Differences in Baseline Lymphocyte Counts and Autoreactivity Are Associated With Differences in Outcome of Islet Cell Transplantation in Type 1 Diabetic Patients

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OBJECTIVE—The metabolic outcome of islet cell transplants in type 1 diabetic patients is variable. This retrospective analysis examines whether differences in recipient characteristics at the time of transplantation are correlated with inadequate graft function.

RESEARCH DESIGN AND METHODS—Thirty nonuremic C-peptide–negative type 1 diabetic patients had received an intraportal islet cell graft of comparable size under an ATG-tacrolimus–mycophenolate mofetil regimen. Baseline patient characteristics were compared with outcome parameters during the first 6 posttransplant months (i.e., plasma C-peptide, glycemic variability, and gain of insulin independence). Correlations in univariate analysis were further examined in a multivariate model.

RESULTS—Patients that did not become insulin independent exhibited significantly higher counts of B-cells as well as a T-cell autoreactivity against insulinoma-associated protein 2 (IA2) and/or GAD. In one of them, a liver biopsy during posttransplant year 2 showed B-cell accumulations near insulin-positive β-cell aggregates. Higher baseline total lymphocytes and T-cell autoreactivity were also correlated with lower plasma C-peptide levels and higher glycemic variability.

CONCLUSIONS—Higher total and B-cell counts and presence of T-cell autoreactivity at baseline are independently associated with lower graft function in type 1 diabetic patients receiving intraportal islet cells under ATG-tacrolimus–mycophenolate mofetil therapy. Prospective studies are needed to assess whether control of these characteristics can help increase the function of islet cell grafts during the first year posttransplantation. ***Diabetes*** **58:2267–2276, 2009**

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Islet cell tranplantation is a promising therapy for type 1 diabetic patients, but its current state faces several limitations and obstacles (1,2). Insulin independence can be achieved during the first year posttransplantation in up to 80% of selected patients in small, single-center cohorts (3–7), but the success rate is lower in larger studies with less stringent criteria for selection of recipients and donor tissue (8,9). Several factors can account for the observed variability in outcome. Their identification is hindered by the difficulty in standardizing protocols and by the small numbers of patients that have so far been included per protocol. Within these limitations, graft and recipient characteristics have been related with the outcome of clinical islet cell transplantation (10–13). A minimal donor tissue mass was reported to induce insulin independence but is in itself not sufficient (3,10,13); administration of more potent immune suppressants can lower this treshold (14,15), which is lowest in autologous transplantation (16). Using cultured β-cell preparations in an ATG-based protocol, we defined the minimal number of β-cells that reproducibly resulted in circulating signs of a surviving graft 2 months after transplantation (17). In the latter study, achievement of insulin independence also depended on the β-cell mass in the graft but appeared counteracted by the presence of an islet-specific T-cell autoreactivity as measured by in vitro lymphocyte stimulation tests against the islet autoantigens GAD and insulinoma-associated protein 2 (IA2) (18). We have now analyzed a cohort of 30 consecutively transplanted recipients in search for a possible correlation between their baseline characteristics and the clinical outcome of defined islet cell grafts that are intraportally injected under the same ATG-based protocol.

RESEARCH DESIGN AND METHODS

Graft recipients and baseline characteristics. Between September 2000 and January 2006, 35 nonuremic type 1 diabetic patients received an islet cell transplant under ATG induction therapy and maintenance immune suppression with mycophenolate mofetil (MMF) and tacrolimus. They were all C-peptide negative, had large within-subject variation of fasted glycemia (coefficient of variation of prebreakfast glycemia [CVfg] >25%), and one or

TABLE 1
Metabolic outcome 6 months after islet cell transplantation

	At posttransplant month 6		
	Insulin independent	Insulin dependent	*P**
C-peptide positive (*n*)	15/15	14/15	
C-peptide (ng/ml)	2.3 (1.9–3.0)	1.0 (0.4–1.2)	<0.001
Fasting glycemia			
Mean (mg/dl)	127 (115–135)	132 (127–153)	0.12
CVfg (%)	9 (8–10)	19 (15–39)	<0.001
A1C (%)	6.1 (5.8–6.4)	6.1 (5.7–6.7)	0.66
Insulin dose (IU · kg^{-1} · day^{-1})	0	0.27 (0.20–0.46)	<0.001
Percentage of baseline insulin dose (%)	0	43 (34–69)	<0.001

Data are medians (IQR). Fasting glycemia was measured at home, and within-subject variation of fasted glycemia (CVfg) was calculated during the preceding month. *Statistical analysis was done with Mann-Whitney *U* test.

more signs of diabetic lesions (hypoglycemic unawareness, microalbuminuria, or retinopathy). The first 24 patients had been included in a phase 1 graft-dose finding study and the last 11 patients in a protocol that aims to assess influence of tapering of tacrolimus after month 12. Graft survival with this immune-suppressive regimen was previously reported for the first 24 patients (17,18). Informed consent had been obtained from all candidate recipients before they were listed as such by the Eurotransplant Foundation. Selection for transplantation occurred on basis of listing date, bloodgroup compatibility with the available graft, and health status. At the time of transplantation, none presented symptoms of acute infectious disease or inflammation. Analysis for cytomegalovirus (PCR and serology) and hepatitis A, B, and C (serology) at baseline excluded active disease. Two patients tested positive for complement-binding HLA antibodies pretransplantation, two patients that discontinued immune suppression during the first 6 months and one patient that died from a cerebral hemoraghe at 18 weeks posttransplant. These five patients were excluded from the current analysis.

Graft characteristics and transplantation procedure. Islet cells were isolated and cultured according to standardized protocols (17,19,20). For all islet cell grafts used, a sample was taken before transplantation and analyzed for its insulin synthesis capacity in the absence and presence of glucose (10 mmol/l). They all fulfilled the set criteria for function (i.e., minimally 20 pmol insulin synthesized per 2 h per million β-cells). Preparations were analyzed for their cellular composition and combined to grafts before being infused into the portal vein using either a laparoscopic (n = 16) (21) or subcutaneous transhepatic approach (n = 14) (22). Donor and graft characteristics are listed in appendix 1 (available at http://diabetes.diabetesjournals.org/cgi/content/full/db09-0160/DC1).

Immune and anticoagulant therapy. Induction therapy consisted of rabbit ATG (Fresenius HemoCare, Redmond, WA) initiated 1–4 days before transplantation. A first dose of 9 mg/kg body wt was followed by 3 mg/kg for 6 days; no injection was given on days with a T-cell count <50 cells/mm^3. Maintenance immunesuppression consisted of MMF (2,000 mg/day Cellcept starting on the day of the first ATG injection; gift of Roche, Brussels, Belgium) and tacrolimus (Prograft, starting 1 day before the last ATG injection, Fujisawa). Tacrolimus troughlevels were maintained between 8 and 10 ng/ml during the first 3 months after an islet cell transplantation and 6–8 ng/ml thereafter. This MMF-tacrolimus treatment was continued when a second islet cell transplant was given, without additional antibody course, but with one injection of 500 mg methylprednisolone 3 h before transplantation. Anticoagulant therapy was started 1 day after infusion and consisted of acetylsalycilic acid 100 mg once daily in all patients and additional low–molecular weight heparin at preventive dosage in 18 subjects (0.3 ml Fraxiparin once daily). No heparin was administered during islet infusion.

