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Research Reports

Immunodepletion in Xenotransplantation

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Abstract *Xenograft transplantation is perhaps the most immunologically difficult problem in transplantation today. An overwhelming hyperacute rejection reaction (HAR) occurs within minutes of organ implantation. Preformed antibodies are thought to initiate this process. We used a pig-to-dog renal xenograft transplant model and investigated methods of decreasing the severity of hyperacute rejection. Female pigs weighing 15–20 kg were used as*

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donors. Recipients were mongrel dogs weighing 15–25 kg. Experimental dogs were all given a number of treatments of IgG depletion using an antibody removal system (Dupont–Excorim). This machine immunoadsorbs plasma against a column containing immobilized staphylococcal protein A, which is known to bind the IgG Fc receptor. An 84% reduction in the IgG levels and a 71% reduction in IgM levels was achieved. Postoperative assessment was made of urine output, time to onset of HAR, and histopathological examination of the rejected kidneys. Although cross-matches between donor lymphocytes and recipient sera remained strongly positive in the treated dogs, there was a two- to fourfold reduction in the titers. The time to onset of HAR was prolonged in the experimental group, and the urine output was increased slightly. The histopathologic changes in the experimental group generally showed signs of HAR, but of less intensity than in the nonimmunodepleted control group.

Keywords: Immunoadsorption, xenotransplantation, immunodepletion, hyperacute rejection.

Currently, one of the major limitations in renal transplantation involves the large number of sensitized dialysis patients. Blood transfusions, prior transplants, or multiple pregnancies can all play an etiologic role, but result in an identical situation. These highly sensitized patients, who have antibodies to most or all HLA antigens, are rendered immunologically untransplantable. At present there is no effective means of dealing with this problem, and these patients can spend years on transplant waiting lists.[1]

Discordant xenograft transplantation, ie, organ transplantation across widely divergent species barriers, confronts the same type of barriers to transplantation seen in the sensitized patient. Preformed antibodies render the donor organ susceptible to a humorally mediated hyperacute rejection, which can occur within minutes. One of the most reliable and violent animal models of hyperacute rejection is evident in pig-to-dog renal xenotransplantation.[2] Various experimental approaches have been attempted without success to obtain long-term survival of the xenografts.

One potential solution to these problems utilizes the ability of the staphylococcal protein A to bind selectively the Fc receptor of IgG[3] and to sites on the Fab region of other immunoglobulin isotypes (eg, IgM)[4] (Figs 1 and 2). A novel technology has been developed whereby plasma can be passed over a column of protein A–Sepharose, and the IgG and IgM, or any moiety containing an Fc receptor, can be adsorbed.[5] This technique of immunodepletion can decrease the level of preformed antibodies and thus should be potentially helpful in the abrogation of the hyperacute rejection response.

Materials and Methods

Immunodepletion

Male or female mongrel dogs weighing between 15 and 25 kg were anesthetized with pentothal, intubated, and maintained on a ventilator with 40% oxygen, ketamine 1.5 mg/kg every 1–2 h intravenously, and pancuronium 0.05–0.1 mg/kg every 1–2 h intravenously. Cutdowns in the neck on both the carotid artery and the external jugular vein were made and the vessels cannulated with 13-gauge Quinton–Scribner shunt cannulas. Arterial blood was fed into a Haemonetics V-50 plasmapheresis unit, which separates the red blood cells from the plasma. Citrate was used as an anticoagulant. The plasma was then fed into the immuno-

