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Xenotransplantation: Role of natural immunity☆

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ABSTRACT

Hyperacute rejection, mediated by natural anti-Galα1,3Galβ1,4GlcNAc (αGal) antibodies and the classically activated complement pathway, was identified as the first major barrier to the survival of porcine organs in humans. Subsequently, discordant pig-to-nonhuman primate and concordant rodent models revealed key roles for T and B lymphocytes in the second form of rejection, *acute vascular rejection* (AVR) or *delayed xenograft rejection* (DXR). As significant progress was made in strategies to circumvent or suppress xenoreactivity of the adaptive immune system, it became clear that, apart from natural antibodies, other innate immune system elements actively participate in AVR/DXR and represent a barrier to xenograft acceptance that may be particularly difficult to overcome. Observations in pig-to-primate and semi-discordant and concordant rodent models indicate that Natural Killer (NK) cells play a more prominent role in xenograft than in allograft rejection. Several mechanisms through which human NK cells recognize porcine endothelial cells have been elucidated and these appear to be more diverse than those involved in NK cell alloreactivity. Further, it has been demonstrated that human macrophages and neutrophils can directly recognize pig derived cells and can mediate direct xenograft damage. Here, we review the recent progress in the understanding of the xenoreactivity of the natural immune system, focussing on preclinical pig-to-(non) human primate systems, and discuss the proposed strategies to overcome these barriers.

1. Introduction

Hyperacute rejection (HAR), which leads to graft rejection within minutes, was identified as the first barrier to pig-to-human xenografting. Mediated by pre-existing anti-αGal natural antibodies (NAb) and the classical complement pathway, HAR is regarded as a barrier of the natural immune system. Subsequently, concordant rodent and discordant pig-to-nonhuman primate models revealed key roles for T and B lymphocytes in the humoral and cellular components of *acute vascular rejection* (AVR), the second form of xenograft rejection that typically occurs within days (reviewed in [1,2]). As experimental approaches became increasingly successful in suppressing or eliminating the barriers of the adaptive immune system, it appeared that several components of the innate immune system contribute to the rejection of concordant and discordant xenografts. In addition to the role of αGal NAb, roles have now been revealed for natural killer (NK) cells, macrophages and neutrophils in pig-to-human xenograft rejection.

2. Natural antibodies and the complement system

Naturally occurring antibodies (NAb) are produced early in life and constitute life-long high titers of circulating antibodies. The majority of complement-fixing xenoreactive Nab in humans are directed against αGal, a carbohydrate residue synthesized by α1,3Galactosyltransferase (αGalT) on cell-surface glycoproteins and glycolipids in lower mammals [3]. The αGal epitope is absent in humans, apes and Old World monkeys, as they lack the αGalT enzyme, and anti-αGal antibodies arise in the first months of life upon encounter of αGal-expressing microorganisms in the gastrointestinal tract [4]. When anti-αGal Nab bind to αGal on pig endothelial cells (pEC), this leads to HAR of the graft through engagement of the classical complement pathway and the coagulation system.

The key role of anti-αGal NAb and complement in discordant xenograft rejection became evident from the observations that anti-αGal NAb depletion and/or inhibition of the complement system can prevent the occurrence of HAR in discordant pig-to-non-human primate (NHP) models [5,6]. As a preventive approach to target the complement pathway, pigs expressing transgenes for human complement regulators, e.g. CD46 (membrane cofactor protein, MCP), CD55 (human decay-accelerating factor, hDAF), and CD59 (membrane-attack complex inhibitor or protectin) and double- and triple-transgenic pigs, combining expression of CD55, CD59 and α-1,2-fucosyltransferase were developed [7,8]. Several groups have shown that the use of these transgene pig organs in NHP can prevent HAR, and can extend xenograft survival [7–9].

