Hepatocyte Growth Factor Gene Therapy for Pancreatic Islets in Diabetes: Reducing the Minimal Islet Transplant Mass Required in a Glucocorticoid-Free Rat Model of Allogeneic Portal Vein Islet Transplantation

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Islet transplantation for diabetes is limited by the availability of human islet donors. Hepatocyte growth factor (HGF) is a potent β-cell mitogen and survival factor and improves islet transplant outcomes in a murine model. However, the murine model employs renal subcapsular transplant and immunodeficient mice, features not representative of human islet transplantation protocols. Therefore, we have developed a more rigorous, marginal-mass rat islet transplant model that more closely resembles human islet transplantation protocols: islet donors are allogeneic Lewis islets; recipients are normal Sprague Dawley rats; islets are delivered intraperitoneally; and immunosuppression is accomplished using the same immunosuppressants employed by the Edmonton group. We demonstrate that 1) surprisingly, the Edmonton immunosuppression regimen induces marked insulin resistance and β-cell toxicity in rats, 2) adenovirus does not adversely affect islet transplant outcomes, 3) the Edmonton immunosuppressants may delay or block rejection of adenovirally transduced islets, and more importantly, 4) pretransplant islet adenoviral gene therapy with HGF markedly improves islet transplant outcomes, 5) this enhanced function persists for months, and 6) HGF enhances islet function and survival even in the setting of immunosuppressant-induced insulin resistance and β-cell toxicity. This approach may enhance islet transplantation outcomes in humans. (Endocrinology 145: 467–474, 2004)
employed in the Edmonton studies: low-dose tacrolimus (FK-506), sirolimus (rapamycin), daclizumab (IL-2 receptor monoclonal antibody) (1, 2).

Materials and Methods

Islet graft recipients

Male Sprague Dawley rats weighing 150–200 g were purchased from Charles River Laboratories (Charlestown, MA). Tap water and chow pellets were provided ad libitum. Animals were rendered diabetic by a single injection of streptozotocin (STZ) (Sigma-Aldrich, St. Louis, MO) at a dose of 65 mg/kg, freshly dissolved in citrate buffer (pH 4.5). Before transplantation, diabetes was confirmed by the presence of hyperglycemia; only those rats with blood glucose levels of more than or equal to 350 mg/dl for at least 5 d were used as recipients. Tail blood was obtained from nonfasted rats, and whole-blood glucose levels were measured with a portable glucose meter (Medisense, Bedford, MA) at the times indicated in the figures. All animals were housed under conventional conditions, and all studies were performed with the approval of, and in accordance with, guidelines established by the University of Pittsburgh Institutional Animal Care and Use Committee.

Islet graft donors

Male Lewis rats (Charles River Laboratories) (6–8 wk old; body weight, 250–300 g) were used as islet donors. Islets were isolated as previously described by Ricordi and Rastellini (9), with some modifications. Briefly, after general anesthesia, a midline abdominal incision was performed, and the pancreas was exposed and injected through the pancreatic duct with 15 ml of 1.7 mg/ml collagenase P (Roche Molecular Biochemicals, Indianapolis, IN) in Hank’s buffered saline solution (HBSS) (Life Technologies, Inc., Long Island, NY). The pancreas was surgically removed and incubated at 37 C for 17 min, and then passed through a 500-µm wire mesh. The digested pancreas was rinsed with HBSS, and islets were separated by density gradient in Histopaque (Sigma-Aldrich). After several washes with HBSS, islets were hand picked under a microscope. To standardize the islet mass to be transplanted, one islet equivalent (IE) was defined as one 125-µm-diameter islet. Before transplantation, islets were placed in culture overnight in RPMI 1640 medium (Life Technologies, Inc.) supplemented with 10% fetal calf serum (HyClone, Logan, UT), 2 mmol/liter l-glutamine, penicillin/streptomycin, and HEPES buffer (Mediatech, Herndon, VA) at 37 C and 5% CO2.

