# Cardiothoracic Transplantation

Cardiac xenotransplantation: Recent preclinical progress with 3-month median survival

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Extra material is available online.

Objectives: Transplantation is limited by a lack of human organ donors. Organs derived from animals, most likely the pig, represent a potential solution to this problem. For the heart, 90-day median graft survival of life-supporting pig hearts transplanted to nonhuman primates has been considered a reasonable standard for entry into the clinical arena. Overcoming the immune barrier to successful cardiac xenotransplantation is most appropriately first explored with the non–life-supporting heterotopic model.

Methods: We performed a series of 7 heterotopic heart transplantations from CD46 transgenic pigs to baboons using a combination of therapeutic agents largely targeted at controlling the synthesis of anti-pig antibodies. Rituximab (anti-CD20) and Thymoglobulin (rabbit antithymocyte globulin [ATG]; SangStat Medical Corp, Fremont, Calif) were used as induction therapy. Baseline immunosuppression consisted of splenectomy, tacrolimus, sirolimus, steroids, and TPC (an anti-Gal antibody therapeutic). Rejection events were not treated.

Results: By using Kaplan-Meier analysis, median graft survival was 96 days (range, 15-137 days; 95% confidence interval, 38-99 days). Only 2 grafts were lost as a result of rejection, as defined by cessation of graft palpation. There was no evidence of a consumptive coagulopathy, infectious complications were treatable, and no posttransplantation lymphoproliferative disorders occurred. No cellular infiltration was observed.

Conclusions: This study reports the longest median survival to date (96 days) of pig hearts transplanted heterotopically into baboons. Duplication of these results in the orthotopic life-supporting position could bring cardiac xenotransplantation to the threshold of clinical application.

Transplantation has become the preferred therapy for the treatment of end-stage organ failure. This success has unmasked a substantial gap between the number of patients who could benefit from a transplant and those who actually receive one. In the United States it has been calculated that on an annual basis 40,000 patients in congestive heart failure could benefit from a heart transplantation, yet fewer than 2500 transplantations are performed, a number that has not substantially changed in the last 10 years.[1,2] This discrepancy between supply

and demand has stimulated interest in alternative solutions, one of which is the use of animals as organ donors. The animal of choice for clinical application of xenotransplantation is currently considered to be the pig because of its size, physiologic compatibility, and breeding characteristics and the potential for genetic modification. The current major limitation to clinical xenotransplantation is immunologic rejection.[3] A 90-day median survival of life-supporting orthotopic pig heart transplants in nonhuman primates has been proposed as a prerequisite for clinical application.[4] A first step in achieving this goal is to define immunosuppressive regimens that control the xenograft immune response by using non–life-supporting heterotopic transplants.

Pig organs, when transplanted to primates, are rapidly rejected because of the presence of recipient antibody that recognizes a carbohydrate epitope, galactose α1-3 galactose (α-Gal), which is present on glycoproteins and glycolipids on the pig endothelium.[5] This rejection process, hyperacute rejection, can be routinely overcome by the removal of anti-Gal antibody[6] or the inhibition of the complement cascade by the use of transgenic pigs that express human complement regulatory proteins.[7-9] When hyperacute rejection is blocked, grafts are usually lost within a few days to weeks through a process called delayed xenograft rejection (DXR).[10-12] DXR is thought to be mediated by both preexisting and induced anti-Gal antibodies and is not controlled by the currently available immunosuppressive agents.[13,14] We have previously shown that an α-Gal–polyethylene glycol conjugate (TPC) can block the anti-Gal immune response in primates.[15,16] This led us to hypothesize that the use of such a conjugate, together with a regimen of immunosuppressive agents targeted to block an antibody-mediated immune response, would result in prolonged graft survival of a transgenic pig heart transplanted heterotopically to the baboon.

In a previous protocol we had used an immunosuppressive regimen consisting of rituximab (anti-CD20 monoclonal antibody), tacrolimus, sirolimus, steroids, enoxaparin (Lovenox), and TPC.[17] This earlier study obtained a median graft survival of 76 days; however, excessive use of ATG to treat presumptive rejection episodes led to the development of infectious complications, including frequent bacterial sepsis, fatal emergence of baboon cytomegalovirus (CMV), and posttransplantation lymphoproliferative disorder (PTLD). To improve on this experience, we have adopted a simplified immunosuppressive regimen using ATG and rituximab only for induction therapy, and rejection episodes were not treated. Our current protocol uses no postoperative anticoagulants and resulted in 96-day median graft survival. This protocol showed no evidence of perioperative consumptive coagulopathy, was free of fatal infectious complications, and showed no evidence of CMV or PTLD. This result is the longest reported median survival of pig hearts transplanted into baboons and represents a significant step toward the onset of clinical xenotransplantation.

