Stimulated Endocrine Cell Proliferation and Differentiation in Transplanted Human Pancreatic Islets

Effects of the *ob* Gene and Compensatory Growth of the Implantation Organ

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Neogenesis is crucial for the maintenance of β-cell mass in the human pancreas and possibly for the outcome of clinical islet transplantation. To date, no studies have reported a stimulation of human β-cell neogenesis in vivo. Therefore, we investigated whether human α-, β-, and duct cell growth can be stimulated when human islets are xenotransplanted to obese hyperglycemic-hyperinsulinemic *ob/ob* mice immunosuppressed with anti-lymphocyte serum. Moreover, we wanted to study whether β-cell growth and duct-to–β-cell differentiation were induced in the hepatocyte growth factor (HGF)-dependent compensatory kidney growth model. For that purpose, we evaluated human islets grafted to nude (*nu/nu*) mice before uninephrectomy of the contralateral kidney for DNA-synthesis and duct cell expression of the β-cell–specific transcription factor Nkx 6.1 as an estimate of differentiation. Human islet grafts were well preserved after 2 weeks when transplanted to *ob/ob* mice during anti-lymphocyte immunosuppression. Both human β-cells ($P < 0.01$) and duct cells ($P < 0.001$) were growth stimulated when islets were transplanted to *ob/ob* mice. We also observed a correlation between increased duct cell proliferation and increased organ donor age ($P = 0.02$). Moreover, duct ($P < 0.05$) and β-cell ($P < 0.05$) proliferation, as well as duct cell Nkx 6.1 expression ($P < 0.05$), were enhanced by the compensatory kidney growth after uninephrectomy. We conclude that it is possible to stimulate human β-cell neogenesis in vivo, provided that the recipient carries certain growth-stimulatory traits. Furthermore, it seems that duct cell proliferation increases with increasing organ donor age. Altogether, these data and previous results from our laboratory suggest that human β-cell neogenesis becomes more dependent on differentiation and less dependent on proliferation with increasing age. *Diabetes* 50:301–307, 2001

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ANOVA, analysis of variance; CBR, cell birth rate; GLP-1, glucagon-like peptide 1; HGF, hepatocyte growth factor; ICC, islet-like cell cluster; LI, labeling index; MALS, mouse anti-lymphocyte serum.

Human β-cell neogenesis (i.e., differentiation from precursor cells and proliferation from preexisting β-cells) is crucial for the maintenance of β-cell mass in the native human pancreas and possibly for the outcome of clinical islet transplantation. Due to the limited supply of human islets for transplantation, any possibility to stimulate the growth and/or the differentiation of β-cells would be of interest. We and others have previously reported that adult human β-cells have a limited capacity to proliferate both in vitro and in vivo (1–3). Because glucose was found to have a stimulatory effect on β-cell proliferation (3,4), investigators have extensively searched for other possible β-cell growth-stimulating agents (5–7). Also, there are a few animal models with extensive islet cell growth, and one of these is the obese hyperglycemic-hyperinsulinemic *ob/ob* mouse, which has an abundance of enlarged native islets (8). The growth factors involved in this model have not been characterized in detail, although it has been suggested that glucose, insulin, glucagon-like peptide 1 (GLP-1), and C-peptide play a role (4,9,10). We have previously observed that this islet enlargement is also induced when islets isolated from lean mice have been transplanted to obese recipients (11,12). In the present study, we investigated whether human α-, β-, and duct cell growth could be further stimulated in vivo, when human islets were transplanted to immunosuppressed obese hyperglycemic-hyperinsulinemic *ob/ob* mice and followed for 2 weeks before killing and evaluating DNA-synthesis.

Presently, the hepatocyte growth factor (HGF) is the most interesting factor in the context of human β-cell growth. In vitro, HGF has been shown to effectively stimulate human fetal β-cell proliferation (13). It has also been reported to stimulate the proliferation of adult human β-cells (2) or the islet-associated ductal cells, which may represent a population of endocrine precursor cells (13). Beattie et al. (14) showed that HGF stimulates human β-cell proliferation, but the cells de-differentiate to pancreatic duodenal homeobox gene 1 (Pdx-1)-positive cells not expressing insulin. Such a de-differentiation of β-cells has also been observed during stimulation with other growth factors (5,6) and is, therefore, important to overcome. HGF also plays an important

