0041-1337/00/6902-208/0
TRANSPLANTATION
Copyright © 2000 by Lippincott Williams & Wilkins, Inc.
Vol. 69, 208–214, No. 2, January 27, 2000
Printed in U.S.A.

IG-THERASORB IMMUNOAPHERESIS IN ORTHOTOPIC XENOTRANSPLANTATION OF BABOONS WITH LANDRACE PIG HEARTS[1,2]

PAOLO BRENNER,[3,4,7] HERMANN REICHENSPURNER,[3] MICHAEL SCHMOECKEL,[3] COSMAS WIMMER,[3] ALEXANDER RUCKER,[3] VERONIKA EDER,[3] BRUNO MEISER,[3] MICHAEL HINZ,[3] THOMAS FELBINGER,[5] JOSEF MÜLLER-HÖCKER,[6] CLAUS HAMMER,[4] AND BRUNO REICHART[3]

Department of Cardiac Surgery and Institute for Surgical Research, Klinikum Grosshadern, Ludwig-Maximilians-University of Munich, 81377 Munich, Germany

Background. **The major problem of xenotransplantation is, that hyperacute xenograft rejection (HXR) causes graft failure within minutes or a few hours because of natural antibodies and activation of the complement system. As a preclinical model we transplanted pig hearts orthotopically into baboons. To prevent HXR after orthotopic xenotransplantation (oXHTx), the immunoglobulins (Ig) and natural antibodies were adsorbed to reusable Ig-Therasorb® immunoadsorption (IA) columns.**

Methods. **We performed three oXHTx of landrace pig hearts into baboons (19±6.8 kg), using extracorporeal circulation (ECC) connected to the IA unit. After separating the recipient's blood into plasma and cellular fraction by a plasma filter, plasma flow was directed to the Ig-Therasorb column coated with polyclonal sheep-antibodies against human IgG, IgM, and IgA. Intraoperative treatment consisted of 4 cycles of IA. For a control, we transplanted one pig heart into a baboon (16.9 kg) without applying IA. Perioperatively, serum concentrations of Ig, anti-pig-antibodies, complement and cardiac enzymes were determined. Tissue samples of myocardium were collected at the end of the study for immunohistochemical examinations, light microscopic examination (LM) and electron microscopic examination (EM). For cardiac monitoring after oXHTx, we used ECG, echocardiography, and invasive measurement of cardiac output. To prevent a mismatch of donor and recipient heart size, the donor pig had a 30–40% lower body weight than the recipient baboon.**

Results. **Four cycles of IA removed >80% of IgG, IgM, and IgA from plasma. The graft of the control animal failed after 29 min. The first oXHTx with IA was intentionally terminated after 100 min, the second oXHTx after 11 hr and the third oXHTx after 21 hr. All xenografts showed no histological signs of HXR. After weaning off ECC, these donor hearts worked in sinus rhythm without electrocardiographic ST-segment elevation. An excellent cardiac output was measured by echocardiography and thermodilution (2 L/min). Serological parameters indicating cardiac damage were significantly lower after IA if compared with the control experiment. Macroscopically, the xenograft of the control animal showed massive hemorrhage in comparison with the almost inconspicuous grafts after IA. The myocardium of the IA group demonstrated fewer deposits of Ig and complement components compared with the control animal.**

Conclusion. **Baboons do not hyperacutely reject a porcine xenograft after antibody depletion by the Ig-Therasorb column. In our experiment only 4 cycles of immunoapheresis effectively prevented HXR after oXHTx of baboons. The Ig-Therasorb column is a reusable device, which can be handled easily in combination with the ECC. IA must be tested in oXHTx long-term survival experiments, especially in combination with transgenic pig organs, which could be a reliable preclinical approach for future clinical xenotransplantation.**

In 1964, Hardy et al. performed the first xenotransplantation (XT*) of an undersized chimpanzee heart into a 68-year-old man in cardiogenic shock. The patient died after 1 hr (*1*). All subsequent clinical trials of XT with pig, sheep, baboon, and chimpanzee donor hearts failed after a maximum of 24 hr. The last orthotopic xenogeneic heart transplantation was performed by Czaplicki in 1992 (Table 1). Only baby Fae, an infant with an incompetent immune system, survived 20 days after XT of a baboon heart in 1984.

A worldwide increasing shortage of human donor organs (*9*), together with recent advances in the field of genetic engineering, have stimulated research in the use of animal organs for clinical transplantation. The major obstacles in a discordant species combination are the rejection mechanisms, especially hyperacute xenograft rejection (HXR). Scientists differentiate between "concordant" XT, for example humans receiving nonhuman primate organs from baboons

[1] Presented in abstract form at the 25th Annual Scientific Meeting of the American Society of Transplant Surgeons (ASTS), May 19–21, 1999, Chicago, Illinois.

[2] This work was supported by a grant from Bavarian Research Foundation and by Baxter/Therasorb Comp., Unterschleissheim, Germany.

[3] Department of Cardiac Surgery
[4] Institute for Surgical Research.
[5] Institute of Anesthesiology.
[6] Pathological Institute.
[7] Address correspondence to: Paolo Brenner, M.D., Department of Cardiac Surgery, Klinikum Grosshadern, Marchioninistr. 15, D-81377 Munich, Germany. E-mail: Paolo.Brenner@hch.med.uni-muenchen.de.

