α_1-Antitrypsin Therapy Downregulates Toll-Like Receptor-Induced IL-1β Responses in Monocytes and Myeloid Dendritic Cells and May Improve Islet Function in Recently Diagnosed Patients With Type 1 Diabetes

Peter A. Gottlieb, Aimon K. Alkanani, Aaron W. Michels, Eli C. Lewis, Leland Shapiro, Charles A. Dinarello, and Danny Zipris

Barbara Davis Center for Childhood Diabetes (P.A.G., A.K.A., A.W.M., D.Z.) and Division of Infectious Diseases (C.A.D.), University of Colorado Denver, Aurora, Colorado 80045; Department of Clinical Biochemistry and Pharmacology (E.C.L.), Faculty of Health Sciences, Ben-Gurion University of the Negev, 84105 Beer-Sheva, Israel; and Department of Medicine (L.S.), Division of Infectious Diseases, Veterans Affairs Medical Center and University of Colorado Denver, Denver, Colorado 80202

Context: Recent studies have implicated proinflammatory responses in the mechanism of type 1 diabetes (T1D).

Objective: Our objective was to evaluate the safety and effects of therapy with the anti-inflammatory serum protein α_1-antitrypsin (AAT) on islet function and innate immunity in recent-onset patients.

Design and Setting: This was an open-label phase I trial at the Barbara Davis Center for Childhood Diabetes, University of Colorado Denver.

Patients: Twelve recently diagnosed subjects with T1D with detectable C-peptides were included in the study.

Intervention: Eight consecutive weekly infusions of 80 mg/kg of AAT were given.

Main Outcome Measures: Patients were monitored for adverse effects of AAT therapy, C-peptide responses to a mixed-meal tolerance test, and toll-like receptor (TLR)-induced cellular IL-1β in monocytes and myeloid dendritic cells (mDCs).

Results: No adverse effects were detected. AAT led to increased, unchanged, or moderately reduced levels of C-peptide responses compared with baseline in 5 patients. The total content of TLR4-induced cellular IL-1β in monocytes at 12 months after AAT therapy was 3-fold reduced compared with baseline ($P < .05$). Furthermore, at baseline, 82% of monocytes produced IL-1β, but at 12 months after therapy, the level decreased to 42%. Similar reductions were observed using TLR7/8 and TLR3 agonists in monocytes and mDCs. Unexpectedly, the reduction in cellular IL-1β was observed only 9 and 12 months after treatment but not in untreated diabetics. Improved β-cell function in the 5 AAT-treated individuals correlated with lower frequencies of monocytes and mDCs producing IL-1β compared with subjects without improvement of islet function ($P < .04$ and $P < .02$, respectively).

Conclusions: We hypothesize that AAT may have a beneficial effect on T1D in recently diagnosed patients that is associated with downmodulation of IL-1β. (*J Clin Endocrinol Metab* **99: E1418–E1426, 2014**)

How type 1 diabetes (T1D) is triggered is unknown; however, studies in humans with T1D and animal models support the hypothesis that microbial infections and the innate immune system play crucial roles in the disease mechanisms (1). This possibility is consistent with the hypothesized role of pancreatropic viruses, such as enteroviruses, in the mechanisms leading to T1D in humans (2–7) and the observation that islet β-cells from patients with T1D coexpressed enterovirus-capsid protein, interferon-γ, CXCL10, and other chemokines (5, 8).

α1-Antitrypsin (AAT) is 52-kDa serum serine-protease inhibitor that is normally produced by hepatocytes and mononuclear phagocytes (9). Emerging evidence suggests that AAT has anti-inflammatory properties. For example, AAT can inhibit the lipopolysaccharide (LPS)-induced production and release of both TNF-α and IL-1β and enhance IL-10 production in human monocytes (10). In whole blood cultures, AAT inhibits the expression of IL-8, IL-6, TNF-α, and IL-1β (11). Moreover, AAT suppresses the activation of nuclear factor (NF)-κB, a transcription factor associated with the expression of proinflammatory cytokines (12). Recently, it was reported that AAT treatment can restore normoglycemia (13) and halt T1D (14) in NOD mice and prevent islet allograft rejection (15).

Our studies implicated IL-1β in the proinflammatory response leading to T1D in animal models of virus-induced T1D (16–19). We recently reported that monocytes and dendritic cells from newly diagnosed patients with T1D and subjects at risk for disease development have dysregulated toll-like receptor (TLR)-induced cellular levels of IL-1β in monocytes and myeloid dendritic cells (mDCs) (20, 21). We hypothesized that this proinflammatory signature is associated with the mechanism of β-cell destruction. In the present study, we examined the hypothesis that treating T1D patients with the anti-inflammatory serum protein AAT is a safe treatment regimen that could downmodulate TLR-induced IL-1β in peripheral monocytes and DCs and preserve endogenous insulin production. To test this possibility, we analyzed IL-1β responses in TLR-activated peripheral monocytes and mDCs and monitored the C-peptide response of persons with diabetes to the mixed-meal tolerance test (MMTT) before and after AAT infusion. We report that

AAT administration is a safe treatment regimen in patients with normal levels of AAT. Furthermore, we show for the first time that AAT can potentially preserve islet function in a subset of patients via a mechanism that could involve downmodulation of IL-1β responses in monocytes and mDCs. Our data support the possibility that an anti-inflammatory therapy may be an efficient clinical strategy to downmodulate inflammation in T1D.

Subjects and Methods

Study participants and AAT study protocol

The clinical trial was designed as an open-label phase I study. Twelve subjects with T1D within ~4 years from disease diagnosis and detectable C-peptide were recruited to the study. The diagnosis of diabetes was established by American Diabetes Association criteria, and the diagnosis of T1D was confirmed by the presence of anti-glutamic acid decarboxylase 65, anti-islet cell autoantigen 512, anti-insulin, and anti- zinc transporter 8 autoantibodies. The subjects were infused with AAT (Aralast; Baxter Inc) at a dose of 80 mg/kg body weight at a rate of 0.08 mL/kg body weight per minute once a week for 8 weeks. Infusions took place at the Barbara Davis Center and Clinical and Translational Research Center-University of Colorado Hospital (UCH) and Children's Hospital. Blood samples were drawn before the treatment (time 0, or baseline) and weeks 1, 3, 7, and 9 and months 3, 6, 9, 12, and 18 after the initiation of the treatment. Blood samples were drawn for immunologic studies and hemoglobin A_{1c} (HbA$_{1c}$), and a 2-hour MMTT was performed per standard protocol at 0, 3, 6, and 12 months. All subjects enrolled in this study underwent intensive management of their diabetes. AAT was well-tolerated, and the only significant adverse event seen was hyperglycemia in 7 of the 12 subjects. The sera and peripheral blood mononuclear cells (PBMCs) were isolated immediately after the blood samples were drawn. Two subjects were rechallenged with a second course of AAT at the same dose and schedule as described for the first course and therefore did not undergo MMTT at 18 months after treatment. The study was approved by the Institutional Review Board at the University of Colorado Denver.