role in organ regeneration after injury (15,16). Previously, we have shown that such compensatory growth processes during organ regeneration stimulate pancreatic islet cell growth. Thus, the growth of mouse islets transplanted to liver or kidney before partial hepatectomy or uninephrectomy is considerably stimulated by the organ resection (17). Because HGF seems to primarily have de-differentiating effects on human β-cells in vitro (14), in contrast to actually increasing the β-cell number in HGF overexpressing mice (18), we also studied whether human β-cell de-differentiation is overcome in vivo, in the HGF-dependent (16) compensatory kidney growth model. Therefore, we transplanted human islets to nude (*nu/nu*) mice and performed uninephrectomy 2 weeks after transplantation. These mice were killed 1–5 days thereafter, and the islet grafts were evaluated for DNA synthesis and the presence of β-cell precursors, i.e., cells positive for both the ductal cell marker cytokeratin 19 and the β-cell–specific transcription factor Nkx 6.1 (19).

RESEARCH DESIGN AND METHODS

Islet isolation and culture. Human islets were isolated from 29 heart-beating organ donors (21 for the *ob/ob* study and 8 for the nephrectomy study) at the Central Unit of the β-cell Transplant, Brussels, Belgium, and were transported by air to Uppsala, Sweden. The age of the organ donors (mean ± SE) was 39 ± 3 years (range 8–59). Islet isolation and characterization by light and electron microscopy regarding cell viability (95 ± 0.6%) and cellular composition (52 ± 2.2% insulin positive cells, 14 ± 1.7% glucagon positive cells, 29 ± 2.6% nongranulated cells, i.e., predominantly duct cells, and 1.1 ± 0.8% exocrine cells) were performed in Brussels as previously described (20). On arrival in Uppsala, islets were kept in culture in RPMI-1640 medium supplemented with 5.6 mmol/l glucose, 10% fetal calf serum, 0.17 mmol/l benzylpenicillin, and 0.17 mmol/l streptomycin (a favorable culture condition for human islets) (20,21) for 4–5 days before further experiments were performed.

Porcine islet-like cell clusters (ICC) were prepared by collagenase digestion from pancreas anlage of 68- to 74-day pig fetuses (full term ~115 days) as described in detail elsewhere (22). After repeated washings, the digest was resuspended and cultured in RPMI-1640 medium (11 mmol/l glucose) supplemented with 10% heat-inactivated pooled human serum (The Blood Center, Huddinge Hospital, Huddinge, Sweden), 10 mmol/l nicotinamide, 0.17 mmol/l benzylpenicillin, and 0.17 mmol/l streptomycin for 4–5 days before transplantation.

Animals. Male lean (+/?) or obese *ob/ob* littermates of both sexes (C57Bl/6 local colony; Biomedicum, Uppsala, Sweden) or male nude *nu/nu* mice (C57Bl/6J; Bomholtgaard, Ry, Denmark) 3–6 months of age were used as islet graft recipients. In the *ob/ob* mice and their littermates, body weight, blood glucose, and serum insulin concentrations were measured the day before transplantation and on the day of killing. Glucose concentrations were analyzed by means of a glucometer (ExacTech; MediSense Sverige, Gothenburg, Sweden) and insulin concentrations by means of a radioimmunoassay (Pharmacia, Uppsala, Sweden).

Immunosuppression. To develop a suitable immunosuppressive regimen for *ob/ob* mice, as well as their littermates, lean C57Bl/6J mice were transplanted with porcine ICC or human islets as detailed below. In the initial studies, porcine ICCs were used because of the limited supply of human islets. Mouse anti-lymphocyte serum (MALS) (Accurate Chemical and Scientific, Westbury, NY), 0.2 ml per animal, titrated on the basis of animal mortality, was injected intraperitoneally the day before transplantation (day –1) and then at different time points during the observation period (Table 1). The grafts were removed and processed for histology, and rejection was evaluated with a semiquantitative ranking after hematoxylin-eosin staining, where 0 represents total rejection with only connective tissue and mononuclear cells present, 1 represents fulminant rejection with massive mononuclear cell infiltration and with very few endocrine or epithelial cells left, 2 represents some endocrine or epithelial cells left with a clear mononuclear cell infiltration, 3 represents mainly an intact graft, but with some mononuclear cells present, and 4 represents no sign of rejection.

