Alternative Approaches to Reducing Gal Expression in Pig Organs

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The major xenoantigen in the pig-to-human combination is the galactose-α1,3-galactose (Gal) epitope[1] which is abundantly expressed on pig endothelium. It has a pivotal role in hyperacute rejection and is likely to be involved in acute vascular rejection. Deletion of the Gal epitope from pigs may not be possible for several reasons.[2] Firstly, the process of gene targeting to 'knock out' (KO) genes using embryonic stem (ES) cells, perfected in the mouse has not been translated to the pig because pig ES cells have proved difficult to isolate. Secondly, gene targeting using differentiated cells requires nuclear transfer technology to produce the modified animal. This 'cloning' technology is currently inefficient and its utilization with gene-targeted pig cells has not been demonstrated. Thirdly, Gal may play an essential role in the pig, given the much higher expression in this species compared to lower order animals such as mice, and its deletion may therefore be lethal. For this reason, transgenic strategies that reduce but do not necessarily eliminate Gal expression may be the only avenue to reducing graft antigenicity.

Competitive Glycosyltransferases

The Gal epitope is synthesized by the enzyme α1,3-galactosyltransferase (α1,3-GT) in the Golgi apparatus.[3] The substrate for α1,3-GT is N-acetyllactosamine which can also be utilized by other glycosyltransferases. It was first suggested by Sandrin[4] that expression of these enzymes could divert substrate from α1,3-GT in a competitive manner leading to a reduction in Gal expression and the production of less immunogenic substances on the cell surface. Examples of these 'competitive' enzymes are α1,2-fucosyltransferase, α1,3-fucosyltransferase, α2,3-sialyltransferase and α2,6-sialyltransferase, which have been shown to reduce Gal expression in transfected pig cell lines.

The human α1,2-fucosyltransferase (α1,2FT, H-transferase) is illustrative of this approach. The product of α1,2FT is human blood group H antigen which is universally tolerated. Pig cell lines transfected with the α1,2FT gene have been shown to exhibit reduced Gal expression and increased resistance to lysis by human serum compared to non-transfected cells.[5] Furthermore, α1,2FT transgenic mice exhibit high levels of H substance and a reduction in Gal expression of approximately 70% and confirming the feasibility of the competitive approach.[6] Hearts from these mice were more resistant than WT hearts to human serum in functional ex vivo perfusion studies but interestingly binding of human antibodies was not completely eliminated. These antibodies were presumably directed against residual Gal, non-Gal xenoantigens, and new 'xenoantigens' uncovered or generated by the genetic modification.[7] As Gal expression in pigs is much higher than in mice, reduction in Gal will probably be the most important determinant in reducing xenoantigenicity of pig organ grafts in humans particularly given the role of Gal in hyperacute rejection. The long term effects of the minor xenoantigens remain unknown.

Other Enzymic Interventions

N-acetylglucosaminyltransferase III (GnT-III) is another enzyme shown to reduce Gal expression in transfected pig endothelial cells.[3] The enzyme increases production of bisecting N-acetylglucosamine residue which in turn attaches and inhibits further processing of oligosaccharides by

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glycosyltransferases. α-galactosidase is an enzyme that can be over expressed on the cell membrane to promote the breakdown of the Gal epitope.[8] The appeal of this enzyme is that it could potentially complement the competitive strategies by eliminating the inevitable residual Gal expression.

Novel Approaches to Reduce Gal Expression

Vanhove has demonstrated that pig cells transfected with recombinant single-chain Fv (ScFv) antibodies directed against pig α1,3-GT can reduce its activity by 70%, reduce Gal expression by the same extent and reduce cell cytotoxicity to anti-Gal antibodies and complement by 90%.[9,10] Another alternative to reducing α1,3-GT activity is to decrease expression at the mRNA level by a dominant negative mutant enzyme or antisense oligonuleotides.

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The Ideal 'Carbohydrate-Remodeled' Pig

α1,2FT transgenic pigs have been reported by several groups but the degree of Gal reduction appears to be much less efficient than in transgenic mice and there have been no functional in vivo studies demonstrating reduction in xenoantigenicity.[11,12] It is possible that a single competitive enzyme will be insufficient to reduce Gal expression in the pig and some investigators have proposed transgenic expression of a combination of enzymes to achieve this. Several groups have demonstrated that co-transfection of pig endothelial cells with various combinations of these enzymes can incrementally improve Gal reduction with cytotoxicity assays with human serum showing similar trends.[3]

There is uncertainty whether a high degree of carbohydrate antigen modification of the cell surface will be deleterious to embryogenesis and cell differentiation. There is increasing evidence that some specific glycosyltransferase activities are involved in the regulation of the formation of tissue boundaries in development.[13,14] It is therefore quite conceivable that the alterations in the balance or these activities by transgenic overexpression of multiple 'Gal reducing' enzymes will be embryonically lethal. There has also been a report of colonic adenocarcinoma in one of three pigs with α1,2FT expression from one group. Therefore, the ideal combination of transgenically expressed enzymes will have the maximum reduction of Gal with minimum effect on cell development and function.

Conclusions

It is conceivable that the generation of a Gal KO pig will never be possible. As Gal expression in pig organs is the major component of their xenoantigenicity in humans, reduction of Gal by the alternative means outline above may become the most important genetic modification to produce the 'ideal' pig organ in this setting. However, none of these strategies alone have succeeded in completely eliminating Gal and a combination of transgenes will probably be required to reduce the epitope sufficiently while preserving cell integrity in the process.

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