Portal vein islet transplantation

A midline laparotomy was performed in the diabetic rats under isoflurane general anesthesia. Islets were then slowly injected into the portal vein via the superior mesenteric vein in a volume of 400 µl PBS solution. After infusion, the syringe was rinsed several times by repeated aspiration and reinjection of portal vein blood. Manual compression of the injection site followed removal of the needle to minimize bleeding. Tail blood glucose levels were measured under nonfasting conditions at d 1, 2, 3, 5, and 7, and then weekly or biweekly, as shown in the figures. Blood samples were weekly obtained from a tail vein for plasma insulin determination and biochemistry. Plasma insulin determinations were performed by RIA per the manufacturer’s specifications (Linco Research, St. Louis, MO).

Immunosuppression regimen

Immunosuppression consisted of low-dose tacrolimus (FK-506) (0.5 mg/kg/d; Fujisawa, Deerfield, IL), sirolimus (rapamycin) (2 mg/kg sc, every 48 h; Wyeth-Ayerst, Princeton, NJ) and daclizumab (2 mg/kg iv at d 0, 1 mg/kg at d 15; Roche, Nutley, NJ), as described in the figures. These doses were selected to mimic the doses used in the human Edmonton protocol. Doses were calculated based on previous reports (10–14). Whole-blood tacrolimus concentrations were measured by microplate enzyme immunoassay technology using the IMX tacrolimus assay (Abbott Laboratories, Abbott Park, IL), and whole-blood rapamycin levels were measured in the Clinical Chemistry Laboratory at the University of Pittsburgh Medical Center using HPLC mass spectrometry.

Adenovirus construction and islet transduction

Ad5 adenoviral vectors encoding murine HGF and β-galactosidase (LacZ) were prepared as we have described in detail previously (8), using the methods of Becker et al. (15). Isolated islets were infected with purified adenovirus in RPMI 1640 for 1 h at 37 C (at a multiplicity of infection of 250, assuming ~1000 cells/islet), rinsed with RPMI 1640 containing 1% penicillin/streptomycin and 10% fetal bovine serum, and incubated in 0.5 ml of the same medium for 18–24 h at 37 C on the day before transplant.

Relative semiquantitative PCR

Total islet RNA was isolated, and PCR was performed as described in detail previously (6–8).

Western blot for HGF

Western blotting of isolated islets for murine HGF was performed as described previously (8).

Histology and insulin staining and insulin determinations

Livers were removed on d 25, fixed overnight in Bouin’s solution, and embedded in paraffin. Five-micrometer sections were obtained and stained with hematoxylin and eosin using standard techniques. Immunostaining for insulin and visualization of the staining was achieved using the species-appropriate avidin-biotin complex system as previously described (6–8).

Results

Edmonton-style immunosuppression induces insulin resistance and β-cell failure in normal rats

We were initially interested in determining the effects of the Edmonton protocol on completely normal rats, and therefore administered the three drugs using the regimen described in Materials and Methods for 21 d. As can be seen in Fig. 1, institution of the Edmonton protocol in normal Sprague Dawley rats (solid line) resulted in the development of frank diabetes by d 10. This contrasted with the findings in normal control rats that remained euglycemic under the same conditions (dotted line). It also contrasted with findings in rats treated with sirolimus plus daclizumab (dashed line), which also remained euglycemic. These findings indicate that the Edmonton protocol, in the doses employed herein, causes diabetes in normal rats. Importantly, the trough tacrolimus concentrations were 6 ± 1.5 ng/ml (mean ± se), in the low therapeutic range for humans, and trough rapamycin concentrations were 8.7 ± 2.5 ng/ml (mean ± se), also in the range reported by the Edmonton group in humans (1, 2).

To determine the cause of the diabetes induced by the Edmonton protocol, plasma insulin levels were determined. As can be seen in Fig. 2, insulin concentrations remained normal in the control, euglycemic control rats. In contrast, insulin levels rose in the sirolimus- plus daclizumab-treated rats. These rats also remained euglycemic (Fig. 1), indicating that the combination of sirolimus and daclizumab in the doses used, causes insulin resistance, but, as a result of compensatory β-cell insulin secretion, did not result in diabetes.

