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CHAPTER 5B

PREDICTIVE FACTORS OF ALLOSENSITIZATION FOLLOWING IMMUNOSUPPRESSANT WITHDRAWAL IN RECIPIENTS OF LONG-TERM CULTURED ISLET CELL GRAFTS


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Chapter 5B

Chapter 5B Consequences of Reducing Immunosuppressive Burden in Islet Transplantation

ABSTRACT

Islet transplantation has been reported to induce allosensitization in the majority of type 1 diabetic recipients of fresh or shortly incubated islet grafts prepared from one to three donors.

We examined the appearance of human leukocyte antigen (HLA) antibodies after withdrawal of immunosuppressants in 35 type 1 diabetic recipients of islet cell grafts prepared from a median of 6 donors (range, 2-11), cultured for longer periods, and characterized for their cellular composition. Immunosuppression consisted of antithymocyte globulin induction followed by mycophenolate mofetil plus calcineurin inhibitors (n=28), with 7 also receiving steroids) or sirolimus with (n=3) or without calcineurin inhibitors (n=4). Both the complement-dependent cytotoxicity (CDC) assay (class I) and the solid-phase flow-based Luminex method (class I and II) were used to identify HLA antibodies.

Immunosuppressant withdrawal resulted in CDC positivity for class I antibodies in only 6% of patients. However, the majority became positive for class I antibodies (72%) or class II antibodies (72%) in the Luminex assay; positivity was not correlated to a higher number of donors or HLA mismatches, but with a lower beta-cell purity; use of steroids reduced de novo positivity for Luminex class I antibodies.

Allosensitization to cultured human islet cell grafts was low when assessed by CDC assay but high in Luminex. No correlation was found with the number of donors but risk was higher for grafts with lower beta-cell purity.

INTRODUCTION

Over the last decade, the success rate of intraportal human islet allotransplantation in nonuremic type 1 diabetic patients has significantly improved, but graft function cannot be maintained in the majority of recipients [90,93,113,151,245,256,257, 300]. The cessation of immunosuppression in recipients with failing implants has been found to result in development of human leukocyte antigen (HLA) antibodies [45,46,193]. Allosensitization was noticed in most patients in the Edmonton protocol, who received fresh or shortly incubated islet grafts prepared from one to three donors under immune therapy consisting of induction with daclizumab at each islet graft injection and maintenance therapy with sirolimus with low-dose tacrolimus. A recent report from the Collaborative Islet Transplant Registry including 303 islet graft recipients also found a significant increase in HLA class I sensitization [193]. The present study examines whether this is also the case in our protocol in which long-term cultured islet cell grafts were prepared from 2 to 11 donors and characterized and standardized for their cellular composition. Our immunosuppression protocol consisted of anti-thymocyte globulin (ATG) induction followed by mycophenolate mofetil (MMF) and calcineurin inhibitors (CNI; n=28) or sirolimus with (n=3) or without (n=4) CNI. Both the standard complement-dependent cytotoxicity (CDC) assay and a solid-phase flow-based method (Luminex) were used to detect and identify HLA antibodies. Their appearance was analyzed against characteristics of patients, grafts, and immunosuppression.

MATERIALS AND METHODS

RECIPIENT, DONOR, AND GRAFT CHARACTERISTICS

Thirty-five type 1 diabetic patients received intraportal islet graft between 1999 and 2007 as described previously [135,163,181]. Patient and graft characteristics are shown in Table 5.2. Patients with plasma creatinine >2 mg/dl, albuminuria ≥1000 mg/24 hours, or histories of (nondiabetic) renal disease were excluded. Nine of 14 female patients had been pregnant before transplantation. Blood transfusion was documented before transplantation in four female patients and one male subject.

Six patients (three males and three females) received leukocyte-depleted blood transfusions for anemia related to immunosuppressive treatment. No platelet transfusions were performed.

