Journal of Diabetes Science and Technology
2014, Vol. 8(6) 1193–1203

DOI: 10.1177/1932296814547096
dst.sagepub.com
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Mechanistic Evidence in Support of Alpha1-Antitrypsin as a Therapeutic Approach for Type 1 Diabetes

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Abstract

Utilizing endogenous molecules as a therapeutic approach is almost unequivocally superior to engineered or synthetic molecules. However, one rarely encounters an anti-inflammatory, cytoprotective, immunomodulatory and wound-healing molecule that has been available for use for decades. α1-antitrypsin (AAT), a circulating protein that rises more than 4-fold during acute-phase responses, has been administered for a rare genetic deficiency at large doses, for life. Aside from advances in insulin therapy, medical research in type 1 diabetes (T1D) has predominantly focused on autoimmunity—controlling the adaptive immune response. However, it is now appreciated that one may need to extend therapeutic targets to incorporate immune responses to *cellular injury*, as well as promote selective control over *excessive inflammation* and *early tissue repair*. Recent data suggest that tissue damage related to lung and renal ischemia-reperfusion injury, stroke, and ischemic heart disease is markedly reduced by AAT. AAT was also shown to protect pancreatic islet β cells at multiple levels. Unlike classic immunosuppressive and anti-inflammatory approaches, AAT exerts some antiviral and antibacterial activities. Based on these and other reports, AAT is under evaluation for treatment of T1D patients in multiple clinical trials. Initial results suggest that AAT therapy could potentially improve insulin production without adverse effects. Up to 50% of individuals displayed improved islet function. It is a rare occurrence in T1D research that a therapy is offered that holds a safety profile equal or superior to that of insulin alone. While placebo-controlled trials are ongoing, the mechanism(s) behind these favorable activities of AAT are still being explored.

Keywords

clinical trials, inflammation, pancreatic islets, tissue injury

The utilization of endogenous, physiological human molecules as a therapeutic approach is almost unequivocally superior to treatment with nonnative engineered or synthetic molecules. However, one rarely encounters a molecule that is well established as a wound-healing agent, and has also been available for use for the past 3 decades.[1-4] α1-Antitrypsin (AAT) is a circulating protein that is controlled mainly by the liver; hepatocytes are responsible for steady-state constitutive circulating levels of AAT (0.9-1.75 mg/ml), which increase more than 4-fold during acute-phase responses. In addition to hepatocytes, AAT is expressed by lung alveolar epithelial cells, monocytes, macrophages, neutrophils, endothelial cells, human intestinal paneth cells, and endometrial cells, as well as by pancreatic islet cells.[5] However, these do not significantly contribute to circulating AAT levels, they supply local AAT during inflammatory conditions.

Evidence suggests that AAT not only possesses the ability to inhibit serine proteases, such as the tissue destructive and inflammatory elastase and proteinase 3 (PR3), but can also modify dendritic cell maturation and promote regulatory T cell (Treg) differentiation, increase the release interleukin (IL)-10[6] and IL-1 receptor antagonist (IL-1Ra),[7] and protect various cell types from cell death. Unlike classic immunosuppressants, AAT allows undeterred isolated T-lymphocyte responses and IL-2 production,[8,9] an apparent prerequisite for the evolvement of inducible Tregs.

Evidence for direct protection of tissues from injury by AAT dates back to early data on lung emphysema, which emerges spontaneously in patients with genetic

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AAT deficiency (AATD). More recent data suggest that hypoxia-related tissue damage (eg, lung and renal ischemia-reperfusion injury,[10,11] stroke,[12] and ischemic heart disease [IHD][13]), as well as tissue damage with an underlying allogeneic background (graft-versus-host disease, GvHD[14,15]) and inflammation that is fueled by danger associated molecular pattern agents (DAMPs) that are released from injured cells and serve as immune adjuvants,[16,17] are inhibited by AAT.

Based on these and other reports, AAT is presently under evaluation in multiple clinical trials for treatment of IHD patients, treatment-resistant GvHD patients, and recently diagnosed type 1 diabetes (T1D) patients.

Current understanding of the natural history of T1D stipulates that individuals with a fixed number of β cells are exposed to a yet unidentified trigger that induces autoimmunity toward β cells. Appropriately, the predominant focus of medical research for finding a cure for T1D has adopted the notion that the immune response is largely *unwanted*, and that islets represent passive targets of immune cell responses.

