Alloantibody and Autoantibody Monitoring Predicts Islet Transplantation Outcome in Human Type 1 Diabetes

Lorenzo Piemonti,[1] Matthew J. Everly,[2] Paola Maffi,[1] Marina Scavini,[1] Francesca Poli,[3] Rita Nano,[1] Massimo Cardillo,[3] Raffaella Melzi,[1] Alessia Mercalli,[1] Valeria Sordi,[1] Vito Lampasona,[4] Alejandro Espadas de Arias,[3] Mario Scalamogna,[3] Emanuele Bosi,[1,5] Ezio Bonifacio,[6] Antonio Secchi,[1,5] and Paul I. Terasaki[2]

Long-term clinical outcome of islet transplantation is hampered by the rejection and recurrence of autoimmunity. Accurate monitoring may allow for early detection and treatment of these potentially compromising immune events. Islet transplant outcome was analyzed in 59 consecutive pancreatic islet recipients in whom baseline and de novo posttransplant autoantibodies (GAD antibody, insulinoma-associated protein 2 antigen, zinc transporter type 8 antigen) and donor-specific alloantibodies (DSA) were quantified. Thirty-nine recipients (66%) showed DSA or autoantibody increases (de novo expression or titer increase) after islet transplantation. Recipients who had a posttransplant antibody increase showed similar initial performance but significantly lower graft survival than patients without an increase (islet autoantibodies $P < 0.001$, DSA $P < 0.001$). Posttransplant DSA or autoantibody increases were associated with HLA-DR mismatches ($P = 0.008$), induction with antithymocyte globulin ($P = 0.0001$), and pretransplant panel reactive alloantibody >15% in either class I or class II ($P = 0.024$) as independent risk factors and with rapamycin as protective ($P = 0.006$) against antibody increases. DSA or autoantibody increases after islet transplantation are important prognostic markers, and their identification could potentially lead to improved islet cell transplant outcomes. ***Diabetes*** **62:1656–1664, 2013**

The setting of islet transplantation is interesting because both allogenic rejection and recurrence of autoimmunity may occur and affect graft survival. Histological evidence of these mechanisms is extremely rare (1,2) because obtaining biopsy specimens from transplanted human islets is difficult (3). Consequently, surrogate markers of allo- and autoimmunity are used to evaluate the adaptive immune response of islet graft recipients (4). Poor islet transplant outcome is associated with the presence of pretransplant autoreactive T cells (5–7) and pretransplant or de novo donor-specific cytotoxic and $CD4^+$ T cells (7–11). This evidence from monitoring cellular immunity strongly suggests that long-term clinical outcome after islet transplantation is hampered by rejection, recurrence of autoimmunity, or both. Although compelling, the practical aspects of monitoring cellular immunity after islet transplantation is challenging. Monitoring of humoral immunity is easier and has now been validated for both alloimmunity (12–14) and islet autoimmunity (15). It is largely accepted that preformed pretransplant autoimmune antibodies only weakly predict posttransplant outcome (5,16–19), whereas preformed alloreactive antibodies are an important negative predictor of islet transplant outcome (20). On the other hand, the relevance of posttransplant de novo autoantibodies (19) and de novo donor-specific alloantibodies (DSA) (11,20–22) to islet transplant outcome is still unclear. In this study, we analyzed a cohort of 59 consecutive transplant recipients in which baseline and de novo posttransplant allo- and autoantibodies were measured prospectively and frequently and show the relevance of de novo responses to transplant outcome.

From the [1]Diabetes Research Institute, San Raffaele Hospital Scientific Institute, Milan, Italy; the [2]Terasaki Foundation Laboratory, Los Angeles, California; [3]Organ and Tissue Transplantation Immunology, Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Milan, Italy; the [4]Center for Translational Genomics and Bioinformatics, Milan, Italy; [5]Vita-Salute San Raffaele University, Milan, Italy; and the [6]Center for Regenerative Therapies and Paul Langerhans Institute Dresden, Dresden University of Technology, Dresden, Germany.

Corresponding author: Lorenzo Piemonti, piemonti.lorenzo@hsr.it.

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E.Bon., A.S., and P.I.T. contributed equally to this study.

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RESEARCH DESIGN AND METHODS

Islet transplant patients and baseline characteristics. Between February 2001 and March 2011, 49 nonuremic patients with type 1 diabetes (islet transplantation alone), 7 patients with type 1 diabetes who had a successful kidney transplant (islet after kidney transplantation), and 3 uremic patients with type 1 diabetes receiving a simultaneous kidney transplantation (simultaneous islet-kidney transplantation) received an islet transplantation under different immunosuppression regimens. Twenty-seven patients received anti-CD25 monoclonal antibody (mAb) induction and tacrolimus/sirolimus (SIR) immunosuppression (Edmonton protocol) (23), 12 were treated with a calcineurin inhibitor (CNI)-free protocol (induction of antithymocyte globulin [ATG] 1.5 mg/kg for 4 days starting at day −1 and immunosuppression with SIR/mycophenolate mofetil [MMF]) (clinical trial reg. no. NCT01346085), and 20 were treated with an SIR-free protocol (ATG or anti-CD25 mAb induction and tacrolimus/MMF immunosuppression).

Seventeen patients (nine Edmonton protocol and eight CNI-free protocol) received rapamycin 0.1 mg/kg monotherapy for at least 30 days (target trough levels 8–10 ng/mL, range 26–314 days) as preconditioning for islet transplantation (24). All islet transplantations were performed at the San Raffaele Scientific Institute in Milan, Italy. In all cases, the patients had a negative complement fixing lymphocyte crossmatch against recipient cells. All patients signed informed consent before enrollment in the islet transplantation program. The ethics committee of the San Raffaele Scientific Institute approved the protocols.

HLA typing. Genomic HLA typing was carried out with PCR sequence-specific primer (Invitrogen, Madison, WI) and reverse dot blot bead array (One Lambda, Inc., Canoga Park, CA) (25), with DNA isolated through the Maxwell 16 Blood DNA Purification System and stored at −70°C until testing. HLA-A, -B, and -DR mismatches were calculated by measuring the total number of mismatches to HLA-A, -B and -DR. Cw and DQB1 typing were available but are not traditionally used in documenting HLA mismatches. A number of the islet recipients received more than one infusion or an infusion from two donors at once, with maximum exposure to islets from four donors. Therefore, the maximum number of HLA mismatches was 24 (8 HLA-A, 8 HLA-B, and 8 HLA-DR). If an HLA antigen was a repeated mismatch, it was only counted as one mismatch.

Percentage of panel reactive alloantibodies. Panel reactive alloantibody (PRA) levels were calculated both by a complement-dependent cytotoxicity (CDC) method and by a Luminex method. Sera were screened by CDC using a whole lymphocyte population comprising a panel of 52 cells from Italian blood donors, incorporating HLA-A, -B, and -DR normally detected in the Italian population (26). A standard CDC protocol was used as previously published (27). For the Luminex method, sera screening and identification of antibody specificity were carried out with LABScreen Mixed and LABScreen PRA (One Lambda), respectively. PRA was evaluated according to the manufacturer's instructions, and the analysis was performed with HLA Visual version 1.1 software (One Lambda).