Methods
Donors and Heterotopic Heart Transplantation
Pig donors were transgenic for the human complement regulatory protein CD46 and have been described in detail.[18] Standard heterotopic abdominal pig-to-baboon heart xenotransplantation was performed.[19,20] All animals were housed and received humane care in accordance with the standards established by the Institutional Animal Care and Use Committee of the Mayo Clinic and Foundation and as described in the "Guide for the Care and Use of Laboratory Animals" prepared by the Institute of Laboratory Animal Resources and published by the National Institutes of Health (NIH publication no. 86-23, revised 1985).

Immunosuppression
The day of transplantation is considered postoperative day (POD) 0. The immunosuppressive regimen consisted of TPC at 50 mg/kg every 3 days for a total of 6 doses before transplantation and daily after transplantation until day 30. After day 30, TPC was administered once every 3 days. Induction therapy consisted of Thymoglobulin (rabbit antithymocyte globulin [ATG]; SangStat Medical Corp, Fremont, Calif) at 1.5 mg/kg starting at day 2 for a total of 5 doses and rituximab (Genentech Inc, South San Francisco, Calif) at 19 mg/kg weekly starting at day −7 for a total of 4 doses. Maintenance immunosuppression consisted of tacrolimus (Prograf; Fujisawa Healthcare Inc, Deerfield, Ill) to achieve a trough

TABLE 1. Summary of transplantation results

Recipient	Duration (d)	Graft failure	Palpation score	DXR grade	IgM	IgG	C5b	Cause of death
1	15	No	5	3	D2	NEG	F1	Atrial rupture
2	38	No	6	1	F2	NEG	NEG	Ventricular rupture
3	54	No	6	1	D2	NEG	NEG	Iatrogenic
4	64	No	6	1	D2	F2	NEG	Renal failure
5	96	Yes	0	4	UR	UR	UR	NA: elective explantation
6	99	No	5	4	D3	D2	NEG	Pulmonary embolism
7	137	Yes	0	4	UR	UR	UR	NA: elective explantation

DXR, Delayed xenograft rejection; *D*, diffuse; *NEG*, negative; *F*, focal; *UR*, unreadable; *NA*, not applicable.
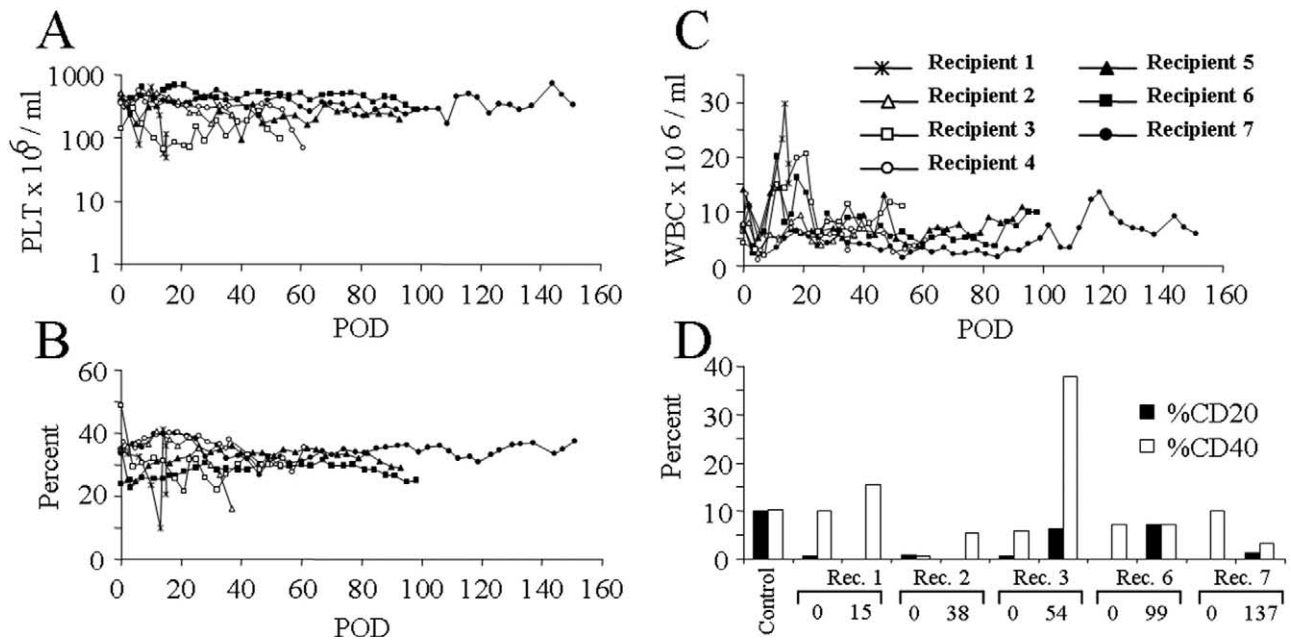
Figure 1. Hematologic analysis of transplant recipients: A, platelet counts; B, hematocrit value; C, total white blood cell counts; D, analysis of CD20$^+$ and CD40$^+$ lymphocytes in mesenteric lymph nodes on POD 0, the day of transplantation, and at the time of rejection or recipient death (Table 1). Data from recipients 1, 2, 3, 6, and 7 are shown. Comparable samples from recipients 4 and 5 were not obtained, and therefore these animals were excluded from this analysis. *Control* represents the proportion of CD20$^+$ and CD40$^+$ lymphocytes from an untreated baboon.

