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Cardiac Xenotransplantation: Progress and Challenges

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Abstract

Purpose of Review—Cardiac xenotransplantation (CXTx) remains a promising approach to alleviate the chronic shortage of donor hearts. This review summarizes recent results of heterotopic and orthotopic CXTx, highlights the role of non-Gal antibody in xenograft rejection, and discusses challenges to clinical orthotopic CXTx.

Recent Findings—Pigs mutated in the α 1,3 galactosyltransferase gene (GTKO pigs) are devoid of the galactose α 1,3 galactose (α Gal) carbohydrate antigen. This effectively eliminated any role for anti-Gal antibody in GTKO cardiac xenograft rejection. Survival of heterotopic GTKO cardiac xenografts in nonhuman primates continues to increase. GTKO graft rejection commonly involves vascular antibody deposition and variable complement deposition. Non-Gal antibody responses to porcine antigens associated with inflammation, complement, and hemostatic regulation and to new carbohydrate antigens have been identified. Their contribution to rejection remains under investigation. Orthotopic CXTx is limited by early perioperative cardiac xenograft dysfunction (PCXD). However, hearts affected by PCXD recover full cardiac function and orthotopic survival up to 2 months without rejection has been reported.

Summary—CXTx remains a promising technology for treating end-stage cardiac failure. Genetic modification of the donor and refinement of immunosuppressive regimens have extended heterotopic cardiac xenograft survival from minutes to in excess of 8 months.

Keywords

Organ transplantation; xenotransplantation; antibody-mediated rejection; α 1,3 galactosyltransferase gene-knockout; complement regulatory proteins; transgenic

Introduction

The chronic shortage of donor organs limits the full benefit of organ transplantation as a therapy for end-stage organ failure. This has spurred interest in alternative therapies including mechanical devices, regenerative medical approaches, and xenotransplantation. Left ventricular assist devices, used as a bridge to transplant or as destination therapy, are effective for patients with end-stage heart failure (1). Changes in device design and patient selection have improved survival, although infections and bleeding complications remain.

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Conflicts of interest

There are no conflicts of interest to report.

As destination therapy, mechanical devices may not provide an equal quality of life compared to organ transplantation. Regenerative medicine promises immunologically compatible organs produced from recipient progenitor cells. This approach has had good success for simple tissues (2), but regenerative medicine for heart transplantation remains at a nascent stage (3). Genetic modification of the porcine genome has transformed xenotransplantation (4). Survival of pig-to-primate cardiac xenotransplantation (CXTx) has increased from a few hours to a median of 3 months (5). If replicated using life-supporting orthotopic transplants, CXTx may warrant clinical testing. This review summarizes recent developments in heterotopic and orthotopic CXTx and highlight the role of non-Gal antibody-mediated endothelial cell (EC) activation as a primary effector of delayed xenograft rejection (DXR).

Elimination of the α Gal antigen

It has been nearly 10 years since the development of pigs with a targeted mutation in the α -galactosyltransferase gene (6, 7) (GGTA-1; GTKO pigs). This gene encodes the enzymatic function to produce the galactose α 1,3, galactose glycan (α Gal). Initially there was concern that residual levels of α Gal might persist in GTKO pigs. Residual α Gal expression was detected on cultured GTKO porcine fetal fibroblasts isolated after in vitro selection of GT^{+/-} cells (8) and a second α -galactosyltransferase, encoding the production of the rat isogloboside iGb3, was shown to induce α Gal expression in human cells (9). This suggested that GTKO pigs might still produce α Gal glycolipids. This is not the case. Cells from GTKO pigs fail to bind the α Gal specific lectin GSIB-4 or any of a series of anti-Gal monoclonal antibodies. ((10) and GWB personal comm.) Comparisons of neutral and acidic glycolipids from Gal-positive and GTKO pig organs found no evidence of α Gal in GTKO tissues (11) and molecular analysis failed to observe expression of the iGb3 α -galactosyltransferase in GTKO pig cells (12). Xenotransplantation of GTKO hearts has no effect on preformed anti-Gal antibody and does not stimulate an anti-Gal antibody response (13, **14). These findings indicate that the GTKO mutation has eliminated the α Gal antigen from pig tissues and marginalized any role of anti-Gal antibody in rejection of GTKO donor organs.

