T cell immunity to islets of Langerhans : relevance for immunotherapy and transplantation to cure type 1 diabetes

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CHAPTER 5B

Allograft-specific cytokine profiles associate with clinical outcome after islet cell transplantation

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ABSTRACT

Islet cell transplantation can cure type 1 diabetes, but allograft rejection and recurrent autoimmunity may contribute to decreasing insulin independence over time. In this study we report the association of allograft-specific proliferative and cytokine profiles with clinical outcome.

Peripheral blood mononuclear cells were obtained of 20 islet recipients. Cytokine values in mixed lymphocyte cultures (MLC) were determined using stimulator cells with graft-specific HLA class II. Qualitative and quantitative cytokine profiles were determined before and after islet transplantation, blinded from clinical outcome. Cytotoxic T Lymphocyte precursor (CTLp) assays were performed to determine HLA class I alloreactivity.

Allograft-specific cytokine profiles were skewed toward a Th2 or regulatory (Treg) phenotype after transplantation in insulin-independent, but not in insulin-requiring recipients. IFNγ /IL10 ratio and MLC proliferation decreased after transplantation in insulin-independent recipients ($p = 0.006$ and $p = 0.01$, respectively). Production of the Treg cytokine IL10 inversely correlated with proliferation in alloreactive MLC ($p = 0.008$) and CTLp ($p = 0.005$). Production of IL10 combined with low-MLC reactivity associated significantly with insulin independence.

The significant correlation between allograft-specific cytokine profiles and clinical outcome may reflect the induction of immune regulation in successfully transplanted recipients. Islet donor-specific IL10 production correlates with low alloreactivity and superior islet function.

INTRODUCTION

Transplantation of islets of Langerhans isolated from a donor pancreas is an elegant and minimally invasive treatment for patients with type 1 diabetes suffering from poor glycemic control[1,2]. Islet isolation and transplantation techniques have gradually improved and immunosuppressive regimens have been implemented that increase short-term success[3]. Yet, despite sustained production of C-peptide in a majority of patients, long-term outcome defined by independence from exogenous insulin is disappointing[4]. Most successfully transplanted patients show a gradual decline in graft function, prompting them to resume insulin injection to control glycemia. The reasons for this progressive loss of graft function are still largely unknown, but can conceivably be attributed to the transplanted islets, the immunosuppressive regimen or the immune system of the recipient[5].

Factors that may contribute to graft failure include insufficient graft size[6], islet apoptosis[7,8], absence of revascularization and forced homing into the liver[9]. Immune factors that may be responsible for graft failure include the immediate blood-mediated immune reaction[10], homeostatic expansion of T cells[11], presence of anti-HLA antibodies[12] and signs of recurrent autoimmunity[13]. We previously demonstrated that the cellular autoimmune status prior to transplantation is predictive for transplantation outcome[14]. Counterintuitively, alloreactivity as defined by CTLp did not provide an independent correlate with islet allograft function or survival in our aforementioned study, in spite of clear and frequent changes in CTLp alloreactivity toward the islet donors. Several explanations for this may apply: first, the alloreactive effect could be overshadowed by islet autoimmunity through 'determinant spreading' of the immune response[15]. Second, avidity rather than precursor frequency of alloreactive CTLs may associate with graft survival, similar to studies in kidney transplantation[16]. Third, the immunosuppressive regimen may effectively suppress allospecific CTLs in vivo, rendering their effect nonsignificant. Finally, HLA class II-restricted $CD4^+$ T cells, rather than HLA class I-specific CTLs, may serve as biomarkers of allograft rejection, and perhaps provide immune correlates of success[17]. In accordance, regulatory T cells (Tregs) supposed to be involved in promoting allograft tolerance are believed to be mainly HLA class II-restricted $CD4^+$ T cells[18,19]. Our present in-depth study was performed to assess the latter possibility. We studied the cohort reported previously[6] for associations between T-cell alloreactivity and graft survival, by determining graft-specific cytokine profiles in supernatants of mixed lymphocyte cultures (MLC) and subsequent correlation with cellular alloreactivity and clinical outcome.

