### Long-term Survival of Transplanted Allogeneic Cells Engineered to Express a T Cell Chemorepellent

Natalia Papeta,<sup>1</sup> Tao Chen,<sup>1</sup> Fabrizio Vianello,<sup>1</sup> Lyle Gererty,<sup>1</sup> Ashish Malik,<sup>1</sup> Ying-Ting Mok,<sup>1</sup> William G. Tharp,<sup>1</sup> Jessamyn Bagley,<sup>2</sup> Guiling Zhao,<sup>2</sup> Liljana Stevceva,<sup>1</sup> Victor Yoon,<sup>1</sup> Megan Sykes,<sup>2</sup> David Sachs,<sup>2</sup> John Iacomini,<sup>2</sup> and Mark C. Poznansky<sup>1,3</sup>

**Background.** Alloantigen specific T cells have been shown to be required for allograft rejection. The chemokine, stromal cell derived factor-1 (SDF-1) at high concentration, has been shown to act as a T-cell chemorepellent and abrogate T-cell infiltration into a site of antigen challenge in vivo via a mechanism termed fugetaxis or chemorepulsion. We postulated that this mechanism could be exploited therapeutically and that allogeneic cells engineered to express a chemorepellent protein would not be rejected.

**Methods.** Allogeneic murine insulinoma  $\beta$ -TC3 cells and primary islets from BALB/C mice were engineered to constitutively secrete differential levels of SDF-1 and transplanted into allogeneic diabetic C57BL/6 mice. Rejection was defined as the permanent return of hyperglycemia and was correlated with the level of T-cell infiltration. The migratory response of T-cells to SDF-1 was also analyzed by transwell migration assay and time-lapse videomicroscopy. The cytotoxicity of cytotoxic T cell (CTLs) against  $\beta$ -TC3 cells expressing high levels of SDF-1 was measured in standard and modified chromium-release assays in order to determine the effect of CTL migration on killing efficacy.

**Results.** Control animals rejected allogeneic cells and remained diabetic. In contrast, high level SDF-1 production by transplanted cells resulted in increased survival of the allograft and a significant reduction in blood glucose levels and T-cell infiltration into the transplanted tissue.

**Conclusions.** This is the first demonstration of a novel approach that exploits T-cell chemorepulsion to induce site specific immune isolation and thereby overcomes allograft rejection without the use of systemic immunosuppression.

Keywords: Chemokines, Pancreatic islets, Rejection, T cells.

(Transplantation 2007;83: 174-183)

**R**ejection of allogeneic grafts, which results in the destruction of transplanted organs, is a significant clinical problem and requires the treatment of allograft recipients with systemic immunosuppressive agents. There is a need for novel therapeutic approaches to solving this problem in view of the fact that the current generation of immunosuppressive therapies for graft rejection while ablating allospecific T-cell responses also increases the risk of opportunistic infections and neoplasia (1-3).

Alloantigen specific T cells have been shown to be re-

<sup>3</sup> Address correspondence to: Mark C. Poznansky, M.D., Ph.D., Partners AIDS Research Center, Massachusetts General Hospital (East), Building 149, 13th St., 5th Floor, Charlestown, MA 02139.

174

DOI: 10.1097/01.tp.0000250658.00925.c8

quired for allograft rejection although other cell types and alloantibodies can participate in this process (4, 5). Infiltration of the allograft with recipient T cells capable of killing allogeneic cells is dependent on the action of several members of a superfamily of 8 to 10 kD proteins called chemokines which are released within the allograft (6-10). The utility of chemokine orthologues and antagonists that interfere with chemokine function has been explored with the aim of inducing site-specific tolerance (11–13). In addition, attempts to physically isolate the allograft from host immune cell infiltration have been made by encapsulating the transplant in biocompatible materials (14, 15). We took a different approach to overcome allograft rejection. It was recently demonstrated that the chemokine, stromal cell derived factor-1 (SDF-1/CXCL12), a known chemoattractant for T cells at concentrations up to 10 nM, could repel CD4 and CD8 T-cells at concentrations above 100 nM via a novel mechanism, termed fugetaxis or chemorepulsion. T-cell fugetaxis plays a physiological role in the exit of mature T cells from the thymus and may contribute to a novel mechanism by which human immunodeficiency virus-1 and certain forms of cancer evade the immune system (16–21). More recently, the chemokines, eotaxin-3, CXCR3 ligands, and interleukin (IL)-8 were

#### Transplantation • Volume 83, Number 2, January 27, 2007

This research was supported by grants from the National Institutes of Health (R21 AI45898-01 and RO1 AI49757) and The Juvenile Diabetes Research Foundation.

N.P. and T.C. contributed equally to this manuscript.

<sup>&</sup>lt;sup>1</sup> Infectious Diseases Medicine Division and Partner AIDS Research Center, Massachusetts General Hospital, Charlestown, MA.

<sup>&</sup>lt;sup>2</sup> Transplantation Biology Research Center, Massachusetts General Hospital, Charlestown, MA.

E-mail: mpoznansky@partners.org

Received 23 August 2006. Revision requested 25 September 2006.

Accepted 26 September 2006.

Copyright © 2007 by Lippincott Williams & Wilkins

ISSN 0041-1337/07/8302-174

shown to act as chemorepellents for human monocytes, dendritic cells, and neutrophils, respectively (22–24).

We proposed that T-cell chemorepulsion could be exploited to generate site-specific immune isolation of donor tissue by excluding recipient T-cells relevant to graft rejection from the allograft. We engineered insulin producing  $\beta$ -TC3 cells and primary islets to express SDF-1 and green fluorescent protein (GFP) and transplanted them into allogeneic mice. Function and growth kinetics of the engineered cells were quantitated in vitro and in vivo and repulsion of T-cells from the  $\beta$ -TC3 cells secreting high levels of SDF-1 was shown. High levels of SDF-1 production by transplanted cells resulted in survival of the allograft, significant reductions in blood glucose levels and levels of T-cell infiltration into the transplanted tissue in contrast to the controls where the transplanted cells were rejected and the mice remained diabetic. In this way we were able to demonstrate that the production of high levels of SDF-1 by allograft cells could abrogate rejection and thereby prolong graft function and survival.

