Long-Term Graft Function of Adult Rat and Human Islets Encapsulated in Novel Alginate-Based Microcapsules After Transplantation in Immunocompetent Diabetic Mice

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We describe the results of the first study to show that adult rat and human islets can be protected against xenogenic rejection in immunocompetent diabetic mice by encapsulating them in a novel alginate-based microcapsule system with no additional permselective membrane. Nonencapsulated islets lost function within 4–8 days after being transplanted into diabetic Balb/c mice, whereas transplanted encapsulated adult rat or human islets resulted in normoglycemia for >7 months. When rat islet grafts were removed 10 and 36 weeks after transplantation, the mice became immediately hyperglycemic, thus demonstrating the efficacy of the encapsulated islets. The explanted capsules showed only a mild cellular reaction on their surface and a viability of >85%, and responded to a glucose stimulus with a 10-fold increase in insulin secretion. Furthermore, transplanted mice showed a slight decrease in the glucose clearance rate in response to intraperitoneal glucose tolerance tests 3–16 weeks after transplantation; after 16 weeks, the rate remained stable. Similar results were obtained for encapsulated human islets. Thus we provide the first evidence of successful transplantation of microencapsulated human islets. In conclusion, we have developed a novel microcapsule system that enables survival and function of adult rat and human islets in immunocompetent mice without immunosuppression for >7 months. *Diabetes* 54:687–693, 2005

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HBSS, Hank's balanced salt solution; HSA, human serum albumin; IPGTT, intraperitoneal glucose tolerance test; NPCC, neonatal pancreatic cell cluster; UW, University of Wisconsin.

As has recently been shown (1), long-term normoglycemia can be achieved in type 1 diabetic patients by allogenic human islet transplantation. However, this technique requires the administration of immunosuppressives, which are well known to be associated with serious side effects. The severe shortage of available human islets is another drawback of this promising therapeutic approach. A bioartificial pancreas consisting of islets immunoprotected by an alginate microcapsule provides an alternative approach to overcoming the rejection problem (2–4). Such immunoprotection is also appealing because it provides improved prospects of obtaining an abundant supply of human islets from ductal tissue (5) and the possibility of using xenogenic islets (e.g., porcine islets) (6). In most studies, islets have been encased in Ca^{2+}–cross-linked alginate-polylysine-alginate microcapsules following the protocol of Lim and Sun (7). However, the results obtained by various groups using this microcapsule formulation in the last two decades have been inconsistent. Most importantly, graft survival is random and generally too limited for therapeutic application of these microcapsules (8–10). The reasons for this are presumably the cytotoxicity of the polyamino acid and the mechanical instability of the microcapsules (2,11,12). Microcapsules made of alginate cross-linked with Ba^{2+} do not share these disadvantages (13). Duvivier-Kali et al. (14) have reported that these microcapsules protect islets against allorejection and autoimmunity. Furthermore, Omer et al. (15) found that Ba^{2+}–cross-linked alginate microcapsules protect neonatal pancreatic cell clusters (NPCCs) when transplanted in the streptozotocin-induced diabetic Balb/c mouse model for 20 weeks. We have recently shown that using highly purified and ultra–high-iscosity alginates when cross-linked internally and externally with Ba (2) results in microcapsules of extremely high stability, particularly when proteins (e.g., human serum albumin [HSA]) are simultaneously incorporated (16,17). A recent study has shown that rat islets encapsulated in these novel microcapsules exhibit a well-preserved insulin secretion in tissue culture over 3 weeks (16).

Here we demonstrate that adult rat as well as adult human islets encapsulated in these novel microcapsules

and transplanted into diabetic mice are protected against xenogenic rejection for extremely long periods of time without the need for immunosuppression.

RESEARCH DESIGN AND METHODS

Isolation of rat islets. Isolation of rat islets was performed according to the protocol previously described (17,18). Briefly, SD rats (Central Animal Facility, University of Mainz) age 8 weeks and weighing 280–330 g were used as islet donors. Rats were anesthetized by pentobarbital administration (60 mg/kg, i.p.). A midline abdominal incision was made and the pancreas was exposed and injected via the pancreatic duct with Hank's balanced salt solution (HBSS; Gibco BRL, Long Island, NY) containing 1.7 mg/ml collagenase (Serva PanPlus, Heidelberg, Germany). After the animal was killed, the pancreatic tissue was surgically removed and incubated for 10 min at 37°C in the collagenase solution. Mechanical disruption of the digested pancreatic tissue was achieved by incubating the tissue in the collagenase solution at 37°C for 10 min, with the incubation being interrupted every 2 min by shaking for 30 s. Digestion was stopped by adding cold (4°C) HBSS plus 10% FCS (Greiner, Frickenhausen, Germany). Islet purification was achieved using a discontinuous three-phase Ficoll density gradient (densities: 1.090, 1.077, and 1.040).

Isolation of human islets. Human pancreases were obtained from brain-dead cadaveric multiorgan donors with the informed consent of their relatives. Tissue procurements were approved by the Human Ethics Committee of the Landesärztekammer Rheinland-Pfalz. Human islets were isolated from two consecutively processed human pancreases after a vascular flush with University of Wisconsin (UW) solution. Briefly, pancreases were intraductally distended with 250 ml HBSS containing Liberase HI (Roche, Mannheim, Germany). Distended pancreases were digested in a continuous digestion-filtration device at 37°C. After a subsequent 60-min cold storage of the digest in UW solution, human islets were purified using a continuous hyperosmolar Hank's-Ficoll gradient (Biochrom KG, Berlin, Germany) on a Cobe 2991.