The subject cohort treated with AAT included 12 subjects with T1D (Table 1). The average age of the treated cohort was 24.6 ± 10.5 years (range, 12–39 years; 4 females and 8 males). The subjects had an average body mass index of 23.3 ± 3.7 kg/m² and an average disease duration of 15.7 ± 14.9 months (range, 3–44 months). All of the treated individuals were non-Hispanic Caucasians. As a control for the flow cytometry analyses, we

Table 1. Clinical Parameters in AAT-Treated Subjects[a]

		Time After Initiation of AAT Therapy				
	Baseline	**3 mo**	**6 mo**	**12 mo**	**18 mo**	**24 mo**
HbA$_{1c}$, %	6.0 ± 0.51 (10)	6.2 ± 0.65 (9)	6.5 ± 0.61 (11)	7.0 ± 1.7 (9)	6.6 ± 0.74 (8)	6.65 ± 0.64 (6)
Blood glucose, mg/dL	119.5 ± 21.4 (10)	124.9 ± 33.1 (9)	137.0 ± 34.2 (10)	148.7 ± 60.3 (10)	152.1 ± 46.4 (9)	129.8 ± 55.5 (5)
Insulin use, U/d	20.2 ± 13.8 (10)	22.5 ± 9.9 (9)	26.0 ± 12.8 (10)	35.1 ± 15.1 (10)	32.4 ± 15.6 (9)	30.7 ± 9.2 (5)
C-peptide AUC, ng/mL	269.9 ± 127.7 (10)	264.5 ± 138.8 (11)	213.6 ± 84.9 (12)	178.3 ± 151.0 (12)	162.43 ± 101.7 (9)	137.1 ± 70.8 (7)

[a] Data are means ± SD. Values in parentheses represent subject number.

used an untreated cohort of 4 T1D patients with an average age of 18.8 ± 3.6 years (range, 16–25 years, 3 males and 1 female) and body mass index of 24.1 ± 3.2 kg/m^2. These subjects were selected based on the disease duration that was similar to that of the treated cohort at 17.3 ± 4.3 (range, 12–23) months.

MMTT and C-peptide and autoantibody measurements

Measurements of C-peptide levels and autoantibody detection were performed by the Clinical Core at the Barbara Davis Center for Diabetes, University of Colorado Denver. HbA$_{1c}$ levels were measured by the DCA-2000 plus methodology (Siemens/Bayer).

PBMC isolation and flow cytometry

PBMCs were rapidly isolated from freshly drawn heparinized blood from autoantibody-positive and autoantibody-negative subjects by Ficoll-Hypaque Plus density centrifugation (GE Health Care). The PBMCs were washed twice with PBS (Invitrogen Life Technologies) and resuspended in endotoxin-free high-glucose DMEM containing 2mM L-glutamine, 100 U/mL penicillin and 100 μg/mL streptomycin (both from Invitrogen Life Technologies), and 10% AB serum (PAA Laboratories). For the flow cytometry analyses, the PBMCs were washed and resuspended in fluorescence-activated cell sorting buffer consisting of PBS (Invitrogen Life Technologies) containing 1% BSA and 0.05% sodium azide (both from Sigma-Aldrich). The frequency of monocytes and mDCs was assessed by flow cytometry as recently described (20, 21).

Activation of PBMCs with TLR ligands and intracellular cytokine staining

PBMCs were added to a 96-well round-bottom microtiter plate at a concentration of 1×10^6 per well in a total volume of 100 μL. For the intracellular cytokine analysis, the PBMCs were incubated in the presence or absence of various purified TLR ligands plus 1 μL/mL brefeldin A (BD Biosciences) for 4 hours and then stained for surface markers and intracellular IL-1β. For intracellular cytokine staining, the PBMCs were cultured in the presence or absence of 100 ng/mL ultrapurified LPS (O111:B4, InvivoGen) and 1 μL/mL R848 (Axxora) for 4 hours and then stained for DC and monocyte surface markers and intracellular IL-1β, as previously described (21). For compensation control, BD CompBeads (BD Biosciences) were used. Instrument performance was verified daily according to the manufacturer's quality control guidelines. Ultra Rainbow particles (3 μm; Spherotech) were used to monitor the optical alignment, laser-delay settings, histogram peak channel stability, and fluidic stability of the cyan. The median fluorescence intensity of IL-1β expression was determined by gating on the monocyte and mDC subsets. The TLR ligands were reconstituted in Dulbecco's PBS and stored in aliquots at −20°C until used.

Statistical analyses

Statistical differences in the frequencies of mDCs, plasmacytoid DCs (pDCs), and monocytes; frequencies of monocytes and mDCs expressing IL-1β after TLR ligation; and serum IL-1β levels at different times after therapy were evaluated using the nonparametric Kruskal-Wallis test with Dunn's multiple comparison. C-peptide response to the MMTT at time intervals of 0,

30, 60, 90, and 120 minutes was expressed as the total area under the response curve. The 2-hour C-peptide area under the curve (AUC) was calculated using the trapezoidal rule over the 2-hour period. The AUC calculation was based on the time points available from the MMTT (22). Statistical differences in the C-Peptide AUC were calculated using the nonparametric Kruskal-Wallis test with Dunn's multiple comparison. Comparisons between 2 samples were performed using the unpaired t test. P values < .05 were considered to be statistically significant.