Islet transplantation. A graft consisting of ~0.6 µl human islets or 1 µl porcine ICC was transplanted to the left renal subcapsular space of lean or *ob/ob* mice as previously described (23). Either 2 or 4 weeks after islet transplantation, the recipients were injected intraperitoneally with 1 µCi/g body wt (200 µl) ^{3}H-thymidine (Amersham Pharmacia Biotech, Uppsala, Sweden) and killed 2 h later by cervical dislocation. The graft-bearing kidneys were dissected, fixed in formalin, embedded in paraffin, and prepared for histology as previously described (3). The *nu/nu* mice received a human islet graft of 0.3–0.6 µl under the renal capsule of the left kidney. Then, 2 weeks posttransplantation, these mice were anesthetized again, and the right kidney was extirpated as described elsewhere (17). Sham-operated controls were anesthetized, opened, and the kidney was handled without being removed. Between 1 and 5 days after the nephrectomy, the animals were injected with ^{3}H-thymidine, killed 2 h later, and the remaining kidney was prepared for histological evaluation as previously described. The sham-operated controls were killed on either the same day after surgery as the nephrectomized animals or on day 5.

Immunohistochemistry and autoradiography. Sections of grafts were stained for insulin (antibovine insulin; ICN, Irvine, CA), glucagon (antiporcine glucagon; NOVO, Copenhagen) or the human pancreatic duct cell marker cytokeratin 19 (24) (Dako, Glostrup, Denmark), the latter after pretreatment with 0.2% trypsin (Sigma, St. Louis, MO) in 0.2% $CaCl_2$ for 10 min. Antibody binding was detected with the LSAB system (Dako) giving a red color precipitate at the antigenic site. Wet slides were then dipped in 50% film-emulsion (autoradiography emulsion; Kodak, New York) in 0.75 mol/l ammonium acetate and stored in a light proof chamber to dry overnight. The films were then exposed for 3 weeks at 4°C before being developed and fixed and counter-stained with Mayer's hematoxylin. Sections were also stained for cytokeratin 19 in combination with Nkx 6.1 (anti–mouse gst-Nkx 6.1; obtained from Dr. O.D. Madsen, Gentofte, Denmark) after pretreatment with 0.1% pepsin (Sigma) in 0.1 mol/l HCl for 5 min and microwaving (500 W) for 5 min in 10 mmol/l citric acid (pH 6.0). Cytokeratin 19 antibody binding was detected with a biotinylated donkey anti-mouse antibody (Jackson ImmunoResearch, West Grove, PA) and the ABC method (Vectastain ABC-AP; Vector Laboratories, Burlingame, CA), which gave a blue color precipitate (Blue Alkaline Phosphatase Substrate Kit III; Vector) at the antigenic sites; Nkx 6.1 antibody binding was detected with a biotinylated donkey anti-rabbit antibody (Jackson ImmunoResearch); the ABC method (Vectastain ABC-HRP) and amino-ethylcarbazole (Sigma), which gave a brownish-red color precipitate.

Microscopical evaluation. Insulin-, glucagon-, and cytokeratin 19–positive cells were counted in a light-microscope (400×) and cells with ≥10 black-silver grains over the nuclei were considered to be in the S-phase of the cell cycle (25). There was a clear difference between background thymidine incorporation (generally <3 grains/nucleus) and labeled cells (≥10 grains/nucleus). During the examinations, the observer was unaware of the sample identity. The fraction of labeled cells at a certain time point was determined and expressed as a labeling index (LI) (number of labeled cells × 100/total number of cells). Because the S-phase of rat β-cells is 6.4 h (26), we believe that the 2 h labeling is short enough to show only the fraction of cells that are entering or have entered the S-phase at that moment. Nkx 6.1 and cytokeratin 19 double-positive cells were also counted in a light microscope, and state of differentiation was expressed as percent of Nkx 6.1–positive duct cells of all duct cells. To obtain accurate measurements of LI and state of differentiation, 4,757 ± 326 β-cells (n = 42), 2,788 ± 375 α-cells (n = 10), and 1,101 ± 147 duct cells (n = 30) in each graft were counted for the LI measurements, and 415 ± 59 (n = 22) duct cells were counted for the measurements of state of differentiation (n reflects both the number of donors and all of the experimental conditions combined).