Heterotopic GTKO cardiac transplants

The initial pig-to-primate GTKO cardiac xenografts observed no hyperacute rejection and obtained a median cardiac xenograft survival of 78 days (13). Recipients in this study were strongly immunosuppressed and showed hyporesponsive cellular reactivity and no notable induction of non-Gal antibody. Despite effective immune regulation and use of recipients with minimal pretransplant non-Gal antibody, DXR of GTKO hearts showed vascular antibody and complement deposition along with platelet and fibrin rich microvascular thrombosis (MT). The extent of MT paralleled increases in antibody and complement deposition, EC expression of tissue factor and von Willebrand's factor and decreased expression of CD39 (15). We recently compared the histopathology of GT⁺;CD46 and GTKO;CD55 cardiac xenograft rejection.(14) Despite depletion of anti-Gal antibody for GT⁺ + graft recipients prior to transplant, both donor types show vascular antibody deposition within 30 minutes of reperfusion and in all subsequent interim biopsies. Complement activation occurred, as evidenced by C3d deposition. Vascular deposition of C5b and C5b-9 were minimal, consistent with the intrinsic regulatory function of CD46 or CD55. Early histopathology showed myocyte vacuolization, generally prior to significant levels of MT, followed by increasing levels of MT, ischemic injury, and myocardial coagulative necrosis. (Figure 1) These later histologic features paralleled decreased cardiac contractility and increased levels of serum troponin. Elevated expression of porcine ICAM-1, VCAM and tissue factor, consistent with EC activation, was observed at explant. This study and others using GT⁺ donors suggests that when early effects of anti-Gal antibody are blocked the

histopathology of DXR is the same in both GT+ and GTKO cardiac xenografts (16, 17). Together these studies strongly indicate that rejection of GTKO organs remains primarily an antibody-mediated process resultant to non-Gal antibody-mediated injury or activation of vascular ECs.

Non-Gal Antigens and Cardiac Xenograft Rejection

The natural antibody repertoire consists of germline encoded polyreactive antibody (IgM) produced in the absence of clear immune challenge (18, 19). Natural antibody also includes reactivity to α Gal and to blood groups A and B glycans which develop in response to intestinal microflora (20). Naïve human and nonhuman primate sera includes antibody reactivity to GTKO pig cells. In primates this is mainly IgM and in humans it is a mixture of IgG and IgM. Preformed non-Gal antibody is cytotoxic to GTKO peripheral blood mononuclear cells in most human sera and about 64% of baboon sera (21, 22). The level of non-Gal antibody and cytotoxicity is generally reduced compared to wild type pig cells and there is a wide range of individual variation.

The impact of preformed non-Gal antibody in CXTx is not evident using recipients with minimal antibody reactivity to GTKO pig cells. Recent studies using recipients with demonstrable preformed non-Gal antibody report early immune injury to GTKO hearts, including an instance of hyperacute rejection (23–25). GTKO cardiac xenograft survival of less than 1 day was observed in the absence of immunosuppression and early graft failure was reported in recipients with variant sub-therapeutic levels of immunosuppression (24, 25). Rejection was characterized by vascular antibody and complement (C4d) deposition, MT, and graft infiltration by neutrophils and macrophages. We reported hyperacute rejection of a GTKO cardiac xenograft in a recipient treated with a full regimen of immunosuppression (23). At explant, 90 minutes after reperfusion, the histology showed extensive intramyocardial hemorrhage, vascular IgM and C5b deposition. (Figure 2A–C) Despite elimination of α Gal antigen, these studies show that preformed non-Gal antibody is commonly present and can induce early graft injury. This suggests that methods to deplete or block preformed non-Gal antibodies may be needed to prevent early graft injury and GTKO xenograft rejection.