DSA. DSA were measured and monitored as graft recipient alloantibodies to donor HLA antigens. All sera were screened for HLA IgG and IgM antibodies by LABScreen Mixed class I and II antibody screening kit (One Lambda). If positive, antibody specificities were determined by LABScreen PRA single-antigen class I or class II (One Lambda). The manufacturer's instructions for testing were followed. Antibody screening included beads that have HLA-A, -B, -C, -Cw, -DR, -DQ, and -DP expressed on their surface. Single-antigen beads were used to test for antibodies against HLA-A, -B, -Cw, -DRB1, -DRB3, -B4, -B5, -DQA1, -DQB1, -DPA1, and -DPB1. Donor typing for HLA-DP was not performed, and therefore, DSA were not attributed to DP. DSA measurements performed with this methodology are highly sensitive and often positive when complement-fixing lymphocyte crossmatch is negative (28). Measurements were obtained at baseline and then at day 14; months 1, 3, 6, 12, 18, and 24; and then every 12 months thereafter for each islet infusion. Posttransplant DSA increase was defined as either *1*) serum conversion, when in a patient with undetectable DSA, at least one DSA became measurable (mean fluorescence intensity >1,000); *2*) increasing titers, when the mean fluorescence intensity of already-positive DSA increased at least 1.5-fold; or *3*) spreading, when the serum conversion of additional DSA occurred.

Islet autoantibodies. Autoantibodies to GAD antibody (GADA), insulinoma-associated protein 2 antigen (IA-2A), and zinc transporter 8 antigen (ZnT8A) were measured by radiobinding and immunoprecipitation assays as previously described (29–31). The thresholds for positivity in each assay was the 99th percentile of control subjects, which was equivalent to 3 arbitrary units for GADA, 1 arbitrary unit for IA-2A, and 5 arbitrary units for ZnT8A. According to the Diabetes Autoantibodies Standardization Proficiency workshop convened in 2009, these assays have the following sensitivities and specificities: GADA 66% and 97%, IA-2A 58% and 98%, and ZnT8A 68% and 99%, respectively (32). Measurements were taken at baseline; at days 1, 3, 5, 7, and 14 and months 1, 3, 6, 12, 18, and 24 after each islet infusion; and then every 12 months thereafter. Posttransplant autoantibody increase was defined as either *1*) serum conversion, when in a patient with no measurable islet autoantibodies, at least one autoantibody became detectable; *2*) increasing titer, when the titer of an already-positive islet autoantibody increased at least threefold; or *3*) spreading, when serum conversion of additional autoantibodies occurred.

Islet transplant outcome measures and definitions. A fasting C-peptide level of 0.3 ng/mL was established as the threshold to define functional islet transplant survival (≥0.3 ng/mL) or failure (<0.3 ng/mL). Islet transplant survival, therefore, was calculated from the date of first islet infusion to the time of failure. Other definitions of islet transplant outcome were as follows: Primary nonfunction was defined as a C-peptide level persisting at <0.3 ng/mL from the initial postinfusion period; early graft loss, as an initial postinfusion increase of C-peptide level ≥0.3 ng/mL followed by a decrease to <0.3 ng/mL within 2 months; partial graft function, as a C-peptide level ≥0.3 ng/mL over the first 2 months after islet infusion associated with a requirement for exogenous insulin or with inadequate glycemic control (see definition next); insulin independence, as no need for exogenous insulin because of adequate glycemic control (defined as glycated hemoglobin <6.5%, fasting glucose <140 mg/dL [7.8 mmol/L] at least three times per week, and 2-h postprandial glucose <180 mg/dL [10 mmol/L] at least four times per week); and gain of insulin independence, as the date of first islet infusion to the time of insulin independence.

TABLE 1
Patient demographics and islet transplant data

Factor	Edmonton protocol	CNI-free protocol	SIR-free protocol	Total
Patients (*n*)	27	12	20	59
Age (y)	39 ± 10	37 ± 8	48 ± 8	41 ± 10
Male/female sex (*n*)	15/12	6/6	8/12	29/30
Duration of diabetes (y)	24 ± 10	22 ± 12	33 ± 8	27 ± 11
Islet equivalents/kg	11,198 ± 4,796	11,269 ± 5,971	7,436 ± 2,631	9,937 ± 4,754
ITA/IAK/SIK (*n*)	27/0/0	12/0/0	10/7/3	49/7/3
No. infusions received by patients (%)				
One	26	42	70	44
Two	48	42	25	39
Three	26	16	5	17
No. donors received by patients (%)				
One	22	8	50	29
Two	30	58	35	37
Three	22	17	10	17
Four	26	17	5	17
HLA mismatches				
HLA-A	3 (0–6)	2.5 (1–5)	2 (0–4)	3 (0–6)
HLA-B	4 (1–7)	3 (1–6)	2.5 (0–5)	3 (0–7)
HLA-DR	3 (1–6)	3 (2–5)	2 (1–4)	3 (1–6)
All	11 (2–17)	9.5 (6–15)	7 (2–12)	9 (2–17)
HLA matches				
HLA-A	1 (0–2)	1 (0–2)	1 (0–2)	1 (0–2)
HLA-B	0 (0–1)	0 (0–1)	0 (0–1)	0 (0–1)
HLA-DR	1 (0–2)	0 (0–1)	1 (0–2)	1 (0–2)
All	2 (0–5)	1 (0–3)	2 (0–5)	2 (0–5)
Graft outcome (intention to treat)				
Early graft loss	11	33	35	24
Partial graft function	26	42	50	39
Insulin independence	63	25	15	39
Graft survival (d) (mean ± SE)	1,471 ± 273	391 ± 130	989 ± 210	1,180 ± 194

Data are mean ± SD, %, or median (range) unless otherwise indicated. IAK, islet after kidney transplantation; ITA, islet transplantation alone; SIK, simultaneous islet-kidney transplantation.

Statistical analyses. Results are expressed as mean ± SD or median with range for continuous variables and number of observations with percentages for categorical variables. Islet transplant survival was analyzed both as per-patient and per-islet infusion. Extended Cox proportional hazards regression model for recurrent events was used to compare islet graft survival among transplant recipients and to identify graft survival risk factors. For the post-transplant increase in islet-specific autoantibodies and DSA, the probability of a functioning islet transplant or for insulin-free survival was estimated by Kaplan-Meier method, with the antibody change as a time-varying covariate. Comparison of graft survival or insulin-free survival probability after a post-transplant increase in antibodies was performed using the Cox proportional hazards regression model. Extended Cox proportional hazards regression model for recurrent events was used to compare antibody increase between the levels of each predictor and to identify independent risk factors for the increase of islet cell autoantibody and DSA events. For all analyses, a two-sided $P < 0.05$ was considered statistically significant. Analyses were performed using Stata 10.1 (Stata Corp., College Station, TX) or SPSS for Windows version 13.0 (IBM, Chicago, IL) statistical software.

