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Limitations of the pig-to-non-human primate islet transplantation model

Martin Wijkstrom¹, Rita Bottino^{1,2}, and David K. C. Cooper¹

Martin Wijkstrom: wijkstrommn@upmc.edu

¹Department of Surgery, Thomas E. Starzl Transplantation Institute, University of Pittsburgh, Pittsburgh, PA

²Division of Immunogenetics, Children's Hospital of Pittsburgh, University of Pittsburgh, Pittsburgh, PA

In a paper which appears in this issue of *Xenotransplantation*, Drs Graham and Schuurman discuss potential limitations in the preclinical pig-to-non-human primate (NHP) islet transplantation model [1]. They summarize the results from five different groups, each of which has achieved moderately long-term islet xenograft function using varying approaches, for example, different immunosuppressive regimens, the transplantation of adult or neonatal islets and of islets from wild-type or genetically engineered pig donors, and the transplantation of free or encapsulated islets. In view of their extensive experience with this model, few, if any, investigators are positioned to evaluate the status of the field as well as the authors, who have been in the forefront of developing this model. They and their colleagues in Minneapolis have contributed significantly to such topics as animal enrichment, the use of vascular access ports, and defining safe and effective methods of inducing diabetes in NHPs.

Consistent and safe reversal of diabetes in NHPs is currently considered a prerequisite for a clinical trial. The authors make the case that the bar has perhaps been set too high, and we are inclined to agree with them on this point, as we discuss in a forthcoming commentary in this journal [2]. For several reasons, it would be expected that humans receiving pig islets would have superior outcomes to those obtained in NHP models [3, 4]. Factors that increase the barriers of the pig-to-NHP model, as compared to pig islet transplantation in humans, include (i) metabolic differences in glucose metabolism (with monkeys maintaining a lower set point for normoglycemia and higher insulin and C-peptide secretion than in pigs or humans); (ii) body weight loss due to nutritional deficiencies and diarrhea, particularly if a total pancreatectomy has been carried out to induce a state of diabetes; and (iii) a species-specific smaller therapeutic "window" of some of the commonly used immunosuppressive agents (e.g., calcineurin and mTOR inhibitors) possibly resulting in unanticipated side effects.

A limitation of the current review is the lack of presentation of recent data from the Minneapolis group. It would have been interesting for the readers to learn whether the authors have modified their protocols since their last report, and, if so, what has been the impact on their results during the past several years.

Several groups have achieved long-term (>6 months) pig islet function in NHPs using different induction and maintenance immunosuppressive regimens. We can realistically anticipate that a safe and effective protocol can be developed, especially with experience gained from the clinical testing of novel agents, for example anti-CD40 monoclonal antibodies, thus obviating the need for the administration of anti-CD154 monoclonal antibodies, which, although effective, may prove unacceptable for clinical application.

The remaining major issue to be addressed is how to minimize loss (and maximize engraftment) of transplanted pig islets from the instant blood-mediated inflammatory reaction (IBMIR), originally described by Bennet et al. [5]. Our group [6] and others [7] have attempted to elucidate the factors operative in immediate graft destruction (reviewed by van der Windt et al. [8]). *In vitro* studies have drawn attention to the significant role in the development of IBMIR played by the innate immune response, which may have been underestimated previously [6]. In *in vivo* studies, the Emory group has investigated the short-term effect of intraportal islet infusion by transplanting islets from different pig sources (e.g., wild type and 1,3-galactosyltransferase gene-knockout) concomitantly into different lobes of the liver, thus allowing comparative analysis [7]. Together, these data are enabling conclusions to be made on the effect of genetic engineering of islet-source pigs on the alleviation of IBMIR.

In addition, alternative sites for islet xeno-transplantation are being explored in NHPs or other experimental animals, some of which may render the islets less susceptible to IBMIR. These sites have included, but have not been limited to, the omental pouch [9], the gastric submucosal space [10], and the subcutaneous tissue [11] sometimes combined with protection by macroalginate encapsulation [12], various scaffolding systems [13], and recently, lymph nodes [14].

It is reasonable to assume that within the fore-seeable future, an effective immunosuppressive protocol and a strategy to minimize immediate graft loss will be optimized and tested in humans. However, if the limitations outlined by Drs. Graham and Schuurman persist, we may never achieve proof in the pig-to-NHP model.

With the above limitations in mind, the International Xenotransplantation Association consensus statement on conditions for undertaking clinical trials of porcine islet transplantation [15, 16] may be too stringent. There may be no need to demonstrate insulin independence of xenogeneic islets for periods in excess of 6 months. A balance has to be sought between (i) the benefit of extending graft and recipient survival to learn more of the rejection process and how it might be avoided; and (ii) the detrimental effect of the limitations and complications of the pig-to-NHP model. Furthermore, the field is likely to develop faster if follow-up of the NHP recipients is limited to 6 months.

One other approach that might facilitate progress in the difficult pig-to-NHP model is the transplantation of the islets into recipient NHPs that have not been rendered diabetic. The management of the recipient would be greatly facilitated, and the risk of complications, including hypoglycemic episodes or infection [17], would be reduced. Islet graft function could be monitored by measurement of porcine C-peptide (before and after glucose or arginine challenge) and, depending on the transplantation site, by serial biopsies. Limited studies in rodent models suggest that this approach would provide a reliable indication of islet graft survival and function [18–20].

Drs Graham and Schuurman have provided a valuable service to the transplant community by drawing attention to the limitations of the pig-to-NHP islet transplantation model. Addressing the problems they raise will undoubtedly result in progress being made and thus drawing us closer to clinical trials.

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