Islet Transplantation with Alemtuzumab Induction and Calcineurin-Free Maintenance Immunosuppression
Results in Improved Short- and Long-Term Outcomes

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Background. Only a minority of islet transplant recipients maintain insulin independence at 5 years under the Edmonton protocol of immunosuppression. New immunosuppressive strategies are required to improve long-term outcomes.

Materials and Methods. Three subjects with unstable type 1 diabetes mellitus underwent islet transplantation with alemtuzumab induction and sirolimus-tacrolimus maintenance for 3 months and then sirolimus-myophenolic acid maintenance thereafter. Follow-up was more than 2 years. Comparison was with 16 historical subjects transplanted under the Miami version of the Edmonton protocol.

Results. Insulin independence was achieved in 2 of 3 alemtuzumab and 14 of 16 historical subjects. Those who did not achieve insulin independence only received a single islet infusion. Insulin-independence rates remained unchanged in the alemtuzumab group, but decreased from 14 of 16 (88%) to 6 of 16 (38%) in the historical group over 2 years. Insulin requirements increased in the historical group while remaining stable in the alemtuzumab group. Comparison of functional measures at 3 months suggested better engraftment with alemtuzumab (P=NS). Further comparison of alemtuzumab versus historical groups, up to 24 months, demonstrated significantly better: Mixed meal stimulation index (24 months, 1.0±0.08 [n=3] vs. 0.5±0.06 pmol/mL [n=6], P<0.01), mixed meal peak C-peptide (24 months, 5.0±0.5 [n=3] vs. 3.1±0.3 nmol/mL [n=6], P<0.05), HbA1c (24 months, 5.4±0.15 [n=3] vs. 6.3±0.12 pmol/mL [n=10], P<0.01). Administration of alemtuzumab was well tolerated. There was no increased incidence of infections in alemtuzumab subjects despite profound, prolonged lymphocyte depletion.

Conclusions. Islet transplantation with alemtuzumab induction was well tolerated and resulted in improved short- and long-term outcomes. Further investigation is underway for validation.

Keywords: Islet transplantation, Alemtuzumab, Calcineurin free, Graft survival and function.

(Transplantation 2008;86: 1695–1701)

The beginning of the last decade witnessed unprecedented advances in the field of islet transplantation (IT) with the results of the Edmonton protocol (1–3). The short-term success has been duplicated in multiple centers; however, longer-term outcomes have been less successful with insulin independence only maintained in the minority of recipients at 5 years (4, 5).

Although several factors may be responsible for the low long-term insulin-independence rates, it is clear that newer immunosuppressive strategies with lower toxicity profiles and aimed toward achieving better long-term outcomes are required.

Currently, maintenance immunosuppression with sirolimus and tacrolimus is the standard in IT. Paradoxically, calcineurin inhibitors (CNI) are associated with β-cell toxicity (6, 7), insulin resistance (8) and are largely responsible for the development of post-transplant diabetes mellitus in the whole organ transplant setting (9–11). Another concern associated with this regimen is the development of renal dysfunction (12).

Lympho-depleting strategies are commonly used in whole organ transplantation for the prevention of acute allograft rejection. Alemtuzumab (Campath®, Genzyme Corporation, Cambridge, MA) is a humanized monoclonal antibody against the CD-52 human antigen present on the surface of mature B/T lymphocytes, natural killer cells, monocytes, and macrophages but absent on lymphoid progenitors (13). It has been used in several whole organ transplant settings with promising results (14–17). Recent data in the literature suggest that alemtuzumab may contribute to expansion of regulatory T cells (13, 18, 19) and this property may favorably modulate the alloimmune response thereby improving long-term survival.

The effect of alemtuzumab on multiple inflammatory cell types, for example, macrophages, may prevent the production of pro-inflammatory mediators by intrahepatic macrophages and endothelial cells, thus reducing early islet losses secondary to the deleterious effects of cytokines at the time of islet infusion.
Although its administration results in profound and long-term lympho-depletion, incidence of opportunistic infections seems comparable with that associated with other induction agents (20).

To improve IT outcomes by addressing two major areas, engraftment and immunosuppression-related toxicity, we developed an immunosuppressive protocol consisting of alemtuzumab induction and long-term CNI-free maintenance immunosuppression. Herein, we report preliminary data on three subjects who underwent IT at our center under this novel regimen.