Results

Effects of AAT on metabolic parameters

To assess the effect of AAT treatments on islet function, we screened the AAT-treated subjects for the expression level of HbA$_{1c}$, glucose, C-peptide AUC, and insulin use. No significant differences were observed in the overall expression of these metabolic parameters in blood samples drawn at 3, 6, 12, 18, and 24 months after the initiation of the treatment compared with the baseline value (Table 1 and Figure 1B). We observed, however, that 5 of the 12 subjects infused with AAT (patients 3, 4, 10, 12, and 15) had either increased or moderately reduced C-peptide levels at 12 to 18 months compared with the baseline value. Specifically, the levels of C-peptide AUC in the blood from subjects 3 and 4 were decreased and increased by 18% and
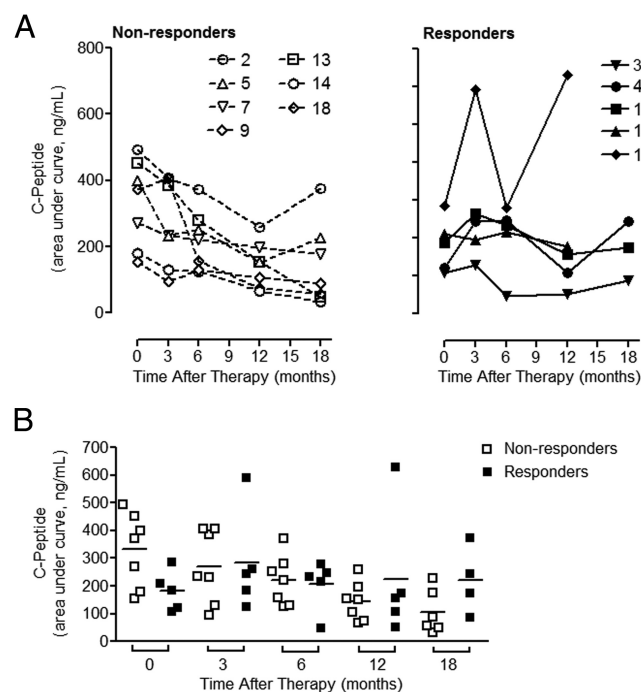

Figure 1. C-peptide responses to MMTT in AAT-treated persons with diabetes. A and B, The individual AUC of the C-peptide during a 2-hour MMTT from nonresponders and responders as indicated (A) and comparison between the C-peptide response of responders and nonresponders (B) before and at different time points during and after the start of therapy (n = 10–12 per time point).

100%, respectively. The levels of C-peptide AUC in sub-jects 10 and 12 were reduced by 8% and 16%, respectively, and that of subject 15 was increased by 120% compared with the baseline level (Figure 1A). In contrast, the other subjects exhibited a reduction of 40% to 80% in their C-peptide AUC levels compared with the baseline (Figure 1A). These data suggest that treatment with AAT could potentially result in improved or stable C-peptide responses in a subset of patients.

Frequencies of monocytes and dendritic cells in the peripheral blood from newly diagnosed T1D patients treated with AAT

We next examined the potential effect of AAT therapy on the proportion of monocyte and DC subsets in the peripheral blood from treated patients. PBMCs were isolated from freshly drawn blood before or during and after the AAT infusions. The data show similar frequencies of monocytes, mDCs, and pDCs in the blood from the subjects before, during the 8-week course of AAT infusions, and after the treatment (Figure 2 and Supplemental Figure 1, published on The Endocrine Society's Journals Online website at http://jcem.endojournals.org). These findings suggest that AAT therapy does not alter the percentage of monocytes and DC subsets from the levels before treatment.

Therapy with AAT reduces the TLR-induced IL-1β fluorescence intensity in monocytes and mDCs

Increased IL-1β levels in the peripheral blood monocytes have been linked to the development of T1D in humans (23–26) and animal models of the disease (16). For example, we recently reported that TLR-induced cellular levels of IL-1β is increased in monocytes and mDCs from newly diagnosed patients with T1D (21) and subjects at risk for the disease (20). In the present study, we tested the hypothesis that therapy with AAT may alter the TLR-induced IL-1β levels in peripheral monocytes and mDCs. For this purpose, freshly isolated PBMCs were obtained from the subjects before and at different times during and after the AAT infusion treatment. The cells were cultured in the presence or absence of LPS (TLR4 ligand) or R848 (ligand of TLR7/8). We used flow cytometry to assess the fluorescence intensity of IL-1β expression in monocytes 4 hours after the activation of TLRs in vitro. Using a cohort of approximately 90 subjects with islet autoimmunity, we recently established that IL-1β is highly expressed in 80% to 90% of the total monocytes and 30% to 75% of mDCs after in vitro TLR ligation (20, 21). Similar median fluorescence intensities (MFIs) were detected in unactivated monocytes and mDCs at all time points (Figure 3, A–C, and Supplemental Figure 2, A and B). The data shown in Figure 3B further indicate that the average cellular level of
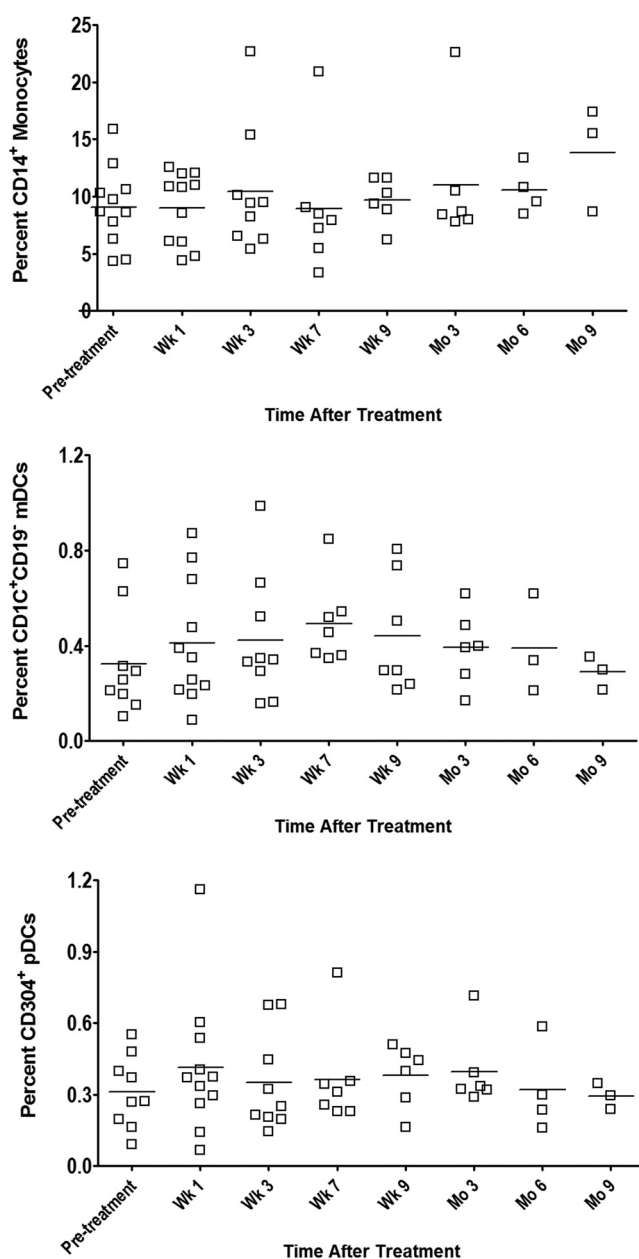

