Diabetologia (2001) 44: 2066–2076

Diabetologia

Blood glucose normalization upon transplantation of human embryonic pancreas into beta-cell-deficient SCID mice

M. Castaing[1], **B. Péault**[2], **A. Basmaciogullari**[1], **I. Casal**[2], **P. Czernichow**[1], **R. Scharfmann**[1]

[1] INSERM U457, Hospital R. Debré, Paris, France
[2] INSERM U506, Hospital Paul Brousse, Villejuif, France

Abstract

Aims/hypothesis. Transplanting human pancreatic islet beta cells could represent a radical new treatment of Type I (insulin-dependent) diabetes mellitus. However, beta cells available for grafting are scarce and finding new sources of such cells would be crucial for any cell therapy for diabetes. Undifferentiated precursor cells present in the human embryonic pancreas could represent such a source.
Methods. We grafted human embryonic pancreases (6–9 weeks of development) that contain very few beta cells onto NOD/*scid* mice.
Results. The human pancreatic tissue grew, increasing in weight 200 times within six months and endocrine cells differentiated, the number of human beta cells being increased by a factor 5000. Finally, the developed human endocrine tissue was mature enough to control the glycaemia of mice deficient in endogenous beta cells.
Conclusion/interpretation. Human embryonic pancreas represent a source of immature cells that can proliferate and differentiate into mass beta cells after transplantation. Transplantation of human embryonic pancreas into NOD/*scid* mice is a useful model for understanding the development of the human pancreas during prenatal life. [Diabetologia (2001) 44: 2066–2076]

Keywords Pancreas, human, embryonic, development, growth, differentiation, islet cells.

Type I (insulin-dependent) diabetes mellitus is due to the destruction by immune mechanisms of pancreatic beta cells, resulting in a lack of insulin production and hyperglycaemia. Cell therapy using beta cells from donors could represent a cure for diabetic patients. However, two major obstacles remain. Firstly, immunosuppressive protocols have to be designed to provide immunologic protection of the graft. Recent reports indicate that progress has been made in this field [1]. Secondly, alternative sources of beta cells have to be found because of the small number of mature beta cells from donors that are available for grafting [2]. During the last few years, it has been proposed that new beta cells for cell Type I diabetes therapy could be produced by understanding and recapitulating beta cell development that occurs during embryonic and fetal life. Huge efforts and progress have thus been made to define the molecular mechanisms that control prenatal pancreatic development in rodents and to clarify the role of specific transcription and growth factors [3–8]. Different tissue sources potentially rich in precursor cells are also currently being tested for their ability to differentiate into mature beta cells. Such cells come either from fetal or neonatal porcine pancreas [9, 10] or from fractions of human adult pancreas enriched in duct cells that are thought to contain precursor cells [11]. New insulin-expressing cells could be generated from these tissues [9–11].

Received: 12 June and in revised form: 17 July 2001

Corresponding author: R. Scharfmann, INSERM U457, Hospital R. Debré, 48 Bd Sérurier, 75019 Paris, France,
e-mail: scharfma@infobiogen.fr
Abbreviations: WD, Weeks of development; NOD/scid, non-obese diabetic/severe combined immunodeficiency; BrdU, 5-bromo-2-deoxyuridine; PC1/PC3, proconvertase 1/proconvertase 3

In the past, prenatal human pancreases have been tested to see if they could be used as a potential source of tissue to produce beta cells. Such prenatal tissues have been extracted from fetuses at late stages of development and were thus already quite mature when used in different assays [12–16]. In this study, we specifically used immature human embryonic pancreases at 6–9 weeks of development. At these stages of development, such embryonic tissues contained no or very few insulin-expressing cells [17]. We here showed that when human embryonic pancreases were grafted onto immuno-incompetent mice, the pancreatic tissue grew with its weight increasing 200 times within six months. At the same time, endocrine cell differentiation occurred, the absolute number of human beta cells being increased by a factor of 5000. Finally, the endocrine tissue that developed was functional, being able to regulate the glycaemia of mice deficient in rodent beta cells.

In conclusion, we demonstrate that functional human beta cells can develop in NOD/*scid* mice from immature human embryonic pancreases. This model of human embryonic pancreases in NOD/*scid* mice that mimics the ontogeny of the human pancreas that occur in vivo can now be used to study the mechanisms that control the development of the human embryonic pancreas, a study that has been difficult until now due to the lack of proper experimental systems. Finally, our data indicate that human embryonic pancreases represent a source of immature cells that can proliferate and differentiate in mass into beta cells when transplanted into an adult animal. This tissue could thus be useful as an alternative source of beta cells for transplantation.

Materials and methods

Human tissues. Human pancreases were extracted from embryonic tissue fragments obtained immediately after voluntary abortions performed mechanically by aspiration between 6 and 9 weeks of development (WD), in compliance with the current French legislation and the guidelines of our institution. The warm ischemia time was less than 30 min. Gestational ages were determined from several developmental criteria: duration of amenorrhea; crown-rump length measured by ultrasound scan; hand and foot morphology. Pancreases were dissected. Six pancreases were directly fixed and embedded in paraffin. Forty-eight pancreases were grafted onto non-obese diabetic/severe combined immunodeficiency (NOD/*scid*) mice as described below. These studies were approved by the French animal ethics committee.

Animals and transplantation into NOD/SCID mice. NOD/*scid* mice were bred in isolators supplied with sterile-filtered, temperature-controlled air. Cages, bedding and drinking water were autoclaved. Food was sterilized by X-ray irradiation. All manipulations were performed under a laminar flow hood. Embryonic pancreases (6–9WD) were implanted (1 pancreas for each transplantation), using a dissecting microscope, under the left kidney capsule of 6- to 8-week-old NOD/*scid* mice that had been anaesthetized with Hypnomidate (Janssen-Cilag). At different time points after the graft (7 days – 9 months), mice were killed and the grafts were removed, weighed, fixed in formalin 3.7% and embedded in paraffin. For cell proliferation analysis, mice were injected with BrdU (50 mg/kg) 2 h before being killed.

Immunohistochemistry. Four μm-thick sections were cut on gelatinized glass slides. For immunostaining, sections were deparaffinized in toluene, rehydrated, microwaved in citrate buffer 0.01 mol/l, pH 6, and permeabilized for 20 min in TRIS-Buffered Saline (TBS) containing 0.1% Triton. Non-specific sites were blocked for 30 min in TBS containing 3% BSA and 0.1% Tween 20 and sections were incubated overnight at 4 °C with primary antibodies. The sections were then washed and incubated for 1 h at room temperature with the appropriate secondary antibodies, labelled with two different fluorochromes. The primary antibodies were: mouse anti-human insulin (Sigma Aldrich, St Quentin Fallavier, France, 1/1000); guinea pig anti-pig insulin (Dako, Trappers, France, 1/2000); mouse anti-human glucagon (Sigma Aldrich, 1/2000); rabbit anti-human pan-cytokeratin (Dako, 1/500); mouse anti-human cytokeratin 19 (Dako; 1/50); mouse anti-pig vimentin (Dako; 1/30); rabbit anti-proconvertase 1/3 (gift from Dr Steiner, Chicago, USA, 1/200); rabbit anti-Pax 6 (gift from Dr S. Saule, Lille, France); rabbit anti-rat Nkx 6.1 (gift from Dr Serup, Gentofte, Denmark); mouse anti-BrdU (Amersham, Saclay, France). Fluorescent secondary antibodies were: fluorescein-anti-guinea pig antibodies (Dako, 1:500); fluorescein-anti-rabbit antibodies (Immunotech, 1/200); fluorescein-anti-mouse antibodies (Immunotech, 1/200); Texas-red-anti-mouse antibodies (Jackson, 1/200); Texas-red-anti-rabbit antibodies (Jackson, 1/200).