The immunological response toward β cells does involve a progressive activation of antigen-specific T and B lymphocytes, but it is preceded and fueled by cellular members of the nonspecific innate immune system.[18] Upon excessive inflammation, β cells initially shutdown insulin production in the context of a physiological response to inflammatory stress, and subsequently undergo cell death. In the absence of sufficient insulin, excessive circulating glucose levels unused by the brain and red blood cells remain high, and the lack of insulin signaling in metabolic tissues causes the liver to assume the hormonal mode of fasting, resulting in glycogenolysis, gluconeogenesis, and fatty acid mobilization.

The inflammatory process, with its consequential outcome of β cell failure and a significant proportion of cell necrosis, further promotes antigen presentation by local cells.[19] Nevertheless, in multiple immunologically oriented clinical studies, the disease has yet to be ablated.

Thus, since its discovery about a century ago, insulin is still the standard treatment for T1D. Both safety and tolerability of insulin treatments have greatly improved, and further advances are expected to appear in as far as different modes of insulin administration.[20] Yet, ultimately, one should consider that treatment with any form of insulin holds some risk for lethal hypoglycemia, particularly when applying tight glucose control,[21] and that exogenous insulin can be considered, at best, only symptomatic.

To date, patients that achieve tight glucose control still might spend up to 60% of the day hyperglycemic. Once hyperglycemia predominates, nonenzymatic glycation of multiple circulating plasma proteins occurs, resulting aberrations in their original function. As expected, this aspect of T1D is also shared with type 2 diabetes (T2D).[22]

Islet β cells are also injured in poorly uncontrolled T2D. There is evidence for T2D being, at least partially of autoinflammatory origin, resulting from an attempt of the pancreatic islets to compensate for increased insulin needs. According to one school of thought, fat tissue–derived adipocytokines contribute to a systemic low-grade inflammatory response, driven particularly by the circulating proinflammatory cytokines, IL-1β and IL-6.[23] These interfere with peripheral tissue responses to insulin, a physiological reflex intended to produce a short-term rise in glucose levels upon stress. To compensate for increased insulin resistance, functional β cell mass initially expands, leading to hyperinsulinemia. Then, islet amyloid polypeptide (IAPP) and DAMPs, such as extracellular ATP, are released from β cells and are believed to aggravate resident islet macrophages; in response, macrophages release local IL-1β, which, in excess, results in β cell demise.[24]

Despite promising preclinical evidence in favor of targeting the inflammatory aspect of insulin resistance, clinical trials for testing specific anti-inflammatory approaches have been generally disappointing.[23] In addition, none have directly addressed the highly challenging component of islet β cell injury.

Islet β cells are also injured by a series of diabetogenic drugs. Posttransplant diabetes mellitus (PTDM) has emerged as a major adverse effect of chronic immunosuppressive drugs, including the not uncommon chronic use of prednisone in the case of asthmatic children. All in all, over 150 drugs from a wide range of classes have the potential to cause β cell injury.[23] There is currently no medical approach to address islet β cell injury under these, usually elective, treatments. Nonetheless, it is possible that once β cell injury is directly addressed, some reprieve may be afforded to β cells during treatment with diabetogenic drugs.

The notion that emerges from these substantial volumes of several decades worth of data strongly suggests that one may need to extend the therapeutic targets so as to incorporate biological processes of immune responses to *cellular injury*, as well as promote selective control of *excessive injurious inflammation* and enhance pathways associated with *tissue repair*.

Deficiency in AAT Activity and Islet Injury: Proposed Mechanisms

Diabetic patients with poor glycemic control display increased nonenzymatic glycation of total plasma proteins. For some proteins this means no particular change in functionality, but circulating AAT is *inactivated* by excessive glycation.[25] For example, the glycated form of hemoglobin, HbA1c, is one of the hallmark circulating markers that individuals with T1D and T2D record periodically. In this spontaneous, nonenzymatic, time- and glucose-dependent covalent chemical modification, hemoglobin is tagged by excessive exposure to glucose—yet its original function is maintained.

Protein glycation has been shown to cause cross-linking of protein molecules, which in some cases may alter their function. The glycation is irreversible in the blood stream,

