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Blocking of human anti-pig xenoantibodies by soluble GALα1–3GAL and Galα1–2GAL disaccharides; studies in a pig kidney in vitro perfusion model

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Abstract Depletion of anti-pig xenoantibodies reduces cell cytotoxicity of human serum to pig endothelial cells and lymphocytes. The aim of this study was to test, in a pig kidney xenoperfusion model, the ability of soluble αGal terminated disaccharides to prevent the hyperacute rejection process in an organ. Porcine kidneys were perfused with whole human blood lacking saccharide and blood supplemented with Galα1–3GAL, Galα1–2Gal and lactose. Parameters evaluated were, urine production, renal blood flow, vascular resistance, renal clearance, blood cell counts, xenoantibody titers, complement activation and histopathology. The blood flow was higher in the Galα1–3Gal (155 ± 31 ml/min × 100 g^{-1} kidney tissue) group compared to Galα1–2Gal (138 ± 16), lactose (92 ± 78) and controls (69 ± 16). When calculated as percent of the blood flow value at 1 min, the blood flow at 30 min was 157% for the Galα1–3Gal and for 187% the Galα1–2Gal. The corresponding values for the lactose and control groups were 102% and 74%, respectively. Urine production in the lactose/control groups was lower (0.7 ml/min × 100 g^{-1} kidney tissue) compared to Galα1–3Gal (3.0) and Galα1–2Gal (3.7). Urine sodium excretion was reduced in the lactose/control groups, compared to the Galαα1- groups during the perfusions. An increase in urine potassium excretion was found in the Galαα1-groups while a reduction occurred in the lactose/control experiments. An initial 40–50% reduction in platelet count was observed in all groups while the leukocyte count showed a continuous decrease. Immunohistochemistry revealed less deposition of IgM, IgG, C3 and C1q in the Galαα1-saccharide groups compared to the lactose/control groups. Soluble Galαa1-disaccharides improved both functional and histological parameters. However, significant pathological changes were still present indicating that this approach to inhibit HAR must be used in combination with additional therapeutic approaches such as solid phase xenoantibody immunoadsorption and blocking of complement activation.

Keywords Xenotransplantation · Hyperacute rejection · Pig kidney · in vitro perfusion · Galα-saccharides

Abbreviations *HAR* Hyperacute rejection · *GFR* Glomerular filtration rate

Introduction

Hyperacute rejection (HAR) of vascularised pig xenografts in human or primate recipients is initiated by preformed xenoantibodies present in the recipient, and is the first major barrier to overcome in order to make xenotransplantation of pig organs clinically applicable [26]. Most of the human anti-pig xenoantibodies binds to the Galα1–3Gal antigens present on cell surface glycoproteins and glycolipids [7, 27, 29]. About 1–2% of total human serum IgG and 4–8% of IgM have been found to react with the Galα1–3Gal epitope [18]. Several approaches to block the HAR initiated by the Galα1–3Gal / anti-Gal antibody interaction have been proposed and tested experimentally. First, removal of the recipients xenoantibodies by non-specific techniques (extracorporeal organ perfusion, plasmapheresis or immunoadsorption by protein A) [1, 25, 35] or specific saccharide immunoadsorbents [33, 38]. Second, intravenous infusion of soluble αGal terminated oligosaccharides functioning as haptens in baboons transplanted with pig xenografts [30, 39]. Third, change of the expression of Galα1–3Gal determinants in the graft by α1–3galactosyltransferase gene knockout [32, 34], diverging the Galα1–3Gal biosynthesis by addition of the α1,2fucosyltransferase gene forming the blood group H determinant [5, 6, 28] or removing the antigen determinant by α-Galactosidase [15, 21, 36]. Fourth, blocking complement activation by infusion of human soluble complement receptor 1 [23] or production of pig strains transgenic for human complement regulatory proteins [9, 11, 14].

The use of soluble blood group A and B oligosaccharides to block preformed antibodies has been successful for the treatment of haemolytic disease of the newborn caused by ABO incompatibility [24] and, experimentally, to prevent HAR in ABO incompatible heart transplantation in baboons [8]. αGal-terminated saccharides have been tested for their ability to block the binding of human xenoantibodies to pig cell cultures and to block the cytotoxic effect of human plasma on pig endothelial and epithelial cell cultures [12, 19, 39]. The possibility to test soluble Galα1–3Gal saccharides in full-scale experiments in primates and organ perfusion with human blood has been hampered by the lack of a sufficient amount of saccharides and by the great costs. To our knowledge, only one single pig organ perfusion experiment with human blood has been reported so far. In that experiment, the tissue damage was evaluated histologically, while kidney function parameters were not studied [4]. The aim of this study is to evaluate the ability of soluble Galα saccharides to block hyperacute rejection and the following functional impairment and tissue damage. Porcine kidneys were perfused with human whole blood supplemented with Galα1–2Gal, Galα1–3Gal, lactose and compared to control experiments, in which pig kidneys were perfused with human blood lacking saccharide.

Materials and methods

Animals and surgical procedure

Domestic pigs of both sexes and of about 40 kg body weight were used. The blood group type of the pigs (A/O) was established by hemagglutination using 3 different monoclonal blood group A antisera. The pigs were bred and maintained in compliance with the principles of laboratory animal care stated in the "Guide for the care and use of laboratory animals", NIH. The study was approved by the University ethical committee for animal investigation. Atropine 0.5 mg/kg and pethidin hydrochloride 2 mg/kg were given as premedication, and anaesthesia was induced by ketamine hydrochloride 25 mg/kg i.m, followed by injection of azaperonum (Janssen Pharmaceutical, Beerse, Belgium) 2 mg/kg, and metomidati chloride (Janssen Pharmaceutical) 4 mg/kg i.v. Anaesthesia was maintained by iteration of azaperonum and metomidati chloride injections. The kidneys were exposed by a median laparotomy, removed after administration of 12000 U of heparin i.v., and immediately flushed with 20 ml of saline containing 5000 U heparin. This was followed by perfusion with 500 ml cold Soltran kidney perfusion solution (Baxter, Norfolk, U.K) at a hydrostatic pressure of about 100 cm H_2O. The kidneys were stored on ice until the perfusion experiment. Warm and cold ischemia times were approximately 2–3 min and 15 min respectively. The kidneys were flushed with 100 ml saline before connection to the perfusion circuit, in order to remove the high potassium concentration present in the Soltran solution.

Preparation of heparinized human blood

450 ml blood from regular blood donors, with the corresponding blood group A/O type as the pigs were collected in transfusion bags containing 63 ml of citrate-phosphate-dextrose, CPD solution (327 mg acid citrate monohydrate/100 ml CPD, Baxter UK). About 3 U of Heparin/ml was added and the anti-coagulating effect mediated by the CPD solution was neutralised by addition of 0.059 mmol Mg^{2+} and 0.24 mmol Ca^{2+} respectively ($MgCl_2$, $CaCl_2$, MERCK, Darmstadt, Germany) per 100 ml plasma.

Blocking of human natural anti-Gal antibodies by soluble saccharides

Galα1–3Galα1-O-C_6H_4-NH-CO-CH_3(*para*) and Galα1–2Galα1-O-C_6H_4-NH-CO-CH_3(*para*) sacharides were from GLYCOREX, Lund Sweden and lactose (Galβ1–4Glc) with non-substituted reducing end was from Sigma (St. Louis MI). The soluble disaccharides were added to the heparinized human blood to a final concentration of 10 mg/ml plasma (about 3 g per experiment).