#### **MATERIALS AND METHODS**

#### **Cell Cultures and Vector Constructs**

Human embryonic kidney cells (HEK 293T) and murine insulinoma  $\beta$ -TC3 cells (H2-b/d) were cultured in Dulbecco's modified Eagles medium (DMEM) supplemented with 10% fetal bovine serum (FBS), penicillin, and streptomycin (Mediatech, Herndon, VA). Murine T cells were isolated from spleens of C57BL/6 mice using a T-cell isolation kit (Miltenyi Biotech, Auburn, CA). Alloreactive cytotoxic T cells (CTLs) were generated by stimulation of  $4 \times 10^{6}$  splenocytes from C57BL/6(H-2b) mice with irradiated (2000 Rad) splenocytes from DBA/2(H-2d) mice for five days. The cDNA of murine SDF-1 $\alpha$  was kindly provided by Dr. G. Dranoff. The coding region of SDF-1 $\alpha$  (82–232 bp) was cloned into the XhoI and EcoRI cloning sites of the encoding enhanced green fluorescent protein (EGFP) bicistronic transfer vector murine stem cell virus 2.2 (MSCV, provided by Dr. N. Carlesso). Subsequently the region, encoding SDF-1, internal ribosomal entry site (IRES), and EGFP, was recloned into the HpaI and Asc I sites of the lentiviral vector FUW.

# Transduction of $\beta$ -TC3 Insulinoma Cells and Cell Sorting

293T HEK cells were cotransfected using the calciumphosphate method with the following vectors: MSCV2.2-SDF-1 or MSCV2.2, pKat, and pCMV-VSV-G (25). Then 48 and 72-hour conditioned media were used for transduction of  $\beta$ -TC3 insulinoma cells as previously described (26). Cell sorting was performed using a FACSVantage cell sorter (BD Biosciences, San Diego, CA), in order to select the brightest MSCV cells and the two subpopulations of MSCV.SDF-1 cells: with high and low levels of fluorescence (termed MSCV.SDF-1 bright and MSCV.SDF-1 dim cells).

#### Detection of the Transgene Expression by Western Blotting

Conditioned media (CM) from transduced and untransduced  $\beta$ TC-3 cells were concentrated by ultrafiltration and analyzed by Western blotting. Proteins were separated in 10–20% SDS-PAGE, transferred onto a nitrocellulose membrane and stained with anti-SDF-1 antibody (R&D Systems, Minneapolis, MN) or anti-green fluorescent protein (GFP) antibody (Clontech, Palo Alto, CA), followed by detection using horseradish peroxidase (HRP)-labeled antimouse antibody and an enhanced chloroluminescence (ECL) kit (Amersham Bio-sciences, Piscataway, NJ).

### Phenotypic Characterization of Subpopulations of $\beta$ -TC3 Cells

Levels of expression of CXCR4, MHC-I, and MHC-II by subpopulations of  $\beta$ -TC3 cells were analyzed by flow cytometry, using the following PE-labeled antibodies: CXCR4, MHC-I H-2Kd (both BD Biosciences) or MHC-II H2-I–Ad (Research Diagnostics, Flanders, NJ). Levels of murine insulin in the conditioned media were measured using an enzyme-linked immunosorbent assay kit (Mercodia, Sweden) (*27*).

### Assays for Detection of the Migrational Response of T-cells to SDF-1 Secreted by $\beta$ -TC3 Cells

The CM of control and SDF-1 producing  $\beta$ -TC3 cells, prepared as above, were analyzed by T-cell transmigration assays using a standard checkerboard analysis (19, 20). For the analysis of T-cell responses in SDF-1 gradients generated by  $\beta$ -TC3 cells, murine T-cells were also added to the wells with SDF-1 secreting and control  $\beta$ -TC3 cells, grown as round 6-mm patches on fibronectin or laminin coated 24well and 48-well plates (BD BioSciences), and incubated for 15 hr at 37°C. Images were then taken every 30 seconds using a digital camera (Hamamatsu, Japan), for 60 min controlled by IPLab software. T-cells were also preincubated with the CXCR4 receptor antagonist, AMD3100 (Sigma, St. Louis, MO) at a concentration of 5  $\mu$ g/mL for 30 min at 37°C as previously described (28). Migratory paths of randomly selected T-cells towards and/or away from transduced  $\beta$ TC3 cells were plotted using Metamorph software and mean chemotropic indices (MCI) were determined as a quantitative measurement of directional movement using Mat-Lab software (29).

#### Mice, Induction of Diabetes, Immunization, and $\beta$ -TC3 Cell Transplantation

C57BL/6 (H-2b) (four to six weeks old) mice were obtained from Jackson Laboratories (Bar Harbor, ME). Hyperglycemia was induced by intraperitonial (i.p.) injections of 200 mg/kg streptozotocin (Sigma). Mice with two consecutive blood glucose readings greater than 300 mg/dL were considered hyperglycemic and used for transplantation. Immunization of mice was achieved by subcutaneous (s.c.) injection of  $5 \times 10^6$  irradiated MSCV cells (4000 Rad).  $\beta$ -TC3 cells (1×10<sup>6</sup> MSCV, MSCV.SDF-1bright or MSCV.SDF-1dim  $\beta$ -TC3 cells (H-2b/d) were transplanted by intramuscular injection. Blood glucose was monitored weekly until normoglycemia was established (two successive blood glucose measurements of less than 200 mg/dL). Mice that became hypoglycemic (two consecutive reading <100 mg/dL), as a result of  $\beta$ -TC3 cell growth and increased insulin production by the transplant, were euthanized. Mice that remained hyperglycemic for up to 90 days post-transplantation were also euthanized.

Alloreactive CTLs were generated by mixed leukocyte tumor cell cultures (MLTC).  $6 \times 10^6$  responder T cells (isolated from spleens using the Miltenyi Biotec kit for pan T cell isolation) were cocultured with  $1.2 \times 10^5$  irradiated  $\beta$ -TC3 MSCV (4.000 Rad) cells for five days. MLTC were established using H-2b splenocytes from mice previously immunized with irradiated  $\beta$ TC3 MSCV ( $1 \times 10^6$  cells subcutaneously). Cytotoxicity of CTLs was measured in a standard chromiumrelease assay using round-bottom 96-well plates and in a modified CTL assay in flat-bottom wells in order to determine the effect of CTL migration on killing efficacy as previously described (21).