Figure 2. Frequencies of monocytes and DC subsets in the peripheral blood. Freshly drawn PBMCs were isolated from recently diagnosed subjects before and after AAT administration and stained for surface markers characteristic of CD14$^+$ monocytes, CD1C$^+$CD19$^-$ mDCs, and CD304$^+$ pDCs, as indicated (n = 3–11 per group). The results are expressed as the percentage of the cell subtypes of all the PBMCs. The bars indicate the mean values. There are no statistically significant differences in the frequencies of monocytes or DC subsets over time.

IL-1β observed in LPS-activated monocytes was reduced from an MFI of 16 detected before AAT treatment to an MFI of 6 at 12 months after treatment ($P < .05$). Likewise, the MFI for IL-1β expression in R848-activated monocytes from the subjects at 9 or 12 months after the initiation of the AAT infusion was significantly reduced compared with that of the subjects before the treatment and at 1, 3, 7, or 9 weeks after the start of therapy (Figure 3B). The MFI for IL-1β in the TLR4-activated monocytes from
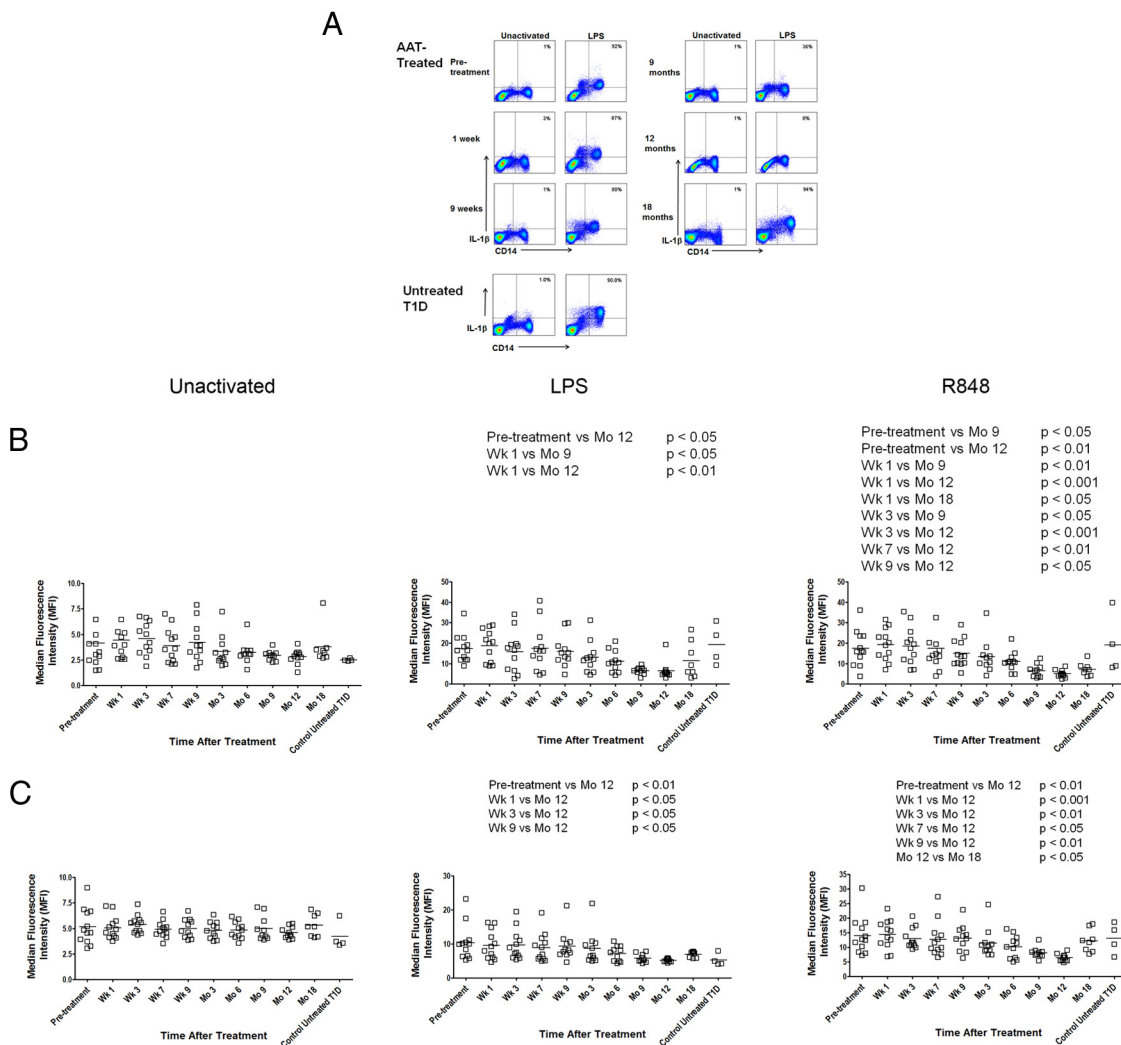
Figure 3. Fluorescence intensity of IL-1β produced in LPS- and R848-activated monocytes and mDCs. A–C, As indicated, PBMCs were left unactivated or stimulated with ultrapurified LPS or R848 in the presence of brefeldin A and then stained with fluorochrome-conjugated monoclonal antibodies directed against surface markers for monocytes (A and B) or mDCs (C). The cells were fixed, permeabilized, and labeled with fluorochrome-conjugated monoclonal antibodies against IL-1β or the appropriate isotype controls. A, Representative flow cytometry images of LPS-induced IL-1β-expressing monocytes. The MFI was determined by gating on the monocyte or mDC populations. Each symbol represents an individual MFI (n = 4–12 per group). The bars indicate the mean values. The indicated P values were determined using the nonparametric Kruskal-Wallis test with Dunn's multiple comparison.

