

α 1-Antitrypsin monotherapy induces immune tolerance during islet allograft transplantation in mice

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Human pancreatic islet transplantation offers diabetic patients tight glucose control but has low graft survival rates. The immunosuppressive drugs that are administered to graft recipients lack the antiinflammatory benefits of corticosteroids because of their diabetogenic effects. The serum protease inhibitor α 1-antitrypsin (AAT) possesses antiinflammatory properties and reduces cytokine-mediated islet damage. In the present study, diabetic mice were grafted with allogeneic islets and treated with AAT monotherapy ($n = 24$). After 14 days of treatment, mice remained normoglycemic and islet allografts were functional for up to 120 treatment-free days. After graft removal and retransplantation, mice accepted same-strain islets but rejected third-strain islets, thus confirming that specific immune tolerance had been induced. Explanted grafts exhibited a population of T regulatory cells in transplant sites. According to RT-PCR, grafts contained high levels of mRNA for foxp3, cytotoxic T lymphocyte antigen-4, TGF- β , IL-10, and IL-1 receptor antagonist; expression of proinflammatory mediators was low or absent. After implantation of skin allografts, AAT-treated mice had greater numbers of foxp3-positive cells in draining lymph nodes (DLNs) compared with control treatment mice. Moreover, dendritic cells in DLNs exhibited an immature phenotype with decreased CD86 activation marker. Although the number of CD3 transcripts decreased in the DLNs, AAT did not affect IL-2 activity *in vitro*. Thus, AAT monotherapy provides allografts with antiinflammatory conditions that favor development of antigen-specific T regulatory cells. Because AAT treatment in humans is safe, its use during human islet transplantation may be considered.

dendritic cells | diabetes | interleukin-1 | interleukin-10 | T-regulatory cells

Islet loss of function in most islet transplant patients steadily progresses after grafting and results in a low 5-year graft survival rate (1, 2). The immunosuppressive agents used for islet transplantation exclude diabetogenic corticosteroids, and the grafted cells lack the benefit of antiinflammatory therapy (3). Because islets are particularly prone to injury during inflammatory conditions (4), the functioning islet mass rapidly decreases after transplantation before antigenic recognition (5). As damage intensifies, necrotic islet cells become a source of damage-associated molecular patterns, which induce the production of injurious cytokines and chemokines that facilitate alloantigen presentation to the recipient (6); the host immune system is thus alerted to the alloantigen-rich site (7).

The favorable state of immune tolerance can be elaborated by a shift in balance between effector T cells and protective regulatory T (Treg) cells (8), a process that requires, contrary to immunosuppression, the uninterrupted activity of IL-2 (9, 10). By reducing the intensity of inflammation while allowing IL-2 activity, one may provide the conditions for prolonged allograft survival and tolerance induction (8).

In addition to its ability to inhibit serine proteases, α 1-antitrypsin (AAT) possesses antiinflammatory properties (11–

13). We reported that AAT prevented the demise of islet beta cells from normal mice, enabling insulin secretion in the presence of IL-1 β and IFN- γ and reducing cytokine and chemokine secretion (13). Administered to animals, AAT reduced the susceptibility of islets to inflammation and prolonged islet allograft survival (13). Importantly, as reported elsewhere (14) and verified in the present study, AAT treatment allows for IL-2 activity while suppressing inflammation.

In the present study, the impact of extended human AAT (hAAT) monotherapy on the process of allograft rejection was examined in the setting of a normal immune system. Unexpectedly, hAAT therapy withdrawal revealed the remarkable induction of treatment-induced strain-specific immune tolerance.

Results

Prolonged Administration of hAAT to Diabetic Islet Allograft Recipient Mice Results in Strain-Specific Immune Tolerance. To examine the outcome of islet allograft transplantation during extended monotherapy with hAAT, mice heterozygous for tissue-specific hAAT [hAAT transgene (hAAT-Tg)], which exhibit levels of circulating hAAT that are below detection, were used as graft recipients. hAAT-Tg mice (H-2^b) were rendered diabetic, transplanted with allogeneic islets (H-2^d), and treated with serial doses of hAAT ($n = 24$) or albumin ($n = 6$). As shown in Fig. 1A, control albumin-treated mice rejected allografts by day 12. In contrast, all hAAT-treated mice that were treated for the various durations indicated exhibited extended normoglycemia. The shortest course of hAAT therapy, 14 days, resulted in delayed loss of function in 50% of the transplants and in graft acceptance in the remainder. A 21-day course resulted in a single delayed graft failure event of the six transplanted islet grafts. All 12 mice that received hAAT treatment for 30 days or more achieved graft acceptance (treatment duration, 30 days, $n = 8$; 41 days, $n = 1$; 52 days, $n = 2$; 60 days, $n = 1$).

