Rapid Publication

Molecular Detection of Circulating β-Cells After Islet Transplantation

Beate Ritz-Laser,[1] José Oberholzer,[2] Christian Toso,[2] Marie-Claude Brulhart,[1] Katerina Zakrzewska,[1] Frédéric Ris,[2] Pascal Bucher,[2] Philippe Morel,[2] and Jacques Philippe[1]

Islet transplantation is a promising treatment for type 1 diabetes. However, islet grafts are submitted to multiple injuries, including immunosuppressive drug toxicity, hyperglycemia, hypoxia, unspecific inflammatory reactions, as well as allo- and autoimmune destruction. Therapeutic approaches to these damage mechanisms require early detection of islet injury, which is currently not feasible because of the lack of efficient markers. Based on the hypothesis of islet dissociation and release of islet cells into the circulation during islet injury, we designed a highly sensitive and specific molecular assay, able to detect two β-cells per milliliter of venous blood by RT-PCR of insulin mRNA. We report that circulating β-cells can be demonstrated up to 10 weeks after intraportal islet transplantation, as assessed after six islet grafts in four type 1 diabetic patients. Furthermore, our results suggest that the time during which circulating islet cells can be detected may depend on the graft environment and the immunosuppressive regimen. This test may allow better estimation of islet cell loss and identification of factors involved in islet graft injury. *Diabetes* 51:557–561, 2002

Replacement of insulin-producing cells through islet transplantation is a promising approach for treatment of type 1 diabetes. This procedure is safe and relatively simple with rare complications, but, up to recently, only moderate success rates have been achieved at 1 year posttransplantation (with 41% graft survival, as defined by C-peptide ≥0.5 ng/ml, and 11% insulin independence) (1). The Edmonton protocol, including an immunosuppressive strategy without steroids, presently leads to 100% graft survival and 75% insulin independence in a median follow-up of 10.2 months (2,3).

Whereas blood flow monitoring and amylase/lipase clearance (in case of bladder drainage), as well as serum amylase/lipase and nitric oxide concentrations, are used to asses pancreatic graft rejection (4), for islet transplantation, no early and reliable markers are available to monitor islet injury. Anti-GAD_{65} antibodies are observed more frequently in patients experiencing an islet graft loss (5), but no systematic correlation can be found. Similarly, monitoring of serum GAD_{65} levels could not be used as a marker of acute islet cell destruction (6). At present, islet graft survival is controlled by monitoring of glycemia, serum C-peptide, and HbA_{1c} levels; however, their sensitivity is low, and onset of hyperglycemia and C-peptide levels <0.5 ng/ml are end-stage events indicating the loss of >85% of the transplant (7,8). Evaluation of the functional reserve of transplanted islets by glucose tolerance tests or calculation of the proinsulin-to-insulin ratio seems to be more indicative for islet dysfunction, but still not sensitive enough for an early detection of graft loss (6,7).

Our aim is to develop a reliable and early marker of the rejection process as well as a diagnostic tool for islet engraftment. As a first study, we set up a highly sensitive and specific molecular assay to investigate whether and for how long transplanted β-cells can be detected in the general circulation. This assay was based on the hypothesis of islet dissociation and release of islet cells into the circulation during graft injury. Amplification of insulin mRNA as a major and specific β-cell transcript by RT-PCR allowed us to detect as few as two β-cells per milliliter of blood. We report here that islet β-cells can be identified in the circulation up to 10 weeks after transplantation, as demonstrated in four type 1 diabetic patients, indicating an early loss of transplanted β-cells through islet dissociation. Furthermore, the time during which circulating β-cells are detected may depend on the graft environment and immunosuppression strategy.

RESEARCH DESIGN AND METHODS

Pancreas procurement and islet isolation. Pancreata were harvested from heart-beating multiorgan donors who were hemodynamically stable, aged between 20 and 70 years, and without previous history of alcohol abuse or pancreatitis. Pancreata were transported at 4°C in University of Wisconsin solution to the isolation laboratory of the Unit of Investigative Surgery, with a mean cold ischemia time of 6 h (range 2–9). Islets were isolated as described previously (9) and kept in culture in CMRL solution (Sigma) supplemented with 10% FCS (Gibco, Paisley, Scotland), 1% glutamine (Merck, Darmstadt, Germany), 1% HEPES, penicillin, and streptomycin (Sigma) at 24°C and 5% CO_2 for <24 h. Before transplantation, islets were washed three times with Hank's solution containing 4% human albumin (Albumin 20%; SRK, Bern, Switzerland), and samples were taken for counting. Only intact dithizone-

From the [1]Diabetes Unit, Department of Internal Medecine, University Hospital Geneva, Geneva, Switzerland; and the [2]Clinic of Digestive and Transplant Surgery, Department of Surgery, University Hospital Geneva, Geneva, Switzerland.