MATERIALS AND METHODS

PATIENTS, DONORS AND TRANSPLANTATIONS

Twenty-two patients were analyzed that received islet cell grafts from multiple donors after signing informed consent. Patient, donor and graft characteristics were extensively described earlier[6]. From this reported cohort, one patient was excluded from this study because of pretransplant immunization with graft-specific HLA antibodies. Another case could not be analyzed because of incomplete samples for determination of alloreactivity.

The remaining 20 patients received either one (n = 8) or two (n = 12) islet cell grafts. The decision to inject a second β-cell graft was based on the C-peptide levels and/or variation of fasting glycemia (CV glucose >25%) after the first engraftment[6]. The total number of donors per patient ranged from 2 to 10 (6 median). Graft recipients were long-term type 1 diabetes patients with plasma C-peptide <0.09 ng/mL, large variation in blood glucose levels (Coefficient of variation [CV] ≥25%),% HbA_{1c} >7and one or more chronic diabetes lesions. Exclusion criteria were: body weight >90 kg, active smoking, pregnancy, disturbed liver function tests, history of hepatic disease, presence of HLA antibodies or negative EBV serostatus.

Donor organs were procured from multiple heart-beating donors through the Eurotransplant Foundation (Leiden, The Netherlands) according to medical, ethical and legal guidelines. They were processed at the Beta Cell Bank in Brussels to β-cell-enriched fractions that were cultured for 2–20 days (median 6 days). The methods for enrichment and culture were described earlier[6,20,21]. Briefly, islet cell-enriched fractions were cultured using serum-free Ham's F10 medium/0.5% human albumin/135 mg/dl glucose/2 mM glutamine. After 2–20 days (median 6 days; IQR 3–11 days) the preparations were analyzed for their β-cell number and purity. The final cellular composition of each β-cell graft was determined on samples that were taken just before implantation. The total number of cells in a fraction was calculated on the DNA content compared to the average cellular DNA content measured in sorted single human adult β-cells and duct cells. The number of β-cells was then determined on the basis of the percentage of insulin-positive cells. At transplantation, β-cells were injected into the portal vein of the recipient as previously described[6,21,22].

Immunosuppressive induction therapy consisted of antithymocyte globulin (ATG, Fresenius, Fresenius Hemocare, WA) with a single infusion of 9 mg/kg and subsequently with 3 mg/kg for 6 days except when T-lymphocyte count was under 50/mm^3. Maintenance immunosuppression consisted of tacrolimus (Prograft, Fujisawa/Pharma Logistics, dose according to trough level: 8–10 ng/mL in the first 3 months posttransplantation, 6–8 ng/mL thereafter) and mycophenolate mofetil (MMF, Roche, 2000 mg/day). Patients were regularly followed

up clinically regarding basal plasma C-peptide (glycemia 120–200 mg/dL) and need for exogenous insulin injection.

MIXED LYMPHOCYTE CULTURES

For MLC experiments, cryopreserved PBMC from islet cell recipients that were isolated before and up to six times in the first 18 months after transplantation were used as responder cells. Stimulator PBMC were selected for expression of HLA specific for the islet cell donors (HLA class I for CTLp assays (see below) and HLA class II for MLC). Three to eight different stimulators were used per patient, depending on the number of donors and HLA mismatches.

The 20 recipients were transplanted with islet cell grafts from in total 127 donors. This accounted for a total of 190 HLA–DR mismatches with the recipients; 133 (70%) of these were tested in our MLC and cytokine assays. A median of 90% of donor grafts were covered per patient. For 67% of the donors, all HLA–DR mismatches with the recipient were tested. Experiment characteristics per patient are available online in Supporting Table S1.

Both stimulator and responder cells were thawed, washed and counted. One-way MLC were set up in triplicates in 96 well V-bottomed plates (Costar, Cambridge, MA) in 150 µL RPMI with 2 mMol/L l-glutamine (Gibco, Paisley, Scotland) and 10% pooled human serum. Responder cells (40 000) were incubated with 50 000 irradiated stimulator cells (irradiated at 3000 rad) per well at 37°C/5% CO_2. After 5 days, ^{3}H-thymidine (1.0 µCi per well) was added and 16 h later ^{3}H-thymidine incorporation was measured on a β plate counter. Proliferation in response to phyto-hemagglutinin was used as positive control.