Pancreases from brain-dead heart-beating donors were procured by hospitals affiliated with the Eurotransplant Foundation (Leiden, The Netherlands) according to local medical, legal, and ethical guidelines for organ donation. The Beta Cell Bank in Brussels, a recognized and monitored transplant tissue bank,

| Patients | 35 |
| Age at transplantation (years) | 40 (33-47) |
| Gender (M/F) | 21/14 |
| Duration of diabetes (years) | 23 (16-30) |
| Grafs | 1 5 (14%) |
| | 2 26 (74%) |
| | 3 4 (11%) |
| Total number of donors | 6 (5-9) |
| Total number of beta cells (million/kg body weight) | 4.5 (3.1-5.6) |

Table 5.2. Patient and graft characteristics.
prepared cultured beta-cell suspensions according to standardized protocols; their cellular composition was determined by immunocytochemistry and electron microscopy [135,137,156]. This methodology allows defining the grafts on the basis of their beta-cell number and purity instead of IEQ [135]. Median donor culture time was 138 hours (interquartile range (IQR), 97-169 hours). For practical reasons, no HLA matching was performed and unacceptable antigens were not listed.

IMMUNOSUPPRESSION
All 35 recipients received ATG induction therapy (Fresenius, HemoCare, Redmond, WA) at the time of the first islet cell implant [135]. No additional ATG was administered at second or third islet cell infusion. The median time interval between the first and second implants was 12 weeks (IQR, 10-14) and 34 weeks (IQR, 31-38) between the first and third transplants. At the time of last transplant, CD3+ T lymphocytes were significantly lower when compared with baseline values (median, 275 cells/mm³; IQR, 195-515 vs. 1362 cells/mm³; IQR, 1126-1805, respectively; p<0.0001, Wilcoxon signed rank test). At the start, maintenance immunosuppression consisted of MMF plus CNI in 28 patients (9 cyclosporine: before 2001 and 19 tacrolimus: after 2001); 7 of 9 patients on MMF plus cyclosporine also received methylprednisolone starting at 40 mg per day and tapering over a median period of 22 weeks. Three patients received sirolimus plus tacrolimus, and four received only sirolimus. This immunosuppressive regimen was chosen for a study comparing sirolimus monotherapy with sirolimus-tacrolimus combination as we have previously published [96]. Sirolimus-related side effects led us to replace this compound by MMF in three of these patients.

WITHDRAWAL OF IMMUNOSUPPRESSION
Immunosuppression was stopped after a median of 83 weeks (IQR, 47-170 weeks). In 27 of 35 (77%), the reason was a failing implant (stimulated plasma C-peptide <0.09 ng/ml) and recurrence of hypoglycemic episodes; in seven, the cause was drug-related side effects in the presence of metabolically insignificant C-peptide levels (<0.20 ng/ml); one patient decided to withdraw immunosuppression for personal reasons despite C-peptide levels >0.5 ng/ml.

HLA ANTIBODY DETECTION AND IDENTIFICATION
Pretransplantation and posttransplantation sera were retrospectively analysed for the presence of HLA antibodies using solid-phase flow-based assays (Luminex, Austin, TX). All patients were sampled using the qualitative bead-based immunoassay LIFECODES LifeScreen Deluxe Kit (Gen-Probe, San Diego, CA) followed by quantitative sampling using LIFECODES Class I ID kit (Gen-Probe) and LIFECODES Class II IDv2 Kit (Gen-Probe) to determine PRA and antibody specificity for class I and II, respectively, in case of positive response on qualitative analysis. The beads in these assays have HLA-A, HLA-B, and HLA-C or HLA-DRB1 and HLA-DQB1 antigens represented. We followed the manufacturer’s instructions for staining and acquiring. Beads were analyzed on a Luminex 100 IS 2.3. Mean fluorescence intensity (MFI) >1000 was used a cutoff for positivity and high MFI antibodies were defined as MFI >5000. Broad sensitization was defined as PRA ≥50% [45].