the subjects at 18 months after the therapy was greater than the values observed at 9 and 12 months, but this difference did not reach a statistically significant level due to the relatively small subject number. As expected, the MFI for IL-1β in TLR4-activated monocytes from untreated control patients with T1D was increased and similar compared with that observed in monocytes from treated subjects at 12 months after the therapy and the baseline level, respectively. As observed in the monocytes, the MFI for IL-1β levels in LPS-activated mDCs from AAT-treated subjects at the 12 months was significantly less than the levels observed before the treatment or those 1, 3, or 9 weeks after the treatment (Figure 3C and Supplemental Figure 2B). The MFI for IL-1β in R848-activated mDCs from patients at 12 months was less than that observed during the pretreatment period or 1, 3, 7, and 9

weeks after the initiation of the therapy (Figure 3C and Supplemental Figure 2B). As observed in the monocytes, the MFI values for cellular IL-1β expression in mDCs from the treated subjects after 18 months and the untreated T1D controls were greater than the values at 12 months. Similar reductions in the MFI of the IL-1β staining were observed in monocytes and mDCs activated with poly(I:C) (Supplemental Figures 3 and 4). Taken together, these data suggest that therapy with AAT results in a reduction in the levels of TLR-induced cellular IL-1β detectable at 12 months after the therapy.

AAT treatment decreases the frequency of TLR-induced IL-1β–containing monocytes and mDCs

Because AAT induces a reduction in the MFI for IL-1β in TLR-activated monocytes and mDCs at 12 months after
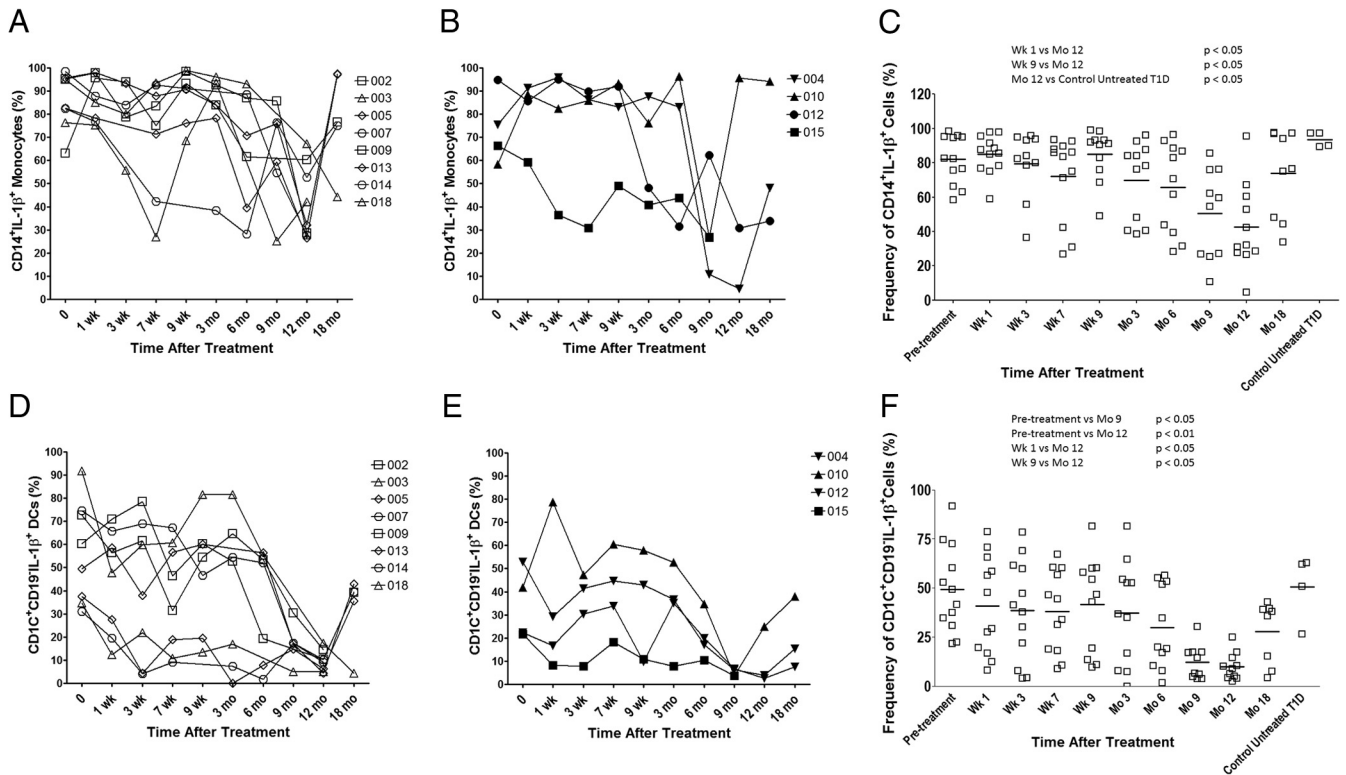
Figure 4. IL-1β production in LPS-activated monocytes and mDCs. A–F, PBMCs were either unactivated or activated with purified LPS in the presence of brefeldin A and subsequently stained with fluorochrome-conjugated monoclonal antibodies directed against IL-1β and surface markers specific for monocytes (A–C) and mDCs (D–F). A, D, B, and E, Individual TLR4 responses from nonresponders (A and D) and responders (B and E) before and at various time points after therapy. C and F, Comparisons between IL-1β responses in monocytes (C) and mDCs (F) from AAT-treated subjects vs control untreated patients with T1D (n = 4–12 per group). The bars indicate the mean values. The *P* values were evaluated using the nonparametric Kruskal-Wallis test with Dunn's multiple comparison.

therapy, we next assessed the effect of AAT on the overall proportion of monocytes and mDCs that contain IL-1β after in vitro TLR ligation. We observed that the frequency of IL-1β–positive monocytes from untreated subjects cultured in the absence of TLR agonists was approximately 3% compared with 2.5% to 6.9% observed in the AAT-treated subjects (data not shown). The data indicate that LPS activation led to the induction of cellular IL-1β in approximately 50% to 100% and 25% to 90% of monocytes (Figure 4, A–C) and mDCs (Figure 4, D–F), respectively, and that this phenotype was overall stable until 9 to 12 months after therapy. Figure 4C demonstrates that at 1 and 9 weeks after the initiation of the treatment, 80% of monocytes, produced IL-1β after LPS activation compared with 40% of monocytes from the subjects at 12 months after the treatment ($P < .05$ for both time points). The frequency of LPS-induced IL-1β–producing monocytes from subjects at 18 months after the treatment was increased compared with the value at 12 months. Similar to our recent data from new-onset patients and at-risk subjects (20, 21), activation of PBMCs from untreated control patients with LPS induced IL-1β in most the monocytes at a frequency similar to that seen in monocytes from subjects before therapy and higher than that seen at 12

months ($P < .05$ for both pretreatment and 12-month values). Comparable data were obtained from mDCs activated with LPS (Figure 4, D–F). Finally, activation of PBMCs with R848 and poly(I:C) led to reduced cellular levels of IL-1β in monocytes and mDCs at 12 and 18 months after therapy (Supplemental Figures 3 and 4). Collectively, these data indicate that AAT therapy results in reduced frequencies of monocytes and mDCs expressing cellular IL-1β after in vitro TLR ligation.