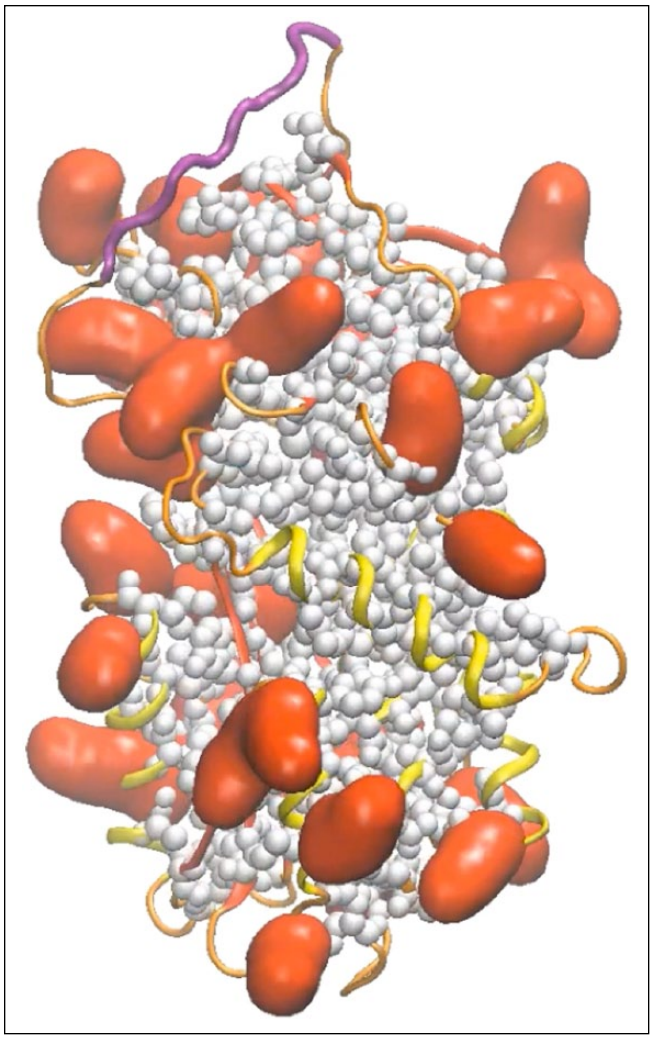

Figure 1. In silico depiction of glycated AAT. Orange, wire-diagram of the protein-sequence with secondary structures highlighted in yellow and red, and the protease-binding domain in purple. Nonexposed amino acids that are positioned under the surface of the molecule are represented by white beads. The remaining wire structure is predicted to be available for access by multiple binding partners. Red, surface lysine residues that interact nonenzymatically with glucose in a time- and concentration-dependent manner.

although specific intracellular enzymes can deglycate some proteins. In a study of total trypsin inhibitory capacity in the serum of diabetic and nondiabetic children, Lisowska-Myjak et al showed that while the *levels* of AAT are *normal* in diabetic children compared to nondiabetic children, total trypsin inhibitory capacity is significantly diminished.[26] Multiple related studies support this phenomenon.[27-29] Circulating AAT is glycated within approximately a week in hyperglycemic individuals (Figure 1) and as a result—AAT turns inactive.[25]

Evidence of Direct Protection of Islet β Cells by AAT

Inflammatory mediators play an important role in β cell loss of function and subsequent demise. In vitro, the combination of IL-1β and IFNγ stimulates inducible nitric oxide synthase (iNOS) expression in islet cells, resulting in increased production of nitric oxide (NO) (Table 1). Although some minimal levels of NO are important for islet function, excess levels of NO cause islet β cell destruction. AAT has been shown to reduce the levels of released NO.[30] In these studies, the degree of NO release was not obliterated, thus allowing for essential functions of NO. Indeed, in the presence of AAT, primary mouse islets that were subject to an inflammatory environment displayed enhanced viability and improved insulin release. Under these conditions, islet cells expressed fewer surface MHC class II molecules involved in immune-mediated islet destruction. Reduction in TNFα release from islet cells was accompanied by an *increase* in membrane-associated TNFα, most probably due to direct blockade of the membrane-bound protease responsible for TNFα release, ADAM17.[31]

AAT readily enters multiple types of cells.[32] Once intracellular, AAT can *function* as a modulator of intracellular molecules. In a series of studies involving incubation of murine insulinoma cells (MIN-6) with labeled-AAT, Zhang et al demonstrated that upon entry, AAT inhibits not only serine proteases but also particular cysteine proteases, including the proapoptotic caspase-3.[32] Indeed, AAT was also shown to protect β cells from cytokine-induced apoptosis.[33] In addition, AAT was found to diminish the activity of caspase-1, resulting in lower levels of IL-1β release,[13] and metalloproteases, such as MMP-9.[34] These attributes may explain the protection of tissues by AAT at time of excess connective tissue degradation.

These are all nonobvious extensions of the current widely perceived mechanism of action of AAT in supporting islet survival, that is, elastase inhibition.