In 11 patients, specificity for HLA class I could not be reliably determined without the use of single antigen beads, which was not performed. This was also the case in two patients for HLA class II. Samples were analysed at four time points: before transplantation, 6 weeks after last transplantation under full-dose immunosuppression (median; IQR, 6-6), and early (median, 7 weeks; IQR, 5-9) and late (median, 38 weeks; IQR, 31-59) after complete withdrawal.

Pretransplantation samples and the last available samples after discontinuation of immunosuppressive drugs were also tested for CDC if positivity for class I HLA antibodies was demonstrated on Luminex. Patient sera were screened for anti-HLA class I antibodies by CDC using a panel of lymphocytes from 60 donors who were typed for HLA-A, HLA-B, and HLA-C. The CDC protocol used was the standard method as described previously [282].

HLA TYPING AND MISMATCHES
Recipient and donor HLA typing was performed in the Eurotransplant Reference Laboratory (Leiden, The Netherlands). The cumulative number of HLA class I mismatches between recipient and the donors was counted. Repeated mismatches with several donors were counted only once. HLA class I differences were further calculated on antibody binding site level using HLAMatchmaker [71]. This program calculates differences of three sequential amino acid residues between HLA molecules expressed on antibody-accessible sites in the groove. If high-resolution HLA typing was unavailable, the most prevalent allele was assumed.

STATISTICAL ANALYSIS
Univariate analysis was performed using Mann-Whitney U test for continuous variables and Fisher’s exact test for dichotomous variables to relate recipient and graft characteristics to HLA antibody prevalence. To determine independent predictor ability of the variables associated with HLA antibody development, we used forward binary logistic regression analysis including all parameters with p<0.05. All p-values reported are two-sided. All analysis were performed using SPSS statistics 19.0.

RESULTS
CDC ASSAY TO DETECT HLA CLASS I ANTIBODIES
Using the CDC assay, 2 of 35 patients presented lymphocytotoxic antibodies against HLA class I before transplantation. One patient had been pregnant, with no history of blood transfusion; her panel-reactive antibody (PRA) level was 3% at start and 5% after withdrawal of immunosuppression with no specificity detected. The other patient tested positive before transplantation (PRA 3%, nonspecific) but was negative at the end of the study.
Two of 33 patients had de novo CDC HLA class I antibodies after withdrawal of immunosuppression (Figure 5.5; 6%)[a] with PRA levels of 5.2% (nonspecific) and 16% (specific), respectively. Both were females with previous pregnancy but without previous blood transfusion.

**SOLID-PHASE ASSAY TO DETECT HLA CLASS I AND II ANTIBODIES**

Using the solid-phase Luminex assay, 32 patients were negative for HLA class I antibodies before transplantation. Twenty-three of these (72%) developed Luminex antibodies after transplantation (Figure 5.5), mostly after withdrawal of immunosuppression (Table 5.3). Ten exhibited high MFI, and 15 had a PRA ≥50%. De novo Luminex HLA class II antibodies were also detected in 72% of patients, but the prevalence of high MFI was lower (Table 5.3). Of the 30 patients who were negative for both class I and II before transplantation, 5 remained negative for both after stopping immunosuppression, whereas 18 became double positive, 3 only for class I and 4 only for class II. Among female patients with previous pregnancy, PRA levels for class I were significantly higher and high MFI antibodies were more frequent when compared with nonpregnant women or male recipients. A similar but nonsignificant difference was noted for class II (p≤0.05 versus on immunosuppression).

Three of 35 patients were positive for HLA class antibodies in the Luminex pretransplantation. Each had antibodies with MFI >5000 and PRA ≥50%, which were maintained under immunosuppression and after withdrawal of immunosuppression.

Table 5.3. Overview of the prevalence of de novo HLA antibodies using solid-phase assays (Luminex). *De novo CDC antibodies for class I were present in two subjects belonging to the annotated groups. PRA>50% was only present in one of these two. **p<0.05 versus pretransplantation. ***p<0.005 versus pretransplantation. ****p<0.005 versus on immunosuppression.