Address correspondence and reprint requests to Beate Ritz-Laser, Diabetes Unit, University Hospital Geneva, CH-1211 Geneva 14, Switzerland. E-mail: beate.laser@medecine.unige.ch.

Received for publication 4 October 2001 and accepted in revised form 8 January 2002. Posted on the World Wide Web at http://diabetes.diabetesjournals.org/rapidpubs.shtml on 4 February 2002.

EIN, equivalent islet number; IAK, islet after kidney; SIK, simultaneous islet-kidney.

TABLE 1
Graft characteristics and immunopressive regimens

Patient ID	Type	EIN	EIN/ kg	Immunosuppression
1	IAK	1,109,826	19,134	aIL2-R, C, MMF, S
2-1	SIK	187,732	3,237	aIL2-R, C, MMF, S
2-2	IAK	277,560	4,785	aIL2-R, C, MMF, S
3-1	IAK	294,000	4,324	aIL2-R, T, Si
3-2	IAK	350,000	5,147	aIL2-R, T, Si
4	SIK	366,750	4,826	aIL2-R, T, Si

AIL2-R, anti–interleukin-2-receptor antibodies; C, cyclosporin; MMF, mycophenolate; S, steroids; Si, sirolimus; T, tacrolimus.

stained islets were considered, and the equivalent islet number (EIN) was calculated by normalizing the islets to a standard islet of 150 μm diameter.

Islet transplantation. Four C-peptide–negative type 1 diabetic patients (three women and one man) with a mean age of 50.5 years (50–55) and a mean weight of 65 kg (58–76) were transplanted with islet allografts according to the approval of the Geneva Hospital ethics board. Two patients had a previous functioning kidney graft and received injection of islets alone, while the two other patients received a combined islet and kidney graft (Table 1). To increase the mass of grafted islets up to a minimum of 8,000 EIN/kg, two patients received two islet injections (patients 2 and 3) while a third patient received only one islet injection and is still waiting for the second graft (patient 4). A mean of 12,209 EIN/kg (range 8,022–19,134) were injected in the three first patients (Table 1).

For islet after kidney (IAK) transplantation, injection was performed in the portal vein after puncture under radiological control. For simultaneous islet-kidney (SIK) transplantation, a right colic vein was cannulated during the kidney operation. After administration of 5,000 units of intravenous heparin, islets were injected in Dulbecco's modified Eagle's medium solution supplemented with 4% human albumin under portal pressure control.

Immunosuppression. Induction therapy was performed with anti–interleukin-2-receptor antibodies. Basiliximab (Simulect; Novartis, Basel, Switzerland) 20 mg i.v. at days 0 and 4 was used for the first two patients and daclizumab (Zenapax; Roche, Basel, Switzerland) 1 mg/kg every 2 weeks for a total of five doses in the other two patients.

The immunosuppressive protocol of the first two patients consisted of oral cyclosporine (Neoral; Novartis, Basel, Switzerland), with trough levels between 150 and 200 ng/ml during the first 3 months, thereafter 100 ng/ml; mycophenolate (Cell Cept; Roche); and steroids (Table 1). The two last patients received sirolimus (Rapamune; Wyeth, Zug, Switzerland), with trough levels between 12 and 15 ng/ml for the first 3 months and 7–10 ng/ml thereafter, and tacrolimus (Prograf; Fujisawa, Kerry, Ireland), with target trough levels between 3 and 6 ng/ml, but no steroids. In all cases, an adjuvant treatment was administrated as follows: 2 g nicotinamide (Nicobion; Astra-France, Monts, France), 800 units vitamin E, and 1,200 mg pentoxifylline (Trental; Hoechst-Marion-Roussel, Zurich, Switzerland) per day for a period of 1 month. When needed, intravenous insulin was administered.