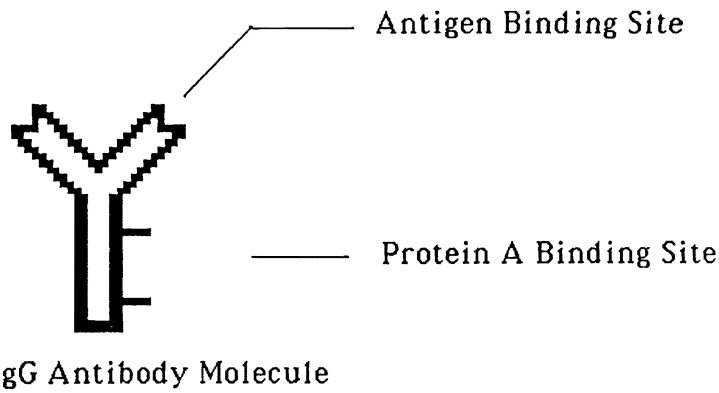

Figure 1. Schematic view of staphylococcal protein A binding to the Fc receptor of IgG and IgM.

adsorption system which contains two columns of protein A attached to Sepharose CL-4B (Immunosorba A) in parallel (Figs 3 and 4). The CITEM 10 (Excorim KG, Lund, Sweden/Dupont) is a computer-controlled device which controls the flow of plasma and various regeneration solutions over the two columns. While plasma was run across one column, the adsorbed IgG and IgM were eluted from the other column with low pH citrate. The immunodepleted plasma was then returned to the plasmapheresis unit, where it was added to the red cells and returned to the dog via the venous cannula. Cycles of adsorption and elution were alternated in the column every 10 min during the treatment procedure under the control of the CITEM 10 monitor. Light absorbance and pH detectors were used to monitor and control blood flow and prevent excessive plasma loss or dilution. Alarms detected and warned of unsafe conditions in the treatment system, eg, air in the line, abnormal pressure changes, and low plasma pH. The alarms were programmed to stop the procedure if there was no operator response within one minute. At the end of the treatment procedure the CITEM 10 automatically regenerated and preserved the two columns by filling them with a merthiolate preservative solution.

Continuous recording of pulse, blood pressure, and temperature was made,

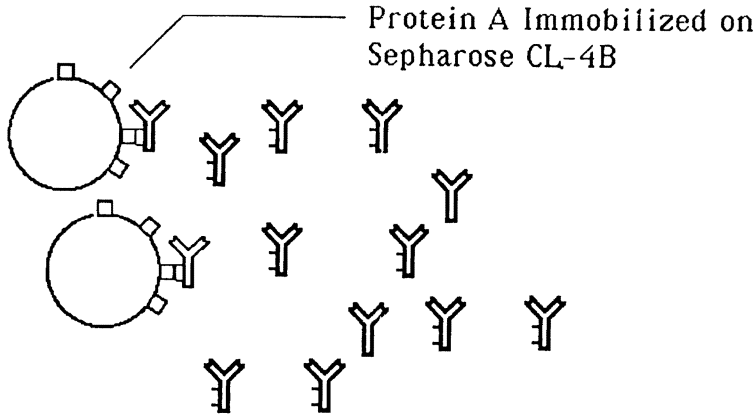

Figure 2. Schematic view of Sepharose-bound protein A binding to antibody.