level of 20 to 30 ng/mL, sirolimus (Rapamune; Wyeth Laboratories Inc, Philadelphia, Pa) to achieve a trough level of 10 to 20 ng/mL, and a tapering dose of steroids. After POD 60, targeted trough levels of tacrolimus were reduced to 10 to 15 mg/mL. Splenectomy was performed on day −7 before the first dose of rituximab.

Graft Monitoring

Graft survival was estimated as either the last day of detectable palpation or animal mortality, whichever occurred first. Palpation was scored on a scale of 0 to 6, with 6 indicating the strongest pulsation. Cessation of palpation was confirmed by means of echocardiography and intramyocardial electrography, as previously described.[21]

Additional methods for histologic analysis, anti-Gal enzyme-linked immunosorbent assay, infectious disease management, and statistical analysis can be found in the online-only Appendix E1.

Results

Duration of Graft Function

Seven heterotopic heart transplantations were performed, and the length of survival ranged from 15 to 137 days (Table 1). Two grafts succumbed to rejection on days 96 and 137, as defined by a cessation of palpation and a lack of contractility at echocardiography. All other grafts (n = 5) were still contracting vigorously, with a palpation score of 6 (n = 3) or 5 (n =

2) confirmed by means of echocardiography near the time of recipient mortality. Two recipients died of hemorrhage (one on day 15 from rupture of a thrombosed atrium and another on day 38 from left ventricular rupture, likely caused by coronary embolism from a thrombosed left heart and donor aorta). One recipient died with a functioning graft on day 64 of renal insufficiency. This recipient exhibited increasing serum creatinine levels at the time of death, and histology of the baboon kidney showed widespread acute tubular necrosis at necropsy. One recipient died from a pulmonary embolism on day 99. The last recipient died on day 54 after gastric feed was injected intravenously in error. Because this recipient had a vigorously contracting pig heart at the time of death and died for reasons clearly unrelated to the immune response to the xenograft, we considered the duration of organ function as censored at the time of death for this animal. The observed durations of organ function ranged from 15 to 137 days, and the Kaplan-Meier estimation of median duration of organ function was 96 days (95% confidence interval, 38-99 days).

Hematology

Immediately after transplantation, there was no evidence of thrombocytopenia associated with consumptive coagulopathy (Figure 1, *A*). Most recipients maintained platelet levels

in excess of 100×10^6/mL throughout the transplantation period. The only exception to this was the graft lost to atrial rupture on day 15 that showed a precipitous platelet decrease associated with the hemorrhage. The recipient that died on POD 54 exhibited chronic low platelet levels beginning on POD 11, with no adverse effects on graft function or histology at necropsy. In general, the hematopoietic function in the baboons remained good, with a hematocrit value of approximately 30% (Figure 1, B). Erythropoietin was not used in any of these recipients. Blood transfusions were given to 4 of the recipients. In 2 instances (recipients 1 and 2) transfusions were in direct response to bleeding episodes that preceded rupture of the graft. In another case a transfusion was given after detecting and repairing an apical aneurysm in the left ventricle of the xenograft in recipient 7 (POD 43). The other transfusion was given to recipient 3 (POD 21) in response to low hemoglobin levels. Circulating white blood cell (WBC) counts decreased early after transplantation, presumably in response to ATG treatment (Figure 1, C). Subsequently, WBC counts rebounded and remained stable at approximately 2 to 8×10^6/mL throughout the transplantation period. Differential analysis of whole WBCs indicated that neutrophils constituted approximately 85% of the cells, with lymphocytes making up approximately 9%.