RESULTS

Islet transplant cases. Ninety-eight islet infusions were performed in 59 recipients; 26 (44%) received 1 islet infusion, 23 (39%) received 2, and 10 (17%) received 3 (Table 1). In 33 infusions, islets from two donors were infused simultaneously and considered as a single infusion.

HLA matching. The median (range) of HLA-A and -B mismatches, HLA-DR mismatches, and HLA-A, -B, and -DR mismatches per recipient was 6 (1–13), 3 (1–6), and 9 (2–17), respectively. The HLA-A, -B, and -DR mismatching was not predictive of islet transplant outcome (data not shown).

Pretransplant antibodies. The majority of recipients were of low immunological risk pretransplant: 18 (30.5%) had PRA <15% when tested by the Luminex method (both IgG and IgM), and 1 (1.6%) had PRA <15% when tested by the CDC method. The detection of pretransplant PRAs per se was not predictive of subsequent islet transplant outcome (Table 2).

DSA were found in 29 of 59 patients (49%) before transplantation. Twenty-one (36%) had IgG and/or IgM DSA class I, 10 (17%) had IgG and/or IgM DSA class II, and 2 (3%) had both class I and class II DSA. The islet transplant outcome was, in general, improved in patients with pretransplant DSA, reaching statistical significance in patients having IgG and/or IgM DSA class I, class II, or both (hazard ratio [HR] 0.43 [95% CI 0.22–0.88], $P = 0.021$) (Table 2).

Islet autoantibodies were found in 26 of 59 patients (44%) before transplantation. Twelve (20.3%) had one autoantibody (4 GADA and 8 IA-2A), 12 had two autoantibodies (7 GADA and IA-2A, 3 GADA and ZnT8A, and 2 IA-2A and ZnT8A), and 2 had all three autoantibodies. Islet transplant survival was not influenced by any of the autoantibodies either alone or in combination (Table 2).

Posttransplant antibodies. Total posttransplant follow-up was 1,420.9 patient-months, with a median of 12.1 (range 0.52–113). During posttransplant follow-up, both DSA and autoantibodies did not increase in 20 of the 59 patients (34%), whereas increases were observed in 39 (66%). Seventeen (28.8%) patients had an increase in DSA only, 12 (20.3%) in autoantibodies only, and 10 (16.9%) in both DSA and autoantibodies. Within the 10 with increases in both DSA and autoantibodies, 4 had DSA increases before autoantibody increases and 6 after autoantibody increases.

Among the 22 patients with an autoantibody increase (Fig. 1), 10 had a serum conversion for GADA ($n = 8$), ZnT8A ($n = 1$) or GADA, and IA-2A ($n = 1$); 3 had spreading

TABLE 2
Univariate extended Cox proportional hazards regression analysis for islet graft failure (fasting C-peptide level <0.3 ng/mL)

	Summary	HR* (95% CI)	*P* value
PRA CDC method (%)	2 ± 11	0.99 (0.96–1.02)	0.61
≤15%	58 (98.4)	—	
>15%	1 (1.6)	—	
PRA Luminex method			
IgG class I (%)	2.9 ± 7.3	1.02 (0.98–1.06)	0.31
≤15%	55 (93.2)	Reference	
>15%	4 (6.8)	1.84 (0.59–5.66)	0.28
IgG class II (%)	3 ± 10	0.98 (0.96–1.01)	0.37
≤15%	55 (93.2)	Reference	
>15%	4 (6.8)	0.57 (0.16–2.035)	0.38
IgM class I (%)	12.9 ± 25	1.003 (0.99–1.015)	0.67
≤15%	46 (78)	Reference	
>15%	13 (22)	1.12 (0.52–2.43)	0.76
IgM class II (%)	0.05 ± 0.4	0.23 (0–132)	0.65
≤15%	59 (100)	—	—
>15%	0 (0)	—	—
PRA class I and II combined			
IgG both ≤15%	51 (86.5)	Reference	
IgG either >15%	8 (13.5)	0.99 (0.41–2.37)	0.99
IgG both >15%	0	—	—
IgM both ≤15%	46 (78)	Reference	
IgM either >15%	13 (22)	1.12 (0.52–2.43)	0.76
IgM both >15%	0	—	
IgG/IgM all ≤15%	41 (70)	Reference	
IgG/IgM any >15%	18 (30)	0.98 (0.46–2.11)	0.97
IgG/IgM >1 >15%	4 (6.8)	1.63 (0.43–6.15)	0.47
DSA class I			
None	38 (64)	Reference	
IgG	8 (14)	0.80 (0.26–2.5)	0.71
IgM	16 (27)	0.68 (0.30–1.45)	0.34
IgG and/or IgM	21 (36)	0.67 (0.32–1.37)	0.27
DSA class II			
None	49 (83)	Reference	
IgG	7 (12)	0.31 (0.09–1.05)	0.06
IgM	3 (5)	10.5 (1.55–71.4)	**0.016**
IgG and/or IgM	10 (17)	0.65 (0.25–1.69)	0.38
DSA class I and II combined			
Both negative IgG and IgM	30 (51)	Reference	
Any positive IgG	15 (25)	0.41 (0.16–1.02)	0.055
Any positive IgM	19 (32)	0.65 (0.31–1.35)	0.25
Any positive IgG and/or IgM	29 (49)	0.43 (0.22–0.88)	**0.021**
No. mismatches			
HLA-A and -B	6 (1–13)	0.94 (0.78–1.15)	0.59
HLA-DR	3 (1–6)	1.19 (0.82–1.71)	0.34
HLA-A, -B, and -DR	9 (2–17)	0.99 (0.86–1.14)	0.96
Autoantibodies			
None	33 (56)	Reference	
GADA	16 (27)	0.87 (0.39–1.93)	0.73
IA-2A	19 (32)	0.74 (0.34–1.6)	0.45
ZnT8A	7 (12)	1.48 (0.48–4.51)	0.48
Any	26 (44)	0.79 (0.4–1.56)	0.52
No. autoantibodies	0 (0–3)	0.95 (0.65–1.39)	0.8

Data are mean ± SD, *n* (%), or median (range) unless otherwise indicated. Boldface data indicate significance at $P < 0.05$. *Extended Cox proportional hazards regression analysis was performed with stratification for different immunosuppression and correction for islet equivalents/kg transplanted.