QUANTIFICATION OF CYTOKINE PRODUCTION IN MLC

Production of different cytokines was measured with Luminex technology using a human Th1/Th2 Bio-plex cytokine kit (BioRad, Veenendaal, the Netherlands), including IL2, IL4, IL5, IL10, IL12p70, IL13, GM-CSF, IFNγ and TNFα, according to the manufacturer's protocol. Briefly, antibody-coated cytokine-specific beads were added to 96 well Millipore plates (BioRad). Supernatants taken at day 5 from graft-specific mixed lymphocyte cultures were added for 45 min at room temperature in the dark under 300 Hz shaking, allowing cytokines to bind to the cytokine-specific beads. Subsequently, plates were washed and incubated with biotinylated anti-cytokine detection antibody for 30 min. After washing, streptavidin PE was added for 10 min to bind to the detection antibody. Fluorescence labeling of cytokine-specific beads was analyzed by a double-laser Bio-plex reader (Bio-Rad). Cytokine concentration was determined based on a standard curve included in each plate, using cytokine standards provided by the manufacturer.

CYTOTOXIC T LYMPHOCYTE PRECURSOR FREQUENCY

The CTLp assay has been described in detail previously[14,23]. Briefly, cryopreserved PBMCs from recipients from before and different time points after transplantation were cultured in

a limiting dilution assay (40 000 to 625 cells/well, 24 wells per concentration) with different irradiated stimulator PBMCs expressing HLA class I antigens that are also expressed on the injected islet cell grafts (50 000 cells/well). These stimulator cells were the same as those used in MLC. HLA class I mismatches were covered as described[14]. Cells were cultured for 7 days at 37°C in 96 well round-bottomed plates in RPMI 1640 medium with 3 mM L-glutamine, 20 U/ml IL-2 and 10% pooled human serum. Next, Europium-labeled graft HLA-specific target cells (5 000 cells/well, 4 to 8 different targets) were added to the stimulator/responder combinations for 4 h. Wells were scored positive if the Europium release through target cell lysis exceeded spontaneous release plus 3x SD. Quantification of CTLp frequencies was performed by specifically designed computer software[24].

STATISTICS

Differences in continuous variables were calculated using Mann–Whitney U test or unpaired t-test for nonpaired observations, based on sample distribution. Wilcoxon signed rank test was used for paired observations. Multiple comparison correction was not appropriate for single cytokine analyses because of the strong interrelationship between cytokines. Binary logistic regression analysis was used to analyze differences in qualitative cytokine profiles between insulin-independent and insulin-requiring patients. Differences in dichotomous variables were calculated using chi-square and Fischer exact test. Significance of correlations was analyzed using Spearman's nonparametric correlation. In the case of inverse correlations, inverse values (1/MLC and 1/CTLp) were correlated with IL10 by Spearman's non-parametric correlation on values that were non-zero. Statistical analyses were performed using GraphPad Prism version 4.0 and SPSS version 14.0. $P < 0.05$ was considered significant.

Importantly, all assays and analyses were performed blinded from clinical outcome and correlated after generation and interpretation of the immunological data.

RESULTS

PATIENT CHARACTERISTICS

Twenty patients were analyzed for MHC class I and class II-restricted alloreactivity as well as class II-mediated cytokine production. No differences in baseline transplantation characteristics were observed between patients eventually achieving or not achieving insulin independence (Table 1). Data points from both before and after transplantation were available for analysis in 16 out of 20 patients, allowing paired analysis when applicable; from the other 4 patients only data from after transplantation were available that were included in comparisons between insulin-independent and insulin-requiring patients posttransplantation.

TABLE 1 Clinical and transplant characteristics of patients achieving (n=12) or not achieving (n=8) insulin independence.