Rituximab (anti-CD20) induction on days −7, 0, 7, and 14 was highly effective in depleting circulating B cells but appeared to be less effective at eliminating B cells within lymphoid tissues. We used anti-CD20 and CD40 antibodies to identify baboon B cells. Reagents that detect baboon CD19 were not available. In a previous analysis, all B cells in unmanipulated baboons were double positive for these markers. After anti-CD20 induction therapy, there was a complete depletion of circulating B cells for the duration of the transplantation (data not shown). Depletion of CD20$^+$ cells was also apparent in the mesenteric lymph nodes on the day of transplantation; however, positive staining with anti-CD40 suggested that B cells were still present and that these cells were either coated with rituximab, thus preventing the binding of a second anti-CD20 monoclonal antibody, or alternatively had modulated expression of the CD20 surface antigen. A similar situation was observed in necropsy samples in which, except for recipient 6, the proportion of CD20$^+$ lymph node cells was reduced, but CD40$^+$ cells were in proportions that are comparable with those seen in untreated lymph nodes (Figure 1, D).

Histologic Analysis

The endpoint of graft failure under these experimental conditions was DXR, which was characterized histologically as coagulative necrosis with large areas of ischemic tissue and microvascular thrombosis (Table 1). Generally, there was no thrombosis of the larger blood vessels, which remained
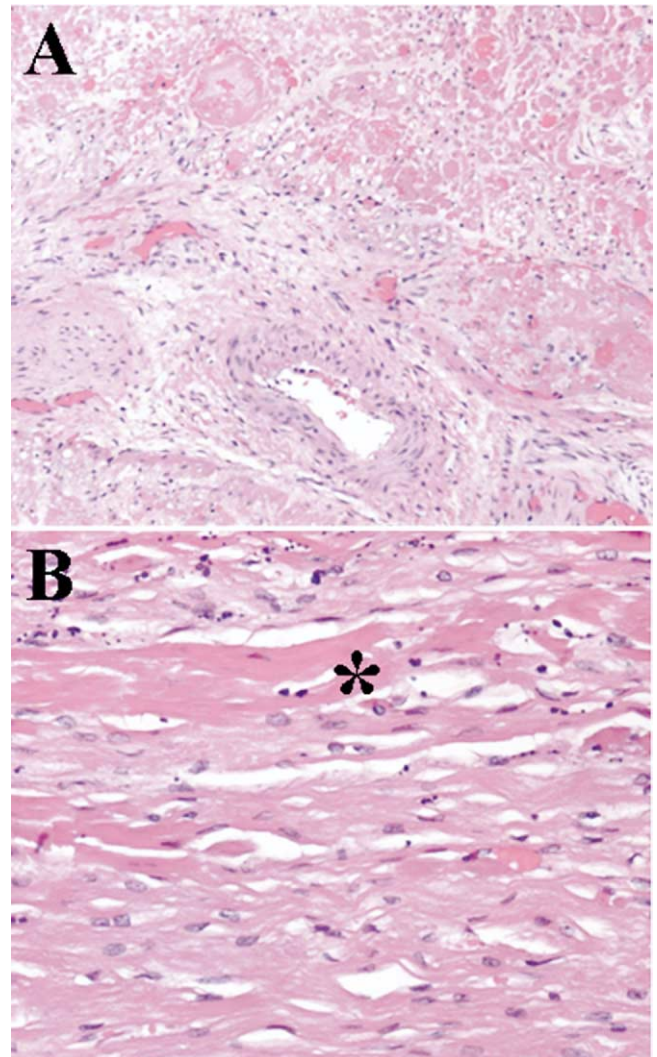

Figure 2. Xenograft histology. A, Xenograft of recipient 6 explanted on POD 99, when the recipient died of a pulmonary embolism. The graft shows extensive DXR with large regions of coagulative myocyte necrosis. B, Xenograft of recipient 4 that died of renal failure on POD 64. The section shows minimal evidence of DXR. There is one small focus of myocardium with coagulative necrosis (*) surrounded by well-preserved myocardium. (Hematoxylin and eosin, original magnification 100×.)

patent (Figure 2, A). There was no evidence of a cellular infiltrate in any graft. Transplant vasculopathy, characterized by intimal thickening of the arterial wall, was not a prominent feature of rejection and was detected in only a single vessel of one recipient. Long-surviving grafts with vigorous contractility exhibited well-preserved myocardium, with only focal regions of tissue damage, ranging from mild edema or ischemia to myocyte necrosis (Figure 2, B).