![Figure 5.5. De novo HLA antibodies in patients withdrawing immunosuppression. Before transplantation 2 of 35 patients had cytotoxic antibodies and 3 of 35 had antibodies on Luminex.](image)

![Figure 5.6. Peak PRA levels for class I antibodies on Luminex according to gender and previous pregnancy. All patients tested negative for both Luminex and CDC at baseline. Left: Peak PRA with threshold for positivity MFI>1000. Right: percentage of high titer class I antibodies (MFI>5000) according to gender and previous pregnancy. *p<0.05, **p<0.005.](image)

**CORRELATIONS WITH ABSENCE OF HLA SENSITIZATION**

The observation that a number of HLA antibodynegative recipients did not develop Luminex class I or II antibodies led us to examine whether this correlated with their characteristics and/or transplant variables. We thus compared the 9 persistently HLA class I antibody negatives and the 23 that became HLA class I antibody positive after transplantation and omission of immunosuppression (Table 5.4). Similarly, the group of 9 class II antibody negatives was compared with the 23 that became HLA class II antibody positive (Table 5.5).

Absence of HLA class I sensitization was associated with steroid inclusion in the immunosuppressive treatment and with a higher beta-cell purity and lower contamination by exocrine and nongranulated duct cells (Table 5.4). Importantly, there was no association with a lower number of donor organs. The number of class I mismatches did not differ between groups, nor did we find any differences in potential antibody binding site frequencies by calculating amino acid triplet mismatches using HLAMatchmaker (Table 5.4). Multivariate analysis indicated that the use of steroids was independently associated with lower risk for HLA class I sensitization (odds ratio, 0.023; 95% confidence interval, 0.002-0.260; p=0.002). Interestingly, the only subject who became CDC class I positive with specificity had not been treated with steroids and had received a graft of low beta-cell purity (21%) and high contamination by exocrine (7%) and nongranulated cells (61%).

Absence of class II sensitization was also correlated with grafts with higher beta-cell purity and lower contamination by exocrine cells (Table 5.5). Multivariate analysis retained beta-cell purity as independent predictor of lower risk for HLA class II sensitization (odds ratio, 0.913; 95% confidence interval, 0.841-0.991; p=0.029).

One of them was also HLA class II antibodies positive maintaining similar characteristics before and after transplantation. The two others were HLA class II positive before transplantation with MFI <5000 and PRA <50%. Under immunosuppression, they had the same characteristics, but after withdrawal of immunosuppression one of them became MFI >5000 and PRA ≥50% positive. Retrospectively, the baseline presence of donorspecific antibodies (DSA) on Luminex was noted in four patients. All of these achieved C-peptide positivity, but none reached insulin independence.

![Chapter 5B](image)
Consequences of Reducing Immunosuppressive Burden in Islet Transplantation

<table>
<thead>
<tr>
<th>Patient</th>
<th>HLA class I antibody negative (n=9)</th>
<th>HLA class I antibody positive (n=23)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age at transplantation (years)</td>
<td>36 (32-46)</td>
<td>40 (33-48)</td>
<td>1.00</td>
</tr>
<tr>
<td>Gender</td>
<td>8M/1F</td>
<td>13M/10F</td>
<td>0.12</td>
</tr>
<tr>
<td>Blood transfusion post-transplantation (n)</td>
<td>0</td>
<td>5</td>
<td>0.29</td>
</tr>
</tbody>
</table>

**Immunosuppression**

| ATG dose (mg/kg) | 21.3 (20.8-27.2) | 23.6 (21.8-24.0) | 0.48 |
| Steroids (n) | 6 | 1 | 0.001* |
| Mycophenolate mofetil (n) | 6 | 19 | 0.37 |
| Calcineurin inhibitor (n) | 8 | 20 | 0.69 |
| Sirolimus (n) | 3 | 4 | 0.30 |

**Graft**

| Donors (n) | 8 (5-10) | 6 (5-9) | 0.32 |
| Class I mismatches (n) | 13 (9-17) | 12 (9-13) | 0.48 |
| Class I epitope mismatches (n) | 40 (29-46) | 38 (35-45) | 0.98 |
| Total number of beta cells (million/kg body weight) | 2.1 (1.3-5.8) | 4.6 (3.4-6.3) | 0.13 |