Assessment of baseline characteristics and transplantation outcome. Efficacy and safety criteria were examined weekly during the first 6 weeks posttransplant, every 2 weeks between posttransplant week 6 and 12, and monthly thereafter. The CVfg was assessed using home glucose monitoring. Plasma C-peptide (TRFIA; Perkin-Elmer, Turku, Finland) with corresponding glycemia, A1C levels (HPLC; Pharmacia Biotech, Upsala, Sweden), and autoantibodies [islet cell antibody, IA2A, GAD antibody, and I(A)A] were assayed in the central laboratory of the Belgian Diabetes Registry (23). There was also a central measurement of lymphocyte subsets CD3+, CD4+, CD8+, CD19+, and NK cells (CD3−CD16+CD56+) (EpicsXl flow cytometer; Beckman Coulter, Miami, FL). Total lymphocyte counts were determined by routine hematology in local centers. Baseline characteristics of graft recipients are shown in online appendix 1. The majority of patients (n = 24) were treated with a subcutaneous insulin pump for at least 2 months prior to transplantation. In one patient, insulin pump was started in the second week after first islet infusion. Insulin tapering was only considered in patients with plasma C-peptide values ≥1.0 ng/ml (at glycemia 120–220 mg/dl), CVfg <25%, and mean fasting glycemia <125 mg/dl and was started after month 2 at a rate of −2 IU every 3–5 days or faster when patients experienced hypoglycemic episodes (<70 mg/dl).

Lymphocyte stimulation test to determine baseline autoreactivity against islet cell antigens. Baseline T-cell autoreactivity to islet cell antigens was assessed at the Leiden University Medical Center and data analyzed blinded from clinical outcome. Blood was drawn before the first ATG administration, and peripheral blood mononuclear cells were isolated and processed as described before (24). Briefly, 150,000 fresh peripheral blood mononuclear cells were cultured in triplicate in 96-well round-bottomed plates in Iscove's modified Dulbecco's medium with 2 mmol/l glutamine (Life Technologies, Paisley, Scotland) and 10% pooled human serum in presence of islet autoantigens IA-2 (10 μg/ml) or GAD65 (10 μg/ml), of interleukin-2 (35 units/ml), or of medium alone. After 5 days, ^{3}H-thymidine (0.5 μCI/well) was added and its incorporation measured after 16 h. Data were expressed as a stimulation index (S_i) by comparison with the medium alone value. An S_i ≥3 for any of the two antigens was considered a sign of T-cell autoreactivity against an islet cell antigen. In three patients, cellular autoreactivitiy could not be assessed because autoantigens were not available for testing of baseline samples.

Histology. In one patient, a third islet cell preparation was injected at posttransplant week 60. During the laparoscopically guided infusion, a liver biopt was taken from a steatotic area on the liver surface. Immunohistochemistry was performed on semiconsecutive paraffin-embedded sections using a rabbit insulin antibody (raised by Dr C. Van Schravendijk, Vrije Universiteit Brussels, Brussels, Belgium) and monoclonal CD3 (NeoMarkers, Freemont, CA) and CD20 (DAKO, Glostrup, Denmark) antibodies. Human tonsils and pancreas was used as control. For epitope retrieval, sections were heated to 98°C with citrate buffer, pH 6.0.

Statistics. All values are expressed as median and interquartile range (IQR), unless indicated otherwise. Baseline and postransplant characteristics were related with status of insulin independence at month 6 as well as β-cell graft function and glycemic variability during the first 6 months. To assess differences between subgroups, we used nonparametric Mann-Whitney *U* test for continuous data and Fisher's exact test for categorical data. Correlations between baseline characteristics and CVfg or mean C-peptide during the first 6 months after transplantation were assessed by calculating Pearson's correlation coefficient.

To determine independent predictor ability of the variables, we used forward stepwise binary logistic regression analysis for insulin independence and a stepwise linear regression model for both CVfg and mean C-peptide. The analysis was performed on 27 subjects. Three subjects could not be included because of missing data. In our multivariate model we included parameters with P value <0.05 in univariate analysis.

All analysis were performed using SPPS (version 16.0), and graphics were computed by GraphPad Prism (version 4.0). All reported P values are two sided, and $P < 0.05$ was considered significant.

RESULTS

Metabolic outcome of islet cell transplantation. All 30 recipients became C-peptide positive after transplantation; 1 of them returned to C-peptide negativity before posttransplant month 6. At posttransplant month 6, 15 patients were insulin independent while the other 15 were on

TABLE 2
Graft and recipient characteristics according to insulin need at 6 months posttransplantation

	At posttransplant month 6		
	Insulin independent	Insulin dependent	*P**
n	15	15	
Graft			
β-Cell number (million/kg)			
First graft	3.0 (2.2–4.2)	2.5 (2.0–2.9)	0.16
Total	4.5 (3.5–5.7)	3.7 (2.6–4.7)	0.11
Cellular composition			
β-Cells	32 (21–38)	30 (20–37)	0.87
α-Cell	9 (4–11)	7 (5–10)	0.34
Nongranulated cells	46 (41–63)	45 (34–62)	0.28
Acinar cells	1 (1–4)	1 (1–5)	0.80
Dead cells	8 (6–11)	10 (7–12)	0.31
Two β-cell grafts	8	9	1.00
Culture time (days)	6 (4–9)	6 (4–10)	0.66
Recipient			
Age (years)	45 (41–49)	40 (32–52)	0.14
Sex (male/female)	10/5	7/8	0.46
Body weight (kg)	68 (64–74)	68 (62–78)	0.72
BMI (kg/m^2)	23 (21–26)	25 (22–26)	0.55
Duration of disease (years)	27 (21–33)	24 (17–32)	0.30
A1C (%)	7.0 (6.5–7.9)	7.8 (7.4–8.1)	0.05
Insulin dose (IU · kg^{-1} · day^{-1})	0.52 (0.39–0.74)	0.67 (0.58–0.85)	0.13
Insulin pump before transplantation	14	10	0.17
Fasting glycemia			
Mean (mg/dl)	146 (129–174)	166 (123–199)	0.65
CVfg (%)	43 (38–46)	46 (35–50)	0.42
Immune status before ATG			
Total lymphocyte count	1,579 (1,380–1,885)	2,065 (1,869–3,005)	0.007
CD3+ count (cells/mm^3)	1,142 (916–1,342)	1,419 (1,160–1,867)	0.049
CD19+ count (cells/mm^3)	247 (141–271)	318 (227–514)†	0.023
Leucocyte count	5,300 (4,400–6,000)	6,400 (5,700–8,100)	0.040
Presence of T-cell reactivity against IA2 and/or GAD	6/13	12/14†	0.046
Positivity for ICA/GAD/IA2-A	4/8/6	2/5/7	0.65/0.46/1.0
Positivity for two or more autoantibodies	5	3	0.68
Immune status at first infusion			
Total lymphocyte count	238 (122–313)	273 (173–748)	0.19
CD3+ count (cells/mm^3)	24 (15–43)	36 (9–83)	0.73
Leucocyte count	4,700 (2,900–11,400)	8,300 (3,225–12,225)	0.36
Immune monitoring 0–6 months posttransplant			
Presence of T-cell reactivity against IA2 and/or GAD	5/13	8/14	0.45
Positivity for ICA/GAD/IA2-A	4/8/6	2/7/9	0.65/1.0/1.0
De novo HLA-Ab (CDC)	1	1	1.0

Data are medians (IQR). *Statistical analysis was done with Mann-Whitney *U* test for continues variables, Fisher exact test for dichotomous variables. †These variables were confirmed as independently associated with insulin independence by multivariate analysis (binary logistic regression).

lower-dose insulin therapy (Table 1). Both groups had similar A1C concentrations and fasting mean glucose levels (Table 1). However, insulin-independent recipients had significantly higher basal C-peptide levels and exhibited a lower variability of fasting glycemia (Table 1).