Parameter	**Insulin-independent (n=12)**	**Insulin-requiring (n=8)**	**p-value**
Number of transplants (1/2)	6/6	2/6	0.37*
Total injected β-cells (10^6 per kg)	4.3 (3.6-5.6)	3.6 (2.7-4.1)	0.12
No. of donors	6 (4-10)	6 (5-8)	0.85
No. of HLA class II mismatches	9 (6-13)	9 (6-13)	0.85
% mismatches tested	74.3 (57.4-95.8)	81.0 (37.5-85.4)	0.68
% donors tested	95.0 (78.5-100)	86.2 (44.8-100)	0.43
Total ATG dosage (mg/kg)	22.4 (19.8-24.6)	23.5 (21.3-24.4)	0.42
Median Tacrolimus level (ng/ml), 0-12 months	9.00 (8.15-9.08)	7.85 (5.85-8.93)	0.16
Median MMF dosage (mg/day) 0-12 months	2000 (1500-2000)	2000 (1500-2000)	0.85

Data is represented as median + interquartile range. Differences between groups are calculated using Mann-Whitney U test. * Calculated by Fischer exact test.

GRAFT-SPECIFIC CYTOKINE PRODUCTION BEFORE AND AFTER TRANSPLANTATION

Because cytokine production in MLC is a reflection of allograft-specific T-cell responses, quantitative differences in cytokine production in response to islet donor HLA were analyzed in patients who reached insulin independence versus insulin-requiring patients. HLA–DR mismatches between islet donors and recipient were tested using multiple graft-specific stimulator cells per patient on samples from before and multiple time points after transplantation (available online in Supporting Table S1). To allow for comparison, the median value of allograft-specific cytokine production of each patient before and after transplantation was used for statistical analysis (Table 2). In insulin-independent recipients, median graft-specific

TABLE 2 Production (in pg/ml) of graft specific cytokines before and after transplantation, stratified for transplantation outcome. 3 to 8 different graft HLA-specific stimulator cells were used in MLC dependent on the number of HLA mismatches between donor and recipient. From these measurements the median value was used for analysis, from a single timepoint before and from several timepoints in the first 18 months after transplantation.

	Insulin-independent (n=10)				Insulin-requiring (n=6)			
Cytokine	Before	After	p-value	W(rank sum)	Before	After	p-value	W(rank sum)
IL2	117.5 (22.6-76.6)	27.3 (14.8-103.6)	0.006	51.0	113.1 (33.6-208.8)	48.9 (45.1-119.0)	0.44	9.0
IL4	1.2 (0.1-2.4)	0.5 (0.2-1.0)	0.04	41.0	1.1 (0.4-1.6)	0.4 (0.1-1.0)	0.06	19.0
IL5	63.8 (1.9-185.1)	28.2 (5.5-49.1)	0.04	41.0	44.5 (13.4-142.9)	25.3 (0.9-108.7)	0.31	11.0
IL10	2.4 (1.2-12.7)	11.5 (4.0-28.0)	0.03	-43.0	2.9 (1.3-6. 3)	2.3 (1.5-12.9)	0.63	-5.0
IL13	413.3 (21.4-1337.0)	381.9 (2.7-535.0)	0.08	35.0	430.1 (85.5-1501.0)	123.9 (20.1-1219.0)	0.22	13.0
IFNγ	16.0 (4.7-172.1)	35.3 (7.0-74.8)	0.77	7.0	75.7 (13.3-338.0)	33.2 (7.7-122.9)	0.69	5.0

Data is represented as median and interquartile range. Differences between paired observations are calculated using Wilcoxon signed rank test.

IL2, IL4 and IL5 production decreased significantly after transplantation (p = 0.006, p = 0.04 and p = 0.04, respectively), whereas allospecific IL10 production increased (p = 0.03). Apart from a trend for IL4, such conversions were not observed in patients failing to reach insulin-independence. Pretransplant cellular autoreactivity, a predictor for graft failure established earlier, was observed in 4 out of 11 insulin independent and 6 out of 7 insulin requiring patients (p = 0.07, Supporting Table S1). Autoreactive and non-autoreactive patients elicited comparable changes in allograft-specific cytokine production, while pretransplantation alloreactive IL2 production tended to be lower in nonautoreactive patients (p = 0.08).

QUALITATIVE INTERPRETATION OF CYTOKINE PROFILES

Alloreactive T cells can produce multiple cytokines upon recognition of alloantigens and analysis of single cytokines may not provide an appropriate representation of actual immune reactivity. The overall cytokine production pattern may give a more complete overview of the alloresponse. For this purpose patients were attributed graft-specific cytokine profiles based on the type and amount of cytokine production, before and after transplantation. Importantly, profiles were independently determined by three persons, blinded from clinical outcome. They were either marked as dominated by Th1-(dominance of IL2 (>50 pg/mL) and/or IFNγ (>50 pg/mL)), Th0-(no dominant cytokine), Th2-(dominance of IL4 (>10 pg/mL), IL5 (>50 pg/mL) and/or IL13 (>500 pg/mL)) or Treg-cytokines (dominance of IL10 (>50 pg/mL) in the absence of IL2 and/or IL4).