**MATERIALS AND METHODS**

Three subjects with type 1 diabetes mellitus have been transplanted to date and have completed at least 2 years follow-up after their final islet infusion. All subjects had hyperglycemia unawareness, glycemic liability, and progressive complications despite intensive insulin therapy. All had stable renal function without evidence of diabetic nephropathy. The protocol was approved by the University of Miami health research ethics board (IRB) and each subject gave written informed consent.

Induction therapy consisted of alemtuzumab 20 mg intravenously (IV) on postoperative day-1 and 0 of initial islet infusion. Each dose required premedication with diphenhydramine (50 mg IV), acetaminophen (650 mg orally), and methylprednisolone (125 mg IV). Steroids were not administered with the second dose of alemtuzumab unless clinically indicated.

Maintenance immunosuppression, commenced postoperative day-1, consisted of sirolimus (Rapamune®, Wyeth, Madison, NJ) titrated to achieve trough levels of 7 to 10 ng/mL and tacrolimus (Prograf®, Astellas Pharma U.S., Inc., Deerfield, IL) titrated to attain a trough level of 4 to 6 ng/mL for 3 months at which time it was discontinued, and mycophenolic acid (Myfortic, Novartis Pharmaceuticals Corporation, NJ) introduced and increased up to a total dose of 720 mg two times per day as clinically tolerated. Subject 3 experienced multiple immunosuppression-related adverse events (AEs), predominantly sirolimus related. Consequently, at the time of the switch to mycophenolic acid she was continued on tacrolimus and mycophenolic acid. Tacrolimus levels have been below target range since that time, instead, dose of mycophenolic acid was increased to 900 mg two times per day by 8 months posttransplant.

The antitumor necrosis factor-alpha agent etanercept (Enbrel®, Immuneex Corporation, Thousand Oaks, CA) was administered as follows: 50 mg IV within 1 hr of islet infusion and 25 mg subcutaneously twice a week for 2 weeks after islet infusion. The anti-inflammatory pentoxifylline, 400 mg orally three times per day, was administered for 3 months posttransplantation.

After initial islet infusion, if insulin independence was not achieved within 2 weeks, a second islet infusion was performed. Induction was with a single 20 mg IV dose of alemtuzumab on the day before islet infusion, if lymphocyte was not achieved within 2 weeks, a second islet infusion was performed. Premedication without steroids was administered before the alemtuzumab dose.

Islet isolation and transplantation were performed as previously described (5, 21–23). There was no difference in the isolation technique between the two groups. In both, DNAse (0.625 mL/L, Pulmozyme [dornase alfa] recombinant Genentech, Inc.) was used throughout the isolation procedure, including digestion, dilution, and wash. Digestion was performed with Liberase enzyme blend (Roche Pharmaceuticals) in both groups. Islet recovery, average purity, islet number, and viability were comparable in both groups. Islets in all subjects underwent a period of culture no less than 24 hr, no more than 72 hr. Postculture islet viability and stimulation index were not different in both groups. IV heparin was given in the infusion bag in both groups (21).

Subjects were followed up for insulin independence, graft function, metabolic control, and immunologically to evaluate the effects of alemtuzumab. Metabolic testing consisted of a 5 hr Mixed Meal Tolerance test (MMTT) and Intravenous Glucose Tolerance test (IVGTT) as previously described (21). Parameters compared during MMTT included peak C-peptide, C-peptide AUC (AUC C-pep), and Mixed Meal Stimulation Index (MMSI) and during IVGTT included acute C-peptide release to glucose and AUC C-pep.

Comparison was with 16 historical islet recipients transplanted under the Miami version of the Edmonton protocol (21). Specifically, induction therapy consisted daclizumab (Zenapax®, Roche, US) 1 mg/kg biweekly for a total of five doses, beginning day of transplant and continued at monthly and bimonthly intervals over the first 2 years post-transplant, respectively. Historical islet infusions were performed between August 2000 and July 2003; alemtuzumab subjects were transplanted between May 2005 and January 2006.

AEs were compared between groups from the time of commencement of immunosuppression up to 2 years after completion of islet infusion. Frequency of events overall was compared and severity of events was measured using the National Cancer Institute criteria of AEs, version 3.0.