Surface quantification and statistical analysis. All images were numerized using a Hamamatsu C5810 cooled tri-CCD camera. Pictures were made at the same magnification and analysed with the IPLab software (version 3.2.4, Scananalytics). For each transplanted tissue, sections were taken at regular intervals throughout the graft and stained for insulin. Altogether 3–4 sections and 3–5 views were analysed for each section. The evolution of the beta-cell mass during the transplantation period was calculated as the product of the surface that stained positive for insulin by the corresponding graft weight.

In situ hybridization. For in situ hybridization, sections were deparaffinized, rehydrated and permeabilized in PBS containing 1% Triton X-100. Prehybridization was done at 70 °C in hybridization buffer (50% formamide, 5X SSC, 5X Denhardts' solution, 250μg/ml yeast RNA, 500μg/ml herring sperm DNA). RNA probes were labelled with DIG-UTP by in vitro transcription using the DIG-RNA labelling kit (Boehringer Mannheim). Hybridization was initiated by the addition of fresh hybridization buffer containing 1μg/ml probe and continued overnight at 70 °C. Thereafter, the slides were washed with decreasing concentrations of *SSC*. Revelation was processed by immunohistochemistry. Non-specific sites were blocked with 2% blocking reagent (Boehringer Mannheim) in TRIS 25 mmol/l pH7.5, NaCl 140 mmol/l, KCl 2.7 mmol/l Tween 20 0.1% for 30 min at RT. Slides were then incubated overnight at 4 °C with alkaline phosphatase-conjugated polyclonal sheep anti-DIG antibody (diluted 1:1000, Boehringer Mannheim). The reaction product was visualized by an enzyme-catalysed colour reaction using nitro-blue tetrazolium and 5-bromo-4-chloro-3-indolyl-phosphate medium (Bohringer Mannheim). Sections were incubated until the coloured reaction product developed at the sites of hybridization. The slides were washed

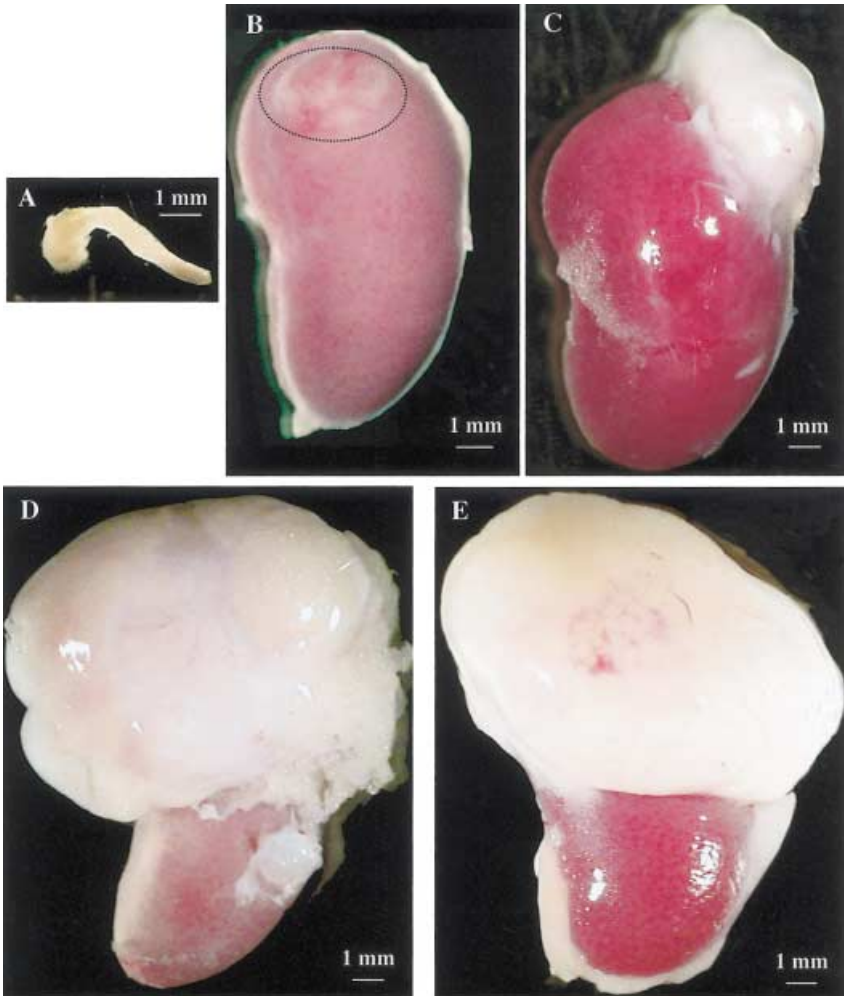


Fig. 1. Development of the human pancreas in NOD/*scid* mice. **A** A pancreas at 8 weeks of development before transplantation. **B–E** The pancreata were grafted under the kidney capsule of NOD/*SCID* mice and analysed 7 days (**B**), 2 months (**C**), 6 months (**D**), and 9 months (**E**) later

in H_2O, mounted and visualized on a Leitz DMRD light microscope (Leica, Heerbrugg, Switzerland). The probe used here corresponded to human proinsulin.

Test of induction of diabetes. To determine the capacity of the graft to regulate the glycaemia of the mouse, grafted and non-grafted NOD/*scid* mice were injected intravenously with alloxan (Sigma-Aldrich, 90 mg/kg body weight), which is known to destroy rodent, but not human, beta cells [18]. Glucose concentrations were measured on blood collected from the tail vein every day during one week, using a portable glucose metre (GlucoMen, A. Menarini diagnostics, Florence, Italy). To confirm the contribution of the graft to the normalization of blood glucose values in the host, grafts were removed by unilateral nephrectomy at different time points (7 days or 42 days) after the injection of alloxan and blood glucose concentrations were measured.

Results

Human embryonic engraftment in NOD/scid mice. In this study, 48 embryonic pancreatic tissues (6–9WD) were grafted onto NOD/*scid* mice. Mice were killed at different time points after transplantation (7 days-9 months) and the grafts were dissected. Altogether 39 grafts were recovered. Among the nine grafts that were not recovered, one grafted mouse died, but the graft was present. In eight cases, mice died for unknown reasons and graft growth was not analysed.