*Abbreviations: αGal, Galα1–3Gal epitope; ECC, extracorporeal circulation; EM, transmission electron microscopic analysis; HXR, hyperacute xenograft rejection; IA, immunoadsorption; Ig, immunoglobulin; LM, light microscopic analysis; oXHTx, orthotopic xenogeneic heart transplantation; PBS, phosphate-buffered saline; XNAb, xenoreactive, natural antibodies; XT, xenotransplantation.

TABLE 1. History of clinical xenotransplantation[a]

Author	Year	Donor species	Technique
Hardy et al. (*1*)	1964	Chimpanzee	Orthotopic
Ross et al. (*2*, *3*)	1968	Pig	Heterotopic
Cooley et al. (*4*)	1968	Sheep	Orthotopic
Marion et al. (*5*)	1969	Chimpanzee	Orthotopic
Barnard et al. (*6*)	1977	Baboon	Heterotopic
Barnard et al. (*6*)	1977	Chimpanzee	Heterotopic
Bailey et al. (*7*)	1984	Baboon	Orthotopic
Czaplicki et al. (*8*)	1992	Pig	Orthotopic

[a] Published reports of clinical xenotransplantation of hearts in concordant and discordant animal species.

TABLE 2. Orthotopic xenotransplantation of pig hearts into baboons after immunoadsorption

Animal	B1	B2	B3	B4
Cycles of IA	4	4	4	0
Body weight baboon (kg)	17	25.8	18.1	16.9
Body weight pig (kg)	13.2	14.2	13.5	13.0
Heart weight baboon (g)	64.4	118.9	85.0	80.0
Heart weight pig (g)	87.1	65.5	67.6	76.2
Anti-pig-titer before IA (1/n)	512	128	256	1024
Anti-pig-titer after IA (1/n)	64	32	32	1024
Ischemic time (hr)	4.5	2.4	2.8	4.0
Graft survival (hr)	1.7	10.8	20.8	1.0

or chimpanzees with a close phylogenetic relationship, from a "discordant" XT between widely divergent species (*10*). The typical xenogeneic rejection mechanism in the discordant system pig-to-primate is the early hyperacute xenograft rejection process (*11*), which occurs within minutes to hours. HXR, which is analogous to rejection in ABO-incompatible allograft transplantation, is primarily caused by xenoreactive, natural antibodies (XNAb) (*12*), by activation of the complement cascade (*13*), and direct endothelial cell activation through monocytes and natural killer cells (*14*). The XNAb of primates (only in humans, apes, and old world monkeys) with no functional α1–3-galactosyltransferase bind to α1–3Gal-epitopes (αGal) of glycoproteins and glycolipids on the vascular endothelium (*15*). Consequently, the most effective procedure to enhance xenograft survival could be a perioperative antibody depletion.

Antibodies and complement can be depleted (*16*) by plasmapheresis, xenogeneic organ perfusion with kidney and liver, treatment with haptens like α1–3-Gal-fragments, and application of penicillamine (*17*). Selective immunoadsorption (IA) techniques based on antigen-antibody-binding, are used today. The aim of our experiment was to test a selective immunoglobulin (Ig)G-, IgM-, and IgA-antibody removal from human blood by immunoadsorption. We used an Ig-Therasorb column. The principle of the reusable Ig-Therasorb column are polyclonal sheep antihuman IgG antibodies conjugated to Sepharose beads, which remove specifically IgG, IgM, and IgA from the baboon plasma. In a working heart model, perfusing pig hearts with human blood IA had the potential to postpone HXR (*18*, *19*). For an analogous in vivo investigation, we applied an orthotopic XT model. Heterotopic models are only of limited relevance, because hearts do not eject against an afterload. Only orthotopic transplantation represents a reliable life-supporting model similar to clinical XT conditions. In the primate model described, we applied IA technique in orthotopic xenotransplantation (oXHTx) of Landrace pig hearts into baboons for the prevention of HXR.

METHODS

Animals and procedure. For orthotopic XT, we used nontransgenic Landrace pigs (body weight: 13–14 kg). Thoracotomies were performed, and the pig hearts were explanted after induction of cardioplegic arrest with 4°C cold Celsior-solution (Imtix, Pasteur Merieux Serums & Vaccines, Lyon, France).

Recipients were adult *Papio anubis* baboons, with a body weight of 17–26 kg (Table 2). We performed three oXHTx of landrace pig hearts into baboons (B1, B2, B3) in Group 1 using extracorporeal circulation (ECC) connected to the IA unit. After separating the recipient's blood into plasma and the cellular fraction by a plasma filter, plasma flow was directed to the Ig-Therasorb column. Intraoperative treatment consisted of 4 cycles of IA. For a control, we performed one oXHTx of a baboon (B4, 16.9 kg), not applying IA. The transplantation technique was that commonly used in allogenic heart transplantation, according to Lower and Shumway. After the thoracotomies, during ECC the recipient hearts were removed and the anastomoses were made between the donor organs and the recipients, beginning with the left atrium, followed by right atrium, pulmonary artery, and finally the ascending aorta. Before releasing the aortic clamp, steroids were given. After reperfusion, weaning from extracorporeal circulation was started, sometimes with an initial use of catecholamines. A mismatch of donor and recipient heart size was prevented by selecting a 30–40% smaller body weight of donor pigs than recipient baboons.

