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

Islet cell transplantation 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 threshold (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 more...
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 hemorrhage at 18 weeks posttransplant. These five patients were excluded from the current analysis.

**Table 1**

| Metabolic outcome 6 months after islet cell transplantation | At posttransplant month 6 | P* |
|-----------------------------------------------------------|---------------------------|----|
| **C-peptide positive (n)**                                | 15/15                     |    |
| **C-peptide (ng/ml)**                                     | 2.3 (1.9–3.0)             | 1.0 (0.4–1.2) | <0.001 |
| **Fasting glycemia (mg/dl)**                              | 127 (115–135)             | 132 (127–153) | 0.12 |
| CVfg (%)                                                  | 9 (8–10)                  | 19 (15–39) | <0.001 |
| **AIC (%)**                                               | 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 |

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

**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 autoantigen IA-2 (10 μg/ml) or GAD65 (10 μg/ml), of interleukin-2 (25 units/ml), or of medium alone. After 5 days, [3H]-thymidine (0.5 μCi/well) was added and its incorporation measured after 16 h. Data were expressed as a stimulation index (SI) by comparison with the medium alone value. An SI ≥3 for any of the two antigens was considered a sign of T-cell autoreactivity against an islet cell antigen. In three patients, cellular autoreactivity could not be assessed because autoantibodies 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 biopit was taken from a static area on the liver surface. Immunohistochemistry was performed on semisecutive 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 posttransplant 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 SPSS (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...
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 million 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 subcutaneous 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 shown in Table 2.
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 patents 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. 1B). 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 <50, while only 1 of 11 patients with T-cell autoreactivity and a B-cell count >50 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. 2A).
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. 2B). 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. 2A and 2B). The number of \( \beta \)-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 \( \beta \)-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. 1A). 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. 1A); 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%).

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**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            | 0.17                      | 0.02                | 0.28                | 0.17                |
| Sex (male/female)      | 0.46                 | 0.44            | 0.48                      | 0.44                | 0.48                | 0.48                |
| Body weight (kg)       | 0.72                 | 0.91            | 0.95                      | 0.91                | 0.95                | 0.95                |
| BMI (kg/m\(^2\))       | 0.55                 | 0.86            | 0.83                      | 0.86                | 0.83                | 0.83                |
| Duration of disease (years) | 0.30              | 0.55            | 0.41                      | 0.55                | 0.41                | 0.41                |
| AIC (%)                | 0.05                 | 0.71            | 0.48                      | 0.71                | 0.48                | 0.48                |
| Insulin dose (IU·kg\(^{-1}\)·day\(^{-1}\)) | 0.13               | 0.02            | 0.04                      | 0.02                | 0.22                | 0.17                |

| Fasting glycemia       | Mean (mg/dl)         | 0.65            | 0.70                      | 0.11                | 0.78                | 0.94                |
|                        | CVfg (%)             | 0.42            | 0.78                      |                     |                    |                    |

| Immune status before ATG | Total lymphocyte count | 0.007            | 0.21                      | 0.001                | 0.02 (\( \beta = 0.370 \)) | 0.006                | 0.001 (\( \beta = 0.437 \)) |
|                        | CD3+ count           | 0.05            | 0.32                      | 0.06                 | 0.13                | 0.34                |
|                        | CD19+ count          | 0.02            | 0.14 (odds ratio 0.989)   | 0.08                 | 0.04                | 0.34                |
|                        | Leucocyte count      | 0.04            | 0.14                      | 0.007                | 0.97                | 0.04                |
|                        | T-cell reactivity against IA2 and/or GAD | 0.05 | 0.06 (odds ratio 0.101) | 0.01                 | 0.02 (\( \beta = 0.352 \)) | 0.001                | \(<0.001 (\beta = 0.652)\) |
|                        | Presence of autoantibodies pretransplant (yes/no) | 0.70 |                   | 0.86                 | 0.88                | 0.88                |
| Immune status at first infusion | Total lymphocyte count | 0.19 |                   | 0.50                 | 0.46                | 0.46                |
|                        | CD3+ count           | 0.73            |                          | 0.81                 | 0.83                | 0.83                |
|                        | Leucocyte count      | 0.36            |                          | 0.004                | 0.03 (\( \beta = 0.363 \)) | 0.02                | 0.17                |
| Immune status posttransplant | days 1–7 | Median CD3+ count | 0.38 |                   | 0.82                 | 0.76                | 0.76                |
|                        | Median CD19+ count   | 0.04            | 0.54                      | 0.02                 | 0.51                | 0.03                |
|                        | Median CD4-to-CD8 ratio | 0.59 |                   | 0.29                 | 0.39                | 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 \( P < 0.05 \).
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 were 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%) \(>p_{50}\) were seen in four patients who never presented T-cell autoreactivity. 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 biopsy was available from a patient who had received...
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/mm³) with a B-cell count (536/mm³) above the 50th percentile of the recipient population before treatment (259 B cells/mm³); despite >1 year of immune suppressive treatment, the mean B-cell count until the time of the biopsy (412/mm³) was still above this 50th percentile. Interestingly, we did not observe a dominant CD20+ infiltrate in a liver biopsy done in the patient who died 18 weeks posttransplant of cerebral hemorrhage. This patient had lower numbers of total lymphocytes (1,776/mm³) and B-cells (185 cell/mm³) at baseline. Last circulating CD19+ cell count was 30 cells/mm³ 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 autoreactivity. In 1 of these 10 patients with elevated baseline immune status and inadequate graft outcome, a biopsy 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 patho-
genic 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 de-
struction of B-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 autoimmune 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
relation between the presence or titer of autoantibod-
ies and the number of circulating B-cells. On the other
hand, alloantibodies can mediate rejection of organ trans-
plants and islet cell grafts have been found to induce
alloantibody formation (35–37). Alloantibodies after trans-
plantation 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 acti-
vated 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 microenvironment as was
noticed in the biopsy. 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
posttransplant weeks. In a study in nonhuman primates, Liu
et al. (44) demonstrated that B-cell–directed immuno-
therapy promotes long-term islet allograft survival in nonhu-
man 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 ritux-
imab, 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 correla-
tion is confined to recipients receiving lower numbers of
β-cells. The role of autoreactive T-cells in β-cell destruc-
tion 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 trans-
plant 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 auto-
reactivity 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 mecha-
nisms we mention above. In a severe lymphopenic envi-
nronment 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 auto-
reactivity remains present at a later time point, demon-
strating the insufficient irradiation of autoreactive T-cells,
which may be susceptible to proliferation by lymphopenia-
induced homeostatic proliferation (50).

In conclusion, this study correlates circulating charac-
teristics 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 associ-
ated 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 biopsy was
available (>1 year after the transplant) B-cells were pre-
dominantly 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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LYMPHOCYTES IN ISLET TRANSPLANTATION
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