The rats that received the full triple-drug regimen displayed higher insulin concentrations than their normal controls, indicating that the triple-drug regimen, too, causes insulin resistance in normal rats. Importantly, these animals displayed lower insulin concentrations than the sirolimus-plus-daclizumab animals, and also displayed frank diabetes (Fig. 2).
These findings indicate that the addition of tacrolimus to the sirolimus-plus-daclizumab combination results in a failure of H9252-cells to compensate for insulin resistance induced by the Edmonton regimen.

The Edmonton protocol protects against portal allograft rejection in rats

We next wanted to assess whether the immunosuppression protocol was indeed immunosuppressive in this rat portal allograft context. As shown in Fig. 3A, rats not receiving tacrolimus plus sirolimus plus daclizumab, or sirolimus plus daclizumab, showed statistically significant (P < 0.001) higher plasma insulin levels compared with the rats that received no immunosuppression. Interestingly, animals receiving the three immunosuppressants displayed lower insulin levels than the group treated with sirolimus plus daclizumab (P < 0.01).

Ad.HGF effectively transduces rat islets

To examine the ability of Ad.HGF and Ad.lacZ to transduce rat islets, we examined HGF expression by RT-PCR in Lewis rat islets exposed to no virus, to Ad.lacZ, or to Ad.HGF for 24 h. Ad.lacZ transduction resulted in strong β-galactosidase staining not present in normal islets (data not shown). As can be seen in Fig. 4A, RT-PCR products representing HGF are present in far greater quantities in Ad.HGF-transduced islets than in normal or Ad.lacZ-transduced islets. Similarly, at the protein level, Ad.HGF effectively induced HGF expression, as shown in Fig. 4B. By 48 h, HGF protein expression could be determined, we further reduced the dose of transplanted islets to 10 IE/g. As can be seen in Fig. 3B, in rats receiving no immunosuppression, as in the prior experiment in A, transplanted islets ceased functioning by d 10, presumably reflecting rejection. In contrast, in rats receiving 10 IE/g, glycemic control was actually worsened by immunosuppression. The results in Fig. 3 extend the observations in Figs. 1 and 2 demonstrating that the three-drug regimen is detrimental not only to endogenous islet function but also to transplanted islets.
Introduction of Ad.HGF into a minimal-islet-mass transplant markedly improves graft function

With the availability of the minimal (10 IE/g)-graft model in Fig. 3B, and the ability to introduce HGF into islets using Ad.HGF, we next combined these approaches. As shown in Fig. 5, normal uninfected and Ad.lacZ-transduced Lewis rat allograft transplants at 10 IE/g had only a transitory effect on blood glucose. These results are almost identical with those in Fig. 3B. In striking contrast, Ad.HGF introduction into this otherwise-inadequate islet mass markedly enhanced graft function. As can be seen in Fig. 3B, random, nonfasting glucose concentrations in the rats receiving the 10 IE/g portal Ad.HGF-transduced allografts rapidly approached normal and remained in the 200–250 mg/dl range for the duration of the experiment. Importantly, this degree of glucose control was comparable with that observed in normal rats receiving the Edmonton agents (Fig. 1), and was superior to the 250–300 mg/dl range achieved using 50% larger (15 IE/g) doses of normal islets in Fig. 3A.

Plasma insulin concentrations in the three groups in Fig. 5 are shown in Fig. 6. As anticipated from the glucose results, plasma insulin concentrations were markedly higher in the rats receiving Ad.HGF than in rats receiving equivalent numbers of either Ad.lacZ or normal islets. Together with the glucose results in Fig. 5, these results indicate that the introduction of HGF using an adenoviral vector markedly improves portal allograft function, not only compared with Ad.lacZ-transduced islets, but also compared with normal islets.