We then examined the evolution of grafted tissues in terms of volume and mass. As shown in Figure 1, the human embryonic tissue developed massively when grafted under the kidney capsule of the SCID mice (Fig. 1). We also studied the evolution of the grafted tissue in term of mass. After 1 week in the mouse, the graft weight was less than 10 mg, in the same range of the ungrafted tissue (2–8mg) but it next increased rapidly with time to reach 100 mg after 8–12 weeks and 1000 mg after 33–38 weeks (Fig. 2). Identical growth rates occurred with embryonic pancreases derived from 6–9 week old embryos.

Immunohistochemistry using anti-cytokeratin and anti-vimentin antibodies was done to study the evolu-

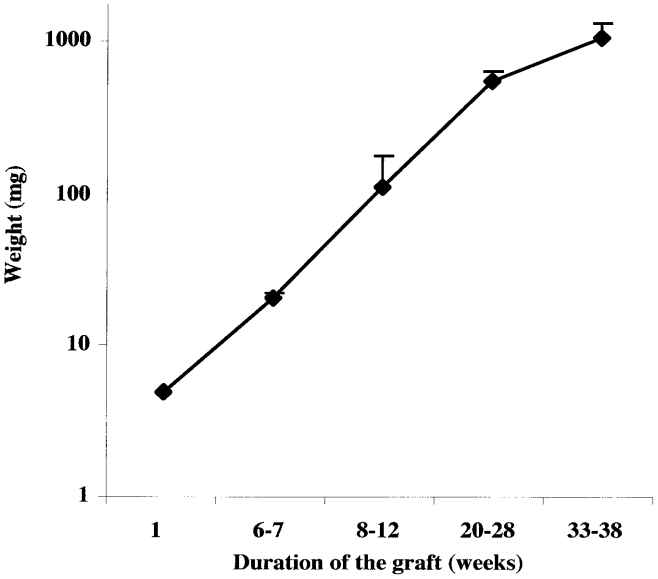
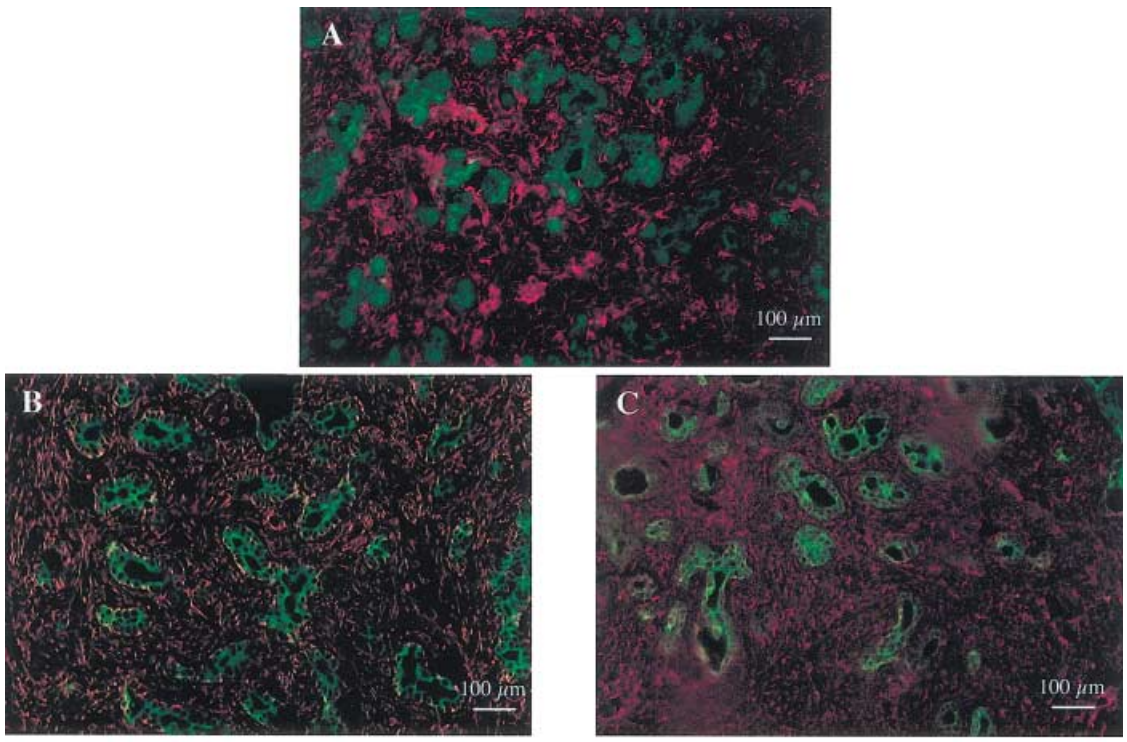


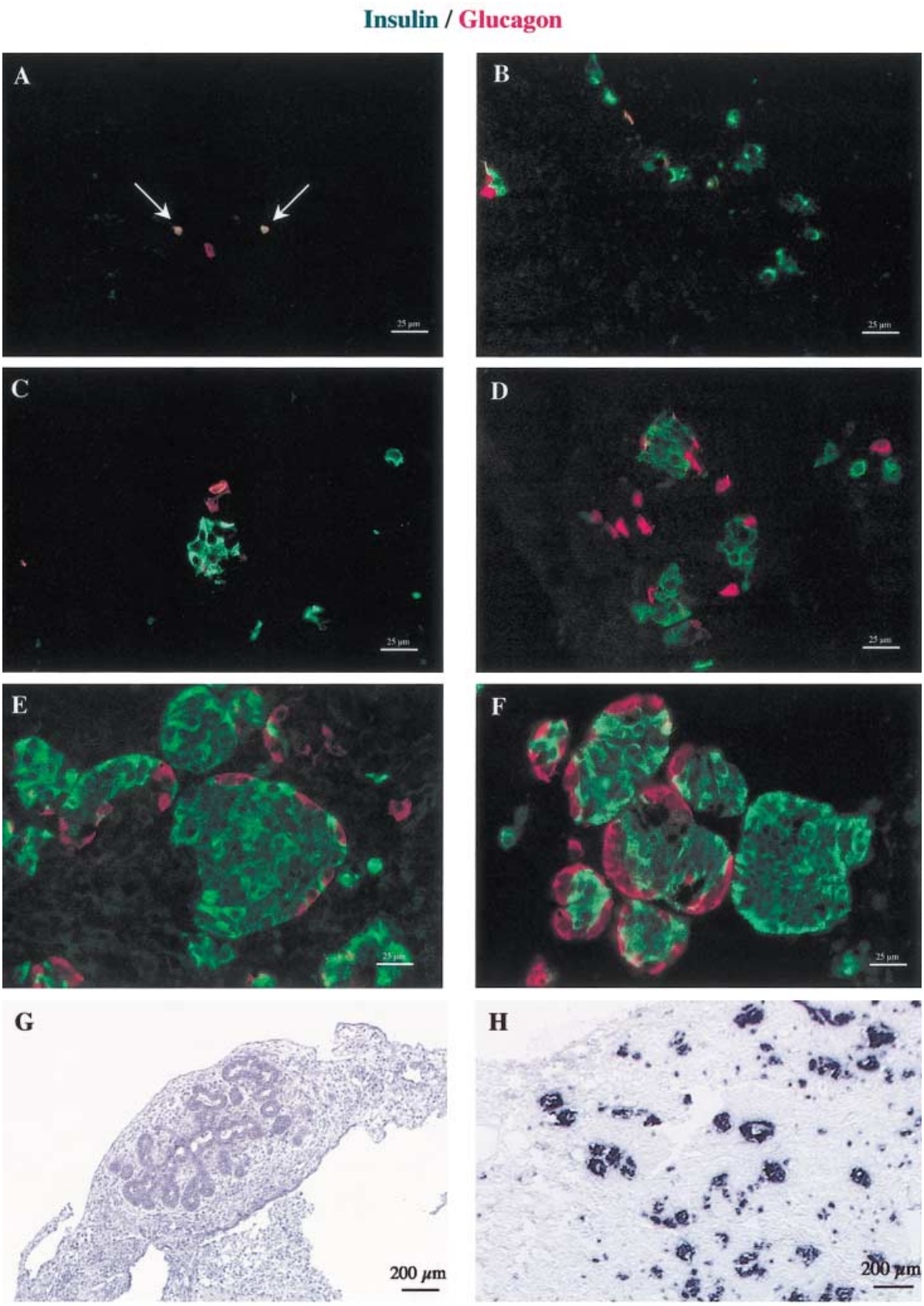
Fig. 2. Evolution of the weight of the transplanted pancreas. Grafts were removed at different time points after transplantation, finely dissected to remove the fat, and weighed. A total of 22 grafts were analysed: 1 after 1 week, 2 after 6–7 weeks, 2 after 8–12 weeks, 8 after 20–28 weeks, 9 after 33–38 weeks of implantation