Calculations. The cell birth rate (CBR), the production of new cells per 24 h, was calculated as previously described (26): $CBR = LI \times 24/s_t$, where s_t is the duration of the S-phase, i.e., 6.4 h. The cell-population doubling time (x), the time it would take for a cell population to double without any reduction in the cell number by cell death, was also calculated: $P = (1 + CBR)^x$, where P is the growth index, i.e., 2 (3).

Ethics. The local animal ethics committee approved all animal experiments, and the Project Management Group of the β-cell Transplant (Brussels), approved the use of human islets for the present study.

Statistical analysis. Data are means ± SE. One human pancreas donor was regarded as one observation, unless otherwise indicated. The paired experiments were analyzed using Wilcoxon's signed-rank test. The coefficients of correlations (R) were obtained using simple linear regression, and the statistical significances of correlations were evaluated using analysis of variance (ANOVA). Mouse characteristics were compared using ANOVA followed by group comparisons using paired Student's t test, and the P values were correlated for multiple comparisons using the Bonferroni method (27).

RESULTS

Immunosuppression. To develop an immunosuppressive regimen for successful islet xenotransplantation, we injected MALS intraperitoneally to lean C57Bl/6 mice grafted with

TABLE 1
Histological evaluation of renal subcapsular islet grafts immunostained for insulin (adult human) or hematoxylin/eosin (fetal porcine), 10–28 days after transplantation to lean C57Bl/6 mice

	Injection days with MALS						
	None		0	–1, 0, 1, 3, 5, 7		–1, 0, 1, 3, 5, 7, 9, 12, 14, 16, 18, 20, 22, 24, 26	
Day of killing	Porcine	Human	Porcine	Porcine	Human	Porcine	Human
10	0 ± 0	0 ± 0	0 ± 0	4 ± 0	4 ± 0	ND	ND
14	ND	ND	ND	ND	ND	2.0 ± 0.7	3.8 ± 0.2
20	ND	ND	ND	1.8 ± 0.4	ND	ND	ND
28	ND	ND	ND	0.2 ± 0.2	ND	1.0 ± 0.4	3.5 ± 0.3

Data are means ± SE (n = 4–12). Day 0 is the day of transplantation. ND, not determined.

porcine ICCs or human islets. Mice receiving no immunosuppression completely rejected the grafts (Table 1). With just one (Table 1) or a few (data not shown) intraperitoneal injections of 0.2 ml during the observation period, there were no signs of tolerance induction. However, a regimen with injections every day from the day before to the day after transplantation and then every second day resulted in an extensive graft preservation for 10 days of porcine ICC grafts and for 14 days of human islet grafts (Table 1 and Fig. 1). With this regimen, human islets were well preserved even up to 4 weeks after transplantation, whereas the porcine ICC grafts were not so well preserved beyond 10 days. The total mortality was low (11%) during the first 2 weeks, but increased to 30% during the following 2 weeks. Based on duration of graft survival in combination with recipient mortality data, we chose to use observation periods of 2 weeks for the cell proliferation studies.

Transplantations to *ob/ob* mice. The *ob/ob* mice were slightly hyperglycemic, severely hyperinsulinemic, and obese throughout the observation period (Table 2). During the 2-week observation period, there were no major changes in body weight or glycemic or insulinemic status due to the MALS regimen or the transplantation, although minor deviations in blood glucose (lean mice) and serum insulin (*ob/ob* mice) were observed (Table 2).

Both β-cell and duct cell proliferation rates were considerably increased in human islets transplanted to *ob/ob* mice when compared with data from the lean recipients (β-cell LI [n = 9–10] 0.10 ± 0.02 lean and 0.17 ± 0.03 obese; duct cell LI [n = 6] 0.57 ± 0.17 lean and 1.25 ± 0.22 obese). On the other hand, α-cell proliferation was unaffected (α-cell LI [n = 5] 0.17 ± 0.04 lean and 0.16 ± 0.03 obese). When paired comparisons were made between growth of cells grafted to obese and to lean recipients, these observations were statistically confirmed (Fig. 2). Moreover, duct cell proliferation seemed to correlate to the age of the human islet donors, i.e., there was an increased proliferation with increased donor age (P = 0.02), whereas β-cell proliferation tended to decrease with increased donor age, but the difference was not statistically significant (Fig. 3).