# Islet Isolation, Transduction, and Transplantation

Primary islets were isolated from female BALB/C (H-2d) donor mice as previously described (30). GFP-encoding lentiviruses FUGW and FUW.SDF-1 were generated as previously described (31) and 48-hour CM were used for islet transduction. Islets transduced with no (mock) or GFPencoding viruses FUGW (control) or FUW.SDF-1 were cultivated for six days in Roswell Park Memorial Institute medium, supplemented with 10% FBS (Mediatech) in order to permit expression of the transgenes. Then 400 islets were transplanted under the capsule of the left kidney of diabetic C57/B6 mice and blood glucose was monitored twice a week. Rejection was defined as the return of hyperglycemia (blood glucose >250 mg/dL on two consecutive measurements). Mice were sacrificed at the point of graft rejection or if they remained normoglycemic for more that 100 days. Removal of the graft by nephrectomy, causing reoccurrence of hyperglycemia, was used to confirm long-term allograft function.

#### **Immune Histochemistry**

Tissue samples were collected at sites of transplantation, formalin-fixed and paraffin-embedded. Five-micron sections were stained with hematoxylin and eosin (H&E), anti-CD3 (DakoCytomation, Carpinteria, CA), anti-GFP (Clontech, Palo Alto, CA), and secondary HRP-labeled En-Vision antibody (DakoCytomation).

### Assessment of Alloreactivity in Transplanted Mice

The alloreactivity and immune competence of mice transplanted with  $\beta$ -TC3 cells was assessed by measuring antibody responses and the survival of allogeneic skin transplants. Alloantibodies were quantitated in immunized mice transplanted with MSCV or MSCV.SDF-1bright cells according to established methods (*32*) using flow cytometry. Mice receiving transplants of MSCV or MSCV.SDF-1bright cells were also transplanted with syngeneic (donor C57BL/6 mice) and allogeneic skin (donor B6D2F1/JH mice–H2-b/d and C3H/HeJ–H2-k) and skin graft survival documented as previously described (*33*).

#### **Statistical Analysis**

The data were analyzed using Kaplan-Mayer and Student *t* tests.

#### RESULTS

### Quantitation and Bioassay of SDF-1 Produced by Transduced $\beta$ -TC3 Cells

MSCV, MSCV.SDF-1bright, and MSCV/SDF-1dim  $\beta$ -TC3 cells were sorted from transduced populations of  $\beta$ -TC3 cells as described above. Mean fluorescence intensity



**FIGURE 1.** Expression of transgenes by transduced  $\beta$ -TC3 cells. (A) Schema of the region of the MSCV.SDF-1 vector, encoding SDF-1 and GFP. (B) Mean fluorescent intensities (MFI) of sorted GFP expressing cells. Dotted line, control nonfluorescent  $\beta$ -TC3 cells; bold line, MSCV cells; solid gray, MSCV.SDF-1dim; black, MSCV.SDF-1bright cells. (C) Detection of SDF-1 in the concentrated CM by immunoblot. Lane 1, recombinant SDF-1 $\alpha$  (10 ng). Lanes 2–4, CM of the following cells. 2, MSCV; 3, MSCV.SDF-1dim; 4, MSCV.SDF-1 bright. (D) Detection of GFP by immunoblot in the following cell lysates. 1, MSCV; 2, MSCV.SDF-1 dim; 3, MSCV.SDF-1 bright. Lanes 5C and 4D, molecular weight markers.



**FIGURE 2.** Demonstration of T-cell repulsion from MSCV.SDF-1bright cells producing high levels of SDF-1 using timelapse videomicroscopy. CD3<sup>+</sup>T cells were randomly distributed in the wells with control MSCV or MSCV.SDF-1bright cells and incubated for 15 hr. (A–C) Final distribution of T cells, which remained random in the wells with control MSCV cells (A) and demonstrated a "zone of clearance" around MSCV.SDF-1bright cells, producing a high level of SDF-1 (B). When T-cells were pretreated with the CXCR4 antagonist, AMD3100, the T cells remained evenly distributed around the MSCV.SDF-1bright cells (C). (D and E) T-cell migration was tracked by time-lapse video microscopy and the migratory paths of randomly selected T-cells are depicted. The start points of migration tracks are distributed along the axes, the end points are marked with crosses (x). In control MSCV wells, T-cell migration was chemokinetic or random (D). In wells containing MSCV.SDF-1bright cells, T-cell migration was initially chemotactic during the first 1.5 hr of tracking and subsequently changed to robust fugetaxis.

of MSCV.SDF-1 bright cells was on average 10-fold greater than that of MSCV.SDF-1 dim cells (Fig. 1B). SDF-1 was detected by Western blotting in CM of MSCV.SDF-1 bright and MSCV.SDF-1 dim cells but not from control MSCV cells (Fig. 1C). The highest level of SDF-1 detected in CM of MSCV.SDF-1 bright and dim cells were 600 ng/mL and 60 ng/mL, respectively. Western blotting with anti-GFP antibody demonstrated a 10-fold difference between MSCV. SDF-1 bright and dim cells in the levels of GFP as well (Fig. 1D). These data confirmed a correlation between the production of SDF-1 and GFP that allowed indirect monitoring of the level of the chemokine in vitro and in vivo.

The functional activity of SDF-1 secreted by transduced  $\beta$ -TC3 cells was analyzed by transwell migration assay. A high level of chemotactic migration was detected for T-cells in response to CM of MSCV.SDF-1 bright and MSCV.SDF-1 dim cells, but not control MSCV cells (data not shown). In this setting, T cells were exposed to a step gradient of SDF-1 in CM from  $\beta$ -TC3 cells generated in 24 hours, which was only

thought to approximate the situation in vivo. Consequently, we developed a second assay for observing cell migration and that allowed us to demonstrate the fugetactic effect of a steep continuous SDF-1 gradient on T-cell migration. In this system, T cells were observed and tracked by time-lapse microscopy and digital image analysis to migrate within the vicinity of patches of MSCV.SDF-1 bright or control cells. T cells were evenly distributed within the wells with MSCV cells or MSCV.SDF-1 bright cells at the beginning of experiments. In the wells with control cells, the distribution of T cells remained random by the end of the experiment and cell migration was documented to be chemokinetic in nature by time-lapse video microscopy (MCI,  $-0.018\pm0.012$ ; Fig. 2A, D). In contrast, T-cells migrated away from MSCV.SDF-1 bright cells and formed zones of clearance of T-cells around the patches of SDF-1 secreting cells at 15 hr of incubation. T-cell migration was predominantly chemotactic towards SDF-1 secreting cells during the first 1.5 hr of incubation (MCI,  $+0.08\pm0.018$ ), but subsequently changed to a che-

morepellent response (MCI,  $-0.268\pm0.019$ ; P<0.05; Fig. 2B, E) where MCI values from -0.05 to -1.0 indicate fugetaxis and +0.05 to +1.0 indicate chemotaxis (24). To confirm that T-cell chemorepulsion was SDF-1-mediated, T cells were pretreated with AMD3100 at a concentration that has previously been demonstrated to inhibit migration mediated by CXCR4 but not to block migration mediated by other receptors (34). Pretreated T cells failed to migrate away from SDF-1 secreting cells and remained randomly distributed (MCI,  $0.06\pm0.01$ ; Fig. 2C). In this way, we demonstrated that MSCV.SDF-1bright cells and not control MSCV cells elicited migrational responses from T-cells, and that the high level of SDF-1 secretion by MSCV.SDF-1 bright cells was sufficient to repel T cells in vitro via a CXCR4-mediated mechanism.