In vitro perfusion procedure

The pig kidney perfusion experiment set up has been described in detail elsewhere [3]. The kidneys were placed in a perfusion chamber and connected to the perfusion circuit by Luer connection nipples inserted in the renal artery and vein. A thin catheter was placed in the ureter. A dialysis pump unit (AK-10, Gambro,

Lund Sweden) equipped with a roller pump, flow and pressure recording units, a diffusion membrane oxygenator (O_2/CO_2, 95/5) with a heater casing (M5 model, Jostra Medizintechnik, Hirrlingen Germany) were used. The different parts of the circuit and the transfusion bag reservoir containing the blood were connected to each other by hemodialysis lines (Gambro) with permeable membrane ports for sample collection and injections of drugs. The perfusions were performed at 38 °C, the venous outflow was drained into an open bubble trap, and the outflow level was adjusted to a pressure of + 3 cm water. The major part of the urine produced was repeatedly re-injected into the system during the perfusion to maintain constant levels of electrolytes, urea, creatinine, PAH and iohexol. Mean weight of the kidneys ($n = 12$) before perfusion was 134 (± 20) g. The kidneys were perfused with whole human blood supplemented with 10 mg/ml of Galα1–3Gal ($n = 3$), Galα1–2Gal ($n = 3$), lactose ($n = 4$). The control kidneys ($n = 2$) were perfused with human blood lacking saccharide. The blood flow rate was continuously adjusted in order to keep artery perfusion pressure at 100 mm Hg and the perfusions were terminated at 30 min.

Laboratory analysis

Blood/plasma and urine samples were analysed by routine techniques used at the laboratory of clinical chemistry, Sahlgrenska Universitets sjukhuset. The leukocyte and platelet counts were established by an automatic cell counter, Technicon H2 (Bayer, Germany).

Clearance analysis

Three hundred mg of iohexol (Omniuepaque, Nycomed Pharma, Oslo, Norway) and 100 mg of PAH (Aminohippurate sodium, Merck && Co Inc, West Point, PA) were injected into the circulating system immediately after the kidneys were connected to the circuit. The concentration of iohexol in plasma was measured with X-ray fluorescence using a Renalyzer PRX90 (Provalid AB, Lund Sweden). PAH concentration in plasma was measured by using a Hitachi model 100–20 spectrophotometer at 450 nm (Hitachi Ltd, Tokyo Japan).

In this study we used a recirculating in vitro perfusion model in which fluid losses occur due to collection of urine samples and to a slight leakage of lymphatic fluid from the kidney hilus. With this experimental set-up, the standard clearance equation ($Cl = Q_U \times C_U / C_A$) used for *in vivo* estimation must be slightly modified. Therefore, the calculation of plasma clearance is based on the A-V differences of iohexol/PAH plasma concentration, and compensating for the differences in arterial and venous blood flow as well as for the A-V differences in hematocrit during the perfusions. Moreover, except for the losses previously described, it is assumed that blood cells, plasma and the amount of iohexol/PAH are conserved in the system (= conservation of mass).

Hence, the arterial blood flow, $Q_{B\text{-}A}$ equals the sum of the venous blood flow, $Q_{B\text{-}V}$ and urine flow, Q_U as follows: $Q_{B\text{-}A} = Q_{B\text{-}V} + Q_U$ (Eq 1). Secondly, conservation of erythrocytes in the system gives the following formula for arterial hematocrit: $Hct_A = Q_{B\text{-}V} / Q_{B\text{-}A} \times Hct_V$ (Eq 2), where the subscript V and A denotes venous and arterial respectively. Combining Eq 1 and 2 gives: $Hct_A = (Q_{B\text{-}A} - Q_U) / Q_{B\text{-}A} \times Hct_V$ (Eq 3). Thirdly, clearance (Cl) for iohexol/PAH can be obtained from the arterial and venous concentrations using the following equation: $Cl = ((C_A \times Q_{B\text{-}A} \times (1\text{-}Hct_A)) - (C_V \times Q_{B\text{-}V} \times (1\text{-}Hct_V)) / C_A$ (Eq 4), where C_A and C_V denotes arterial and venous plasma concentration of iohexol/PAH respectively. Combining Eqs 1, 3 and 4 gives the following equation used for calculation of clearance values per 100 g of kidney weight, W:

$$Cl = \frac{\left[\frac{1-(Q_{B\_A}-Q_U)}{Q_{B\_A}} \cdot Hct_V\right] \cdot Q_{B\_A} \cdot C_A - \left[\left[\frac{1-(Q_{B\_A}-Q_U)}{Q_{B\_A}} \cdot Hct_V\right] Q_{B\_A} - Q_U\right] \cdot C_V}{C_A \cdot \frac{W}{100}}$$

Flow cytometric analysis of human anti-pig IgG and IgM antibodies

Porcine lymphocytes were separated on Lymphoprep (Nycomed Pharma) from fresh defibrinated pig blood. Perfusate samples were collected at different times during the pig kidney perfusions, from 6 healthy volunteers. Thirty µl of resuspended porcine lymphocytes in PBS were mixed with 30 µl of each sample and incubated for 30 min at room temperature. The cells were washed with PBS (pH 7.5 with 0.1 % sodium azide) and centrifuged at 670 × *g* for 5 min. The cells were incubated with fluorescein (FITC)-conjugated AffiniPure F(ab') fragment goat anti-human IgG + IgM (H + L), (Jackson ImmunoReseach Laboratories, Inc, West Grove, PA) in PBS/sodium azide for 20 min on ice, washed, and resuspended in 300 µl PBS/sodium azide. Flow cytometric analysis was performed using a FACScan (Becton Dickinson, San Jose CA) instrument. Five thousand events were collected and the median fluorescence intensity (MFI) was recorded.

Analysis of complement activation

The samples for complement measurements were drawn in precooled tubes containing 0.1 ml/10 ml of ethylene diamine tetraacetic acid (EDTA, 0.47 mol/l) stored on ice, centrifuged at + 4 °C within 2 h and stored at –70 °C until analysis. The C3a anaphylatoxin was analysed using an ELISA kit detecting C3a-desArg (Quidel, San Diego, CA), and the soluble non-lytic terminal complement complex using a SC5b-9 ELISA kit (Progen, Biotechnik, Heidelberg, Germany).

Histopathological analysis

Immediately after the perfusions were terminated, kidneys were detached from the perfusion circuit and, 3–5 mm thick slices were fixed in 4 % paraformaldehyde in 0.1 M sodium cacodylate buffer, pH 7.4 overnight at + 4 °C. The samples were embedded in paraffin. As controls, non-perfused, pig kidneys ($n = 4$) were processed similarly as for the perfused kidneys. To detect bound immunoglobulins and complement compounds, the linked streptavidin biotin (LSAB, K675 DAKO A/S, Glostrup Denmark) technique was used as described in [30]. Briefly, deparaffinated 5 µ thick sections were digested for 30 min with protease (type XXIV, product P-8038, Sigma) 0.5 mg/ml in PBS, at 37 °C followed by washings in PBS. Endogenous peroxidase was blocked with 3 % H_2O_2 in Tris-HCl buffer for 10 min. Antibodies used were: rabbit anti human IgA, IgG, IgM, C1q, C3c, CD 68, MAC 378, HLA-DR, fibrin/fibrinogen and mouse anti human GpIIIa, all obtained from DAKO. Positive reaction was visualized with diaminobenzidine (DAB, DAKO). Immunolabeling of each tissue was performed three times using an automatic immunostainer (TechMate TM 500, DAKO). Control sections were consistently negative.