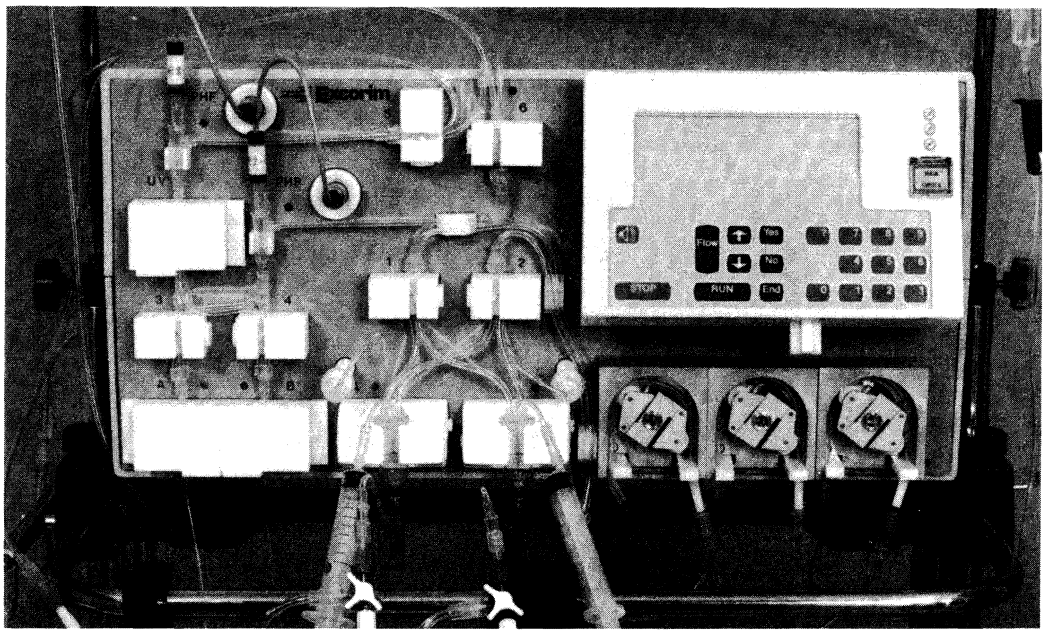

Figure 3. CITEM 10 (Excorim, Dupont), a computer-controlled device to remove IgG and IgM.

and serial measurements of arterial blood gases, hematocrit, sodium, potassium, and ionized calcium were taken every 0.5 h. A variable number of plasma volume exchanges were performed, ranging from 5 vol/day to 15 vol/5 days, prior to the xenograft transplantation (see Table 1).

Recipients, Control Group

With one exception (see below), control animals were treated identically with the experimental group, with the exception that they did not undergo pretransplant immunodepletion.

Donors

Female white outbred SPS pigs that weighed 15–20 kg were purchased from Shadyside Farms, Ohio, and served as donors. They were anesthetized with pentothal and were maintained with 30% oxygen through an endotracheal tube. Continuous EKG monitoring was used. The donor pigs received intravenous Ringer's lactate (1000 mL) and furosemide (10 mg). After systemic heparinization (5000 units) and in situ cooling with cold 4°C Ringer's lactate through an aortic cannula, the kidneys were removed en bloc. The kidneys were then separated, and one was transplanted heterotopically into the treated dog; the other kidney was transplanted into the paired control dog not treated with immunodepletion.

Surgical Technique

The operative technique involved the exposure of the right common iliac artery and vein. The venous anastomosis was performed end-to-side with fine continuous monofilament suture. The artery was then anastomosed end-to-end and the cross-clamps released. Total ischemia time was less than 2 h in every case. Warm ischemia time ranged from 30 to 45 min, with no more than 7 min difference between experimental and control animals.

Measurements

The time to onset of hyperacute rejection, urine output, and histopathology served as end points in the treated and control dogs. Time to onset of hyperacute rejection was calculated as the time until the first evidence of xenograft mottling. Urine output was calculated by placing a plastic catheter in the distal ureter and measuring the volume of urine every 15 min for the first 2 h after cross-clamp release. Histopathologic examination was performed on one section from each kidney after it was harvested at the end of the experiment. Sections were processed routinely with light microscopy and evaluated for percent normal, congested, and thrombosed glomeruli; tubular vacuolization or ATN; and interstitial hemorrhage, status of vessels, and overall impression. For immunofluorescence