Comparison of insulin-independent and insulin-treated recipients for their baseline graft and recipient characteristics. No differences between both patient groups were noticed in terms of graft characteristics (Table 2); respectively, 87 and 80% of their subjects had received at least 2 milion β-cells per kg body wt in the first graft.

Baseline recipient characteristics such as age, sex, body weight–BMI, duration of disease, autoantibody positivity, metabolic control, and insulin dose were also similar (Table 2); a tendency to higher A1C concentrations was noticed in patients who did not become insulin independent ($P = 0.054$). After first transplantation, 14 patients who would achieve insulin independence continued treatment with insulin pump for a median time of 15 weeks (IQR 10–18). Among patients not achieving insulin independence, seven were treated with insulin pump during the 6-month follow-up. In four patients, insulin pump was discontinued after a median of 20 weeks because of low daily insulin need and replaced by subcutanuous injections four times a day. Metabolic control during the first 2 months did not differ between groups with median A1C values of 5.0% (IQR 4.4–5.9) in insulin-independent subjects vs. 5.2% (IQR 4.7–5.9) in insulin-requiring patients ($P = 0.48$). The number of patients treated with low–molecular weight heparin after transplantation did not differ between groups.

On the other hand, a significant difference was observed in the baseline immune state (before ATG treatment), as

TABLE 3
Immune status before first and second islet infusion according to insulin need at 6 months posttransplantation among recipients receiving two islet grafts

	At posttransplant month 6		
	Insulin independent	Insulin dependent	*P**
n	8	9	
Grafts			
Immune status before ATG			
Total lymphocyte count	1,567 (1,044–2,023)	2,065 (1,906–2,721)	0.03
CD3+ count (cells/mm^3)	1,134 (839–1,328)	1,419 (1,189–1,761)	0.07
CD19+ count (cells/mm^3)	223 (119–255)	267 (225–430)	0.04
Leucocyte count	5,250 (3,950–6,925)	6,400 (5,700–8,400)	0.08
Presence of T-cell reactivity against IA2 and/or GAD	5/12	7/12	0.57
Positivity for ICA/GAD/IA2-A	3/5/2	1/4/4	NS
Positivity for two or more autoantibodies	3	2	0.62
Immune status at first infusion			
Total lymphocyte count	189 (97–584)	272 (154–779)	0.25
CD3+ count (cells/mm^3)	27 (12–134)	52 (12–80)	1.0
Leucocyte count	3,350 (2,525–7,700)	8,700 (2,725–13,350)	0.29
Immune status before second infusion			
Total lymphocyte count	499 (357–593)	636 (501–833)	0.15
CD3+ count (cells/mm^3)	186 (169–267)	277 (224–341)	0.05
CD19+ count (cells/mm^3)	213 (84–269)	182 (152–311)	0.50
Leucocyte count	3,150 (2,250–3,825)	3,900 (3,300–4,900)	0.08
Presence of T-cell reactivity against IA2 and/or GAD	2	3	0.62
Positivity for ICA/GAD/IA2-A	1/5/2	1/6/4	NS
Positivity for two or more autoantibodies	2	3	0.62

Data are median (IQR). *Statistical analysis was done with Mann-Whitney *U* test for continues variables, Fisher exact test for dichotomous variables. NS, not significant.

expressed by the absolute number of lymphocytes, of CD3+ cells, and of CD19+ cells, with higher initial counts in the recipient group that would not become insulin independent (Table 2). Of four patients with a baseline lymphocyte count >3,000 cell/mm^3 (range 3,005–3,455), none became insulin independent. The higher initial number of T-cells in the insulin-dependent group was associated with a higher number of CD8+ cells (547/mm^3 [IQR 462–657] vs. 393/mm^3 [305–503] in insulin-independent patients; $P = 0.021$) but not of CD4+ T-cells (850/mm^3 [742–1,134] and 716/mm^3 [570–936], respectively; $P = 0.093$) or of NK cells (184/mm^3 [147–302] vs. 174/mm^3 [144–271], respectively; $P = 0.72$). At the day of islet infusion, a similar tendency was noted (Table 2). B-cell and T-cell autoreactivity were not measured at the day of islet infusion.

At the time of a second infusion (Table 3) we observed slightly higher CD3+ counts in insulin-dependent patients. It is important to note that in these 17 patients we still observed a significant difference in lymphocyte subsets in samples taken before ATG administration. There were no differences in immune profile and reactivity between patients receiving one or two grafts.

Both groups also differed in their baseline T-cell autoreactivity. This in vitro test could be performed in 27 of 30 subjects listed in Table 2. In the group that did not become insulin independent, 12 of 14 patients scored positive for IA2 and/or GAD65, whereas this was only the case in 6 of 13 patients who would become insulin independent ($P = 0.046$; Table 2). The role of baseline T-cell autoreactivity remained confined to a subgroup of recipients receiving amounts of β-cells below the median, as we previously reported (18; online appendix 2). On the other hand, no difference was seen in baseline autoantibody status or in the presence of multiple autoantibodies prior to transplantation (Table 2). Of 19 patients positive for IA2 and/or GAD65 antibodies at baseline, 10 became insulin independent, whereas this was the case for 5 of 10 patients that were negative for these antibodies ($P = 1.0$ by Fisher's exact test). This was also the case for islet cell antibody positivity. The number of HLA mismatches did not differ between groups (data not shown).

Univariate and multivariate analysis of associations between baseline recipient and graft characteristics and clinical outcome parameters. The observed correlation between baseline immune status of the recipient and the achievement of insulin independence at posttransplant month 6 was further examined by multivariate analysis (Table 4). Baseline B-cell count and T-cell autoreactivity were found to be independently correlated with the ability to achieve insulin independence (odds ratio 0.989 [95% CI 0.979–0.999] and 0.101 [0.009–1.067], respectively). Of nine patients without T-cell autoreactivity at start, seven became insulin independent, whereas this was only the case for 6 of 18 patients that tested positively (Fig. 1*B*). When these patients were further stratified according to their baseline B-cell count (i.e., under or above the 50th percentile [259 B-cells/mm^3]), insulin independence was seen in seven of eight patients without baseline T-cell autoreactivity and a B-cell count <p50, while only 1 of 11 patients with T-cell autoreactiviy and a B-cell count >p50 became insulin independent ($P = 0.001$ by Fisher's exact test).