Immunoadsorption. The sterile and pyrogen-free Ig-Therasorb® glass column contains polyclonal sheep anti-human IgG antibodies (heavy chain- and light chain-specific) conjugated to cyanogen bromide-activated sepharose beads (Fig. 1). The anti-Ig column, with a total volume of 300 ml, removes specifically IgG (subclasses 1–4), IgM, IgA, circulating immune complexes and fragments of Ig with an average Ig reduction of 60–70% per cycle. The column is loaded with a storage buffer containing phosphate-buffered saline (PBS) and 0.01% sodium acid at pH 7.2 at 4°C until use. The IA system consists of a pair of reusable Ig-Therasorb® columns and a hemopump with a plasma filter, which separates human whole blood (500 ml) into plasma and the cellular fraction. A second circuit ensures a constant plasma flow directed to the first column. After passage through the

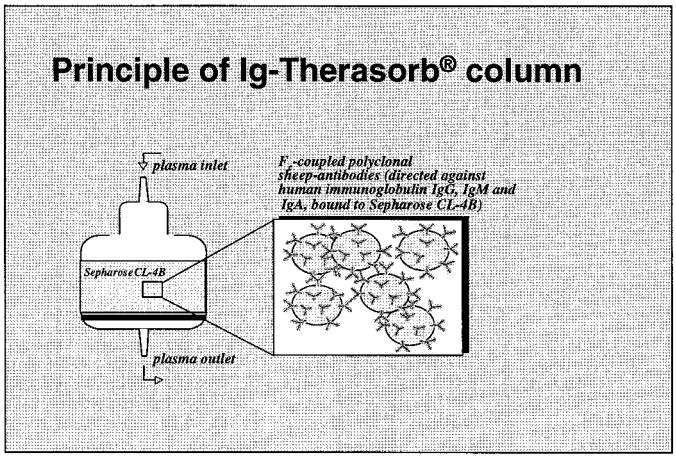


FIGURE 1. Principle of Ig-Therasorb column. Human plasma passes through a sterile and pyrogen-free column containing polyclonal sheep anti-human IgG (heavy chain- and light chain-specific) conjugated to cyanogen bromide-activated sepharose beads, with a total column volume of 300 ml. The column is filled with phosphate-buffered saline, 0.01% sodium acide, pH 7.2, at 4° C until use.

first column, the bound Ig are released from the sepharose beads by glycine (pH: 2.8) and PBS buffer solution (pH: 7.2). The column is regenerated by glycine and PBS solution, whereas the second column is loaded.

Serological examination. Blood samples were collected from baboons at fixed intervals before, during, and after IA and XT. After centrifugation at 4°C, the plasma was stored at −70°C. Efficiency of IA was controlled by measuring the levels of IgA, IgG, IgM, and anti-pig-antibodies as well as complement C3 and C4. Complement activity was quantified by the hemolytic CH50 assay (classical pathway) and the AP50 assay (alternative pathway). For the anti-pig-antibody-test, porcine plasma (0.5 ml) was serially diluted. Washed pig red blood cells were incubated for 30 min, and hemagglutination was titrated under a light microscope. The anti-pig-antibody titer was the highest dilution level of baboon plasma when erythrocytes still agglutinated. As markers for myocardial damage, creatine kinase, lactate dehydrogenase, and aspartate aminotransferase measurements were determined by standard methods.

Histological examination. After cardiac arrest, tissue from both atria and ventricles of the xenograft were sampled for immunohistochemical examination and light (LM) and electron microscopic examination (EM). Frozen tissue sections of 4–6 μm were stained using hematoxylin and eosin and subjected to LM. For EM, tissue sections from both the right and left ventricle and atrium were taken at the end of perfusion, embedded in tissue tek (Miles, USA), snap-frozen in liquid nitrogen, and stored at −70°C until use. Other tissue samples were fixed in glutaraldehyde 6.25% and stored until further saccharose (0.2 mol/l) processing. Hemithin 0.5 μm sections prepared with epon resin were first colored with toluidine-methylene blue to gain an overview by LM. Ultrathin tissue sections of interest (100 nm) were laid on copper grids and stained with uranylacetate and lead. EM (Philips 300) was performed at two magnifications (×10.000 and ×16.000). For immunohistochemical analysis, cryostat-prepared tissue specimens were stained with FITC-conjugated goat antibodies specific for C3, C4, and C5b-9. Tissue deposits of IgM, IgG, and IgA were stained according to the avidin-biotin method. Monoclonal antibodies were obtained from Dako (Hamburg, Germany) and Immunotech Diagnostics (Marseille, France).

Hemodynamic examination. For cardiac monitoring after oXHT, ECG was registered and echocardiography was used for measurement of ejection fraction (percent) and fraction shortening (percent). During surgery a Swan-Ganz catheter was positioned in the pulmonary artery for invasive measurement of cardiac output.

RESULTS

Ischemic time was 2.5–4.5 hr (Table 2). All baboons had high anti-pig-antibody levels (titer in hemagglutination test: B1: 1:512, B2: 1:128, B3: 1:256, B4: 1:1024), and immunoadsorption was sufficient in Group 1 (titer after IA: B1: 1:64, B2: 1:32, B3: 1:32). Selecting a 31% smaller body weight of donor pigs (13.5±0.29 kg) than recipient baboons (19.45±2.13 kg) resulted in an 15% smaller heart weight of pig hearts (74±4.9 g) compared with baboon hearts 87.1±11.5 g).