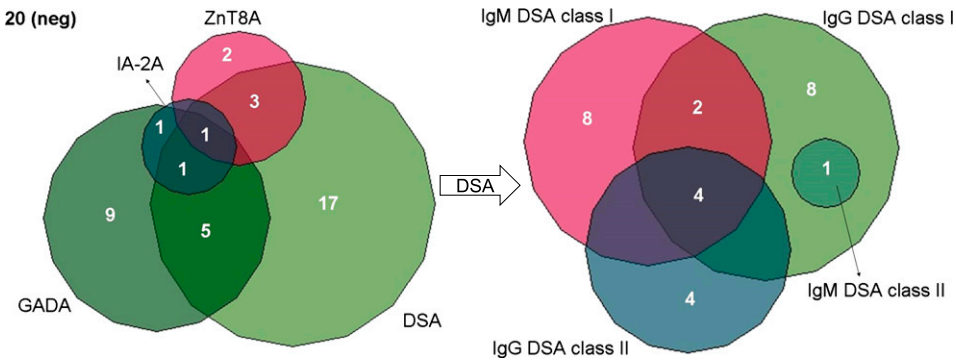

FIG. 1. Venn diagrams of antibody increase after islet transplantation. Significant antibody increase after transplantation was observed in 39 of 59 patients (66%). Twenty-two of 59 patients (37.3%) had an increase in autoantibodies (*left*). Single and overlapping GADA, IA-2A, ZnT8A, and DSA increases are reported, with the percentage of patients in each group shown. Twenty-seven of 59 patients (45.7%) had an increase in DSA (*right*). Single and overlapping IgG DSA class I, IgG DSA class II, IgM DSA class I, and IgM DSA class II increases are reported, with the percentage of patients in each group shown.

from IA-2A only to GADA (n = 1) or ZnT8A (n = 2); one had spreading from IA-2A only to GADA and a concomitant increase of IA-2A; one had spreading from IA-2A and ZnT8A to GADA and a concomitant significant increase of IA-2A and ZnT8A; and the remaining 7 had a significant increase of GADA (n = 4) or ZnT8A titers (n = 3). Among the 27 (45.7%) patients with DSA change, 11 had a serum conversion, 13 had spreading, and 3 had increasing titers (Fig. 1).

The median time between first islet infusion and antibody increase was 16 (95% CI 6.8–25.1) days, with antibodies developing in 27 (69%) patients within 3 months after the first islet infusions (Fig. 2). Within the 39 patients with antibody increases, GADA (7 [5.6–8.3] days) was the first antibody to increase followed by IA-2A (16 [12.7–19.2] days), IgM DSA (30 [26.3–33.6] days), IgG DSA (82 [27–136] days), and ZnT8A (90 [0–306] days).

Islet transplant patients who had posttransplant antibody increases showed similar time to gain of insulin independence (Supplementary Fig. 1) but significantly lower graft survival than patients with no antibody changes (Fig. 3*A*) (HR 5.23 [95% CI 2.46–11.12], $P < 0.001$). The median time to graft loss after antibody increase was 304 (95% CI 54.9–553) days and was faster if the increase occurred within 3 months of infusion (P = 0.032 vs. >3 months) (Fig. 3*B*). Any antibody increase was also predictive of a shorter duration of insulin independence (6.46 [1.98–21.05], P = 0.002) (Fig. 3*C*), and linear regression analysis showed a strong association between antibody modification–free time and insulin-free time (R = 0.87, $P <$ 0.001) (Fig. 3*D*).

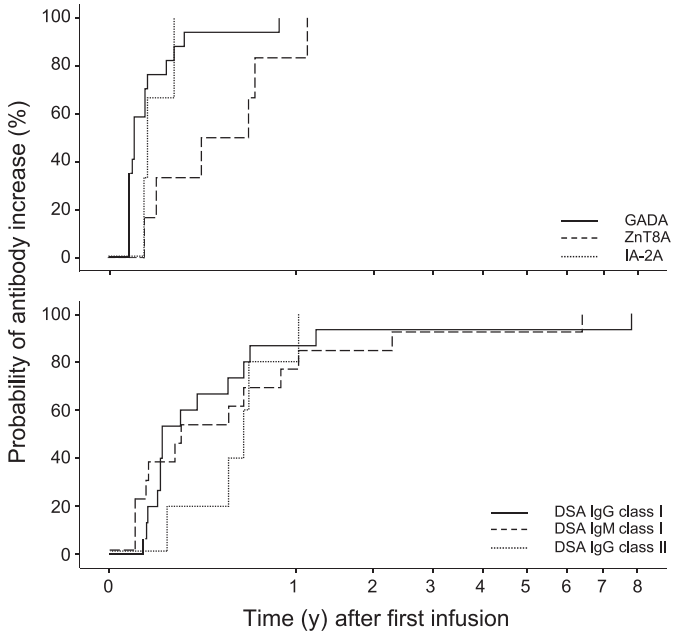

FIG. 2. Time of antibodies appearance. Time course of autoantibody (*top*) or DSA (*bottom*) increase after first islet infusion.

When analyzed separately, both DSA and autoantibody increases were associated with reduced graft survival compared with follow-up without antibody increase (HR 5.26 [95% CI 2.23–12.40] and 5.21 [2.30–11.79], respectively, both $P < 0.001$) (Fig. 4*A*). Reduced graft survival was also observed when analyses were restricted to patients who only had DSA increases (5.12 [2.1–12.4] compared with no increase, $P < 0.001$) or only islet autoantibody increases (5.3 [2.1–12.8] compared with no increase, $P < 0.001$). The median time to graft loss was 318 (95% CI 156–479) days after DSA increase and 117 (0–308) days after autoantibody increase. Concordantly, DSA or autoantibody increases were predictive of a shorter duration of insulin independence (Fig. 4*B*).

Risk factors for posttransplant antibody increases. The identification of variables associated with posttransplant antibody increases was performed based on a per-infusion analysis using Cox proportional hazards regression. Significant antibody increases were observed in 49 of the 98 islet infusions (50%). Of these, 24 (24.5%) had an increase in DSA only, 16 (16.3%) had an increase in autoantibodies only, and 9 (9.2%) had increases in both. The risk of posttransplant antibody increase was associated with a pretransplant insulin requirement, pretransplant PRA >15% in either class I or class II, HLA-DR mismatches, HLA class I matches, number of islet donors, the use of ATG as induction of and MMF as maintenance for immunosuppression (Fig. 5). Conversely, the use of anti-CD25 mAb as induction of or rapamycin as maintenance for immunosuppression was associated with a decreased risk of antibody increase. The Cox proportional hazard regression model included HLA-DR mismatches (P = 0.013), ATG as induction (P = 0.006), and pretransplant PRA >15% in either class I or class II (P = 0.028) as independent risk factors for and rapamycin as protective (P = 0.023) against antibody increases (Table 3).

