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

JUAN CARLOS LOPEZ-TALAVERA, ADOLFO GARCIA-OCAÑA, IAN SIPULA, KAREN K. TAKANE, IRENE COZAR-CASTELLANO, AND ANDREW F. STEWART

Division of Endocrinology and Metabolism, University of Pittsburgh School of Medicine, Pittsburgh, Pennsylvania 15213

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 intraportally; 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)

HUMAN PANCREATIC ISLET allograft transplantation has recently received attention because of advances in the isolation of human islets, in the survival of pancreatic allografts, and in the immunosuppression regimens employed in preventing allograft rejection (1, 2). One important advance achieved by the so-called “Edmonton protocol” is the reduction or avoidance of β-cell toxic and insulin resistance-inducing immunosuppressive agents such as glucocorticoids and high-dose tacrolimus (FK-506) (1–3). One of the main barriers to widespread application of the Edmonton protocol is the limited availability of human pancreatic islets.

Hayek, Otonkoski, and colleagues (4, 5) have demonstrated that the addition of hepatocyte growth factor (HGF) to rodent or human islets in culture induces islet cell proliferation. We have shown that, when delivered to the pancreatic β-cell of transgenic mice, HGF increases β-cell number, islet number, islet size, and overall islet mass (6, 7). Moreover, HGF gene transfer increased the expression of three key β-cell genes, glucokinase, Glut-2 (the β-cell glucose transporter), and insulin (6, 7). These coordinated events led to enhanced glucose-stimulated insulin secretion in vitro, mild fasting hypoglycemia, and superior glucose tolerance in HGF transgenic mice compared with their normal littermates (6). More recently, we have demonstrated that delivery of HGF using adenovirus to otherwise normal murine islets markedly improves the outcomes and function of islets transplanted into diabetic, immunodeficient, severe combined immunodeficiency (SCID) mice (8). In that study, we also demonstrated that HGF has potent prosurvival effects on transplanted murine islets (8). This prosurvival effect of HGF is mediated by phosphatidylinositol 3-kinase (8). Thus, HGF appears to be an ideal growth factor for enhancing islet transplant outcomes: it increases β-cell proliferation (4–7); enhances β-cell survival in a transplant setting (8); increases the expression of glucokinase, Glut-2, and insulin, and thereby enhances glucose-stimulated insulin secretion (7); and has demonstrated efficacy in two transplant models in mice (7, 8).

Our prior studies were performed in immunodeficient mice, in which allograft, immunosuppressant, and adenoviral immunological problems with islet allograft gene therapy were attenuated or not operative. The main question addressed by the current study was the following: What is the efficacy of adenoviral delivery of HGF in a setting that more closely resembles that which occurs in humans? We therefore developed a rat model that mimics several key features of the currently employed Edmonton protocol. First, we employed an allogeneic system, using Lewis rat islets as donors and Sprague Dawley rats as recipients. Second, we employed the portal delivery of islets, as is employed in human islet transplants (1, 2), instead of the renal subcapsular graft we have employed in prior studies (7, 8). Third, we used the same glucocorticoid-free combination of immunosuppressive agents

Abbreviations: HBSS, Hanks’ buffered saline solution; HGF, hepatocyte growth factor; IE, islet equivalent; SCID, severe combined immunodeficiency; STZ, streptozotocin.

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467
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 (Charles River, 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 RPMI 1640 medium (Life Technologies, Inc.) supplemented with 10% fetal calf serum, and 25 mmol/liter 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 isofluorane 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 intravenously; 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).
2). These findings indicate that the addition of tacrolimus to the sirolimus-plus-daclizumab combination results in a failure of $\beta$-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, Lewis rat islets at a dose of 15 IE/g were portally transplanted into STZ-diabetic Sprague Dawley rats randomly assigned to receive either the Edmonton agents or no immunosuppressive drugs. As can be seen in Fig. 3A, rats not receiving immunosuppression progressed transiently toward normal blood glucose levels for approximately 1 wk, and then became diabetic, consistent with rejection by the Sprague Dawley rats of the allogeneic Lewis islets. In contrast, islet allografts in the setting of the three-drug immunosuppression regimen functioned until the end of the experiment on d 25. These results indicate that the Edmonton immunosuppression regimen was indeed effectively immunosuppressive.

However, an additional goal was to develop a platform or minimal model in which glucose control was sufficiently poor that we could observe clear improvements with HGF gene therapy from a diabetic baseline. In the 15 IE/g model shown in Fig. 3A, the blood glucose concentrations were so close to normal that an improvement would be difficult to clearly discern. To develop a platform from which a clear improvement 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.

**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 $\beta$-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 is easily visible in Ad.HGF-transduced islets, and by 72 h, HGF protein expression is increased further. In contrast, as shown, neither islets infected with Ad.lacZ nor uninfected islets displayed visible HGF expression under these conditions. Collectively, these results indicate that Ad.HGF transduction at the multiplicity of infection and under the conditions employed can lead to expression of HGF in rat 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.

Histologic sections of liver from Ad.HGF-transduced islet allografts are shown in Fig. 7. This figure illustrates several
points. 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-
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.

FIG. 8. Quantitation of the number of islets per liver section in the
Ad.HGF vs. Ad.lacZ livers at d 25. The number of islets was signif-
icantly higher in the livers that received Ad.HGF-transduced islets.
These numbers underestimate the true total mass of insulin-produ-
ing cells present within the livers, because islet size was not taken into
account.

FIG. 9. Long-term function of islet grafts and normal islets as as-
sumed using random blood glucose values in normal Sprague Dawley
rats or Sprague Dawley rats transplanted with Lewis islets, all
mixed 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 regi-
men 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 immunosup-
pressive 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 im-
munosuppressive 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 mel-
itus over the long term. Equally importantly, we have de-
vloped 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 cur-
cently 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 hu-
mans; 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 im-
unosuppressive agents; and 7) it allows the assessment of
the efficacy of adeno virus as a gene delivery vector. A suc-
cessful 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 enhance-
ment 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, adeno virus-
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 ana-
phylaxis, as well as host immune system-driven destruction
of the target cells that express adenoviral proteins. Moreover,
adeno virus 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 con-
siderations in some settings, in the relatively unique setting
of islet transplantation, they may be positive attributes. Be-
cause islet transplantation requires immunosuppression, im-
mune 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 trans-
plantation (17), transient expression of an agent that pro-
motes 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 ex vivo, the viral load delivered to

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the patient would be small, reducing concerns regarding anaphylaxis. Finally, immunosuppression would 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 cells 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 con-
ditions of exposure to Ad.HGF, to identify more effective and
less toxic immunosuppressive strategies, and to identify ad-
ditional 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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Address all correspondence and requests for reprints to: Andrew F.
Stewart, M.D., Division of Endocrinology, BST E-1140, University
of Pittsburgh School of Medicine, 3550 Terrace Street, Pittsburgh, Pennsylva
nia 15213. E-mail: stewart@mxs.dept-med.pitt.edu.

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