Genetically engineered pigs and target-specific immunomodulation provide significant graft survival and hope for clinical cardiac xenotransplantation

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Objectives: Cardiac transplantation and available mechanical alternatives are the only possible solutions for end-stage cardiac disease. Unfortunately, because of the limited supply of human organs, xenotransplantation may be the ideal method to overcome this shortage. We have recently seen significant prolongation of heterotopic cardiac xenograft survival from 3 to 12 months and beyond.

Methods: Hearts from genetically engineered piglets that were alpha 1-3 galactosidase transferase knockout and expressed the human complement regulatory gene, CD46 (groups A-C), and the human thrombomodulin gene (group D) were heterotropically transplanted in baboons treated with antithymocyte globulin, cobra venom factor, anti-CD20 antibody, and costimulation blockade (anti-CD154 antibody [clone 5C8]) in group A, anti-CD40 antibody (clone 3A8; 20 mg/kg) in group B, clone 2C10R4 (25 mg/kg) in group C, or clone 2C10R4 (50 mg/kg) in group D, along with conventional nonspecific immunosuppressive agents.

Results: Group A grafts (n = 8) survived for an average of 70 days, with the longest survival of 236 days. Some animals in this group (n = 3) developed microvascular thrombosis due to platelet activation and consumption, which resulted in spontaneous hemorrhage. The median survival time was 21 days in group B (n = 3), 80 days in group C (n = 6), and more than 200 days in group D (n = 5). Three grafts in group D are still contracting well, with the longest ongoing graft survival surpassing the 1-year mark.

Conclusions: Genetically engineered pig hearts (GTKOhTg.hCD46.hTBM) with modified targeted immunosuppression (anti-CD40 monoclonal antibody) achieved long-term cardiac xenograft survival. This potentially paves the way for clinical xenotransplantation if similar survival can be reproduced in an orthotopic transplantation model. (J Thorac Cardiovasc Surg 2014;148:1106-14)

Patients with end-stage organ failure waiting for donor organs have limited treatment options. For those with cardiac failure, mechanical assist devices provide one solution, but various complications associated with these devices have reduced their effectiveness.[1] Until we learn to grow organs via tissue engineering, which is unlikely in the near future, xenotransplantation seems to be a valid approach to supplement human organ availability. Despite many setbacks over the years, 2 major recent developments have helped revitalized progress in the xenotransplantation field. First is the ability to produce genetically engineered (GE) pigs[2,3] in which certain genes that are immunogenic to humans are knocked out and human transgenes, such as complement regulatory proteins (human complement regulatory protein) and human thromboregulatory protein, are expressed on pig cells.[4] The second achievement is the development of target-specific immunosuppression that could be used clinically in place of generalized immunosuppression.[4] With these developments, cardiac xenograft survival has been prolonged to more than 1 year.[5] Because of the cost of these experiments and scarce research funding, it is not feasible to address each genetic and immunosuppressive manipulation individually. Therefore, laboratories performing experiments in the field of xenotransplantation have selectively picked specific genetic modifications in pigs and immunosuppressive drug combinations to perform xenotransplantation experiments. In this report, we have summarized our results from multiple experiments to show the impact of these GE pigs and target-specific immune suppression focusing on recipient B-cell depletion and costimulation blockade.

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Abbreviations and Acronyms

ACT = activated clotting time
CMV = cytomegalovirus
EKG = electrocardiogram
GE = genetically engineered
Ig = immunoglobulin
IV = intravenously
LVP = left ventricular pressure
mAb = monoclonal antibody
SPF = specific pathogen-free
TBM = thrombomodulin
TM = thrombotic microangiopathy
WBC = white blood cell

METHODS

Animal Models and Genetic Modifications

Specific pathogen-free (SPF) baboons weighing 7 to 15 kg from the University of Oklahoma (Norman, Okla) were housed in a clean pathogen-free facility. These SPF baboons are known to have lower levels of both anti–non-Gal immunoglobulin (Ig)G and IgM.[6,7] Genetically modified pigs (aged 4-8 weeks) that were alpha galactosidase transferase knockout and hCD46 transgenic (GTKO.hCD46), with or without thrombomodulin (TBM) expression (Revivicor Inc, Blacksburg, Va), were used as heart donors. The weights of donor pigs were matched to the baboon recipient to ensure adequate accommodation of the heterotopic heart. All animals were used in compliance with guidelines provided by the National Heart, Lung, and Blood Institute Animal Care and Use Committee. All transplant procedures were performed at a National Heart, Lung, and Blood Institute core surgical facility.