Removal of grafts by nephrectomy restored hyperglycemia. Shown as an example in Fig. 1C *Left* and *Right*, by day 12 after transplantation the albumin-treated mouse (broken line) had mounted an acute allograft rejection response and developed overt hyperglycemia, whereas the hAAT-treated mice maintained normoglycemia for the duration of therapy. After with-

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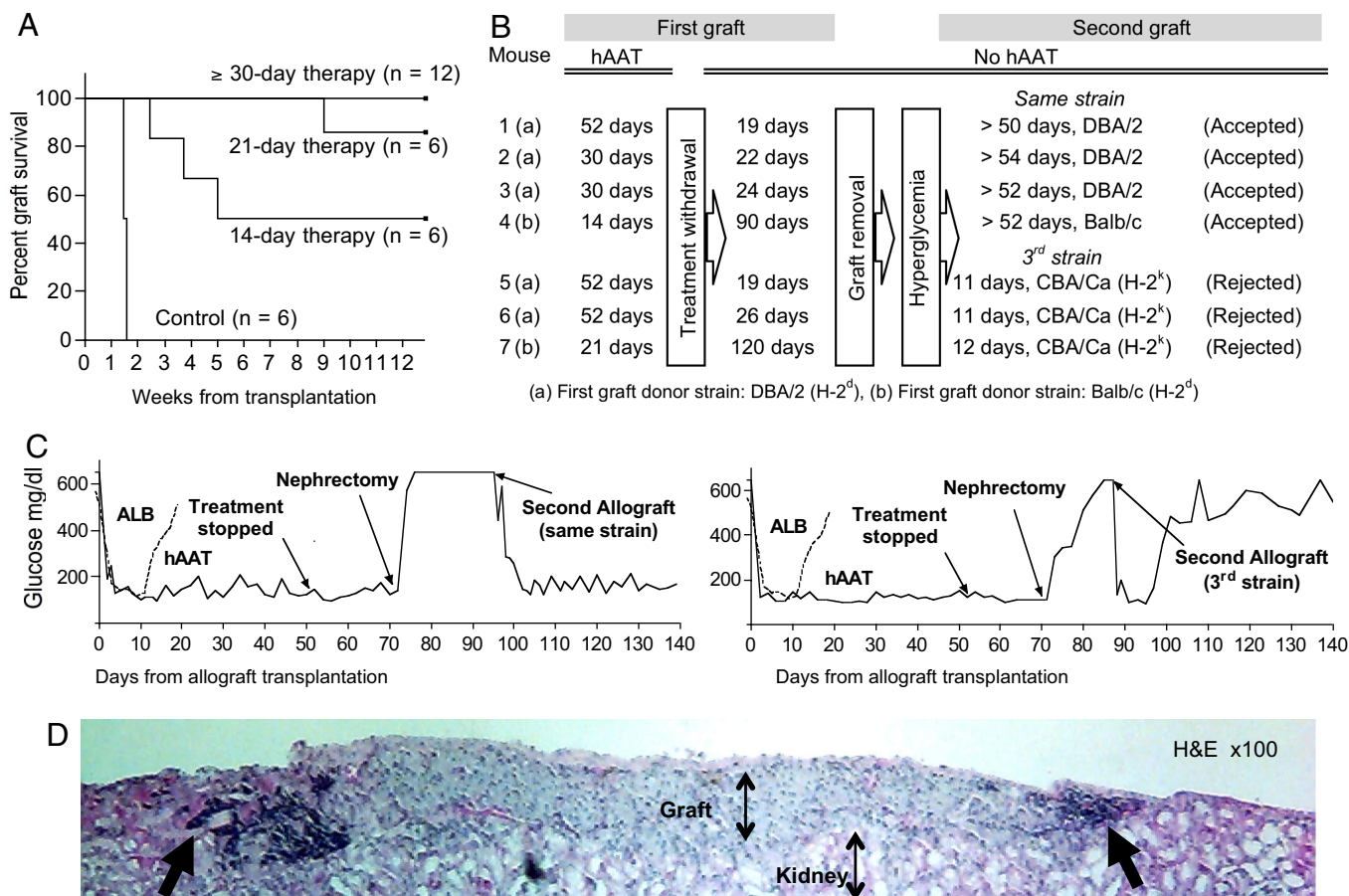