Fig. 3. Histological analysis. Human pancreas at 8 weeks of development before transplantation (**A**), and 1 month (**B**) and 6 months (**C**) after transplantation stained with an anti-pan cytokeratin antibody (green) or with an anti-vimentin antibody (red)

tion of the epithelial and mesenchymal cells which had been present in the human embryonic pancreas before transplantation during the grafting period. As shown in Figure 3A, before grafting, an 8WD pancreas is composed of epithelial cells forming ducts and mesenchymal cells. Both 1 month and 6 months after transplantation, the tissue was also composed of epithelial cells that stained positive for cytokeratin and mesenchymal cells positive for vimentin, indicating that both cell types did develop during the graft (Fig. 3B and C).

Development of the endocrine tissue. Before transplantation, only a few endocrine cells were detected by immunohistochemistry that stained positive either for glucagon or for both insulin and glucagon. Such cells were dispersed in the pancreatic tissue and were not associated into islets of Langerhans (Fig. 4A). Once transplanted, the endocrine tissue started to develop and the number of endocrine cells increased with time (Fig. 4B-F). In panels G and H are shown representative hybridizations for proinsulin before and after transplantation for 6 months of an 8-week old pancreas. Huge increases in the number of cells that express proinsulin mRNA could be clearly visualized. Evolution of the insulin-positive cell mass was quantified after immunohistochemistry. As shown in Figure 5, the absolute surface occupied by insulin-expressing cells multiplied by 300 after 8–12 weeks in the mouse and by 5000 after 21–28 weeks. The evolution in the insulin cell mass




Insulin / Glucagon
A
B
C
D
E
F
G
H
25 µm
200 µm
Proinsulin mRNA

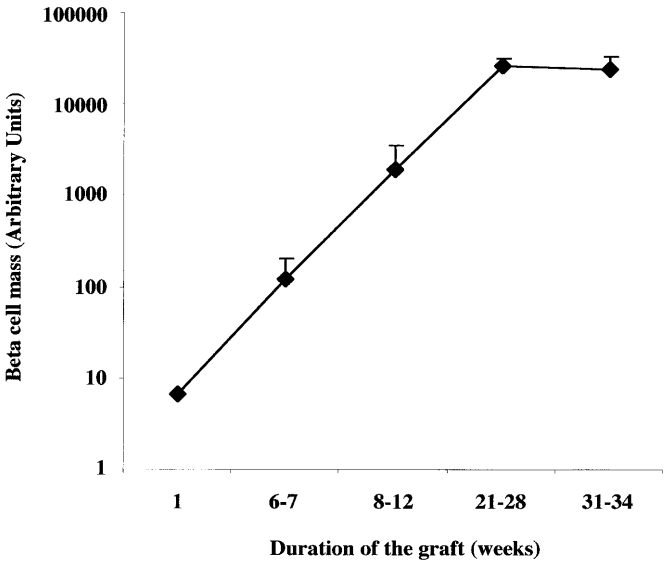


Fig. 5. Evolution of the endocrine cell mass during the transplantation period. The absolute mass of insulin-expressing cells is presented in arbitrary units. A total of 16 grafts were analysed: 1 after 1 week, 2 after 6–7 weeks, 2 after 8–12 weeks, 3 after 20–28 weeks, 8 after 33–38 weeks of implantation

was the same when pancreata derived from 6–9 week old embryos were transplanted.

Human undifferentiated epithelial cells but not endocrine cells, proliferated during the engraftment period. To clarify whether the increase in the absolute number of endocrine cells was due to the differentiation of precursor cells or to the proliferation of the few endocrine cells that were present before grafting, engrafted mice were injected with BrdU 2 h before being killed and immunohistological analysis was performed. Both at 1 and 3 months of development in the mouse, when cells that stained positive for both cytokeratin and BrdU were frequently detected, cells positive for both insulin and BrdU were very rarely found (less than one beta cell out of 1000) (Fig. 6). These results strongly suggest that increases in the endocrine cell mass was due to the differentiation of precursor cells, rather than to the proliferation of rare pre-existing endocrine cells.

Fig. 4. Development of the pancreatic endocrine tissue in NOD/*scid* mice. Eight-week pancreas before grafting (**A**), and 7 days (**B**), one month (**C**), 2 months (**D**), 6 months (**E**) and 9 months (**F**) after transplantation. Insulin (green) and glucagon (red) immunostainings. The arrows in **A** represent two cells that stain positive for both insulin and glucagon. **G** and **H** show representative hybridizations of a proinsulin probe on sections from 8-week human pancreas before grafting (**G**) and after 6-months engraftment (**H**)

Human endocrine cells developed in mice resemble mature endocrine cells. To determine whether human endocrine cells that developed in NOD/*scid* mice express markers known to be present in human beta cells developed in vivo, we tested a series of antibodies by immunohistochemistry. All beta cells developed in NOD/*scid* mice express specific transcription factors such as Nkx 6.1 and Pax 6 (Fig. 7A, B), as well as PC1/3, an enzyme necessary for the processing of proinsulin into insulin (Fig. 7C, D). Moreover, endocrine cells in the grafts are frequently associated in islets of Langerhans with a core of insulin-expressing cells surrounded by glucagon-expressing cells (Figs. 4E and F). Finally, while the first endocrine cells found in human embryonic pancreas stained positive for cytokeratin as described previously [19], the human insulin-expressing cells that developed in NOD/*scid* mice did not express cytokeratin 19 (Fig. 7E and F).