In univariate analysis, similar correlations were found with other clinical outcome parameters, such as the average coefficient of variation of prebreakfast glycemia (CVfg) and the mean plasma C-peptide levels during the first 6 months (Table 4). After multivariate analysis, baseline positivity for T-cell autoreactivity and total lymphocyte counts correlated positively with the values of CVfg and negatively with the mean C-peptide levels (Fig. 2*A*). A

TABLE 4
Univariate and multivariate analysis of recipient characteristics associated with clinical outcome

	Insulin independence		CVfg 0–6 months		Mean C-peptide 0–6 months	
	Univariate analysis*	Multivariate analysis†	Univariate analysis‡	Multivariate analysis§	Univariate analysis‡	Multivariate analysis§
	P	*P*	*P*	*P*	*P*	*P*
Age (years)	0.14		0.02	0.28	0.17	
Sex (male/female)	0.46		0.44		0.48	
Body weight (kg)	0.72		0.91		0.95	
BMI (kg/m^2)	0.55		0.86		0.83	
Duration of disease (years)	0.30		0.55		0.41	
A1C (%)	0.05		0.71		0.48	
Insulin dose (IU · kg^{-1} · day^{-1})	0.13		0.02	0.22	0.04	0.17
Fasting glycemia						
Mean (mg/dl)	0.65		0.70		0.11	
CVfg (%)	0.42		0.78		0.94	
Immune status before ATG						
Total lymphocyte count	0.007	0.21	0.001	0.02 (β 0.370)	0.006	0.001 (β 0.437)
CD3+ count	0.05	0.32	0.06		0.08	
CD19+ count	0.02	0.03 (odds ratio 0.989)	0.08		0.13	
Leucocyte count	0.04	0.14	0.007	0.97	0.04	0.34
T-cell reactivity against IA2 and/or GAD	0.05	0.06 (odds ratio 0.101)	0.01	0.02 (β 0.352)	0.001	<0.001 (β −0.652)
Presence of autoantibodies pretransplant (yes/no)	0.70		0.86		0.88	
Immune status at first infusion						
Total lymphocyte count	0.19		0.50		0.46	
CD3+ count	0.73		0.81		0.83	
Leucocyte count	0.36		0.004	0.03 (β 0.363)	0.02	0.17
Immune status posttransplant days 1–7						
Median CD3+ count	0.38		0.82		0.76	
Median CD19+ count	0.04	0.54	0.02	0.51	0.03	0.89
Median CD4-to-CD8 ratio	0.59		0.29		0.39	

*Mann-Whitney *U* test for continuous variables, Fisher exact test for categorical data. †Independent predictor ability of the variables studied by forward stepwise binary logistic regression analysis. ‡Pearson's correlation for continuous variables and Mann-Whitney *U* test for categorical variables. §Independent predictor ability of the variables studied by stepwise linear regression analysis, inclusion criteria $P < 0.05$.

correlation with leukocytes at the day of infusion was maintained after multivariate analysis for CVfg but not C-peptide. Recipients with baseline T-cell reactivity had significantly higher CVfg (24% [IQR 20–35]) and lower C-peptide (1.0 ng/ml [0.5–1.3]) than those without (18% [15–24], $P = 0.014$, and 1.9 ng/ml [1.3–2.2; $P = 0.001$], respectively) (Fig. 2*B*). These differences became more pronounced when a further stratification was made according to presence or absence of a baseline total lymphocyte count >p50. Thus, a negative test for T-cell autoreactivity and a lymphocyte count <p50 was associated with a lower CVfg and a higher C-peptide, whereas a positive test and a count >p50 appeared to predispose for a high CVfg and a low C-peptide (Fig. 2*A* and *B*). The number of β-cells transplanted per kilogram bodyweight in the first graft had a tendency to correlate with CVfg in univariate analysis ($R = -0.34$; $P = 0.07$) but not with C-peptide levels during the first 6 months posttransplant ($R = 0.27$; $P = 0.15$). This could not be retained when it was added to the multivariate model ($P = 0.56$ and $P = 0.42$, respectively). The total number of β-cells transplanted or the number of donors used did not affect CVfg nor C-peptide levels.

Comparison of insulin-independent and insulin-treated recipients in terms of posttransplant lymphocyte counts and immune status. One week after start of the immune therapy, the number of CD3+ cells had markedly dropped in both groups, reaching similar low values that were maintained until posttransplant month 6 (Fig. 1*A*). On the other hand, baseline CD19+ counts did not decrease during the first week, thus remaining higher in the patient group that did not become insulin independent (Fig. 1*A*); at later time points, CD19+ counts had decreased to similar levels in both groups. Total ATG dose in insulin-independent patients was similar to that used in insulin-requiring patients (21.9 mg/kg body wt [IQR 20.0–24.7] and 23.6 mg/kg body wt [22.0–26.2], respectively, $P = 0.11$). Mean tacrolimus trough levels (9.2 ng/ml [8.6–9.5] and 9.2 ng/ml [7.4–9.7]) and MMF dose (2,000 mg/day [IQR 1,500–2,000] and 2,000 mg/day IQR [1,500–2,000]) during the first 6 months posttransplant were also comparable between both groups ($P = 0.66$ and $P = 0.45$, respectively). A similar observation was made for CVfg and mean C-peptide levels during the first 6 months (data not shown).

We were unable to demonstrate any correlations between posttransplant immune measures and clinical outcome in the first 6 months posttransplant (Table 2). Interestingly, we observed a clear difference between subjects showing T-cell reactivity posttransplant when these results were combined with baseline T-cell reactivity. In eight subjects where T-cell reactivity was present both at baseline and during the first 6 months, only one achieved insulin independence. C-peptide levels were low in these patients (mean 0.74 ng/ml) and CVfg high (mean 33%).

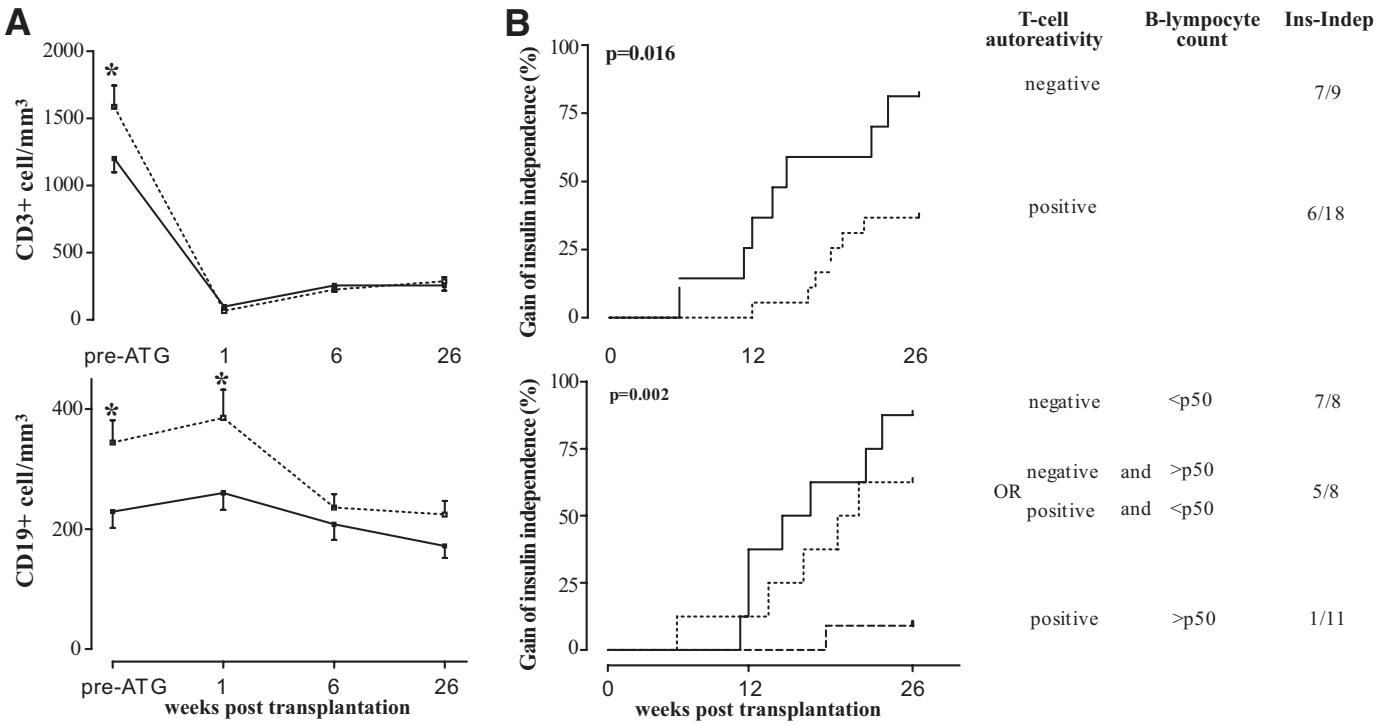

FIG. 1. T- and B-cell count at baseline and during 6 months after transplantation (*A*) in islet graft recipients gaining insulin independence (solid line) and remaining insulin independent (dotted line). Data represent means ± SE; **P* < 0.05. *B*: Gain of insulin independence according to baseline in vitro T-cell reactivity against islet autoantigens. The *lower panel* shows gain of insulin independence in three different groups according to both T-cell autoreactivity and baseline B-cell count.