Immunohistochemistry of frozen sections was interpretable in 5 of the 7 grafts and consistently showed the depo-
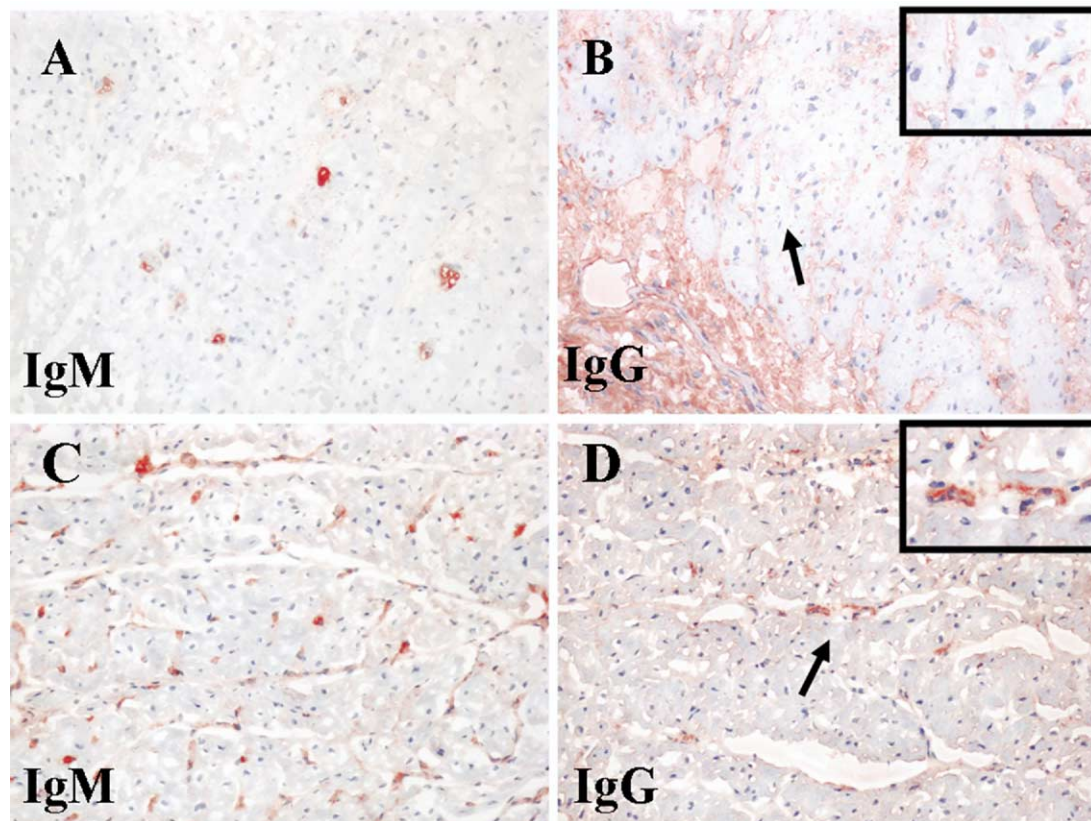
Figure 3. Immunohistochemical staining of explanted cardiac xenografts. A and B are from recipient 6, the same animal shown in Figure 2, *A*, with severe DXR, and C and D are from recipient 4, the same animal shown in Figure 2, *B*, with minimal DXR. Intense diffuse staining of endothelial cells in remaining viable myocardium by IgM and IgG is seen in A and B. Diffuse moderate staining of endothelial cells with IgM is seen in C, and focal moderate staining of endothelial cells by IgG is seen in D. *Arrows* highlight areas shown as *insets* (Original magnification 100× for each panel, 200× for IgG insets.)

sition of IgM (Figure 3, *A* and *C*), ranging from a focal to a diffuse staining with a trend toward more intense labeling in the grafts that showed evidence of greater damage (Table 1). Staining for IgG (Figure 3, *B* and *D*) ranged from negative in 3 of the grafts to a diffuse staining in 1 graft. The trend again was to more intense and widespread staining in the more damaged grafts. Complement deposition was either absent (n = 4) or weakly positive (n = 1).