Correlation between C-peptide levels and TLR-induced IL-1β responses

We next determined whether the C-peptide levels observed after the MMTT correspond to the magnitude of the TLR-induced IL-1β responses in monocytes and mDCs. For this analysis, we evaluated the LPS-induced IL-1β responses from subjects stratified into C-peptide responders and nonresponders. The data shown in Figure 5 demonstrate that AAT-treated subjects who had improved islet function also had significantly lower frequencies of IL-1β–positive monocytes and mDCs compared with the nonresponders 9 months after the AAT administration ($P < .01$ for monocytes and $P < .003$ for mDCs). Although not statistically significant, we found a similar
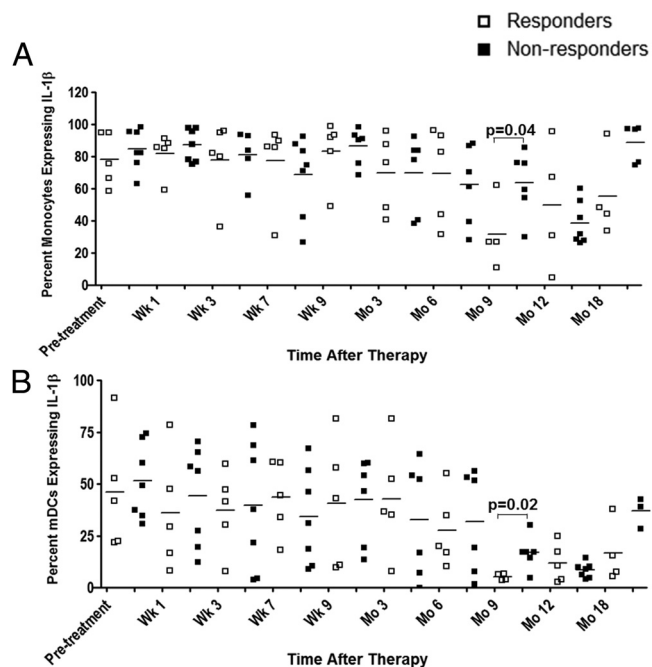
Figure 5. TLR-induced IL-1β responses in C-peptide responders vs nonresponders. IL-1β responses were evaluated in subjects stratified based on their response to the MMTT (responders vs nonresponders) at different times in the course of the trial. Each symbol represents an individual frequency of IL-1β–expressing monocytes (A) and mDCs (B) after LPS activation. The bars represent the mean values (n = 3–8 per group). The P values were evaluated using an unpaired t test.

trend toward reduced frequencies of IL-1β–positive monocytes and mDCs after TLR7/8 ligation in subjects who had improved islet function compared with nonresponders (data not shown). These findings suggest that improved β-cell function after AAT therapy could be linked to a downmodulation in the TLR-induced IL-1 production in monocytes and mDCs.

Discussion

Published data from animal models and humans with islet autoimmunity have led to the recognition that proinflammatory responses play a key role in triggering T1D (4, 5, 8, 27, 28). Therefore, effective therapeutic interventions for disease prevention and maintenance of endogenous insulin production will likely have to include modalities that downmodulate proinflammatory responses associated with disease progression (29). In this exploratory phase I clinical study, we tested the safety and efficacy of the anti-inflammatory compound AAT in recently diagnosed patients with T1D. We report that AAT is well-tolerated by subjects with T1D and provide evidence for the first time that AAT may potentially be beneficial to islet β-cell function in some patients via mechanisms that could be linked with downregulation of IL-1β responses.

How AAT modulates the function of peripheral monocytes and mDCs remains to be identified. A mechanism that explains the reduction in TLR-induced IL-1β production observed at 9 and 12 months after AAT infusion must take into consideration a potential effect of AAT on bone marrow precursors at a specific stage of differentiation of myeloid precursors resulting in altered signaling upstream or downstream of TLR activation. Others have reported that a subpopulation of normal bone marrow cells releases a complex of AAT and a 31-kDa fragment of proteinase-3 (PR3), which can inhibit the proliferation of leukemic cells (30, 31). AAT is a potent inhibitor of PR3 as well as elastase and cathepsins. Experiments with highly purified CD34+ bone marrow cells suggested that PR3 acts directly on the progenitor cells, implicating a role for PR3 in regulating myelopoiesis (30). Thus, AAT therapy could result in PR3 inhibition during myeloid maturation. Such an effect, however, would be detectable only 9 to 12 months after therapy because these cells exit the bone marrow to the periphery as mature monocytes. Another potential mechanism for the AAT-induced reduction in IL-1β production could be linked with reduced TLR expression because AAT was shown to suppress the surface expression of TLR2 and TLR4 (32, 33). AAT can also bind the heat-shock protein gp96, and such interaction could lead to reduced TLR expression (34).

One major problem in evaluating the outcome of therapies targeting the innate immune system is the lack of biomarkers that can be used to assess in vivo drug efficacy. Using a flow assay system established in our laboratory (20), we demonstrate that patients with T1D treated with AAT had lower TLR-induced IL-1β responses in monocytes and mDCs observed at 9 or 12 months after therapy. Moreover, this alteration correlated with improved C-peptide responses after MMTT. These data are in accordance with serum data indicating a trend toward reduced serum IL-1β levels 6 to 9 months after AAT therapy (Supplemental Figure 5). Additional studies are required to determine whether TLR-induced IL-1β in monocytes and mDCs could be used as a biomarker for monitoring the in vivo effect of anti-inflammatory therapies.