Fig. 1. Extended AAT monotherapy induces strain-specific immune tolerance toward islet allografts in mice. Islet allograft transplantation was performed and blood glucose was followed in mice that received albumin (ALB) ($n = 6$) or hAAT monotherapy ($n = 24$) for various periods of time. (A) Islet graft survival curve. (B) Summary of uninterrupted normoglycemic intervals achieved during and after hAAT monotherapy ("First graft") and during a second grafting procedure that was carried out in explanted animals in the absence of therapy ("Second graft") ($n = 7$). Double-underlined headings indicate number of hAAT monotherapy and therapy-free days. The outcome of the second grafting procedure is indicated for individual mice. (C) Representative blood glucose follow-up. Albumin (ALB)-treated animals are represented by a dashed line. Day of hAAT treatment withdrawal is indicated. Treatment-free glucose levels were determined during the ensuing days. Graft removal by nephrectomy, resulting in hyperglycemia, is indicated. A second grafting without further hAAT treatment was performed with same strain islet allograft (Left) or third strain islet allograft (Right). Transplantation outcome of the second grafting is monitored for 50 days. (D) Histology. Representative day 72 explanted graft from hAAT-treated mice 20 days after withdrawal of hAAT treatment. H&E stain, image of entire islet graft site, is shown. Islet mass appears flanked by a dense mononuclear cell population (thick arrows).

drawal of hAAT, continued graft-derived insulin production was observed (Fig. 1 B, second column, and C, days 52–72), raising the possibility that allospecific immune tolerance was achieved. To examine this possibility, grafts were removed and a second grafting procedure was undertaken in the subcapsular space of the remaining kidney without further treatment, using the same strain of islets that had been originally transplanted ($n = 4$; H-2^d) (Fig. 1B and illustrated in an example in Fig. 1C Left). In each case, after reengraftment, recipient mice remained normoglycemic for >50 days. To ascertain that antigen-specific immune tolerance had been induced, islets from a third strain (H-2^k) were used as the source of the second graft in three hyperglycemic mice without further hAAT treatment (Fig. 1 B and C Right). As shown, all three mice exhibited acute rejection of third-strain allografts.

According to histology, a "cuff" of mononuclear cells surrounded the entire islet mass in all explanted grafts (Fig. 1D). The mononuclear cells were located at the intersection between the renal parenchyma and capsule, flanking an intact islet graft mass. By staining for several cell-specific markers, we found the near absence of activated macrophages (CD11b) (data not

shown), and a predominance of CD4- or CD8-positive cells, interspersed with CD25-positive cells (data not shown).

Inflammatory and Antiinflammatory Gene Expression in Islet Allografts. We next examined the steady-state levels of inflammation-related genes in explanted islet grafts. Fig. 2 depicts a comparison between mRNA patterns present early after transplantation in albumin-treated mice (days 1, 3, 5, and 7) and those present in long-lasting hAAT-treated islet grafts (representative day 72). As shown, transcripts of genes coding for islet-injurious ligands were low in grafts from hAAT-treated mice. These include IL-1 β , in addition to CD14, a marker for invading macrophages, IL-2 from invading T cells, and intercellular adhesion molecule-1 (ICAM-1). In addition, mRNA transcripts that encode for the pronutrophilic CXC chemokines, KC and macrophage inflammatory protein-2 (MIP-2), were undetectable in long-lasting islet allografts. Islet allograft explants from hAAT-treated mice also exhibited elevated expression of IL-1 receptor antagonist (IL-1Ra) and isoforms of IL-18-binding protein (IL-18BP), both reported to protect islet allografts (15, 16). In contrast, explants of albumin-treated mice exhibited

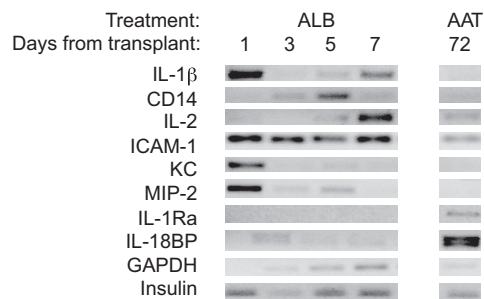


Fig. 2. Effect of hAAT monotherapy on gene expression profile in islet allografts. RT-PCR of explanted islet allografts from albumin (ALB)-treated control and hAAT-treated mice. The left four columns show the initial days after islet transplantation into control mice. The right column shows day 72 after islet transplantation into hAAT-treated mice (see Fig. 1). Data are representative of $n = 6$ (ALB) and $n = 3$ (hAAT; time points between days 30 and 72 after transplantation).

either low or undetectable expression of IL-1Ra and IL-18BP (Fig. 2, days 1, 3, 5, and 7).