Xenograft survival and hemodynamic features. The xenograft of the control animal failed after 29 min of reperfusion during extracorporeal circulation with a fibrillating ventricle. This experiment was terminated after 1 hr of reperfusion. The study was not authorized as a long-term survival experiment. To obtain specimens for histological examination during HXR normally occurring within the first 2 hr, the first oXHTx (B1) with IA was deliberately terminated after weaning from ECC and stabilization of circulation after 100 min. The second experiment (B2) was terminated after 11 hr and the third oXHTx (B3) after 21 hr. All hearts were without signs of HXR. After weaning off ECC, donor xenografts of the IA group displayed sufficient function in echocardiography (ejection fraction: 65±13%, fraction shortening: 32±6%), sinus rhythm without electrocardiographic ST-segment elevation, and arrhythmia in ECG. The cardiac output measured invasively was 1.93±0.035 l/min.

Serological features. Four cycles of IA removed 85.5% of IgG (from 9.43±0.76 g/L to 1.37±0.23 g/L), 94% of IgM (from 0.6±0.19 g/L to 0.1±0.01 g/L) (Fig. 2a) and 83.5% of IgA (from 0.97±0.39 g/L to 0.17±0.08 g/L) from plasma. Four cycles of IA reduced total anti-pig-antibodies by 85% (Fig. 2b). Specific porcine antibodies of the control animal were completely absorbed to the xenograft itself, leading to xenograft failure. In parallel, complement factors were eliminated by IA (C3: Group 1: from 1.27±0.22 g/L to 0.47±0.19 g/L after IA; Group 2: from 0.52 to 0.41 g/l; C4: Group 1: from 0.2±0.037 g/L to 0.075±0.02 g/L after IA; Group 2: from 0.058 to 0.049 g/L).

Serological enzymatic parameters indicating cardiac damage were lower after IA compared with the control experiment. (ASAT: Group 1: 107.1±39.8 U/L, Group 2: 254.6 U/L).

Hematological features. Hemodilution during IA and ECC turned out to be a problem because of hemoglobin levels decreasing to a minimum of 6 mg/dl (Fig. 3a). In further experiments, this problem was solved by homologous and autologous blood transfusion. White blood cell count increased after XT to 20–30 G/L (Fig. 3b). The number of platelets was reduced by 50% (Fig. 3c).

Histological features. Macroscopically, the xenograft of the control animal showed massive hemorrhage compared with the almost inconspicuous graft 20 hr after IA. The myocardium of the control animal showed typical signs of HXR, like occluded vessels and interstitial hemorrhage, under LM analysis (hematoxylin/eosin staining). This was in clear contrast to open vessels in a nearly inconspicuous tissue of the IA group.

It is surprising that EM of investigated areas showed no difference between both groups. The immunohistochemical investigation of the myocardium of the IA group demonstrated less depositing of IgM, IgG, and IgA and complement components C3, C4, and C5b-9 than in the control animal.

