Early islet cell loss is a significant problem in clinical islet cell transplantation. Diverse stress stimuli induce innate immune responses in islets that contribute to β-cell dysfunction, inflammation, and loss. Here, we show that cytokine-stimulated murine islets express multiple inflammatory chemokines that recruit T-cells and thereby impair islet function in vitro and in vivo. Both nonislet ductal and exocrine elements and the individual islet cellular components contribute to this innate immune response. CD4⁺CD25⁺ regulatory T-cells inhibit islet chemokine expression through a cell contact–dependent, soluble factor–independent mechanism and inhibit effector T-cell migration to the islet. Regulatory T-cells can also migrate to stimulated islets. Cotransfer of regulatory T-cells with islets in a transplantation model prevents islet innate immune responses and inflammation and preserves normal architecture and engraftment. Regulatory T-cell inhibition of multiple components of innate immune responses may be a fundamental aspect of their function that influences ischemia-reperfusion injury and adaptive immunity. Diabetes 55:1011–1021, 2006

Islet transplantation offers a potential curative treatment for type 1 diabetes. However, in addition to the antialloantigen adaptive response, antigen-independent innate responses initiated during the peritransplant period contribute to islet cell loss and β-cell dysfunction. Isolated islets constitutively produce chemokines, and chemokine production is stimulated by local and systemic factors such as brain death, surgical manipulation, ischemia-reperfusion, cytokines, and gene transfer vectors (1–3). These stimuli impair survival of allogeneic and even syngeneic islet grafts, and decreased survival is associated with increased inflammation in the graft, due in large part to chemokines produced by the islets (4–7). The role of innate responses in transplant immunity and the benefit of regulating innate immune responses to graft survival have recently been explored (8,9). Modulating innate immunity and chemokine responses may prove to be more effective in preventing detrimental adaptive responses than protocols that rely solely on targeting adaptive immunity. Moreover, because of the redundancy and degeneracy of chemokine and other innate responses, interventions that focus on single molecular pathways are unlikely to successfully control inflammation. Rather, it is important to define mechanisms that simultaneously regulate multiple aspects of the broad innate response.

CD4⁺CD25⁺, anergic, and Foxp3⁺ regulatory T-cells have emerged as major cellular elements that regulate immune responses (10,11) and have clinical potential to treat diverse immune and inflammatory diseases. Regulatory T-cells have been shown to prevent or even reverse autoimmunity in vivo (12) and play important roles in islet alloimmune responses and tolerance to alloantigen (13). In autoimmune diabetes models, regulatory T-cells are able to migrate to the pancreas, the islet, and peripancreatic lymph nodes, showing the importance of chemokines and chemokine responses in their suppressive function (12,14). Regulatory T-cells use a variety of mechanisms to modulate immune responses. In vivo studies suggest that interleukin (IL)-10, CTLA-4, and transforming growth factor (TGF)-β play important roles in regulatory T-cell effector function (15–17). In vitro studies show that regulatory T-cells inhibit the activation and proliferation of other T-cells, either directly through a cell contact–dependent mechanism (18) or indirectly by downregulating antigen-presenting cell activity (19), although regulatory T-cell effector mechanisms remain mostly undefined. Whereas much is known about regulatory T-cell inhibition of adaptive immunity, little is known about their role in other inflammatory events, although recent studies show that regulatory T-cells can inhibit some aspects of innate immunity (20,21). In the current report, we explored the role of regulatory T-cells in modulating islet innate immune responses. The results demonstrate a significant role for regulatory T-cells in modifying many aspects of pancreatic cell innate immunity with subsequent effects on inflammation, islet dysfunction, and graft survival.

**RESEARCH DESIGN AND METHODS**

Inbred BALB/c (H-2b) male mice were purchased from The Jackson Laboratories (Bar Harbor, ME). C57BL/6 mice on a BALB/c background (22) were a gift from Dr. Craig Gerard (Harvard Medical School, Boston, MA). Mice were housed under specific pathogen-free conditions. Animals were treated in strict compliance with regulations and all experiment protocols were approved by the institutional animal care and use committee.
CDC4/225\(^{+}\) or CDC4/225\(^{+}\) cells (5 \times 10^5) were added to stimulated islets at a ratio of one lymphocyte to four islet single cells (assuming one islet contains 1,000 single cells). Islets and T-cell populations were cocultured in serum-free RPMI 1640 for 2 days for in vitro experiments or for 2 h followed by cotransplantation to diabetic mice.