AAT was recently shown to have binding partners irrespective of its protease-capturing domain. For example, AAT binds the DAMP molecule gp96, a heat-shock protein that is involved in autoimmune pathologies and is found elevated in the circulation of patients with T1D. AAT was shown to diminish gp96-induced islet injury.[16] This novel function of AAT is in perfect timing with the recent appreciation of the role of DAMPs in immune responses to damaged tissues, normal intracellular molecules that adopt an inflammatory function once they leak out of injured cells. In effect, they behave as immune adjuvants. HSP70 shares the same properties as gp96 in that it acts as a chaperon inside cells, then turns to become an inflammatory agent once leaked out of dead cells; it too was found to be elevated in patients with T1D and is found bound to AAT.[17] Their inactivation by AAT

Table 1. Experimental Support for In Vitro Islet-Protective AAT Activities.

	Trigger	Response	AAT conc./dose	Outcome	Reference
β cell protection					
MIN-6 (mouse β cell line)	TNFα or STZ	Apoptosis	0.5 mg/ml	Apoptosis rates reduced by 10-20%; caspase-3 activity inhibited	32
		Viability	0.5 mg/ml	Increased by up to 20%	32
INS-1 (rat β cell line)	IL-1β, IFNγ and TNFα	Apoptosis	0.125-1 mg/ml	Apoptosis rates reduced by 27-40%	33
	Clonidine	Glucose stimulated insulin secretion capacity	0.5 mg/ml	Insulin release increased by up to 60%; further increase in the presence of the insulin-release agents, GLP-1 and forskolin	33
	None	Intracellular cAMP levels	0.5 mg/ml	cAMP levels increased by up to 70%	33
Pancreatic islet protection					
Mouse islets	IL-1β and IFNγ	Apoptosis	0.25 mg/ml	Islet viability increased by 20%	30
		Nitric oxide release	0.25 mg/ml	Inhibited by 36%	30
		TNFα release	0.25 mg/ml	Reduced by up to 99% while resulting membrane-associated TNFα levels were 8-fold higher	30
		MIP-1α release	0.25 mg/ml	Reduced by up to 82%	30
		Glucose stimulated insulin secretion capacity	0.5 mg/ml	Islets resumed insulin release	30
		MHC class II expression	0.25 mg/ml	Reduced	30
	Heat-shock protein gp96	Survival	0.5 mg/ml	Improved	16
Rat islets	IL-1β, IFNγ and TNFα	Glucose stimulated insulin secretion capacity	0.125-1 mg/ml	Insulin release increased by up to 60%; further increase in the presence of the insulin-release agents, GLP-1 and forskolin	33
		Apoptosis	0.125-1 mg/ml	Apoptosis rates reduced by 27-40%	33
	None	Intracellular cAMP levels	0. 5 mg/ml	cAMP levels increased by up to 70%	33
Pig islets	Isolation-related islet injury	Isolation yield	0.5 mg/ml	Improved islet yield	40
Insulin protective assays					
Human islet purification	Impure human islet fractions	Proteolytic insulin breakdown	0.5 mg/ml	Intact insulin levels rise from 58% to 98%	41,71

Selected studies with direct evidence for protection of islet β cells from injury in the presence of AAT. Reports relate to plasma-purified AAT, unless otherwise indicated.

is an act of blunting the immune response to an injured tissue. Whether this binding to DAMP molecules is hampered by glycation of AAT is currently under investigation.

The *function* of β cells is also compromised under inflammatory conditions. In a study by Kalis et al, AAT was found to increase insulin release capacity.[33] The effect of AAT on insulin secretion was accompanied by an increase in intracellular cAMP levels, suggesting that AAT may advance some of its activities through the cAMP pathway. Of note, it is through elevated cAMP levels that the anti-inflammatory cytokine, IL-10, is induced, one of the most consistent outcomes of AAT activity in multiple experimental systems. Indeed, AAT was shown to elevate cAMP levels and IL-10 production in human blood cells.[35] Thus, the microenvironment afforded by AAT includes better functioning islet β cells, an added beneficial layer to its modulation of the immune response.

Protection of islets by AAT is consistent across multiple diabetes models. Findings from multiple experimental animal models provide clear evidence that AAT possesses broad

anti-inflammatory and immunoregulatory activities, promoting tissue recovery processes in the context of immune-mediated β cell destruction (see Table 2). The beneficial effect of AAT on diabetes was first demonstrated using virus-mediated gene delivery of AAT to nonobese diabetic (NOD) mice.[36] The study was intended to explore the possibility that an IL-10 plasmid would protect islets, and had added an AAT-expressing vector as a control protein; nonetheless, a single injection of the AAT-expressing DNA plasmid reduced insulitis in these mice. This success occurred in spite of antibodies generated against human AAT, being that it is a foreign antigen to the mouse. Although the study did raise the possibility of a potential therapeutic role for AAT in T1D, it focused on a mouse model that is currently attracting criticism as to its translatability.[37] Of note, the NOD mouse is the only mouse strain to exhibit steady-state low levels of circulating murine AAT, compared to other strains.