Infectious Complications
In our previously published transplantation series, we had observed widespread activation of baboon CMV with severe clinical manifestations.[17] In the current series of transplantations, there was no evidence of CMV. Whether this was due to a lack of activation of the virus or the presence of effective prophylaxis with ganciclovir cannot be established. No PTLD, a transplant-related malignancy associated with infection by a γ-herpesvirus, was seen either

grossly or histopathologically at necropsy. No fungal infections were seen. Bacterial infections (detected in 3/7 recipients) were reversible with appropriate antibiotic therapy.

Anti-Gal Antibody
Serum anti-Gal antibodies were measured throughout the course of the transplantation procedure and also after explantation. Before transplantation, TPC reduced both anti-Gal IgG and IgM to background levels, and these levels were maintained during the transplantation (data not shown). Postexplantation induction of anti-Gal antibodies could be determined in only one animal (recipient 7) that rejected the xenograft on POD 137 (Figure 4). In the absence of TPC but during basal immunosuppression, there was no increase in postexplantation anti-Gal IgG consistent with a lack of sensitization. Serum anti-Gal IgM levels rebounded slowly after graft removal; however, they only attained approximately 40% of the baseline level. These observations are consistent with our previous

results with a similar immunosuppression regimen in which induction of an anti-Gal antibody response was severely restricted.[22]

Discussion

In this study long-term graft function of heterotopic pig-to-baboon cardiac transplants was achieved with only 2 of the 7 grafts lost because of rejection. Median graft survival in this prospectively defined group of animals was 96 days (range, 15-137 days; n = 7), showing progress from our previous study[17] and a substantial improvement over previously reported median survivals seen by others.[23,24] For xenotransplantation to progress to clinical application, acceptable immunosuppressive regimens capable of controlling the immune response to the graft must be defined. We have used basal immunosuppression consisting of tacrolimus, sirolimus, and steroids. This combination of drugs has shown efficacy in preclinical primate allograft models[25] and is used clinically for presensitized patients.[26] It is important to note, however, that the efficacy range for tacrolimus and sirolimus in baboons is not established. In our earlier studies that did not use ATG at the time of transplantation, the blood trough levels for tacrolimus and sirolimus were maintained within the range normally used in human subjects. Under these conditions, anti-Gal immune responses were minimized, but median organ survival was limited to 15 days (range, 4-53 days).[21,22] In a subsequent study, basal immunosuppression was increased to the range used in the current study, resulting in a median duration of xenograft heart function of 76 days (range, 55-113 days; n = 9).[17] These experiments, however, also included weekly dosing with rituximab and the treatment of multiple presumptive rejection episodes with ATG. As a result, the overall level of immunosuppression in these transplants was considered excessive and resulted in significant bacterial[27] and viral infections and 2 cases of PTLD. In the present study we omitted the treatment of presumptive rejection episodes and used ATG and rituximab as induction therapy only. This reduction of immunosuppression had no negative effect on graft survival and appears to have reduced the significant infectious complications seen previously, although more effective antiviral prophylaxis might also have contributed to the absence of CMV emergence. This suggests to us that the presumptive rejection episodes treated in our previous study were probably falsely identified and underscores the need for effective diagnostic and therapeutic modalities for DXR. Furthermore, although we cannot as yet determine whether the increased levels of tacrolimus and sirolimus, the induction therapy with ATG, or a combination was the key to longer graft survival, it seems likely that repeated ATG depletion of lymphocytes is not required under these conditions.
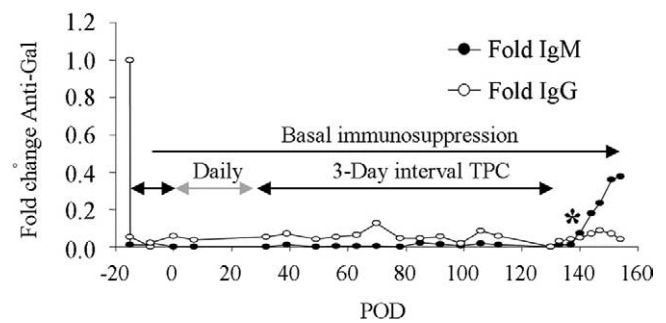

Figure 4. Serum anti-Gal antibodies in the one animal that survived organ removal on day 137. The graph depicts the fold change in anti-Gal IgM and IgG relative to pretransplantation baseline serum values. The day of organ explantation (137) is indicated by an *asterisk*. TPC was administered every 3 days before transplantation and after day 30. Daily TPC dosing was given from days 0 to 30, as indicated by the *horizontal arrows*. The duration of immunosuppression with tacrolimus and sirolimus is indicated by a *horizontal arrow* extending from POD −6 to 151.