Islet grafts were retrieved from individual animals and embedded in OCT, and consecutive 8-\(\mu\)m sections of the islet grafts were immunostained with anti–CD4, anti–CD45, anti–Ly-6G, and anti–F4/80-like receptor. The number of positively stained cells (brown in each case) were selected, and the number of cells per square millimeter in the islet grafts was counted (27). Quantification data present 10 nonoverlapping fields per graft from two individual mice per group.

**RNA isolation, cDNA synthesis, and quantitative real-time PCR.** RNA isolation, cDNA synthesis, and quantitative real-time PCR were performed as previously described (23,24). The PCR primers were designed to be intron-spanning; sequences are available upon request.

**Multiplex chemokine assay.** Conditioned islet media were assayed for CCL2, CCL5/MIP-1\(\alpha\), CXCL1/KC, CXCL9, and CXCL10/IP-10 with a chemokine 5-Plex bead immunoassay kit (Biosource International, Camarillo, CA), according to the manufacturer’s instructions.

**Migration assays.** Cells (1–5 \times 10^5) were added to the top chamber of a 24-well transwell plate, and chemokine-driven migration was quantified as previously described (28). The results are expressed as the percentage of migrated cells. Controls showed that all migration was chemotactic and not chemokinetic (data not shown).
Migration and insulin release results represent mean values of duplicate to triplicate samples. Analysis for statistically significant differences was performed with Student’s t test. P/H11021 0.05 was considered a significant difference. Results are expressed as means ± SE.

RESULTS

Islets produce chemokines in response to inflammatory cytokines. To characterize islet innate immune responses, we identified constitutive and cytokine-stimulated islet chemokines in vitro. Isolated islets were incubated without or with a well-characterized combination of cytokines (TNF-α, IL-1β, and IFN-α) that resemble inflammatory stimuli (1,2,7). After culture for 1 h, islets were washed and cultured for 2 days, and a comprehensive panel of chemokines was screened using quantitative real-time RT-PCR. Figure 1A shows that only some homeostatic chemokines, such as CCL25 and CCL28, have relatively high expression in untreated islets (>5% of ubiquitin). Similar to our previous findings with a cDNA array (7), cytokine stimulation upregulated inflammatory chemokines, such as CCL2, -3, and -5 and CXCL1, -2, -9, -10, and -11 but not homeostatic chemokines. Cytokine-induced upregulation of chemokines was dose dependent (Fig. 1B), and single islet cells produced more than intact islets, probably reflecting the more vigorous chemical and physical manipulation required to make single islet cell suspensions and also more complete exposure of single cells to the cytokine stimuli. These findings were confirmed at the protein level by multiplex chemokine assay of cultured medium (Fig. 1C). Migration assays with conditioned media from cytokine-stimulated islets showed enhanced recruitment of T-cells (Fig. 1D).

Dendritic cells, endothelial cells, and ductal and exocrine debris are major contributors to islet chemokine responses. It is technically inevitable that exocrine and ductal cells will be present as residual debris in isolated islets. These nonislet cells may affect islet chemokine expression. To test this, basal and adenovirus vector-induced cytokine and chemokine expression in islets of 90 (standard islet purity after isolation), 75, and 50% purity (by adding exocrine debris) were assayed by RT-PCR. Adenovirus vector transduction is another innate immune response model efficient in both islet and solid organ...
Data represent results from three experiments.

CXCL10 in islets with debris than in purer islets (Fig. 2). Production resulted in greater expression of CCL5 and compared with purer islets (Fig. 2).

Islets of 75% and 50% purity express more proinflammatory cytokines (TNF-α) and similar chemokines at baseline compared with purer islets (Fig. 2A). Adenovirus vector transduction resulted in greater expression of CCL5 and CXCL9 in islets with debris than in purer islets (Fig. 2A), showing that debris exaggerate the chemokine response synergistically with other inflammatory stimuli.