Human preformed non-Gal antibody includes antibody reactivity to terminally linked N-glycolylneuraminic acid (anti-Neu5Gc) (26). The Neu5Gc sialic acid modification is produced by hydroxylation of N-acetylneuraminic acid (Neu5Ac) through CMP-Neu5Ac hydroxylase (CMAH) (27). A mutation of the CMAH locus eliminates this pathway in humans, but not nonhuman primates. Anti-Neu5Gc antibody is estimated to constitute 7–13% of the preformed non-Gal human antibody repertoire (28). The potential for anti-Neu5Gc antibody to affect cardiac xenograft rejection remains unclear since they are not present in nonhuman primates, and binding of anti-Neu5Gc may be highly context dependent (27). The development of a targeted CMAH mutation in mice will be useful to define the potential of anti-Neu5Gc antibody to mediated organ rejection (29).

Several studies have attempted to identify non-Gal antigenic targets detected after CXTx. Yeh and colleagues (30) used sensitized baboon sera to screen a panel of 15 prospective glycan polyacrylamide conjugates. These neoconjugates included blood group A and B glycans, α Gal, α - and β -lactoasamine, Forssman disaccharide, sulphated derivatives of β -lactosamine, Neu5Gc, Neu5Ac, P1, Pk, and Lewis A, B and C glycans. GTKO sensitized baboon sera showed no specific induced immune response to any of these glycans. In contrast Diswall and colleagues (11) detected an induced antibody response to an undefined acidic glycolipid present in GTKO tissue. We have examined the specificity and diversity of induced non-Gal IgG using proteomic and expression library screening methods (23, **31).

These studies identified both protein and putative carbohydrate non-Gal antigens. (Table 1) Expression library screening identified a porcine glycosyl transferase homologous to human and murine β 1–4 N-acetylgalactosaminyl transferase 2 (B4GALNT2) (32, 33). Lectin and antibody binding suggests that human cells expressing the porcine B4GALNT2 homologue produces a CAD-like glycan(32), which may be consistent with the acidic glycolipid response reported by Diswall et al (11).

Our analysis suggests that the induced non-Gal antibody response is directed to a limited number of non-Gal antigens composed of members of the heat shock and annexin protein families, porcine complement and thromboregulatory proteins. Induced antibody responses to porcine fibronectin, the endothelial cell protein C receptor, CD46 and the B4GALNT2 produced glycan are frequently observed (23, **31). Antibody responses to these non-Gal antigens may contribute to xenograft rejection by blocking key EC functions which directly or indirectly contribute to MT. This may occur if non-Gal antibody blocks the function of annexin A2 (ANAX2) which regulates fibrin deposition (34), porcine CD46 and CD59 which control complement amplification, porcine GRP78 and ECPCR involved in regulating thrombosis and inflammation (35, 36) and CD9 involved in platelet activation (37). If this is the case then substitution of the human gene for these non-Gal targets could eliminate the induced antibody response and maintain key EC functions to promote graft survival. Likewise elimination of the glycan produced by the porcine B4GALNT2 homologue could further reduce the antigenicity of pig organs.

Orthotopic Cardiac Xenotransplantation

Pig-to-primate CXTx has mainly been studied using the abdominal heterotopic transplantation model. This is an immunological model as the xenograft is perfused and beating but does not contribute to circulation. Median heterotopic cardiac xenograft survival of 3 months has been achieved (5) with individual survival beyond 8 months (25). Replicating these results with life supporting orthotopic transplantation might justify future clinical applications (38). The number of reported orthotopic pig-to-primate cardiac transplants is limited (39–**46) with survival ranging from 1 to 57 days. In this difficult model recipient death was mainly from postoperative complications with several cases explanted between 9 and 57 days showing little histologic evidence of DXR. This suggests that the efficacy of life sustaining CXTx is limited by postoperative complications and not cardiac function.