#### High Levels of SDF-1 Do Not Augment the Growth, Function, or Survival of Allografts in Naïve Recipients

The growth kinetics of control and SDF-1 producing cells in vitro were equivalent and no statistically significant differences were found (mean doubling times were within 1.93±0.06 days; for MSCV.SDF-1 dim and MSCV.SDF-1 bright cells vs. MSCV cells, respectively (P=0.44 and 0.22)). Analysis of CXCR4, MHC-I and MHC-II expression in the  $\beta$ -TC3 subpopulations indicated no effect of SDF-1 production on levels of CXCR4 and no downregulation of MHC-I or MHC-II by high level of SDF-1 (data not shown). To investigate the effect of SDF-1 secretion on the tumorigenicity of cells, 1×10<sup>6</sup> MSCV, MSCV.SDF-1 bright, or MSCV.SDF-1dim cells were transplanted into the flank of naïve diabetic C57BL/6 mice. Only control mice injected with PBS remained hyperglycemic for up to 80 days (mean=62±13 days) and average glucose level at euthanization was 409±83 mg/dL. In contrast, mice transplanted with all three transduced cell types reached normo- or hypoglycemia within 31.7 $\pm$ 6.6 days, which correlated with the survival of allografts and formation of tumors. Average glucose levels in the groups of mice transplanted with MSCV, MSCV.SDF-1 bright and MSCV.SDF-1 dim cells decreased from 547 $\pm$ 35.3, 476.5 $\pm$ 32.5, and 554.5 $\pm$ 45.5 mg/dL to 90 $\pm$ 24.2, 72 $\pm$ 19.6, and 88 $\pm$ 25.3 mg/dL, respectively (MSCV.SDF-1 bright vs. MSCV: P=0.91; MSCV.SDF-1 dim vs. MSCV: P=0.33). There was no significant decrease in the time to normoglycemia in naive mice transplanted with MSCV.SDF-1 bright or dim cells when compared to MSCV cells (31.4 $\pm$ 1.8 days and 43 $\pm$ 10 days vs. 24.3 $\pm$ 5.3 days, respectively). These data suggest that SDF-1 secreted by transplanted transduced cells did not increase the insulin production or the proliferation rate of  $\beta$ TC3 cells in this animal model.

#### High Levels of SDF-1 Secretion Facilitate Allograft Survival and Function in Immunized Mice

Survival of allogeneic cells expressing Tag in naïve mice and rejection of these cells in immunized mice has been shown previously (35). Having obtained similar results, we immunized mice with irradiated control MSCV cells prior to transplantation. The results of transplantation of immunized mice with control or SDF-1 secreting cells were strikingly different from data obtained in naive mice (Fig. 3). In all, 89% and 90% of mice transplanted with MSCV and MSCV.SDF-1 dim cells remained hyperglycemic indicating destruction of allograft cells. In contrast, 75% of mice transplanted with MSCV.SDF-1 bright cells reached normoglycemia as a result of the survival of allografts. Blood glucose levels were significantly lower in mice at sacrifice from the MSCV.SDF-1 bright group  $(79\pm40 \text{ mg/dL})$  excluding the single hyperglycemic outline compared to the MSCV (447±76 mg/dL) or MSCV.SDF-1 dim groups (423±87 mg/dL) and at sacrifice (MSCV.SDF-1 bright vs. MSCV: P=0.018; MSCV.SDF-1 dim cells vs. MSCV: P=0.768). The average times to sacrifice



**FIGURE 3.** Glucose levels in the immunized diabetic mice, transplanted with the transduced  $\beta$ -TC3 cells, producing various levels of SDF-1. (A) Comparison of glucose levels prior to transplantation and at euthanization. Only mice transplanted with MSCV.SDF-1bright cells, secreting high level of SDF-1, demonstrated a statistically significant reduction in glucose level, while the mice remained hyperglycemic in all other groups (MSCV.SDF-1 bright vs. MSCV: P=0.018; MSCV.SDF-1 dim vs. MSCV: P=0.768). (B) Survival curve for allografts posttransplantation. Return to normoglycemia was considered as an indicator of allografts survival, confirmed by detection of tumors, while persistent hyperglycemia and lack of tumor formation were considered as indicators of rejection (Kaplan-Mayer log rank test, P=0.001).



**FIGURE 4.** High level SDF-1 expression by MSCV.SDF-1 bright cells reduced infiltration of T-cells into allograft tissues as compared to control and MSCV.SDF-1 dim cells. Paraffin-embedded sections were stained with H&E (A–D), anti-CD3 (E–H), or anti-GFP antibody (I–L). The samples represent tissues collected at the sites of injection with ovalbumin (A, E, I) or (B, F, J) control MSCV cells; (C, G, K) MSCV.SDF-1 dim cells; (D, H, L) MSCV.SDF-1 bright cells. Control MSCV and MSCV.SDF-1 dim cells expressing low level of SDF-1 induced significant infiltration of T-cells, while only occasional T-cells indicated by an arrow (4h) were detected among the MSCV.SDF-1 bright cells, producing a high level of SDF-1. Negative staining with isotope control antibody (I) confirms specificity of anti-GFP staining. Magnification:  $400 \times$ .

or death in persistently hyperglycemic mice of the MSCV and MSCV.SDF-1dim groups were  $57.6 \pm 14$  and  $66.6 \pm 15.7$  days, respectively, as compared to  $25 \pm 9.8$  days in the MSCV.SDF-1 bright group that reached normoglycemia. The survival of MSCV.SDF-1 bright cell allografts was significantly increased in comparison to MSCV and MSCV.SDF-1dim allografts (*P*=0.018, MSCV.SDF-1 bright cells vs. MSCV cells, Kaplan-Mayer log rank test: *P*=0.001).