Islets contain several cell types: a majority of β-cells, a few α- and δ-cells, vascular endothelial cells, and some immune cells (resident macrophages, dendritic cells, and natural killer). Non-β-cells could be separated from single islet cell suspensions by cell sorting using β-cell autofluorescence (26). The enriched endocrine β-cells express less basal and cytokine-stimulated chemokines than non-β-cells (Fig. 2B). Basal and cytokine-stimulated chemokine expression is significantly decreased after depletion of vascular endothelial cells (CD31+) and dendritic cells (CD11c+) by cell sorting (Fig. 2C). Intact islets were cultured for 5 days to permit intraislet immune cells to migrate out (data not shown) (29). Basal and viral vector-stimulated expression of IFN-β and inflammatory chemokines (CCL2, CCL5, and CXCL9) is lower after a 5-day culture (Fig. 2D).

Together, these results demonstrate that different cellular components of islets produce or stimulate chemokines in response to proinflammatory stimuli and suggest that leukocytes, endothelial cells, and ductal and exocrine elements are all major contributors.

Innate immune responses impair islet function directly or by recruiting effector leukocytes. We next characterized the effects of these induced chemokines on islet function. Groups of 150 islets were treated with a mixture of cytokines (TNF-α, IFN-β, and IL-1β) for 1 h, washed, and cultured in a transwell plate. Splenocytes (5 x 10⁵) were loaded in an insert on top of these cultures to allow splenocytes to migrate to islets for 3 h. Islets were then incubated with migrated splenocytes for 24 h. Additional groups of 150 freshly isolated islets were incubated with a mixture of chemokines (5 ng/ml TNF-α, 100 units/ml IFN-β, and 1 ng/ml IL-1β) for 1 h, a mixture of chemokines (0.2 μg/ml CCL2, 0.5 μg/ml CCL19, and 0.1 μg/ml CXCL9), or 5 x 10⁶ splenocytes for 24 h. Islet function in each group was determined by insulin release in response to 5.5 and 20 mmol/l glucose stimulation. *P < 0.05 vs. 20 mmol/l stimulated control. Data represent results from three experiments.

Regulatory T-cells inhibit islet innate chemokine responses in vitro. Because there must be mechanisms that normally regulate islet responses to the many environmental challenges that impinge upon the pancreas, we explored the effect of regulatory T-cells on islet innate immunity by evaluating cytokine-stimulated chemokine induction. Purified islets were treated with cytokines as above and then cocultured for 48 h with natural CD4⁺CD25⁺ regulatory T-cells, TGF-β-driven de novo regulatory T-cells (24), or control CD4⁺CD25⁻ T-cells. Fresh or cultured natural CD4⁺CD25⁺ regulatory T-cells and TGF-β-driven de novo regulatory T-cells used in the experiments were routinely tested for Foxp3 expression and suppressive function. Foxp3 mRNA expression was at least 100 (natural regulatory T-cells) to 50 (de novo regulatory T-cells) times higher than in control cells, and regulatory T-cells significantly inhibited anti-CD3 monoclonal antibody (mAb)–stimulated T-cell proliferation at a ratio of one regulatory T-cell to four T-cells by at least 70%. Islets were handpicked to separate them from the T-cells at the end of the coculture, islet RNA was isolated for RT-PCR, and culture supernatants were saved for protein and migration assays. Fresh or cultured natural CD4⁺CD25⁺ regulatory T-cells and TGF-β-driven de novo regulatory T-cells inhibit many inflammatory chemokines induced by cytokines, such as CCL2, CCL5, CXCL9, and CXCL10, on the transcriptional (Fig. 4A) and translational (Fig. 4B) levels. Coculture with control T-cells did not have an inhibitory effect on chemokine mRNA expression, protein expression, or migration activity. In migration assays (Fig. 4C), media from cytokine-stimulated islets recruited more splenocytes, and leukocyte recruitment activity was partially reversed by coculture with natural regulatory T-cells or TGF-β-driven de novo regulatory T-cells. Chemokine induction could be suppressed by natural regulatory T-cells in all experiments, but only CCL2 and CXCL9 of the five tested chemokines were inhibited by TGF-β-driven de novo regulatory T-cells in some experiments.