Histologic sections of liver from Ad.HGF-transduced islet allografts are shown in Fig. 7. This figure illustrates several
First, abundant insulin-staining β-cell clusters can be observed in the livers of the 10 IE/g Ad.HGF animals. Second, in the presence of immunosuppression, there is no evidence of an inflammatory response to either the islet allograft or the adenovirus used to transduce the islets. Third, livers from animals transplanted with Ad.HGF-transduced islets, compared with Ad.lacZ islets, contained larger numbers of islets. This is shown quantitatively in Fig. 8, where the number of islets per liver section is seen to be more than 2-fold higher in those livers that received Ad.HGF-transduced islets than in controls. This estimate is of course an underestimate if one considers that the average size of the residual transplanted islets in the Ad.HGF livers is significantly larger than that in the Ad.lacZ controls.

The studies described thus far indicate that pretransplant delivery of Ad.HGF improves islet transplant outcomes, but only for a relatively brief period of time. Therefore, we next asked how long this salutary effect might last. To do this, the experiments described earlier (Figs. 1 and 5) were repeated and combined using the following three groups of rats, each of which received the Edmonton agents as in Figs. 1 and 5: 8 normal, nondiabetic rats treated with the Edmonton immunosuppressive regimen, and 5 Ad.lacZ and 10 Ad.HGF Lewis marginal (10 IE/g)-islet allografts into STZ-diabetic Sprague Dawley recipients. However, instead of sacrificing the animals on d 25, they were observed indefinitely. As can be seen in Fig. 9, the new control group of STZ-diabetic animals receiving 10 IE/g, the marginal islet mass, and Ad.lacZ (open squares) remained diabetic, confirming the results of briefer studies shown in Figs. 3B and 5. All five of these animals had died by wk 17 of the experiment. In marked contrast, pretreatment of this same marginal islet mass with Ad.HGF (Fig. 9, filled squares) again dramatically and rapidly improved blood random blood glucose values, as had been observed in earlier experiments (Fig. 5) over a 25-d time period. However, it can now be appreciated that the duration of this improvement compared with the controls is long-lasting: the Ad.HGF animals displayed blood glucose concentrations some 200 mg/dl lower than the Ad.lacZ animals until wk 16–18, by which time all of the Ad.lacZ animals had died. Thus, the improvement in control lasted at least five months, approximately one fifth of the 2-yr life span of a rat.

Finally, as can be seen in Fig. 9, normal rats exposed to the Edmonton reagents (open circles) again promptly developed diabetes as had occurred in Fig. 1 during the 25-d experiment. However, as seen in Fig. 9, with more prolonged exposure to the Edmonton regimen, the diabetes became even more severe, such that by 16 wk, these animals had non-

FIG. 6. Plasma insulin levels under nonfasting conditions in diabetic rats after allotransplantation with ex vivo-infected islets and receiving the Edmonton immunosuppression regimen. Diabetic rats transplanted with Ad.HGF-infected allogeneic islets showed a significant \( P < 0.05 \) increase in plasma insulin levels compared with rats receiving uninfected or Ad.lacZ-infected islets.

FIG. 7. Insulin immunostaining of liver sections obtained at d 25 post-islet transplantation. A and B, Liver sections from Ad.HGF-transduced islets at low- and high-power magnification. The arrow in A indicates the islet that is further magnified in B. C and D, Livers from Ad.lacZ-transduced islets, at the same low- and high-power magnification. The arrow in C indicates a sole islet. As can be seen, the livers from the Ad.HGF-transduced islet transplants contain far more as well as larger islets than the Ad.lacZ controls.
superiorly to the Ad.lacZ animals. See animals behaved indistinguishably from the normal controls and far triple-drug regimen developed severe diabetes, and that the Ad.HGF study was discontinued. Note that the normal rats receiving the wk 17, and all but one of the Ad.HGF had died by wk 22 when the study was discontinued. Note that the normal rats receiving the triple-drug regimen developed severe diabetes, and that the Ad.HGF animals behaved indistinguishably from the normal controls and far superiorly to the Ad.lacZ animals. See Results for details.