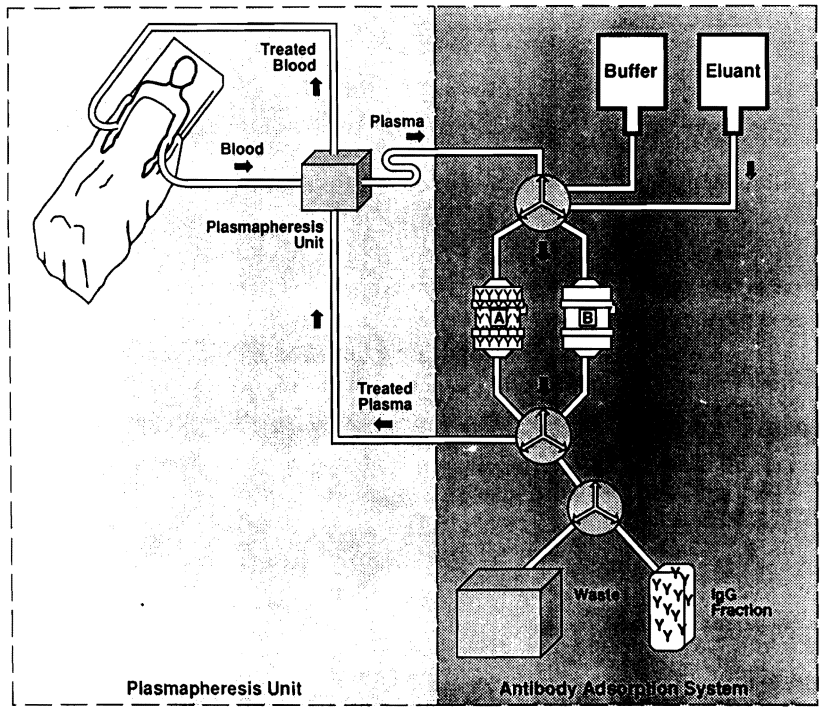

Figure 4. Immunodepletion. Blood from patient is separated into plasma and red cells; plasma is passed over Staph-A column in CITEM 10; immunodepleted plasma is returned with red cells to patient.

Table 1
A Summary of Protein A Depletion Studies Done on Canine Xenograft Recipients

Dog Number	Number of Treatments	Plasma Volume/ Treatment	Total Plasma Volume	Additional Treatment
1	3	2.7	8	None
3	2	3	6	None
4	3	3	9	None
7	5	2.8	14.2	None
9	5	3	15	Cyclophosphamide/methylprednisolone iv 2–5 mg/kg · day.
10	1	5	5	None
12	1	5	5	PGE_1 5 μg/kg · min
13	1	5	5	Cyclosporine 10 mg/kg · day + azathioprine 2 mg/kg · day + prednisone 1 mg/kg · day
14	1	5	5	Cyclosporine 10 mg/kg · day + azathioprine 2 mg/kg · day + prednisone 1 mg/kg · day + prostacyclin 0.4 μg/kg · min (intraarterial)
15	1	5	5	Cyclosporine 10 mg/kg · day + azathioprine 2 mg/kg · day + prednisone 1 mg/kg · day + prostacyclin 0.4 μg/kg · min (intraarterial)
17	1	5	5	Cyclosporine 10 mg/kg · day + azathioprine 2 mg/kg · day + prednisone 1 mg/kg · day + synthetic prostacyclin iv

studies, frozen sections from the harvested kidneys were stained using fluoresceinated goat anti-dog IgG (1/20) and IgM (1/20) (Research Plus). Sections were evaluated for location and degree of positive immunostaining. Slides were read blindly. Cytoxic matching with titers was performed between recipient serum and donor lymphocytes pre- and postimmunoadsorption. IgG and IgM levels were assayed by an radial immunodiffusion technique using a commercially available kit (ICN Immunobiologicals). Plasma procoagulant activity was determined as the 1/500 dilution of the activated partial thromboplastin time.