Functional development of human pancreas grafts. To determine whether the human beta cells that developed in the grafts were functional, 15 NOD/*scid* mice were engrafted with human embryonic pancreas. Three months later, grafted or non-grafted NOD/*scid* mice were injected with alloxan, a drug known to be toxic for murine, but not for human beta cells [18]. Before alloxan injection, blood glucose concentrations were not statistically different in the transplanted and non-transplanted mice. After alloxan treatment, the glycaemia of all non-grafted mice increased up to 6 g/l. Conversely, the glycaemia remained stable in 12 out of 15 engrafted mice. To demonstrate that glycaemia regulation in engrafted mice injected with alloxan is indeed due to the development of the graft, unilateral nephrectomies were done to remove the grafts in six mice and blood glucose concentrations were monitored. After removal of the graft by unilateral nephrectomy, either 7 or 43 days after alloxan injection the mice became hyperglycaemic (Fig. 8A). Murine pancreases and grafts were also analysed for the presence of insulin-expressing and glucagon-expressing cells before alloxan injection or at the end of the experiments when the mice were killed. Very few insulin-expressing cells were detected in the murine pancreas of grafted NOD/*scid* mice that had been injected with alloxan, compared to NOD/*scid* mice that had not been treated with alloxan (Fig. 8). On the other hand, a huge number of insulin-producing cells were present in the human graft after alloxan treatment.

Discussion

In this work, we show that human early embryonic pancreas can develop when engrafted under the kidney capsule of NOD/*scid* mice. The size and weight

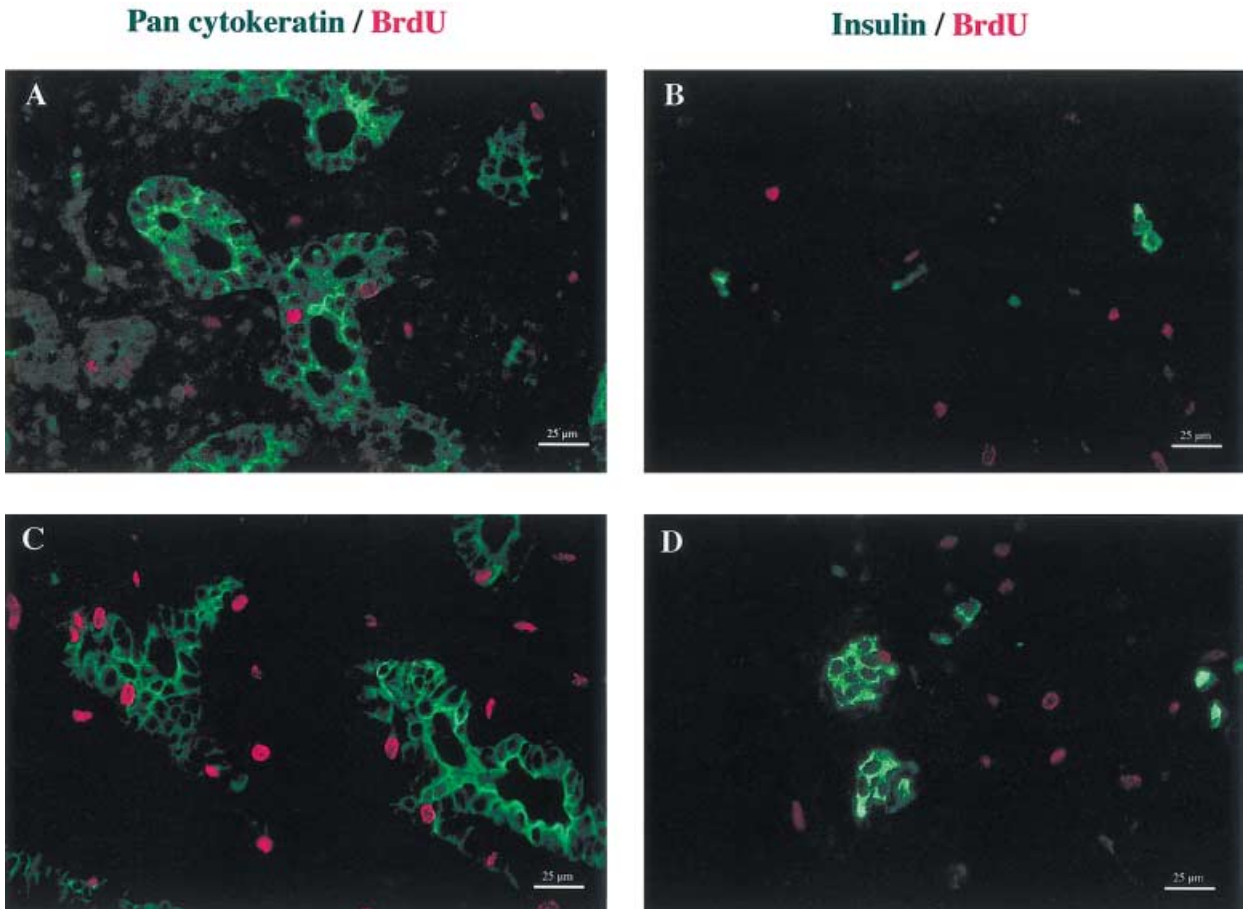


Fig. 6. Cell proliferation analysis. Double immunostaining for BrdU (red) and pan-cytokeratin (green) (**A**, **C**) or BrdU (red) and insulin (green) (**B**, **D**) on sections of human embryonic pancreas that developed in SCID mice during 1 month (**A**, **B**) or during 3 months (**C**, **D**). Mice were killed 2 h after BrdU injection

of the grafts increased considerably and endocrine cells differentiated, organizing themselves into islets of Langerhans and meeting numerous criteria of maturity. Finally, the human endocrine pancreatic tissues that developed could normalize blood glucose concentrations in diabetic mice, underlying their functionality.