Sample harvesting, RNA isolation, and RT-PCR. Samples of venous blood (3 ml) were collected before ($t = 0$) and at various time points (up to 20 months) after transplantation. They were drawn into vacutainer EDTA tubes (Becton Dickinson), kept at 4°C, and processed within 12 h. RNA isolation was performed using the QIAmp RNA blood minikit (Qiagen). Briefly, after selective erythrocyte lysis, remaining cells were concentrated by centrifugation and disrupted under highly denaturing conditions. After DNaseI treatment, RNA was purified on silica-gel columns. RT was performed in a total volume of 20 μl using 3 μg total RNA, random hexamer primers, and Superscript II reverse transcriptase (Life-Technologies). Insulin cDNA (255-bp fragment) was amplified using one-tenth of the RT reaction and Goldstar *Taq* DNA polymerase (Eurogenetech) for 45 cycles (1 min 94°C, 1 min 55°C, 1 min 72°C; sense 5′ CCTTTGTGAACCAACACCTG, exon 2; antisense 5′ AGTAGT TCTCCAGCTGGTAG, exon 3). For most samples, two independent amplifications were performed. PCR products were analyzed by gel electrophoresis and Southern blot hybridization with rat insulin cDNA. Negative and positive controls were cDNA from blood samples of healthy control subjects without and with addition of 10–20 dissociated nonsorted human islet cells per milliliter of blood, respectively. All RT reactions were controlled by amplification of the G protein α-subunit ($G_s\alpha$), a low-expressed gene in blood cells, for 30 cycles (1 min 94°C, 1 min 50°C, 1 min 72°C) (sense 5′ ACTTCTG GAATCTTTGAGACCAAG, exon 8; antisense 5′ TTAAAGGCTTTAATTAATTT GGGGGTTCC, exon 13).

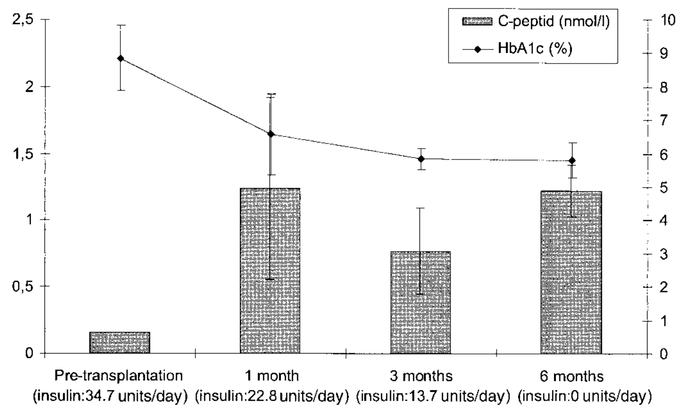

FIG. 1. Metabolic evaluation after islet allotransplantation. Data are means ± SD of C-peptide and HbA_{1c} levels, as well as insulin requirements of four patients.

Student's t test was used for statistical analysis. $P < 0.05$ was considered significant.

RESULTS

Patient follow-up and metabolic evaluation. Mean follow-up was 8.5 months (range 3–20) after the last transplantation. The first C-peptide measurement was performed 12 h after the injection and demonstrated a transient peak reaching 1.79 ng/ml ($P < 0.05$ vs. 1-month values). At 1 month, mean ± SD C-peptide levels were measured at 1.24 ± 0.7 ng/ml and remained stable during the follow-up (Fig. 1). HbA_{1c} reached 8.9 ± 1 ng/ml before transplantation and decreased to 5.85 ± 0.3 and 5.8 ± 0.5 ng/ml at 3 and 6 months after graft, respectively ($P \leq 0.01$). The first two patients decreased their insulin requirements according to the doses of steroids administered. They stopped exogenous insulin treatment at 5 and 6 months, respectively, after the last graft. Patient 3 became insulin free a few hours after the last islet injection. Patient 4 received only the first injection and still needs exogenous insulin at present time. He was not included in the 6-month outcome because of insufficient follow-up.