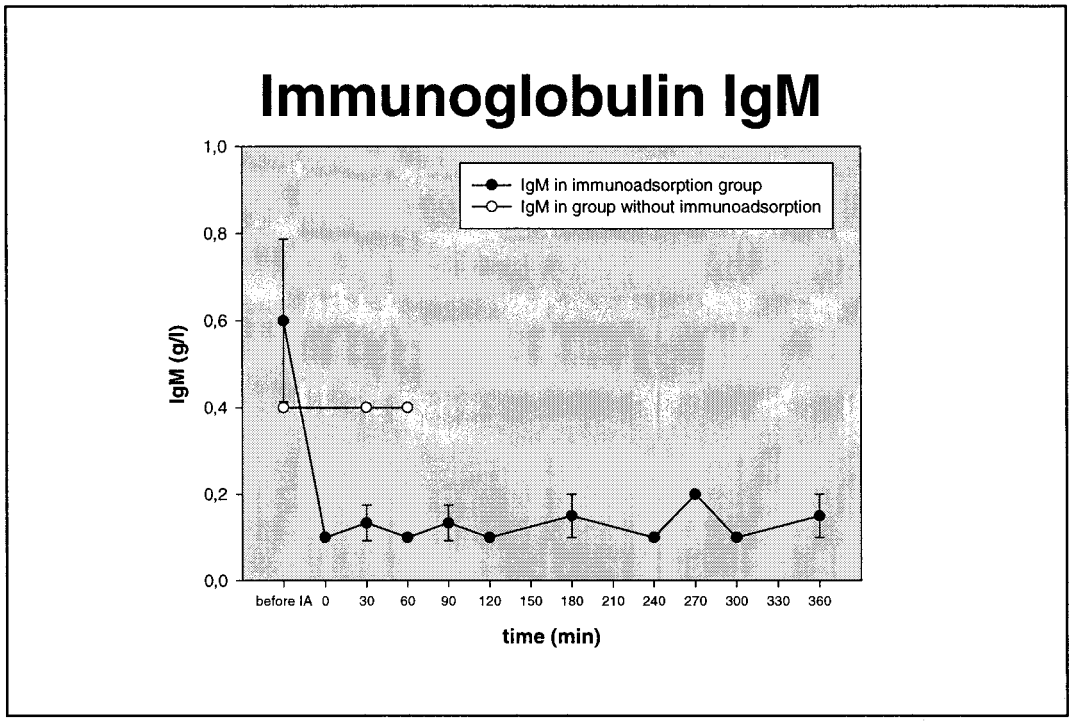
DISCUSSION

HXR triggered by xenoreactive antibodies is a central mechanism in xenograft rejection, which causes microvascular thrombosis, interstitial edema, hemorrhage, and cell necrosis (*20*). Therefore a great variety of therapeutic strategies for removal of XNAb and complement depletion (*21*) was investigated, like plasma exchange, plasmapheresis, xenogeneic organ perfusion, unspecific antibody absorbents, and the use of haptens like αGal1–3Gal-fragments and penicillamine (*17*). Plasma exchange and organ perfusion result in a loss of coagulatory and plasma proteins and, therefore, are clinically unattractive.

Alternatively, trials with IA (immunoapheresis) using affinity columns of immobilized staphylococcal proteins A and G have been highly effective in the treatment of autoimmune diseases, renal transplant patients with anti-HLA antibodies (*22*), and a pig-to-dog renal transplant model (*23*).

In the first animal experiment in 1981, using antibody-based immunoadsorption as a very specific depletion technique, sheep antibodies against LDL-cholesterol in pig

2a



2b

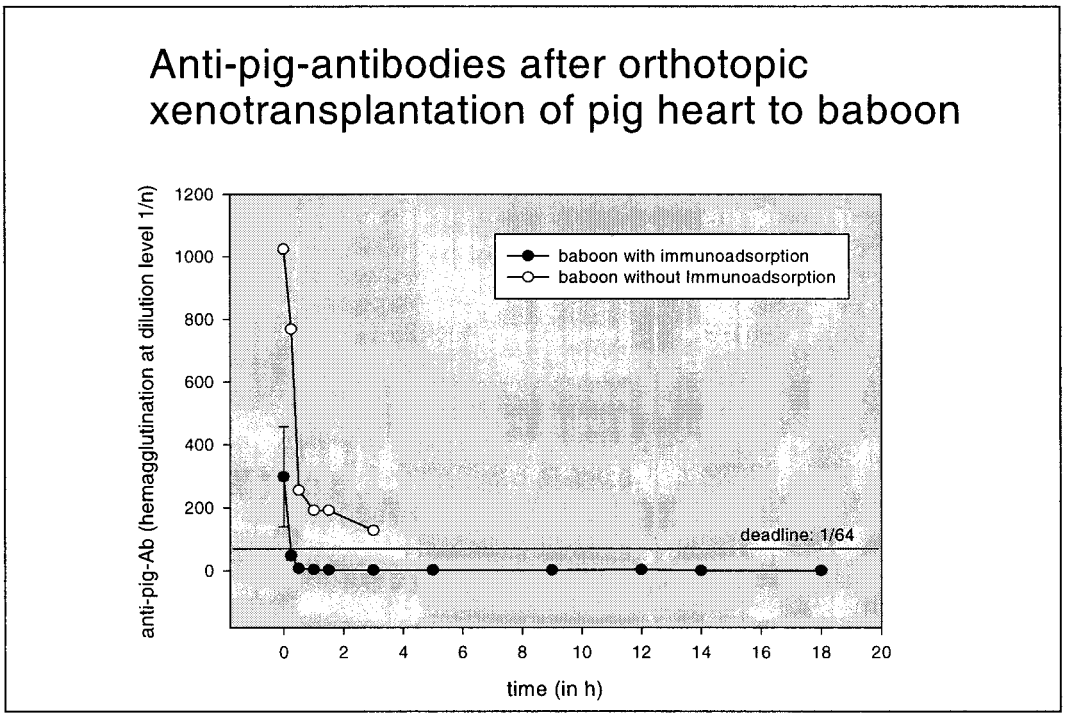
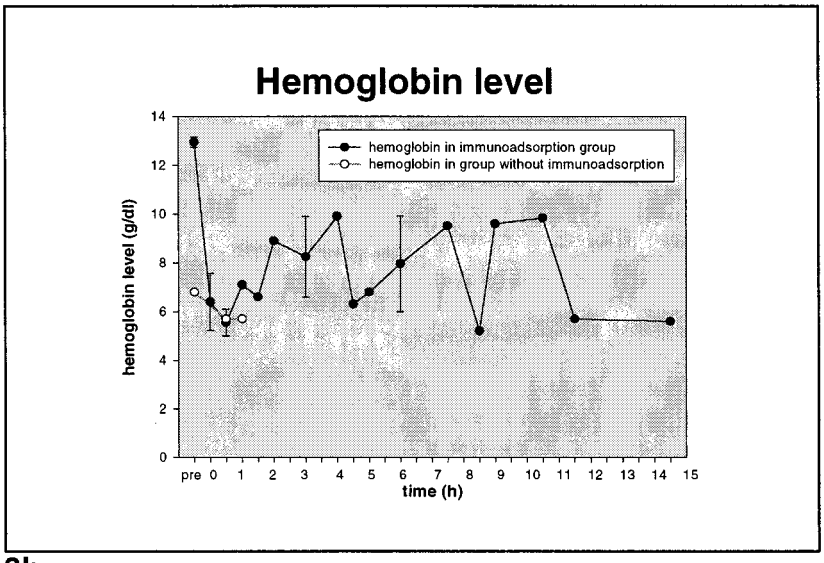
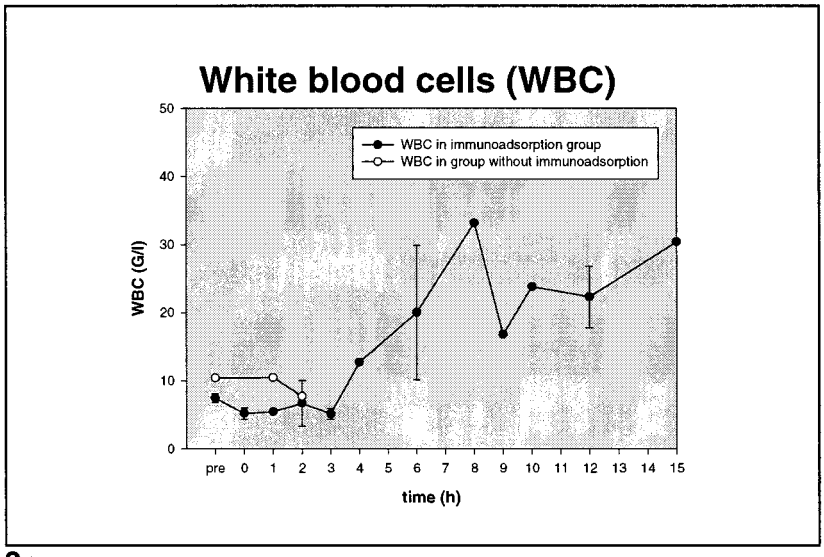