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Studies with hDAF-transgenic pig organs in NHP have shown that, whereas local complement activation can be controlled, fluid-phase complement activation still significantly contributes to antibody-mediated AVR [10]. Complement inhibition in these models, using C1-inhibitor or soluble complement receptor-type 1, combined with immunosuppression resulted in prevention of AVR [11,12]. More recent studies have shown that dextran sulfate, which inhibits the classical and alternative complement pathway, can delay HAR in ex vivo perfused pig lungs [13,14]. Temporary complement depletion by cobra venom factor has been shown in concordant rodent models to prevent AVR and to induce expression of protective genes in the xeno-endothelium, allowing for accommodation [15]. Similarly, Laumonier et al. showed that dextran sulfate binds to pEC and thus protects the endothelium from human complement-mediated lysis [16].

With the development of $\alpha GalT^{-/-}$ pigs, donor organs became available in which αGal expression was completely eliminated [17], and in preclinical baboon models, HAR of $\alpha GalT^{-/-}$ pig kidneys or hearts could also efficiently be prevented [18,19]. Recent studies however indicate that human naïve sera contain significant titers of preformed anti-porcine but anti-non-αGal IgM and IgG antibodies, capable of inducing complement lysis or antibody dependent cellular cytotoxicity (ADCC) of $\alpha GalT^{-/-}$ pig cells [20]. As these may represent a relevant barrier to grafting of $\alpha GalT^{-/-}$ pig organs, studies are directed at identifying the target xenoantigens, and Saethre have shown that anti-HD antibodies make up a considerable proportion of anti-non-αGal Xab in human sera [21]. Also, data from $\alpha GalT^{-/-}$ rodents indicate that isoglobotriaosylceramide-3 synthase (iGb3s), an alternative galactosyltransferase, can give rise to low level αGal expression [22]. However, although iGB3s mRNA has been detected in selected porcine tissues [23], residual αGal expression has so far not been documented in pEC derived from $\alpha GalT^{-/-}$ pigs [20]. Finally, *N*-acetyllactosamine is a carbohydrate that in $\alpha GalT^{-/-}$ mice is higher expressed than in WT mice [24], but its role in antibody-mediated porcine xenograft rejection is so far unclear [25].

3. Natural killer cells

The role of NK cells in rejection of hematopoietic stem cell allografts is well established in mice, and the role of KIR-ligand mismatches—in particular HLA-C mismatches—has recently gained attention in HLA-mismatched allogeneic stem cell transplantation, in particular with respect to graft rejection, graft-versus-host disease and graft-versus-leukemia effects [26]. The contribution of NK cells to solid organ allograft rejection is increasingly being acknowledged [27]. Recent evidence indicates that NK cells play an even more prominent role in rejection of xenografts, and that the mechanisms through which they recognize xenogeneic cells are more diverse than those involved in NK cell alloreactivity. Evidence that NK cells contribute to xenograft failure is derived from the observations that human NK (huNK) cells can lyse pEC in vitro [28–31], that NK cells could be documented in rejected xenografts in rodent [32–34] and pig-to-nonhuman primate models [35,36], and that NK cell infiltrates are present in pig organs perfused with human blood [37,38]. Using a depletion approach in fibrinogen-depleted rats, Chen et al. directly showed that NK cells can reject mouse hearts [34].

Part of the interaction between huNK cells and pEC is based on the molecular incompatibility between porcine MHC molecules and MHC INK cell receptors, while another part is based on a cross-species interaction between activating NK cell receptors and pEC-associated ligands, and between pEC- and huNK-associated adhesion molecules As demonstrated by gene sequence analysis, most of the residues critical for human KIR recognition are absent in the swine leucotye antigen (SLA) class-I genes, indicating that SLA-I fail to establish KIR signaling [39]. However, some studies have delivered evidence that SLA-I can provide some negative signaling through huKIR [40,41]. In addition, cytolysis of pig cells can be triggered by ligation of activating NK receptors. HuNK cytotoxicity against porcine cells is triggered by the C-type lectin receptor NKG2D and the natural cytotoxicity receptor NKp44 [42]. Porcine ULBP-1, expressed on pEC has been identified as the ligand of NKG2D, whereas that of NKp44 is as yet unknown [42,43]. Also, it has recently been demonstrated on mouse cells that the C-type lectin receptor NKPR1A directly recognizes αGal, and following enzymatic removal of αGal on pig cells, also binds to *N*-acetyllactosamine, the expression of which is increased in $\alpha GalT^{-/-}$ pig cells [44]. This confirms earlier reports demonstrating reactivity of huNK cells to αGal containing ligands [45,46], but is in apparent contradiction with other studies [47–49]. The role of direct αGal recognition by huNK cells in xenogeneic interactions, and with N-acetyllactosamine in $\alpha Gal^{-/-}$ animals is therefore as yet not clear.