Regulatory T-cells migrate to cytokine-stimulated islets. The above results demonstrate that the direct addition of regulatory T-cells to islets inhibits cytokine-stimulated innate chemokine responses. It was next important to determine whether regulatory T-cells can migrate to islets, whether this migration is mechanistically similar to that used by control T-cells, and whether migrated regulatory T-cells have the same inhibitory effect as directly added regulatory T-cells. The chemokine-receptor expression profiles of freshly isolated CD4⁺CD25⁺ and CD4⁺CD25⁻ T-cells were examined by RT-PCR. Most chemokine receptors are expressed at comparable levels, except that CCR6 expression is higher on CD4⁺CD25⁻ regulatory T-cells, whereas CXCR6 expression is higher on CD4⁺CD25⁻ cells (Fig. 5A). In migration assays, fresh, cultured, and TGF-β-driven de novo regulatory T-cells all have similar abilities to migrate to islet-conditioned media,
comparable with control fresh CD4⁺CD25⁻ T-cells (Fig. 5B). Antibodies to CCL17 (CCR4 ligand) and CCL20 (CCR6 ligand) partially blocked the migration of both control T-cells and regulatory T-cells to cytokine-stimulated islets. Blocking major inflammatory chemokine and receptor pathways by using CXCR3⁻/⁻/⁻ T-cells along with anti-CCL2 (CCR2 ligand) and anti-CCL5 (CCR5 ligand) antibodies inhibited CD4⁺CD25⁻ control T-cell but not regulatory T-cell migration (Fig. 5C), suggesting that regulatory T-cells use a different but overlapping array of chemokine-receptor interactions to migrate to islets.

We next tested the suppressive effect of regulatory T-cells that migrated to stimulated islets. Purified islets were treated with cytokines for 1 h and washed, and $5 \times 10^5$ fresh regulatory T-cells or control T-cells were placed in the upper chamber of the transwell system and allowed to migrate to the stimulated islets overnight. Approximately 10% of the input cells, i.e., $5 \times 10^5$ cells, migrated to the islets, equivalent to the number of T-cells placed directly into the islet cultures. After migration, islets were cultured with the migrated T-cells for an additional 24 h.

The migrated regulatory T-cells inhibit cytokine-stimulated inflammatory chemokine expression in islets as potently as directly applied regulatory T-cells (Fig. 5D).

**Regulatory T-cells inhibit islet chemokine responses through a cell contact–dependent, soluble factor–independent mechanism.** Although most investigators have found that the suppressive properties of CD4⁺CD25⁻ regulatory T-cells in vitro are strictly cell contact dependent and independent of soluble agents, studies in vivo suggest that IL-10 and TGF-β are important for their suppressive effects (15–17). To determine whether regulatory T-cells suppress chemokine responses by direct contact or soluble mediators in the islet cultures, a 0.4-μm transwell system was used to prevent direct contact of regulatory T-cells and islets during coculture. The results in Fig. 6A show that direct contact of regulatory T-cells and cytokine-stimulated islets is required to inhibit chemokine expression. To determine whether soluble mediators might also contribute to regulatory T-cell activity, anti-TGF-β, anti-IL-10, anti-IL-10R1, and anti-CTLA-4 mAbs were added to islet–regulatory T-cell cocultures.
separately or as a mixture. The results in Fig. 6B and C demonstrate that these antibodies do not abrogate regulatory T-cell inhibition of cytokine-stimulated chemokine induction in islets, suggesting these molecules play a limited role in regulatory T-cell function in this model.

**Regulatory T-cells inhibit IL-6 production by stimulated islets.** In addition to expressing inflammatory chemokines in response to innate immune stimulation, islets are also able to express a variety of other molecules. In particular, we evaluated IL-6, another acute inflammatory cytokine expressed by islets (30) that has potent chemotactic activities (31). The results in Fig. 7A show that cytokine-stimulated islets express IL-6 mRNA, and IL-6 is inhibited by coculture with regulatory T-cells. The inhibitory effect of regulatory T-cells on IL-6 expression is cell contact dependent and IL-10, CTLA4, and TGF-β independent (Fig. 7B). Transwell migration assays showed that IL-6 is able to recruit splenocytes (Fig. 7C). Thus, regulatory T-cells modulate islet innate immunity by suppressing both chemokines and cytokines, limiting their effect on effector leukocyte recruitment. Additional experiments showed that IL-6 is a better chemoattractant for CD4^+^CD25^+^ T-cells than for CD4^+^CD25^−^ regulatory T-cells (Fig. 7D). This along with the finding that blockade of inflammatory chemokine-receptor pathways inhibit effector T-cell but not regulatory T-cell migration (Fig. 5C) suggests that regulatory T-cells may preferentially inhibit the recruitment of effector leukocytes but not the influx of additional regulatory T-cells.

