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Review

Recent investigations into pig antigen and anti-pig antibody expression

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HIGHLIGHTS

- Gal-free GTKO pigs have significantly altered xenograft rejection which is now directed to non-Gal antigens.
- Data on human non-Gal antibody responses to pig tissue is rare, but, shows variable induction of anti-Neu5Gc antibody.
- Nonhuman primates do not make anti-Neu5Gc antibody so the pathogenicity of this specificity remains unknown.
- Nonhuman primates induce antibody to pig EC proteins and an Sd^a-like glycan made by porcine B4GALNT2.

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ABSTRACT

Genetic engineering of donor pigs to eliminate expression of the dominant xenogeneic antigen galactose α1,3 galactose (Gal) has created a sea change in the immunobiology of xenograft rejection. Antibody mediated xenograft rejection of GGTA-1 α-galactosyltransferase (GTKO) deficient organs is now directed to a combination of non-Gal pig protein and carbohydrate antigens. Glycan analysis of GTKO tissues identified no new neo-antigens but detected high levels of N-acetylneuraminic acid (Neu5Gc) modified glycoproteins and glycolipids. Humans produce anti-Neu5Gc antibody and in very limited clinical studies sometimes show an induced anti-Neu5Gc antibody response after challenge with pig tissue. The pathogenicity of anti-Neu5Gc antibody in xenotransplantation is not clear however as non-human transplant models, critical for modelling anti-Gal immunity, do not produce anti-Neu5Gc antibody. Antibody induced after xenotransplantation in non-human primates is directed to an array of pig endothelial cells proteins and to a glycan produced by the pig B4GALNT2 gene. We anticipate that immune suppression will significantly affect the T-cell dependent and independent specificity of an induced antibody response and that donor pigs deficient in synthesis of multiple xenogeneic glycans will be important to future studies.

Contents

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1. Introduction

Xenotransplantation using pig organs has in recent years made significant advances in vascularized graft survival with median pig-to-baboon heterotopic cardiac xenograft survival beyond 6 months and individual survival in excess of 1 year [1]. Increased cardiac xenograft survival is based on progressive improvements in genetically engineered donor organs and improvements in chronic immune suppression [2]. Antibody mediated rejection (AMR) is the predominant form of vascularized xenograft rejection in which terminal galactose α1,3 galactose (Gal) saccharide is the dominant xenogeneic antigen. Humans and Old World non-human primates (NHP) do not make Gal but instead produce high levels of anti-Gal antibody [3] resulting in hyperacute rejection (HAR). HAR can be prevented by depletion or blocking anti-Gal antibody prior to transplant [4,5]. Pigs were engineered with a mutation in the GGTA-1 alpha-galactosyltransferase gene (GalT-KO pigs) to eliminate the Gal antigen. Extensive biochemical studies of GalT-KO porcine glycolipids and glycoproteins [6–9], the loss of tolerance and spontaneous expression of anti-Gal antibody in GalT-KO pigs [10], and the absence of an induced anti-Gal antibody response after GalT-KO organ xenotransplantation [11] all support the full elimination of the Gal antigen from GalT-KO pigs.

The advent of GalT-KO pigs did not completely eliminate AMR but instead revealed the significance of a less abundant and more diverse set of antibody which mediates GalT-KO xenograft rejection by binding to “non-Gal” pig antigens. This review summarizes our current understanding of non-Gal antibodies (NGal-Ab) and antigens in NHP, the major xenotransplantation model, and in humans.

2. Non-Gal antibody and antigen: definition

NGal-Ab has been defined based on the technologies available at the time. Lam et al. [12] first identified a pathogenic role for NGal-Ab by correlating the emergence of non-Gal IgM and IgG with humoral cardiac xenograft rejection. Their analysis identified NGal-Ab by immunoabsorbing serum using Gal-coated Sepharose beads. Prior to the availability of GalT-KO pigs similar strategies of immune absorption, soluble Gal competition, or antigen depletion were commonly used to measure serum NGal-Ab [13–15]. These studies were unable to fully eliminate the possibility of residual anti-Gal reactivity, however, their observations accurately presaged the role of NGal-Ab mediated graft rejection confirmed in later GalT-KO donor organ studies [11,16–18]. For this review NGal-Ab is defined as human and NHP antibody which binds to GalT-KO pig cells [19].

3. Preformed non-Gal antibody: abundance and pathogenicity

Cytotoxic NGal-Ab is present in both human and NHP serum. Rood et al. [20] surveyed human, baboon and cynomologus monkey serum for antibody binding and cytotoxicity to conventional Gal-positive ($GalT^+$) and GalT-KO pig peripheral blood mononuclear cells (PBMNCs) and showed approximately 50% of human and baboon serum samples and 75% of cynomologus monkey serum exhibited significant cytotoxicity to GalT-KO PBMNCs. NGal-Ab cytotoxicity to porcine aortic endothelial (PAEC) and liver sinusoidal endothelial cells (LSEC) has also been reported [21,22].

In NHPs pre-existing NGal-Ab is clearly pathogenic. In a comparison of GalT-KO and GalT-KO:CD55 donor organs Byrne et al. [23] reported a case of HAR for a GalT-KO pig-to-baboon heterotopic cardiac xenograft. Rejection occurred after 90 min with widespread intramyocardial haemorrhage, vascular antibody and complement deposition. While HAR of GalT-KO organs is rare, early immune injury has been reported [24,25] and interim biopsies 7 days post transplant detect vascular antibody and complement deposition presaging myocardial injury [17].

The very limited number of clinical xeno-studies, performed several years ago, have all used $GalT^+$ pig kidneys [26,27], livers [28–30] or porcine hepatocytes [31]. Therefore, no information regarding the contribution of NGal-Ab to the extensive tissue injury is available.

4. Baboon non-Gal antibodies

Two general approaches have been used to identify potential non-Gal antigens, profiling serum antibody reactivity to identify immunoreactive porcine antigens and biochemical studies comparing the antigenic profile of GalT-KO porcine and human tissues. Biochemical studies have largely focused on identifying porcine specific carbohydrate antigens.

Sensitized baboon sera, obtained after xenotransplantation of various pig $GalT^+$ or GalT-KO organs, has been used to profile the specificity of NGal-Ab [6,15,25,32–34]. An early study used Gal-specific immune absorption to measure NGal-Ab binding to $GalT^+$ pig red blood cells (RBCs). The induced NGal-Ab level was about 4% the level of induced anti-Gal antibody and non-Gal binding to RBCs was not diminished after treating cells with α-galactosidase or other exoglycosidases. This suggested that the NGal-Ab was mainly directed to pig proteins. Yeh et al. [32]. used an ELISA assay to measure antibody reactivity to a series of neo-glycoconjugates representing suspected glycan antigens (Forssman, A/B-blood group tri-saccharides, Lewis antigens, P1, Pk, Gal, α and β lactosamine and sulphated lactosamine). Naïve human and baboon serum reacted primarily with blood group A/B antigens, Gal, α-lactosamine, Forssman, P1 and Pk antigens. Highly sensitized baboon serum showed significantly higher IgG binding to GalT-KO cells, but did not show increased reactivity to any of the neo-glycoconjugates. These results demonstrated that some prospective glycans (Forssman, and lactosamine) were unlikely NGal-Ab targets and further supported a predominantly protein directed NGal-Ab response in baboons.

A glycan reactive NGal-Ab response was initially reported by Diswall et al. [6] after GalT-KO cardiac xenotransplantation. Post transplant baboon serum showed increased binding to a trace, acidic glycolipid extracted from GalT-KO heart tissue. In a recent