Cutting Edge: Pig Islet Xenografts Are Susceptible to "Anti-Pig" But Not Gal ω(1,3)Gal Antibody Plus Complement in Gal o/o Mice

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Hyperacute rejection due to Galα(1,3)Gal (Gal) Ab plus complement (C') is a major problem in xenografting vascularized organs from pigs to primates, but the fate of neovascularized xenografts is unclear. Nonendocrine islet cells are Gal', and there is a large rise in Gal Abs after transplantation, but graft remnants persist for many days in monkeys and humans. To define the role of αGal Ab plus C' in porcine islet graft rejection, cultured porcine fetal islets were transfused to mice lacking the α(1,3)galactosyltransferase gene. Anti-Gal Ab plus C' did not cause islet damage or rejection in mice lacking the α(1,3)galactosyltransferase gene, even when additional Ab plus C' was given; in addition, hyperimmune mice (titer >1/20,000) did not reject pig islets, showing that islets are resistant to Gal Ab plus C'. However, islets can be destroyed by polyclonal mouse anti-pig Abs. Thus, the focus of islet xenografting should not be on Gal Ab plus C'. The Journal of Immunology, 1998, 161: 5116–5119.

Major problem in grafting vascularized pig organs to primates is hyperacute rejection due to Galα(1,3)Gal (Gal) Abs. These Abs react with the Gal carbohydrate present on the endothelial cells of the pig organ, fix complement (C'), and cause hyperacute rejection (1). However, the fate of neovascularized pancreatic islet xenografts in the first few days after xenotransplantation is not so clear. Like other tissues, the ducts and blood vessels of the pig pancreas are Gal', whereas the α, β, and δ endocrine cells (ECs) are Gal' (2, 3). When such islets are cultured, they express large amounts of Gal on ducts but not on ECs (4); also, a large increase of Gal Abs occurs when pig islets are transplanted to humans (5, 6). Thus, although the important ECs do not express Gal, the transplant itself does; consequently, there is the potential for Ab plus C'-mediated damage that may secondarily destroy the Gal' ECs. However, the immediate fate of pig islets transplanted to humans is unknown, for such grafts were not monitored in the first hours or days during the time when Ab-mediated rejection might occur; rather, these grafts were examined some weeks later, when graft tissue was found in one recipient. It is not known whether grafts are damaged by Gal Ab plus C' diffusing into the fetal pig pancreas (FPP) within the first few days or whether islets are entirely resistant to a Ab plus C'-mediated damage. Furthermore, it is possible that the rise in Gal Ab after pig-to-human transplantation, which gives a T cell-dependent ~60-fold rise in amount and an ~100-fold increase in the affinity of Gal Ab (5, 6), could cause delayed rejection. To answer these questions and determine whether Gal Abs are as important in islet transplantation as in the rejection of other tissues, we examined Ab plus C'-mediated rejection of pig islets in Gal o/o mice, that, like humans, lack a functional α(1,3)galactosyltransferase gene and have naturally occurring Gal Abs (7, 8).

We monitored the islet grafts for damage and cell infiltration at frequent intervals over the first 120 h after transplantation, used additional Gal Ab plus C' approximately as used in other systems to cause xenograft rejection to ensure that these were not limiting (9, 10), and used hyperimmune mice in which the amount of Gal Ab present was in excess of that seen in patients after transplantation. Finally, as the studies showed pig islets to be resistant to Gal Ab plus C', we used a polyclonal mouse anti-pig Ab that was able to destroy the islets in 3–5 days. Thus, Gal' ECs are resistant to direct insult by Gal Ab plus C' but can be destroyed by anti-pig Abs directed against other determinants.