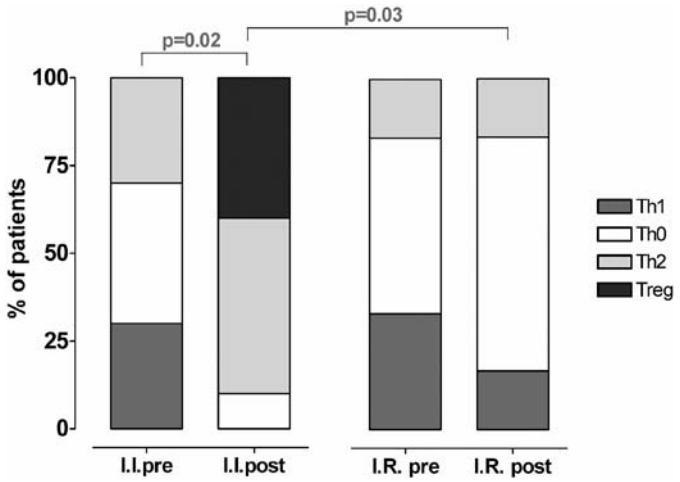

FIGURE 1 Qualitative interpretation of cytokine profiles. Shown are patients with Th1 (dark grey), Th0 (white), Th2 (light grey) and Treg (black) cytokine profiles, stratified for transplantation outcome. Insulin-independent recipients (I.I., n = 10) experience a significant change in cytokine profile (p = 0.02, Wilcoxon matched pairs test) after transplantation. Their profile after transplantation is significantly different from insulin-requiring recipients (I.R., n = 6) (p = 0.03, binary logistic regression). Cytokine profiles were interpreted blinded from clinical outcome.

To compare cytokine patterns prior to and after transplantation, the 16 patients from whom all data points were available were included in this analysis. Allospecific cytokine profiles prior to transplantation were similar for insulin-independent and nonindependent patients (Figure 1). After transplantation, patients who became insulin independent showed a significant skewing toward Th2 and Treg cytokine profiles in response to alloantigen ($p = 0.02$), whereas transplanted patients still requiring insulin did not.

Cytokine profiles after transplantation differed significantly between insulin-independent and insulin-requiring patients ($p = 0.03$). This difference remained significant ($p = 0.03$) after adding those patients to the groups from whom only posttransplant profiles were available (two in both groups, all four with Th0 profile).

QUANTITATIVE INTERPRETATION OF CYTOKINE PRODUCTION AND MLC

The IFNγ/IL10 ratio has previously been reported as an important measure of immune reactivity in type 1 diabetes and transplantation[25,26]. Therefore, this alternative quantitative assessment was performed comparing this ratio before and after transplantation, demonstrating a similar pattern in changes. The median IFNγ /IL10 ratio decreased significantly after islet transplantation in recipients reaching insulin independence, ($p = 0.006$, $W = 51.0$), while insulin-requiring recipients displayed decreases as well as stable or even increased ratios ($p = 0.56$, $W = 7.0$) (Supporting Figure S1, available online). IFNγ and IL10 genotype polymorphisms were not different between the groups (data not shown). Similarly, another measure of $CD4^+$ T-cell-mediated alloreactivity, the median MLC stimulation index (SI), also decreased significantly in insulin-independent ($p = 0.01$, $W = 47.0$) but did not reach significance in insulin-requiring recipients ($p = 0.44$, $W = 7.0$).