Additional Treatment (Table 1)

In experiments 1, 3, 4, 7, and 10, no additional treatment was given beside immunodepletion. In experiment 9, the experimental animal only was given a 4-day course of intravenous cyclophosphamide 2–5 mg/kg · day and intravenous methylprednisolone 1–3 mg/kg · day. In experiment 22, both experimental and control recipients received PGE, 5 μg/kg · h and the xenograft kidneys were flushed with 500 μg of PGE, on the back table. In experiments 13–17, both experimental and control dogs received cyclosporine 10 mg/kg · day, azathioprine 2 mg/kg · day, and prednisone 1 mg/kg · day, for 12 days (experiment 13), 10 days (experiments

14 and 15), and 21 days (experiment 17) preoperatively. In addition, in experiments 14 and 15, both experimental and control dogs received intra arterial prostacyclin 0.4 μg/kg · min, immediately after xenotransplantation. In experiment 17, both experimental and control dogs received synthetic prostacyclin intravenously during and immediately after xenotransplantation.

Results

Eleven experiments were completed successfully. Six experiments could not be completed successfully. The causes of failure to complete a given experiment are listed in Table 2. In most of the cases, technical errors were responsible—catheter placement problems, inadvertent disconnection from the ventilator, and overreplacement of potassium. In two cases, a picture of coagulopathy was seen. The latter two cases were done with the original venous cannulas system in which clotting of the cannulas was a recurring problem.

IgG and IgM Immunodepletion

Significant lowering of both serum IgG and IgM was achieved in all experimental animals treated with Staph-A immunodepletion (Figs 5–8). IgG levels were lowered by 84% from baseline and IgM levels were lowered 71% from baseline. In those experiments in which animals received immunodepletion treatment over more than one day, an overnight rebound was noted, presumably a reflection of reequilibration from the interstitium. Both IgG and IgM are known to be partitioned between the intravascular and extravascular spaces. The CITEM 10/antibody removal system was highly efficient in removing both the IgG and IgM in all cases.

Table 2
Causes of Failure to Complete Experiment

Experiment Number	Cause
2	Experimental dog died during catheter placement (probable right ventricular perforation)
5	Experimental dog died on 2d day of Staph-A depletion—coagulopathy, decreased platelets, hematocrit, BP
6	Experimental dog died on 3d day of Staph-A depletion—coagulopathy, decreased platelets, ? DIC
8	Experimental dog died during catheter placement after inadvertant disconnection from ventilator
11	Control dog died during transplantation—hypotension, hypoxia
16	Experimental dog died during Staph-A depletion—hyperkalemia