Culture conditions. Islets were cultured in RPMI 1640 medium (Biochrom KG) supplemented with 100 mg/dl glucose, 25 mmol/l HEPES (Greiner), 0.2 g/l Glutamax (Gibco BRL, Paisley, Scotland), 10% FCS (Greiner), and antibiotics (100 units/ml penicillin, 100 μg/ml streptomycin [Gibco BRL, Paisley, Scotland], and 10 μg/ml ciprofloxacin [Ciprobay; Aventis, Frankfurt, Germany]). Incubation lasted 12 h at 37°C. Islets were then transferred into a slightly modified culture medium containing 3% HSA (Pharmacia, Erlangen, Germany) instead of FCS and an elevated concentration of glucose (300 mg/dl). After being cultured for 12 h at 37°C, the islets were used for encapsulation or transplantation.

Islet encapsulation. Microencapsulation was performed using ultra-high–viscosity alginate of clinical grade (viscosity of a 0.1% wt/vol solution in distilled water was 20–30 mPa · s). The extraction and purification protocol has been described elsewhere (19,20). For microcapsule formation, the alginate was dissolved in sterile, endotoxin-free 0.9% NaCl solution at a concentration of 0.7% (wt/vol). Then 3% HSA (Pharmacia) was added to the alginate solution before it was cross-linked. For microcapsule formation, a two-channel air jet droplet generator was used. The islet-containing microcapsules were dropped into a 20-mmol/l $BaCl_2$ solution adjusted to 290 mOsm with appropriate amounts of NaCl and buffered to pH 7.0 with 5 mmol/l histidine. After 20 min, the islet-containing microcapsules were washed three times with 0.9% NaCl solution and treated with 6 mmol/l Na_2SO_4 saline solution for 30 min at 37°C to precipitate excess Ba^{2+} ions (20). The microcapsules obtained by this encapsulation procedure had a diameter of ~700–800 μm and on average contained one islet or, in rare cases, two or three.

Animals. Male Balb/c mice (Central Animal Facility) age 6–8 weeks were used as recipients for nonencapsulated (controls) or encapsulated islets. Mice were rendered diabetic with intraperitoneal injections of streptozotocin (250 mg/kg body wt; Sigma, St. Louis, MO) freshly dissolved in citrate buffer 2 weeks before islet transplantation. Only animals exhibiting blood glucose concentrations >350 mg/dl in four consecutive measurements were used as recipients.

Transplantation of microencapsulated islets. Transplants were made in a Class 100 biological safety cabinet under sterile conditions. Encapsulated rat ($n = 8$) or human ($n = 3$) islets were injected into the peritoneal cavity with a 20-gauge needle (Vasofix; Braun, Melsungen, Germany). Before treatment, the Balb/c mice were anesthetized with ketamine (65 mg/kg i.p.; Pfizer, Karlsruhe, Germany) and xylazine hydrochloride (13 mg/kg i.p.; Bayer, Leverkusen, Germany). Each mouse received 1,800 microencapsulated islets. Control animals ($n = 4$) received the same number of nonencapsulated rat or human islets, respectively.

Intraperitoneal glucose tolerance test. After islet transplantation, animals' body weight was determined once a week and blood glucose levels of islet recipients were monitored biweekly. Blood samples were taken from the tail vein under nonfasting conditions (8:00 A.M.; standard laboratory diet ad libitum overnight) and determined using a glucometer (Accutrend sensor, Roche Diagnostics, Germany). The mice were considered to be normoglycemic when blood glucose levels were <200 mg/dl. An intraperitoneal glucose tolerance test (IPGTT) was performed in mice transplanted with encapsulated rat islets 3 ($n = 8$), 9 ($n = 7$), 16 ($n = 5$), and 36 ($n = 2$) weeks after transplantation, using a 5% glucose solution (1 g/kg body wt). In mice transplanted with encapsulated human islets, an IPGTT was carried out at the same times ($n = 2$ at 3, 9, and 16 weeks; $n = 1$ at 36 weeks). IPGTTs were also performed in all diabetic animals 3 days before islet transplantation and in body weight–matched nondiabetic Balb/c mice. Blood glucose levels were measured at 0, 10, 20, 30, 60, and 120 min.

Microcapsule explantation. Grafts were retrieved from two animals that had received encapsulated adult rat islets 10 ($n = 1$) and 36 weeks ($n = 1$) after islet transplantation. Under anesthesia, a 2-cm incision was made in the abdomen and capsules were explanted by repeated flushes with warm 0.9% NaCl solution. The abdomen was sutured and the animals were checked daily for blood glucose concentrations. Explanted microcapsules were evaluated for fibrotic overgrowth under a Wilowert S microscope (Hund GMBH, Wetzlar, Germany), as previously described (21). Images were captured with a digital camera and stored as jpg files. The viability of the encapsulated islets was assessed using the fluorescein diacetate method, as described elsewhere (16,22).