### High Levels of SDF-1 Secretion by Allografts Prevent T-cell Infiltration

Histopathology and T-cell infiltration were analyzed in the tissues excised at the site of transplantation. Tissue sections were stained with hematoxylin and eosin (Fig. 4A–D), anti-CD3 (Fig. 4E–H), or anti-GFP antibodies (Fig. 4I–L). Positive control tissues, obtained from mice immunized against ovalbumin and subsequently challenged with OVA, demonstrated an extensive CD3+ T-cell infiltrate (Fig. 4A, E) at the site of antigen challenge. Similarly, immunized mice receiving MSCV cells (Fig. B, F) or MSCV.SDF-1 dim cells (Fig. 4C, G) demonstrated dense infiltration of the allograft with CD3+ T-cells. Occasional GFP positive cells were seen in infiltrated tissues indicating the presence of transplanted MSCV (Fig. 4J) and MSCV.SDF-1dim cells (Fig. 4K). In contrast, only scanty CD3+ T cell infiltration was observed among GFP-positive MSCV.SDF-1 bright cells (Fig. 4D, H, and L). Tissues immunostained with isotype control antibodies were negative for all groups of transplanted cells (Fig. 4I and data not shown). These data demonstrated that expression of transgenes was maintained in the transduced cells in vivo and that high-level expression of SDF-1 abrogated T-cell infiltration into allogeneic tissues.

# High-Level Expression of SDF-1 by $\beta\text{-TC3}$ Cells Impairs CTL Killing

Having shown in vivo that high levels of SDF-1 facilitated survival of the allograft, we examined if it could protect

allogeneic cells from CTL-mediated lysis in vitro. We tested this hypothesis by measuring the killing activity of H-2dallospecific H-2b effector cells using standard and modified <sup>51</sup>Cr-release assays (Fig. 5A) (21). In the standard assay, where target and effector cells are pelleted together, MSCV.SDF-1bright and MSCV cells were lysed by allospecific CTLs with the same efficacy (P=0.14). When tested in a modified assay, which takes into account the effect of CTL migration on killing activity, the efficacy of allospecific CTLs was significantly lower for MSCV.SDF-1 bright cells as compared to MSCV cells (P=0.0009; Fig. 5B). High levels of SDF-1 secretion by target cells therefore impairs the efficacy of CTL killing in an assay in which migration of effector cells plays a critical role.

#### SDF-1 Secretion by Primary Allogeneic Islets Facilitates Allograft Survival and Reduces T-cell Infiltration

In view of the possibility that an intrinsic characteristic of the  $\beta$ -TC3 cell line may play a role in SDF-1 mediated escape from an immune response, primary islets from BALB/C mice were mock transduced or engineered to express GFP or GFP and SDF-1. Transduced islets were then transplanted under the renal capsule of diabetic C57BL/6 mice. Mean pretransplant levels of blood glucose were equivalent in all groups (mock transduced, 358±35 mg/dL; FUGW transduced, 369±28 mg/dL; FUW.SDF-1 transduced, 419±43 mg/dL). Normoglycemia was initially restored by islet transplantation in all groups. Mice receiving mock transduced or islets expressing GFP alone became hyperglycemic within  $13.8\pm2.2$  and  $14.7\pm4$  days post-transplantation, respectively. In contrast, hyperglycemic mice receiving allogeneic islets (H2-d) transduced with the vector encoding GFP and SDF-1 remained normoglycemic on average for 57.4±12.2 days post-transplantation (P=0.01; Kaplan-Mayer log-rank test). In addition, analysis of mean blood glucose levels posttransplantation and just prior to euthanization revealed that mice transplanted with mock-transduced islets ( $317.0\pm120.1$  mg/dL) or FUGW-transduced islets ( $288.3\pm43.6$  mg/dl) had significantly higher blood glucose levels than those in mice transplanted with SDF-1 expressing islets ( $167.0\pm38.59$  mg/ dl) (mock versus FUW.SDF-1 P=0.023; FUGW vs. FUW. SDF-1; P=0.004, Student *t* test; Fig. 6A). Furthermore, blood glucose levels in normoglycemic mice that had received islets transduced with FUW.SDF-1 under the left kidney capsule rose significantly and became hyperglycemic following removal of the kidney bearing the transplanted and functioning islets (mean prenephrectomy blood glucose= $149\pm7$  mg/dL; postnephrectomy blood glucose= $249\pm62$  mg/dL; P=0.019).

Following nephrectomy or sacrifice, immunohistochemical staining techniques were used to analyze T-cell infiltration at the site of islet transplantation under the renal capsule (Fig. 6). Mock- or FUGW-transduced islets were densely infiltrated with CD3+ T-cells. In contrast, only scanty or no T cells were detected among SDF-1 secreting FUW.SDF-islets. T cells were occasionally observed at the periphery of the SDF-1 secreting islet mass (Fig. 6B). In addition, SDF-1 was detectable by immunohistochemical staining throughout the transplanted FUW.SDF-1 islets and not in FUW or control untransduced islets or in adjacent renal tissue (Fig. 6E–G).

#### SDF-1 Does Not Affect Alloantibody Generation

Mice receiving FUW and FUW.SDF-1 bright cells were shown to generate allotypic antibodies at 14 days posttransplantation. Known negative controls and untransplanted mice generated mean fluorescent intensities of  $8.4\pm1.8$ , whereas positive controls in which anti-H2d IgG2b antibodies were induced gave an MCI of  $82.2\pm9.9$ . Mice transplanted with islet cells transduced with the FUW and FUW. SDF-1 were considered to be weakly positive with an MCI of  $20.3\pm3.8$ . These data indicated that as expected recipient



**FIGURE 5.** High level of SDF-1 production by MSCV.SDF-1bright cells reduced cytolytic activity of allospecific CTL. Cytotoxicity of allospecific-CTL against control MSCV ( $\blacklozenge$ ) or MSCV.SDF-1bright ( $\blacksquare$ ) cells were compared by Cr release assays using round-bottom (A) and flat-bottom (B) wells. No difference in the level of cytotoxicity between control MSCV and MSCV.SDF-1bright cells was detected in round-bottom wells (P=0.14), in contrast to the assay performed in flat-bottom wells where efficacy of killing of SDF-1 producing cells was reduced as compared to MSCV controls (P=0.0009). Results represent mean values  $\pm$  SEM of four independent experiments in each setting.