Influence of nephrectomy on β-cell neogenesis. In the *nu/nu* mice, human β-cell LI in the subcapsular islet grafts increased 3 days after contralateral nephrectomy and remained elevated 2 days later, when compared with values for the sham-operated controls (Fig. 4). Duct cell LI increased 1 day after nephrectomy, an increase that was further exaggerated 3 days after nephrectomy (Fig. 4). Differentiation of duct cells to cells expressing a β-cell–specific transcription factor was studied with double immunostaining for the duct cell marker cytokeratin 19 and the β-cell transcription factor Nkx 6.1 (Fig. 5). It was found that the frequency of Nkx 6.1–positive duct cells in the grafts was 2.2 ± 0.36% in sham-operated controls, 4.3 ± 1.2% 1 day after nephrectomy, 6.4 ± 1.4% (P < 0.05 vs. sham) 3 days after nephrectomy, and 7.0 ± 1.6% (P < 0.05 vs. sham) 5 days after nephrectomy (n = 4–7).

DISCUSSION

Discordant xenotransplantation of human islets to C57Bl/6 mice requires constant immunosuppression to achieve long-term survival of the grafted islets. Because administration of cyclosporine and steroids has been demonstrated to negatively affect islet cell function and proliferation (4,28,29), we wanted to use an immunosuppressive regimen without such side effects. Previously, anti-lymphocyte serum has been successfully used in allogeneic transplantation studies obtaining 2-week graft survival after one single injection (30). In concordant and discordant xenogeneic islet transplantation, multiple injections have been used with varying results (31,32). In the present study, we used MALS with low recipient mortality and good human islet graft preservation for at least 2 weeks. It is unlikely that the MALS treatment alone affects islet cell proliferation, because the antibodies are specific for lymphocytes and should not bind to other cell types.

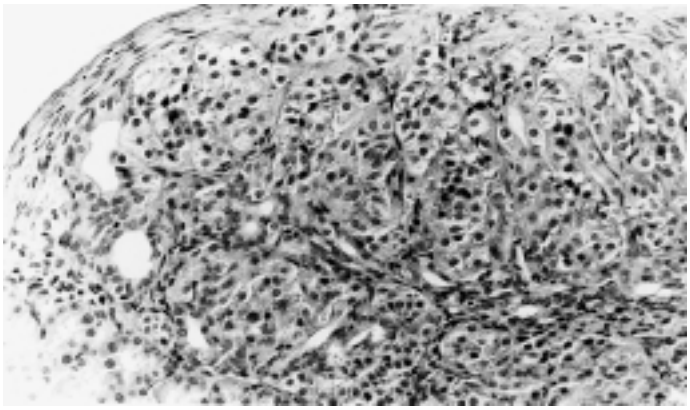

FIG. 1. Micrograph of a discordant xenograft of human islets 14 days posttransplantation to the renal subcapsular space of a C57Bl/6 mouse treated with 0.2 ml MALS intraperitoneally on days –1, 0, 1, 3, 5, 7, 9, and 12 (original magnification ×200).

TABLE 2
Body weight, blood glucose and serum insulin concentrations of the obese (ob/ob) and lean human islet graft recipients

	Body weight (g)		Blood glucose (mmol/l)		Serum insulin (μU/ml)	
	Day –1	Day 14	Day –1	Day 14	Day –1	Day 14
ob/ob	47.2 ± 1.6*	47.2 ± 2.53*	11.1 ± 0.8†	9.0 ± 0.8†	2,087 ± 372*	3,046 ± 579*‡
Lean	28.8 ± 0.6	28.0 ± 0.7	7.5 ± 0.3	6.4 ± 0.2‡	126 ± 16	154 ± 23

Data are means ± SE (n = 9–22). Day 0 is the day of transplantation. *Significant differences from lean mice at the same day (P < 0.001); †significant differences from lean mice at the same day (P < 0.01); ‡significant differences from –1 for the same type of mice (P < 0.05); ANOVA was followed by a paired Student's t test and Bonferroni's correction.

However, we cannot rule out that secondary effects from the immunosuppression might affect the islets in some way. Indeed, we observed minor changes in recipient blood glucose and serum insulin concentrations during the 2-week observation period, but this did not seem to antagonize the stimulation of islet cell neogenesis.