The serial tissue sections obtained from perfused and control porcine kidneys were coded and analyzed blindly. The localization of immunolabeled material was defined in glomerular capillaries (gc), glomerular basement membrane (gbm), peritubular capillaries (ptc), arterioles (aa) and arteries (A). In each cellular compartment, the staining was considered as either generalized (g) or focal (f). The intensity of the immunolabeling was graded as strong (+ +), weak (+) or absent (0).

Results

The aim of this work was to evaluate, in a full-scale experimental set-up, the ability of soluble Galα1- terminating saccharides to inhibit the HAR process and the following functional impairment and tissue damage by in vitro perfusion of pig kidneys with human blood. The amount of saccharides used (10 mg/ml plasma) was selected from earlier studies [24] to reach a maximal antibody binding inhibition. The perfusion time of 30 min was selected based on two factors. First, the requirement of saccharides limited the perfusate volume to about 500 ml, which restricted the number of samples collected for monitoring. Second, the HAR process occurs within minutes in this discordant species combination, and to obtain an optimal morphological analysis, the experiments were terminated at 30 min. In total, 12 pig kidneys were perfused with whole human blood supplemented with soluble Galα1–3Gal ($n = 3$), Galα1–2-Gal ($n = 3$), lactose ($n = 4$) and the control perfusions ($n = 2$) with human blood lacking saccharide. In addition, the perfusion circuit (omitting the kidney) was perfused with human blood to obtain baseline values ($n = 4$) for the changes in red blood cell, leukocyte and platelet counts as well as complement activation.

Renal blood flow, vascular resistance and urine production

In all perfusion experiments, the perfusion pressure (arterial side) was kept stable at 100 mm Hg by pump speed regulation. The experiments using the Galα1–3-Gal disaccharide showed the highest blood flow 155 ± 31 ml/min × 100 g^{-1} kidney tissue at 30 min of perfusion, which was higher, compared to the Galα1–2Gal disaccharide (138 ± 16). The blood flow for lactose and the control experiments lacking saccharides were 92 ± 78 and 69 ± 16, respectively. When calculated as percentage of the blood flow value at 1 min, the blood flow for the Galα1–3Gal was 157% and for the Galα1–2Gal 187% (Fig. 1A). The corresponding values for the lactose and control groups were 102% and 74%, respectively. The vascular resistance, calculated as percent of the vascular resistance value at 1 min (Fig. 1B), of the kidneys perfused with human blood supplemented with Galα1–3Gal and Galα1–2Gal were at 30 min 60% and 54%, respectively. The corresponding values for the lactose and the controls lacking saccharide were 131% and 132%, respectively. The urine production (Table 1) in the lactose and control groups showed a distinct decrease and was less than 1 ml/min x 100 g^{-1} kidney tissue at 30 min. The Galα1–3Gal and Galα1–2Gal groups showed a stable level of urine production of 3.0 and 3.7 ml/min × 100 g^{-1} at 30 min (Table 1). No macroscopic hematuria was observed in any of the perfusion experiments.

	1 min	10 min	20 min	30 min
-■-	99 (37)	133 (49)	151 (31)	155 (31)
-◆-	74 (7)	116 (36)	116 (48)	138 (16)
-▲-	93 (30)	82 (4)	67 (3)	69 (16)
-●-	90 (24)	103 (76)	93 (78)	92 (78)

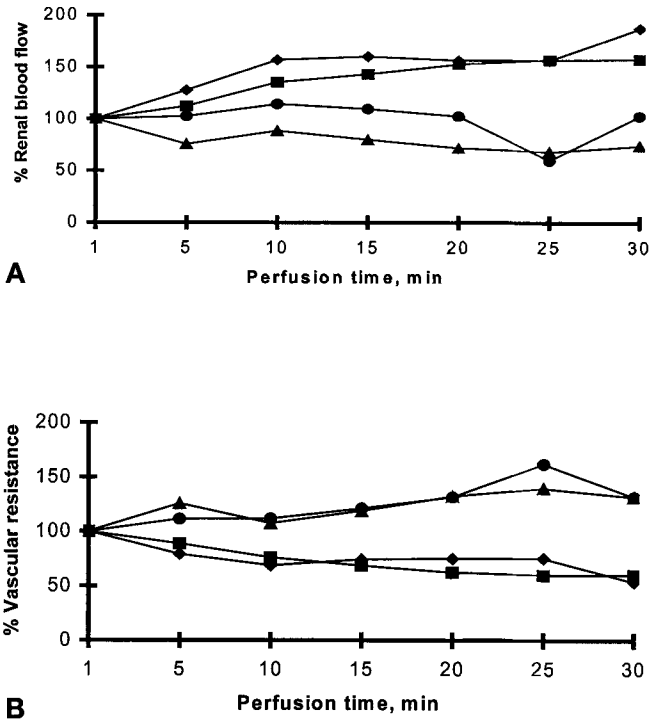


Fig. 1 A, B Renal blood flow (**A**) and vascular resistance (**B**), calculated as percent of the blood flow and vascular resistance value at 1 min, of porcine kidneys perfused with human blood at a pressure of 100 mmHg. A table of the absolute renal blood flow values is inserted in **A**. Galα1–3Gal ($n = 3$) ■, Galα1–2Gal ($n = 3$) ◆ and lactose ($n = 4$) ● were added to the human blood to a final concentration of 10 mg/ml plasma. Control experiments with human blood lacking saccharide ($n = 2$) are represented by ▲. Each point measured represent the mean value and is calculated per 100 g kidney tissue weight

Table 1 Diuresis, Iohexol/ Aminohippurate sodium clearance, plasma flow, filtration fraction, and urine sodium and potassium excretion during perfusion of porcine kidneys with heparinized fresh human blood, supplemented with 10 mg/ml of Galα1–2, Galα1–3, lactose and control lacking saccharides. Samples were collected at 10 and 30 min, respectively. Filtration fraction = Iohexol clearance / plasma flow. All values, mean (± SD), were calculated per 100 g kidney tissue weight

	Experimental set-up			
	Galα1–3 ($n = 3$)	Galα1–2 ($n = 3$)	Lactose ($n = 4$)	Control ($n = 2$)
Diuresis (ml/ min)				
10 min	3.1 (2.2)	3.7 (2.4)	3,2 (2,2)	2.8 (1.5)
30 min	3.0 (1.8)	3.7 (2.5)	0,7 (0.7)	0.7 (0.3)
Iohexol clearance (ml/min)				
10 min	7.9 (5.0)	9.9 (3.5)	14.7 (3.6)	9.8 (2.5)
30 min	10.1 (8.4)	7.6 (7.4)	9.7 (3.2)	5.9 (0.4)
PAH clearance (ml/min)				
10 min	54.4 (36.5)	32.4 (14.5)	28.4 (20.8)	29.0 (1.0)
30 min	39.7 (17.3)	38.5 (29.8)	29.6 (31.2)	23.9 (3.4)
Plasma flow (ml/min)				
10 min	88.1 (32.6)	77.8 (25.9)	68.9 (54.2)	52.7 (1.8)
30 min	97.6 (12.6)	74.0 (31.6)	60.0 (52.8)	44.3 (13.1)
Filtration Fraction (%)				
10 min	8.6 (4.8)	13.0 (3.4)	17.6 (7.0)	18.6 (5.3)
30 min	10.0 (7.6)	10.7 (7.9)	8.7 (5.6)	13.8 (3.1)
Na^+ excretion (μmol/min)				
10 min	475 (318)	583 (375)	483 (336)	448 (243)
30 min	333 (148)	482 (292)	96 (95)	114 (59)
K^+ excretion (μmol/min)				
10 min	39 (27)	54 (34)	45 (35)	29 (9)
30 min	50 (13)	69 (9)	19 (18)	16 (2)