**FIGURE 6.** Secretion of SDF-1 by transduced primary islets facilitated survival of the allografts and reduced T-cell infiltration. (A) Survival curve for allografts posttransplantation. Return to hyperglycemia was considered as an indicator of rejection, while sustained normoglycemia was considered as an indicator of allograft survival, (Kaplan-Mayer log rank test, FUGW versus FUW.SDF-1; P=0.01). (B–G) Reduction of T-cell infiltration into SDF-1 producing allografts. Paraffin-embedded sections were stained with H&E (B–D) and anti-CD3 (E–G) antibody. Dense infiltration of T-cells was detected in the samples with control mock (B, E) and FUGW (C, F) islets. In contrast, SDF-1 secreting FUW.SDF-1 islets were not infiltrated with T-cells, which were found outside allograft tissues (D, G). A sample of FUW.SDF-1 allograft shown was collected after 95 days of persistent normoglycemia. Immunohistochemical staining for SDF-1 expression was also performed on sections of transplanted islets; these are shown in insets to (E–G) and demonstrate positive staining for the chemokines exclusively in islets transduced with the FUW.SDF-1 vector (G) and not in transplanted islets or adjacent renal tissue, mock transduced or transduced with the FUGW vector (E and F), respectively.

mice remained immunocompetent and capable of weak allogeneic humoral responses despite the secretion of high levels of SDF-1 by concurrently transplanted FUW.SDF-1 transduced islets.

#### Effect of SDF-1 Expression on Alloimmune Responses In Vivo

Mice transplanted with MSCV and MSCV.SDF-1bright  $\beta$ -TC3 cells were also concurrently transplanted with allogeneic (H-2b/d and H-2k) and syngeneic (H-2b) skin. Mice in this experiment rejected allogeneic skin on average in 19.4±6 days while syngeneic skin was not rejected in this setting. As expected recipient mice remained immunocompetent and capable of allogeneic humoral and cell mediated responses despite the secretion of high levels of SDF-1 by concurrently transplanted MSCV.SDF-1 bright cells. These data support the thesis that the

secretion of high levels of SDF-1 by transplanted cells induced immune isolation of the allograft.

#### DISCUSSION

In this study, we show site-specific immune isolation in which donor allograft tissue is protected from the host immune system through the induction of effector T cell chemorepulsion as a result of the secretion of chemorepellent concentrations of the chemokine, SDF-1 by the allograft. Murine allogeneic insulin secreting  $\beta$ -TC3 cells or primary allogeneic islets were engineered to constitutively express a high level of SDF-1. The  $\beta$ -TC3 insulinoma cell line originating from a transgenic mouse of the hybrid strain B6D2F1/J and transformed with SV-40 T large antigen, has been shown to have characteristics similar to primary  $\beta$ -cells and has been

previously used in studies of diabetes (*36*, *37*). When control and SDF-1 secreting  $\beta$ -TC3 (H-2b/d) cells were used for transplantation into allogeneic diabetic C57BL/6 (H-2b) mice we found that secretion of high levels of SDF-1 could consistently delay allograft rejection. This result was recapitulated in a model where primary islets expressing high levels of SDF-1 using a lentiviral vector escaped rejection and demonstrated persistent function in association with minimal T cell infiltration. Prolongation of islet survival was observed up to 95 days post-transplantation into an allogeneic recipient in the current study. The persistence of this effect beyond this time point is currently under investigation.

The role of high levels of SDF-1 in the survival of allografts is further supported by the demonstration of T-cell migration away from cells secreting high levels of SDF-1 in vitro and the finding that high levels of SDF-1 secretion by target cells impaired the efficacy of CTL killing. It is known that CD8+ T cells are sufficient to mediate acute allograft islet rejection and autoreactive CD8+ T cells have been reported to play a major role in the pathogenesis of type I diabetes (38–40). Allorecognition and rejection of islet allografts in C57BL/6 mice, used in our study, have been previously shown by depletion with CD4 or CD8 antibody, to be CD8+ T cell mediated (35, 41). Therefore, we suggest that in our model the principle effect of SDF-1 is on CD8+ T-cell chemorepulsion although an effect on CD4+ T-cells is conceivable based on previously published data (42, 43).

In view of the fact that SDF-1 has been shown to have effects beyond its actions on cell migration, we examined whether these mechanisms were operational in our system. We showed that SDF-1 did not affect levels of CXCR4 expression, insulin production or the growth kinetics of  $\beta$ -TC3 cells in vitro and in immune naive mice. High levels of SDF-1 did not appear to affect T cell apoptosis and activation or downregulate expression of MHC-I or MHC-II by target cells, which was concordant with our data showing equivalent efficacy of allo-specific CTL killing of SDF-1 secreting and control cells in the standard CTL assay (18). Transplantation with MSCV.SDF-1 bright cells or SDF-1 expressing islets did not impair the immunocompetence of the H-2b recipient, as measured by allo-specific antibody production and rejection of allogeneic skin. Previous and current data are consistent with a bimodal action of SDF-1, acting as a T-cell chemorepellent and chemoattractant at high (600 ng/mL) and low (15-70 ng/mL) concentrations, respectively (44, 45). The identification of a fugetactic protein or fugetaxin which acts only as a T-cell chemorepellent is currently underway in our laboratory.

It has been shown that tumors producing SDF-1 can dysregulate immunity by inducing migration of immature plasmacytoid dendritic cells (PDCs) into a tumor microenvironment (46, 47). The presence of immature PDCs could be responsible for defective local priming or activation of intratumoral T cells. Our data do not support a defective activation of a brisk T-cell infiltrate into a graft expressing high levels of SDF-1. It is also important to note that we observed a failure to reject an SDF-1 secreting graft in the  $\beta$ -TC3 model in which antigen presenting cell-T cell interactions would be expected to be less affected by SDF-1 since mice were initially primed against  $\beta$ -TC3 by immunization with mock-transfected cells in absence of SDF-1 (48). Intriguingly, a correlation between levels of endogenous SDF-1 and long term survival of murine allogeneic islets has been recently demonstrated (Reza Abdi, personal communication).