Cell-Specific Effects of AAT. The expression of antiinflammatory molecules observed here belongs to grafts that were explanted several weeks after withdrawal of hAAT treatment (see Fig. 1). It is therefore more likely that the intra-graft antiinflammatory gene expression profile reflects the cellular components that accumulated in the antigen-rich site. To examine the effects of hAAT on major cell subpopulations, we performed *in vitro* assays for lymphocytic and nonlymphocytic responses. As shown in Fig. 3, IL-2-stimulated human peripheral blood mononuclear cells (PBMCs) were able to produce IFN- γ and proliferate, as expected, in the presence of hAAT. Similarly, mouse splenocytes responded to Con A with secretion of IFN- γ , as well as increased cell proliferation and cell clumping, each response unaffected by hAAT [supporting information (SI) Fig. S1a]. In contrast to lymphocytic responses, peritoneal macrophages responded to hAAT by secreting significantly less IFN- γ -induced nitric oxide in a concentration-dependent manner (Fig. S1b).

Treg-Related Gene Expression in hAAT-Treated Islet Allografts. In the unique set of genes expressed within grafts of hAAT-treated recipient mice, we also observed the expression of genes indicative of Treg cells (Fig. 4). As shown, grafts from hAAT-treated mice (Fig. 4A, representative day 72) exhibit a significantly elevated expression of foxp3, TGF- β , and cytotoxic T lymphocyte antigen-4 (CTLA-4), representing the expected phenotype of Treg cells (17–20). In contrast, the expression of these genes was either below detection or terminated early in grafts from albumin-treated mice (days 1, 3, 5, and 7). As depicted in Fig. 4B, the presence of foxp3-positive cells was observed as early as day 14 of hAAT therapy in sections that contained the graft site (Fig. 4B, G). Notably, in renal tissue from kidney portions that did not

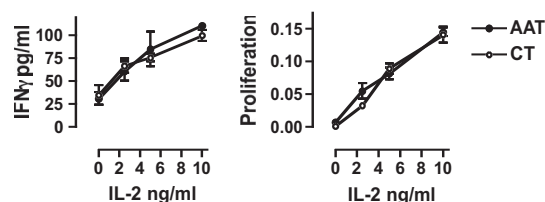


Fig. 3. Cell-specific effects of hAAT. Inducible IFN- γ levels (Left) and cell proliferation (Right) assessed in Con A-primed PBMCs that were stimulated with increasing concentrations of IL-2 in the presence of 0.5 mg/ml hAAT or albumin (CT). Data are mean \pm SEM of three individual donors.

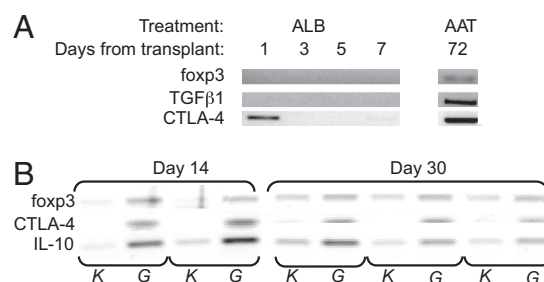


Fig. 4. Identification of hAAT-induced IL-10-expressing Treg cells in nonrejecting islet allografts. (A) RT-PCR of explanted islet allografts in albumin (ALB)-treated graft recipients during acute allograft rejection (left four columns; days 1–7) and hAAT-treated graft recipient 20 days after withdrawal of hAAT treatment (right column; day 72; see Fig. 2). Data are representative of $n = 6$ (ALB) and $n = 3$ (hAAT; representative time point between days 30 and 72 after transplantation). (B) Intra-graft gene expression profile throughout hAAT therapy. RT-PCR of explanted islet allografts in hAAT-treated graft recipients during hAAT treatment. K, tissue from pole opposite to the grafting site; G, intra-graft gene profile.

contain the grafted islets (Fig. 4B, K), foxp3-positive cells were also observed. CTLA-4 expression was present only inside the graft (G). Of particular importance, IL-10 transcript levels were closely associated with foxp3 expression, suggesting that the identified Treg cells are also inducers of IL-10.