Clearance values and urine electrolyte excretion

The values for iohexol clearance and filtration fraction after 30 min of perfusion with Galα1–3Gal supplemented blood increased, while in the Galα1–2Gal group a slight decrease were observed when compared to the values achieved at 10 min (Table 1). The mean values for iohexol clearance and filtration fraction for the lactose and control groups showed a 40% decrease. The Galα1–2Gal experiments showed an increase in PAH clearance while the Galα1–3Gal and control groups showed a decrease. In the lactose group, stable PAH clearance, values were achieved. Urine sodium excretion was reduced in all groups during the perfusions (Table 1). In the lactose and control groups, the sodium excretion at 30 min was reduced to about 20–25% of the levels seen at 10 min. In contrast, the Galα1–3Gal and Galα1–2Gal groups showed a reduction to about 70–80%. The urine potassium excretion in the Galα1–3Gal and Galα1–2Gal saccharide groups increased about 25%, whereas a decrease of about 50% was seen for the lactose and control groups. The changes in electrolyte excretions during the different perfusions indicate that the tubular cell function was improving in the Galα-groups compared to the controls.

Haematological parameters

The changes in absolute haemoglobin concentration, leukocyte and platelet counts in the blood samples collected before the start and at different times during the perfusion are listed in Table 2. The haemoglobin concentration was stable in the base line experiments without kidneys, showing that no destruction of red blood cells occurred in the perfusion circuit. A slight continuous increase of haemoglobin concentration was seen for all pig kidney perfusion experiments. This is explained by a small loss of fluids from the circuit, due to a slight leakage of lymphatic fluid from the kidney hilus and the collection of urine samples. A quick drop in platelet number was seen during the first 5 min while the leukocyte count gradually decreased in all kidney perfusions experiments. Perfusion of the perfusion circuit without kidneys showed only a slight decrease in platelet and leukocyte number (Table 2).

Flow cytometric analysis

The amount of functionally active human anti-pig antibodies (IgM + IgG) in perfusate samples collected at different times during the perfusions were tested by flow cytometric analysis of porcine lymphocytes and the results are presented as MFI in Table 3. Two different controls were used; the amounts of anti-pig antibodies in undiluted human serum and in the control

Table 2 Haemoglobin concentration, leukocyte and platelet count during perfusion of porcine kidneys with heparinized fresh human blood. The blood was supplemented with 10 mg/ml of Galα1–3, Galα1–2 and lactose. Control experiments without saccharides and without kidney and saccharides (perfusion circuit) are also shown. Samples were collected at the venous side of the kidneys at different times as indicated. Values are given as mean (± SD)

	Experimental set-up				
	Galα1–3 ($n = 3$)	Galα1–2 ($n = 3$)	Lactose ($n = 4$)	Control ($n = 2$)	Perfusion circuit ($n = 4$)
Haemoglobin (g/l)					
Start	109.7 (7.2)	110.0 (6.9)	114.8 (8.3)	110.5 (12.0)	109.5 (7)
After 5 min	115.7 (10.0)	110.7 (3.2)	121.0 (6.5)	120.5 (17.7)	111.8 (5.6)
After 10 min	116.7 (8.6)	117.3 (1.5)	121.8 (8.0)	122.0 (14.1)	110.8 (6.4)
After 20 min	123.3 (10.4)	121.3 (5.0)	125.3 (7.9)	123.5 (12.0)	110.3 (5.6)
After 30 min	130.0 (10.1)	124.5 (9.2)	127.5 (7.1)	125.5 (10.6)	1 10.8 (5.0)
Leukocyte count ($\times 10^9$/l)					
Start	5.2 (0.6)	5.2 (0.5)	3.6 (0.5)	3.2 (1.1)	4.3 (0.8)
After 5 min	4.6 (0.9)	4.4 (0.5)	2.2 (0.2)	2.4 (0.9)	4.4 (0.9)
After 10 min	3.4 (1.0)	4.0 (0.4)	2.2 (0.6)	1.8 (0.2)	4.4 (0.8)
After 20 min	2.8 (0.9)	2.9 (0.3)	1.4 (0.3)	1.4 (0.4)	4.4 (1.0)
After 30 min	2.2 (0.5)	2.0 (0.1)	1.3 (0.3)	1.4 (0.3)	4.2 (0.9)
Platelet count ($\times 10^9$/l)					
Start	195.0 (25.1)	197.0 (19.7)	172.5 (26.7)	153.5 (12.0)	210.0 (16.3)
After 5 min	80.3 (33.6)	109.7 (17.2)	111.5 (34.3)	131.0 (18.4)	188.8 (15.7)
After 10 min	96.3 (27.6)	96.3 (19.9)	102 (36.3)	133.0 (19.1)	182.8 (13.8)
After 20 min	104.7 (23.6)	95.0 (24.8)	94.5 (33.2)	101.5 (27.6)	177.0 (13.3)
After 30 min	104.0 (11.8)	86.0 (11.3)	93.3 (34.5)	113.0 (17.0)	175.0 (8.3)

Table 3 The amount of functionally active anti-porcine xenoantibodies in plasma during perfusion of pig kidneys with human blood. Plasma samples were collected before, at 10, 20 and 30 min of perfusion for each experimental set up and incubated with porcine lymphocytes, followed by incubation of FITC conjugated anti-human IgG/IgM and analysed by FACS. The median fluorescence intensity (*MFI*) is presented as mean (± SD)

	Experimental set-up				
	Galα1–3 ($n = 3$)	Galα1–2 ($n = 3$)	Lactose ($n = 4$)	Control ($n = 2$)	Undiluted human serum ($n = 6$)
MFI					
Start	876 (246)	1808 (788)	1762 (1340)	1662 (1000)	2392 (1083)
After 10 min	620 (81)	658 (151)	545 (265)	522 (76)	
After 20 min	688 (132)	557 (48)	353 (232)	485 (93)	
After 30 min	714 (216)	541 (112)	376 (143)	490 (25)	

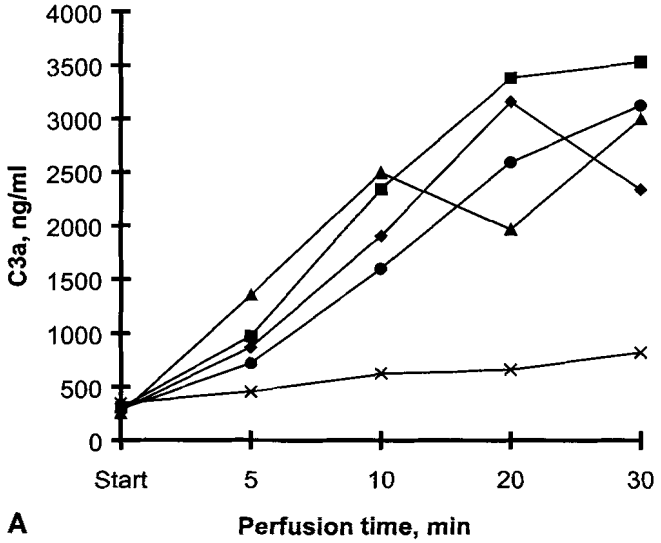
perfusate without saccharides added. The mean value for the 6 undiluted human serum controls was 2392 ± 1083, and in the control group lacking saccharide, the MFI value was 1662 ± 1000 before perfusion. Addition of Galα1-3Gal saccharide reduced the MFI value to 876 ± 246, while after addition of Galα*1*-2Gal and lactose MFI values of 1807 ± 787 and 1762 ± 1340 respectively were achieved. After 10 min of perfusion, the MFI values were all considerably reduced, and the difference between the Galα1-3Gal saccharide group and the other experimental groups was eliminated.

