EDITORIAL

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Xenotransplantation 2.0

Will targeted immunosuppressants and new tools in genome engineering be enough to finally give xenopigs wings?

The idea of making pigs into human organ factories has been slow to take flight. Despite steady scientific progress, xenotransplantation has suffered years of neglect from funding agencies. The feasibility of the approach has been called into question, given the daunting challenge of engineering pigs to elude the gamut of host rejection mechanisms. The work is expensive and laborious and requires specialized facilities and expertise. Little in the experimental experience of the past two decades counters doubters: there are few successful examples of long-term organ xenografts in nonhuman primates (beyond a few months), while in humans even the simplest cellular xenografts (such as pig islets or dopaminergic neurons) usually are rejected or fail to function.

And yet, pigs may still fly as human organ donors. An expanded set of gene synthesis and genome engineering tools has opened new avenues for research. In recent weeks, a gene editing *tour de force* has addressed one nagging concern—the potential for cross-species transmission of infections to human recipients. Furthermore, the drugs available to suppress the human immune system and address graft rejection are no longer few and blunt but increasingly diverse and targeted. Commercial interest and private investment are growing, with established companies quietly bankrolling xenotransplantation programs and new ventures being founded.

Few would dispute the need for new transplantation options. In the United States alone, >124,000 patients are on waiting lists for organ transplantation, and yet only 28,000 transplants from human cadavers are carried out every year. The problem may in fact be much bigger. Transplant surgeons have relaxed criteria for marginal donors and increased stringency for recipients in response to the shortage: some estimates put the total number of US patients in need of transplants at one million.

Pigs have been seen as a potential source of transplant organs because their physiology is similar to that of humans and their organs are comparable in size. They are inexpensive to keep, are easy to breed, have large litters, reproduce quickly and can be reared in pathogen-free conditions. But farming is not medicine. Humanized organs require genome engineering—relatively facile in mice but far from straightforward in pigs: porcine embryos are notoriously fragile to manipulate, and porcine embryonic stem cells have yet to be isolated.

Research over the past two decades has clarified the immunological barriers to xenotransplantation. These include, within hours, hyperacute rejection (mediated mainly by complement via antibodies against α1,3Gal), acute vascular rejection (which occurs within days and is mediated by antibodies against α1,3Gal and other antigens, activated complement and coagulation systems, and innate immune cells) and, in the following weeks, chronic xenograft rejection (mediated by macrophages and $CD4^+$ T cells).

Armed with this knowledge, two main lines of attack to thwart rejection have emerged; one targets the organ recipient, the other the animal donor.

In the recipient, *ex vivo* hemoperfusion can deplete xenoreactive antibodies or complement components from blood; alternatively, xenogeneic bone marrow cell transplants (mixed chimerism) can help induce B cell tolerance. But most current interest is focusing on new targeted immunosuppressants, such as T cell co-stimulation blockers (for example, anti-CD40 antibodies and anti-CTLA4–Fc fusions) or agents that sequester cells in lymph nodes (for example, fingolimod). These targeted agents prolong graft survival more effectively than standard pharmacological immunosuppressants such as tacrolimus or cyclosporine.

In donor animals, over 40 modifications have now been carried out, including reduction of α1,3Gal (via knockout of α1,3-galactosyltransferase) and expression of human complement regulatory proteins, anti-inflammatory proteins or coagulation-regulatory proteins. Using pronuclear injection of fertilized oocytes, the majority of these modifications have been introduced painstakingly, one by one, and knockouts created via homologous recombination. Engineered pig cells can be regenerated as whole animals through somatic cell nuclear transfer. Chimeric pigs, with organs originating from human cells, also represent an intriguing option, although residual animal cells in such organs would represent a challenge.

Today, there is also the possibility of applying DNA oligo synthesis to stitch together large numbers of genes in the same construct and the coexpression of strings of transgenes using multicistronic vectors containing 'self-cleaving' 2A peptides. CRISPR-Cas9 technology is ratcheting up the speed with which pigs with multiple genetic modifications can be created and their harvested organs tested in nonhuman primates.

In a recent advance (p. 46), 62 genes that encode porcine endogenous retroviruses were simultaneously edited, producing engineered pig kidney epithelial cells that were 1,000-fold less infectious than unmodified cells. This and similar feats will help build confidence that multiple modifications can be carried out in pigs; indeed, a commercial venture termed eGenesis has been formed around the work (p. 3). Elsewhere, public biotech company United Therapeutics is combining its financial muscle and xenotransplantation capabilities (acquired from Revivicor) with Synthetic Genomics' expertise in a research collaboration.

All of which suggests that the coming years promise much for the field of xenotransplantation. This will benefit not only basic studies of the mechanisms of immune rejection but also current human-to-human transplantation procedures and future off-the-shelf stem cell–derived treatments, both of which need to evade patient immune responses.

In the 1990s, concerns about potential transfer of porcine retroviruses to xenotransplant recipients shut down commercial programs, even though transmission had not (and still has not) been demonstrated *in vivo*. Given the continuing shortage of human organs for transplant, the return of commercial funding to xenotransplantation is encouraging. Government funders should take note. Yes, stem cell–derived therapies offer great long-term promise for degenerative diseases. But xenotransplants represent an additional intriguing option—one with potentially shorter horizons to the clinic. Humanized pigs are certainly not yet ready to go to market. But their day may come sooner rather than later.

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