In this study, we used NOD/*scid* mice as recipients for transplantation. NOD/*scid* mice were generated by crossing the *scid* mutation from C.B-17-*scid/scid* mice onto the NOD background. These animals are lacking T- and B-lymphocytes, [20] and fail to generate either humoral or cell-mediated immunity. Because of the absence of xenograft rejection in *SCID* mice, they were previously used as recipients for human or fetal hematolymphoid tissues and cells [21]. Non-haematopoietic human tissues such as ovarian cortex [22], thyroid [23], skin [24] and airway [25] have also been successfully transplanted in this model. However, the capacity of these tissues to develop functional properties and to replace a physiological function has only rarely been demonstrated. One example of physiological function replacement is the formation of adrenocortical tissue by transplantation of bovine adrenocortical cells which replace the essential functions of the mouse adrenal gland [26]. However, in this study, the transplanted tissue was not of human but of bovine origin. Moreover, the tissue had been expanded from a primary culture of bovine adrenocortical cells. Donor cells were thus already fully differentiated at the time of grafting. In our study, we show that immature human embryonic pancreas can develop and acquire functional properties in SCID mice.

In previous studies, human pancreatic fragments (14–24 weeks) were engrafted into immunoincompetent mice with the aim of studying endocrine tissue development [12, 14, 16, 27]. After a few weeks or months in recipient mice, all endocrine cell types were found when human tissues were removed [13, 14]. However, it is important to remember that between 14 and 24 weeks of development (the age of the tissue at the time of the transplantation), endocrine cells are already present and associated into islets of Langerhans [19, 28–30]. Moreover, in these experiments using late human fetal tissues no clear increase in the beta cell mass was detected when the quantity of insulin-expressing cells present in the

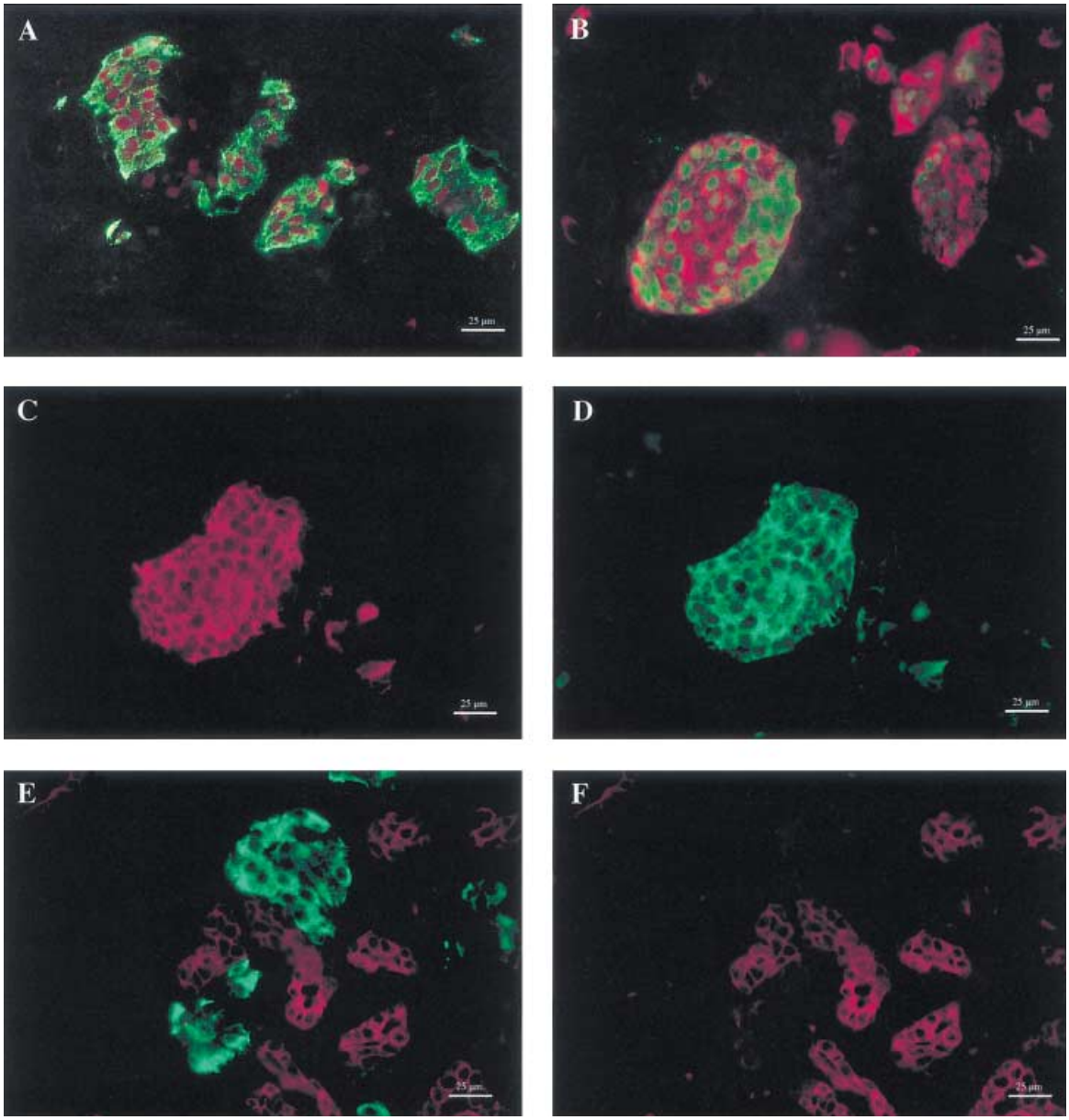


Fig. 7. Human endocrine cells developed in mice resemble mature endocrine cells. Sections of a human embryonic pancreas 6 months after transplantation. (**A**) Insulin (green) and Pax 6 (red); (**B**) Insulin (red) and Nkx 6.1 (green); (**C**, **D**) Insulin (red) and PC1/3 (green); (**E**) Insulin (green) and Cytokeratin-19 (red); (**F**) Cytokeratin 19 alone (red)

graft was compared before and after transplantation [13]. It is consequently difficult to determine whether the human endocrine cells that were present after a few weeks or months in the mouse were newly formed endocrine cells or cells that existed before transplantation and that survived. In this study, we systematically used embryonic pancreas no older than 9 weeks. At this stage of development, very few endocrine cells are present as previously described (Fig. 4) [17, 28]. We thus grafted immature rudiments that contained undifferentiated epithelial cells and mesenchymal tissue and almost no insulin-expressing cells. Moreover, the absolute mass of insulin-expressing cells was multiplied by nearly 5,000 after 6 months in the mouse. The fact that very few endocrine cells were present before transplantation, while a massive amount of endocrine cells was detected a few weeks later, clearly indicates that neoformation of endocrine cells occurred in the present model.