Our observations indicate that therapy with AAT downmodulates proinflammatory responses in monocytes and mDCs, lowers the levels of serum IL-1β, and may also improve islet β-cell function in a subset of recent-onset patients. Our islet function data, however, must be interpreted with caution due to the small sample size of our trial and the absence of a placebo group. Previous data have shown that some patients with T1D can maintain their C-peptide levels after disease onset (22). Our data are consistent with earlier data obtained from the NOD mouse model showing that AAT therapy prevented insu-

litis and T1D via mechanisms hypothesized to be linked to downmodulation of proinflammatory pathways (13, 14). However, our data are in contrast to a previous report suggesting that AAT treatment does not protect NOD mice from islet autoimmunity (35). In any case, AAT was shown to suppress TLR-induced expression of TNF-α and IL-1β and enhance IL-10 production in human monocytes (10). AAT can suppress the expression of IL-8, IL-6, TNF-α, and IL-1β in whole blood cultures (11) and inhibit the activation of NF-κB, a transcription factor associated with the expression of proinflammatory cytokines (12).

How AAT-induced IL-1β responses improves islet function is not yet clear. This effect could partially be a result of reduced levels of proinflammatory cytokines such as IL-1β in the microenvironment of islets leading to lesser β-cell inflammation. The findings that AAT downmodulates IL-1 production and improves islet function lend support to the hypothesis that IL-1β could potentially be involved in islet inflammation and insulin deficiency in recently diagnosed patients. Observations from a number of animal models implicate IL-1 in the mechanisms of islet destruction. We recently demonstrated that IL-1β is upregulated in virus-induced T1D in rat models of the disease (16, 17), and therapy with the IL-1 blocker anakinra can prevent disease development (18). IL-1, either alone or in conjunction with other proinflammatory cytokines such as interferon-β, can cause β-cell destruction in the islets from humans and animals and in a perfused pancreas model via mechanisms involving MAPK and NF-κB (reviewed in Refs. 36–38).

Although we found that improved islet function in AAT-treated subjects correlated with downmodulation of IL-1β production, recent clinical trials demonstrated that therapies specifically targeting IL-1 with IL-1 receptor antagonist (anakinra) or anti–IL-1β monoclonal antibodies did not exert a beneficial effect on islet function in new-onset patients (39, 40). One potential explanation for these seemingly disparate results is that IL-1–targeted monotherapy was not sufficient to block islet destruction. Data from humans and animal models suggest that the proinflammatory response associated with islet destruction involves, in addition to IL-1, other proinflammatory cytokines and chemokines including IL-6, IL-12, IL-18, and CXC chemokine ligand 10 (4, 5, 8, 27, 28). It could therefore be that the beneficial effect of AAT on islet autoimmunity seen in subjects with T1D is linked with its known ability to suppress, in addition to IL-1, a number of other proinflammatory molecules such as TNF-α, IL-8, IL-6, and NF-κB and upregulate IL-10 production (10–12).

In summary, we found that therapy with AAT either increased or inhibited the decline in C-peptide responses in

some patients, and this effect correlated with lower TLR-induced IL-1β production. Given these data, we suggest that targeting inflammation with inhibitors of the innate immune system might represent an efficient therapeutic strategy for disease prevention. Future trials will have to be conducted in at-risk and recent-onset subjects to assess the full therapeutic potential of AAT in T1D.

Acknowledgments

References

1. **Gianani R, Eisenbarth GS.** The stages of type 1A diabetes: 2005. *Immunol Rev.* 2005;204:232–249.

2. **Dotta F, Censini S, van Halteren AG, et al.** Coxsackie B4 virus infection of β-cells and natural killer cell insulitis in recent-onset type 1 diabetic patients. *Proc Natl Acad Sci U S A.* 2007;104(12):5115–5120.

3. **Dotta F, Galleri L, Sebastiani G, Vendrame F.** Virus Infections: Lessons from Pancreas Histology. *Curr Diab ReP.* 2010;10(5):357–361.

4. **Richardson SJ, Willcox A, Bone AJ, Foulis AK, Morgan NG.** The prevalence of enteroviral capsid protein vp1 immunostaining in pancreatic islets in human type 1 diabetes. *Diabetologia.* 2009;52(6):1143–1151.

5. **Roep BO, Kleijwegt FS, van Halteren AG, et al.** Islet inflammation and CXCL10 in recent-onset type 1 diabetes. *Clin ExP Immunol.* 2010;159(3):338–343.

6. **Ylipaasto P, Klingel K, Lindberg AM, et al.** Enterovirus infection in human pancreatic islet cells, islet tropism in vivo and receptor involvement in cultured islet β-cells. *Diabetologia.* 2004;47(2):225–239.

7. **Yoon JW, Austin M, Onodera T, Notkins AL.** Isolation of a virus from the pancreas of a child with diabetic ketoacidosis. *N Engl J Med.* 1979;300(21):1173–1179.

8. **Tanaka S, Nishida Y, Aida K, et al.** Enterovirus infection, CXC chemokine ligand 10 (CXCL10), and CXCR3 circuit: a mechanism of accelerated β-cell failure in fulminant type 1 diabetes. *Diabetes.* 2009;58(10):2285–2291.

9. **Breit SN, Wakefield D, Robinson JP, Luckhurst E, Clark P, Penny R.** The role of α1-antitrypsin deficiency in the pathogenesis of immune disorders. *Clin Immunol ImmunoPathol.* 1985;35(3):363–380.

10. **Janciauskiene S, Larsson S, Larsson P, Virtala R, Jansson L, Stevens**

T. Inhibition of lipopolysaccharide-mediated human monocyte activation, in vitro, by α1-antitrypsin. *Biochem BioPhys Res Commun*. 2004;321(3):592–600.

11. **Pott GB, Chan ED, Dinarello CA, Shapiro L.** α1-Antitrypsin is an endogenous inhibitor of proinflammatory cytokine production in whole blood. *J Leukoc Biol*. 2009;85(5):886–895.

12. **Shapiro L, Pott GB, Ralston AH.** α1-Antitrypsin inhibits human immunodeficiency virus type 1. *FASEB J*. 2001;15(1):115–122.

13. **Koulmanda M, Bhasin M, Hoffman L, et al.** Curative and β-cell regenerative effects of α1-antitrypsin treatment in autoimmune diabetic NOD mice. *Proc Natl Acad Sci U S A*. 2008;105(42):16242–16247.