**Materials and Methods**

Mouse anti-pig serum (MAPS) was produced by immunizing (BALB/c × CBA)F1 mice with the pig endothelial cell line PIEC, emulsified in CFA, and administered repeatedly over 8 wk. Normal human serum (NHS), which was obtained from healthy volunteers, was used immediately or kept at 4°C for up to 5 days. Normal rabbit serum was used as a source of C' (RC'); rabbits were bled, blood was allowed to clot at 4°C, and serum was collected and stored at ~70°C. Gal o/o mice were immunized with 200 μl of packed rabbit RBCs (which have high levels of Gal (11)) administered i.p. weekly for 3 wk; Gal Ab levels were measured by ELISA using plates coated with Gal (12). Recipients were inbred young-adult Gal o/o mice (7, 10), which have a mixed background of C57BL/6, 129, and DBA/2 strains;
the other mice used were C57BL/6 and SCID mice. Donor tissue was obtained from an outbred Landrace pig (gestational age of ~85 days). The FPP was dissected and placed into organ culture in 37°C at a gas/medium interface for 3 days in 90% O2/10% CO2 and for 1 day in 90% air/10% CO2 (13). Grafts were then transplanted under the left kidney capsule to seven groups of five mice each: 1) Gal/o/o controls; 2) 0.3 ml (i.p.) of MAPS on day 0; 3) 1 ml (i.p.) of NHS on day 0; 4) 0.5 ml (i.p.) of RC1 on day 0; 5) 1 ml (i.p.) of NHS plus 0.5 ml (i.p.) of RC1 on day 0; 6) hyperimmunized mice (Gal Ab titer >1/20,000); and 7) hyperimmunized mice receiving 0.5 ml (i.p.) of RC1 on day 0. Animals received the depleting anti-CD4 mAb GK1.5 (14) (0.5 mg/i.p./mouse) on day 0 to prevent acute cellular rejection. Grafts were then transplanted under the left kidney capsule to seven groups of five mice each: 1) Gal/o/o controls; 2) 0.3 ml (i.p.) of MAPS on day 0; 3) 1 ml (i.p.) of NHS on day 0; 4) 0.5 ml (i.p.) of RC1 on day 0; 5) 1 ml (i.p.) of NHS plus 0.5 ml (i.p.) of RC1 on day 0; 6) hyperimmunized mice (Gal Ab titer >1/20,000); and 7) hyperimmunized mice receiving 0.5 ml (i.p.) of RC1 on day 0. Animals received the depleting anti-CD4 mAb GK1.5 (14) (0.5 mg/i.p./mouse) on day 0 to prevent acute cellular rejection during the observation. Grafts were removed at 1, 2, 4, 6, 8, 10, 12, 24, 48, 72, 96, and 120 h posttransplantation, fixed in Bouin’s solution, and processed. Paraffin-embedded tissue that had been sectioned at 4–5 μm was stained with hematoxylin and eosin for the assessment of infiltration and destruction and cell infiltrate by granulocytes.

Table I. FPP grafts to Gal o/o mice

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<tr>
<th>Recipient Treatment</th>
<th>Results</th>
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<tbody>
<tr>
<td>Gal o/o controls*</td>
<td>-b</td>
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<tr>
<td>Gal o/o + MAPS</td>
<td>++</td>
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<tr>
<td>Gal o/o + RC1</td>
<td>-</td>
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<tr>
<td>Gal o/o human serum</td>
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<td>Gal o/o + human serum + RC1</td>
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*a Gal o/o mice (n = five in each group) received the various treatments listed; grafts were examined at 12 different times (~1–120 h).

b –, cell infiltration or damage; ++, destruction and cell infiltrate by granulocytes.

Results and Discussion

We have shown previously in T cell immunosuppressed mice that FPP xenografts could survive for ≤28 days (16); similar survival occurs in Gal o/o mice that have anti-Gal Abs. The grafts appeared to be histologically normal in the inbred mice, but it was not clear how much of the islet tissue had been rejected, as early monitoring had not been performed. Any destruction could have been obscured by the extensive regeneration that occurs after the transplantation of fetal islets. Therefore, it was important that pig islet xenografts be examined in Gal o/o mice in the presence of Gal Abs. We have shown previously in T cell immunosuppressed mice that FPP xenografts could survive for ≤28 days (16); similar survival occurs in Gal o/o mice that have anti-Gal Abs. The grafts appeared to be histologically normal in the inbred mice, but it was not clear how much of the islet tissue had been rejected, as early monitoring had not been performed. Any destruction could have been obscured by the extensive regeneration that occurs after the transplantation of fetal islets. Therefore, it was important that pig islet xenografts be examined in Gal o/o mice in the presence of Gal Abs and, further, that excess Ab plus C′ be provided to ensure that neither were limiting. Also, monitoring was performed frequently in the first 120 h to detect any features of Ab-mediated rejection (Table I).

**Pig islet rejection Gal o/o mice examined at ≤5 days postgrafting**

Cultured FPPs were transplanted to Gal o/o mice and monitored by histology at 1, 2, 4, 6, 8, 10, 12, 24, 48, 72, 96, and 120 h. Over this time, there was no cellular infiltration of granulocytes, which are indicative of Ab-mediated events, or of mononuclear cells, which are indicative of cell-mediated rejection (Fig. 1, A and B). However, it was possible that pig islets were intrinsically resistant to Ab-mediated mechanisms or that insufficient Ab and/or C′ was available in the Gal o/o mice to cause damage; these possibilities were examined.