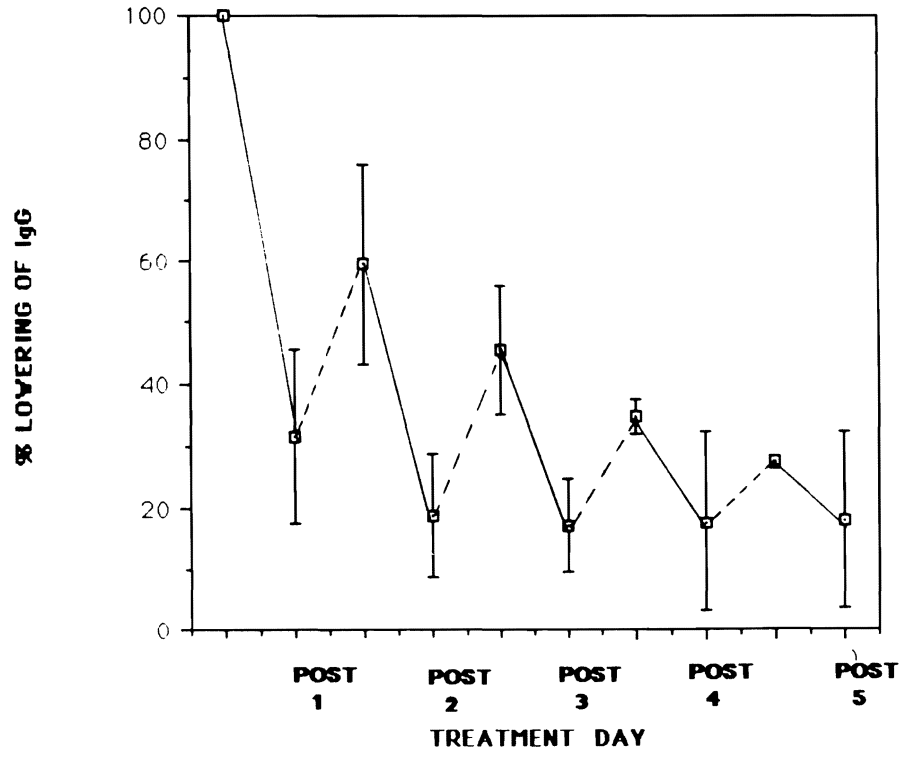

Figure 5. IgG depletion in five dogs given multiple treatments of immunodepletion.

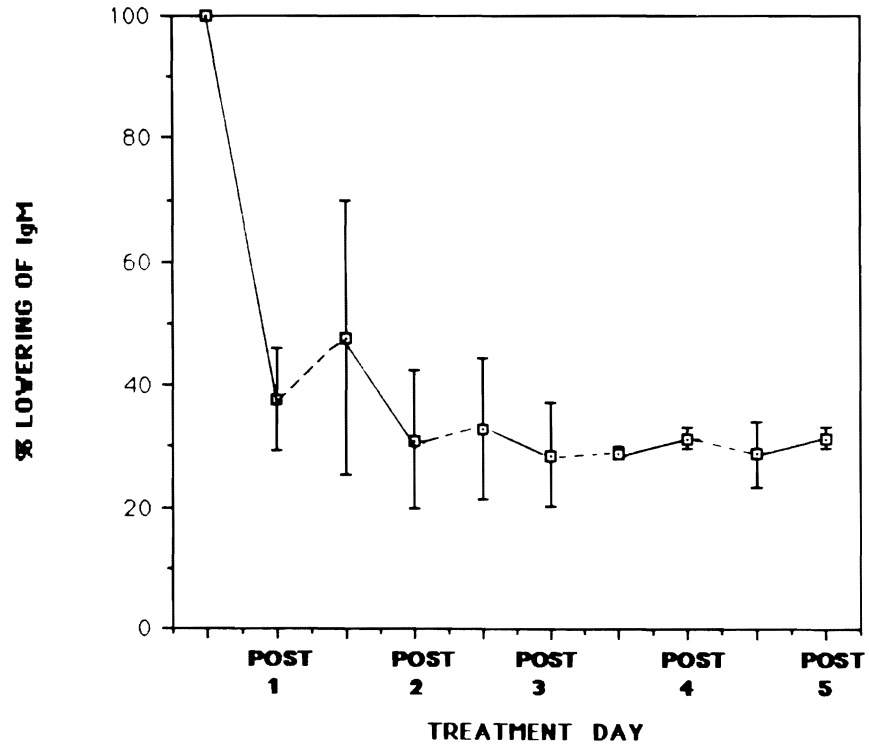

Figure 6. IgM depletion in five dogs given multiple treatments of immunodepletion.