Surgical Procedure

Donor pig hearts were transplanted into recipient SPF baboons in a heterotopic position, as described previously.[8] Briefly, the recipient baboon's infrarenal aorta and inferior vena cava were exposed through a midline abdominal incision. Side-biting clamps were applied; an aortotomy and venotomy were made, and the end-to-side anastomosis was performed between the donor and recipient aorta and the donor pulmonary artery with the recipient inferior vena cava.

Immunosuppressive Regimen

A detailed description of the immunosuppressive regimen is shown in Figure 1. In brief, it includes induction with antithymocyte globulin, anti-CD20 antibody (Rituxan; Genentech, Inc, South San Francisco, Calif), and costimulation blockade with anti-CD154 (5C8) or anti-CD40 (3A8 or 2C10R4) monoclonal antibody (mAb). Cobra venom factor was used to inhibit the complement activation. Mycophenolate mofetil and costimulation blockade antibody were also used daily and weekly as described in Figure 1 to prevent immune rejection. All recipient baboons received continuous heparin infusion to maintain the activated clotting time (ACT) level at twice the baseline. Ganciclovir was administered intravenously (IV) daily to prevent any potential viral infections. Erythropoietin (200 U/kg) was administered IV daily from day −7 to 7, and cefazolin (250 mg) IV was administered twice per day for 7 days.

Experimental Groups

The experimental groups are described in Table 1, and the duration of immunosuppression is illustrated in Figure 1. The main difference among the 4 groups was the type, strength, and duration of antibody used for costimulation blockade. In group A, anti-CD154 (5C8) (20 mg/kg) IV was used for the entire period of graft survival. In group B, anti-CD40 (3A8) (20 mg/kg) IV was used for a maximum of 60 days. In group C, anti-CD40 (2C10) (20 mg/kg) was tapered off in 60 days. In group D, anti-CD40 (2C10) (50 mg/kg) was continued for 1 year (n = 2) or reduced (25 mg/kg) after 100 days (n = 2).

Rescue Therapy

If rejection was suspected by diminution of xenograft function, rescue therapy was initiated with intravenous methyl prednisolone (10-15 mg/kg) for 6 days. Heparin was also used to prevent thrombus formation, and activated clotted time (ACT) was maintained at twice the baseline.

Measurement of Graft Survival

Telemetry, manual palpation, and noninvasive ultrasonography were used to monitor the xenograft function. A telemetry device was implanted at the time of transplantation to monitor the baboon recipient's temperature, graft left ventricular pressure (LVP), and electrocardiogram (EKG). The telemetry device data were transmitted wirelessly to a receiver attached to the animal's cage (RMISS, Wilmington, Del). The parameters were recorded and included the peak systolic pressure, end-diastolic pressure, LVP, heart rate based on LVP, EKG, heart rate based on EKG, and recipient's body temperature. Heart function was continuously evaluated by telemetry, and a decrease in LVP to less than 60 mm Hg was correlated with the initiation of the rejection process, which affected the graft contractility. An LVP less than 10 mm Hg was an indicator of complete cessation of graft contractility.

Recipients were sedated weekly for the first 2 months post-transplant and biweekly thereafter for blood collection. Palpation and ultrasound were also done on this schedule. On the basis of xenograft palpation, contractility of the heart was scored as ++++ (fully functional) to 0 (nonfunctional). Blood flow and wall motion were analyzed by echocardiography.

Hematology

White blood cell (WBC) count, hematocrit, red blood cell count, hemoglobin, platelets, neutrophils, and monocytes were analyzed. Blood chemistry was performed weekly for the first 2 months and then biweekly until the graft was explanted or rejected. ACT, prothrombin time, and troponin levels were measured at the same intervals.

Histology

Paraffin sections from biopsies and explanted xenografts were stained with hematoxylin–eosin for light microscopy. Sections were analyzed for cardiomyocyte viability and the presence of hemorrhage, microvasculature thrombosis, and cellular infiltrates.

RESULTS

Graft Survival

Graft survival curves for all groups are shown in Figure 2, *A*. Median graft survivals of the 4 groups are shown in Figure 2, *B*, and the longest survival in each group is shown in Figure 2, *C*. Among all groups, group D, receiving the high dose of anti-CD40mAb, had the longest median and individual survival. In group A, most of the grafts were still contracting at the time of recipient death or euthanasia because of various complications. All grafts in groups B and C were rejected. Two of 5 grafts in group D stopped contracting on postoperative days 146 and 159, but the other