**Composition**

| Beta cells (%) | 49 (31-60) | 32 (26-37) | 0.02 |
| Exocrine cells (%) | 0 (0-1) | 2 (1-5) | 0.008 |
| Non-granulated cells (%) | 31 (16-52) | 48 (41-52) | 0.04 |
| Dead cells (%) | 8 (6-11) | 6 (7-11) | 0.77 |

**Table 5.4** Graft and patient characteristics in relation to the prevalence of HLA class I antibodies after immunosuppression withdrawal in subjects who were HLA class I antibody negative (Luminex) at baseline. *Multivariate analysis with inclusion criteria p<0.05 retained steroid administration (odds ratio 0.023; 95% confidence interval 0.002-0.260; p=0.002). Independent predictor ability was assessed by forward binary logistic regression analysis. Data are median (interquartile range). Mann-Whitney U test was used for continuous variables and Fisher’s Exact test for dichotomous variables.

The median donor culture time in recipients without class II sensitization was significantly longer (185 hours; IQR, 127-278) when compared with sensitized subjects (136 hours; IQR, 93-160; p=0.019). This difference was not observed for class I sensitization (141 hours; IQR, 132-256 vs. 136 hours; IQR, 93-160; p=0.019). The total number of HLA class I antibodies detected in 11 of 14 (79%) patients for class I. The number of DSA per patient ranged from 1 to 5 (median, 2), whereas the number of non-DSA ranged from 1 to 6 (median, 3). Within the 14 patients, the total number of HLA class I antibodies identified on Luminex was 67, of which 27 (40%) were DSA. Antibody identification was performed in all 23 patients with de novo class II antibodies. Class II DSA were detected in 18 of 23 (78%) patients. DSA were lower under immunosuppression for both class I (n=3, 13%) and class II (n=5, 22%).

**DISCUSSION**

In human islet allotransplantation, HLA matching is not routinely performed because of the scarcity of donors. Within our transplant program, beta-cell grafts are prepared from up to 11 donors, raising the question whether the wide range of unmatched allogeneic antigens increases the risk of allosensitization, especially when immunosuppression is stopped. We therefore retrospectively analyzed data from 35 islet cell recipients under our protocol. Grafts had been prepared from
long-term cultured islet cell isolates and characterized for their cellular composition. Immune therapy consisted of ATG induction and, for most patients, maintenance doses of a CNI plus MMF; a subgroup also received steroids during 22 weeks after ATG. The appearance of donor-specific lymphocytotoxic antibodies in islet cell recipients could form an obstacle if they would require an organ transplant later in life [53,54,143,205,280]. In our series of patients, immunosuppression withdrawal induced positivity for CDC HLA class I antibodies in only 6%, which is markedly lower than the 58% in the study of Cardani et al [46]. The reason for this difference is uncertain. Whereas they used the Edmonton protocol, we used an ATG-based regimen, which may have contributed to a lower number of CDC-positive patients. Furthermore, analysis of our data suggests that use of cultured islet cell grafts with higher alpha-cell purity might be responsible for a lower alloreactivity in CDC.

Interestingly, the prevalence of lymphocytotoxic antibodies in our study is also significantly lower than that after loss of whole-organ transplants [20,75,106,162]. Graft rejection is considered to be an important risk factor for allosensitization in whole-organ transplantation; its impact cannot yet be determined in islet cell recipients where the process cannot be assessed in histopathologic sections. However, according to plasma C-peptide levels, islet cell implants appear to progressively deteriorate rather than being acutely rejected. This might be in part related to the low number of donor endothelial cells, which are known to be a major target for alloantibodies [55]. A lower prevalence of CDC positivity may thus be related to the composition of cell grafts and reflect the associated mechanism of graft loss.

