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 allo antibodies (DSA) were quantified. Thirty-nine recipients (69%) 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 allo antibody $> 15\%$ in either class I or class II ($P = 0.024$) as independent risk factors and with rapamycin as protection ($P = 0.006$) 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.

The setting of islet transplantation is interesting because both allogeneic 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 autoantibodies (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.

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 0 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 $\sim 70^\circ$C until testing. HLA-A, -B, and -DR mismatches were calculated by measuring the total number of mismatches to HLA-A, -B, and -DR. Cs 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 (5 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 Single-antigen 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). 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, -C, -Cw, -DRB1, -DRB3, -B4, -B5, -DQA1, -DQB1, -DPB1, and -DPB2. 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), insulinnoma-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.

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**TABLE 1 Patient demographics and islet transplant data**

| Factor | Edmonton protocol | CNI-free protocol | SIR-free protocol | Total |
|--------|-------------------|-------------------|-------------------|-------|
| Patients (n) | | | | |
| 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 ± 5,976 | 7,436 ± 2,631 | 9,937 ± 4,754 |
| ITA/IAK/ SIK (n) | 27/0/0 | 12/0/0 | 10/7/3 | 48/7/3 |

| 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) | 1 (0–2) | 1 (0–2) | 1 (0–2) |
| All | 2 (0–5) | 1 (0–5) | 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 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 <5% 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 (0.59–6.66) | 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 (%) | 12.5 ± 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 (%) | 0.06 ± 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 (0.46–2.2) | 0.97 |
| IgG/IgM >15% | 4 (6.8) | 1.03 (0.45–0.63) | 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 |

Summary HR* (95% CI) \( P \) value

Data are mean ± SD, \( n \) (%), and 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.
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 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 postransplant 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. 3A) (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. 3B). Any antibody increase was also predictive of a shorter duration of insulin independence (6.46 [1.98–21.05], P = 0.002) (Fig. 3C), and linear regression analysis showed a strong association between antibody modification–free time and insulin-free time (R = 0.87, P < 0.001) (Fig. 3D).

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. 4A). 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. 4B).

Risk factors for postransplant antibody increases.

The identification of variables associated with postransplant 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 postransplant 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).
When analyzed separately, DSA and autoantibody changes were associated with different risk factors. Pretransplant PRA 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. 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
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. 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, 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 immunoglobulin (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. 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.
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.I.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.

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