Assessment of insulin secretion by a static incubation assay. The static incubation assay was used to assess the insulin secretion response of nonencapsulated and encapsulated rat or human islets after a glucose challenge both before transplantation and after retrieval, as previously described (16,17). For each animal, 10 samples of 10 nonencapsulated or encapsulated islets of equal size and shape were hand picked and transferred into a culture insert (membrane pore diameter 12 μm; Millicell PCF, Millipore, France). The insert was put into a well of a 24-well culture-plate (Falcon Multiwell; Becton, Dickinson). Basal insulin secretion was measured after a 1.5-h incubation at 37°C in RPMI 1640 + 1 g/l D-glucose + 3% HSA. The inserts with islets were transferred into hyperglycemic culture medium (RPMI 1640 + 3 g/l D-glucose + 3% HSA) for an additional 1.5 h. Aliquots of the medium were stored at −20°C after inserts were removed. Rat insulin was measured with the rat-insulin enzyme-linked immunosorbent assay (Mercodia, Uppsala, Sweden) and human insulin was determined with a chemiluminiscent immunoassay (ADVIA Centaur insulin assay; Bayer). Results are expressed as nanograms per islet per 1.5 h for rat islets and as microunits per islet per 1.5 h for human islets.

Statistics. Data are given as means ± SE. Statistical significance of differences was calculated with an unpaired Student's t test (two-sided).

RESULTS

Graft survival. Before encapsulated islets were transplanted, their insulin secretion capacity after being cultured for 1 day was determined. As shown in Table 1, encapsulated rat or human islets responded well to a high-glucose stimulus. The insulin increase after a glucose challenge was comparable with that of the corresponding nonencapsulated islets, even though the absolute amount of insulin was lower, presumably due to diffusion restrictions within the cross-linked alginate matrix (16). Transplantation of 1,800 nonencapsulated rat islets into the peritoneal cavity of diabetic Balb/c mice resulted in normoglycemia for ~4–8 days. Similar results were obtained for nonencapsulated human islets (Table 2). In contrast, when encapsulated rat and human islets were transplanted under the same conditions, normoglycemia was achieved for >270 days (Fig. 1). As indicated in Table 2, part of the transplantation experiments are still going on. Loss of graft function was observed in only one case. This happened 69 days after transplantation of encapsulated rat islets and was due to a severe infection of the tail as a result of repeated blood collection. Normoglycemic blood glucose levels were generally reached within 24 h of transplantation; normoglycemia was not established in

TABLE 1
Insulin secretion of nonencapsulated and encapsulated islets in a static incubation assay before and 10 and 36 weeks after transplantation

	Rat islets to Balb/c				Human islets to Balb/c			
	Nonencapsulated		Encapsulated		Nonencapsulated		Encapsulated	
	Basal	Stimulated	Basal	Stimulated	Basal	Stimulated	Basal	Stimulated
Before transplant (control)	3.9 ± 1.2	22.3 ± 5.8	2.1 ± 0.3	16.1 ± 2.4	4.0 ± 0.6	9.3 ± 5.0	0.8 ± 0.3	3.7 ± 0.9
10 weeks posttransplant	—	—	1.5 ± 0.2*	10.1 ± 2.4*	—	—	NR	NR
36 weeks posttransplant	—	—	1.3 ± 0.4*	9.5 ± 3.5*	—	—	NR	NR

Data are means ± SE. Rat islets are expressed as ng · islet^{-1} · 1.5 h^{-1} (n = 8); human islets are expressed as μU · islet^{-1} · 1.5 h^{-1} (n = 2). NR, not retrieved. *Measurements were performed after retrieval; 10 samples of 10 encapsulated islets per animal were analyzed; $P < 0.001$ (in relation to pretransplant values).

only one animal that had received encapsulated human islets. This was presumably due to a heavy infection at the abdominal wall induced by the transplantation procedure. Graft explantation from two animals 10 and 36 weeks after transplantation resulted in the immediate reappearance of hyperglycemia associated with blood glucose concentrations > 500 mg/dl. In addition, transplantation of microencapsulated rat or human islets led to an increase in body weight, as depicted in Fig. 2.

Intraperitoneal glucose tolerance test. Figure 3 illustrates the blood glucose profiles of Balb/c mice transplanted with encapsulated rat islets during IPGTTs at different times (3, 9, 16, and 36 weeks) after transplantation. During the IPGTTs, the blood glucose concentration profiles were always significantly lower compared with those of diabetic controls (Fig. 3*A–D*). As indicated in Fig. 3*A*, 3 weeks after receiving transplanted encapsulated rat islets, mice showed the same blood glucose concentration after overnight fasting (t = 0) as the nondiabetic controls. Interestingly, the subsequent glucose clearance kinetics were much faster and the final glucose value reached after 120 min was considerably lower than in nondiabetic mice (Fig. 3*A*). In contrast, 9 weeks after transplantation, blood glucose concentration and kinetics in rat islet–transplanted mice were similar to those observed of nondiabetic control mice (Fig. 3*B*). The fasting and peak glucose levels of the rat islet–transplanted mice during IPGTT 16 and 36 weeks after transplantation were similar to those of the controls, but their subsequent glucose clearance rate was significantly delayed and, in turn, their blood glucose levels remained higher than those of the nondiabetic controls at the end of the IPGTT (Fig. 3*C* and *D*).

Because of the limited number of mice transplanted with encapsulated human islets, the blood glucose kinetics during the IPGTT could not be determined as thoroughly as for encapsulated rat islets. However, as shown in Fig. 4, the blood glucose kinetics of these mice were apparently not significantly different from those of the nondiabetic control mice 3, 9, and 16 weeks after transplantation. Consistent with the finding for mice with encapsulated rat islets, 36 weeks after transplantation, glucose clearance in human islet–transplanted mice was delayed, but the blood glucose concentration at t = 0 (after fasting) and the glucose peak value were comparable with the corresponding values measured in nondiabetic controls.