Time-Dependent Distribution of Treg Cells Between Draining Lymph Nodes (DLNs) and Allografts. To examine the effect of hAAT on Treg cell development during transplantation, foxp3-GFP knock-in mice were used as graft recipients (C57BL/6 background, H-2^b). A vigorous allogeneic response was evoked by implanting wild-type skin grafts (H-2^d) under the surface of both left and right thighs. Animals were treated with hAAT ($n = 13$) or albumin ($n = 13$) using the same dosing schedule used in the islet transplantation protocol (see Fig. 1). Inguinal DLNs were removed on various days after grafting and CD4⁺-sorted cells were examined by FACS and by RT-PCR for foxp3-positive cells. As shown in Fig. 5A, between transplantation and 3 days after engraftment of islets, the number of foxp3-positive cells in the DLNs unvaryingly decreases in both the albumin control and hAAT-treated graft recipient mice. However, between days 4 and 9, DLNs from hAAT-treated mice had more foxp3-positive cells. In the days that followed, the gap in the size of the Treg population was restored. Gene expression analysis corroborated FACS findings (Fig. 5A Inset).

By using foxp3-GFP knock-in mice as Matrigel skin graft recipients, we were able to observe Treg cells infiltrating allografts by day 10 after transplantation. This model offers a particular advantage because invading fluorescent cells can be directly identified in freshly obtained, unstained specimens by using fluorescent microscopy. As shown in Fig. 5B, invading foxp3-positive cells localized to grafts in hAAT-treated animals (Fig. 5B Lower). Fur is autofluorescent and can be observed. Total intensity of infiltrating cells can be appreciated by DAPI counterstaining. Similarly, as shown in Fig. 5C, islet allografts that had been transplanted into foxp3-GFP knock-in recipient mice also contained foxp3-positive cells in the cuff site.

Early Local and Systemic Effects of hAAT. We next studied the events that might precede the changes observed in the DLNs. Islets embedded into Matrigel allow for examination of islet-driven cellular invasion during the first 48 h after implantation. Allogeneic islets were introduced into hAAT-containing Matrigel plugs (12.5–100 μ g of hAAT per graft) and implanted s.c. into mice. Grafts were retrieved 48 h after transplantation and

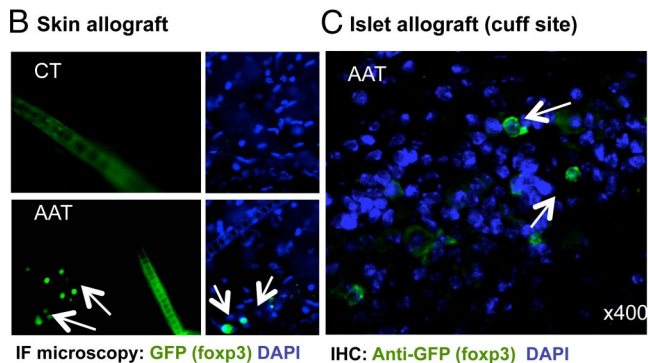
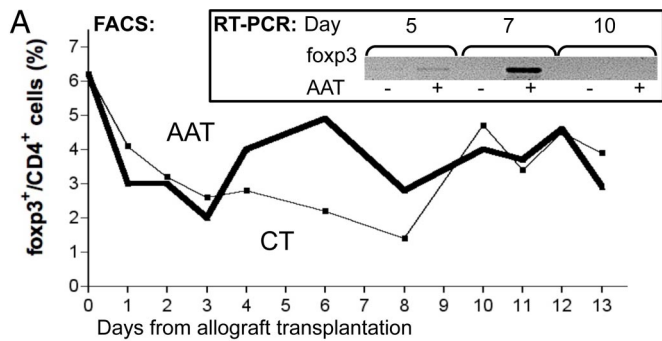


Fig. 5. Time-dependent hAAT-induced distribution of Treg cells between DLNs and allograft. *foxp3*-GFP knock-in mice (H-2^b) were grafted with wild-type BALB/c tissue (H-2^d). Mice received a 10-day hAAT treatment or albumin protocol (see Fig. 1). (A) Inguinal DLN. FACS analysis of CD4⁺-sorted *foxp3*-GFP-positive DLN cells. (Inset) RT-PCR for *foxp3* mRNA transcripts in DLNs. Shown are representative time points. (B) Matrigel-skin graft. Treg cells in Matrigel grafts on day 10 identified by fluorescent microscopy of unstained material (Left) plus DAPI-counterstained material (Right). (C) Islet graft. Day 14 Treg cells identified in the cuff site (see Fig. 1D). Anti-GFP antibody immunostaining and DAPI counterstaining is shown. Representative image of three hAAT-treated grafts is shown. Grafts from albumin-treated mice contained no cuff (data not shown).