14. **Ma H, Lu Y, Li H, et al.** Intradermal α1-antitrypsin therapy avoids fatal anaphylaxis, prevents type 1 diabetes and reverses hyperglycaemia in the NOD mouse model of the disease. *Diabetologia*. 2010;53(10):2198–2204.

15. **Lewis EC, Mizrahi M, Toledano M, et al.** α1-Antitrypsin monotherapy induces immune tolerance during islet allograft transplantation in mice. *Proc Natl Acad Sci U S A*. 2008;105(42):16236–16241.

16. **Wolter TR, Wong R, Sarkar SA, Zipris D.** DNA microarray analysis for the identification of innate immune pathways implicated in virus-induced autoimmune diabetes. *Clin Immunol*. 2009;132(1):103–115.

17. **Londono P, Komura A, Hara N, Zipris D.** Brief dexamethasone treatment during acute infection prevents virus-induced autoimmune diabetes. *Clin Immunol*. 2010;135(3):401–411.

18. **Hara N, Alkanani AK, Dinarello CA, Zipris D.** Modulation of virus-induced innate immunity and type 1 diabetes by IL-1 blockade [published online September 23, 2013]. *Innate Immun*. doi:10.1177/1753425913502242.

19. **Hara N, Alkanani A, Dinarello C, Zipris D.** Histone deacetylase inhibitor suppresses virus-induced proinflammatory responses and type 1 diabetes. *J Mol Med*. 2013;1–10.

20. **Alkanani AK, Rewers M, Dong F, Waugh K, Gottlieb PA, Zipris D.** Dysregulated Toll-like receptor-induced interleukin-1β and interleukin-6 responses in subjects at risk for the development of type 1 diabetes. *Diabetes*. 2012;61:2525–2533.

21. **Meyers AJ, Shah RR, Gottlieb PA, Zipris D.** Altered toll-like receptor signaling pathways in human type 1 diabetes. *J Mol Med*. 2010;88(12):1221–1231.

22. **Greenbaum CJ, Beam CA, Boulware D, et al.** Fall in C-peptide during first 2 years from diagnosis: evidence of at least two distinct phases from composite type 1 diabetes TrialNet data. *Diabetes*. 2012;61(8):2066–2073.

23. **Dogan Y, Akarsu S, Ustundag B, Yilmaz E, Gurgoze MK.** Serum IL-1beta, IL-2, and IL-6 in insulin-dependent diabetic children. *Mediators Inflamm*. 2006;2006(1):59206.

24. **Erbaǧci AB, Tarakçioǧlu M, Coşkun Y, Sivasli E, Sibel Namiduru E.** Mediators of inflammation in children with type I diabetes mellitus: cytokines in type I diabetic children. *Clin Biochem*. 2001;34(8):645–650.

25. **Nicoletti F, Conget I, Di Marco R, et al.** Serum levels of the interferon-γ-inducing cytokine interleukin-18 are increased in individuals at high risk of developing type I diabetes. *Diabetologia*. 2001;44(3):309–311.

26. **Perez F, Oyarzun A, Carrasco E, Angel B, Albala C, Santos JL.** Plasma levels of interleukin-1β, interleukin-2 and interleukin-4 in recently diagnosed type 1 diabetic children and their association with β-pancreatic autoantibodies [in Spanish]. *Rev Med Chil*. 2004;132(4):413–420.

27. **Zipris D.** Toll-like receptors and type 1 diabetes. *Adv ExP Med Biol*. 2010;654:585–610.

28. **Zipris D.** Innate immunity in type 1 diabetes. *Diabetes Metab Res Rev*. 2011;27(8):824–829.

29. **Herold KC, Vignali DA, Cooke A, Bluestone JA.** Type 1 diabetes: translating mechanistic observations into effective clinical outcomes. *Nat Rev Immunol*. 2013;13(4):243–256.

30. **Sköld S, Rosberg B, Gullberg U, Olofsson T.** A Secreted Proform of Neutrophil Proteinase 3 Regulates the Proliferation of Granulopoietic Progenitor Cells. *Blood*. 1999;93(3):849–856.

31. **Sköld S, Zeberg L, Gullberg U, Olofsson T.** Functional dissociation between proforms and mature forms of proteinase 3, azurocidin, and granzyme B in regulation of granulopoiesis. *ExP Hematol*. 2002;30(7):689–696.

32. **Jonigk D, Al-Omari M, Maegel L, et al.** Anti-inflammatory and immunomodulatory properties of α1-antitrypsin without inhibition of elastase. *Proc Natl Acad Sci U S A*. 2013;110(37):15007–15012.

33. **Subramaniyam D, Zhou H, Liang M, Welte T, Mahadeva R, Janciauskiene S.** Cholesterol rich lipid raft microdomains are gateway for acute phase protein, *SERPINA1*. *Int J Biochem Cell Biol*. 2010;42(9):1562–1570.

34. **Ochayon DE, Mizrahi M, Shahaf G, Baranovski BM, Lewis EC.** Human α1-antitrypsin binds to heat-shock protein gp96 and protects from endogenous gp96-mediated injury in vivo. *Front Immunol*. 2013;4:320–333.

35. **Grant CW, Moran-Paul CM, Duclos SK, Guberski DL, Arreaza-Rubin G, Spain LM.** Testing agents for prevention or reversal of type 1 diabetes in rodents. *PLoS One*. 2013;8(8):e72989.

36. **Eizirik DL, Mandrup-Poulsen T.** A choice of death–the signal-transduction of immune-mediated β-cell apoptosis. *Diabetologia*. 2001;44(12):2115–2133.

37. **Mandrup-Poulsen T.** The role of interleukin-1 in the pathogenesis of IDDM. *Diabetologia*. 1996;39(9):1005–1029.

38. **Pickersgill LM, Mandrup-Poulsen TR.** The anti-interleukin-1 in type 1 diabetes action trial–background and rationale. *Diabetes Metab Res Rev*. 2009;25(4):321–324.

39. **Mandrup-Poulsen T, Pickersgill L, Donath MY.** Blockade of interleukin 1 in type 1 diabetes mellitus. *Nat Rev Endocrinol*. 2010;6(3):158–166.

40. **Moran A, Bundy B, Becker DJ, et al.** Interleukin-1 antagonism in type 1 diabetes of recent onset: two multicentre, randomised, double-blind, placebo-controlled trials. *Lancet*. 2013;38(9881):1905–1915.