TABLE 2
Graft function of nonencapsulated and encapsulated islets transplanted into the peritoneal cavity of diabetic Balb/c mice

Islet donor	Treatment	Time of graft function (days)
Rat	None	6.2 ± 1.5
Rat	Microcapsules	65,* 70,† 90,‡ 150,‡ 156,‡ 175,‡ 250,† 270‡
Human	None	6.3 ± 1.2
Human	Microcapsules	0,* 115,‡ 268‡

Data are means ± SE. *Lost by infection; †time of explantation; ‡ongoing graft function.

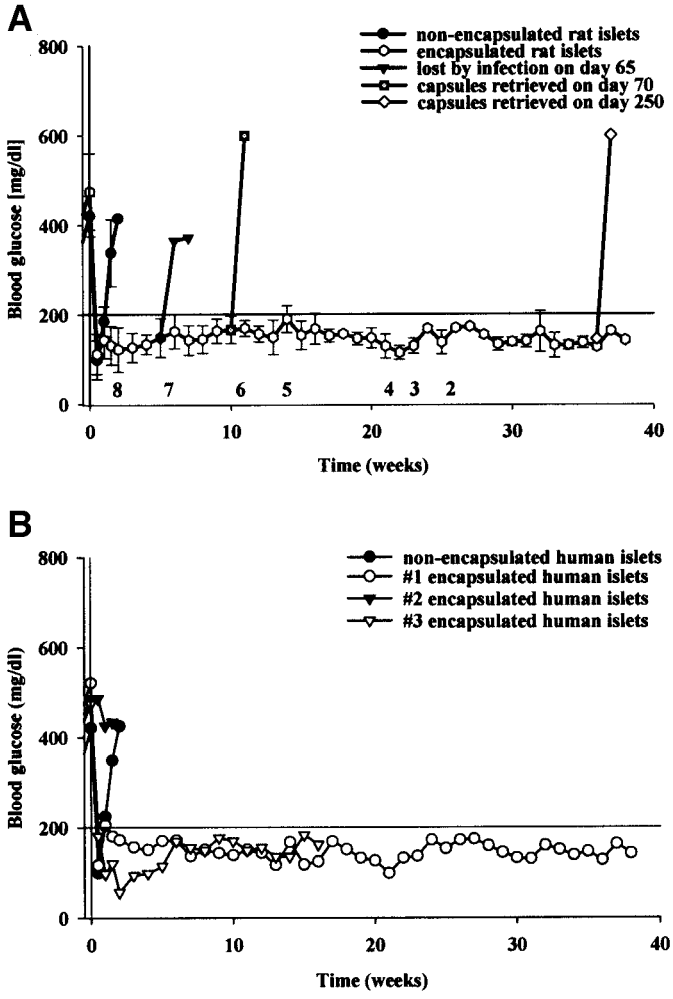

FIG. 1. In vivo function of 1,800 nonencapsulated and encapsulated islets transplanted into diabetic Balb/c mice. *A*: Nonencapsulated and encapsulated adult rat islets. *B*: Nonencapsulated and encapsulated adult human islets. Data are means ± SE.

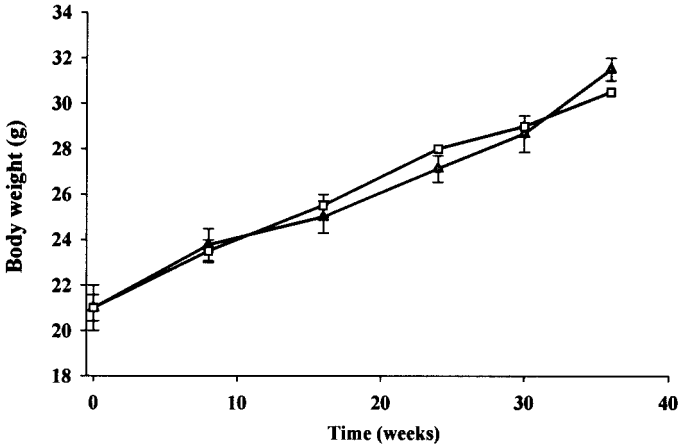

FIG. 2. Weight increase of Balb/c mice transplanted with encapsulated adult human (□, $n = 2$) or adult rat (▲, $n = 8$) islets. Data are means ± SE.

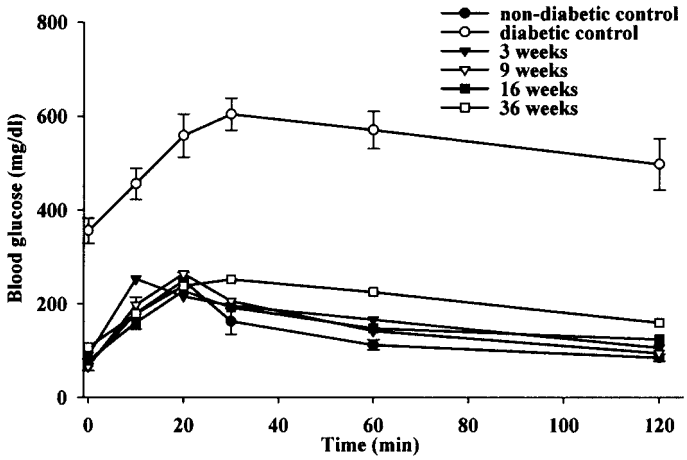

FIG. 4. Intraperitoneal glucose tolerance tests. Blood glucose profile of one mouse transplanted with 1,800 encapsulated adult human islets 3, 9, 16, and 36 weeks after transplantation in comparison with the glucose clearance kinetics of nondiabetic and diabetic controls.