In the present study, we have demonstrated that stimulation of human β-cell neogenesis can be achieved not only in vitro (2,3,14), but also in vivo. Thus, both human β-cells and duct cells were growth stimulated when islets were transplanted to *ob/ob* hyperglycemic-hyperinsulinemic mice during MALS immunosuppression. Moreover, duct and β-cell proliferation, as well as duct cell differentiation, was enhanced by the compensatory kidney growth after uninephrectomy. An increased proliferation is expected to be accompanied by an increased volume of the cell population studied, provided that cell death is presumed to be negligible. The human islet graft volume 2 weeks after transplantation to *ob/ob* mice was estimated to be 4% greater than in grafts residing in lean littermates. Such calculations (and those performed below) were based on LI values from the present study combined with previously published islet cell cycle data (26) and formulas (3,26). It would not be possible to demonstrate this small increase with an estimated bias of ~10% in the original volume of the grafts at the day of transplantation. A measurable volume increase might have been induced by 4 weeks. However, it was not possible to carry out such studies because of the increased recipient mortality and the decreased immunosuppressive efficiency at that time (previously described). Nephrectomy leads to a pulse of growth-factor production and release for only a few days (33). Again, the short time-span of the growth stimulation probably induces only a minute increase that is difficult to assess in islet volume. However, if persistent, an LI increase of 100–500%, as seen in the obese or nephrectomized animals, should lead to an impressive increase of the β-cell mass. Thus, assuming β-cell growth could be constantly stimulated over time, the β-cell population doubling time (see RESEARCH DESIGN AND METHODS) would dramatically decrease (Fig. 6).

Concerning the underlying mechanisms of the presently demonstrated human β-cell growth stimulation, one might anticipate that the leptin deficiency of the *ob/ob* mice (34) might have beneficial effects on islet cell growth. To test this hypothesis, we aimed to transplant human islets to leptin receptor–deficient *db/db* mice that overexpress the functional leptin protein (35,36). However, the MALS regimen designed for the *ob/ob* mice was not applicable in the *db/db* mice. Thus, the overall survival of the recipients was markedly decreased, and the graft survival was markedly attenuated. However, it is likely that the metabolic syndrome in the *ob/ob* mice is responsible for the observed growth stimulation of human β- and duct cells. Besides the leptin deficiency, the combination of high serum concentrations of glu-

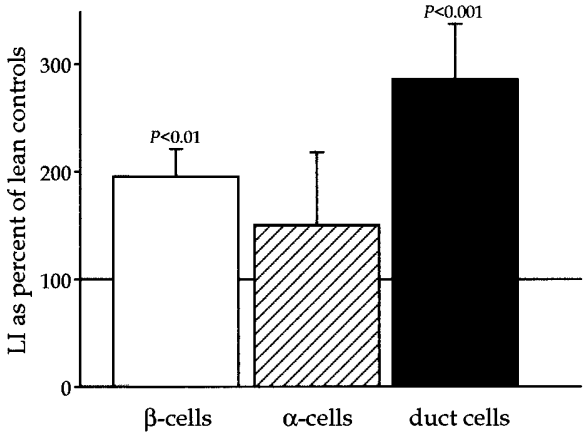

FIG. 2. LI of human β-, α-, and duct cells 14 days after transplantation to lean or obese *ob/ob* C57Bl/6 mice. LI expressed as percent of control (lean mice) and compared with paired Wilcoxon's signed-rank test.

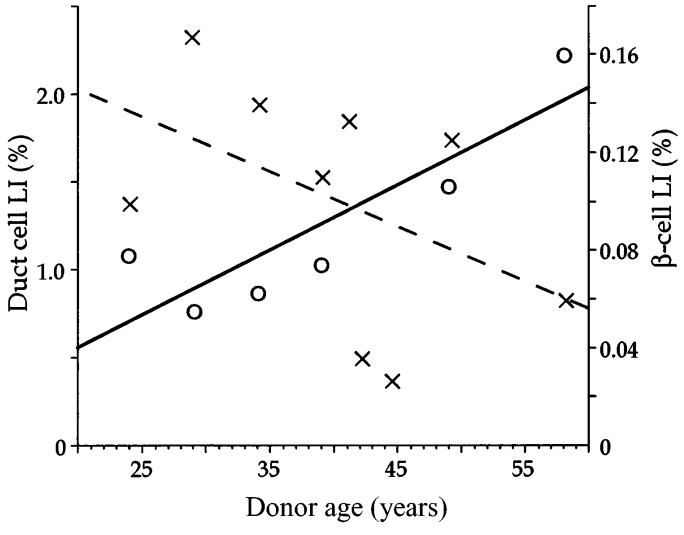

FIG. 3. Duct and β-cell LI correlated to age of the human islet donors 2 weeks after transplantation to C57Bl/6 mice immunosuppressed with MALS according to the regimen described in Table 1. The statistical significances of correlation were evaluated with ANOVA (○ with continuous line, duct cell LI versus age, R = 0.88, P = 0.02 and × with dashed line, β-cell LI versus age, R = 0.54, P = 0.1)

