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Immune Regulatory Mechanism of Alpha1-Antitrypsin in Type 1 Diabetes and Its Clinical Application Progress

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Abstract:

Type 1 Diabetes (T1D) is an autoimmune disease-causing selective destruction of pancreatic β-cells, requiring lifelong insulin. Current treatments cannot prevent β-cells loss or restore immune tolerance, highlighting the need for diseasemodifying therapies. Alpha-1 antitrypsin (AAT) has attracted attention due to its multiple mechanisms of action in T1D. It can regulate NKDC interactions, boost Treg function, and inhibit Th1 and CD8+ T cells. It also suppresses the NFκB/IL-1β pathway and upregulates IL1Ra. These actions reduce β-cell apoptosis and increase β-cell mass. Clinical studies show safety of AAT in new-onset T1D and islet autotransplantation, but efficacy outcomes have not been consistently met, partly due to insufficient dosing (90 mg/kg/week) and compensatory endogenous AAT elevation. Different reactions related to gender have been observed in female patients. Future larger-dose, placebo-controlled trials with gender are needed. Engineered mesenchymal stem cells overexpressing AAT and gene delivery strategies provide an effective way to achieve sustained and localized AAT expression, and are expected to overcome the current challenges in clinical translation.

Keywords: Alpha-1 antitrypsin (AAT); Type 1 diabetes (T1D); Immunomodulation; Clinical trial.

1. Introduction

Type 1 Diabetes (T1D) is a chronic metabolic disease caused by selective destruction of islet β-cells by autoimmune reaction. Current mainstream treatment is insulin injection. However, this method fails to prevent the continuous loss of β-cells, and reverse the immune imbalance. Therefore, finding a method possesses immune modulating function and β-cells protective effect has become plume of T1D research. Alpha1-antitrypsin (AAT) is an acute phase response protein which can protect β-cells on multiple levels. Currently in medical research on T1D shows a growing tendency to find therapeutic targets, which can make an immune response to cell damage, achieving selective control of early tissue repair and excessive

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inflammation. The preliminary results from existing studies show that AAT therapy has an expectation of improving insulin secretion function in the absence of adverse reactions. This makes AAT therapy a potential treatment option [1].

In animal models AAT inhibits the natural killer (NK) cell degranulation against β-cells effectively [2]. Short-term AAT therapy can let blood glucose levels in 21 out of 24 newly generated non-obese diabetic (NOD) mice restore normal (effect sustaining for over 270 days without continued treatment) [3]. AAT reduces the production of IL-15 by dendritic cells (DCs), and downregulate NKp-46 ligands on β-cells, diverting NK cell cytotoxicity away from islets [2]. AAT enhances the function of regulatory T cells (Tregs), inhibiting the expression of helper T cells 1 (Th1) and CD8+ cells and inducing the exhaustion phenotype in CD8+ cells [3,4,5]. AAT inhibits the expression of IL-1β (induced by TLR) and suppress the activation of NF-κB and upregulate the expression of IL-1Ra, so apoptosis of β-cells can be reduced and β-cells are stimulated to regenerate (increase to five times) [3,6,7].

In clinical applications, AAT is well tolerated and reduces HbA1c without adverse events in children with new-onset T1D, though large trials are needed to confirm efficacy [8]. In autologous islet transplantation, perioperative AAT (90 mg/kg×6) failed to improve two-year islet function; endogenous AAT levels were significantly elevated in all groups, peaked on day 7 and remained above normal levels until day 28, which may confound efficacy assessment; insulin secretion parameters showed an upward trend in women [9,10]. Phase I retention trials further demonstrated that a weekly dose exceeding 90 mg/kg is required to maintain therapeutic AAT concentrations above 2.0 g/L for several consecutive days [11]. Another trial using 60 mg/kg weekly confirmed safety but found no improvement in 12-month C-peptide area under the curve [12].

AAT has significant effects in preclinical studies, but inconsistent results. Total Pancreatectomy with Islet Autologous Transplantation (TPIAT) has not demonstrated therapeutic efficacy. In the surgical conversion process also presents numerous challenges. Therefore, finding systematic reviews of existing evidence is crucial for optimizing future trial designs. This article will discuss following aspects: the regulatory role of AAT on NK/DC/T cell immune mechanism and inflammatory pathways; clinical research advancements (including safety, efficacy, and perioperative data) of AAT in T1D and islet transplantation; sex-specific responses in trial, and emerging delivery strategies including AAT-overexpressing mesenchymal stem cells (MSCs) and gene therapy.

2. Immune Pathogenesis and Treatment Challenge of T1D

T1D results from broken immune tolerance, causing autoimmune β-cell destruction [2,3]. Both innate (NK cells, DCs) and adaptive ($CD4^+$ Th1, $CD8^+$ T cells) immunity contribute, while Tregs fail to control the response [3,4]. Proinflammatory cytokines from macrophages and DCs damage βcells via NFκB and induce apoptosis [6,7]. This inflammation also causes insulin resistance [3]. Current insulin therapy cannot prevent β-cells loss or restore tolerance [1,8]. This chapter discusses T1D immunopathology and treatment challenges, providing a rationale for AAT as a potential diseasemodifying therapy.

2.1 Autoimmune Characteristics of T1D

During the autoimmune process of T1D, adaptive immunity and innate immunity work in concert. $CD4^+$ cells (particularly the Th1 subset) activate macrophages and $CD8^+$ cytotoxic T lymphocytes by secreting IFN-γ; $CD8^+$ T cells recognize self-antigen peptides presented by MHC calss I molecules on the surface of β-cells, and then mediate β-cells killing through the perforin and granzyme pathways [3]. Meanwhile, because of the lack of the function of Tregs, Tregs can not inhibit these pathogenic T cells effectively [3,4].