FIGURE 2. (A) Four cycles of IA removed >85.5% of IgG, 94% of IgM, and 83.5% of IgA from plasma. (B) 85% of all anti-pig-antibodies were removed in the IA group. Specific porcine antibodies of our control animal were eliminated by binding to the xenograft. In further experiments, we could evaluate a kind of "deadline" in anti-pig-antibodies at a 1:64 dilution level. Xenografts in untreated primates with higher antibody levels could not survive HXR.

3a



3b



3c

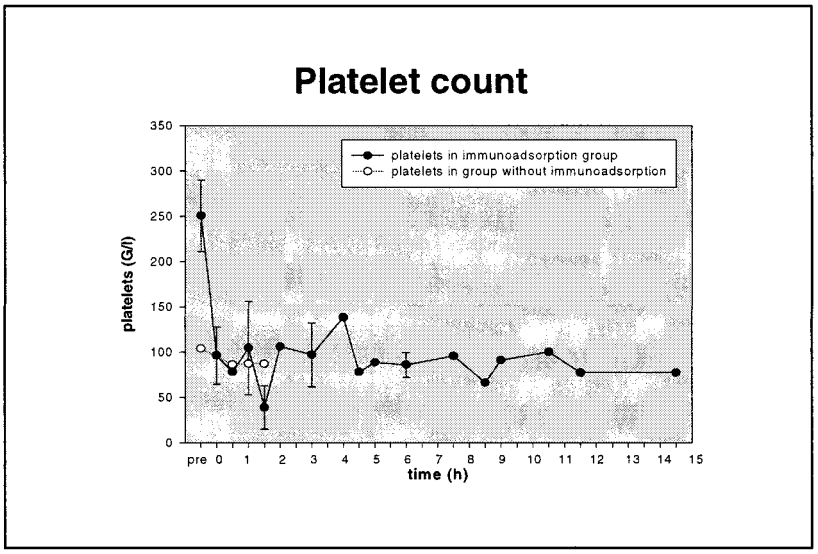


FIGURE 3. (A) A major problem was hemodilution during immunoadsorption and ECC because of decreasing hemoglobin levels. (B) The white blood cell count increased after XT. (C) The number of platelets decreased by 50% to critical values <100 G/L.

plasma were used (*24*). The first successful clinical trial was performed in 1983 with the LDL-Therasorb column (*25*). Columns of polyclonal antibodies directed against human Ig were extremely effective for removing human IgG and IgM XNAbs from plasma without a significant impact on coagulatory and plasmatic proteins (*19*). In our experiments, we investigated immunoapheresis performed by a reusable antihuman Ig-Therasorb column (Therasorb, Baxter Corp., Unterschleissheim, Germany) in an orthotopic in vivo XT model.

The total number of α1–3Gal-epitopes causing HXR is very large and it is not generally known in detail. The same applies to the distribution between IgG, IgM, and IgA antibodies. Our "unspecific" method removes all Ig and shows in clinical applications (autoimmune diseases and presensitized transplant patients) only minor side-effects with regard to infections (which also were not observed in our baboon long-term survival experiments). This has proved to be a safe method for xenoreactive antibody elimination. Moreover, it has been shown that removal of α1–3Gal-epitopes by using knockout mice leads to the presentation of additional epitopes, like the Forssman antigen (*26*), which again induces the binding of XNAb.