CORRELATIONS BETWEEN CYTOKINE PRODUCTION AND CELLULAR ALLOREACTIVITY

To further understand the mechanisms underlying the relation between graft-specific cytokine production and cellular alloreactivity, production of IL2, IFNγ and IL10 were correlated for each posttransplant MLC with graft specific $CD4^+$ T-cell proliferation (MLC SI). These analyses were performed using data from all 20 patients. The production of IL2 was positively correlated with MLC SI ($r = 0.46$, $p < 0.0001$, Figure 2A). No such correlation was observed between IFNγ and MLC SI (not shown). In contrast, the production of IL10 was inversely correlated with MLC SI ($r = 0.23$, $p = 0.008$, Figure 2B). An inverse correlation was also observed between CD4-mediated IL10 production and cytotoxic T lymphocyte precursor frequency ($r = 0.25$, $p = 0.005$, data not shown).

Production of IL10 larger than 50 pg/mL was seen in 19 assays, of which 16 were observed in experiments with MLC responses below the median (SI = 8.6) and only three in experiments with MLC responses above the median (Figure 2B, $p = 0.001$ by Fischer exact test). High IL10 combined with low MLC responses was observed more often in insulin-independent

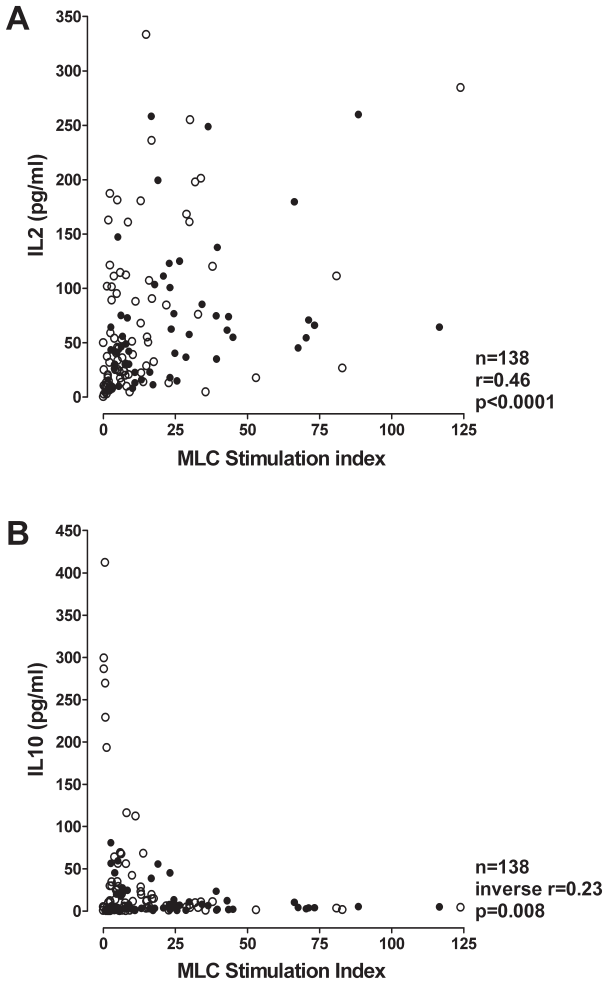

FIGURE 2 Correlation of graft-specific cytokine production with cellular alloreactivity in PBMC from patients reaching insulin independence (open circles) and recipients who still required insulin (black circles). (A) MLC SI per experiment is significantly correlated with production of IL2. (B) Correlation between MLC SI and production of IL10: high proliferation is only observed in the absence of IL10 (lower right), whereas high IL10 production is only observed in patients with low proliferation (upper left). A significant inverse correlation exists between MLC SI and IL10 (n = 138, r = 0.23, p = 0.008). Median MLC SI value = 8.6.

cases than insulin-requiring cases (13/85 MLCs, 15% vs. 3/53 MLCs, 6%). In contrast, MLC responses above the median with low IL10 production was observed less frequently in insulin-independent cases than insulin-requiring cases (30/85 MLCs, 35% vs. 36/66 MLCs, 68%). The difference between these proportions was statistically significant (p = 0.003 by Fischer exact test). Consequently, alloreactivity characterized by production of IL10 associates with insulin independence, while alloreactivity characterized by high proliferation associates with continued insulin requirement.

