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It is well-documented that Galα1-3Gal (Gal) epitopes play a major role in the development of graft rejection after pig-to-primate xenotransplantation (discussed elsewhere in this issue). Now that cloning technology in pigs has been established, the production of knockout pigs for α1,3galactosyltransferase, which is responsible for Gal expression, is likely to be realized. If the problems related to Gal could be completely resolved, the next subject for investigation would be the immunological response to other (non-Gal) epitopes. Are natural non-Gal epitopes involved in hyper-acute rejection or acute vascular rejection? If not, can antibody against non-Gal epitopes be elicited after the transplantation of a pig organ?

What Non-Gal Epitopes Can Be Bound By Human Natural Xenoreactive Antibodies?

This question has been discussed previously,[1] and various candidates are listed in Table 1. However, no conclusive results have been reported, although the character of human natural antibodies directed against non-Gal epitopes expressed on pig red blood cells has recently been clarified by Zhu.[2]

Zhu has suggested that non-Gal antigen-antibody interactions can contribute to complement-mediated hemolysis, but that neither terminally α- or β-linked Gal residues, nor GalNAc residues, are involved in antibody binding. Furthermore, a novel protein of 45 kDa was isolated as a major protein antigen recognized by human anti-non-Gal antibodies. Zhu also demonstrated that human anti-non-Gal antibodies had approximately only one-tenth the cytotoxicity of anti-Gal antibodies. Although these observations are of considerable interest, a similar examination of pig endothelial cells will be required, as the nature of the epitopes on endothelial cells may differ from those on red blood cells.

To date, however, no porcine non-Gal epitopes that are clearly and consistently bound by human or nonhuman primate xenoreactive antibodies have been determined.

Will Non-Gal Epitopes Be Involved in Hyperacute or Acute Vascular Rejection?

When Gal-knockout mice became available,[3] it became evident that elimination of Gal epitopes resulted in only approximately 60% reduction in the binding of human xenoreactive antibodies. Gal-knockout mouse cells still remained susceptible to antibody-mediated rejection. Further studies using αgalactosidase-treated porcine endothelial cells or primate sera after removal of anti-Gal antibodies (by immunoadsorption using Gal oligosaccharide immunoaffinity columns) have been carried out in an effort to detect the effects of anti-non-Gal antibody.

It has been estimated that >85% or >90% of human natural anti-pig antibodies are directed against Gal epitopes.[4,5] In contrast to anti-Gal antibodies that have a high affinity and cytotoxicity, anti-non-Gal antibodies do not appear to cause hyperacute rejection, but might be associated with the development of antibody-dependent cell-mediated cytotoxicity (ADCC) or acute vascular rejection following pig-to-primate organ transplantation. In contrast, another report indicated that anti-non-Gal antibodies were involved in hyperacute rejection, particularly of pig lungs.[6] The contribution of anti-non-Gal antibodies to rejection might be different from organ to organ.

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Table 1 **Non-Gal Carbohydrate Antigens Against which Human Xenoreactive Antibodies can be Directeda**

1.	A: GalNAcα1-3(Fuc α1-2)Galβ1-4GlcNAcβ-R[b]
2.	B: Galα1-3(Fucα1-2)Galβ1-4GlcNAcβ-R
3.	Thomsen-Friedenreich (T or TF) Galβ1-3GalNAcα1-R
4.	Tn (TF precursor) GalNAcα-R
5.	Sialosyl-Tn: NeuAcα2-6GalNAcα1-R
6.	P[k]: Galα1-4Galβ1-4Glcβ1-R
7.	Other P antigens
8.	Sulfatide: SO_4-3Gal-R
9.	Forssman: GalNAcα1-3GalNAcβ1-3Galα1-4Galβ1-4Glcβ1-R
10.	i: Galβ1-4GlcNAcβ1-3Galβ1-4GlcNAcβ-R[c]
11.	I: Galβ1-4GlcNAcβ1-3(Galβ1-4Glcβ1-6)Galβ1-4GlcNAcβ-R[c]
12.	αRhamnose-containing oligosaccharides L-Rhm-α-Rhm L-Rhm-α1-3GlcNAcβ1-2L-Rhm-α-R
13.	βGlcNAc-containing oligosaccharides GlcNAcβ-R GlcNAcβ1-4GlcNAcβ-R
14.	Galα1-3Lewis[x]: Galα1-3Galα1-4(Fucα1-3)GlcNAcβ1-3Galβ1-4Glcβ1-R
15.	Hanganutziu-Deicher: N-Glycolylneuraminic acid (NeuGc)