The results show, that baboons are able to survive the period of HXR after XT. The antibody depletion by Ig-Therasorb column improved xenograft function to sustain circulation in a life-supporting way in a primate model. Only 4 cycles of IA effectively prevented HXR in oXHTx of baboons. The anti-pig-antibody monitoring using a hemagglutination test was a reliable method for controlling efficiency of IA. This test was already used in earlier studies of ex vivo working heart perfusion of pig hearts as an exact monitoring for xenoreactive hemagglutinating anti-pig-antibodies and for evaluating a critical antibody level causing HXR (*27*). In further long-term survival experiments in heterotopic and orthotopic models (Brenner *et al.,* 1999 unpublished data), we established a "critical limit" for anti-pig-antibodies necessary to induce HXR at a 1:64 dilution level, if body weight of adult baboons is between 15 and 25 kg. Xenografts in primates with higher antibody levels would not survive HXR without IA treatment. In 1 of our last 20 experiments after heterotopic XT (Brenner *et al.,* 1999 unpublished data), a xenograft of a small baboon (17 kg) with a 1:64 antipig-antibody level was rejected after 11 hr. In oXHTx, xenograft failure because of HXR occurred always within 1 hr.

The Ig-Therasorb column is a re-usable device, which allows easy handling and a combination with the extracorporeal circulation. The increase in white blood cell count seems to be the result of steroid application before opening the aortic clamp (Fig. 3b). The decrease in platelets was probably caused by the plasma filter of the IA unit (Fig. 3c). IA reduced myocardial damage in histological, immunohistochemical, and serological parameters.

Further experiments are necessary to evaluate the possible application of immunoadsorption also in delayed xenograft rejection after oXHTx and chronic humoral rejection mechanisms. Lin et al. achieved good results with IA in overcoming acute vascular rejection in a heterotopic pig-to-baboon cardiac xenotransplant model (*28*). A combination of IA, the use of donor pigs transgenic for hDAF, and other complement inhibitors, together with an ideal combination of immunosuppressive drugs may be able to realize a safe, long-term survival of xenografts, not only in the primate model, but eventually in future clinical XT programs.

REFERENCES

1. Hardy JD, Chavez CM, Kurrus FD, et al. Heart transplantation in man. JAMA 1964; 188: 1132.
2. Ross DN. In: Shapiro H, ed. Experience with human heart transplantation. Durban, South Africa: Butterworths, Durban, 1969: 140, 228.
3. Cooper DKC. The first heart transplants in the United Kingdom, 1968–80: the role of Guy's graduates. Part 2. Guy's Hospital Gazette 1995; 109: 114.
4. Cooley DA, Hallman GL, Bloodwell RD, et al. Human heart transplantation: experience with 12 cases. Am J Cardiol 1968; 22: 804.
5. Marion P, Lapeyre D, Estanove S, et al. Clinical attempts at circulatory assistance. Ann Chir Thorac Cardiovasc 1969; 8(4): 411.
6. Barnard CN, Wolpitz A, Losman JG. Heterotopic cardiac transplantation with a xenograft for assistance of the left heart in cardiogenic shock after cardiopulmonary bypass. S Äfr Med J 1977; 52: 1035.
7. Bailey LL, Nehlsen-Cannarella SL, Concepcion W, et al. Baboon-to-human cardiac xenotransplantation in a neonate. JAMA 1995; 254: 3321.
8. Czaplicki J, Blonska B, Religa Z. The lack of hyperacute xenogeneic heart transplant rejection in a human. J Heart Lung Transplant 1992; 11: 393.
9. Hosenpud JD, Bennett LE, Keck BM, et al. The Registry of the International Society for Heart and Lung Transplantation: fourteenth official report. J Heart Lung Transplant 1997; 16: 691.
10. Calne RY. Organ transplantation between widely disparate species. Transplant Proc 1970; 2: 550.
11. Bach FH. Xenotransplantation: a step into the next century. Chimera 1995; 7: 12.
12. Lawson JH, Platt JL. Molecular barriers to xenotransplantation. Transplantation 1996; 62: 303.
13. Lu CY, Khair-El-Din TA, Dawidson IA. Xenotransplantation. FASEB J 1994; 8: 1122.
14. Inverardi L, Clissi B, Stoltzer AM, et al. Overlapping recognition of xenogenic carbohydrate ligands by human natural killer lymphocytes and natural antibodies. Transplant Proc 1996; 28: 552.
15. Galili U, Shohet SB, Korbin E, et al., Man, apes and old world monkeys differ from other mammals in the expression of α-galactosyl epitopes on nucleated cells. J Biol Chem 1988; 52: 17755.
16. Shapiro R, Tzakis AG, Scantlebury V, et al. Immunodepletion in xenotransplantation. J Invest Surg 1990; 3: 39.
17. Bach FH, Platt JL, Cooper DKC. Accomodation: the role of natural antibody and complement in discordant xenograft rejection. In: Cooper DKC, et al., eds Xenotransplantation. Heidelberg, Germany: Springer, 1991: 81.
18. Brenner P, Schmoeckel M, Huber H, et al. Influence of antibody removal by Ig-Therasorb column immunoadsorption on hyperacute xenograft rejection in a xenogeneic perfusion model. Langenbecks Arch Chir 1998 (suppl); 1: 645.
19. Kroshus TJ, Dalmasso AP, Leventhal JR, et al. Antibody removal by column immunoabsorption prevents tissue injury in an ex vivo model of pig-to-human xenograft hyperacute rejection. J Surg Res 1994; 59: 42.
20. Platt JL, Fischel RJ, Matas AJ, et al. Immunopathology of hyperacute xenograft rejection in a swine-to-primate model. Transplantation 1991; 52: 214.
21. Shapiro R, Tzakis AG, Scantlebury V, et al. Immunodepletion in xenotransplantation. J Invest Surg 1990; 3: 39.