Serum panel-reactive antibody (PRA) activity to human leukocyte antigen (HLA)-I and HLA-Ⅱ was determined by complement-dependent microlymphocytotoxic technique using a commercial kit (Lambda Cell Tray, OneLambda, Canoga Park, CA) before 2006, thereafter, PRA activity was determined by ELISA using OneLambda antigen tray-mixed standardized HLA-ELISA (OneLambda) or by flow cytometry (Luminex Corporation, Austin, TX).

Two-tailed *t*-tests analyses were performed between groups at all follow-up time points. Results are expressed as mean±SEM. Statistical significance was reached at *P* less than 0.05. Subjects in the historical group were no longer considered if they withdrew from immunosuppression or underwent a supplemental infusion, hence the number of subjects in this group decreases over time.

**RESULTS**

Alemtuzumab and historical groups were compared for demographic variables and no significant differences were found (Table 1).

In the alemtuzumab group, two subjects received two islet infusions and both achieved insulin independence after the second. The third subject developed sirolimus toxicity and was switched to tacrolimus-mycophenolic acid mofetil (MMF) at 4 months post-infusion. Despite resolution of side effects of alemtuzumab. Metabolic testing consisted of a 5 hr Mixed Meal Tolerance test (MMTT) and Intravenous Glucose Tolerance test (IVGTT) as previously described (21). Parameters compared during MMTT included peak C-peptide, C-peptide AUC (AUC C-pep), and Mixed Meal Stimulation Index (MMSI) and during IVGTT included acute C-peptide release to glucose and AUC C-pep.

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TABLE 1. Demographics t tests were performed between groups to denote statistical significance

<table>
<thead>
<tr>
<th>Variable</th>
<th>Alemtuzumab subjects (n=3)</th>
<th>Historical subjects (n=16)</th>
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<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>SD</td>
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<tr>
<td>Age (yr)</td>
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<tr>
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<td>Wt (kg)</td>
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<td>Ins/kg/d</td>
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</tr>
<tr>
<td>HbA1c (%)</td>
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<td>IEQ</td>
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</tbody>
</table>

SD, standard deviation; T1DM, type 1 diabetes mellitus; BMI, body mass index; IEQ, islet equivalent.

**Effects:** The patient refused a second infusion and did not achieve insulin independence although did have a more than 50% reduction in insulin requirements. In the historical group, 13 subjects received two infusions and all achieved insulin independence three subjects received only one infusion and one of which achieved insulin independence, the other two subjects were withdrawn, prior to a second infusion because of complications of immunosuppression. The number of islet equivalents in single- and double-induction subjects was comparable.

Insulin requirements were considered as the mean of all subjects, including those who were insulin independent, at all time points. Insulin requirements were initially lower in the historical group (P=NS) (Fig. 1A). However, by 12 months the requirements were identical and thereafter requirements in the historical group continued to increase while in the alemtuzumab group, requirements remained stable over 2 years.

In an attempt to evaluate engraftment, outcome measures including fasting plasma glucose (FPG), C-peptide, and C-peptide/glucose ratio (CPGR) were compared at 1 and 3 months postinfusion.

At 1 month, FPG and C-peptide were significantly lower in the alemtuzumab group (FPG, 4.9±0.2 vs. 6.2±0.3 mmol/L, P<0.01; C-peptide, 0.37±0.04 vs. 0.57±0.07 mmol/L, P<0.05); however, there was no difference in CPGR (1.2±0.14 vs. 1.5±0.17, P=0.28). By 3 months all results were similar (FPG, 5.1±0.3 vs. 5.9±0.3 mmol/L, P=0.13; C-peptide, 0.43±0.1 vs. 0.47±0.03 mmol/L, P=0.65; CPGR, 1.3±0.25 vs. 1.4±0.09, P=0.98). HbA1c, however, was significantly lower in the alemtuzumab group (5.3±0.15% vs. 6.0%±0.17%, P<0.05) (Fig. 1B).

Longitudinal assessment of graft function demonstrated lowered HbA1c values at all time points in the alemtuzumab group reaching statistical significance again at 24 months (5.1%±0.15% vs. 6.3%±0.12%, P<0.005) (Fig. 1B). FPG was lower in the alemtuzumab group at all time points, specifically in the normal range compared with historical subjects who demonstrated impaired fasting glucose at all time points (Fig. 1C). C-peptide levels were slightly higher in the alemtuzumab group despite lower glucose levels (Fig. 1D).