Our study confirms previous observations by others showing de novo HLA antibody formation detected by Luminex after islet transplantation with a further increase after withdrawal of immunosuppression [45,46,193]. We detected de novo class I and II antibodies in 19% and 25% of immunosuppression recipients and in 72% after immunosuppression withdrawal, respectively. This is in line with a recent report in renal graft recipients where solid-phase HLA antibodies were found in 17% at the time of graft loss but in significantly higher proportions when immunosuppression was withdrawn [251]. The significance of Luminex HLA class I and II antibodies for short- and long-term function of allotransplants is still a matter of debate and falls outside the scope of this discussion [52]. Their presence is considered a risk when MFI is high, leading to longer waiting times with broader sensitization. HLA antibodies with high MFI are often associated with CDC positivity. However, in our study, we did not see such a correlation. High MFI antibodies were detected in 10 of 32 patients for class I, but only 2 of these patients demonstrated CDC positivity. This suggests that the majority of antibodies we detected lack the capacity to bind to the antibody isotype or low affinity for the target antigen. Future studies assessing antibody isotype and affinity may provide additional insight into the clinical relevance of these high MFI, CDC-negative antibodies. During the period of immunosuppression, high MFI (>5000) and broad sensitization (PRA ≥50%) were rare. After immunosuppression withdrawal, one of three patients developed high MFI antibodies and one of two presented a broad sensitization. Scornik and Kriesche performed a study after renal graft failure demonstrating high MFI in 64% of patients, which is considerably higher than in our cohort [251]. As mentioned earlier for CDC antibodies, the mechanism of graft loss may determine the appearance and characteristics of HLA antibodies developing after graft failure and immunosuppression withdrawal.

Most of our patients who developed Luminex HLA antibodies had DSA. However, 60% of all HLA antibodies detected were not specific for the donors; their origin is unknown. Among women, non-DSA was slightly more frequent than among men (75% vs. 53%; p=0.11). Interestingly, female patients with previous pregnancy demonstrated significantly higher PRA values for class I after immunosuppression withdrawal and all these patients had high class I MFI antibodies. This was in sharp contrast with nonpregnant females and male graft recipients. These findings are in line with reports of increased allosensitization risk after blood transfusions in women with past pregnancies. In those cases, cytotoxic antibodies can be found in up to 40% of female patients [252]. In our cohort, six females with de novo class I antibodies on Luminex had previous pregnancy, 2 (33%) of which developed CDC antibodies. A recent report from Honger et al. detected HLA antibodies on Luminex after pregnancy in more than 45% of women [119]. Although such circulating antibodies may disappear, memory B lymphocytes remain present. Antibodies to paternal HLA often reappear after kidney transplantation in case of rejection either due to a bystander response during an inflammatory reaction or due to sharing of antibody epitopes between donor and child. Because sharing of antibody epitopes between donor HLA and paternal HLA of the child is very likely considering our multiple donor approach, we suggest that previous pregnancy may have contributed to the development of non-DSA in women. Thus, previous pregnancy may increase the risk of the development of cytotoxic antibodies, broad sensitization, and high MFI HLA antibodies after islet cell transplantation. Other factors that contribute to the development of non-DSA remain to be identified. It is not inconceivable that dying donor cells or injury to their cell wall may provide antigens that contribute to the development of non-DSA. Because islet cell grafts are injected directly into a blood vessel, exposure to the immune system may differ from other organ transplants. Finally, the clinical relevance of non-DSA toward a future transplantation remains unknown and is an important subject for further studies.