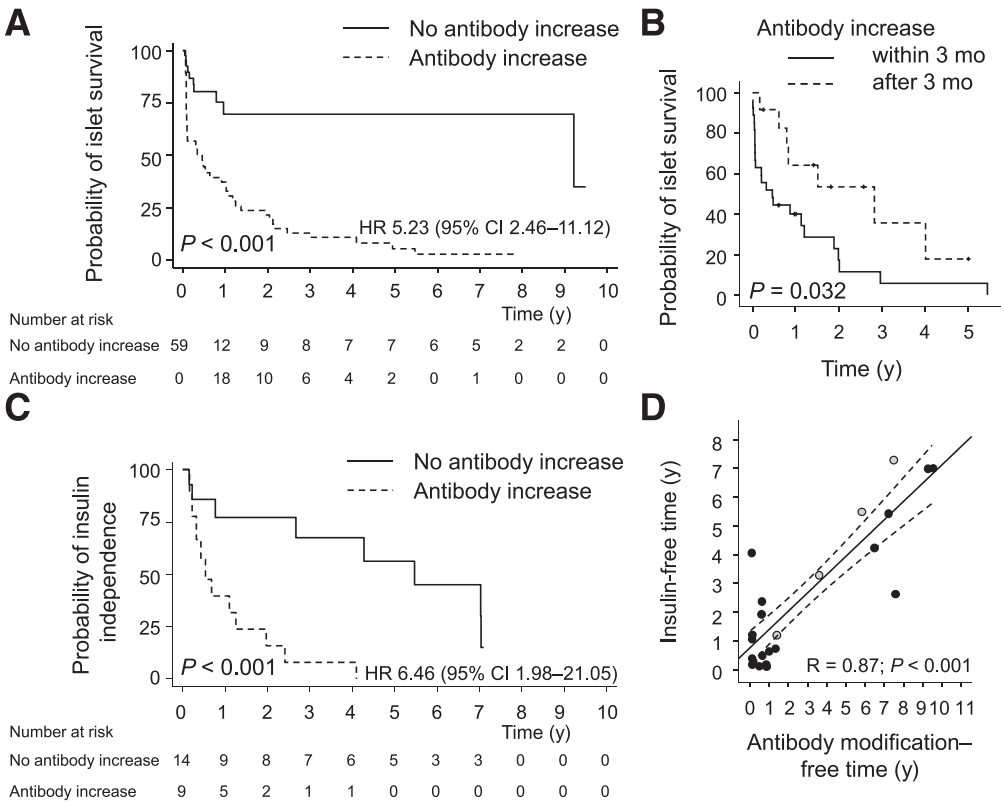

FIG. 3. Antibody increase and graft function. *A*: Probability of islet survival according to Kaplan-Meier method, with the increase of antibody as a time-varying covariate by Cox proportional hazards regression model. *B*: Survival of islet graft functions after increase of antibodies. *C*: Probability of insulin independence loss according to Kaplan-Meier method, with the increase of antibody as a time-varying covariate by Cox proportional hazards regression model. *D*: Association between antibody modification–free time and insulin-free time by linear regression analysis. Measurements are shown with dots, linear regression with solid line, and 95% mean prediction interval with dashed lines. Gray dots, still insulin free at last follow-up; black dots, not insulin free at last follow-up.

When analyzed separately, DSA and autoantibody changes were associated with different risk factors. Pretransplant PRA >15% in either class I or class II and number of islet donors were relevant for DSA but not for autoantibody increases. HLA class I and II mismatching were risk factors for DSA increase, and HLA class I mismatching was a protective factor against autoantibody increase. Pretransplant DSA were a risk for posttransplant DSA increase but were protective against autoantibody increases. Finally, ATG and MMF treatments were risk factors for both DSA and autoantibody increases, whereas anti-CD25 mAb and rapamycin treatments were mainly protective factors against autoantibody changes (Fig. 5).

DISCUSSION

Islet transplantation represents a model in which the alloimmune response and recurrence of autoimmunity coexist, jeopardizing long-term islet function and possibly contributing to graft loss. The findings indicate that both alloimmune and autoimmune responses can be detected and monitored by antibody measurements and that both predict the clinical outcome of islet transplantation.

In the setting of islet and pancreas transplantation, the use of antibodies as diagnostic markers has been reported in a number of studies (19,33,34), but their significance and prognostic role remain controversial (4). To our knowledge, the present study is unprecedented in the frequency and extensiveness of alloantibody and islet autoantibody measurements used. The findings from these measurements unequivocally demonstrate that important increases (de novo appearance or titer increase) in these antibodies are common after islet transplantation. The relatively high frequency of antibody increases is attributed to the highly sensitive methodology for DSA measurement (28) and the frequency of measurement and has yielded respectable numbers of cases on which to study outcome. The limitations of the study include the fact that a large number of variables is likely to influence islet transplant outcome; that many of these factors occur concurrently within transplants; and, as a consequence, that the antibody increases can only be regarded as markers and not considered pathologically relevant. Moreover, cell-based immune responses posttransplant, which may better identify posttransplant immune response to graft (7–11), were not included.

The present findings partly confirm and partly refute previous observations. First, we found no evidence that pretransplant autoantibody status influences functional outcome of islet transplantation. Graft function was unaffected by the presence of islet autoantibodies, their titers, or possible combinations of different autoantibodies before transplantation. This observation agrees with our previous report (19) and reports of others (4,5) but not all (17). We were also unable to find correlations with clinical outcome for preformed alloantibodies measured as PRAs, which is in contrast with an earlier claim that pretransplant PRA >15% is associated with an accelerated posttransplant loss of islet function (20). This discrepancy might be explained by differences in patient immunosuppression and methods used to analyze the data. We performed a per-patient analysis that evaluated preformed alloantibodies only before the first infusion, whereas