These studies also identify a problem in orthotopic CXTx which was not apparent in heterotopic CXTx. Each research group reported variable perioperative mortality ranging from 40 to 60% within the first 48 hours. (Figure 3) Xenograft failure was not due to hyperacute rejection as the explanted hearts showed vascular antibody deposition but otherwise mainly normal myocardial histology. (Figure 2D–F) Early graft failure was mainly due to primary organ dysfunction. This primary organ dysfunction which we have called perioperative cardiac xenograft dysfunction (PCXD) is a significant barrier to the clinical application of CXTx.

PCXD may occur due to xenotransplantation-specific factors, such as the use of young donor organs, the inflammatory effects of preformed non-Gal antibody, and incompatibility between porcine and primate plasma including known dysfunctions in thromboregulation and may be further effected by pig-specific factors, most notably the sensitivity of the pig heart to cardiopulmonary bypass and ischemia-reperfusion (I/R) injury (47, 48). If this is the case then a combination of pretransplant immunoabsorption, cardiac preconditioning (49) or pharmacological treatments to improve cardiac resistance to I/R injury (50, 51) may modulate PCXD. Genetic modification to increase adenosine receptor (52) and CD39

expression (53) in the heart or to increase the number of collateral arteries (54) might also alleviate PCXD. It is important to note that PCXD is not an insurmountable barrier as 20 – 50% of current orthotopic xenografts recover from the initial effect. Over a period of 1 – 2 weeks these hearts recover full cardiac function and can go on to support the life of the primate for nearly 2 months.(43)

Recently there was the first report of pig-to-baboon heterotopic intrathoracic cardiac xenotransplantation (HTCXTx) (*55). The HTCXTx model is a load-bearing heterotopic transplant in which both the donor and recipient heart contribute to circulation. Reichart and colleagues demonstrated the feasibility of this complex surgical procedure and show good GTKO;CD46 porcine cardiac function for 45 days using clinical immunosuppression. Since circulation of the recipient is not fully dependent on xenograft function, the HTCXTx model may be an important model to study PCXD and to define diagnostic criteria and methods to treat and reverse DXR. HTCXTx may also be an alternative clinical application.

Gene Expression during Orthotopic Cardiac Xenotransplantation

We recently reported a genome wide scan of changes in porcine cardiac gene expression after orthotopic CXTx (**56). In cardiac xenografts subject to PCXD, DXR, or recovered from recipients which died of transplant complications in the absence of strong histologic evidence of rejection (Surviving grafts) we identified a set of 260 genes with widely variant changes in gene expression compared to control not transplanted pig hearts. This analysis showed that all orthotopic samples, including those from hearts not subject to DXR, showed evidence of inflammation and myocardial injury consistent with the effects of cytokines and antibody-mediated inflammation. There was also evidence of altered gene expression indicative of a cardioprotective response. Since surviving hearts were fully supporting the circulation of the recipient and not rejected by DXR despite vascular antibody deposition, this analysis suggests that for long periods of time there is a dynamic EC response which balances haemostasis against the prothrombotic effects of EC activation. DXR with MT and coagulative necrosis may be induced when this balance is pushed towards thrombosis. This might occur after an induced antibody response, when key EC functions are blocked, or as a result of species specific dysfunctions in coagulation.