Theoretically, the observed increase in the human beta-cell mass could be due either to the proliferation of rare pre-existing insulin-expressing cells or to the differentiation of precursor cells. It is thought that during prenatal life, increases in the beta-cell mass is mainly due to the differentiation of precursor cells rather than to the proliferation of pre-existing beta cells. A large number of experiments have been performed in rodents that indicate that the increase in

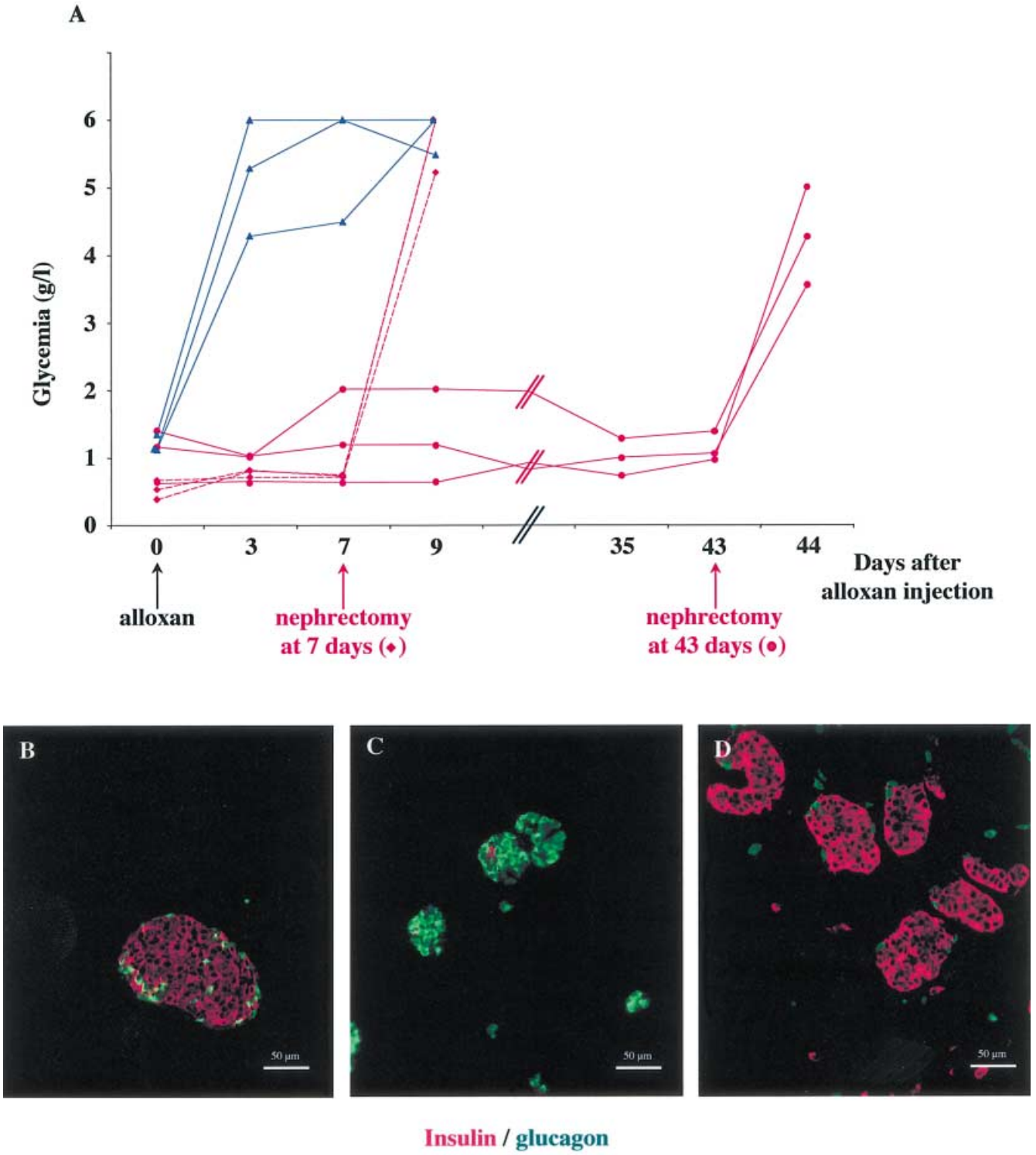


Fig. 8. Functional development of the human pancreas graft. (**A**) 3 months after transplantation, SCID mice (red lines) were injected with alloxan. Non-grafted mice (blue line) also received alloxan. While the glycaemia of the non-grafted mice increased rapidly, that of the grafted mice remained stable. When grafts were removed by nephrectomy at day 7 (◆) or day 43 (●), glycaemia increased rapidly. (**B**) Mouse pancreas before alloxan treatment and at the end of the experiments (**C**) (day 43 after alloxan) stained for insulin (red) and glucagon (green), indicating that alloxan has destroyed the vast majority of host insulin-expressing cells. (**D**) Section of the human graft at the end of the experiment (day 43 after alloxan) stained for insulin (red) and glucagon (green), indicating that alloxan had no effect on human beta cells that developed

the endocrine cell mass observed during fetal life cannot be explained by the proliferation of pre-existing endocrine cells [31]. This also seems to be the case in humans, where, during embryonic/fetal life, insulin-expressing cells rarely stain positive for Ki67, and hence are rarely or not cycling [17, 19]. Our data indicate that when chimeric mice are injected with BrdU, a large number of cytokeratin-positive cells present positive in the graft stain for BrdU, while no or very few insulin-positive cells stain positive. Thus, it can be postulated that in the grafts' newly formed beta cells derived from precursor cells present in the duct epithelium which proliferated and differentiated during engraftment rather than from the proliferation of the few endocrine cells present in the rudiment. Beta cell development in the graft does thus recapitulate normal development that occurs in vivo.

Several arguments indicate that the human beta cells which developed in vivo in NOD/*scid* mice were mature. Firstly, these insulin-expressing cells did not co-express glucagon and were thus different from the first insulin-expressing cells detected in the human pancreas at early stages of development [17, 32, 33]. Secondly, the insulin-expressing cells present in the pancreas before 16 weeks of development express cytokeratin 19, while the insulin-expressing cells found later during development stain negative for this marker [19]. Our data indicate that the human beta cells that develop in NOD/*scid* mice stain negative for cytokeratin 19 and thus resemble adult mature beta cells. Thirdly, human beta cells that develop in NOD/*scid* mice express the prohormone convertase PC1/PC3, an enzyme that is necessary for the processing of proinsulin into insulin [34, 35]. Finally, our data indicate that the human endocrine cell mass that developed in NOD/*scid* mice is able to regulate perfectly the glycaemia of NOD/*scid* mice deficient in endogenous beta cells, and hence is functional. These human endocrine cells remain functional and can regulate the glycaemia of the mice for at least 43 days, the longest period tested before removing the graft.

In conclusion, we show here that newly differentiated human beta cells that are able to regulate the glycaemia of the host deficient in beta cells can be produced from human early embryonic pancreas. The human embryonic pancreas does thus represent an alternative source of tissue needed to generate functional human beta cells for transplantation. Moreover, the model of mice grafted with human embryonic pancreas can now be used to study the development of the human pancreas, a type of study that has been difficult to perform due to the lack of human embryonic pancreases and of proper experimental systems.