Complement activation during perfusion

Complement cascade activation was measured as formation of the anaphylatoxin C3a and the soluble terminal complement complex (C5b-9) in the perfusate samples, and the results are shown in Fig. 2. In all four experimental groups, C3a increased considerably during the perfusion. Concerning SC5b-9, a different pattern was observed. A moderate increase was found for the Galα1–3Gal, Galα1–2Gal and control groups while the lactose group showed the highest values. Baseline control experiments, where the circuit was perfused without kidney, showed a minor formation of C3a and no increase of SC5b-9 (Fig. 2).

Histopathological analysis

No apparent differences were noted between the four experimental groups when examined by light microscopy using conventional histochemical stains. Ischemic changes, observed in all groups, were hydropic degeneration of the tubular epithelium and blebbing of the brush border. These changes were predominantly seen



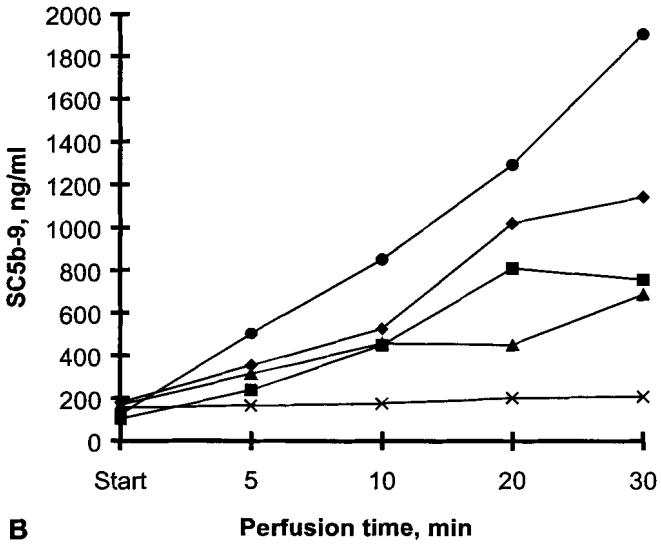


Fig. 2 Formation of C3a (**A**) and SC5b-9 (**B**) during perfusion of pig kidneys with human blood. Galα1–3Gal ($n = 3$) ■, Galα1–2-Gal ($n = 3$) ◆ and lactose ($n = 4$) ● were added to the human blood to a final concentration of 10 mg/ml plasma. Control experiments with human blood lacking saccharide ($n = 2$) and perfusion circuit without kidney ($n = 4$) are represented by ▲ and × respectively. Each point measured represents the mean value

in the proximal tubular epithelium, while in glomeruli, distal tubuli and medullary structures the changes were inconspicuous. No necrosis, massive extravasation of red blood cells or marked accumulation of neutrophilic granulocytes was seen. However an increased amount of inflammatory cells (mononuclear cells and granulocytes) was observed in glomerular and peritubular capillaries in all experimental groups.

Table 4 Immunohistochemical distribution of C1q, C3, IgM, Fibrin and GpIIIa in glomeruli of pig kidneys perfused with human blood. A similar evaluation for each cellular compartment glomerular basement membrane (gbm), peritubular capillaries (ptc), arterioles (aa) and arteries (A) were performed and the result summarized in the text section. The immunostaining was defined as general (*g*) or focal (*f*). The staining intensity was graded as *0*, + and + + . Examples of staining pattern are illustrated in Fig. 3

	Immunological markers				
Experimental set-up	C1q	C3	IgM	Fibrin	GpIIIa
Galα1–3 ($n = 3$)	+ g	+ + g	+ g	+ g	+ g
	+ f	+ f	+ f	+ f	+ f
	+ f	+ f	+ f	+ f	0
Galα1–2 ($n = 3$)	+ g	+ g	+ + g	+ g	+ g
	+ g	+ f	+ f	+ g	+ f
	+ f	+ f	+ f	+ + g	0
Lactose ($n = 4$)	+ f	+ f	+ f	+ f	+ f
	+ g	+ + g	+ g	+ f	+ f
	+ + g	+ + g	+ + g	+ f	+ f
	+ f	+ g	+ f	+ f	+ f
Control ($n = 2$)	+ + g	+ + g	+ g	+ + g	+ g
	+ + g	+ g	+ g	+ + g	+ f

To further evaluate possible tissue damage in the perfused pig kidneys, immunohistochemical staining for human complement (C1q, C3c), immunoglobulins (IgA, IgG, IgM), fibrin/fibrinogen, platelets (GpIIIa) and the accumulation of inflammatory cells (HLA-DR, CD68, Mac387) were used. The distribution and intensity of the immunological markers for tissue damage was semiquantitatively evaluated, as described in the materials and methods section. Table 4 shows the detailed evaluation of glomerular capillaries (gc), and selected illustrations of staining patterns are given in Fig. 3. An identical evaluation of the other cellular compartments of the vascular tree (ptc, aa and A) was performed (not shown) and summarised below. Depositions of complement, immunoglobulins, and fibrin were seen in all four experimental groups but were absent in the unperfused reference kidneys (not shown). The depositions were mainly observed in endothelial cells of arteries, arterioli, glomerular and peritubular capillaries (exemplified in Fig 3). A weak mesangial labelling was observed in all groups for complement and immunoglobulins, and a membranous distribution of C3c and IgM was seen in some kidneys, particularly in the control group lacking saccharides. In addition, a weak but significant labelling was observed in the interstitium, but no depositions were detected in the epithelial cells. Depositions of C1q, C3c, immunoglobulins (particularly IgM), fibrin/fibrinogen and platelets in control experiments with blood lacking saccharides were observed in most capillaries, all glomeruli, the majority of arteries and arterioli