22. Palmer A, Taube D, Welsh K, et al. Removal of anti-HLA antibodies by extracorporeal immunoadsorption to enable renal transplantation. Lancet 1989; 1: 10.
23. Auchincloss H. Xenogeneic transplantation. Transplantation 1988; 46: 1.
24. Stoffel W, Demant TH. Selective removal of apolipoprotein B-containing serum lipoproteins from blood plasma. Proc Natl Acad Sci USA 1981; 78: 611.
25. Richter WO, Suehler K, Schwandt P. Extracorporeal elimination by immunoadsorption: side effects and influences on other serum lipoproteins and serum parameters. Gotto AM, ed. Treatment of severe hypercholesterolemia in the prevention of coronary heart disease. Basel, Switzerland: Karger, 1990; 1835.
26. Shinkel TA, Chen CG, Salvaris E, et al. Changes in cell surface glycosylation in alpha1,3-galactosyltransferase knockout and alpha1,2-fucosyltransferase transgenic mice. Transplantation 1997; 64: 197.
27. Brenner P, Hinz M, Huber H, et al. The influence of antibody and complement removal with Ig-Therasorb column in a xenogeneic working heart model. Eur J Cardiothorac Surg 1999; 15: 672.
28. Lin SS, Weidner BC, Byrne GW, et al. The role of antibodies in acute vascular rejection of pig-to-baboon cardiac transplants. J Clin Invest 1998; 101: 1745.

Received 19 May 1999.
Accepted 2 August 1999.

0041-1337/00/6902-214/0
TRANSPLANTATION
Vol. 69, 214–221, No. 2, January 27, 2000
Printed in U.S.A.
Copyright © 2000 by Lippincott Williams & Wilkins, Inc.

HEPATOCYTE GROWTH FACTOR IS ESSENTIAL FOR AMELIORATION OF HYPERGLYCEMIA IN STREPTOZOTOCIN-INDUCED DIABETIC MICE RECEIVING A MARGINAL MASS OF INTRAHEPATIC ISLET GRAFTS[1,2]

MASAHIKO NAKANO,[3,6] YOHICHI YASUNAMI,[3,4] TAKANOBU MAKI,[3] SHOUTA KODAMA,[3] YASUTO IKEHARA,[3] TOSHIKAZU NAKAMURA,[5] MASAO TANAKA,[6] AND SEIYO IKEDA[3]

Department of Surgery I, Fukuoka University School of Medicine, Fukuoka 814-0180; Division of Biochemistry, Biomedical Research Center, Osaka University, Osaka 565-0871; and Department of Surgery I, Faculty of Medicine, Kyushu University, Fukuoka 812-8582, Japan

Background. **It is crucial for clinical islet transplantation to find a procedure to improve the success rate of insulin independence after islet transplantation. In the present study, we determined whether hepatocyte growth factor (HGF) has a favorable effect on amelioration of hyperglycemia in streptozotocin (STZ, 200 mg/kg)-induced diabetic mice (C57BL/6) receiving a marginal mass of intrahepatic islet isografts.**

Methods. **Isolated syngeneic islets were transplanted into the liver of recipients. HGF with dextran sulfate (DS) was administered intraperitoneally once a day at day 0, 2, 4, 6, and 8 relative to islet transplantation. DS has been known to enhance the effect of HGF.**

Results. **It was found that the number of 250 islets was a marginal mass as donor islets in this model, in which 2 out of 14 diabetic mice receiving 250 islets became normoglycemic by 90 days after transplantation. The treatment with HGF (100 μg) in conjunction with DS (200 μg) produced normoglycemia in all mice (n=5). Morphological study as well as intraperitoneal glucose tolerance test revealed the beneficial effects of HGF. To our surprise, six out of nine mice receiving 250 islets and treated with DS alone became normoglycemic. Additional anti-HGF antibody treatment (100 μg, day −1, 0, 2, 4, 6, and 8) abolished the effects of DS, indicating that the effect by DS is mediated via the endogenous HGF. The effects of DS were not observed when the renal subcapsular space was the site of islet transplantation. There was a significant increase in plasma HGF levels in mice after the intrahepatic grafts but not the renal subcapsular one.**

Conclusions. **These findings demonstrate that HGF is essential for amelioration of hyperglycemia in STZ-induced diabetic mice when a marginal mass of islets**

[1] Presented in abstract form at the 25th Annual Meeting of the American Society of Transplant Surgeons, May 19–21, 1999, Chicago, IL.

[2] This work was supported by a Grant-in-Aid for Highly Advanced Medical Technology and a Grant-in-Aid for Scientific Research from the Ministry of Education, Science, Sports and Culture of Japan, and by funds from the Central Research Institute of Fukuoka University.

[3] Department of Surgery I, Fukuoka University School of Medicine.

[4] Address correspondence to: Yohichi Yasunami, M.D., Associate Professor, Department of Surgery I, Fukuoka University School of Medicine, 7-45-1, Nanakuma, Jonan-ku, Fukuoka 814-0180, Japan. Tel.: 81-92-801-1011 (ext. 3422); Fax: 81-92-863-9759; E-mail: yasunami@fukuoka-u.ac.jp

[5] Division of Biochemistry, Biomedical Research Center, Osaka University.

[6] Department of Surgery I, Faculty of Medicine, Kyushu University.