During MMTT, the peak C-peptide stimulation was significantly higher, at all time points except 3 months, in the alemtuzumab group (3 months, 1.57±0.29 nmol/L [n=3] vs. 1.17±0.06 nmol/L [n=14], P=0.3; 24 months, 1.67±0.17 nmol/L [n=3] vs. 1.17±0.10 nmol/L [n=6], P<0.05) (Fig. 2A).

MMSI was higher in the alemtuzumab group at all time points after 3 months, significantly so at 6, 18, and 24 months (3 months, 1.1±0.19 [n=3] vs. 0.7±0.06 pmol/mL [n=14], P=0.22; 24 months, 1.0±0.08 [n=3] vs. 0.5±0.06 pmol/mL [n=6], P<0.01) (Fig. 2B). AUC C-peptide was higher in the alemtuzumab group at all time points although this did not reach statistical significance (Fig. 2D).

During IVGTT, acute C-peptide release to glucose was higher in the alemtuzumab group at all time points although this never reached statistical significance. Similar findings were noted with the AUC C-peptide during IVGTT (data not shown).

Administration of alemtuzumab resulted in marked leucopenia and lymphopenia. White blood cell (WBC) count in all patients fell to 1000 to 3000 cells/L, (normal range 4800–10,800 cells/µL) after a single dose and this lasted 3 to 4 months, regardless of a single- or double-induction dose. Initial peak at the time of alemtuzumab infusion is secondary to steroid administration during premedication (Fig. 3A,B). WBC in subject 3 increased at 5 months because of previously described changes in immunosuppression, lymphocyte counts remained unchanged (Fig. 3B,D). Peak in WBC in subject 2 in the second month is because of granulocyte colony-stimulating factor (G-CSF) administration at the time of alemtuzumab infusion.
subject continues to demonstrate stable function. PRA levels have remained below 5% in all subjects in both groups. The administration of alemtuzumab was well tolerated in all subjects, the worst side effect being a skin rash that occurred in all subjects. Despite the profound decrease in WBC, particularly lymphocytes, there has been no increased incidence of infections.

The total number of AEs was similar in both groups (alemtuzumab 36.3 ± 4.4 (SE) vs. historical 40.3 ± 6 events, P=NS). Similarly, the number of each level of severity and frequency of infections were also similar.

**DISCUSSION**

The data presented above from three subjects in a novel islet transplant protocol demonstrate a striking improvement in long-term function and stability of transplanted islets.

In these two groups of subjects, differences favoring the alemtuzumab group were already evident at 1 and 3 months postinfusion although they did not reach statistical significance. FPG levels at 1 and 3 months were lower and in the normoglycemic range in the alemtuzumab group, whereas the historical group maintained FPG levels in the impaired fasting glucose range suggesting a higher functional mass secondary to improved engraftment in the alemtuzumab group.

Stimulation testing by MMTT demonstrated a higher \( \beta \)-cell secretory capacity in the alemtuzumab group. A similar trend was observed in response to IVGTT although differences between groups were not significant.

Functional islet mass at any time point is dependent on two factors, the surviving mass of islets at the time of engraftment and the survival of functional islet mass over time. Large losses occur at implantation because of a relatively hostile environment and vulnerability before establishment of an ad-
equate blood supply, triggering of the innate immune system secondary to events such as IBMIR (24, 25) or activation of other components secondary to cytokine release.

The surviving mass is typically marginal which likely contributes to chronic loss over time because of over stimulation and islet exhaustion. In these two groups the differences over time become increasingly pronounced. The number of subjects maintaining insulin independence, the insulin requirements, and the functional parameters all consistently demonstrate stability in alemtuzumab subjects, whereas historical subjects demonstrate a progressive deterioration. This finding is a clear indicator that different immunosuppressive therapies such as this can play an important role in changing the long-term outcomes of islet grafts.

In the long term, these data may indicate that chronic islet loss is not occurring because of the long-term depletion of effector T cells (Teff). New data also suggest that the cell populations that return first may have a regulatory effect rather than a rejection effect. In a trial of renal transplantation using alemtuzumab there was an increase in CD4+CD25+FOXP3+ lymphocytes skewing the regulatory T cell-to-effector T cell (Tregs/Teff) ratio for years whereas induction with basiliximab, an anti CD-25 agent, resulted in a sustained decrease in Tregs (26). Expansion of Tregs may contribute in regulating rejection responses and allowing long-term survival of islet allografts. In historical subjects, CD25+ cells remained undetectable throughout the entire initial 2 years postcompletion because of continued daclizumab treatment. The profound reduction in lymphocytes secondary to alemtuzumab administration occurs within hours and remains striking even at 2 years postadministration even taking into consideration that humans take considerably longer to reconstitute compared with data in smaller mammals. Although alemtuzumab does not affect stem-cell populations, it might cause destruction of a long-lived population of cells.