**Destruction of pig islets by polyclonal mouse anti-pig Abs**

Polyclonal MAPS (0.3 ml) was given i.p. to mice (either inbred C57BL/6 or Gal o/o), and pig xenografts were performed and monitored in the same 12 time intervals as described above. Little change was seen in the grafts until 48 h, when a cell infiltrate consisting mostly of polymorphs occurred. The infiltrate subsequently increased, and islet tissue was mostly destroyed over the next 48–72 h; i.e., by 96–120 h, the islets had been almost totally destroyed, showing central necrosis and a heavy infiltrate of granulocytes in the graft (Fig. 1, C and D). Immunoperoxidase staining for insulin and somatostatin showed a major reduction or disappearance of these cells (Fig. 1, E–H). Clearly, islets can be destroyed by MAPS in a manner similar to the destruction of rat skin or heart grafts by mouse anti-rat Ab (9, 10). There was no noticeable difference in the Ab-mediated destruction in inbred Gal+ or Gal o/o mice. It was of interest that >24–48 h had to elapse before any noticeable infiltrate with polymorphs was observed. Thus, islets are not intrinsically resistant to destruction by Ab-mediated mechanisms; islet destruction can occur if the target cells contain the Ag and there is sufficient Ab plus C′ present. Clearly, pig islets are susceptible to Ab-mediated damage (rejection), although not by intrinsic Gal Ab plus C′; this mechanism must be considered in pig islet graft rejection.

**Fate of pig islet xenografts in Gal o/o mice given additional Gal Ab plus C′**

FPPs were grafted to Gal o/o mice; NHS containing a high titer of Gal Ab (17, 18) was given to ensure that sufficient Ab was present. When 1 ml of Gal polyclonal Ab was administered, no signs of cell infiltration or tissue damage were seen for ≤120 h (Fig. 1, A and B). Furthermore, when additional RC′ was provided, there was also no evidence of rejection; RC′ alone had no effect. Thus, it appears that islets can be destroyed by the appropriate Ab (see above), whereas Gal Abs, even with additional C′, have no such effect. However, it was possible that the Abs in human serum were too low in amount and/or affinity to cause rejection; therefore, hyperimmune mice were used.

**Pig islet cell transplantation to hyperimmune Gal o/o mice**

To examine whether larger amounts of Gal Ab could cause rejection, Gal o/o mice were hyperimmunized and had Gal Ab titers of >1:20,000. FPP transplants were performed and monitored over 5 days; no damage occurred, but an occasional granulocyte was noted in the graft. When additional RC′ was provided, there was also no damage noted. These studies are important, as Groth and colleagues have shown that after pig islet xenotransplants to humans, even with heavy immunosuppression, a large rise of Gal Ab occurred (5, 6), in excess of a 60-fold increase in amount and affinity; to study Ab-mediated rejection, it is necessary to examine grafts in the first few days posttransplant, and also later, when a large rise in Ab occurs. The amount of Ab present in the hyperimmune mice was in excess of that which is seen in humans after islet transplantation; if this delayed rise in Gal Ab was likely to cause rejection, it should have been seen in these mice. We can conclude that pig islets are resistant to destruction by Gal Abs (both to the amount of Gal Ab seen at first exposure and to the secondary rise in Gal Abs that occurs after transplantation); i.e., pig islets xenografts are entirely resistant to rejection by Gal Ab plus C′ but are not resistant to other non-Gal Ab-mediated mechanisms.

Perusal of the existing data on islet xenotransplantation in the presence of anti-Gal Abs does not give a clear indication of the fate of such grafts during the first few days posttransplant. In Groth’s patients, remnants of pig tissue were found in one patient; however, whether the graft was mostly destroyed by Gal Ab plus C′ was not apparent (5). In Mandel’s studies in immunosuppressed cynomolgous monkeys, healthy islets were observed at 42 days; however, it was still not apparent whether these were remnants left...
after Gal Ab plus C’ destruction and then regeneration (19). From the present studies, we can conclude that Gal Ab plus C’ was unlikely to have directly damaged the graft in the first few days.

It is clearly important to determine what molecules are involved in the MAPS-mediated damage and whether molecules these could be relevant to human anti-pig reactions. In addition, as Ab-mediated rejection can occur, transgenic pigs expressing human C’ regulatory molecules (currently being evaluated in vascularized organ transplants) could be useful in islet transplants, although preliminary studies (T.E.M., unpublished observations) indicated
that islets from decay-accelerating factor transgenic pigs fared no better than nontransgenic islets. We conclude that pig islets are not destroyed by Gal Abs plus C' but are susceptible to other Abs. In addition, pig to human/primat xenotransplantation graft rejection mechanisms must include: 1) non-Gal Ab plus C'; 2) Ab (via Ab-dependent cell-mediated cytotoxicity) involving FcR' cells; 3) macrophages (without Ab); or 4) T cells of different types. The observation that encapsulated xenografts but not allografts are destroyed in vivo (20) would tend to exclude cells and very large molecules but would allow for damage caused by other means, including prostaglandins, cytokines, superoxides, nitric oxide, and other substances. Other than excluding Gal Abs, most mechanisms must considered important in the rejection of pig xenografts in Gal o/o individuals.

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References