In this study, we demonstrate for the first time that the delivery of an islet growth factor, in this case HGF, using gene therapy strategies, can improve the efficacy of portal pancreatic islet allografts in the treatment of diabetes mellitus over the long term. Equally importantly, we have developed a rat model system with which to determine or define the efficacy of this and other such treatments under conditions that closely mimic human islet transplantation. The attractive features of this Edmonton rat model are the following: 1) it is an allograft model of transplantation for insulin-deficient diabetes; 2) it is an intraportal transplant system; 3) it employs a well-defined marginal or minimal islet mass; 4) it employs immunosuppressive agents currently in use in clinical trials for human islet transplantation; 5) it requires that transplanted islets overcome the β-cell toxic effects of immunosuppressive agents as might occur in humans; 6) it requires that the transplanted islets not only produce normal amounts of insulin, but produce quantities sufficient to overcome the insulin resistance induced by immunosuppressive agents; and 7) it allows the assessment of the efficacy of adenovirus as a gene delivery vector. A successful gene therapy strategy for enhancing human islet graft survival would need to overcome each of these barriers.

HGF is an ideal candidate for enhancing islet function for the reasons summarized in the introductory section. These prior findings in immunodeficient mice beg three important additional questions: 1) Could this adenoviral HGF enhancement be applied to a model employing the portal delivery site employed in human islet transplantation? 2) Would the improvement in function also be apparent under the more adverse conditions of allograft immunity, adenovirus-directed immunity, and the potential diabetogenic toxicities of immunosuppressive agents? and 3) Will the improvement be maintained over the long term?

The first concern was to select a vector for use in islet gene delivery. Adenovirus has been associated with a number of adverse features, and is viewed by many as a vector that can be used to provide proof of principle regarding the efficacy of gene therapy, but is unlikely to be useful in ultimate human gene therapy applications (16). Negative features are that it evokes a host immune response that can cause anaphylaxis, as well as host immune system-driven destruction of the target cells that express adenoviral proteins. Moreover, adenovirus is a nonintegrating vector, and expression of the gene product in question would therefore have a limited duration of expression. Although these may be negative considerations in some settings, in the relatively unique setting of islet transplantation, they may be positive attributes. Because islet transplantation requires immunosuppression, immune attack on adenovirus-transduced islet grafts will also likely be attenuated. Moreover, because the vast majority of transplanted islet cells die within the first day after transplantation (17), transient expression of an agent that promotes islet engraftment during the first week may be all that is necessary. The lack of integration may be an advantage as well, because random integration into tumor suppressor or other key cellular genes will not occur. Importantly, because the virus is administered \textit{ex vivo}, the viral load delivered to

\begin{figure}[h]
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\includegraphics[width=0.5\textwidth]{fig8.png}
\caption{Quantitation of the number of islets per liver section in the Ad.HGF \textit{vs.} Ad.lacZ livers at d 25. The number of islets was significantly higher in the livers that received Ad.HGF-transduced islets. These numbers underestimate the true total mass of insulin-producing cells present within the livers, because islet size was not taken into account.}
\end{figure}

\begin{figure}[h]
\centering
\includegraphics[width=0.5\textwidth]{fig9.png}
\caption{Long-term function of islet grafts and normal islets as assessed using random blood glucose values in normal Sprague Dawley rats or Sprague Dawley rats transplanted with Lewis islets, all treated with the three Edmonton immunosuppressive agents. The studies shown in Figs. 1 and 5 were repeated, combined, and extended for 22 wk. STZ-diabetic Sprague Dawley rats were transplanted with 10 IE/g either Ad.lacZ-transduced islets (open squares; n = 5) or Ad.HGF-transduced islets (filled squares; n = 10), and were compared with normal Sprague Dawley rats (open circles; n = 8). All three groups were treated with the triple-drug immunosuppressive regimen for the entirety of the study. All of the Ad.lacZ rats had died by wk 17, and all but one of the Ad.HGF had died by wk 22 when the study was discontinued. Note that the normal rats receiving the triple-drug regimen developed severe diabetes, and that the Ad.HGF animals behaved indistinguishably from the normal controls and far superiorly to the Ad.lacZ animals. See Results for details.