**Regulatory T-cells protect islet grafts from innate immune injury and enhance islet graft function.** We next explored the utility of regulatory T-cells in an islet transplant model to determine whether these cells could regulate leukocyte infiltration and protect grafts at early stages after transplantation. To focus on innate immunity, we used a marginal mass, syngeneic islet transplantation model in BALB/c mice (3). As shown in Fig. 8A, untreated control but not cytokine-stimulated islets reverse hyperglycemia 24 h after transplantation. Cotransfer of regulatory T-cells but not control T-cells with the islets re-establishes diabetes cure in cytokine-stimulated islets.

Islet grafts were harvested 1 and 4 days after transplantation and assayed for chemokine expression by RT-PCR and inflammatory infiltrates by immunohistochemistry. Inflammatory CCL5 and CXCL10 expression predominates at day 1 and decreases by day 4 in controls, confirming that this group of chemokines contributes to the very early, innate response. Cytokine-stimulated islets express higher
FIG. 6. Regulatory T-cells inhibit islet chemokine responses through cell contact–dependent mechanisms. A: Groups of 200 islets were exposed to a mixture of cytokines (5 ng/ml TNF-α, 100 units/ml IFN-β, and 1 ng/ml IL-1β) for 1 h and then washed. Five × 10⁴ of fresh CD4⁺CD25⁺ regulatory T-cells or CD4⁺CD25⁻ control T-cells were placed directly into islet cultures or put into a transwell insert with a 0.4-μm-pore-size membrane to separate them from lower chamber islets and then cultured in serum-free media for 2 days. Chemokine mRNA expression in islets was determined by real-time RT-PCR. Data are representative of three similar experiments. B and C: Groups of 200 islets were exposed to a mixture of cytokines (5 ng/ml TNF-α, 100 units/ml IFN-β, and 1 ng/ml IL-1β) for 1 h, washed, and then cocultured with 5 × 10⁴ fresh CD4⁺CD25⁺ regulatory T-cells or CD4⁺CD25⁻ control T-cells for 2 days. Ten micrograms per milliliter anti–CTLA-4, anti–IL-10, anti–IL-10R1, and anti–TGF-β2 antibodies were added individually (B) or as a mixture (C) to islet–regulatory T-cell cocultures. Chemokine mRNA expression in islets was determined by real-time RT-PCR. Data are representative of two similar experiments.
CXCL10 and similar CCL5 levels as controls. Their cognate receptors, CCR5 and CXCR3, known to be expressed on activated Th1 cells (32), are expressed at higher levels in cytokine-treated islets on both days 1 and 4, suggesting recruitment of more inflammatory cells in these islets. Transplanted control islets express higher levels of CCL5 and CXCL10, compared with control islets in vitro (Fig. 8A), suggesting that surgical manipulation and ischemia induced inflammatory chemokine expression in islets. Cotransfer of regulatory T-cells with cytokine-treated islet grafts reversed inflammatory chemokine and leukocyte infiltration with preservation of islet structure. Because there were few islet-associated lymphocytes in the regulatory T-cell group, this suggests that the cells may have apotosed or migrated to draining lymph nodes.

**DISCUSSION**

In this study, we characterized the islet innate immune response using a cytokine-stimulated model that resembles the inflammatory events that impinge on donor islets and explored the potential role of regulatory T-cells in regulating innate immunity and facilitating islet engraftment. We found that islets respond to multiple stimuli, which act through cytokine receptors, toll-like receptors, and probably other receptor pathways, by producing inflammatory chemokines. These chemokines attract lymphocytes, amplify inflammation, impair islet engraftment, and decrease graft survival. These chemokines are produced both by ductal and exocrine contaminants and by multiple different cellular components of islets, including β-cells, dendritic cells, and vascular endothelial cells. Regulatory T-cells are able to migrate to inflamed islets and inhibit islet chemokine expression and production, thereby decreasing inflammation and improving graft survival.

Recent studies demonstrate that suppressive properties of regulatory T-cells are not limited to effects on T-cell responses but also include inhibition of pathology mediated by the cells of innate immune system (20,21,33). In our study, regulatory T-cells directly interact with pancreatic islets, in the absence of effector T-cells, to inhibit the innate response of islets for chemokine production. These findings may partly explain the current failure to fully define regulatory T-cell effector function because most investigations have focused only on regulatory T-cell interactions with effector T-cells and antigen-presenting cell in adaptive immunity. The results here suggest direct interactions between regulatory T-cells and parenchymal cells, so investigations of regulatory T-cell mechanisms will now have to focus on interactions that can be sustained by parenchymal cells. Given recent considerations that adaptive immunity is highly dependent on innate immunity and pathogenesis often results from the inability of regulatory T-cells to shut down inflammation, these studies will be critical to understanding islet engraftment and improving graft survival.
immunity (34), the results here suggest that manipulation of regulatory T-cells to modulate innate immunity will not only decrease acute injury but also decrease innate amplification of subsequent adaptive immunity.