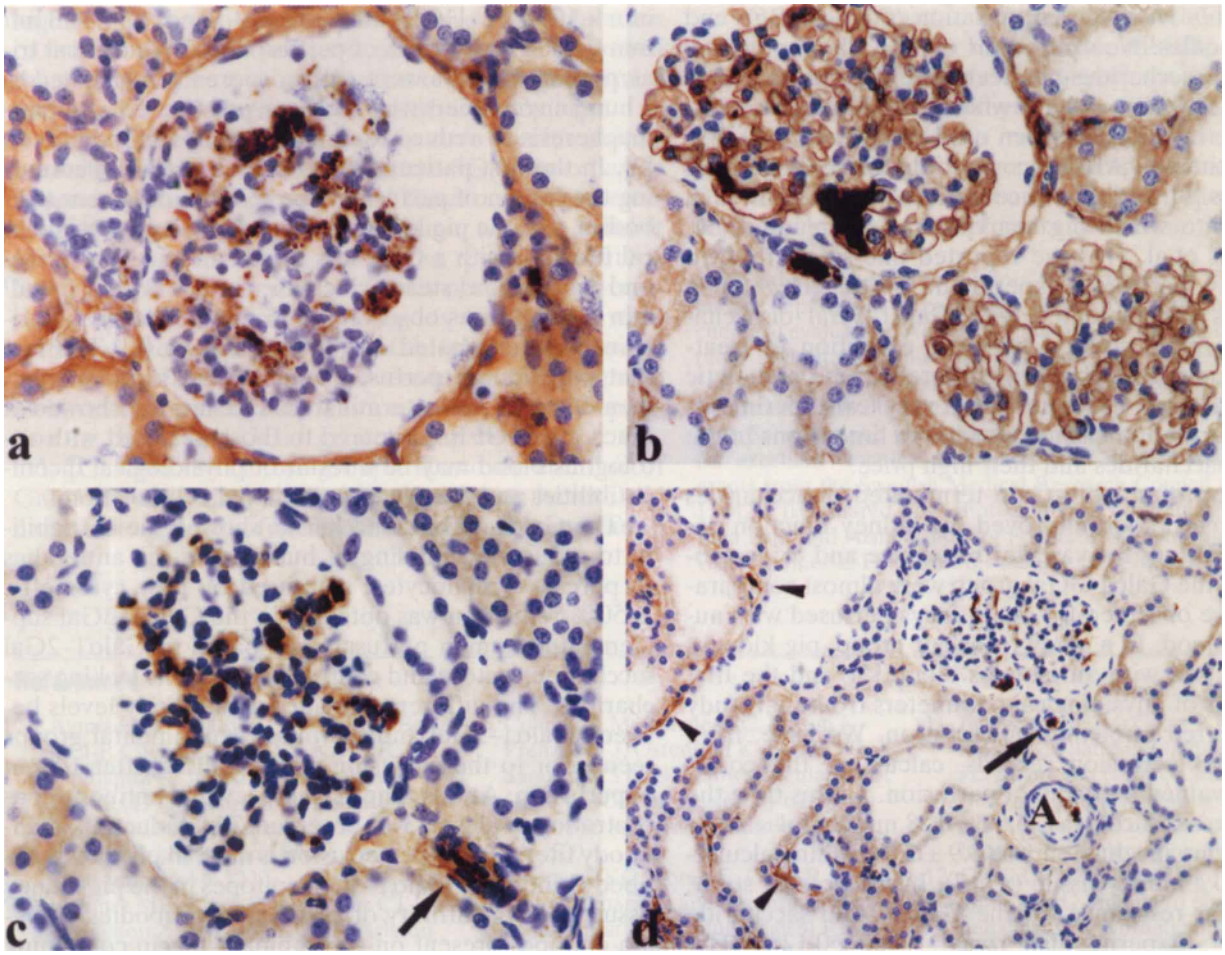


Fig. 3 a–d Light microscopic examinations of pig kidneys perfused with human blood. **a** Platelets (GpIIIa) are observed in most glomerular capillaries in a kidney from the Galα1–3Gal saccharide group. **b** Focal distribution of human IgM in glomerular and peritubular capillaries in a kidney from the control group with human blood lacking saccharides. Note that the glomerular basement membranes are labelled in a membranous pattern. **c** Deposition of C1 q in an arteriole (*arrow*), in glomerular capillaries and in the mesangium of a kidney from the control group. **d** Focal depositions of fibrin in glomerular capillaries, peritubular capillaries (*arrowheads*), an arteriole (*arrow*) and in an artery (*A*) in a kidney from the lactose group

and focally in peritubular capillaries with a regional pattern of distribution. In the lactose group, a less generalised and weaker labelling were seen, and, in addition, there was a large variation between the four different kidneys (illustrated for gc in Table 4). In the Galα1–3-Gal and Galα1–2Gal saccharide groups, the depositions were less dense and predominantly focal in arteries and weaker in glomeruli, but still seen in the majority of glomeruli. Furthermore, fewer deposits were seen in the Galα1–3Gal group, but the labelling varied between the perfused kidneys and the number of experiments was too low to justify any statistical evaluation of labelling intensity and distribution. To summarise the immunohistological observations, the lowest amount of depositions were seen in the Galα1–3Gal group followed by Galα1–2Gal and lactose groups, while the most dense and widespread depositions were seen in the control perfusions with blood lacking saccharides. A high number of inflammatory cells were seen in glomerular and peritubular capillaries, and a large portion of these cells were identified as macrophages (CD68 and/or Mac387 positive). These cells were in addition HLA-DR positive. The remaining cells were identified as neutrophilic granulocytes and occasional lymphocytes. The number of cells was about the same in the saccharide supplemented groups and slightly higher in the control group lacking saccharide.

Discussion

The use of soluble oligosaccharides, acting as haptens against preformed anti-carbohydrate antibodies have

been used *in vivo* for neutralisation of both ABO- and xenoantibodies. Neutralisation of ABO-antibodies by infusion of saccharides has been used therapeutically in haemolytic disease of the newborn [24]. Experimentally, saccharide infusion has been used to reduce the ABO-antibody titers in ABO-incompatible heart allografting in baboons [8], and to reduce the xenoantibody titers in baboons into which pig hearts were transplanted [30, 39]. Cairns et al. [4] have reported one single in vitro pig kidney perfusion experiment where the human blood was supplemented with Galα1–3Gal disaccharides. The cited studies, with the exception of treatments in new-born children, suffered from haemolytic disease [24], have in common that very few experiments were performed in each study, due to limitations in the access to saccharides and their high price.

Addition of soluble Galα-terminated disaccharides to the human blood improved pig kidney function parameters. Values for vascular resistance and urine production in the Galα1–3Gal group were almost comparable to those observed for pig kidneys perfused with autologous blood. In a study from our group, pig kidneys were perfused with autologous blood [3], and the first evaluations of physiological parameters from that study were reported at 60 min of perfusion. We have, from the original perfusion records, calculated the corresponding values at 30 min of perfusion. At this time the vascular resistance was 0.69 ± 0.28 mmHg/ml × min^{-1} and the urine production was 3.9 ± 2.0 ml/min calculated per 100 g kidney tissue ($n = 7$). In the present study, the vascular resistance for the Galα1–3Gal saccharide perfusion experiments was 0.66 ± 0.12 mmHg/ml × min^{-1} × 100 g^{-1}. For the Galα1–3Gal and the Galα1–2Gal saccharide perfusions, a stable level of urine production was observed, 3.0 and 3.7 ml/min respectively, while the lactose and control groups showed a decrease in urine production. Storck et al [31] showed that hDAF transgenic pig kidneys, perfused in vitro with heparinized human blood, had a total urine production of 3.1 ± 0.2 ml/min at 15 min. Grinyo et al. [13] observed no difference in urinary output (range 0.5 to 2.4 ml urine/min × 100 g^{-1}) for porcine kidneys perfused with complement inactivated human blood compared to those perfused with autologous blood. In the same study they reported that no urine was produced in pig kidneys perfused with untreated human blood.