Amplification of insulin mRNA by RT-PCR. To detect circulating pancreatic β-cells with high sensitivity, we amplified insulin mRNA as the major β-cell transcript by RT-PCR. We first analyzed the assay sensitivity by isolating total RNA from whole-blood samples to which increasing

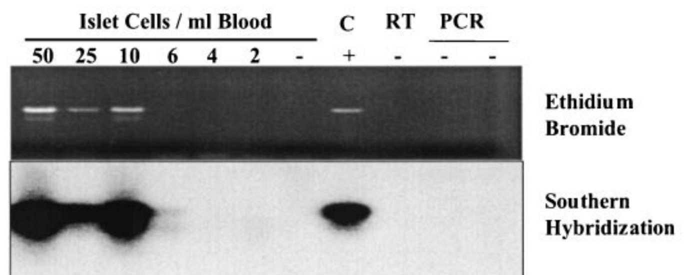

FIG. 2. Insulin RT-PCR assay sensitivity. Dissociated human islet cells were added to whole blood before RNA isolation. Using ethidium bromide staining and Southern blot hybridization with a rat insulin cDNA probe, 10 and 6 islet cells, respectively, per milliliter of blood can be detected by insulin RT-PCR analysis. Considering the proportion of β-cells of the islet preparation (~30%), approximately two β-cells per milliliter of blood can be detected. cDNA from whole blood with addition of 20 human islet cells/ml blood was used as positive control (C+), and RT and PCR steps were controlled with H_2O blank reactions.

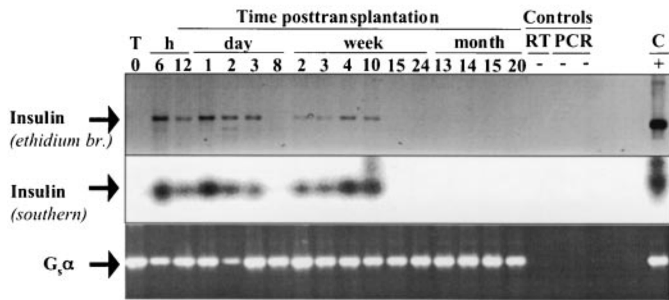

FIG. 3. Circulating β-cells can be detected during 10 weeks after intraportal islet transplantation. Follow-up of a patient (ID no. 1 in Tables 1 and 2) during 20 months after islet transplantation is shown. Circulating β-cells in the venous blood can be detected during 10 weeks posttransplantation, but not in later blood samples (upper and middle panel). PCR product identity confirmation by Southern blot hybridization with a rat insulin cDNA probe is shown in the middle panel. The success of all RT reactions was tested with by amplification of $G_s\alpha$ cDNA (lower panel). cDNA from blood samples of healthy control subjects with addition of 20 human islet cells/ml blood served as positive control (C+), and one RT and two PCR H_2O blank reactions served as negative controls.

numbers of dissociated, nonsorted, human islet cells had been added. After gel analysis and Southern blot hybridization of the PCR products, a minimum of six islet cells per milliliter of blood could be detected (Fig. 2), whereas samples of eight healthy control subjects were negative (data not shown). Since the relative proportion of β-cells in our islet preparation was ~30%, a value corresponding to the β-cell proportion observed in other studies (2), we conclude that approximately two β-cells per milliliter of blood can be detected. A minimum of 10,000 β-cells thus have to be present in the general circulation to generate a positive result in our qualitative, but not quantitative, RT-PCR assay.

Clinical evaluation of RT-PCR assay for insulin mRNA after transplantation. This evaluation was based on the hypothesis that islet injuries are accompanied by a release of islet cells into the circulation. Since RNA isolation was done from nonerythrocyte cells present in the peripheral blood, detection of insulin mRNA by RT-PCR indicates indeed the presence of β-cells in the blood and not only the release of β-cell content due to local cell lysis in the liver. Synthesis of cDNA was successful in all cases, as determined by similar amplification of $G_s\alpha$ cDNA in each sample (Fig. 3 and data not shown). Patient 1 was followed during 20 months after transplantation; whereas circulating β-cells were detected in the venous blood during 10 weeks posttransplantation, further samples were found negative (Fig. 3).

Similar results were obtained with patients 2–4, all of whom exhibited circulating β-cells early after transplantation for a limited period of up to 7 weeks (Table 2). These data indicate that a portion of intraportally transplanted islets dissociates with the release of single or aggregated islet cells into the blood. The fact that not all blood samples analyzed during the circulation period of individual patients produced positive insulin RT-PCR assays (Table 2) might be due to varying amounts of islet cells released per day.