However, among five recipients who only tested positive after transplantation, four achieved insulin independence ($P = 0.03$) with mean C-peptide levels of 1.9 ng/ml ($P = 0.01$) and low mean CVfg (20%; $P = 0.02$). Among 10 recipients with baseline T-cell autoreactivity who where negative during the first 6 months posttransplant, results were intermediate. Five reached insulin independence; C-peptide levels were 1.1 ng/ml with CVfg 24%. Highest C-peptide levels (1.7 ng/ml) and lowest CVfg (18%) were seen in four patients who never presented T-cell autoreativity. Three of them reached insulin independence.

Presence of B-cells in long-term islet cell implant in the liver of a recipient with high blood B-cell count. A liver biopt was available from a patient who had received

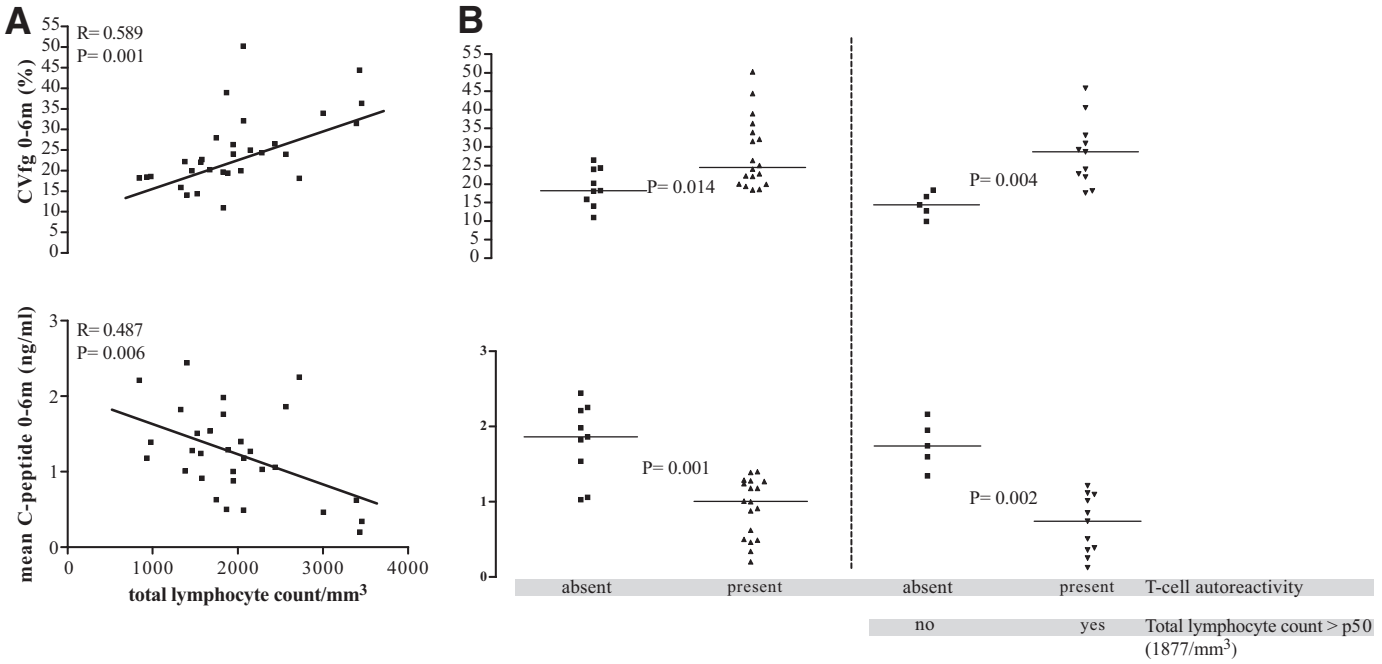

FIG. 2. The effect of baseline total lymphocyte count (*A*), in vitro T-cell reactivity against islet autoantigens or both (*B*) on glycemic stability and C-peptide release.