Retrieved microcapsules. The majority of rat islet–containing microcapsules retrieved 10 or 36 weeks after transplantation exhibited only a mild cellular reaction on their external surface (Fig. 5*C* and *F*). As assessed by fluorescein diacetate staining, encapsulated rat islets retrieved 10 as well as 36 weeks after transplantation exhibited good viability (>85%) (Fig. 5*A*, *B*, *D*, and *E*). Consistent with the results of the IPGTTs, the retrieved encapsulated rat islets responded well to a glucose challenge. Their insulin secretion capacity, however, was slightly reduced compared with their initial insulin secretion capacity before transplantation (Table 1). The reduction in insulin secretion apparently occurred during the first two months of transplantation, as the insulin secretion capacity of encapsulated rat islets retrieved after 36

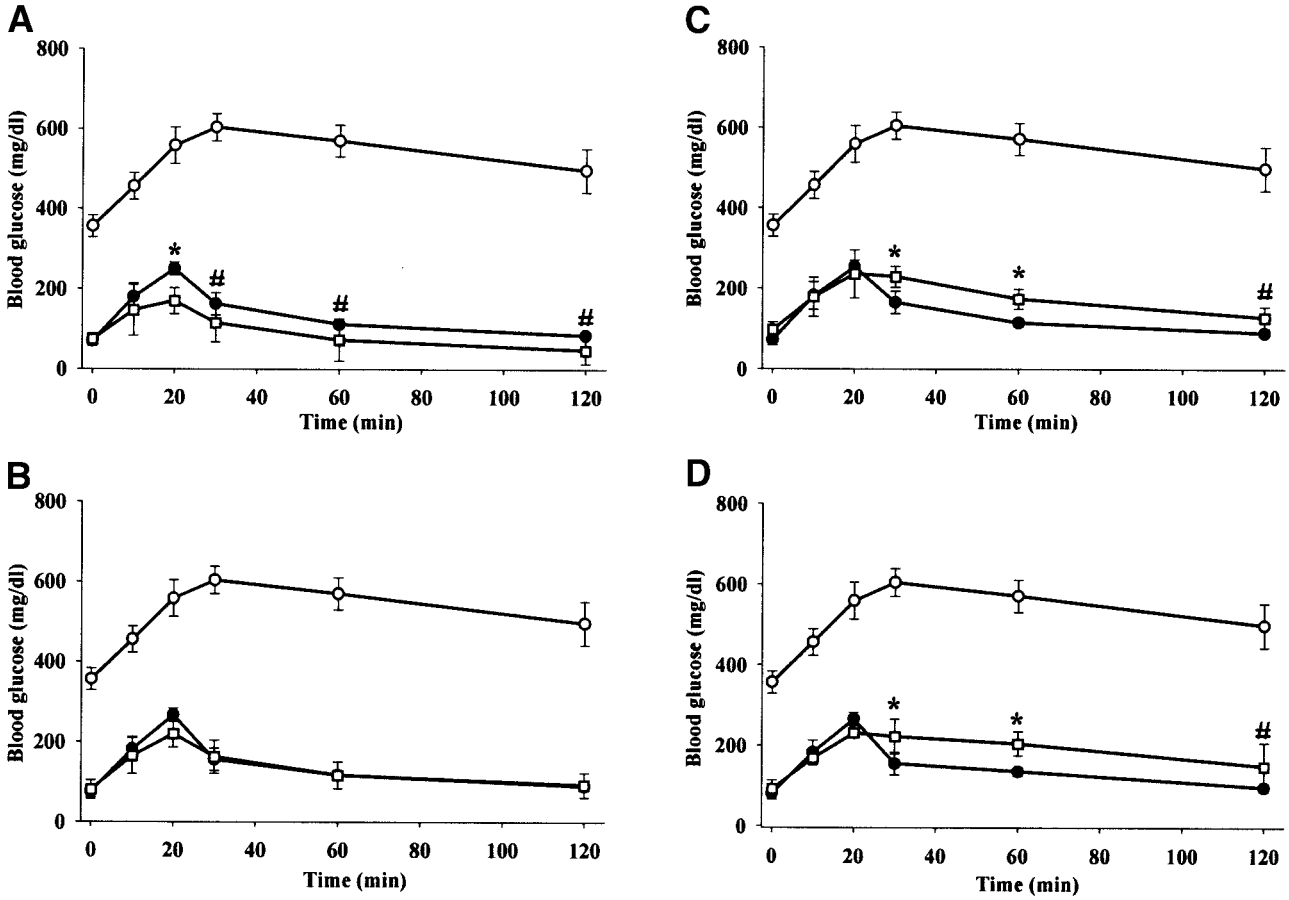

FIG. 3. Intraperitoneal glucose tolerance tests. Blood glucose profiles of mice transplanted with 1,800 encapsulated adult rat islets (□) after transplantation in comparison with glucose clearance kinetics of nondiabetic (●) and diabetic controls (○) at 3 (*A*; $n = 8$), 9 (*B*; $n = 7$), 16 (*C*; $n = 5$), and 36 (*D*; $n = 2$) weeks. Data are means ± SE. *$P < 0.001$ vs. nondiabetic controls; #$P < 0.05$ vs. nondiabetic controls.