Renal function was also measured by the plasma clearance capacity. Iohexol clearance was used as a marker for the glomerular filtration rate (GFR) which in our different experimental set-ups ranged from 5.9 ± 0.4 for the control to 10.1 ± 8.4 ml/min × 100 g^{-1} for the Galα1–3Gal group at 30 min. A decline in GFR was observed during the perfusion for all groups, except for the Galα1–3Gal group in which a small increase was noted. GFR values reported using similar perfusion models and autologous blood were, about 33 ml/min × 100 g^{-1} [10] at 30 min, and 54 ± 13 ml/min × 100 g^{-1} at 60 min of perfusion [3]. In a clinical trial, porcine kidneys were extracorporeally connected to 2 human volunteers who had been pre-treated with plasmapheresis to reduce the anti-pig xenoantibody levels [2]. In the first patient, a HAR process developed during the 65 min of perfusion, due to remaining xenoantibodies, and the pig kidney did not show any physiological function with a GFR less than 4 ml/min. In the second patient, a steady increase in GFR to 7.8 ml/min × 100 g^{-1} was observed at 15 min when the experiment was terminated due to side effects [2]. The fact that pig kidneys perfused with human blood supplemented with Galα-terminated saccharides showed a much lower GFR compared to those perfused with autologous blood may be a result of physiological incompatibilities and decreased endothelial cell function.

The Galα1–3Gal disaccharide showed the best ability to reduce the binding of human anti-pig antibodies to porcine lymphocytes, measured by flow cytometry. A 50% reduction was obtained in the Galα1–3Gal supplemented human perfusate compared to Galα1–2Gal saccharide, lactose and control experiments lacking saccharides. The difference in anti-pig antibody levels between Galα1–3Gal and the other experimental groups seen prior to the perfusion was equalised after 10 min of perfusion. At this time, a stable level of antibody concentration in plasma was observed. The reduction of antibody titer during the perfusion is most likely due to antibody binding to Galα1–3Gal epitopes in the pig kidney tissue since the affinity of anti-Galαa antibodies to Galαa epitopes present on recombinant mucin containing glycoprotein is higher compared to the affinity for Galα- terminated solid phase matrix [16]. For the Galα1–2Gal saccharide, lactose and control experiments without saccharides more than a 70% reduction of the antibody level was seen at 10 min of perfusion. Tuso et al. [35] reported an anti-pig antibody reduction of 67% for IgM and 55% for IgG after 60 min perfusion of pig kidney with 500 ml human blood. The observed difference in capacity to reduce anti-pig IgG/IgM antibodies in human blood between Galα1–3Gal and Galα1–2Gal disaccharides may be partly explained by the difference in steric configuration following the α1–3 or α1–2 linkage positions. This is in accordance with other groups presenting Galα1–3Gal to be the major epitope recognised by human anti-pig antibodies [7, 12, 18, 29]. However presence of heterogeneity in the human anti-pig antibody repertoire has been suggested [7, 18, 22], and McKane et al. reported about polymorphism in the repertoire of human anti-pig antibodies [17]. A weak crossreactivity between Galα1–2Gal and Galα1–3Gal disaccharides in the reduction of cytotoxicity mediated by human serum on PK15 cells has been described by Neethling et al. [20]. The concentration of Galα1–2Gal disaccharide, however, must be about twenty times

higher than for Galα1–3Gal, to achieve 50% inhibition of the cytotoxicity. Wieslander et al. [37] have suggested that there may be a subpopulation of human preformed natural antibodies against the Galα1–2Gal epitope.

Our results showed that in an in vitro porcine kidney perfusion model, addition of Galα1–3Gal disaccharide to human blood improved renal functional parameters considerably, compared to perfusion without soluble saccharides added. For some of the renal parameters, levels almost comparable to results observed in perfusion experiments using autologous pig blood were achieved. Depositions of C1q, C3, IgM, fibrin/fibrinogen and platelets were most severe in the control group lacking saccharide. There was a tendency to less deposition in the Galα1–3Gal group compared to the Galα1–2Gal and the lactose groups. However, addition of Galα1–3Gal, Galα1–2Gal disaccharides or lactose did not affect the level of complement activation measured as C3a.

We conclude that addition of soluble Galα1–3Gal disaccharides to human blood partly inhibits the hyperacute rejection of vascularised pig organs. However, significant pathological changes were still present, indicating that this approach to inhibit HAR must be used in combination with additional therapeutic approaches, such as solid phase xenoantibody immunoadsorption and/or blocking of complement activation.

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References

1. Azimzadeh A, Meyer C, Watier H, Beller J-P, Chenard-Neu M-P, Kieny R, Boudjema K, Jaeck D, Cinqualbre J, Wolf P (1998) Removal of primate xenoantibodies by extracorporeal perfusion of pig kidneys and livers. Transplant Immunol 6: 1322
2. Breimer ME, Björk S, Svalander CT, Bengtsson A, Rydberg L, Lie-Karlsson K, Attman P-O, Aurell M, Samuelsson BE (1996) Extracorporeal ("ex vivo") connection of pig kidneys to humans. I. Clinical data and studies of platelet destruction. Xenotransplantation 3: 328–339
3. Breimer ME, Svalander CT, Haraldsson B, Björck S (1996) Physiological and histological characterisation of a pig kidney in vitro perfusion model for xenotransplantation studies. Scand J Urol Nephrol 30: 213–221
4. Cairns T, Lee J, Goldberg L Cook T, Simpson P, Spackman D, Palmer A, Taube D (1995) Inhibition of the pig to human xenograft reaction, using soluble Galα1–3Gal and Galα1–3Galβ1–4GlcNAc. Transplantation 60: 1202–1207
5. Chen CG, Salvaris EJ, Romanella M, Aminian A, Katerelos M, Fisicaro N, d'Apice AJF, Pearse MJ (1998) Transgenic expression of human α1,2-fucosyltransferase (H-transferase) prolongs mouse heart survival in an ex vivo model of xenograft rejection. Transplantation 65: 832–837
6. Cohney S, McKenzie IFC, Patton K, Prenzoska J, Ostenreid K, Fodor WL, Sandrin MS (1997) Down-regulation of Galα(1,3)Gal expression by α1,2-fucosyltransferase. Transplantation 64: 495–500
7. Cooper DKC, Koren E, Oriol R (1994) Oligosaccharides and discordant xenotransplantation. Immunological Reviews 141: 31–58
8. Cooper DKC, Ye Y, Niekrasz M, Kehoe M, Martin M, Neethling FA, Kosanke S, Debault LE, Worsley G, Zuhdi N, Oriol R, Romano E (1993) Specific intravenous carbohydrate therapy. A new concept in inhibiting antibody-mediated rejection, experience with ABO-incompatible cardiac allografting in the baboon. Transplantation 56: 769–777
9. Cozzi E, Tucker AW, Langford GA, Pino-Chavez G, Wright L, O'Connell M-J, Young VJ, Lancaster R, McLaughlin M, Hunt K, Bordin MC, White DJG (1997) Charactarization of pigs transgenic for human decay-accelerating factor. Transplantation 64: 1383–1392
10. Cruzado JM, Torras J, Riera M, Lloberas N, Herrero I, Condom E, Martorell J, Alsina J, Grinyo JM (1998) Effect of a platelet-activating factor (PAF) receptor antagonist on hyperacute xenograft rejection; evaluation in a pig kidney-human blood xenoperfusion model. Clin Exp Immunol 133: 136–144
11. Diamond LE, McCurry KR, Martin MJ, McClellan SB, Oldham ER, Platt JL, Logan JS (1996) Characterization of transgenic pigs expressing functionally active human CD59 on cardiac endothelium. Transplantation 61: 1241–1249
12. Galili U, Matta KL (1996) Inhibition of anti-gal IgG binding to porcine endothelial cells by synthetic oligosaccharides. Transplantation 62: 256–262
13. Grinyo JM, Cruzado JM, Riera M, Martorell J, Condom E, Borobia FG, Muñoz J, Herrero I, Lloberas N, Gimeno A, Alsina J, Torras J (1997) Functional and pathologic outcome after complement inactivation in a pig kidney-human blood xenoperfusion model. Transplant Proc 29: 2318–2321
14. Kroshus TJ, Bolman RM III, Dalamasso AP, Rollins SA, Guilmette ER, Williams BL, Squinto SP, Fodor WL (1996) Expression of human CD59 in transgenic pig organs enhances organ survival in an ex vivo xenogenic perfusion model. Transplantation 61: 1513–1521
15. LaVecchio JA, Dunne AD, Edge ASB (1997) Enzymatic removal of alpha-galactosyl epitopes from porcine endothelial cells diminishes the cytotoxic effect of natural antibodies. Transplantation 60: 841–847
16. Liu J, Qian Y, Holgersson J (1997) Removal of xenoreactive human anti-pig antibodies by absorption on recombinant mucin-containing glycoproteins carrying the Galα1,3Gal epitope. Transplantation 63: 1673–1682