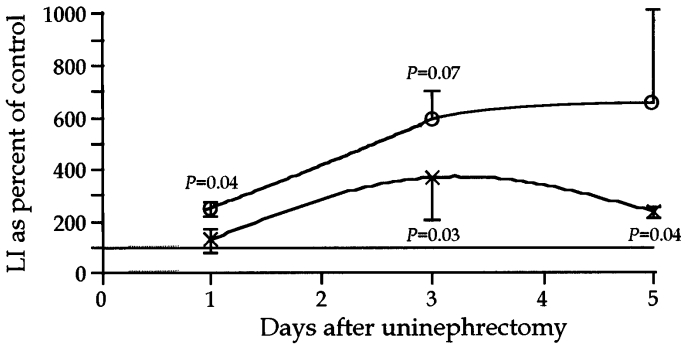

FIG. 4. LI of human β-cells (×) and duct cells (○) transplanted to the renal subcapsular space of *nu/nu* mice. Contralateral nephrectomy (uninephrectomy) was performed 2 weeks after transplantation, and the mice were killed 1, 3, or 5 days thereafter. LI is expressed as percent of sham-operated controls and compared with paired Wilcoxon's signed-rank test (in the controls, β-cells LI was 0.15 ± 0.03% and duct cell LI was 0.65 ± 0.10%; *n* = 3–8).

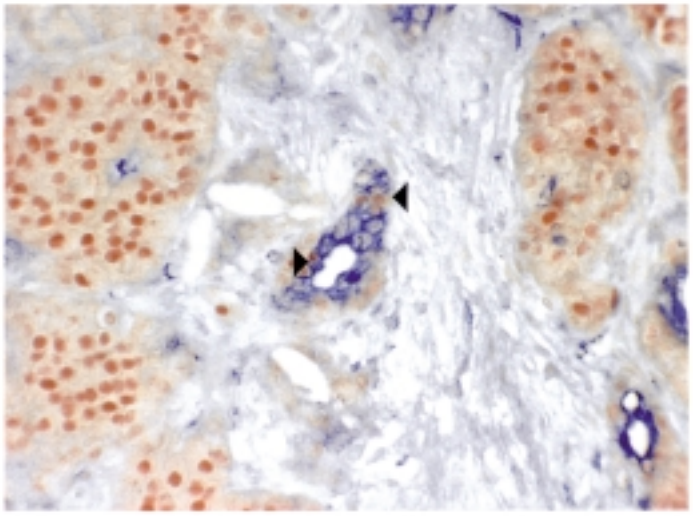

FIG. 5. Part of a human islet graft 3 days after nephrectomy, with some cells (▲) stained for both cytokeratin 19 (blue) and Nkx 6.1 (red). Original magnification ×200.

cose (4) and insulin (9), perhaps combined with overproduction of proposed growth factors such as GLP-1 and C-peptide (10), are likely to be of importance. The *ob/ob* environment might also have affected the differentiation state of the duct cells. However, due to the lack of specimens, this was not possible to assess.

Shortly after an organ injury, a regeneratory process is initiated in which both hyperplasia and hypertrophy of different cell types continue for several days (15,37). Under such circumstances, HGF is of great importance, particularly in kidney and liver regeneration (15,16). Thus, after injury (partial hepatectomy, uninephrectomy, or toxin-induced injury), HGF is rapidly mobilized from the extracellular matrix of stromal and epithelial cells (38,39), followed by 6–12 h postnephrectomy upregulation of HGF mRNA transcription and translation (40). The HGF receptor (c-Met) is known to be expressed in various cells of epithelial origin, among other pancreatic islets (13) and duct cells (41). The growth stimulatory effects of HGF on both fetal and adult human islet cells have been scrutinized in vitro (2,13,14,42,43). However, the present report is the first to suggest that a process involving HGF upregulation (16) in vivo is associated with a stimulation of human islet cell growth and differentiation.