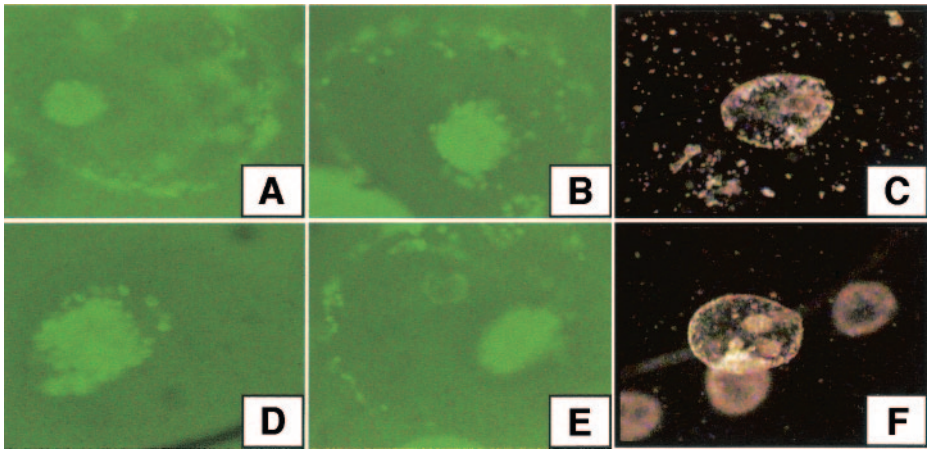

FIG. 5. Viability and cellular reactions on the surface of retrieved encapsulated adult rat islets. Fluorescein diacetate staining gave no indications of cell death 10 (*A* and *B*) or 36 (*D* and *E*) weeks after transplantation. The corresponding cellular reaction on the surface of the microcapsules was weak to moderate (*C* = 10 weeks; *F* = 36 weeks).

weeks was not significantly different from that of microcapsules retrieved after 10 weeks.

DISCUSSION

Over the past two decades, many different hydrogels and synthetic polymers have been proposed for immunoisolation and encapsulation of cells (23). Among these, alginate is one of the most promising natural polymers, as demonstrated by animal studies and small-scale clinical trials (24,25). One of the main requirements for long-term alginate-based transplantation is the stability of the microcapsules. This is achieved by cross-linking ultra-high–viscosity alginate of clinical grade (i.e., free of mitogenic and cytotoxic ingredients as well as of gram-negative and gram-positive bacterial spores) (19,20) with Ba^{2+} and by simultaneously incorporating stabilizing agents such as proteins into the microcapsules (17). As shown elsewhere (16), HSA can preserve insulin secretion for up to 3 weeks in tissue culture when this microcapsule design is used for encapsulation of rat islets. In contrast to FCS, HSA meets the safety criteria of the U.S. Food and Drug Administration and the American Society for Testing and Materials (26). The animal studies reported here confirm the enormous clinical potential of HSA-stabilized alginate-based microcapsules. The encapsulated islets of adult rats or humans maintained insulin secretion for >7 months, implying the absence of xenogenic rejection and the stability of the microcapsules. This finding was also confirmed by studies of insulin secretion of transplants explanted after 10 and 36 weeks. Failure of one graft was obviously due to severe infection of the tail induced by the blood collection procedure. Similarly, the finding that normoglycemia in one mouse was not established after transplantation of encapsulated human islets can most likely be traced back to a heavy infection of the abdominal wall.

Although the number of experiments with human islet cells were limited due to the restricted availability of intact human pancreatic tissue, our human islet cell data are in good accordance with the results obtained with rat islet cells. As depicted in Figs. 3 and 4, the pattern of glucose clearance differed from that of the nondiabetic control mice and changed with time after transplantation. Glucose clearance was faster in rat islet–transplanted mice than in controls 3 weeks after transplantation. This was likely due to the different secretory properties of rat islets. It is well known that rat islets have a lower set point than mouse islets (27,28), as evidenced by the lower fed and fasted blood glucose levels measured in normal rats. In contrast, a decrease in glucose clearance was observed 3–16 weeks after transplantation, as indicated by the considerably higher glucose values recorded 120 min after intraperitoneal glucose injection. It was interesting to observe that a further decrease in the glucose clearance rate with ongoing transplantation did not occur. A possible explanation for this finding is the restricted diffusion of nutrients and oxygen by an intense fibrotic overgrowth of the microcapsules during the first weeks of transplantation. However, although microcapsules retrieved 10 and 36 weeks after transplantation exhibited a reduced insulin secretion after glucose challenge, a strong fibrosis was not observed. The retrieved microcapsules showed only a weak cellular reaction close to the surface. β-Cell injury by complement-dependent components can also be excluded as a possible cause for the reduction in the glucose clearance rate, as the microcapsules used here were protected against these cytotoxic components, as shown recently by in vitro studies (16). The most likely explanation for these findings is that the microcapsules float freely in the peritoneal cavity without any vascularization. The supply of the encapsulated islets with nutrients and oxygen is, therefore, rather limited as evidenced by ^{19}F nuclear magnetic resonance imaging (29). Therefore, compromised nutrient and oxygen supply to the islets might lead to mass loss (8) and, in turn, a reduction in insulin secretion. This could also explain the fact that rather high numbers of encapsulated rat and human islets (1,800 islets) were needed to achieve a stable graft function in this study as compared with the lower numbers of nonencapsulated rat (400) and human (1,200) islets reported elsewhere (30,31). However, the decrease in islet mass was obviously stopped a few weeks after transplantation when the encapsulated islets equilibrated to their unfavorable environment.