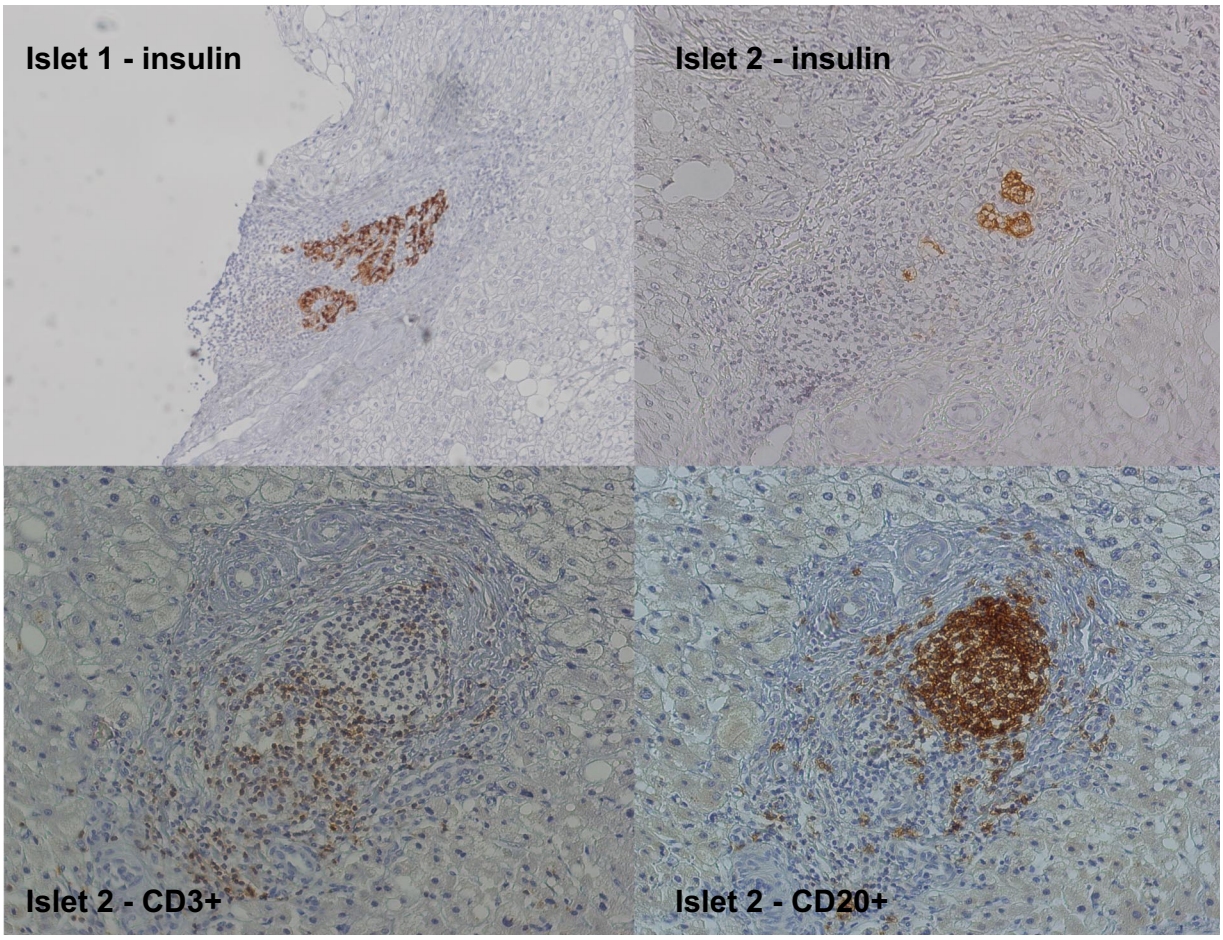

FIG. 3. Two islets were identified in the portal tract. Semiconsecutive sections of islet two show inflammatory cells located around the graft, which are dominated by CD20+ B-cells. (A high-quality digital representation of this figure is available in the online issue.)

two intraportal islet cell injections >1 year earlier. Two islets were identified in a portal tract. Endocrine cell clusters were identified by their positivity for chromogranin, proinsulin, and insulin (Fig. 3). They were surrounded by CD20+ and CD3+ cells; there were only few CD8+ cells detected. The CD20+ cells were dominant in the examined sections. The patient had not become insulin independent, and his posttransplant month 6 mean plasma C-peptide was low (0.62 ng/ml) and CVfg high (32%). At baseline, he did present a cellular autoreactivity against IA2, a high total lymphocyte count (3,393/mm^3) with a B-cell count (536/mm^3) above the 50th percentile of the recipient population before treatment (259 B cells/mm^3); despite >1 year of immune suppressive treatment, the mean B-cell count until the time of the biopsy (412/mm^3) was still above this 50th percentile. Interestingly, we did not observe a dominant CD20+ infiltrate in a liverbiopsy done in the patient who died 18 weeks posttransplant of cerebral hemorrhage. This patient had lower numbers of total lymphocytes (1,776/mm^3) and B-cells (188 cell/mm^3) at baseline. Last circulating CD19+ cell count was 30 cells/mm^3 and mean C-peptide level 2.2 ng/ml in the last 4 weeks before she died.

DISCUSSION

The outcome of islet cell transplantation in type 1 diabetic recipients is variable, ranging from rapid failure to return of insulin independence. This variation can be caused by differences in graft and in recipient characteristics, some of which can be anticipated such as an inadequate size and/or viability of the donor β-cell mass and some being more difficult to distinguish. We have reduced graft variability by standardizing the composition of cultured islet cell grafts (17,20). In a series of 30 C-peptide–negative recipients of such graft, we retrospectively searched for possible recipient characteristics that are associated with an inadequate outcome during the first 6 months posttransplantation. The present data show a correlation with the baseline immune status at the time of the implantation. A higher blood B-cell count was associated with an inability to induce an insulin-independent state, as was also the case for a baseline T-cell autoreactivity against islet antigens, both characteristics being independent variables after multivariate analysis. A higher total lymphocyte count and a baseline T-cell autoreactivity were correlated with lower plasma C-peptide levels and a higher variability in fasting blood glucose.

Of 11 patients with a baseline B-cell count >p50 and a T-cell autoreactivity, only 1 became insulin independent, while this occurred in 7 of 8 with a B-cell count <p50 and negativity for T-cell autoreactivty. In 1 of these 10 patients with elevated baseline immune status and inadequate graft outcome, a biopt of the implant during posttransplant year 2 showed massive B-cell accumulations in the vicinity of insulin-positive cell aggregates; CD3+ cells were also present but less abundant, at least in the examined sections. Throughout follow-up, this patient had maintained a mean B-cell count above the 50th percentile of the recip-

ient population before treatment. The biologic or pathogenic significance of these observations is unclear. Interestingly, no such infiltrate was observed around the graft of a patient who died 18 weeks posttransplant with a well-functioning graft and low B-cell counts. In animal models of diabetes, B-cells appear implicated in the destruction of β-cells (25–29). However, their role in the development of human disease is uncertain. That type 1 diabetes can occur in a patient with severe hereditary B-cell deficiency is seen as evidence that the disease can occur without participation of B-cells (30) but does not exclude their involvement in other patients. In islet cell transplantation, it is not yet clear whether B-cells play a role through production of antibodies and/or through antigen presentation. Islet cell autoantibodies have been correlated to recurrence of autoimmunity and clinical outcome (8,31–34), but this was not confirmed by others (18,20) or by the present study; we did not detect a correlation between the presence or titer of autoantibodies and the number of circulating B-cells. On the other hand, alloantibodies can mediate rejection of organ transplants and islet cell grafts have been found to induce alloantibody formation (35–37). Alloantibodies after transplantation were detected in only two recipients in the current study, one of which became insulin independent. Besides being a source of antibodies, B-cells can also operate as antigen-presenting cells that activate auto- and/or alloimmunity (38–41). This mechanism has been proposed in rejecting kidneys with a B-cell infiltration (42,43). Whether B-cells can interfere with engraftment and/or early graft function of intraportal islet cell grafts by one of these mechanisms cannot be concluded from the present data. Intravenous injection of the donor cells can nevertheless be expected to immediately subject them to influences of circulating lymphocytes and more so to populations that are more abundant and/or rapidly activated by β-cell autoantigens. It is then also conceivable that an early local activation of B-cells may result in their sustained presence in the islet microenvironement as was noticed in the biopt. To replace speculation by evidence, an immune therapy protocol will be needed in which B-cell depletion is achieved just before and during the first posttansplant weeks. In a study in nonhuman primates, Liu et al. (44) demonstrated that B-cell–directed immunotherapy promotes long-term islet allograft survival in nonhuman primates that also received T-cell–depleting ATG antibodies and a maintenance dose of rapamycin. It would thus be interesting to examine whether addition of rituximab, which selectively targets CD20+ B-cells, to our ATG-based protocol increases the percent recipients that become insulin independent.

Islet culture is known to help survival in rat allograft by enriching islet isolates in endocrine cells (45), but this has not yet been demonstrated in humans. Furthermore, islet culture can be expected to reduce inflammatory and/or immune reactivity in the implant in rodents (46). We systematically used islet culture, and a comparative study between freshly isolated and cultured islet cells has not yet been possible. Therefore, we are unable to assess whether these factors may have influenced graft survival, but it cannot be excluded.

Our data confirm in a larger cohort the previously reported correlation between a baseline T-cell reactivity against islet cell antigens and the failure of the implant to induce an insulin-independent state (18). They extend this correlation to other signs of inadequate graft function such as low plasma C-peptide levels and a high variability in fasting glycemia. As we previously reported, this correlation is confined to recipients receiving lower numbers of β-cells. The role of autoreactive T-cells in β-cell destruction is well accepted (47–49). It is conceivable that the ATG-mediated lymphopenia induces a proliferation of autoreactive memory T-cells (50), which appear present or more abundant at baseline in a number of patients and might therefore predispose to a more rapid or extensive immune destruction. Patients rejecting a pancreas transplant were also found to present memory CD4+ T-cells that were specific for the islet autoantigen GAD65 (51). Indeed, we were able to demonstrate in the current study that in those patients where baseline T-cell autoreactivity persisted in the first 6 months posttransplant, clinical outcome was very poor. Although recipients who lost baseline-detectable T-cell reactivity after transplantation had slightly better outcome, both are still in contrast with the well-functioning grafts in recipients where T-cell autoreactivity was only detectable after transplantation.