Nevertheless, it is worth noting that in the compensatory kidney growth after nephrectomy, HGF is not the only growth factor involved. For instance, insulin-like growth factors, vascular endothelial growth factors, platelet-derived growth factors, neural growth factors, fibroblast growth factors, and transforming growth factor α are also produced and released locally by different cells in the kidney (33). All of these may stimulate β- or duct cell proliferation and/or differentiation (5–7,13,44). Although the effects of some of these growth factors have only been studied in β-cell lines or fetal/neonatal islets, it is likely that this complex picture of growth stimulants causes the accelerated adult human β-cell neogenesis observed after nephrectomy.

Interestingly, the relation between duct cell proliferation and the age of the organ donors seems to be the reverse of that of β-cells. In the present report, human pancreatic duct cells showed an increased proliferative capacity when the organ donor age increased, whereas human β-cells revealed a decreased capacity to proliferate when the organ donor age increased. The latter could not be statistically confirmed in this study, probably due to a small number of observations. However, in a previous study we showed a clear correlation between decreased human β-cell growth and increased donor age (3). Nevertheless, these observations might suggest that human β-cell neogenesis is becoming more dependent on differentiation and less dependent on proliferation in older ages, as duct cell proliferation seems to parallel the differentiation of duct cells to β-cells (45). The latter is confirmed in the present study by an increased number of duct cells expressing the β-cell–specific transcription factor Nkx 6.1 (19) after nephrectomy. There is a possibility that the observed increase in Nkx 6.1 staining could instead reflect a de-differentiation process of the human β-cells toward duct cells, as suggested by Yuan et al. (46). However, we could not find any cytokeratin 19/Nkx 6.1–double positive cells scattered

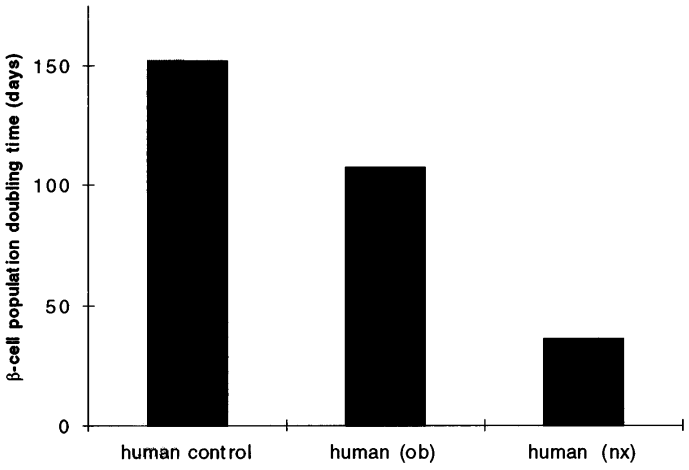

FIG. 6. Estimated β-cell population doubling times (see RESEARCH DESIGN AND METHODS). Average β-cell LIs were used from human islets transplanted to lean C57Bl/6 or sham-operated *nu/nu* mice (human control LI 0.12%), *ob/ob* C57Bl/6 mice (human [*ob*] LI 0.17%), and *nu/nu* mice 3 days after nephrectomy (human [nephrectomy {nx}] LI 0.53%). The estimations were based on the presumption that these LIs were constant over time.

among the β-cells, which most likely would have been the case if the β-cells were de-differentiating. Instead, the ductal cells were consistently found in duct-like clusters.

In summary, we have demonstrated that human pancreatic α-, β- and duct cells display signs of active proliferation in vivo when transplanted to MALS immunosuppressed C57Bl/6 mice or *nu/nu* mice. Moreover, human duct and β-cell proliferation is enhanced after transplantation to *ob/ob* mice and when these cells are situated in the remaining kidney of *nu/nu* mice undergoing contralateral nephrectomy. Finally, human duct to β-cell differentiation is also enhanced in the latter transplantation model. Although human β-cell proliferation is quite low compared with the growth rate of most cell types, the proliferative capacity observed in this study might have significant effects on the outcome of clinical islet transplantations, provided that proper methods can be developed to transfer the beneficial effects of these models to human islet grafts.

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