The data presented here demonstrate that similar to rat islets, encapsulated human islets can reverse diabetes for a long period of time without the need for immunosuppression. This is a very promising finding, because long-term graft function of human islets has not been reported in the literature. Andersson et al. (32) found that the function of human islets entrapped in a chamber device and transplanted in nondiabetic nude mice was limited to ~8 weeks. Even significantly shorter survival times of transplants were reported by Scharp et al. (33). These

authors showed that 150–200 allogeneic adult human islets encapsulated in permselective hollow fibers provided protection against the immune response of diabetic and nondiabetic patients for up to 2 weeks. Treatment of a type 1 diabetic patient with Ca^{2+}-alginate/polylysine-based, intraperitoneally transplanted allogeneic adult islets resulted in insulin independence for only 30 days, despite administration of cyclosporin (34). Even though the literature is extremely limited, the data reported here suggest that microcapsules made of ultra-high−viscosity, clinical-grade alginate cross-linked with Ba^{2+} are superior to macrocapsule systems as well as alginate/polylysine microcapsules cross-linked with Ca^{2+}. Our data extend the results obtained by Omer et al. (15), who established normoglycemia in streptozotocin-induced diabetic mice for up to 20 weeks using NPCCs immunoisolated by Ba^{2+}−cross-linked alginate. However, in this study, diabetes could be reversed only by transplantation of 10,000 islet equivalents, and normoglycemia was observed relatively late, as NPCCs contain large amounts of precursor cells and immature islets that limit initial insulin production. In clinical practice, however, fetal human islets are not available in sufficient amounts; for clinical application of xenotransplantation, the use of adult xenogenic islets has the advantage of rapid onset of normoglycemia and reduction of the transplant volume (6).

In conclusion, our study demonstrated that microcapsules made up of ultra-high−viscosity alginate cross-linked with Ba^{2+} and stabilized with HSA protect adult rat and human islets against xenorejection for long periods after transplantation. These data are promising as they provide the first evidence that human islets can be protected by alginate-based microcapsules without an additional permselective membrane.