NK cells may contribute to xenograft injury through mechanisms other than direct cytotoxic lysis. NK cells are potent cytokine producers, and upon direct interaction, huNK cells activate pEC with induction of adhesion molecules, promoting invasion of immune cells, transformation from an anti- to a procoagulant state and enhanced cytokine secretion such as TNF-α and IFN-γ [28,50,51]. These effects are enhanced in the presence of IgG XAb, as a result of NK cell FcγRIII cross-linking [28,50,51]. Fc crosslinking also gives rise to ADCC and enhanced direct killing [28,52]. In particular, Yin et al. demonstrated in a concordant rodent model that NK cells play a critical role in the HAR response initiated by moderate complement fixing IgG1 xenoantibodies [52,53].

In addition to the direct interaction of NK cells with the vascular endothelium giving rise to endothelial damage, NK cells transmigrate into the graft provided cross-species interaction between NK and pEC associated adhesion molecules can take place. As has been shown for huT cells [54] selected adhesion receptor–ligand interactions have been shown to be functional between huNK cells and pEC, supporting early observations that huNK cells readily adhere to pEC [55]. In vitro adhesion studies have demonstrated roles for CD2, CD11a (LFA-1 integrin-[alpha]L chain), CD11b (Mac-1 integrin-[alpha]M), CD18 (integrin-[beta]2), and CD49d (VLA-4 integrin-[alpha]4) on huNK cells and CD106 on pEC (reviewed in [56])[57]. In addition, in studies addressing the effect of artificial HLA expression by pEC, it has been shown that the KIR2DL1 and ILT2 receptors regulate NK cell adhesion through interaction with HLA-Cw4 and HLA-G [58,59].

Finally, NK cells can contribute to xenograft rejection indirectly, through interaction with the adaptive xenoreactive immune response. Xu et al. have demonstrated in vitro that activated huNK cells can augment huT cell xenoreactivity [60], and Li et al. have demonstrated in a concordant rodent model of xenohearttransplantation that NK cells provide help in the rapidly induced T-independent xenoantibody response [61].

It is clear that interaction between huNK cells and pig endothelium consists of a variety of activating or failing inhibitory interactions, and control of NK xenoreactivity may therefore prove difficult. With this respect, it has been shown that pEC derived from $\alpha GalT^{-/-}$ pigs were more resistant to αGalXab-dependent ADCC, but equally susceptible to hu leucocyte adhesion and huNK cell cytotoxicity [47,48,62], and that resistance to $\alpha GalT^{-/-}$ cells could be readily overcome in TNF-α activated EC or IL-2-activated NK cells [63]. Strategies that aim at inhibiting huNK xenoreactivity can target either the absence of appropriate KIR-signaling, the cross-species recognition of pEC ligands by activating NK cell receptors, or NK cell adhesion molecules. Selected promising approaches have recently been reported on. First, several groups have shown in vitro that artificial expression of hu MHC I molecules in pig cells, including HLA-B27, HLA-CW3, HLA-Cw4, HLA-G and HLA-E can reduce huNK cytotoxicity towards pig target cells [59,64,65]. Specifically, it has been shown that construction of an HLA-E single chain trimer, consisting of the mature HLA-E heavy chain, the mature human B2-microglobulin, and—most importantly—an HLA-E-binding peptide, ensures stable expression in porcine cells and provides partial protection against huNK cytotoxicity [66,67]. For