Interestingly, time during which β-cells were detected in the circulation varied among the four patients analyzed. Patients 2 and 3 received a second islet transplantation at 7 and 13 weeks, respectively, after the first graft. In both

TABLE 2
Follow-up of four patients during 3–20 months after islet transplantation

Patient	T	h	days												
ID	0	6	1	2	3	4	5	6	7	8	9	10	11	12	13
1	−	+	+	+	+					−					
2-1	−	+	+	+	−	+	+	+	+				+	+	
2-2	−	+	+	+	+	−	−	+							
3-1		+	+	−	+	−	−	−	−	+				+	
3-2	−	+	+	+	−	−	−	−					−		
4	−	−	+	−	+	−	−	+	−	−			−	−	

Patient	weeks																									
ID	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27
1	+	+	+						+					−									−			
2-1	+		+	+	+	−																				
2-2	−	−		−		+	−				−						−				−			−		
3-1		−	−	−	−							−														
3-2		−					−	−	−	−	−	−	−													
4	+	−	−		−	−				−																

Patient	months													
ID	7	8	9	10	11	12	13	14	15	16	17	18	19	20
1							−	−	−					
2-1														
2-2	−	−												
3-1														
3-2														
4														

Patients 2 and 3 have received two successive intraportal islet injections. All patients have positive venous blood samples for insulin RNA, indicating the presence of circulating β-cells during an initial period of 2–10 weeks posttransplantation, but negative reactions in the longer follow-up. '+' and '−' indicate positive and negative insulin RT-PCR assays, respectively.

cases, circulating β-cells were detected less systematically and/or during a shorter period of time after the second transplantation (Table 2), suggesting that previous injection of functioning β-cells might protect new transplanted islets.

A second important observation was the correlation between the time during which β-cells were detected in the circulation with the immunosuppressive regimen: patients 1 and 2 received classical immunosuppression with cyclosporine and corticoids, drugs known to exert toxic effects on β-cells (10,11), whereas patients 3 and 4 followed a modified strategy with sirolimus and tacrolimus but no steroids. Interestingly, the duration of the presence of circulating β-cells in the second group was markedly shortened and did not exceed 2 weeks (Table 2).

DISCUSSION

We report the development of a highly sensitive qualitative insulin RT-PCR assay for detection of circulating β-cells after islet transplantation. Similar approaches have been used to monitor circulating cancer cells in the blood of patients with metastatic disease (12,13) or renal allograft rejection in urine samples (14), and the detection limit reached in our assay (two β-cells per milliliter of blood) is excellent, as compared with those described.

Islet transplantations monitored in the present study resulted in immediate graft function and insulin independence of all patients receiving a sufficient islet mass with a mean follow-up of 8.5 months. Surprisingly, we observed the presence of circulating β-cells in venous blood during a period of 6 days to 10 weeks posttransplantation. Although vulnerability of intraportally injected human islets is well known, previous studies have focused on local cell death due to peri- and posttransplant injuries (11,15–17), and this is the first report of intact islet cell release into the circulation. The presence of circulating β-cells may be a marker for acute or chronic islet injury, although this needs to be clearly established; however, it suggests islet loss through dissociation. Isolated β-cells can be regularly observed in liver sinusoids of biopsies immediately after islet transplantations (data not shown), and our data indicate that cell shedding continues in the early posttransplant period. To reach the peripheral circulation, these cells have to pass through the pulmonary bed by a yet-unknown mechanism that might, however, be similar to metastatic cancer cells (12,13). Furthermore, we can not exclude that at least a portion of the released islet cells may be subject to phagocytosis by lymphoid cells (e.g., in the lung or spleen) and that insulin RNA is detected in these cells. However, this RNA should be in part degraded, rendering its amplification less likely.

Beside circulating β-cells, a transient serum C-peptide peak was observed 12 h after islet graft. This phenomenon is consistent with in vitro experiments, demonstrating the incompatibility between human blood and isolated islets of Langerhans (18), and suggests lysis of islets immediately after injection. An important factor leading to islet injury is hypoxia due to the requirement of graft revascularization that is completed only after 10–14 days (16,17). Islet dissociation has been shown to be experimentally induced by islet amyloid polypeptide (19), and the cytotoxic factors leading to local necrosis and apoptosis (10,11,15,20,21) might also be implicated in the islet cell release observed in this study. Regarding our initial purpose to evaluate the insulin RT-PCR assay as a potential marker for graft rejection or autoimmune recurrence, detection might only be feasible after the early islet cell circularization period.