intra-graft mRNA levels were assessed. As shown in Fig. 6, a dose-dependent decrease in CD14 mRNA levels had occurred, reflecting hAAT-dependent decrease in macrophage population. The recipient mice carry the hAAT genomic insert, and invading host cells can thus be identified. Indeed, the amount of invading cells decreased in the presence of hAAT, as corroborated by histological examination of the explanted Matrigel at 48 h (data not shown). Copies of insulin mRNA transcripts correlated with the amount of added hAAT, representing improved hAAT-mediated beta cell viability. hAAT treatment also resulted in a dose-dependent increase in VEGF mRNA levels. VEGF mRNA in the Matrigel-islet graft is likely to be of islet cell

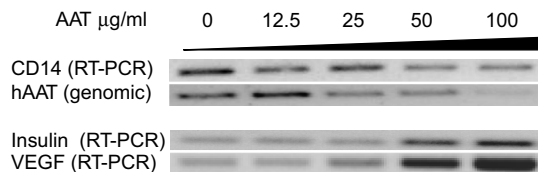


Fig. 6. Early local and systemic effects of hAAT. Wild-type islet-Matrigel grafts containing increasing concentrations of hAAT (indicated, amount per Matrigel) were explanted 48 h after transplantation into hAAT-Tg recipients. (Upper) Identification of CD14-positive cells (RT-PCR) and identification of host cells inside the graft (genomic). (Lower) RT-PCR depiction of insulin and VEGF intra-graft transcripts.

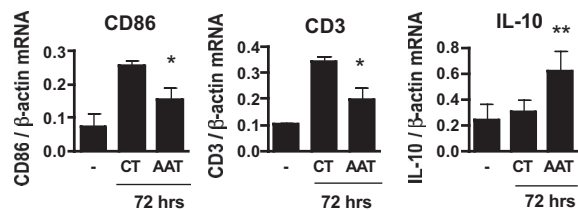


Fig. 7. Effect of AAT on dendritic cell migration and maturation. CD86, MHC class II, and IL-10 expression in renal DLNs. Seventy-two hours after allogeneic skin grafting under the renal capsule, DLNs were harvested and examined by RT-PCR. DLN from nongrafted mice (first bar on left) is compared with 72-h DLN gene expression from untreated (CT) and hAAT-treated (AAT) mice. Shown are mean \pm SEM from three experiments. *, $P < 0.05$; **, $P < 0.01$ between CT and AAT.

origin because VEGF mRNA copies coincided with near absence of host genomic DNA (Fig. 6).

In addition to local events that reflect a dampened inflammatory response and suboptimal antigen presentation environment, we searched for other indications that may support the generation of Tregs. Mice were subjected to 10 days of hAAT treatment to reproduce the circulating cytokine environment of a treated islet graft recipient. Control mice received albumin, and serum levels of cytokines were measured. As shown in Fig. S2, serum levels of Th17-related cytokines, IL-17 and IL-23, were 3-fold lower compared with levels in control mice. Serum IL-6 and monocyte chemoattractant protein-1 were also decreased. However, serum IL-10 levels increased 2-fold and the levels of I-309, a chemoattractant for Treg cells, increased 2-fold. To examine the circulating cytokines evoked during a vigorous inflammatory response, mice were treated for 10 days with hAAT or albumin and then challenged with LPS. Serum cytokines were assessed after 2 h (see Fig. S2). Once again, I-309 and MIP-2 levels exhibited the same changes observed in hAAT-treated but nonchallenged mice. Most strikingly, circulating IL-1Ra levels increased 3-fold. The effect of hAAT treatment on serum IL-6 and IL-10 levels were also measured after a sterile inflammatory response (Fig. S2). In this procedure, the inflammatory response results in increased levels of IL-1 β -dependent IL-6 (21). However, mice treated with hAAT exhibited a 30% decrease in serum IL-6 protein levels and a 27% increase in serum IL-10 protein levels.

Effect of AAT on Dendritic Cell Migration, Maturation, and Function.