In another study that used the NOD mouse,[9] treatment course consisted of 14 days of clinical-grade AAT, so as to evade the mounting of mouse-antihuman antibodies. When examined more than a month later, instead of the islets being invaded by lymphocytes, they were surrounded by a cuff of apparently benign lymphocytes. Upon dissection of the draining pancreatic lymph nodes, a shift in the balance of proinflammatory to anti-inflammatory cytokines was observed. Furthermore, insulin-positive β cell mass appeared to have expanded in AAT-treated mice.

How does AAT relate to inhibition of pathogenic pathways in T2D? An imbalance between AAT levels and neutrophil elastase (NE) has been shown to contribute to the development of obesity and insulin resistance in mice.[38] Increased NE activity and decreased serum levels of AAT were found to occur in leptin-deficient obese mice, as well as high fat diet–induced obese wild-type mice and obese human subjects. The possibility that NE/AAT imbalance might be related to the pathological changes observed in obesity was investigated by comparing NE-knockout mice and AAT-transgenic mice. Both strains were resistant to high fat diet–induced metabolic changes, as well as macrophage infiltration into the adipose tissue.[38]

AAT protects pancreatic islets in the context of islet transplantation. Human islet transplantation has been under clinical evaluation since 2000, displaying impressive 1-year graft survival rates but a disappointing erosion of islet function by the fifth year after transplantation.[39] The state of islet cells during and after isolation renders the grafts highly immunogenic, being that the cells are injured and inflamed—from the point of pancreatic procurement and right up until their embolization into the recipient; this time frame alone is responsible for the common requirement of more than 1 donor per transplant patient, as more than half of the embolized mass of islets is destroyed within the first 48 hours of the procedure, in an antigen-independent manner.

Due to donor shortage, the use of porcine islets for xenotransplantation is of considerable interest and has recently been approved for clinical trials by the FDA. However, porcine islets are particularly difficult to isolate because of an apparently weak islet capsule. Shimoda et al demonstrated that introducing AAT during the procedure of pancreatic digestion markedly improves porcine islet isolation;[40] fortunately, AAT does not inhibit the mixture of collagenases that is required for the isolation of islets from pancreatic acinar tissue. Indeed, impure islet grafts that contain residual exocrine tissue display low recovery profile. Yet, this is improved by addition of AAT (see Table 3). Improving islet cultures may increase the proportion of human islet preparations that meet the criteria for islet transplantation.[41]

Once islets are grafted, AAT protects them from immune destruction and induces active long-standing immunologic tolerance.[8,9,30] In mouse islet transplant models, the very same treated recipient mice are still able to destroy islets that were transplanted later on, and that originated from a third strain of mice, proving that the immune system was not suppressed by temporary AAT therapy. Long-lasting grafts were surrounded by a cellular cuff containing Tregs and an elevated expression level of the anti-inflammatory cytokines IL-1Ra, TGFβ, and IL-10, several weeks after treatment withdrawal.

Prolongation of allogeneic islet graft survival in NOD mice with AAT was demonstrated by Pileggi et al, although the group unfortunately encountered an issue inherent to the NOD mouse colony in a facility-specific manner: a hyperimmune response.[42] Later, Ma et al from the same group showed that anaphylaxis in this strain is avoided by administering AAT intradermally.[43]

Is systemic AAT delivery required for islet graft protection? Could locally produced AAT reach the same outcome? By transplantation of an insulinoma cell line, NIT, transfected with AAT (NIT-AAT) into NOD mice, local AAT was shown to have immunomodulatory properties that delayed the onset of autoimmune diabetes, reduced diabetes incidence, inhibited insulitis, reduced β cell apoptosis rates, and dampened transplant site inflammation. However, no long-term specific immune tolerance was evident.[39] The possibility of β cells displaying increased survival rates in an autoimmune mouse model, without the immune system "waking up" and attacking them, is of particular interest for islet β cell regeneration approaches.[44]

Current State of Clinical Trials With AAT for T1D Patients

Out of 6 clinical trials for T1D patients with AAT treatment, 3 have recently been completed (NIH clinical trial registries NCT01183455, NCT01319331, NCT01183468) and 3 are ongoing (NCT02005848, NCT01304537 and NCT01661192). The first trial to open was an open-label phase I study at the Barbara Davis Center for Childhood Diabetes in Denver, Colorado.[45] The Colorado group also explored the possibility that AAT may reduce inflammatory

Table 2. Experimental Support for Islet-Protective AAT Activities, Preclinical Data.