Time during which β-cells were detected in the circulation was highly variable among patients but had no consequences on graft success inasmuch as with the present follow-up, all patients who received sufficient islet mass were insulin-free. However, fewer islets would probably be required to achieve insulin independence if the posttranplant management could be further optimized; our assay may be useful in monitoring this process. We did not observe any correlation between the number of injected islets and the duration of positive testing for insulin-mRNA. This observation renders unlikely that cells are only released at the time of implantation and then circulate for days or weeks; there is rather an ongoing release of cells as long as the islets are prone to injury.

Importantly, our data indicate a correlation between the duration of β-cell shedding and the type of immunosuppression. The longest times of β-cell circulation were observed with patients receiving classical immunosuppression with cyclosporine, mycophenolate, and steroids, drugs that are known to be toxic to pancreatic islets (11) and/or to inhibit the process of islet revascularization (4,10,11,22). Diabetic microangiopathy and delay of revascularization could thus explain the prolonged duration of β-cell shedding in this group of patients. A modified strategy with relatively low doses of sirolimus and tacrolimus and no steroids resulted in markedly shortened circulation times, suggesting lower islet toxicity and better implantation. Furthermore, the β-cell circulation times appeared shortened in the second islet injection in patients harboring a functional islet graft, suggesting that the presence of the first graft may exert a protective effect for the second injection. Our data are in agreement with reports demonstrating that residual native β-cells contribute to the survival or function of intraportally transplanted islets (15,23). The final fate of the β-cells released into the general circulation is unknown; whether they are eliminated or able to colonize other organs needs to be addressed in further studies.

ACKNOWLEDGMENTS

This study was supported by grants from the University Hospital Geneva, the Institute of Human Genetics and Biochemistry, and Novo Nordisk Switzerland, as well as by the Swiss National Science Foundation (no. 3200-061873.00 to J.O. and P.M.), the Association Genevoise des Diabétiques (to P.M., J.O., and J.P.), and the Foundation Carlos et Elsie de Reuter (to P.M.).

We are indebted to A. Mamin for experimental assistance and to Raymond Mage, Cathy Cametti, Corinne Sinigaglia, and David Matthey for their skillful assistance for islet isolation and preparation. We thank the University Hospitals of Switzerland for their collaboration for the multiple organ procurements needed for our islet transplantation program.