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REFERENCES

1. Ryan EA, Lakey JR, Paty BW, Imes S, Korbutt GS, Kneteman NM, Bigam D, Rajotte RV, Shapiro AM: Successful islet transplantation: continued insulin reserve provides long-term glycemic control. *Diabetes* 51:2148–2157, 2002
2. Zimmermann U, Mimietz S, Zimmermann H, Hillgärtner M, Schneider H, Ludwig J, Hasse C, Haase A, Rothmund M, Fuhr G: Hydrogel-based non-autologous cell and tissue therapy. *Biotechniques* 29:564–581, 2000
3. Zimmermann U, Cramer H, Jork A, Thürmer F, Zimmermann H, Fuhr G, Hasse C, Rothmund M: Microencapsulation-based cell therapy. In *Biotechnology*. Reed G, Rehm HJ, Eds. Weinheim, Wiley-VCH, 2001, p. 548–571
4. Zimmermann U, Hasse C, Rothmund M, Kühtreiber W: Biocompatible encapsulation materials: fundamentals and application. In *Cell Encapsulation Technology and Therapeutics*. Kühtreiber WM, Lanza RP, Chick WL, Eds. Boston, Birkhäuser, 1999, p. 40–52
5. Bonner-Weir S, Taneja M, Weir GC, Tatarkiewicz K, Song KH, Sharma A, O'Neil JJ: In vitro cultivation of human islets from expanded ductal tissue. *Proc Natl Acad Sci U S A* 97:7999–8004, 2000
6. Groth CG, Tibell A, Wennberg L, Korsgren O: Xenoislet transplantation: experimental and clinical aspects. *J Mol Med* 77:153–154, 1999
7. Lim F, Sun AM: Microencapsulated islets as bioartificial endocrine pancreas. *Science* 210:908–910, 1980
8. De Vos P, Van Straaten JF, Nieuwenhuizen AG, de Groot M, Ploeg RJ, De Haan BJ, Van Schilfgaarde R: Why do microencapsulated islet grafts fail in the absence of fibrotic overgrowth? *Diabetes* 48:1381–1388, 1999
9. King A, Lau J, Nordin A, Sandler S, Andersson A: The effect of capsule composition in the reversal of hyperglycemia in diabetic mice transplanted with microencapsulated allogeneic islets. *Diabetes Technol Ther* 50:653–663, 2003
10. Krestow M, Lum ZP, Tai IT, Sun A: Xenotransplantation of microencapsulated fetal rat islets. *Transplantation* 51:651–655, 1991
11. Clayton HA, James RF, London NJ: Islet microencapsulation: a review. *Acta Diabetol* 30:181–189, 1993
12. De Vos P, Wolters GH, Fritschy WM, Van Schilfgaarde R: Obstacles in the application of microencapsulation in islet transplantation. *Int J Artif Organs* 16:205–212, 1993
13. Geisen K, Deutschländer H, Gorbach S, Klenke C, Zimmermann U: Function of barium alginate-microencapsulated xenogenic islets in different diabetic mouse models. In *Frontiers in Diabetes Research: Lessons from Animal Diabetes*. Vol. III. Shafrir E, Ed. London, John Libbey, 1990, p. 142–148
14. Duvivier-Kali VF, Omer A, Parent RJ, O'Neil JJ, Weir GC: Complete protection of islets against allorejection and autoimmunity by a simple barium alginate membrane. *Diabetes* 50:1698–1705, 2001
15. Omer A, Duvivier-Kali VF, Trivedi N, Wilmot K, Bonner-Weir S, Weir GC: Survival and maturation of microencapsulated porcine neonatal cell clusters transplanted into immunocompetent mice. *Diabetes* 52:69–75, 2003
16. Schneider S, Feilen P, Cramer H, Hillgartner M, Brunnenmeier F, Zimmermann H, Weber MM, Zimmermann U: Beneficial effects of human serum albumin on stability and functionality of alginate microcapsules fabricated in different ways. *J Microencapsulation* 20:627–636, 2003
17. Zimmermann H, Hillgartner M, Manz B, Feilen P, Brunnenmeier F, Leinfelder U, Weber M, Cramer H, Schneider S, Hendrich C, Volke F, Zimmermann U: Fabrication of homogeneously cross-linked, functional alginate microcapsules validated by NMR-, CLSM- and AFM-imaging. *Biomaterials* 24:2083–2096, 2003
18. Schneider S, Feilen PJ, Slotty V, Kampfner D, Preuss S, Berger S, Beyer J, Pommersheim R: Multilayer capsules: a promising microencapsulation system for transplantation of pancreatic islets. *Biomaterials* 22:1861–1870, 2001
19. Zimmermann U, Thürmer F, Jork A, Weber M, Mimietz S, Hillgärtner M, Brunnenmeier F, Zimmermann H, Westphal I, Fuhr G, Nöth U, Haase A, Steinert A, Hendrich C: A novel class of amitogenic alginate microcapsules for long-term immunoisolated transplantation. *Ann N Y Acad Sci* 944:199–215, 2001
20. Leinfelder U, Brunnenmeier F, Cramer H, Schiller J, Arnold K, Vasquez JA Zimmermann U: A highly sensitive cell assay for validation of purification regimes of alginates. *Biomaterials* 24:4161–4172, 2003
21. Schneider S, Feilen PJ, Kraus O, Haase T, Sagban TA, Lehr HA, Beyer J, Pommersheim R, Weber MM: Biocompatibility of alginates for grafting: impact of alginate molecular weight. *Artif Cells Blood Substit Immobil Biotechnol* 31:383–394, 2003
22. Mach MA, Schlosser J, Weiland M, Feilen PJ, Ringel M, Hengstler JG, Weilemann LS, Beyer J, Kann P, Weber MM, Schneider S: Size of pancreatic islets of Langerhans: a key parameter for viability after cryopreservation. *Acta Diabetol* 40:123–129, 2003
23. Hunkeler D: Polymers for bioartificial organs. *Trends in Polymer Science* 5:286–293, 1997
24. Orive G, Hernandez RM, Gascon AR, Calafiore R, Chang TM, De Vos P, Hortelano G, Hunkeler D, Lacik I, Shapiro AM, Pedraz JL: Cell encapsulation: promise and progress. *Nat Med* 9:1104–1107, 2003
25. Hasse C, Klock G, Schlosser A, Zimmermann U, Rothmund M: Parathyroid allotransplantation without immunosuppression. *Lancet* 351:1296–1297, 1997
26. Dornish M, Kaplan D, Skaugrud O: Standards and guidelines for biopolymers in tissue-engineered products: ASTM alginate and chitosan standard guides. American Society for Testing and Materials. *Ann N Y Acad Sci* 944:388–397, 2001
27. Tatarkiewicz K, Hollister-Lock J, Quickel RR, Colton CK, Bonner-Weir S, Weir GC: Reversal of hyperglycemia after transplantation of macroencapsulated islets. *Transplantation* 67:665–671, 1999
28. Tatarkiewicz K, Garcia M, Omer A, Van Schilfgaarde R, Weir GC, De Vos P: C-Peptide response after meal challenge in transplanted mice with microencapsulated rat islets. *Diabetologia* 44:646–653, 2001
29. Zimmermann U, Nöth U, Gröhn P, Jork A, Ulrichs K, Lutz J, Haase A: Non-invasive evaluation of the location, the functional integrity and the oxygen supply of implants: ^{19}F nuclear magnetic resonance imaging of perfluorocarbon-loaded Ba^{2+}-alginate beads. *Artif Cells Blood Substit Immobil Biotechnol* 28:129–146, 2000
30. Gorczynski RM, Hu J, Chen Z, Kai Y, Lei J: A CD200FC immunoadhesion

prolongs rat islet xenograft survival in mice.*Transplantation* 73:1948–1953, 2002
31. Nakano M, Matsumoto I, Sawada T, Ansite J, Oberbroeckling J, Zhang HJ, Kirchhof N, Shearer J, Sutherland DE, Hering BJ: Caspase-3 inhibitor prevents apoptosis of human islets immediately after isolation and improves islet graft function. *Pancreas* 29:104–109, 2004
32. Andersson A, Eizirik DL, Bremer C, Johnson RC, Pipeleers DG, Hellerstrom C: Structure and function of macroencapsulated human and rodent islets transplanted into nude mice. *Horm Metab Res* 28:306–309, 1996
33. Scharp DW, Swanson CJ, Olack BJ, Latta PP, Hegre OD, Doherty EJ, Gentile FT, Flavin KS, Ansara MF, Lacy PE: Protection of encapsulated human islets implanted without immunosuppression in patients with type 1 or type 2 diabetes and in nondiabetic control subjects. *Diabetes* 43:1167–1170, 1994
34. Soon-Shiong P, Heintz RE, Merideth N, Yao QX, Yao Z, Zheng T, Murphy M, Moloney MK, Schmehl M, Harris M: Insulin independence in a type 1 diabetic patient after encapsulated islet transplantation. *Lancet* 343:950–951, 1994