	Trigger	Response	AAT conc./dose	Outcome	Reference
Islet protection					
β cell toxicity-induced hyperglycemia	STZ-induced hyperglycemia	Hyperglycemia	60 mg/kg on day -1 and 0	Increased insulin-content and β cell viability	30
			Recombinant AAT:Fc fusion 60 mg/kg	Increased β cell viability	70
Spontaneous diabetes	NOD mouse	Diabetes onset and reversal	60 mg/kg every 3 days for 14 days	Diabetes prevented and reversed	9
			Transgenic plasmid-derived muscle-expressed AAT	Diabetes prevented and reversed	72
			Intradermal, 60 mg/kg every 3 days for 14 days	Diabetes prevented and reversed	43
Islet transplantation					
Mouse islet allografts into STZ-induced hyperglycemic recipients	Alloimmune response	Graft survival	60 mg/kg every 3 days from day -1 for 21-52 days	Prolongation of islet allograft survival	30
		Intragraft gene expression profile	60 mg/kg every 3 days from day -1 for 21-52 days	Reduced levels of IL-1β, IL-2, ICAM-1, CD40, MIP-2, IL-17, IL-23, IL-6, MCP-1; elevated levels of IL-1Ra, CTLA-4, foxp3, TGFβ and IL-10	8,30
		Graft survival	Transgenic plasmid-derived liver-expressed AAT	Prolongation of islet allograft survival	6
Mouse β cell allografts into STZ-induced hyperglycemic recipients	Alloimmune response	Graft survival	β cell transgenic AAT expression	Graft protected	73
Mouse islet allografts into NOD recipients	Alloimmune response in an autoimmune mouse	Graft survival	60 mg/kg every 3 days for 14 days	Graft protected	42
NOD mouse islet grafts into syngeneic STZ-induced hyperglycemic NOD recipients	Autoimmune response	Diabetes recurrence	60 mg/kg every 3 days for 14 days	Diabetes recurrence prevented	9
Xenograft islet transplantation (rat to mouse)	Xenoimmune response	Graft survival	240 mg/kg every 3 days from day -10 in combination with temporary T cell depletion on day -3	Improved survival, only in combination therapy	74
		Intragraft gene expression and immune cell content	240 mg/kg every 3 days from day -10 in combination with temporary T cell depletion on day -3	Reduced content of inflammatory markers and CD14; elevated rat insulin expression, also in AAT monotherapy	74
Syngeneic islet grafts	Graft-related angiogenesis	Graft neovessel maturation	60 mg/kg every 3 days from day -1 for 9 days	Vessel maturity increased; elevated content of CD31 and VEGF; reduced levels of antiangiogenic factors Thbs-1 and PA-1	2
	Graft-related inflammation	Submass graft function	60 mg/kg every 3 days from day -1	Improved graft function; gene array revealed TNFα as strongest responder	75

Selected studies with direct in vivo evidence for protection of islet β cells from injury during AAT therapy. Reports relate to plasma-purified AAT, unless otherwise indicated.

Table 3. Experimental Support for Islet-Protective AAT Activities, Human Data.

	Trigger	Response	AAT conc./dose	Outcome	Reference
Human islet isolation					
Human islet purification	Impure human islet fractions	Proteolytic insulin breakdown	0.5 mg/ml	Improved intact insulin levels from 58% to 98%	[41]
Clinical trials					
Phase IIa (Barbara Davis Center for Childhood Diabetes, USA)	Recent onset T1D	C-peptide production	80 mg/kg IV every 7 days for 8 weeks; 18-month follow-up	5 out of 12 individuals exhibited an increase in c-peptide levels	[45]
Phase IIa NCT01304537 (Schneider Children's Hospital and Assaf Harofe Medical Center, Israel)			40, 60, and 80 mg/kg IV every 7 days for 12 weeks; 24-month follow-up	Completed, not yet reported	[49]
Phase IIa NCT01183468 (Joslin Center for Diabetes Research, USA)			45 then 90 mg/kg IV every 7 days for 12 weeks; 24-month follow-up	Completed, not yet reported	—

Completed clinical studies using plasma-purified AAT for patients with recent-onset T1D.

responses in the patient's white blood cells. The study enrolled 12 subjects with recently diagnosed T1D who had detectable c-peptide levels, ages 12 to 39. Subjects received 8 slow-drip weekly infusions of plasma-derived AAT (80 mg/kg body weight) and then were monitored for adverse events and for the degree of c-peptide response to a mixed-meal tolerance test; blood samples were also collected for analysis of toll-like receptor (TLR)–induced IL-1β expression in circulating cells, periodically, over the stretch of 18 months.