DISCUSSION

Transplantation of isolated islet of Langerhans cells to cure type 1 diabetes has been implemented with increasing success in recent years[2]. The definition and implementation of standardized islet cell grafts warrants identification and if possible adaptation of immune factors impairing as well as promoting graft survival[1,5]. We previously identified the detrimental consequences for clinical outcome of cellular autoreactivity before and after islet transplantation, whereas alloreactive CTLs were not informative. We here report distinct associations of other alloimmune end points with favorable transplantation outcome: skewing of both qualitative and quantitative cytokine profiles as well as decreasing $CD4^+$ T-cell-mediated proliferation are associated with insulin independence. Production of IL10 was inversely correlated with proliferative alloreactivity against the islet donors and was observed mainly in insulin-independent recipients. Acknowledging that transplantation success is not limited to independence from insulin injections, this criterion has been used previously to define correlates of graft function[6,12,13].

We report on a relatively large and extensively studied cohort of islet cell transplantation recipients. Despite a large number of alloantigens per transplantation resulting from the number of islet donors per implant, we succeeded in testing the vast majority of HLA mismatches between donors and recipients for graft specific immune responses. Earlier analysis of HLA class I-related CTLp frequency, a predictor for graft failure in other transplant settings[16,27–29], did not reveal a significant association with outcome in this patient group[14]. This may be due to the presence of autoimmunity, the immunosuppressive protocol or the difference between high and low avidity of CTLs, but our present results suggest an additional role for mainly HLA class II-related alloreactive cells.

Cytokine profiles before transplantation were not different between insulin-independent and insulin-requiring recipients, implying comparable immune reactivity in immunocompetent type 1 diabetic candidate recipients naïve for the allograft and precluding prediction of outcome on basis of baseline alloreactivity. Yet, after islet implantation, insulin independence was associated with a decrease in proliferation and IL2 production. Concomitant skewing of cytokine profiles toward a Th2/Treg phenotype in insulin-independent patients, exemplified by a significant increase in IL10 production, provides support for the proposed functional role of IL10-producing cells in protective immunity in clinical transplantation[30–32].

Group size differed between insulin-independent and insulin-requiring patients. Although this may partly explain why significant changes were observed in insulin-independent and not in insulin-requiring patients (especially regarding the proliferation data), this does not take aside that the reduction in MLC was more pronounced and with higher rank sum values in patients reaching insulin independence. This was corroborated by profound changes in cytokine profiles that were rare in insulin-requiring patients.

IL10 is generally considered one of the major cytokines associated with active suppression of immune reactivity[33]. The immunoregulatory potential of either naturally occurring or antigen-induced IL10-producing Tregs has been shown in the context of autoimmunity[34,35] and transplantation[26,36]. The inverse correlation of IL10 with $CD4^+$ and $CD8^+$ T-cell-mediated alloreactivity, combined with the increase in IL10 if insulin independence is reached, would suggest an important role for this cytokine in the regulation of islet allograft rejection, as observed before with antigen-induced Tregs (Tr1 cells)[36].

Other inhibitory pathways, mediated by, for example, TGFβ, IDO or 'negative' costimulation, may contribute to immunological tolerance[19,37,38]. In addition, peripheral tolerance may be achieved by other mechanisms such as anergy, ignorance or apoptosis[39]. Recently, a role was suggested for soluble IL2-receptor in regulating alloreactivity after islet transplantation[8]. Induction of *auto*antigen-specific tolerance must be considered an additional important immunological prerequisite for lasting islet allograft protection in a background of type 1 diabetes. Interestingly, a recent report on expansion of autoreactive memory T cells after islet transplantation suggests a role for IL7 and IL15 in recurrence of autoimmunity[11]. The memory T-cell data corroborate with earlier observations in our cohort[14] that suggested insufficient suppression of preexistent autoimmunity after islet transplantation, associated with worse graft survival. This study focused on primary alloreactivity rather than preexistent autoimmunity. Unfortunately, expansion of alloreactive memory T cells nor production of cytokines IL7 and IL15 was studied in this cohort, but the differences in cytokine production between successful and less successful transplants emerging after transplantation confirms the relevance of such analyses in the future.

In conclusion, we report the identification of immune correlates for clinical outcome of islet transplantation. T-cell proliferation and cytokine profiles were defined that correlate with need for exogenous insulin, representing biomarkers for immune regulation versus loss of function in clinical transplantation. Discovery and validation of these and other immune correlates of clinical outcome may prove valuable in the continuous effort to improve islet allograft survival and function.

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