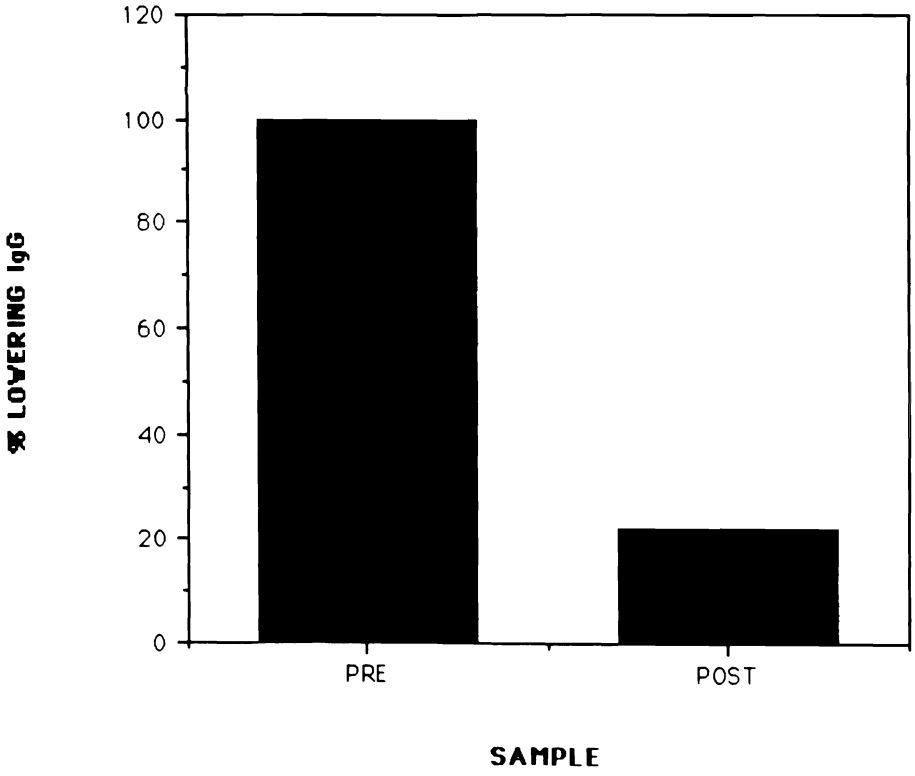

Figure 7. IgG depletion in six dogs given one treatment of immunodepletion.

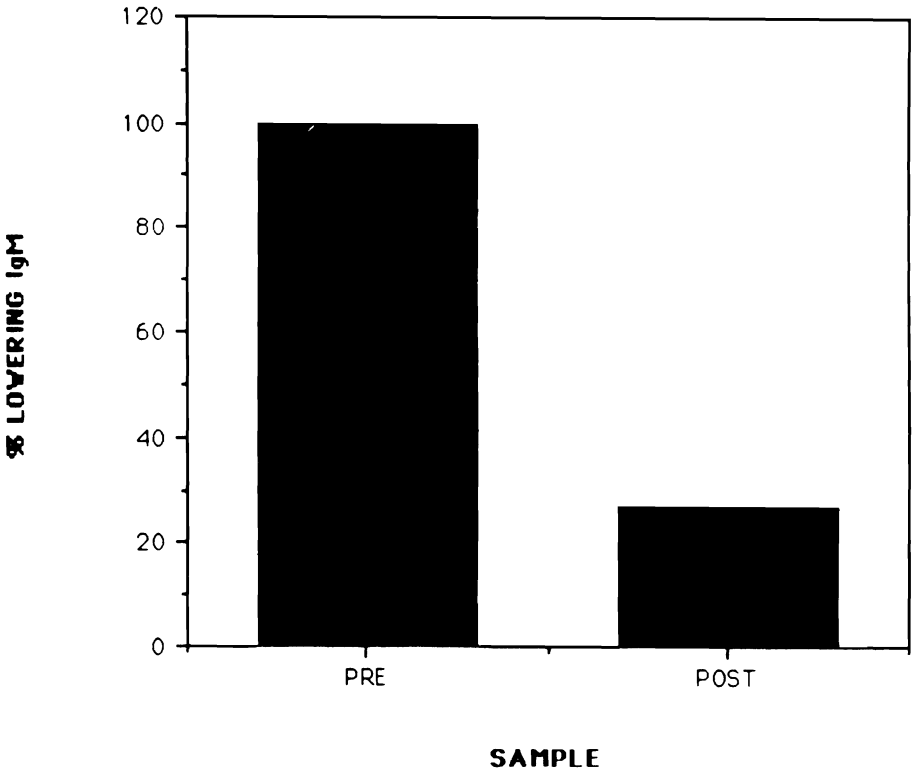

Figure 8. IgM depletion in six dogs given one treatment of immunodepletion.