Study subjects tolerated the treatment well. After 12 to 18 months from treatment, 5 subjects had either increased or unchanged levels of c-peptide, compared to their own individual baseline, as opposed to the remainder of the cohort that had c-peptide progressively decline. In the absence of a placebo group at this stage, the Rituximab trial was used to extrapolate a comparison as it had a similar course of treatment in a matching placebo group.[46] Responsive subjects were distinct in that their c-peptide values upon recruitment were lower. This finding may indicate that the benefit of AAT is most pronounced in *actively damaged* pancreata, but larger studies are required to ascertain such conclusions. Nonetheless, this intriguing outcome agrees with the working hypothesis that the major benefit of AAT may exceed the mere inhibition of inflammation, and lean heavily on its ability to address the immune response to cell and tissue injury. For example, in a preclinical model of ischemic heart disease, which is not an immune disorder and also not an isolated inflammatory pathology as it contains the element of necrotic cell death, AAT treatment results in reduced heart muscle damage and increased contractile functionality.[13] Similarly, one can deduce from the predominant pathological entity found in individuals with genetic deficiency in AAT, as well as smoke-induced alveolar wall degradation, emphysema, that the parameter of tissue injury is one that AAT readily addresses in a seemingly nonredundant manner.

In addition to superior metabolic control, a clear aspect of immunomodulation arose from the Colorado trial. The beneficial effect of AAT in T1D patients correlated with less inflammatory myeloid cells compared to subjects that showed no improvement in islet function. This is a highly intriguing aspect of the consequence of AAT therapy, as the most significant changes were documented not *during* AAT injections, but rather 9 months *later*. This particular time point agrees with the concept that bone marrow myeloid cells employ specific proteases to complete one of several stages of differentiation; of these enzymes, PR3 is inhibited by AAT.[47] In addition, these outcomes strengthen the view that the effects of AAT are both islet-directed, as well as immunomodulatory, with the latter suggesting that other autoimmune disorders may benefit from AAT therapy.[48]

The second of 6 trials in T1D and AAT was a phase I/II open-label study was conducted in Israel by Kamada, Ltd, at Assaf Harofeh Medical Center and Schneider Children's Medical Center (NCT01304537).[49] The 24 subjects had an age range of 9 to 17 years and time from diagnosis was 65 ± 44 days (mean ± SD). Subjects were assigned to 1 of 3 dosage groups of Glassia©, a liquid preparation of AAT[50] at 40, 60, or 80 mg/kg, and each subject received 18 infusions at the assigned dosage.

According to study results, all 24 subjects completed the treatment course; safety and tolerability of AAT treatment were excellent, and the few adverse events were strictly infusion-related (mild and transient fatigue and headache). Mean levels of HbA1c decreased significantly, from 8.82 ± 1.78% at baseline to 7.16 ± 0.82% upon completion ($P < .001$). Although slope analysis of c-peptide did not show significant

changes from baseline, 12 to 15 months c-peptide had decreased from 0.69 ± 0.42 pmol/ml at baseline to only 0.51 ± 0.40 pmol/ml upon completion. AUC% had similarly decreased in a strikingly moderate manner, 23% from baseline, a smaller reduction compared to the ~50% decrease normally expected at 12 to 15 months from diagnosis and compared to the Rituximab study, in which placebo group c-peptide declined by ~50% at 12 to 15 months. Upon study conclusion, 48% of patients exhibited reduced insulin requirement, and the entire cohort displayed a marked reduction in anti-GAD65 and anti-islet antibody titers.

Nonetheless, one should consider that the lack of a placebo arm in both AAT T1D trials precludes statistical significance of the efficacy of therapy, and that comparison with placebo controls from other trials is precarious; the emphasis of the 2 trials would be that the approach is safe, well-tolerated, and worthy of further investigation. None of these parameters were obvious upon approaching a pediatric population of individuals that have normal AAT levels.

The improved metabolic control and β cell function found in both studies, long after the course of AAT infusions was completed, may indicate that AAT interferes with disease progression. Considering the lack of viral recurrence in this cohort and the compelling evidence for antibacterial attributes of AAT,[51-53] it may be concluded that the effect of AAT on the immune system is not immunosuppressive, but rather immunomodulatory, allowing intact host defense.