As a functional unit, islets are composed of multiple cellular components, although few studies have explored the individual contribution of the different components to the islet innate immune response. In the current study, we demonstrated that dendritic cells, vascular endothelial cells, and ductal and exocrine debris contribute to a large degree to islet chemokine production and the innate immune response (Fig. 2). Because islet revascularization is critical to islet engraftment and survival (25,35) and because dendritic cells can function both as initiators of the immune response and as inducers of peripheral tolerance (36), it will be necessary to modulate islet endothelial cell and dendritic cell function rather than simply deplete these cells as strategies to improve islet transplantation. This may be achieved via the introduction of regulatory T-cells into islets or modification of dendritic cells or vascular endothelial cells to support the migration, expansion, or differentiation of regulatory T-cells.

The ability of regulatory T-cells to inhibit a range of immune responses and target a variety of cells suggests that they use multiple different effector mechanisms. Many in vivo studies show that IL-10, CTLA4, and TGF-β play important roles in regulatory T-cell function (15–17). There is evidence that PD-1/PD-L1 (37), CD30 (38), TRANCE-RANK (39), cytolytic activity through granzyme A (40) or granzyme B (41), Lag-3 (42), and production of indoleamine 2,3-dioxygenase by dendritic cells and HO-1 by regulatory T-cells (43) are also involved in regulatory
T-cell–suppressive function. In the current study, we found that the suppressive function of regulatory T-cells on islets is cell contact dependent and IL-10, TGF-β, or CTLA4 independent (similar observations rev. in 11). Our findings suggest investigations of regulatory T-cell–parenchymal cell, –dendritic cell, or –vascular endothelial cell interactions as novel avenues of experimental inquiry. Because parenchymal and vascular endothelial cells would not be expected to express the ligands for most of the mechanisms cited above, it is likely that there are other important regulatory T-cell molecular mediators remaining to be characterized.

As for other effector T-cells, regulatory T-cells must migrate to the proper location, traffic to a microdomain within that location, adhere to stromal cells, and deploy effector functions. Although a number of studies have examined regulatory T-cell chemotaxis, migration, and receptor expression, there is little agreement among the reports. Regulatory T-cells have been variously described by different groups as bearing exclusively CCR4 and CCR8 (44) or higher CCR6 (45). We found that most chemokine receptors are expressed at comparable levels on both CD4+CD25+ and CD4+CD25− cells (Fig. 5A). However, similar chemokine-receptor expression profiles do not necessarily mean identical receptor usage for chemotaxis. In the current study, we found that regulatory T-cells and CD4+CD25− T-cells migrate similarly to mixed islet chemokines but not to IL-6, blockade of CCR4 and CCR6 partially inhibited the migration of both control T-cells and regulatory T-cells, and yet blockade of CCL2, CCL5, and CXCR3 inhibited only control T-cells. Together, the findings suggest that regulatory T-cells and effector T-cells use an overlapping array of chemokine-receptor interactions for migration and trafficking. Thus, depending on the nature of the inflammatory stimuli, different populations of regulatory or effector T-cells may traffic to a region of tissue injury. Regulatory T-cells can also change receptor or integrin expression patterns to home to lymphoid organs or exit to inflamed tissue (46). As demonstrated here, regulatory T-cells migrate to islets, interact with islet parenchymal cells or intraislet leukocytes, and suppress islet immune responses. Regulatory T-cells can respond to CCL19 and CCL21 (47,48) and migrate to peripancreatic lymph nodes (14). In the current study, cotransferred regulatory T-cells prevent islet graft inflammation, but they were not present in large numbers under the kidney capsule, even 1 day after transfer, suggesting that they may have had an initial effect on the islets, and then migrated to draining lymph nodes or even efferent lymphatics and the general circulation to have more widespread regional or systemic effects on inflammation. Our current studies are directed to determine whether cotransferred regulatory T-cells do migrate to draining lymph nodes to inhibit effector cell recruitment.

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