FIGURE 2. Results of stimulation testing. Mixed meal peak C-peptide (A) and stimulation index (B) demonstrate significantly higher levels in the alemtuzumab group (peak C-peptide 24 months, 1.67 ± 0.17 nmol/L [n = 3] vs. 1.17 ± 0.10 nmol/L [n = 6], P < 0.05; MMSI 24 months, 1.0 ± 0.08 [n = 3] vs. 0.5 ± 0.06 pmol/mL [n = 6], P < 0.01). IVGTT acute C-peptide release (C) and MMTT C-peptide AUC (D), up to 24 months postcompletion islet infusion are consistently better in the alemtuzumab group.
that is responsible for repopulation. The absolute suppression of lymphocyte seems to allow for graft survival even when immunosuppression levels are subtherapeutic. Clinically, it is of concern to have counts this low and our concern led to the use of a colony-stimulating factor on two occasions in the same subject when neutrophil counts approached life threatening levels, and we noted a preserved neutrophil response to G-CSF without changes in lymphocyte levels. The frequency of G-CSF use was not different to historical controls. We also observed subjects closely for infectious complications but did not see any increased frequency or severity compared with historical controls. We agree that this is a small number to concluding that devastating complications will not occur; however, our findings concur with those in the literature and experiences at other centers using this agent. Long-term follow-up will further allow us to evaluate leukocyte repopulation and the potential underlying risk to recipient health.

Steroids were also administered in this protocol the day before initial islet infusion to avoid the cytokine storm associated with alemtuzumab cell lysis, but could have been beneficial for engraftment. Interestingly, it has been recently suggested that brief exposure of human islets in vivo and in vitro to methylprednisolone leads to a reduction in mRNA and protein levels of tissue factor, macrophage chemoattractant protein-1 and IL-8 and enhances ATP content without resulting in permanent effects on insulin secretion and metabolism (27).

Removal of CNI in the long term has subjectively improved the tolerability of long-term immunosuppression although objective analysis of this is ongoing. Although the detrimental effects of CNI on islet function are well known (6, 7), sirolimus is not without its limitations, particularly the prevention of proliferation (28). One subject in the alemtuzumab group who received a single islet infusion developed severe toxicity to sirolimus and was switched to tacrolimus-MMF. The subject is currently on 7 U of Lantus daily with excellent glycemic control with 5.6% HbA1c. Kaplan et al. (29) recently reported the case of an insulin-independent islet transplant recipient who was switched from sirolimus-tacrolimus maintenance to low-dose tacrolimus-MMF and monthly daclizumab infusions because of several sirolimus-associated

**FIGURE 3.** Hematological effects of alemtuzumab induction. White blood cell count 0 to 150 days (A) and up to 2 years (B) and absolute lymphocyte count 0 to 150 days (C) and up to 2 years (D), after initial alemtuzumab administration demonstrate profound long-lasting depletion. Changes in subject 3 noted at 4 to 5 months posttransplant coincide with change in immunosuppression (see text).
toxicities. By 12 months postimmunosuppression conversion, the patient remained insulin independent. While both regimens are effective, individual subjects can have unique responses to different immunosuppressive agents. As one of the goals in IT is to find a regimen that is more tolerable for recipients, this may be guided more by the individual side effect profile than the theoretical long-term effect on islet function. An analysis of the side-effect profile of each regimen is ongoing.

These results raise our optimism that both short- and long-term outcomes of IT can match those of solid organ transplantation while minimizing risks. Clearly, the statistical analysis of these subjects is limited because of low numbers; however, these promising results suggest that more attention should be focused in this area and further investigation should help to prove the validity of these results. Stimulation data in the historical group are limited by the fact that subjects are lost during follow-up secondary to withdrawal or supplemental infusion. Nevertheless, even with the “best of the best” remaining in the historical group, the differences remain significant. Induction with alemtuzumab seems to have a beneficial effect on mass preservation at the time of engraftment and during follow-up. Careful selection of subjects should still be performed while the IT community continues to realize the potential of this therapy.

REFERENCES