Table 3
Time to Hyperacute Rejection and Urine Output

Parameter	Experimental	Control
Time to HAR (min)	37.9 ± 36.3	10.6 ± 5.8*
Total urine output (mL)	35.9 ± 39.2	10.8 ± 8.3
first hour	26.9 ± 32.6	9.1 ± 8.0
second hour	9.0 ± 9.6	1.7 ± 2.6*

* $p < .05$.

Time of Hyperacute Rejection (Table 3)

The time to the onset of hyperacute rejection was prolonged in the experimental group, with a mean of 37.9 ± min as opposed to 10.6 ± min in the control group ($p < .05$). In two cases, the experimental kidney never appeared to have gross evidence of hyperacute rejection throughout the period of observation.

Urine Output (Table 3)

Animals in the experimental group tended to produce more urine than animals in the control group during the period of observation. This finding held during the first hour, second hour, and the total urine output, with a mean of 26.9, 9.0, and 35.9 mL in the experimental group and 9.1, 1.7, and 10.8 mL in the control group, respectively. Because of variability, three differences only approached statistical significance ($p < .11$ at 1 h, $p < .05$ at 2 h, and $p < .08$ for 1 and 2 h combined).

Cross-match

Pre- and posttreatment cross-matches were performed using recipient serum and donor lymphocytes. Serial dilution of sera demonstrated positive cross-matches to 1/256–1/512, prior to immunodepletion. Posttreatment cross-matches remained positive to dilutions of 1/64–1/128. Antibody levels declined, but not enough to abrogate HAR.

Pathology

There were no qualitative differences between the control and experimental groups in the appearance of the glomeruli and the tubules. The overall subjective impression was that there was a higher percentage of thrombosed glomeruli and thus a more severe degree of hyperacute rejection in the control animals, but statistically there was no significant difference. Positive immunostaining for IgG and IgM was in the mesangium in all kidneys. IgG was the same in experimental and control kidneys in all ten pairs of kidneys examined. In eight pairs IgM appeared less in the experimental kidney. The overall impression was of slightly diminished HAR in the experimental group, although there was no statistical difference between experimental and control animals.

Coagulation and Platelet Changes

In both experimental and control animals, HAR was accompanied by a fall in the platelet count and a shortening of the plasma procoagulant activity (Table 4).

Table 4
Coagulation and Platelet Changes

Parameter	Time after Transplant		
	15 min	45 min	90 min
% Shortening of 1/500 APTT	9	15	23
p value	NS	.08	.001
% Fall in platelet count	31	46	69
p value	NS	.01	.001

Electrolyte Changes During Immunodepletion

Immunodepletion produced significant perturbations of specific electrolytes. A marked fall in ionized calcium was seen regularly, to the extent that each animal required infusion of 3–4 g of $CaCl_2$ during each treatment. This effect may have been a result of citrate being used as an anticoagulant. Potassium replacement on the order of 60 mEq treatment was also required.

Discussion

The pig-to-dog renal xenotransplantation model reliably leads to a violent hyperacute rejection in 2–10 min. It is thus an extreme analogue of the hyperacute rejection seen in a renal transplant recipient sensitized to a given donor kidney. This rejection is thought to be antibody mediated. With the new technology of column employing immobilized protein A, immunodepletion of IgG and IgM can be used to decrease the antibody level and thus possibly decrease or eliminate the hyperacute rejection response. We have demonstrated a 65–90% reduction of antibody levels, with corresponding changes in preformed cytotoxic antibody titers. This reduction was associated with a modest decrease in the severity of HAR. Obviously, more progress will need to be made with this technology before effective abrogation of HAR can be expected. Research is continuing in our laboratories to assess the efficacy of immunodepletion in combination with other immunosuppressive and pharmacologic protocols. If successful, the protein A column may be used in the future to immunodeplete the highly sensitized recipient prior to transplantation.

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