REFERENCES

1. Brendel MD, Hering BJ, Schulz AO, Bretzel RG: *International Islet Transplant Registry Report*. Giessen, Germany, 2001
2. Shapiro AM, Lakey JR, Ryan EA, Korbutt GS, Toth E, Warnock GL, Kneteman NM, Rajotte RV: Islet transplantation in seven patients with type 1 diabetes mellitus using a glucocorticoid-free immunosuppressive regimen. *N Engl J Med* 343:230–238, 2000
3. Ryan EA, Lakey JR, Rajotte RV, Korbutt GS, Kin T, Imes S, Rabinovitch A, Elliott JF, Bigam D, Kneteman NM, Warnock GL, Larsen I, Shapiro AM: Clinical outcomes and insulin secretion after islet transplantation with the Edmonton protocol. *Diabetes* 50:710–719, 2001
4. Hakim NS: Recent developments and future prospects in pancreatic and islet transplantation. *Diabetes Obes Metab* 3:9–15, 2001
5. Jaeger C, Brendel MD, Hering BJ, Eckhard M, Bretzel RG: Progressive islet graft failure occurs significantly earlier in autoantibody-positive than in autoantibody-negative IDDM recipients of intrahepatic islet allografts. *Diabetes* 46:1907–1910, 1997
6. Shapiro AM, Hao EG, Lakey JR, Yakimets WJ, Churchill TA, Mitlianga PG, Papadopoulos GK, Elliott JF, Rajotte RV, Kneteman NM: Novel approaches toward early diagnosis of islet allograft rejection. *Transplantation* 71: 1709–1718, 2001
7. Davalli AM, Maffi P, Socci C, Sanvito F, Fresci M, Bertuzzi F, Falqui L, Di Carlo V, Pozza G, Secchi A: Insights from a successful case of intrahepatic islet transplantation into a type 1 diabetic patient. *J Clin Endocrinol Metab* 85:3847–3852, 2000
8. Schulak JA, Franklin W, Reckard CR: Morphological and functional changes following intraportal islet allograft rejection: irreversibility with steroid pulse therapy. *Surg Forum* 28:296–299, 1977
9. Oberholzer J, Triponez F, Mage R, Andereggen E, Bühler L, Crérin N, Fournier B, Goumaz C, Lou J, Philippe J, Morel P: Human islet transplantation: lessons from 13 autologous and 13 allogeneic transplantations. *Transplantation* 69:1115–1123, 2000
10. Liu EH, Herold KC: Transplantation of the islets of Langerhans: new hope for treatment of type 1 diabetes mellitus. *Trends Endocrinol Metab* 11:379–382, 2000
11. Drachenberg CB, Klassen DK, Weir MR, Wiland A, Fink JC, Bartlett ST, Cangro CB, Blahut S, Papadimitriou JC: Islet cell damage associated with tacrolimus and cyclosporine: morphological features in pancreas allograft biopsies and clinical correlation. *Transplantation* 68:396–402, 1999
12. Katz AE, Olsson CA, Raffo AJ, Cama C, Perlman H, Seaman E, O'Toole KM, McMahon D, Benson MC, Buttyan R: Molecular staging of prostate cancer with the use of an enhanced reverse transcriptase-PCR assay. *Urology* 43:765–775, 1994
13. Ringel MD, Ladenson PW, Levine MA: Molecular diagnosis of residual and recurrent thyroid cancer by amplification of thyroglobulin messenger ribonucleic acid in peripheral blood. *J Clin Endocrinol Metab* 83:4435–4442, 1998
14. Li B, Hartono C, Ding R, Sharma VK, Ramaswamy R, Qian B, Serur D, Mouradian J, Schwartz JE, Suthanthiran M: Noninvasive diagnosis of renal-allograft rejection by measurement of messenger RNA for perforin and granzyme B in urine. *N Engl J Med* 344:947–954, 2001
15. Bretzel RG, Brandhorst D, Brandhorst H, Eckhard M, Ernst W, Friemann S, Rau W, Weimar B, Rauber K, Hering BJ, Brendel MD: Improved survival of intraportal pancreatic islet cell allografts in patients with type-1 diabetes mellitus by refined peritransplant management. *J Mol Med* 77:140–143, 1999
16. Menger MD, Jaeger S, Walter P, Feifel G, Hammersen F, Messmer K: Angiogenesis and hemodynamics of microvasculature of transplanted islets of Langerhans. *Diabetes* 38 (Suppl. 1):199–201, 1989
17. Menger MD, Yamauchi Ji, Vollmar B: Revascularization and microcirculation of freely grafted islets of langerhans. *World J Surg* 25:509–515, 2001
18. Bennet W, Sundberg B, Groth CG, Brendel MD, Brandhorst D, Brandhorst H, Bretzel RG, Elgue G, Larsson R, B, Korsgren O: Incompatibility between human blood and isolated islets of Langerhans: a finding with implications clinical intraportal islet transplantation? *Diabetes* 48:1907–1914, 1999
19. Ritzel RA, Sultana C, Butler PC: Human islet amyloid polypeptide (IAPP) oligomers disrupt integrity of human islets: a proposed novel mechanism of hIAPP toxicity (Abstract). *Diabetes* 50 (Suppl. 2):A412, 2001
20. Davalli AM, Scaglia L, Zangen DH, Hollister J, Bonner-Weir S, Weir GC: Vulnerability of islets in the immediate posttransplantation period: dynamic changes in structure and function. *Diabetes* 19:1160–1167, 1996
21. Davalli AM, Ogawa Y, Ricordi C, Scharp DW, Bonner-Weir S, Weir GC: A selective decrease in the beta cell mass of human islets transplanted into diabetic nude mice. *Transplantation* 59:817–820, 1995
22. Rooth P, Dawidson I, Lafferty K, Diller K, Armstrong J, Pratt P, Simonsen R, Taljedal IB: Prevention of detrimental effect of cyclosporin A on vascular ingrowth of transplanted pancreatic islets with verapamil. *Diabetes* 38 (Suppl. 1):202–205, 1989
23. Hughes SJ, Powis SH, Press M: Surviving native beta-cells determine outcome of syngeneic intraportal islet transplantation. *Cell Transplant* 10:145–151, 2001