aModified from data collected by Cooper (ref. 1). bR are glycolipid or glycoprotein carrier molecules anchored in the cell membrane.
cThe core structures of the ABH antigen system, which are fucosylated by H transferase to generate H substance.

Even if the effects of natural anti-non-Gal antibodies could be ignored, newly-synthesized anti-non-Gal antibodies may prove to be a problem.

In our previous study comparing the efficacy of antibody removal in pig-to-baboon renal xenotransplantation and in clinical ABO-incompatible renal allotransplantation,[7] pig organs that underwent acute vascular rejection showed immunoglobulin deposition as early as one hour after transplantation, while ABO-incompatible allografts survived long-term without immunoglobulin deposition. For xenotransplantation to be successful, therefore, antibody binding to the graft would need to be maintained at an undetectable level. In contrast, for the development of rejection, binding of anti-non-Gal antibodies to the graft will be essential. With regard to antibody binding, it is sometimes difficult to predict what might happen in vivo from in vitro tests, because some antibodies bind to porcine cells but not to an organ xenograft.[8]

Will the Immunogenicity of Non-Gal Epitopes be Significant?

Even if the effects of natural anti-non-Gal antibodies could be ignored, newly-synthesized anti-non-Gal antibodies may prove to be a problem. In pig-to-nonhuman primate experimental models, antibody production against non-Gal epitopes has been reported to be inhibited by pharmacologic immunosuppression[9] or, particularly, by an anti-CD40L monoclonal antibody.[4,10] Compared with Gal epitopes, which elicit a potent immune response,[11] the response to non-Gal epitopes may therefore be more readily controlled.

For example, Hanganutziu-Deicher (H-D) antigens (with N-glycolylneuraminic acid),[12] are widely expressed on endothelial cells of mammalian species with the exception of humans, and are expressed at a similar level to Gal epitopes in porcine aortic endothelial cells. As baboons and monkeys also express H-D antigens, a pig-to-nonhuman primate experimental model cannot clarify the immunogenicity of H-D antigens. Therefore, we examined the anti-H-D antibody level in the sera from patients who had been previously exposed to porcine tissue. No significant elevation of IgG or IgM against H-D antigens was observed in these patients, suggesting that the immunogenicity of H-D antigens is not as great as that of the Gal antigens.

Are Anti-HLA Antibodies Reactive against Swine Leukocyte Antigens?

Although anti-HLA antibodies are not related to natural anti-pig or anti-Gal antibodies,[13,14] some anti-HLA antibodies seem to be reactive to swine

leukocyte antigens.[15-17] This is an important question that has to be answered, as highly HLA-sensitized patients may be candidates for xenotransplantation. (This topic is discussed elsewhere in this issue.)

Comment

Pretreatment by antibody removal and immunosuppressive therapy may be necessary to inhibit the immunological response to non-Gal epitopes, although it is unclear how effective these strategies will be. Until Gal-knockout pigs are available, it may not be possible to reach a definitive conclusion on whether non-Gal epitopes, including cryptic epitopes that may only become immunogenic when Gal has been removed from pig tissues, can cause antibody-mediated rejection. However, at this stage, it is important to explore the potential problem of non-Gal antigens in order to develop further genetic engineering approaches to xenotransplantation. The first step is, of course, to continue our search for the targets against which these antibodies are directed. To date, the nature of their targets, or even whether these are carbohydrates or proteins, remains uncertain.

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