To investigate the implications of a dampened antigen presentation process during transplantation, we studied dendritic cell activation *in vitro* and *in vivo*. By using transgenic GFP-positive donor skin grafts and subsequent PCR amplification of DNA isolated from DLNs, we evaluated the migration of graft-derived cells toward DLNs *in vivo* (Fig. S3a). Mice received hAAT 1 day before grafting, as during islet allograft transplantation. Graft-derived DNA was present in DLNs after transplantation in both control and hAAT-treated mice.

We next examined the effect of hAAT on the transcript levels of CD86, CD3, and IL-10 in the DLNs of kidneys in mice receiving skin grafts into the renal subcapsular space (Fig. 7). For background gene expression, DLNs from nontransplanted mice were examined. Seventy-two hours after transplantation, CD86 mRNA transcript levels were reduced 2-fold by hAAT treatment. At the same time point, DLNs contained 2-fold less total CD3 mRNA transcripts. Notably, a 2.5-fold rise in IL-10 gene expression was observed in DLNs from hAAT-treated grafted mice.

To examine the direct effect of hAAT on dendritic cell activation and maturation, dendritic cells were cultured *in vitro* with LPS in the absence or presence of hAAT (Fig. S3b). By

FACS analysis, LPS stimulation in the presence of hAAT resulted in a marked decrease in the levels of inducible surface MHC class II and CD86.

To address the possibility that hAAT treatment had specifically induced IL-10 production in Treg cells *in vivo*, hAAT or albumin was administered 3 days before LPS challenge to foxp3-GFP knock-in mice. Sixteen hours later, spleens were harvested and splenocytes were isolated to examine IL-10 release in a cytometric secretion assay. LPS administration alone resulted in foxp3-positive cells that released IL-10 ($6.1 \pm 0.1\%$, compared with $0.2 \pm 0.1\%$ without LPS). The number of IL-10-secreting Treg cells increased in hAAT-treated mice to $10.6 \pm 1.2\%$ (mean \pm SEM; $P = 0.0167$).

Discussion

In the present study, we demonstrate that monotherapy with hAAT during islet allograft transplantation results in the induction of antigen-specific immune tolerance. Therapy comprised a 1-day preconditioning dose with hAAT followed by serial maintenance injections every 3 days extending for a minimum of 14 days. A population of mononuclear cells that included a subpopulation of Treg cells surrounded the islet mass in the form of a cellular cuff, which had high expression levels of foxp3, TGF- β , CTLA-4, and IL-10, but decreased levels of inflammatory genes. In early days after transplantation, islets embedded into Matrigel plugs that contained hAAT exhibited a dose-dependent improvement in islet viability and a marked decrease in monocyte/macrophage infiltration. These data suggest that immunogenicity of implanted islets is considerably reduced by hAAT. By using skin allografts to evoke a vigorous immune reaction, we observed that the number of Tregs rises in the draining lymph nodes of hAAT-treated graft recipients.

Several studies suggest that Tregs participate in controlling allograft stability (reviewed in ref. 22). To generate Tregs, it is essential that IL-2 activity be maintained. Indeed, as demonstrated *in vitro* and as supported by other studies (23), IL-2 activity is unaffected by the presence of hAAT. Also, pivotal to the differentiation of naive T cells into Treg cells in the DLNs, we confirm that the process of cell migration from the site of transplantation into the DLNs occurs without interruption during hAAT therapy.

It has been shown that dendritic cell-expanded T regulatory cells restore normoglycemia in diabetic NOD mice (24). In a noninflammatory environment, dendritic cells remain immature and promote antigen-specific immune tolerance (25); dendritic cells that migrate to the DLNs express cell surface molecules such as CD86 (also known as B7) (26). Here, we show that soon after grafting of islets into hAAT-treated animals, CD86 and MHC class II are diminished in the DLNs. Our findings coincided with a decrease in total CD3 transcript levels in the associated DLNs and a marked increase in IL-10 expression. We previously reported that hAAT reduces isolation-induced CD45⁺-islet-cell surface MHC class II levels (13). We further show in the present study that hAAT inhibits dendritic cell maturation *in vitro*. As we described in ref. 13, the administration of hAAT before day 3 is essential for the prolongation of islet allograft survival. Here, cellular infiltration into islet grafts within the initial 3 days of engraftment was blocked by hAAT in a dose-dependent manner. AAT has been described as blocking phagocytic cell invasion (13) and both production (27) and activity (28) of IL-8 in human cells. Importantly, IL-8 is produced in large amounts by human islets during and after islet isolation and thus contributes to early graft destruction (29). Administration of AAT before inoculation of foreign cells into the peritoneal cavity reduced the number of infiltrating CD3-positive cells by 50% (13). Conversely, in the same model, by allowing neutrophil and macrophage invasion to reach completion and administering AAT 3 days after the inoculation of

foreign cells, uninterrupted CD3-positive cell infiltration occurred, suggesting that the isolated event of lymphocyte activation is, indeed, intact in the presence of AAT.