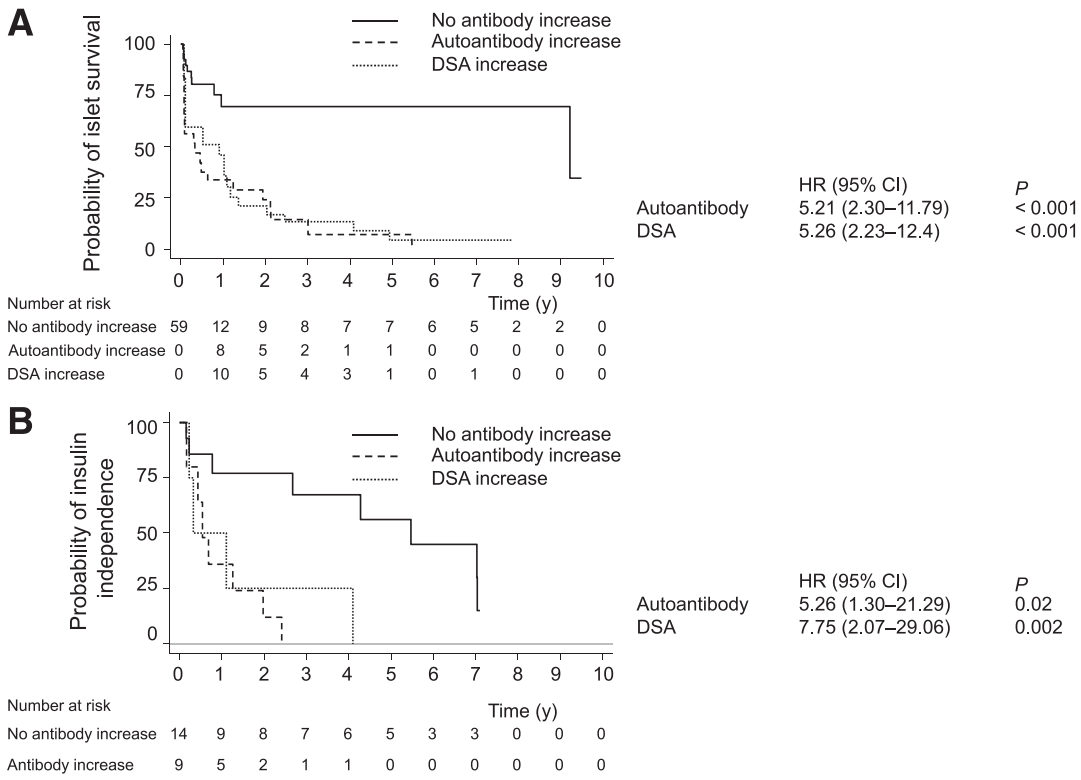

FIG. 4. Autoantibody and DSA increase and graft function. *A*: Probability of islet survival according to Kaplan-Meier method, with the increase of antibody as a time-varying covariate by Cox proportional hazards regression model. *B*: Probability of insulin independence loss according to Kaplan-Meier method, with the increase of antibody as a time-varying covariate by Cox proportional hazards regression model.

Campbell et al. (20) performed a per-infusion analysis, potentially considering PRA increase induced after the first infusion as preformed alloantibodies for subsequent graft follow-up. In the present cohort, the per-infusion analysis revealed that PRA >15% was associated with DSA change, which indirectly speaks in favor of this possibility.

The prevalence of pretransplant IgG DSA observed (25%) was similar to that reported for other organ recipients, such as kidney (range 24–35%) (35–38), whereas we have no reference points for IgM DSA (observed prevalence 32%) because IgM normally is not evaluated. The high prevalence of both isotypes is justified by the high sensitivity of the detection technique (28) used. Additionally, we show that islet transplant outcome was improved in patients with pretransplant DSA, particularly IgM against major histocompatibility complex II, a finding not reported so far, to our knowledge, for islet transplantation but consistent with previously reported beneficial effects of recipient sensitization to donor-specific transplant antigens (39–42).

The present study also strengthens the evidence for posttransplant autoantibody increases (defined as serum conversion, spreading, or increasing titers) that predict future islet pancreatic transplant failure (5,16–19). The addition of ZnT8A to GADA and IA-2A in the screening panel appear useful because there were five patients in whom ZnT8A was the only antibody to appear or increase. The increased sensitivity was also recently reported in pancreas transplantation (33). Posttransplant autoantibody changes most likely reflect the recurrence or recrudescence of type 1 diabetes–associated autoimmunity, although the formal confirmation by graft biopsy studies is lacking in this series and, in general, is almost impossible to obtain in islet transplantation. However, a recent report on documented biopsy cases of recurrent diabetic insulitis after simultaneous pancreas-kidney transplantation showed autoantibody changes similar to those observed in the present study that preceded the loss of β-cell function mediated by autoreactive T cells (43).

In addition to the islet autoantibodies, major DSA responses were frequent and predictive of future islet pancreatic transplant failure. Previous studies reported the development of de novo posttransplant antibody against HLA class I and II antigens after islet transplantation (11,44–46), but antibodies developed in several of the patients after immunosuppression withdrawal and the occurrence of side effects or complete islet graft failure. In the present study, 46% of recipients had important posttransplant DSA increases while receiving immunosuppression therapy, and this was almost always associated with a direct decline in islet graft function. This evidence was previously reported as case reports in two patients (21,22). Of note, in one case, islet function recovered after treatment with anti-CD20 antibody and intravenous immunoglobin (21), suggesting that the identification of a posttransplant DSA response could help in tuning the level of posttransplant immunosuppression. Particularly interesting is the evidence of a chronology of the different antibody changes, with GADA being the earliest marker, and before IgM DSA. This evidence was also observed in individual patients and reinforces the need to monitor both auto- and alloimmune responses.

Finally, we attempted to identify transplant factors that may influence the likelihood of an allo- or autoimmune humoral response. The degree of HLA class I and II mismatching increased the risk of DSA responses, whereas HLA class mismatching appeared to decrease the likelihood

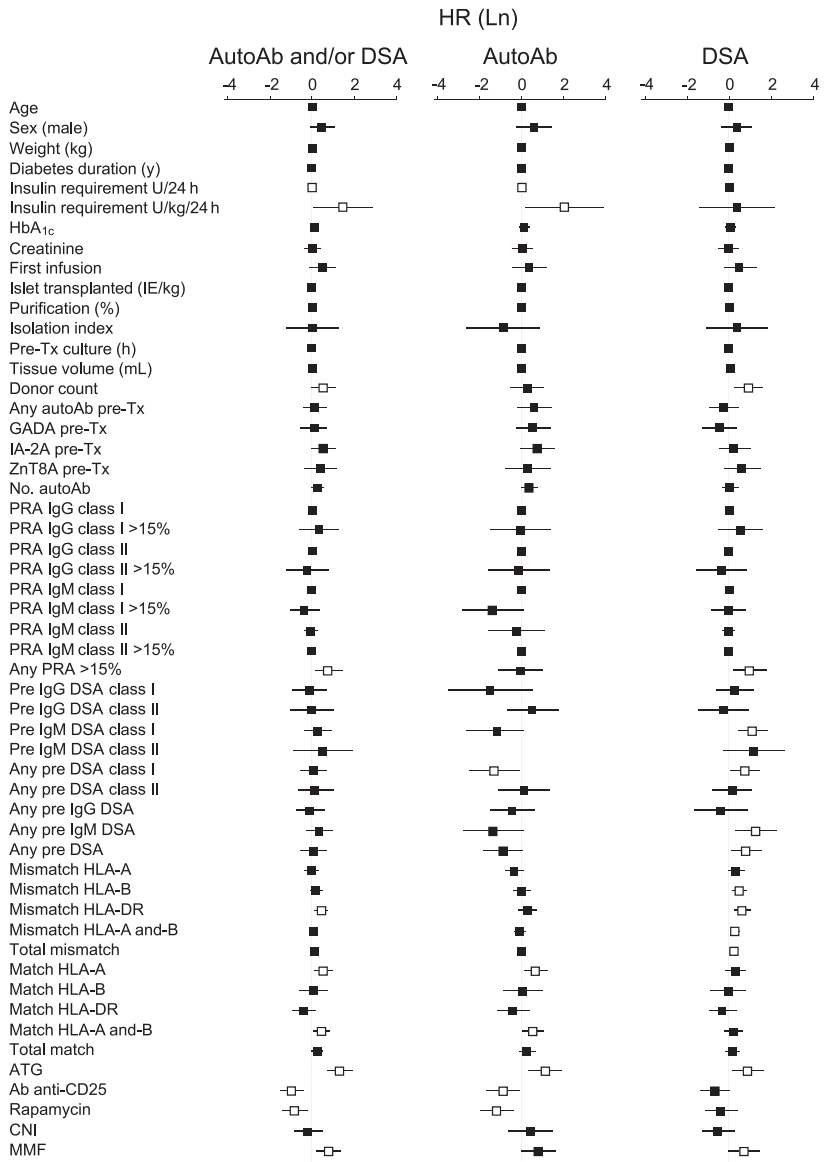

FIG. 5. Univariate HRs for antibody increase. All factors analyzed are depicted. ■, HR; line, 95% CI; □, $P < 0.05$. Ab, antibody; IE, islet equivalents; Ln, natural logarithm; pre-Tx, pretransplant.