17. McKane W, Lee J, Preston R, Hacking A, Simpson P, Lynds S, Goldberg L, Cairns T, Taube D (1998) Polymorphism in the human anti-pig natural antibody repertoire. Implications for antigen-specific immunoadsorption. Transplantation 66: 626–633
18. McMorrow IM, Comrack CA, Sachs DH, Dersimonian H (1997) Heterogeneity of human anti-pig natural antibodies cross-reactive with the Gal(alpha1,3)Galactose epitope. Transplantation 64: 501–510
19. Neethling FA Koren E, Ye Y, Richards SV, Kujundzic M, Oriol R, Cooper DKC (1994) Protection of pig kidney (PK15) cells from the cytotoxic effect of anti-pig antibodies by α-galactosyl oligosaccharides. Transplantation 57: 959–963
20. Neethling FA, Joziasse D, Bovin N, Cooper DKC, Oriol R (1996) The reducing end of α-Gal oligosaccharides contributes to their efficiency in blocking natural antibodies of human and baboon sera. Transpl Int 9: 98–101
21. Osman N, McKenzie IFC, Ostenried K, Ioannou YA, Desnick RJ, Sandrin MS (1997) Combined transgenic expression of α-galactosidase and α1,2-fucosyltransferase leads to optimal reduction in the major xenoepitope Galα(1, 3)Gal. Proc Natl Acad Sci USA 94: 14677–14682
22. Parker W, Lateef J, Everett ML, Platt JL (1996) Specificity of xenoreactive anti-Galα1–3Gal IgM for α-galactosyl ligands. Glycobiology 6: 499–506
23. Pruitt SK, Kirk AD, Bollinger RR, Marsh HC, Bradley BH, Levin JL, Mault JR, Heinle JS, Ibrahim S, Rudolph AR, Baldwin III WM Sanfilippo F (1994) The effect of soluble complement receptor type 1 on hyperacute rejection of porcine xenografts. Transplantation 57: 363–370
24. Romano EL, Soyano A, Montaño RF, Ratcliffe M, Olson M, Suarez G, Martínez N, Worsley G (1994) Treatment of ABO hemolytic disease with synthetic blood group trisaccharides. Vox Sang 66: 194–199
25. Rydberg L, Hallberg E, Björck S, Magnusson S, Strokan V, Breimer ME (1995) Studies on the removal of anti-pig xenoantibodies in the human by plasmapheresis / immunoadsorption. Xenotransplantation 2: 253–263
26. Saadi S, Platt JL (1998) Immunology of xenotransplantation. Life Sci 62: 365–387
27. Samuelsson BE, Rydberg L, Breimer ME, Bäcker A, Gustavsson M, Holgersson J, Karlsson E, Uyterwaal A-C, Cairns T, Welsh K (1994) Natural antibodies and human xenotransplantation. Immunol Rev 141: 151–168
28. Sandrin MS, Fodor WL, Mouhtouris E, Osman N, Cohney S, Rollins SA, Guilette ER, Setter E, Squinto SP, McKenzie IFC (1995) Enzymatic remodelling of the carbohydrate surface of a xenogenic cell substantially reduces human antibodiy binding and complement-mediated cytolysis. Nature Med 1: 1261–1267
29. Sandrin MS, McKenzie IFC (1994) Galα(1,3), the major xenoantigen(s) recognised in pigs by human natural antibodies. Immunological Reviews 141: 169–190
30. Simon PM, Neethling FA, Taniguchi S, Goode PL, Zopf D, Hancock WW, Cooper DKC (1998) Intravenous infusion of Galα1–3Gal oligosaccharides in baboons delays hyperacute rejection of porcine xenografts. Transplantation 65: 346–353
31. Storck M, Abendroth D, Prestel R, Pino-Chavez G, Müller-Höoker J, White DJG, Hammer C (1997) Morphology of hDAF (CD55) transgenic pig kidneys following ex-vivo hemoperfusion with human blood. Transplantation 63: 304–310
32. Tange MJ, Tearle RG, Aminian A, Romanella M, Adam WR, Pearse MJ, d'Apice AJF (1997) Demonstration of the functional importance of the Gal epitope in an ex vivo model of xenotransplantation. Xenotransplantation 4: 20–24
33. Taniguchi S, Neetheling FA, Korchagina EY, Bovin N, Ye Y, Kobayashi T, Niekrasz M, Li S, Koren E, Oriol R, Cooper DKC (1996) In vivo immunoadsorption of antipig antibodies in baboons using a specific Galα1–3Gal column. Transplantation 62: 1379–1384
34. Tearle RG, Tange MJ, Zannettino ZL, Katerelos M, Shinkel TA, Van Denderen BJW, Lonie AJ, Lyons I, Nottle MB, Cox T, Becker C, Peura AM, Wigley PL, Crawford RJ, Robins AJ, Pearse MJ, d'Apice AJF (1996) The α-1,3-Galactosyltransferase knockout mouse. Transplantation 61: 13–19
35. Tuso PJ, Cramer DV, Yasunaga C, Cosenza CA, Du Wu G, Makowka L (1993) Removal of natural human xenoantibodies to pig vascular endothelium by perfusion of blood through pig kidneys and livers. Transplantation 55: 1375–1378
36. Watier H, Guilaumin J-M, Piller F, Lacord M, Thibault G, Lebranchu Y, Monsigny M, Bardos P (1996) Removal of terminal α-galactosyl residues from xenogenic porcine endothelial cells. Transplatation 62: 105–113
37. Wieslander J, Månsson O, Kallin E, Gabrielli A, Nowack H, Timpl R (1990) Specificity of human antibodies against Galα1–3Gal carbohydrate epitope and distinction from natural antibodies reacting with Galα1–2Gal or Galα1–4-Gal. Glycoconjugate J 7: 85–100
38. Xu Y, Lorf T, Sablinski T, Gianello P, Bailin M, Monroy R, Kozlowski T, Awwad M, Cooper DKC, Sachs DH (1998) Removal of anti-porcine natural antibodies from human and nonhuman primate plasma in vitro and in vivo by a Galα1–3Galβ1–4βbGlc-x immunoaffinity column. Transplantation 65: 172–179
39. Ye Y, Neethling FA, Niekrasz M, Koren E, Richards SV, Martin M, Kosanke S, Oriol R, Cooper DKC (1994) Evidence that intravenously administrered α-galactosyl carbohydrates reduce baboon serum cytotoxicity to pig kidney cells (PK15) and transplanted pig hearts. Transplantation 58: 330–337