Our data demonstrate an important predictive role for immune measures taken at baseline, before initiation of immunosuppression, but not for samples from the day of islet infusion. However, we did not measure B-cell and T-cell autoreactivity at the day of infusion. We could therefore not fully assess the correlations between those parameters at the day of infusion and clinical outcome, although the available data point in the same direction. However, it is evident that B-cells are more abundant in insulin-dependent patients in the peritransplant period, which, we suggest, may impair graft function via mechanisms we mention above. In a severe lymphopenic environment such as during ATG treatment, assessment of T-cell reactivity is impossible because the frequency of circulating lymphocytes is too low (24). Yet, T-cell autoreactivity remains present at a later time point, demonstrating the insufficient irradication of autoreactive T-cells, which may be susceptible to proliferation by lymphopenia-induced homeostatic proliferation (50).

In conclusion, this study correlates circulating characteristics of the baseline immune state with the metabolic outcome of intraportal islet cell transplants in type 1 diabetic patients. A higher number of total and of B-cell and a T-cell reactivity against β-cell antigens are associated with a lower return to insulin independence, lower plasma C-peptide levels, and/or a higher variability in fasting glycemia; in the one case where a liver biopt was available (>1 year after the transplant) B-cells were predominantly present around the β-cell aggregates. These data support the rationale of targeting also B-cells during the induction phase of immune therapy.

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REFERENCES

1. Lacy PE, Scharp DW. Islet transplantation in treating diabetes. Annu Rev Med 1986;37:33–40
2. Robertson RP. Islet transplantation as a treatment for diabetes: a work in progress. N Engl J Med 2004;350:694–705
3. Froud T, Ricordi C, Baidal DA, Hafiz MM, Ponte G, Cure P, Pileggi A, Poggioli R, Ichii H, Khan A, Ferreira JV, Pugliese A, Esquenazi VV, Kenyon NS, Alejandro R. Islet transplantation in type 1 diabetes mellitus using cultured islets and steroid-free immunosuppression: Miami experience. Am J Transplant 2005;5:2037–2046
4. 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 2002;51:2148–2157
5. 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 2001;50:710–719
6. 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 2000;343:230–238
7. Warnock GL, Meloche RM, Thompson D, Shapiro RJ, Fung M, Ao Z, Ho S, He Z, Dai LJ, Young L, Blackburn L, Kozak S, Kim PT, Al-Adra D, Johnson JD, Liao YH, Elliott T, Verchere CB. Improved human pancreatic islet isolation for a prospective cohort study of islet transplantation vs best medical therapy in type 1 diabetes mellitus. Arch Surg 2005;140:735–744
8. Shapiro AM, Ricordi C, Hering BJ, Auchincloss H, Lindblad R, Robertson RP, Secchi A, Brendel MD, Berney T, Brennan DC, Cagliero E, Alejandro R, Ryan EA, DiMercurio B, Morel P, Polonsky KS, Reems JA, Bretzel RG, Bertuzzi F, Froud T, Kandaswamy R, Sutherland DE, Eisenbarth G, Segal M, Preiksaitis J, Korbutt GS, Barton FB, Viviano L, Seyfert-Margolis V, Bluestone J, Lakey JR. International trial of the Edmonton protocol for islet transplantation. N Engl J Med 2006;355:1318–1330
9. Ault A. Edmonton's islet success tough to duplicate elsewhere. Lancet 2003;361:2054
10. Ryan EA, Paty BW, Senior PA, Bigam D, Alfadhli E, Kneteman NM, Lakey JR, Shapiro AM. Five-year follow-up after clinical islet transplantation. Diabetes 2005;54:2060–2069
11. Ahmad SA, Lowy AM, Wray CJ, D'Alessio D, Choe KA, James LE, Gelrud A, Matthews JB, Rilo HL. Factors associated with insulin and narcotic independence after islet autotransplantation in patients with severe chronic pancreatitis. J Am Coll Surg 2005;201:680–687
12. Hering BJ, Kandaswamy R, Harmon JV, Ansite JD, Clemmings SM, Sakai T, Paraskevas S, Eckman PM, Sageshima J, Nakano M, Sawada T, Matsumoto I, Zhang HJ, Sutherland DE, Bluestone JA. Transplantation of cultured islets from two-layer preserved pancreases in type 1 diabetes with anti-CD3 antibody. Am J Transplant 2004;4:390–401
13. Markmann JF, Deng S, Huang X, Desai NM, Velidedeoglu EH, Lui C, Frank A, Markmann E, Palanjian M, Brayman K, Wolf B, Bell E, Vitamaniuk M, Doliba N, Matschinsky F, Barker CF, Naji A. Insulin independence following isolated islet transplantation and single islet infusions. Ann Surg 2003;237:741–749; discussion 749–750
14. Hering BJ, Kandaswamy R, Ansite JD, Eckman PM, Nakano M, Sawada T, Matsumoto I, Ihm SH, Zhang HJ, Parkey J, Hunter DW, Sutherland DE. Single-donor, marginal-dose islet transplantation in patients with type 1 diabetes. JAMA 2005;293:830–835
15. Gangemi A, Salehi P, Hatipoglu B, Martellotto J, Barbaro B, Kuechle JB, Qi M, Wang Y, Pallan P, Owens C, Bui J, West D, Kaplan B, Benedetti E, Oberholzer J. Islet transplantation for brittle type 1 diabetes: the UIC protocol. Am J Transplant 2008;8:1250–1261
16. Gruessner RW, Sutherland DE, Dunn DL, Najarian JS, Jie T, Hering BJ, Gruessner AC: Transplant options for patients undergoing total pancreatectomy for chronic pancreatitis. J Am Coll Surg 2004;198:559–567; discussion 568–559
17. Keymeulen B, Gillard P, Mathieu C, Movahedi B, Maleux G, Delvaux G, Ysebaert D, Roep B, Vandemeulebroucke E, Marichal M, In't Veld P, Bogdani M, Hendrieckx C, Gorus F, Ling Z, van Rood J, Pipeleers D. Correlation between beta cell mass and glycemic control in type 1 diabetic recipients of islet cell graft. Proc Natl Acad Sci U S A 2006;103:17444–17449
18. Huurman VA, Hilbrands R, Pinkse GG, Gillard P, Duinkerken G, van de Linde P, van der Meer-Prins PM, Versteeg-van der Voort Maarschalk MF, Verbeeck K, Alizadeh BZ, Mathieu C, Gorus FK, Roelen DL, Claas FH, Keymeulen B, Pipeleers DG, Roep BO. Cellular islet autoimmunity associates with clinical outcome of islet cell transplantation. PLoS One 2008;3:e2435
19. Ling Z, Pipeleers DG. Prolonged exposure of human beta cells to elevated glucose levels results in sustained cellular activation leading to a loss of glucose regulation. J Clin Invest 1996;98:2805–2812
20. Keymeulen B, Ling Z, Gorus FK, Delvaux G, Bouwens L, Grupping A, Hendrieckx C, Pipeleers-Marichal M, Van Schravendijk C, Salmela K, Pipeleers DG. Implantation of standardized beta-cell grafts in a liver segment of IDDM patients: graft and recipients characteristics in two cases of insulin-independence under maintenance immunosuppression for prior kidney graft. Diabetologia 1998;41:452–459
21. Movahedi B, Keymeulen B, Lauwers MH, Goes E, Cools N, Delvaux G. Laparoscopic approach for human islet transplantation into a defined liver segment in type-1 diabetic patients. Transpl Int 2003;16:186–190
22. Maleux G, Gillard P, Keymeulen B, Pipeleers D, Ling Z, Heye S, Thijs M, Mathieu C, Marchal G. Feasibility, safety, and efficacy of percutaneous transhepatic injection of beta-cell grafts. J Vasc Interv Radiol 2005;16:1693–1697
23. Decochez K, Tits J, Coolens JL, Van Gaal L, Krzentowski G, Winnock F, Anckaert E, Weets I, Pipeleers DG, Gorus FK. High frequency of persisting or increasing islet-specific autoantibody levels after diagnosis of type 1 diabetes presenting before 40 years of age: the Belgian Diabetes Registry. Diabetes Care 2000;23:838–844
24. Roep BO, Kallan AA, Duinkerken G, Arden SD, Hutton JC, Bruining GJ, de Vries RR. T-cell reactivity to β-cell membrane antigens associated with β-cell destruction in IDDM. Diabetes 1995;44:278–283
25. Noorchashm H, Noorchashm N, Kern J, Rostami SY, Barker CF, Naji A. β-Cells are required for the initiation of insulitis and sialitis in nonobese diabetic mice. Diabetes 1997;46:941–946
26. Wong FS, Wen L, Tang M, Ramanathan M, Visintin I, Daugherty J, Hannum LG, Janeway CA Jr, Shlomchik MJ. Investigation of the role of β-cells in type 1 diabetes in the NOD mouse. Diabetes 2004;53:2581–2587
27. Wong FS, Wen L. B cells in autoimmune diabetes. Rev Diabet Stud 2005;2:121–135
28. Hu CY, Rodriguez-Pinto D, Du W, Ahuja A, Henegariu O, Wong FS, Shlomchik MJ, Wen L. Treatment with CD20-specific antibody prevents and reverses autoimmune diabetes in mice. J Clin Invest 2007;117:3857–3867
29. Brodie GM, Wallberg M, Santamaria P, Wong FS, Green EA. β-Cells promote intra-islet CD8+ cytotoxic T-cell survival to enhance type 1 diabetes. Diabetes 2008;57:909–917
30. Martin S, Wolf-Eichbaum D, Duinkerken G, Scherbaum WA, Kolb H, Noordzij JG, Roep BO. Development of type 1 diabetes despite severe hereditary B-lymphocyte deficiency. N Engl J Med 2001;345:1036–1040
31. Bosi E, Braghi S, Maffi P, Scirpoli M, Bertuzzi F, Pozza G, Secchi A, Bonifacio E. Autoantibody response to islet transplantation in type 1 diabetes. Diabetes 2001;50:2464–2471
32. 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 1997;46:1907–1910
33. Palmer JP, Fleming GA, Greenbaum CJ, Herold KC, Jansa LD, Kolb H, Lachin JM, Polonsky KS, Pozzilli P, Skyler JS, Steffes MW. C-peptide is the