In this study, we exploit a novel mechanism to abrogate rejection. Previous approaches include attempts to inhibit chemokine-mediated recruitment of immune cells into the allograft by targeting chemokines and their receptors (30, 49-51). T-cell depletion and the establishment of a mechanical barrier represent partially effective strategies to overcome rejection but the lack of biocompatibility, hypoxia, and severe immune suppression are problematic (14, 52). In addition, islet transplants for patients with type 1 diabetes face two distinct types of immune destruction: one generated by the allogeneic T-cell response to foreign tissues and the other resulting from the recurrence of the tissue-specific T-cell autoimmune process that caused the disease (42, 53-55). Therefore, these immunomodulatory therapeutic approaches may not be able to completely block the multitude of factors that contribute to the infiltration of transplanted islets with Tcells. We would propose that our approach, involving the engineered expression of a T cell chemorepellent by transplanted tissues, would be equally effective in abrogating the infiltration and destruction of the graft by auto or alloreactive T cells. This hypothesis is currently being tested in the context of islet transplantation in NOD mice in our laboratory.

In conclusion, we propose that SDF-1-mediated T-cell chemorepulsion, previously demonstrated for CD4+ and CD8+ T-cells (19, 20), may be uniquely capable of inducing site specific immune isolation by excluding auto- and alloreactive CD4+ and CD8+ T-cells from transplanted tissue. At this early stage of development of this work, this approach may at least be contemplated as being useful in the future for affording local protection of cell, tissue, or solid organ transplants from rejection without the use of systemic immunosuppressive agents. This study broadens our understanding of the therapeutic applications of chemokines and opens the way for an assessment of the principle in a large animal model, that site-specific immune isolation can be achieved by engineering transplanted tissues, such as pancreatic  $\beta$ -islets, to express a chemorepellent agent.

#### ACKNOWLEDGMENTS

Thanks to Phillip Bardwell for help with assays of allospecific antibody production.

#### REFERENCES

- 1. Monaco AP. The beginning of clinical tolerance in solid organ allografts. *Exp Clin Transplant* 2004; 2(1): 153.
- Denton MD, Magee CC, Sayegh MH. Immunosuppressive strategies in transplantation. *Lancet* 1999; 353(9158): 1083.
- Lechler RI, Sykes M, Thomson AW, Turka LA. Organ transplantation– how much of the promise has been realized? *Nat Med* 2005; 11(6): 605.
- Game DS, Lechler RI. Pathways of allorecognition: implications for transplantation tolerance. *Transpl Immunol* 2002; 10(2–3): 101.
- Gould DS, Auchincloss H Jr. Direct and indirect recognition: the role of MHC antigens in graft rejection. *Immunol Today* 1999; 20(2): 77.
- Tan J, Zhou G. Chemokine receptors and transplantation. *Cell Mol Immunol* 2005; 2(5): 343.
- DeVries ME, Hosiawa KA, Cameron CM, et al. The role of chemokines and chemokine receptors in alloantigen-independent and alloantigendependent transplantation injury. *Semin Immunol* 2003; 15(1): 33.
- Nelson PJ, Krensky AM. Chemokines, chemokine receptors, and allograft rejection. *Immunity* 2001; 14(4): 377.
- 9. Moench C, Uhrig A, Wunsch A, et al. Chemokines: reliable markers for

diagnosis of rejection and inflammation following orthotopic liver transplantation. *Transplant Proc* 2001; 33(7–8): 3293.

- Yun JJ, Fischbein MP, Laks H, et al. Early and late chemokine production correlates with cellular recruitment in cardiac allograft vasculopathy. *Transplantation* 2000; 69(12): 2515.
- 11. Vincenti F. What's in the pipeline? New immunosuppressive drugs in transplantation. *Am J Transplant* 2002; 2(10): 898.
- DeBruyne LA, Magee JC, Buelow R, Bromberg JS. Gene transfer of immunomodulatory peptides correlates with heme oxygenase-1 induction and enhanced allograft survival. *Transplantation* 2000; 69(1): 120.
- Jiang X, Shimaoka T, Kojo S, et al. Cutting edge: critical role of CXCL16/CXCR6 in NKT cell trafficking in allograft tolerance. *J Immu*nol 2005; 175(4): 2051.
- de Groot M, Schuurs TA, van Schilfgaarde R. Causes of limited survival of microencapsulated pancreatic islet grafts. *J Surg Res* 2004; 121(1): 141.
- 15. Lanza RP, Hayes JL, Chick WL. Encapsulated cell technology. *Nat Biotechnol* 1996; 14(9): 1107.
- 16. Vianello F, Kraft P, Mok YT, et al. A CXCR4-dependent chemorepellent signal contributes to the emigration of mature single-positive CD4 cells from the fetal thymus. *J Immunol* 2005; 175(8): 5115.
- Vianello F, Olszak IT, Poznansky MC. Fugetaxis: active movement of leukocytes away from a chemokinetic agent. *J Mol Med* 2005; 83(10): 752.
- Vianello F, Papeta N, Chen T, et al. Murine B16 melanomas expressing high levels of the chemokine stromal-derived factor-1/CXCL12 induce tumor-specific T cell chemorepulsion and escape from immune control. *J Immunol* 2006; 176(5): 2902.
- 19. Poznansky MC, Olszak IT, Foxall R, et al. Active movement of T cells away from a chemokine. *Nat Med* 2000; 6(5): 543.
- Poznansky MC, Olszak IT, Evans RH, et al. Thymocyte emigration is mediated by active movement away from stroma-derived factors. *J Clin Invest* 2002; 109(8): 1101.
- Brainard DM, Tharp WG, Granado E, et al. Migration of antigenspecific T cells away from CXCR4-binding human immunodeficiency virus type 1 gp120. *J Virol* 2004; 78(10): 5184.
- Kohrgruber N, Groger M, Meraner P, et al. Plasmacytoid dendritic cell recruitment by immobilized CXCR3 ligands. *J Immunol* 2004; 173(11): 6592.
- Ogilvie P, Paoletti S, Clark-Lewis I, Uguccioni M. Eotaxin-3 is a natural antagonist for CCR2 and exerts a repulsive effect on human monocytes. *Blood* 2003; 102(3): 789.
- Tharp WG, Yadav R, Irimia D, et al. Neutrophil chemorepulsion in defined interleukin-8 gradients in vitro and in vivo. *J Leukoc Biol* 2006; 79(3): 539.
- 25. Pear WS, Nolan GP, Scott ML, Baltimore D. Production of high-titer helper-free retroviruses by transient transfection. *Proc Natl Acad Sci U S A* 1993; 90(18): 8392.
- 26. Carlesso N, Aster JC, Sklar J, Scadden DT. Notch1-induced delay of human hematopoietic progenitor cell differentiation is associated with altered cell cycle kinetics. *Blood* 1999; 93(3): 838.
- Zalzman M, Gupta S, Giri RK, et al. Reversal of hyperglycemia in mice by using human expandable insulin-producing cells differentiated from fetal liver progenitor cells. *Proc Natl Acad Sci U S A* 2003; 100(12): 7253.
- Hatse S, Princen K, Bridger G, et al. Chemokine receptor inhibition by AMD3100 is strictly confined to CXCR4. FEBS Lett 2002; 527(1–3): 255.
- 29. Nossal R, Zigmond SH. Chemotropism indices for polymorphonuclear leukocytes. *Biophys J* 1976; 16(10): 1171.
- Abdi R, Means TK, Luster AD. Chemokines in islet allograft rejection. Diabetes Metab Res Rev 2003; 19(3): 186.
- Lois C, Hong EJ, Pease S, et al. Germline transmission and tissuespecific expression of transgenes delivered by lentiviral vectors. *Science* 2002; 295(5556): 868.
- Lee LA, Sergio JJ, Sachs DH, Sykes M. Mechanism of tolerance in mixed xenogeneic chimeras prepared with a nonmyeloablative conditioning regimen. *Transplant Proc* 1994; 26(3): 1197.
- Tomita Y, Nishimura Y, Harada N, et al. Evidence for involvement of clonal anergy in MHC class I and class II disparate skin allograft tolerance after the termination of intrathymic clonal deletion. *J Immunol* 1990; 145(12): 4026.
- 34. Matthys P, Hatse S, Vermeire K, et al. AMD3100, a potent and specific