Conclusion

On the basis of knowledge gained from other autoimmune diseases and from transplantation studies, *immunotherapeutic* approaches have been used to halt the progression of T1D for decades. The first immune-intervention studies used immunosuppressive drugs such as cyclosporine A, which were effective, but only as long as the treatment was maintained, and severe side effects precluded completion of the trials. Therapies such as CTLA4-Ig (Abatacept),[54] an agent that blocks co-stimulation, or CD20-monoclonal antibody (Rituximab) that reduces B lymphocyte contribution to autoimmunity[46] also resulted in significant improvement of β cell function, yet were, again, predominantly short of holding on to a long-term outcome. Pilot trials with anti-inflammatory drugs such as anti-TNFα and IL-1Ra have shown promising effects, and are awaiting results from large ongoing trials. Nonetheless, these all carry side effects directly attributed to their intended activities, that is, blocking of essential molecules and inhibition of important immune cells for a significant period of time. These disadvantages should be compared with positive patient compliance during AAT therapy and the impressive safety record at relevant doses in the past 3 decades, since its approval by the FDA for use to treat individuals with genetic AAT deficiency. In AAT trials, all recruited individuals were included in the outcome assessment; for comparison, the Rituximab trial had 78 individuals in the primary outcome assessment—out of 87 recruited participants (and had also exhibited reactivation of latent viruses, such as polyomaviruses[55]); the T cell–directed anti-CD3 trial had 77 participants included in the primary outcome assessment—out of 83 recruited participants.[56]

Whether AAT therapy can be combined with such strategies and perhaps achieve therapeutic synergy is currently being evaluated. Similarly, the combination of an immunomodulator and β cell regeneration may reduce the recurrence of autoimmunity toward newly regenerated β cells.

Lack of severe side effects in any treatment is a strong positive parameter. This was indeed part of the rationale just over a decade ago for studying AAT as a therapeutic candidate for individuals with T1D: it will have to be safer than insulin alone, possibly for many years of treatment in kids, and a naturally occurring molecule in considering the autoimmune predisposition of the underlying disease. There are no adverse effects related to physiologically elevated plasma levels of AAT in the context that individuals encounter, no doubt, at least once in their lifetime, that is, acute phase response, third trimester of pregnancy[57-60] or advanced age.[61] The infused material in the present trials reaches comparable circulating levels to those measured under physiologically induced AAT serum concentrations, whether the individuals are genetically deficient in AAT or not. As an arbitrary example, a deficient individual with as low as 0.5 mg/ml circulating AAT and a nondeficient individuals with 1.0 mg/ml circulating AAT[62] both reach the levels of 4-6 mg/ml upon infusion of AAT in the first day postinfusion, then rapidly decline thereafter until the subsequent weekly infusion.[63] If any, one observes the accumulation of pathologies in AAT *deficiency*, whether the deficiency is genetic or cigarette-smoke-induced,[64] glycation-related,[25] or by way of excessive proteolytic degradation by bacterial proteases.[65]

Taken together, perceivable limitations of AAT infusions would be related to it being plasma-derived,[66] and can be overcome with the rapidly expanding recombinant technology[67] and gene therapy approaches.[68] When compared to multiple other attempts for T1D therapy,[69] AAT appears to be the only approach to date that offers a safety profile that is equal or superior to that of insulin alone, and, under the currently explored treatment protocols, may reach a 50% success rate in reinstating islet function.

Is AAT available for use on the scale of demand associated with newly diagnosed T1D cases? Recombinant AAT-Fc protein was generated and expressed in Chinese hamster ovary cells. While the recombinant protein does not share all of the biological activities of plasma-derived AAT, such as inhibition of elastase, it did block the development of hyperglycemia in mouse models of diabetes, agreeing with the particular newly appreciated unnecessity of the antielastase function in AAT.[70] Other groups have also been developing recombinant versions of AAT, as well as advanced in gene therapy.[68] Considering the differences from a regulatory point of view between plasma-purified AAT and recombinant alternatives,

it is predicted that FDA approval for clinical use of recombinant forms of AAT for T1D will require several more years.

Abbreviations

AAT, α1-antitrypsin; AATD, AAT deficiency; DAMP, danger associated molecular pattern; GLP-1, glucagon like peptide-1; GvHD, graft-versus-host disease; IAPP, islet amyloid polypeptide; IHD, ischemic heart disease; IL-1, interleukin 1; NE, neutrophil elastase; NOD, nonobese diabetic; PR3, proteinase 3; PTDM, post-transplant diabetes mellitus; T1D, type 1 diabetes; T2D, type 2 diabetes; TLR, toll-like receptor.

Acknowledgments

We thank Anne Walton for her excellent assistance in manuscript preparation.

Declaration of Conflicting Interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Funding

The author(s) received no financial support for the research, authorship, and/or publication of this article.

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