Coagulation and Xenograft Rejection

Fibrin and platelet rich MT and myocardial coagulative necrosis are the prominent histologic features of GTKO cardiac xenograft rejection. Similar pathology is seen only in severe manifestations of antibody-mediated allograft rejection (57). It is unclear if this difference is the consequence of higher concentrations and greater diversity of non-Gal antibody, or due to known disparities in haemostatic regulation between pigs and humans (*58). Dysfunction of porcine thrombomodulin, and von Willebrand factor, spontaneous porcine EC dependent aggregation of human platelets, and loss of CD39 expression from activated ECs would all promote intragraft thrombosis (59–62). These disparities may also contribute to systemic derangement of coagulation parameters sometimes reported after xenotransplantation (63). Systemic therapies using clinical antiplatelet and antithrombotic drugs or using activated protein C fail to prevent MT, fibrin deposition, or prolong xenograft survival (64–68). This result may be due to the level of redundancy in haemostatic regulation, the intensity of non-Gal antibody-mediated rejection, or a failure to durably control haemostasis at the surface of the vascular EC. Numerous transgenic donor pigs designed to effect coagulation have recently been reported (69–75) but their ability to prevent MT, systemic coagulopathies or prolong graft survival remains to be tested.

Conclusion

GTKO organs have eliminated the role of anti-Gal in DXR but DXR remains an aggressive form of antibody-mediated rejection involving chronic non-Gal antibody induced EC activation and injury and possible species restricted dysfunctions in haemostasis. New methods to block the effects of preformed and induced non-Gal antibody may be required for durable xenograft survival. Orthotopic cardiac xenotransplantation has demonstrated the ability of the porcine heart to support life in the nonhuman primate model for up to 2 months; however the efficacy of this approach is limited by early xenograft dysfunction. If improvements in early cardiac xenograft function can be achieved then cardiac xenotransplantation has the potential to make a significant impact in future cardiovascular care.

Key Points

1. GTKO donor organs eliminate the role of anti-Gal antibody in xenograft rejection.
2. Cytotoxic non-Gal antibody is commonly present in nonhuman primates. Preformed non-Gal antibody contributes to early cardiac xenograft injury and is sufficient to induced hyperacute rejection at low frequency.
3. Survival of GTKO and GTKO;hCRP organs has improved, but organ rejection remains an antibody-mediated process with chronic vascular antibody deposition and variable complement activation.
4. Orthotopic cardiac xenotransplantation is limited by early perioperative cardiac xenograft dysfunction (PCXD), not observable in heterotopic xenotransplantation. The origin of PCXD may be related to porcine cardiac ischemic injury and the effects of preformed non-Gal antibody.
5. Further progress towards clinical use of cardiac xenotransplantation will require techniques to mitigate PCXD and new therapies to control both preformed and induced non-Gal antibody.

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Figure 1. Common histopathologic changes observed in DXR of GTKO organs. The illustration depicts the relative intensity and timing of major histologic features based on analysis of interim biopsies from Gal-positive and GTKO heterotopic cardiac xenografts (14). IgM, vascular antibody binding; MV, myocyte vacuolization; MT, microvascular thrombosis; CN, coagulative necrosis

Figure 2. Immunohistopathology comparison of GTKO hyperacute rejection after heterotopic cardiac xenotransplantation and perioperative cardiac xenograft dysfunction (PCXD) after orthotopic cardiac xenotransplantation. Hyperacute rejection (A – C). PCXD (D – F). Panels show hematoxylin and eosin staining (A and D), vascular IgM deposition (B and E) and vascular C5b deposition (C and F). Panels A – C (Byrne GW, McGregor CGA, et al, 2012 Transplantation, accepted pending revision)

Figure 3. Orthotopic cardiac xenograft survival. The graph summarizes the published results for pig-to-primate cardiac xenotransplantation based on recipient survival. Values are based on the data reported in references (39–46).

Table 1

Candidate Non-Gal antigens involved in Cardiac Xenograft rejection

Antigen	Function
Fibronectin	Extracellular matrix adhesion
MG-160	Golgi membrane
ORP150	Stress response
HSP70RY	Stress response
HSP90B1	Stress response
GRP75	Stress response
GRP78	Stress response
HSC70	Stress response
HSP60	Stress response
TCP1	Stress response
Annexin A6	Inflammation
Annexin A2	Inflammation
Annexin A1	Inflammation
Vimentin	Cytoskeleton
CD9	Platelet and EC activation
CD46	Complement regulation
CD59	Complement regulation
PROCR	Haemostasis
B4GALNT2	Glycosylation