Fasting blood glucose concentrations of approximately 400 mg/dl. These studies confirm and underscore the observations in Fig. 1 indicating that these agents, even when used in doses that are associated with normal therapeutic blood levels, are diabetogenic in rats. Importantly, the juxtaposition of the Ad.HGF rats and the normal Sprague Dawley rats in Fig. 9, together with earlier data in the SCID mouse HGF transplant model (7, 8), which does not receive immunosuppressive drugs and that does not develop diabetes, makes it clear that the diabetes in the Ad.HGF and the normal Sprague Dawley rats in Figs. 5 and 9 is a result of the immunosuppressive drugs.

### Discussion

In this study, we demonstrate for the first time that the delivery of an islet growth factor, in this case HGF, using gene therapy strategies, can improve the efficacy of portal pancreatic islet allografts in the treatment of diabetes mellitus over the long term. Equally importantly, we have developed a rat model system with which to determine or define the efficacy of this and other such treatments under conditions that closely mimic human islet transplantation. The attractive features of this Edmonton rat model are the following: 1) it is an allograft model of transplantation for insulin-deficient diabetes; 2) it is an intraportal transplant system; 3) it employs a well-defined marginal or minimal islet mass; 4) it employs immunosuppressive agents currently in use in clinical trials for human islet transplantation; 5) it requires that transplanted islets overcome the β-cell toxic effects of immunosuppressive agents as might occur in humans; 6) it requires that the transplanted islets not only produce normal amounts of insulin, but produce quantities sufficient to overcome the insulin resistance induced by immunosuppressive agents; and 7) it allows the assessment of the efficacy of adenovirus as a gene delivery vector. A successful gene therapy strategy for enhancing human islet graft survival would need to overcome each of these barriers.

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the patient would be small, reducing concerns regarding anaphylaxis. Finally, immunosuppression would also presumably attenuate host allergic reactions. Thus, we believe that adenovirus is worthy of consideration in human islet transplant settings.

One could reasonably ask why one must deliver adenovirus using gene therapy approaches to islets, rather than by systemic injection in the setting of islet transplant. HGF has been administered using such an approach (18), but systemic administration and generalized overexpression using the metallothionein promoter in mice is limited by host toxicity, and local doses to the islet obtained by viral delivery would be higher than those achievable using systemic administration routes. Thus, viral delivery to islets before transplant would appear to be more attractive than systemic administration of HGF to recipients.

One reason for moving from standard immunosuppressive regimens to the three agents that comprise the Edmonton protocol was the need to avoid β-cell toxicity and insulin resistance induced by the high-dose tacrolimus and glucocorticoids (1, 2, 19–21). Our studies clearly demonstrate, to our surprise, that low-dose tacrolimus and sirolimus, with plasma levels comparable with those desired in humans, is indeed associated with β-cell toxicity and marked insulin resistance in rats. We cannot be certain from this study in rats whether induction of diabetes might occur in normal humans treated with the Edmonton cocktail, or whether this may be a rat-specific effect. This question merits consideration in humans. We presume, but have not proven, that the diabetogenic and immunosuppressive effects observed in the current study resulted from sirolimus and tacrolimus, because daclizumab is an antihuman CD25 antibody, and was presumably inert in the current study. These observations suggest that there is room for further improvement in designing and implementing immunosuppressive strategies. However, from the perspective of developing model systems with which to rigorously test islet transplant approaches, these results are ideal, because they allow one to test the efficacy of genetically enhanced islets in an adverse, potentially real-world situation.