Acknowledgements. We would like to thank Drs. Steiner, Serup and Saule for antibodies. We are indebted to the medical staff of the Department of Gynaecological Surgery at R. Debré hospital, directed by Prof. Blot, and to Dr Benifla of the Department of Gynaecological Surgery at Bichat Hospital for providing human embryonic tissues. This work was supported by INSERM (Contrat APEX), by grants from the Juvenile Diabetes Foundation International, by the Lilly-France Institute (Contrat de Recherche en Diabétologie ALFEDIAM-LILLY), and by the French Association for Diabetes (Association Francaise des Diabetiques). M. Castaing was successively supported by the Medical Research Foundation (Fondation pour la Recherche Médicale), and by the Association for the Assistance of Young Diabetics (l'Association d'Aide aux Jeunes Diabétiques).

References

1. Shapiro A M, Lakey J R, Ryan E A et al. (2000) Islet transplantation in seven patients with type 1 diabetes mellitus using a glucocorticoid-free immunosuppressive regimen. N Engl J Med 343: 230–238
2. Weir G C, Bonner-Weir S (1997) Scientific and political impediments to successful islet transplantation. Diabetes 46: 1247–1256
3. Edlund E (1998) Transcribing pancreas. Diabetes 47: 1817–1823
4. St-Onge L, Wehr R, Gruss P (1999) Pancreas development and diabetes. Curr Opin Genet Dev 9: 295–300
5. Wells J M, Melton D A (1999) Vertebrate endoderm development. Annu Rev Cell Dev Biol 15: 393–410
6. Scharfmann R (2000) Control of early development of the pancreas in rodents and humans: implications of signals from the mesenchyme. Diabetologia 43: 1083–1092
7. Grapin-Botton A, Melton D A (2000) Endoderm development: from patterning to organogenesis. Trends Genet 16: 124–130
8. Kim S K, Hebrok M (2001) Intercellular signals regulating pancreas development and function. Genes Dev 15: 111–127
9. Yoon K H, Quickel R R, Tatarkiewicz K et al. (1999) Differentiation and expansion of beta cell mass in porcine neonatal pancreatic cell clusters transplanted into nude mice. Cell Transplant 8: 673–689
10. Otonkoski T, Ustinov J, Rasilainen S, Kallio E, Korsgren O, Hayry P (1999) Differentiation and maturation of porcine fetal islet cells in vitro and after transplantation. Transplantation 68: 1674–1683
11. Bonner-Weir S, Taneja M, Weir G C et al. (2000) In vitro cultivation of human islets from expanded ductal tissue. Proc Natl Acad Sci U S A 97: 7999–8004
12. Tuch B E, Ng A B, Jones A, Turtle J R (1984) Histologic differentiation of human fetal pancreatic explants transplanted into nude mice. Diabetes 33: 1180–1187
13. Tuch B E, Grigoriou S, Turtle J R (1986) Growth and hormonal content of human fetal pancreas passaged in athymic mice. Diabetes 35: 464–469
14. Sandler S, Andersson A, Schnell A et al. (1985) Tissue culture of human fetal pancreas. Development and function of B- cells in vitro and transplantation of explants to nude mice. Diabetes 34: 1113–1119
15. Hayek A, Beattie G M (1997) Experimental transplantation of human fetal and adult pancreatic islets. J Clin Endocrinol Metab 82: 2471–2475
16. Goldrath A, Chen K, Weide L, Pour P, Lebkowski J, Alters S (1995) Retention of endocrine function in the Scid-Hu pancreas mouse- a model for the development of human fetal islet tissue. Transplantation 59: 1497–1500

17. Polak M, Bouchareb-Banaei L, Scharfmann R, Czernichow P (2000) Early pattern of differentiation in the human pancreas. Diabetes 49: 225–232
18. Eizirik D, Pipeleers D, Ling Z, Welsh N, Hellerstrom C, Andersson A (1994) Major species differences between humans and rodents in the susceptibility to pancreatic β-cell injury. Proc Natl Acad Sci USA 91: 9253–9256
19. Bouwens L, Lu W U, De Krijger R (1997) Proliferation and differentiation in the human fetal endocrine pancreas. Diabetologia 40: 398–404
20. Shultz L D, Schweitzer P A, Christianson S W et al. (1995) Multiple defects in innate and adaptive immunologic function in NOD/LtSz-scid mice. J Immunol 154: 180–191
21. Roncarolo M, Namikawa R, Péault B (1995) Human hematopoiesis in SCID mice. Landes, Georgetown
22. Weissman A, Gotlieb L, Colgan T, Jurisicova A, Greenblatt E M, Casper R F (1999) Preliminary experience with subcutaneous human ovarian cortex transplantation in the NOD-SCID mouse. Biol Reprod 60: 1462–1467
23. Martin A, Valentine M, Unger P et al. (1993) Preservation of functioning human thyroid organoids in the scid mouse: 1. System characterization. J Clin Endocrinol Metab 77: 305–310
24. Levy L, Broad S, Zhu A J et al. (1998) Optimised retroviral infection of human epidermal keratinocytes: long- term expression of transduced integrin gene following grafting on to SCID mice. Gene Ther 5: 913–922
25. Delplanque A, Coraux C, Tirouvanziam R et al. (2000) Epithelial stem cell-mediated development of the human respiratory mucosa in SCID mice. J Cell Sci 113: 767–778
26. Thomas M, Northrup S R, Hornsby P J (1997) Adrenocortical tissue formed by transplantation of normal clones of bovine adrenocortical cells in scid mice replaces the essential functions of the animals' adrenal glands. Nat Med 3: 978–983
27. Povlsen C, Skakkebaek N, Rygaard J, Jensen G (1974) Heterotransplantation of human foetal organs to the mouse mutant nude. Nature 248: 247–249
28. Stefan Y, Grasso S, Perrelet A, Orci L (1983) A quantitative immunofluorescent study of the endocrine cell populations in the developing human pancreas. Diabetes 32: 293–301
29. Fukayama M, Ogawa M, Hayashi Y, Koike M (1986) Development of human pancreas. Immunohistochemical study of fetal pancreatic secretory proteins. Differentiation 31: 127–133
30. Miettinen P J, Heikinheimo K (1992) Transforming growth factor-alpha (TGF-alpha) and insulin gene expression in human fetal pancreas. Development 114: 833–840
31. Swenne I (1992) Pancreatic beta-cell growth and diabetes mellitus. Diabetologia 35: 193–201
32. Larsson L I, Hougaard D M (1994) Coexpression of islet hormones and messenger RNAs in the human fetal pancreas. Endocrine 2: 759–765
33. De Krijger R R, Aanstoot H J, Kranenburg G, Reinhard M, Visser W J, Bruining G J (1992) The midgestational human fetal pancreas contains cells coexpressing islet hormones. Dev Biol 153: 368–375
34. Kaufmann J E, Irminger J C, Mungall J, Halban P A (1997) Proinsulin conversion in GH3 cells after coexpression of human proinsulin with the endoproteases PC2 and/or PC3. Diabetes 46: 978–982
35. Furuta M, Carroll R, Martin S et al. (1998) Incomplete processing of proinsulin to insulin accompanied by elevation of Des-31,32 proinsulin intermediates in islets of mice lacking active PC2. J Biol Chem 273: 3431–3437