antagonist of the stromal cell-derived factor-1 chemokine receptor CXCR4, inhibits autoimmune joint inflammation in IFN-gamma receptor-deficient mice. *J Immunol* 2001; 167(8): 4686.

- Pericin M, Althage A, Freigang S, et al. Allogeneic beta-islet cells correct diabetes and resist immune rejection. *Proc Natl Acad Sci U S A* 2002; 99(12): 8203.
- Efrat S, Fejer G, Brownlee M, Horwitz MS. Prolonged survival of pancreatic islet allografts mediated by adenovirus immunoregulatory transgenes. *Proc Natl Acad Sci U S A* 1995; 92(15): 6947.
- Efrat S, Linde S, Kofod H, et al. Beta-cell lines derived from transgenic mice expressing a hybrid insulin gene-oncogene. *Proc Natl Acad Sci* USA 1988; 85(23): 9037.
- Desai NM, Bassiri H, Kim J, et al. Islet allograft, islet xenograft, and skin allograft survival in CD8+ T lymphocyte-deficient mice. *Transplantation* 1993; 55(4): 718.
- Diamond AS, Gill RG. An essential contribution by IFN-gamma to CD8+ T cell-mediated rejection of pancreatic islet allografts. *J Immunol* 2000; 165(1): 247.
- Vizler C, Bercovici N, Cornet A, et al. Role of autoreactive CD8+ T cells in organ-specific autoimmune diseases: insight from transgenic mouse models. *Immunol Rev* 1999; 169: 81.
- Makhlouf L, Yamada A, Ito T, et al. Allorecognition and effector pathways of islet allograft rejection in normal versus nonobese diabetic mice. J Am Soc Nephrol 2003; 14(8): 2168.
- 42. Guo Z, Wu T, Kirchhof N, et al. Immunotherapy with nondepleting anti-CD4 monoclonal antibodies but not CD28 antagonists protects islet graft in spontaneously diabetic nod mice from autoimmune destruction and allogeneic and xenogeneic graft rejection. *Transplantation* 2001; 71(11): 1656.
- Friedman T, Smith RN, Colvin RB, Iacomini J. A critical role for human CD4+ T-cells in rejection of porcine islet cell xenografts. *Diabetes* 1999; 48(12): 2340.
- 44. Nomura T, Hasegawa H, Kohno M, et al. Enhancement of anti-tumor immunity by tumor cells transfected with the secondary lymphoid tissue chemokine EBI-1-ligand chemokine and stromal cell-derived factor-1alpha chemokine genes. *Int J Cancer* 2001; 91(5): 597.
- Dunussi-Joannopoulos K, Zuberek K, Runyon K, et al. Efficacious immunomodulatory activity of the chemokine stromal cell-derived factor 1 (SDF-1): local secretion of SDF-1 at the tumor site serves as T-cell chemoattractant and mediates T-cell-dependent antitumor responses. *Blood* 2002; 100(5): 1551.
- Zou W, Machelon V, Coulomb-L'Hermin A, et al. Stromal-derived factor-1 in human tumors recruits and alters the function of plasmacytoid precursor dendritic cells. *Nat Med* 2001; 7(12): 1339.
- 47. Vermi W, Bonecchi R, Facchetti F, et al. Recruitment of immature plasmacytoid dendritic cells (plasmacytoid monocytes) and myeloid dendritic cells in primary cutaneous melanomas. *J Pathol* 2003; 200(2): 255.
- van Stipdonk MJ, Lemmens EE, Schoenberger SP. Naive CTLs require a single brief period of antigenic stimulation for clonal expansion and differentiation. *Nat Immunol* 2001; 2(5): 423.
- 49. Melter M, McMahon G, Fang J, et al. Current understanding of chemokine involvement in allograft transplantation. *Pediatr Transplant* 1999; 3(1): 10.
- Lee I, Wang L, Wells AD, et al. Blocking the monocyte chemoattractant protein-1/CCR2 chemokine pathway induces permanent survival of islet allografts through a programmed death-1 ligand-1-dependent mechanism. *J Immunol* 2003; 171(12): 6929.
- Hancock WW, Gao W, Csizmadia V, et al. Donor-derived IP-10 initiates development of acute allograft rejection. J Exp Med 2001; 193(8): 975.
- 52. Gray DW. An overview of the immune system with specific reference to membrane encapsulation and islet transplantation. *Ann N Y Acad Sci* 2001; 944: 226.
- Makhlouf L, Kishimoto K, Smith RN, et al. The role of autoimmunity in islet allograft destruction: major histocompatibility complex class II matching is necessary for autoimmune destruction of allogeneic islet transplants after T-cell costimulatory blockade. *Diabetes* 2002; 51(11): 3202.
- Stegall MD, Lafferty KJ, Kam I, Gill RG. Evidence of recurrent autoimmunity in human allogeneic islet transplantation. *Transplantation* 1996; 61(8): 1272.
- 55. Bartlett ST, Schweitzer EJ, Kuo PC, et al. Prevention of autoimmune islet allograft destruction by engraftment of donor T cells. *Transplantation* 1997; 63(2): 299.