IL-1Ra transcripts were elevated in the explanted long-lasting islet allografts. IL-1 β has been shown to break tolerance (30) and facilitate islet antigen presentation (6). In other studies, IL-1Ra inhibited mouse islet allograft rejection (15), increased islet beta cell function during transplantation (31), and improved disease parameters in patients with type 2 diabetes (32). AAT was shown to induce IL-1Ra in human PBMCs (33). The explanted hAAT-treated grafts also exhibited elevated CTLA-4 mRNA levels. The engagement of CTLA-4 participates in the induction of peripheral tolerance (34) and induces the production of TGF- β (35). In addition, AAT treatment reduced the production of IL-6, as shown here and reported by others (36). In the presence of TGF- β , high levels of IL-6 interfere with Treg differentiation in favor of Th17 cells, as reported by Bettelli *et al.* (37). Because IL-1 β is required for Th17 survival (38), it is possible that AAT provides a cytokine milieu that actively supports Treg predominance. Indeed, one of the most significant changes observed in sera from hAAT-treated mice in a protein-blotting array of 36 cytokines was reduced levels of circulating IL-17 and IL-23.

Although AAT inhibits caspase-3 in a rat beta cell line (39), an antiapoptotic function of AAT *per se* is probably incapable of inducing tolerance, because global inhibition of apoptosis during an inflammatory and/or immune response would promote an expanded reactive T cell population. We therefore propose that the cytoprotection observed in the presence of AAT is by virtue of its antiinflammatory properties.

In support of the inclusion of AAT therapy for transplant patients are the long-term safety studies, extending over several years of weekly AAT infusions to patients with various degrees of AAT deficiency (40–42); no evidence of compromised host defense can be demonstrated. We therefore suggest that AAT can be considered for testing in human islet transplantation.

Methods

Mice. hAAT-Tg mice, background strain C57BL/6, were engineered as described in ref. 43 and studied as detailed in *SI Methods*. Circulating levels of hAAT in heterozygous hAAT-Tg mice were determined by a specific ELISA for human AAT, as described in ref. 13. Serum levels were below the limit of detection (10 ng/ml). Foxp3-GFP knock-in mice were kindly provided by T. B. Strom (Harvard Institutes of Medicine, Boston, MA) (37). Wild-type BALB/c, CBA/Ca, and DBA/2 mice were purchased from The Jackson Laboratory. Experiments were approved by the University of Colorado Institutional Animal Care and Use Committee.

Islet Allograft Transplantation. Renal subcapsular islet transplantation was performed as described in ref. 13. Briefly, hAAT-Tg heterozygote mice weighing 25–30 g were rendered diabetic by a single i.p. injection of streptozotocin (225 mg/kg; Sigma-Aldrich). Donor islets were isolated and collected on 100- μ m cell strainer (Falcon; BD Biosciences Discovery Labware), as described in ref. 13. A total of 450 hand-picked isolated islets from DBA/2, BALB/c, C57BL/6, or CBA/Ca donor mice were grafted under the renal subcapsular space. hAAT treatment was initiated 1 day before transplantation and every third day (2 mg per mouse; Aralast; Baxter). Control hAAT-Tg mice received the same amount of human serum albumin (Abbott). In the experiments in which monotherapy exceeded 14 days, the amount of hAAT was increased by 0.5 mg every third day until a 6-mg maintenance dose was reached. Islet allograft rejection was defined as the day blood glucose exceeded 300 mg/dl after a period of at least 3 days of normoglycemia.

Skin Allografts. As donor tissue, 1 mm³ of freshly prepared skin derived from shaved avascular portion of the abdominal midline was used. A graft was inserted into the s.c. space of each thigh in foxp3-GFP knock-in mice through a 1-mm-long incision. Incision site was sealed with a 3-0 suture.

Immuncyte Responses *in Vitro*. PBMCs were isolated from healthy individuals, as described in ref. 44. Studies of human blood were approved by the Colorado Multiple Institutional Review Board. Splenocytes and resident peritoneal

