How to Kill Two Birds With One Transgenic Pig

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Islet transplantation promises to provide safe, long-lasting insulin independence to individuals with diabetes. Yet the need for lifelong immunosuppression coupled with a vastly insufficient supply of donor tissue continues to prevent the widespread application of this approach to treating diabetes. A study (1) in this issue of *Diabetes* describes significant progress in addressing both of these issues.

Porcine islets have long been touted as a potentially unlimited source of transplantable islet tissue. Porcine and human insulin are highly homologous, and until the late 1970s, with the advent of recombinant protein technology, porcine insulin was routinely injected into humans with diabetes. However, the transplantation of porcine tissue has not proven to be so straightforward. Over the past decade, nonhuman primate studies demonstrated that a strong immunosuppression protocol was required to avoid rejection of porcine islet xenografts (2,3). Consequently, although the use of porcine islets would overcome the issue of tissue supply, their use would be tantamount to patients trading insulin injections for strong immunosuppression—not an attractive clinical solution for many.

In the current study, Klymiuk et al. (1) developed transgenic pigs that express an immunomodulatory fusion protein under the control of the insulin promoter. These animals produce the immunosuppressant protein locally upon activation of the insulin promoter within β-cells, but do not appear systemically immunosuppressed, thereby curbing potential complications arising from increased susceptibility of these animals to infections. The fusion protein used in these experiments, LEA29Y, inhibits T-cell activation by preventing costimulation signals from antigen-presenting cells (4). Under the trade name NULOJIX (belatacept), LEA29Y was recently approved by the U.S. Food and Drug Administration for prophylaxis of organ rejection in adult kidney transplant recipients (5), making the transgenic pigs described by Klymiuk et al. of particular clinical relevance.

The authors chose to evaluate how neonatal islet tissue grafts from LEA-tg pigs developed and functioned following transplantation into diabetic mice. Neonatal porcine tissue is an attractive source for islet transplantation because the isolation procedure is less cumbersome than adult islet isolation, and there is no need to maintain the animals over long periods of time—they can be used within 1–2 days of birth. However, neonatal islet tissue requires a maturation period following transplantation before it becomes a fully functional graft. As a result, both wild-type and LEA-tg neonatal islet cell clusters required 6–7 weeks to restore normoglycemia in streptozotocin-induced diabetic immunodeficient mice. Following maturation, the LEA-tg grafts showed strong LEA29Y immunostaining, which colocalized with insulin staining. Therefore in this model, neonatal porcine LEA-tg islet tissue was fully competent to mature into functional islet tissue capable of reversing diabetes.

To test the ability of these LEA-tg islets in the human immunological context, Klymiuk et al. used humanized mice. Human peripheral blood mononuclear cells (PBMCs) were injected into transplanted NOD/Lt-scid IL2Rγ^{null} mice, a strain of mouse known to permit high levels of human immune cell engraftment (6). Some PBMCs were also primed against porcine antigens in culture and injected 6 days following the initial PBMC injection. Over the ensuing 3-week period following reconstitution, 80% of wild-type grafts were rejected, while all LEA-tg grafts survived. No difference in human immune cell engraftment was observed between mice receiving wild-type or LEA-tg islets. Furthermore, the authors found similar evidence of graft-versus-host disease in animals receiving wild-type or LEA-tg tissue, which arises in this humanized mouse model due to the development of a human-versus-mouse immune response. In addition, mononuclear cells were observed surrounding the surviving grafts without evidence of infiltration into the grafts. These observations suggest that the islets were protected by local, and not systemic, immunosuppression.

This is the first demonstration of prolonged islet xenograft function due to local immunosuppression from transgenic porcine islet tissue. Importantly, this work provides evidence for local protection of porcine tissue against human immune responses.

The authors aptly underscored the need for further studies to evaluate the extent to which LEA-tg cells are able to withstand a more competent immune system. Clearly, more rigorous preclinical testing lies ahead for these transgenic islets, including nonhuman primate studies. In addition, the long-term stability of LEA29Y expression is not known. Are there any fluctuations in LEA29Y expression, particularly following exposure to stressors known to perturb insulin levels? Systemic LEA29Y was detectable at low levels in the blood of recipient mice, the consequences of which are not clear. Furthermore, the LEA-tg islets do not readily permit modulation of LEA29Y levels. Future modifications to the transgene, such as use of a ligand-inducible expression system, could be employed to optimize LEA29Y levels. It also remains to be seen if LEA29Y expression alone will be adequate to afford lasting protection for porcine cells in the face of a diabetic human immune system. Indeed, some potential limitations of costimulatory blockade have been highlighted (5), and LEA29Y expression may not be sufficient for all recipients. However, the generation of

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DOI: 10.2337/db12-0201

See accompanying brief report, p. 1527.

transgenic animals expressing combinations of multiple immunomodulatory proteins may allow for recipient-specific immunosuppression strategies.

The developmental stage at which these islets would be most useful clinically needs to be established. Although use of embryonic or neonatal tissue may be faster and more cost-effective to produce, it is not clear if LEA29Y levels would be produced at effective levels from immature β-cells. This point was not addressed in the mouse transplant model used by Klymiuk et al., which allowed for islet maturation prior to immunological challenge. Nonetheless, even the prospect of short-term immunosuppression during the islet maturation phase would be a major improvement compared with life-long immunosuppression. The use of adult porcine islets, on the other hand, provides a significant advantage over human donors as the major variables in islet isolation success (cold ischemia time, brain death, and organ quality) can be controlled or eliminated. Importantly, adult islets are fully developed and do not require a maturation period to attain maximal insulin, and hence LEA29Y, expression.

In conclusion, this work serves as a proof-of-principle study demonstrating that local production of an immunomodulatory protein from transgenic porcine islet tissue can overcome the human-versus-porcine xenogenic barrier. This study marks significant progress in bringing transgenic immunomodulation toward a clinical reality.

ACKNOWLEDGMENTS

No potential conflicts of interest relevant to this article were reported.

The author thanks Steven Paraskevas, Tarek Boutros, and Sarita Negi of the Department of Surgery, McGill University, for critically reviewing the manuscript.

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