:294.
11. Tarkowski A, Czerkinsky C, Nilsson LÅ. Simultaneous induction of rheumatoid factor and antigen-specific antibody-secreting cells during secondary immune response in man. Clin Exp Immunol 1985; 61:379.
12. Bengtsson A, Svalander C, Mölne J, Rydberg L, Breimer ME. Extracorporeal ("ex vivo") connection of pig kidneys to humans. III Studies of plasma complement activation and complement deposition in the kidney tissue. Xenotransplantation 1998; 5:176.
13. Rydberg L, Björck S, Hallberg E, et al. Extracorporeal ("ex vivo") connection of pig kidneys to humans II. The anti-pig antibody response. Xenotransplantation 1996; 3:340.

14. Holgersson J, Breimer ME, Samuelsson BE. Basic biochemistry of cell surface carbohydrates and aspects of the tissue distribution of histo-blood group ABH and related glycosphingolipids. Acta Pathol Microbiol Immunol Scand Suppl 1992; 27:18.
15. Takahashi K. ABO-incompatible kidney transplantation. Elsevier Science, Amsterdam, the Netherlands, 2001.
16. Rieben R, Korchagina EY, von Allmen E, et al. In vitro evaluation of the efficacy and biocompatibility of new, synthetic ABO immunosorbents. Transplantation 1995; 60:425.
17. Rydberg L, Breimer ME, Holgersson J, et al. Characterization of the anti-A antibody response following an ABO (A_2 to O) kidney transplantation. Residual anti-A type 1 chain specific antibody activity after adsorption with A_1 erythrocytes and Synsorb-A. Mol Immunol 1992; 29:547.
18. Dodd BE, Lincoln PJ, Boorman KE. The cross-reacting antibodies of group O sera. Immunological studies and possible explanation of the observed facts. Immunology 1967; 12:39.
19. Contreras M, Armitage SE, Hewitt PE. Response to immunisation with A and B human glycoproteins for the procurement of blood grouping reagents. Vox Sang 1984; 47:224.
20. Hed J, Johansson M, Lindroth M. Complement activation according to the alternate pathway by glass and plastic surfaces and its role in neutrophil adhesion. Immunol Lett 1984; 8:295.
21. Hyllner M, Arnestad JP, Bengtsson JP, Rydberg L, Bengtsson A. Complement activation during storage of whole blood, red cells, plasma and buffy coat. Transfusion 1997; 37:264.
22. Åvall A, Hyllner M, Bengtsson JP, Carlsson L, Bengtsson A. Greater increase in cytokine concentration after salvage with filtered whole blood than with washed red blood cells, but no difference in post-operative hemoglobin recovery. Transfusion 1999; 39:271.
23. Nelson PW, Helling TS, Shield CF, Beck M, Bryan CF. Current experience with renal transplantation across the ABO barrier. Am J Surg 1992; 164:541.