Without the use of anti-Gal therapeutics, such as TPC, graft survival is typically from a few days to weeks and is accompanied by a large induction of anti-Gal antibodies at the time of graft rejection.[28] In this study no anti-Gal antibodies were detected during the course of the transplantation, and in the one animal that we were able to follow after explantation, there was no induction of anti-Gal IgM or IgG, although there was a gradual return to 40% of baseline values for anti-Gal IgM over a 14-day period. Although TPC is generally effective at controlling the effects of anti-Gal antibody, it is unlikely to be 100% effective, and the postexplantation return of anti-Gal IgM in recipient 7 would be consistent with an anti-Gal–mediated DXR. It is interesting, however, that the survival outcomes in this study are similar to those of recent transplantations using donor pigs that lack the Gal epitope.[29] In a pig-to-baboon heterotopic transplantation protocol, Kuwaki and associates[29] reported a median graft survival of 78 days (range, 59-179 days; n = 5) if animals lost because of nonimmunologic causes are excluded from the analysis. Although their immunosuppressive protocol is distinct, by using T-cell depletion and chronic costimulation blockade, both the survival of the organs and the histology of the graft at rejection showing microvascular thrombosis and ischemic injury are consistent with our observations. The immunohistologic analysis of rejected Gal-deficient xenografts showed antibody and complement deposition. We, too, observe antibody binding to vascular endothelial cells but find less evidence of complement deposition. This suggests indirectly that even in the absence of anti-Gal antibody (eg, using Gal deficient donor organs), transgenic expression of human CD46 or other human complement regulatory pro-

teins might still contribute to organ protection and that the generation of pigs that are transgenic for human complement regulatory proteins and lack the Gal epitope might be of benefit. It is clear in this study that TPC can effectively control anti-Gal responses under these conditions; however, we expect that in a clinical context the use of Gal-deficient donor organs would be preferable because their use would simplify postoperative care.

The microvascular thrombosis and ischemic injury to the myocardium we observed at rejection has been ascribed to an antibody-induced endothelial cell activation, resulting in the development of a prothrombotic vasculature.[30] Consistent with this hypothesis, we observe focal-to-diffuse baboon IgM and IgG binding to the graft endothelium. Determination of the specificity of these antiendothelial cell antibodies might help elucidate the mechanism of graft failure and would facilitate strategies to diagnose DXR. We have not observed a cellular infiltrate in cardiac xenografts using histology or immunohistology. This is consistent with our previous experience and that of others but is at odds with in vitro data that suggest that a robust cellular immune response will occur.[31] Given that we do detect anti-pig IgG antibody on some grafts, there is likely to be a T-cell component to the xenograft rejection process that is not manifest as a cellular infiltrate under these conditions. The inability to completely deplete B cells in lymph nodes with rituximab suggests that therapy targeting T-cell help to the xenoreactive B cells might further extend xenograft survival.

Perioperative thrombocytopenia and consumptive coagulopathies have been reported after solid organ and cellular xenotransplantation.[32-35] These early coagulopathies have been at least partially ascribed to the known molecular incompatibilities of the coagulation cascade between pigs and primates.[36-38] As prophylactic therapy, we and others have administered heparin[17,33-35]; however, our recent experience with warfarin sodium (Coumadin), low-molecular-weight heparin, or combination therapy with aspirin and clopidogrel (Plavix) did not support a role for anticoagulation in prolonging cardiac xenograft survival. For that reason, we omitted postoperative enoxaparin in this study and saw no perioperative decreases in platelets levels, no evidence of an induced thrombocytopenia after transplantation, and no histologic evidence of systemic coagulopathies in the recipient organs. This suggests that acute coagulopathies might result from a strong rejection response and not necessarily from molecular incompatibilities in coagulation control.