of islet autoantibody increases. The influence of HLA class I and II matching on the risk of DSA increases is not surprising, whereas the opposite effect of HLA class I matching on autoantibody risk is likely to reflect the need for correct presentation of self-antigen by self- and matched HLA and the importance of CD8 T cells in recurrent islet autoimmunity. In the reported cases of recurrent diabetes after twin (47), related (49), or cadaveric (43) donor pancreas transplantation, the predominant phenotype of islet-infiltrating T cell was CD8, recognizing (auto)antigens through major histocompatibility complex class I, which is consistent with the present finding. One can speculate, therefore, that mismatching at HLA class I loci to reduce the risk of aggravating islet autoimmunity while matching at HLA class II to avoid DSA may benefit islet transplant outcome in patients with autoimmune diabetes. To our surprise, ATG induction therapy was associated with an increased risk of antibody increases posttransplant compared with anti-CD25 treatment, whereas rapamycin, which acts on mammalian target of rapamycin and hinders IL-2–mediated transcription, appeared to be protective. Thus, it seems that specific blocking of IL-2 pathways may be helpful for preventing allo- and autoantibody responses. When analyzed as an independent factor, preconditioning did not appear to be relevant for the protective effects of rapamycin on antibody increases (data not shown).

TABLE 3
Factors associated with autoantibody and DSA increase resulting from a multivariate extended Cox proportional hazards regression analysis

	HR (95% CI)	*P* value
Rapamycin vs. no rapamycin	0.308 (0.112–0.85)	**0.023**
Any PRA >15%	2.62 (1.11–6.23)	**0.028**
MMF vs. no MMF	0.42 (0.12–1.44)	0.17
Mismatch HLA-DR	1.65 (1.11–2.45)	**0.013**
ATG vs. other induction	4.38 (1.51–12.6)	**0.006**

Boldface data indicate significance at $P < 0.05$.

In conclusion, we demonstrate that immune monitoring with frequent posttransplant assessment of allo- and autoantibodies could be helpful in clinical islet transplantation. The immunological tests used in this study were validated in other clinical settings, relatively easy to perform, and readily available. This approach to active immune monitoring should allow for the use of more-tailored (and potentially milder) immunosuppression combinations and prompt intervention for acute immunological events. In addition, such monitoring may provide a better understanding and characterization of the various mechanisms of destruction involved in the loss of islet grafts. Overall, we believe that antibody immune monitoring has the potential to significantly improve islet transplant outcomes. The development and use of such tests should be promoted.

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L.P. promoted the study, researched data, and wrote the manuscript. M.J.E. (alloantibody detection), P.M. (clinical data), F.P. (HLA typing), R.N. (islet isolation), M.C. (PRAs), R.M. (islet isolation), A.M. (islet isolation), V.S. (autoantibody detection), V.L. (autoantibody detection), and A.E.d.A. (HLA typing) researched data. M.Scav. provided statistical analysis and edited the manuscript. M.Scal. and A.S. contributed to discussion. E.Bos. contributed to discussion and reviewed and edited the manuscript. E.Bon. initiated the study, contributed to discussion, and reviewed and edited the manuscript. P.I.T. researched data and reviewed and edited the manuscript. L.P. is the guarantor of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