appropriate outcome measure for type 1 diabetes clinical trials to preserve β-cell function: report of an ADA workshop, 21–22 October 2001. Diabetes 2004;53:250–264
34. Jaeger C, Brendel MD, Eckhard M, Bretzel RG. Islet autoantibodies as potential markers for disease recurrence in clinical islet transplantation. Exp Clin Endocrinol Diabetes 2000;108:328–333
35. Campbell PM, Senior PA, Salam A, Labranche K, Bigam DL, Kneteman NM, Imes S, Halpin A, Ryan EA, Shapiro AM. High risk of sensitization after failed islet transplantation. Am J Transplant 2007;7:2311–2317
36. Cardani R, Pileggi A, Ricordi C, Gomez C, Baidal DA, Ponte GG, Mineo D, Faradji RN, Froud T, Ciancio G, Esquenazi V, Burke GW 3rd, Selvaggi G, Miller J, Kenyon NS, Alejandro R. Allosensitization of islet allograft recipients. Transplantation 2007;84:1413–1427
37. Colvin RB, Smith RN. Antibody-mediated organ-allograft rejection. Nat Rev Immunol 2005;5:807–817
38. Crawford A, Macleod M, Schumacher T, Corlett L, Gray D. Primary T cell expansion and differentiation in vivo requires antigen presentation by B cells. J Immunol 2006;176:3498–3506
39. Noorchashm H, Reed AJ, Rostami SY, Mozaffari R, Zekavat G, Koeberlein B, Caton AJ, Naji A. B cell-mediated antigen presentation is required for the pathogenesis of acute cardiac allograft rejection. J Immunol 2006;177:7715–7722
40. Browning JL. B cells move to centre stage: novel opportunities for autoimmune disease treatment. Nat Rev Drug Discov 2006;5:564–576
41. Shlomchik MJ, Craft JE, Mamula MJ. From T to B and back again: positive feedback in systemic autoimmune disease. Nat Rev Immunol 2001;1:147–153
42. Sarwal M, Chua MS, Kambham N, Hsieh SC, Satterwhite T, Masek M, Salvatierra O Jr.: Molecular heterogeneity in acute renal allograft rejection identified by DNA microarray profiling. N Engl J Med 2003;349:125–138
43. Tsai EW, Rianthavorn P, Gjertson DW, Wallace WD, Reed EF, Ettenger RB. CD20+ lymphocytes in renal allografts are associated with poor graft survival in pediatric patients. Transplantation 2006;82:1769–1773
44. Liu C, Noorchashm H, Sutter JA, Naji M, Prak EL, Boyer J, Green T, Rickels MR, Tomaszewski JE, Koeberlein B, Wang Z, Paessler ME, Velidedeoglu E, Rostami SY, Yu M, Barker CF, Naji A. B lymphocyte-directed immunotherapy promotes long-term islet allograft survival in nonhuman primates. Nat Med 2007;13:1295–1298
45. Pipeleers DG, Pipeleers-Marichal M, Vanbrabandt B, Duys S. Transplantation of purified islet cells in diabetic rats: II. immunogenicity of allografted islet β-cells. Diabetes 1991;40:920–930
46. Lacy PE, Davie JM, Finke EH. Prolongation of islet allograft survival following in vitro culture (24 degrees C) and a single injection of ALS. Science 1979;204:312–313
47. Bach JF. Insulin-dependent diabetes mellitus as an autoimmune disease. Endocr Rev 1994;15:516–542
48. Roep BO, Arden SD, de Vries RR, Hutton JC. T-cell clones from a type-1 diabetes patient respond to insulin secretory granule proteins. Nature 1990;345:632–634
49. Pinkse GG, Tysma OH, Bergen CA, Kester MG, Ossendorp F, van Veelen PA, Keymeulen B, Pipeleers D, Drijfhout JW, Roep BO. Autoreactive CD8 T cells associated with beta cell destruction in type 1 diabetes. Proc Natl Acad Sci U S A 2005;102:18425–18430
50. Monti P, Scirpoli M, Maffi P, Ghidoli N, De Taddeo F, Bertuzzi F, Piemonti L, Falcone M, Secchi A, Bonifacio E. Islet transplantation in patients with autoimmune diabetes induces homeostatic cytokines that expand autoreactive memory T cells. J Clin Invest 2008;118:1806–1814
51. Laughlin E, Burke G, Pugliese A, Falk B, Nepom G. Recurrence of autoreactive antigen-specific CD4+ T cells in autoimmune diabetes after pancreas transplantation. Clin Immunol 2008;128:23–30