We examined whether characteristics of patients, immunosuppression, and grafts might represent risk factors for Luminex HLA antibody formation. In this small series of patients, use of methylprednisolone was associated with a reduced development of class I antibodies: only one of seven of these patients developed HLA antibody. This is in line with observations by the Geneva group who did not see allosensitization in islet after kidney patients receiving lowdose steroid treatment [81]. Other factors that were significant were the use of preparations that contained a higher beta-cell purity and lower contamination by exocrine and nongranulated cells. In contrast to a common opinion, the number of donors or the number of HLA mismatches was not associated with increased of Luminex HLA antibody formation. This finding was confirmed after amore detailed comparison using HLAMatchmaker to calculate amino acid triplet differences. Interestingly, these results are in contrast with previous studies in renal graft recipients where the number of triplet mismatches strongly correlated with the development of DSA and CDC antibodies. Risk associated
with triplet mismatches would be expected to be more specific, because it takes partial overlap of the donor HLA molecule with the recipient HLA into account. A possible reason for not finding a relationship in our cohort is the high number of HLA and triplet mismatches in all patients, which precludes any additional quantitative relationship. Indeed, the strongest relationship between triplet mismatching and antibody production was found in a well HLA-matched cohort of renal transplant recipients [61]. Another possible explanation would be that islets have a different level of immunogenicity compared with kidney transplant.

Our results on class I sensitization are in line with a recent Collaborative Islet Transplant Registry report that could not observe increased risk of sensitization with an increased number of mismatches [193].

De novo formation of class II antibodies was significantly higher for islet cell grafts with lower beta-cell purity. This observation is in line with absence of class II antigens on endocrine islet cells and their presence and cytokine-induced up-regulation in duct cells [206]. Human duct cells may contribute to aggravation of the immune response via production of inflammatory cytokines able to activate dendritic cells [182]. Also, transplants in rats have shown that contamination of the graft with nonendocrine tissue increases its immunogenicity and accelerate graft rejection [128, 215]. Our findings thus support the view that lower contamination of islet grafts with nonendocrine cells, and thus increased beta-cell purity, is an important quality-control criterion in islet cell transplantation. Antigen-presenting cells (APC) such as donor-derived dendritic cells are another potential source of class II antigens.

It has been demonstrated that APCs are present in islet cell grafts, mostly with a mature phenotype. They may therefore contribute to increased immunogenicity of the graft and a subsequent increase in the risk of allosensitization [85]. At present, prolonged culture of human islet cell preparations is the only procedure to allow standardization of the graft composition and increase graft purity, which implies a subsequent decrease in nonendocrine tissue such as exocrine cells, nongranulated cells, damaged cells, and APCs [85, 137, 156]. Indeed, we found that median donor culture time in recipients without class II sensitization was significantly longer when compared with sensitized subjects. Although prolonged culture may relate to a lower risk for class II sensitization via one of these possible mechanisms, it also leads to lower isolation yield in terms of numbers of islet cells that can be transplanted, subsequently increasing the need for multiple donors. It is therefore an important finding that, despite this inherent consequence of cultured grafts, the higher number of donors did not associate with increased risk for allo-sensitization.

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In conclusion, we did not observe a major increase in lymphocytotoxic HLA antibodies in patients receiving longterm cultured islet cell grafts prepared from a large number of donors and implanted under our immunosuppression protocol. However, we identified a high prevalence of newly formed solid-phase detected HLA antibodies after withdrawal of immunosuppressants. Patients willing to undergo islet transplantation should be informed about the significance of these antibodies. Also, renal function needs to be assessed critically before transplantation because the observed sensitization may make it difficult to find an adequately matched kidney if the renal function would deteriorate. If such deterioration occurs, which we did not observe in our study, it might be considered to maintain low-dose immunosuppression to prevent the development of HLA antibodies. However, we should be aware that long-term treatment with tacrolimus might impair renal function and its continuation is therefore not preferable. MMF may be a better alternative, although the risks associated with longterm immunosuppression should always be inferior to the potential benefit for a pending future graft. Although these precautions are relevant for all islet cell graft candidates, our data illustrate that additional attention should be paid to women with previous pregnancy. Diabetic patients with decreased renal function or proteinuria should not be considered for islet transplantation but listed for a combined kidney-pancreas transplant if no cardiovascular or other contraindications. Finally, long-term cultured islet cell grafts, despite their need for a higher number of donors, may be associated with decreased allo-sensitization risk through standardization of the graft composition and minimizing the contamination of the graft with nonendocrine cells.