In this study we observed 2 organ failures caused by rupture of the atrium or ventricle. We and others have observed such graft failures under a variety of immunosuppressive conditions, including with the use of systemic anticoagulation. With echocardiographic monitoring, we observe intrachamber blood stasis, particularly on the left side of the heart. Such stasis promotes thrombosis, which likely contributes to thrombotic complications, such as coronary embolism and myocardial infarction, as well as pulmonary embolism. In addition, kinking of the anastomosis caused by external or internal abdominal compression might further restrict graft blood flow. These failures would not occur after orthotopic transplantation. It remains possible, however, that coagulation incompatibilities alone or in combination with innate rejection, such as with pre-existing non-Gal antibody, might contribute to such thrombosis, although there is a paucity of evidence for such high levels of pre-existing non-Gal antibody.[39,40] Transgenic endothelial cell expression of anticoagulants has been shown to improve xenograft survival in the mouse-to-rat model, suggesting that incorporation of similar genetic strategies in the pig might alleviate problems with thrombosis and further extend pig-to-primate cardiac xenograft survival.[41]

The median graft survival of more than 3 months in this study with only 2 organ rejections is encouraging and suggests that cardiac xenograft rejection can be largely controlled with an immunosuppressive regimen consisting of currently clinically available drugs, with the exception of TPC. The limited evidence of renal impairment, the absence of serious bacterial infections, and the absence of PTLD suggest that this immunosuppressive regimen was not excessive compared with earlier cyclophosphamide-based regimens. If a continued T cell–dependent antibody response persists under these conditions, however, there remains a variety of additional approaches, such as plasmapheresis, costimulation blockage, coreceptor blockage, and others, that could be used to augment this current regimen. Duplication of these results in the orthotopic life-supporting model is an essential next step to fully gauge the effectiveness of the current regimen.

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Appendix E1

Histology

For histology, sections of the right and left ventricles were routinely processed and stained with hematoxylin and eosin. For immunohistochemistry, biopsy samples were embedded in OCT (Miles Laboratories, Inc, Elkhart, Ind), snap-frozen in isopentane and dry ice, and cut on a cryostat into 4-μm-thick sections. Before staining, tissue sections were briefly air-dried, fixed with acetone, and rinsed with 3 changes of PBS, pH 7.2. Sections were stained for human IgM (DAKO, Glostrup, Denmark), human IgG (DAKO), and human C5b neoantigen (Research Diagnostics Inc, Flanders, NJ). Antibody binding was detected with appropriate horseradish peroxidase–conjugated secondary antibodies and diaminobenzidine stain.

A semiquantitative grading system was used to describe the pathologic observations. The grade of DXR was scored on the basis of the percentage of total myocardium identified as damaged by means of examination of hematoxylin and eosin–stained sections as follows: grade 1, 0% to 25%; grade 2, 26% to 50%; grade 3, 51% to 75%; and grade 4, greater than 75%. In its mildest form, the damage was characterized by myocyte vacuolization and interstitial edema, and its severest form was characterized by coagulative necrosis. For immunohistochemistry, the deposition of antibody or complement was separated into 2 components. First, the distribution of staining was assessed on the basis of the percentage of capillaries and small vessels staining and was given a grade of 0 (none), F (focal; 1% to 25%), and D (diffuse; 26% to 100%). The intensity of staining was none (grade 0), mild (grade 1), moderate (grade 2), and marked (grade 3).

At the termination of the experiment, all animals underwent complete autopsy.

Anti-Gal Enzyme-linked Immunosorbent Assay

Sera from blood samples were analyzed preoperatively and once or twice each week postoperatively by means of enzyme-linked immunosorbent assay for detection of α-Gal antibodies.

Infectious Disease Management

Two days of perioperative intravenous antibiotics were given: ciprofloxacin (10 mg/kg; Bayer Corp, West Haven, Conn) and vancomycin (10 mg/kg; Abbott Laboratories, North Chicago, Ill), both twice a day, and fluconazole (1.5 mg/kg once daily; Diflucan; Pfizer Inc, New York, NY). Intravenous ganciclovir (10 mg/kg twice daily; F. Hoffmann-LaRoche Inc, Nutley, NJ; target levels 5-6 ng/mL) and itraconazole (200 mg orally twice daily; Sporanox; Janssen Pharmaceutical Inc, Titusville, NJ) were given prophylactically for the duration of the experiment.

Blood cultures were done for a recipient temperature of greater than 101.5°C, leukocytosis, or unexplained clinical deterioration. Empiric therapy with twice-daily intravenous vancomycin (10 mg/kg) and cefepime (250 mg; Maxipime; Bristol-Myers Squibb, Princeton, NJ) was begun pending blood culture results when antibiotic therapy was guided by in vitro susceptibilities.

Statistical Analysis

The probability of graft failure as a function of time was estimated by using Kaplan-Meier analysis.