REFERENCES

1. Stegall MD, Lafferty KJ, Kam I, Gill RG. Evidence of recurrent autoimmunity in human allogeneic islet transplantation. Transplantation 1996;61:1272–1274
2. Worcester Human Islet Transplantation Group. Autoimmunity after islet-cell allotransplantation. N Engl J Med 2006;355:1397–1399
3. Toso C, Isse K, Demetris AJ, et al. Histologic graft assessment after clinical islet transplantation. Transplantation 2009;88:1286–1293
4. Lacotte S, Berney T, Shapiro AJ, Toso C. Immune monitoring of pancreatic islet graft: towards a better understanding, detection and treatment of harmful events. Expert Opin Biol Ther 2011;11:55–66
5. Hilbrands R, Huurman VA, Gillard P, et al. Differences in baseline lymphocyte counts and autoreactivity are associated with differences in outcome of islet cell transplantation in type 1 diabetic patients. Diabetes 2009;58:2267–2276
6. Huurman VA, Hilbrands R, Pinkse GG, et al. Cellular islet autoimmunity associates with clinical outcome of islet cell transplantation. PLoS ONE 2008;3:e2435
7. Roelen DL, Huurman VA, Hilbrands R, et al. Relevance of cytotoxic alloreactivity under different immunosuppressive regimens in clinical islet cell transplantation. Clin Exp Immunol 2009;156:141–148
8. Roep BO, Stobbe I, Duinkerken G, et al. Auto- and alloimmune reactivity to human islet allografts transplanted into type 1 diabetic patients. Diabetes 1999;48:484–490
9. van Kampen CA, van de Linde P, Duinkerken G, et al. Alloreactivity against repeated HLA mismatches of sequential islet grafts transplanted in non-uremic type 1 diabetes patients. Transplantation 2005;80:118–126
10. Huurman VA, Velthuis JH, Hilbrands R, et al. Allograft-specific cytokine profiles associate with clinical outcome after islet cell transplantation. Am J Transplant 2009;9:382–388
11. Mohanakumar T, Narayanan K, Desai N, et al. A significant role for histocompatibility in human islet transplantation. Transplantation 2006;82:180–187
12. Caro-Oleas JL, González-Escribano MF, Gentil-Govantes MA, et al. Clinical relevance of anti-HLA donor-specific antibodies detected by Luminex assay in the development of rejection after renal transplantation. Transplantation 2012;94:338–344
13. Hirai T, Kohei N, Omoto K, Ishida H, Tanabe K. Significance of low-level DSA detected by solid-phase assay in association with acute and chronic antibody-mediated rejection. Transpl Int 2012;25:925–934
14. Hoshino J, Kaneku H, Everly MJ, Greenland S, Terasaki PI. Using donor-specific antibodies to monitor the need for immunosuppression. Transplantation 2012;93:1173–1178
15. Achenbach P, Bonifacio E, Koczwara K, Ziegler AG. Natural history of type 1 diabetes. Diabetes 2005;54(Suppl. 2):S25–S31
16. Jaeger C, Hering BJ, Hatziagelaki E, Federlin K, Bretzel RG. Glutamic acid decarboxylase antibodies are more frequent than islet cell antibodies in islet transplanted IDDM patients and persist or occur despite immunosuppression. J Mol Med (Berl) 1999;77:45–48
17. 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
18. Hering BJ, Kandaswamy R, Ansite JD, et al. Single-donor, marginal-dose islet transplantation in patients with type 1 diabetes. JAMA 2005;293:830–835
19. Bosi E, Braghi S, Maffi P, et al. Autoantibody response to islet transplantation in type 1 diabetes. Diabetes 2001;50:2464–2471
20. Campbell PM, Salam A, Ryan EA, et al. Pretransplant HLA antibodies are associated with reduced graft survival after clinical islet transplantation. Am J Transplant 2007;7:1242–1248
21. Kessler L, Parissiadis A, Bayle F, et al.; GRAGIL Study Group. Evidence for humoral rejection of a pancreatic islet graft and rescue with rituximab and IV immunoglobulin therapy. Am J Transplant 2009;9:1961–1966
22. Rickels MR, Kamoun M, Kearns J, Markmann JF, Naji A. Evidence for allograft rejection in an islet transplant recipient and effect on beta-cell secretory capacity. J Clin Endocrinol Metab 2007;92:2410–2414
23. Shapiro AM, Ricordi C, Hering BJ, et al. International trial of the Edmonton protocol for islet transplantation. N Engl J Med 2006;355:1318–1330
24. Piemonti L, Maffi P, Monti L, et al. Beta cell function during rapamycin monotherapy in long-term type 1 diabetes. Diabetologia 2011;54:433–439
25. Kellar KL, Iannone MA. Multiplexed microsphere-based flow cytometric assays. Exp Hematol 2002;30:1227–1237
26. Colombo MB, Haworth SE, Poli F, et al. Luminex technology for anti-HLA antibody screening: evaluation of performance and of impact on laboratory routine. Cytometry B Clin Cytom 2007;72:465–471
27. Terasaki PI, McClelland JD. Microdroplet assay of human serum cytotoxins. Nature 1964;204:998–1000
28. Gebel HM, Bray RA. The evolution and clinical impact of human leukocyte antigen technology. Curr Opin Nephrol Hypertens 2010;19:598–602
29. Bonifacio E, Genovese S, Braghi S, et al. Islet autoantibody markers in IDDM: risk assessment strategies yielding high sensitivity. Diabetologia 1995;38:816–822
30. Bonifacio E, Lampasona V, Genovese S, Ferrari M, Bosi E. Identification of protein tyrosine phosphatase-like IA2 (islet cell antigen 512) as the insulin-dependent diabetes-related 37/40K autoantigen and a target of islet-cell antibodies. J Immunol 1995;155:5419–5426
31. Lampasona V, Petrone A, Tiberti C, et al.; Non Insulin Requiring Autoimmune Diabetes (NIRAD) Study Group. Zinc transporter 8 antibodies complement GAD and IA-2 antibodies in the identification and characterization of adult-onset autoimmune diabetes: Non Insulin Requiring Autoimmune Diabetes (NIRAD) 4. Diabetes Care 2010;33:104–108
32. Törn C, Mueller PW, Schlosser M, Bonifacio E, Bingley PJ; Participating Laboratories. Diabetes Antibody Standardization Program: evaluation of assays for autoantibodies to glutamic acid decarboxylase and islet antigen-2. Diabetologia 2008;51:846–852
33. Occhipinti M, Lampasona V, Vistoli F, et al. Zinc transporter 8 autoantibodies increase the predictive value of islet autoantibodies for function

loss of technically successful solitary pancreas transplant. Transplantation 2011;92:674–677
34. Braghi S, Bonifacio E, Secchi A, Di Carlo V, Pozza G, Bosi E. Modulation of humoral islet autoimmunity by pancreas allotransplantation influences allograft outcome in patients with type 1 diabetes. Diabetes 2000;49:218–224
35. Otten HG, Verhaar MC, Borst HP, Hené RJ, van Zuilen AD. Pretransplant donor-specific HLA class-I and -II antibodies are associated with an increased risk for kidney graft failure. Am J Transplant 2012;12:1618–1623
36. Eng HS, Bennett G, Tsiopelas E, et al. Anti-HLA donor-specific antibodies detected in positive B-cell crossmatches by Luminex predict late graft loss. Am J Transplant 2008;8:2335–2342
37. Riethmüller S, Ferrari-Lacraz S, Müller MK, et al. Donor-specific antibody levels and three generations of crossmatches to predict antibody-mediated rejection in kidney transplantation. Transplantation 2010;90:160–167
38. Amico P, Hönger G, Mayr M, Steiger J, Hopfer H, Schaub S. Clinical relevance of pretransplant donor-specific HLA antibodies detected by single-antigen flow-beads. Transplantation 2009;87:1681–1688
39. McAlister CC, Gao ZH, McAlister VC, et al. Protective anti-donor IgM production after crossmatch positive liver-kidney transplantation. Liver Transpl 2004;10:315–319
40. Fradet Y, Roy R, Lachance JG, Noël R. Kidney graft survival: role of blood transfusions and lymphocytotoxic antibodies. Clin Nephrol 1982;18:69–73
41. Melero J, Tarragó D, Núñez-Roldán A, Sánchez B. Human polyreactive IgM monoclonal antibodies with blocking activity against self-reactive IgG. Scand J Immunol 1997;45:393–400
42. Kerman RH, Susskind B, Buyse I, et al. Flow cytometry-detected IgG is not a contraindication to renal transplantation: IgM may be beneficial to outcome. Transplantation 1999;68:1855–1858
43. Vendrame F, Pileggi A, Laughlin E, et al. Recurrence of type 1 diabetes after simultaneous pancreas-kidney transplantation, despite immunosuppression, is associated with autoantibodies and pathogenic autoreactive CD4 T-cells. Diabetes 2010;59:947–957
44. Ferrari-Lacraz S, Berney T, Morel P, et al. Low risk of anti-human leukocyte antigen antibody sensitization after combined kidney and islet transplantation. Transplantation 2008;86:357–359
45. Campbell PM, Senior PA, Salam A, et al. High risk of sensitization after failed islet transplantation. Am J Transplant 2007;7:2311–2317
46. Cardani R, Pileggi A, Ricordi C, et al. Allosensitization of islet allograft recipients. Transplantation 2007;84:1413–1427
47. Sibley RK, Sutherland DE, Goetz F, Michael AF. Recurrent diabetes mellitus in the pancreas iso- and allograft. A light and electron microscopic and immunohistochemical analysis of four cases. Lab Invest 1985;53:132–144
48. Sibley RK, Sutherland DE. Pancreas transplantation. An immunohistologic and histopathologic examination of 100 grafts. Am J Pathol 1987;128:151–170