One key feature of the current rat model is that it employs or defines a minimal mass or marginal mass of islets. This is critically important, because, as described by Inverardi, Ricordi, and others (22–25), one needs to define the limits of adequacy to improve upon them. Thus, in the current model, the 15 IE/g model (Fig. 3A) allows one to clearly define the efficacy of the immunosuppression in protecting islet allografts, and therefore the relevance of the immunosuppressive regimen employed, but the dose of islets is too high to clearly examine enhanced efficacy. Therefore, the 10 IE/g dose, which in the setting of immunosuppression (Fig. 3B) leads to almost no improvement in glycemic control, serves as an ideal platform or minimal model from which to assess improved efficacy.

With this background, Ad.HGF gene delivery was strikingly effective, as shown in Figs. 5–9. Through the addition of Ad.HGF to the 10 IE/g model, glycemic control was far superior to that observed in diabetic control rats receiving normal or Ad.lacZ islets at a dose of 10 IE/g, and was comparable with otherwise completely normal rats treated with the Edmonton regimen (Figs. 1 and 9). Indeed, the outcome in these rats was far superior even to rats receiving 15 IE/g. Although it is difficult to quantitate the improvement in efficacy from these observations, one could reasonably argue that they are at least twice as effective as normal islets. These results are similar to those observed in the rat insulin promoter-HGF islet transplant model, where 250 rat insulin promoter-HGF islets functioned as well as 500 IE from normal islets (7). In addition, the improvement compared with control Ad.lacZ islets was long-lasting, up to 5 months. Given that the life span of a rat is 2 yr, this is approximately one fifth of the life span of a rat, and conceivably could correspond to 10–20 yr in a human. The long-term findings may also indicate that a self-renewing population of β-cells or their precursors has been established. Importantly, it seems likely that the duration of efficacy of the Ad.HGF therapy was limited by the diabetogenic effect of the immunosuppressive agents, and not by intrinsic failure of the Ad.HGF strategy, because SCID mice receiving HGF-expressing islet grafts, once normalized, do not appear to develop diabetes (7, 8). Thus, moving from calcineurin inhibitors to other immunosuppressive regimens in the future may permit even longer efficacy of the HGF effects.

We do not know from these studies which cells within the islet are transduced by the Ad.HGF virus, or which cells secrete HGF. We have previously reported that, in mouse islets infected with Ad.lacZ, approximately 30% of the islets are transduced (8), and we assume that this applies to the Ad.HGF-transduced islets as well. Importantly, because HGF is a secreted factor, and because the promoter used is the cytomegalovirus promoter, it is not necessary that the Ad.HGF transduce β-cells specifically. Any cell within the islet environment—endothelial cells, α-cells, fibroblasts, β-cells, ductal cells, or others—could in theory serve as a local producer of HGF.

Because the islets employed in the current study were delivered to the liver, one might ask whether the delivery of Ad.HGF to the liver might account for some of the improvement in glucose control observed. We believe that this is not a liver-specific effect, for the same degree of improvement in glycemia was observed when the Ad.HGF-transduced islets were delivered to the renal capsule in the SCID mouse model described previously (8). Moreover, the efficacy of the Ad.HGF appears to result from direct effects on the islet graft and not on the residual β-cells in the host pancreas, because we have previously demonstrated in the mouse renal capsule graft model that unilateral nephrectomy completely reverses the effect (7, 8).

As described in the introductory section, HGF stimulates β-cell proliferation, enhances β-cell survival, and improves β-cell function (glucose-stimulated insulin secretion). We cannot determine from the studies described herein which of these potential mechanisms, or what combination, is responsible for the enhanced engraftment and function observed.

In summary, adenoviral delivery of HGF enhances the function of transplanted islets in vivo in an Edmonton rat model that faithfully captures the key elements of human islet transplantation: portal delivery of islets, allograft immunity, immunosuppression, β-cell toxicity, and insulin resistance. Further studies are necessary to determine whether
this approach can be applied to human islets, to accurately quantitate the extent of improvement, to optimize the conditions of exposure to Ad.HGF, to identify more effective and less toxic immunosuppressive strategies, and to identify additional factors such as epidermal growth factor, betacellulin, glucagon-like peptide-1, PTHrP, or others that may display similar effects in this system, or that may act synergistically with HGF to further enhance islet graft performance.

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