In addition, amounts of NK cells lay in the islets in the NOD mice and patients in T1D. Activated receptor NKp46, which be activated by NK cells, can recognize unknown ligand expressed on β-cells surface [2]. In the local islets, (DCs) can capture self-antigens (released by apoptotic β-cells). Then these antigens move to draining lymph nodes, being presented to T cells to initiate adaptive immune responses. DCs activate NK cells by secreting cytokines such as IL-15, and the activated NK cells promote DC maturation, accelerating β-cell damage [2].

Activated immune cells release IL-1β and Tumor necrosis factor (TNF-α). These factors induce iNOS in β-cells and give excess nitric oxide (NO) by making the NF-κB signaling pathway active. This suppresses insulin secretion and induces β-cell apoptosis [6,7]. After β-cell death, released selfantigens and DAMPs further activate antigen-presenting cells, giving chronic inflammation fuel. These inflammatory factors also cause insulin resistance, worsening hyperglycemia [3]. Therefore, T1D is a complex disease driven by both immunity and inflammation.

2.2 Inflammatory Microenvironment and β-cell Apoptosis

Activated macrophages and dendritic cells in islets make IL-1β and TNF-α. These bind to β-cells, turn on NFκB,

and cause iNOS to make too much NO. NO hurts β-cells, lowers insulin release, and kills them. Additionally, IL-1β can stimulate β-cells to produce more chemokines (such as Monocyte Chemoattractant Protein-1), which bring more immune cells into islets, creating a loop that worsens damage [7]. Dying beta cells release their own antigens and damage-associated molecular patterns, which further activate nearby antigen-presenting cells, thus perpetuating the inflammatory response [3]. These inflammatory factors also block insulin action in fat and muscle, leading to insulin resistance and worse high blood sugar [3]. Therefore, the T1D inflammatory microenvironment directly damages βcells and drives long-lasting autoimmunity and metabolic problems.

2.3 Limitations of Current Treatment

Insulin therapy fails to stop β-cell loss or autoimmune attacks, so β-cell function keeps declining year after year [1,3]. Although strict blood sugar control can delay complications, it is accompanied by the risk of hypoglycemia, especially more prominent among children and adolescents [8]. Researchers have attempted a variety of immune intervention strategies but still face with limitations. These limitations suggest that suppressing the immune system or a single target is insufficient. There is an urgent need to develop new drugs with high safety, anti-inflammatory and immunomodulatory functions, and the ability to promote β -cell repair [1,8].

3. Immunoregulatory Mechanism and Clinical Application of AAT in T1D

AAT was initially recognized for its ability to inhibit serine proteases, but recent studies have revealed extensive anti-inflammatory and immunomodulatory activities that are independent of protease inhibition. In T1D and islet transplantation models, AAT exerts multi-level protective effects by acting on natural immune cells (NK cells, DCs), adaptive immune cells (T cells) and β-cells themselves.

3.1 Immunomodulatory Mechanism of AAT

Regulation of NK cells by AAT is not a direct inhibition but is achieved indirectly by altering the characteristics of DCs and β-cells. Scientists found the production of IL-15 in bone marrow-derived DCs treated with AAT was significantly reduced, and IL-15 is a key molecule for DCs to activate NK cells. Therefore, the ability of DCs pretreated with AAT to induce degranulation (killing activity) of NK cells when co-cultured with NK cells subsequently decreased significantly [2]. More importantly, after administration of AAT, the expression level of NKp46 ligand on the pancreatic islet β -cell membrane of mice decreased by approximately 45%. This change causes the cytotoxic activity of NK cells to deviate from that of β-cells, while retaining their normal response ability to other targets such as tumor cells [2]. This selective immunomodulatory property enables AAT to protect β-cells without damaging the normal anti-infection and anti-tumor immunity.

3.2 Clinical Application Progress

3.2.1 Safety and preliminary efficacy in newly diagnosed T1D

Several clinical trials have showed the good safety and preliminary efficacy of AAT in new-onset T1D, particularly pronouncing in children. According to the research, significant improvement in HbA1c was observed among newly diagnosed pediatric patients with T1D. However, due to the lack of placebo-controlled trial, the efficacy of AAT and its definitive protective function of β-cell need to be proved further.

Rachmiel et al. [8] conducted an open-label Phase I/II trial in 24 children with new-onset T1D to evaluate the safety and tolerability of AAT and its impact on glycemic control in children and adolescents with new-onset T1D. They were administered intravenous injections of 40, 60 or 80 mg/kg AAT once weekly for a totoal of 18 doses. HbA1c decreased significantly from 8.43% to 7.09% ($p < 0.001$). 75% of the subjects exhibited a C-peptide peak ≥ 0.2 pmol/mL at the end of study. The author noted this trial shows the safety and tolerability of AAT in children with new-onset T1D. Shorter disease duration was associated with better responses [8].

To explore the safety, optimal dosage of AAT in adults and children with new-onset T1D, the RETAIN Phase I trial (Weir et al. [11]) studied 16 patients (8 adults and 8 children with a course of less than 100 days). They first received 45 mg/kg (6 weeks), then followed by 90 mg/kg (6 weeks). No treatment-related serious adverse events reported. C-peptide was relatively stable at 52 weeks in the adult group (+0.013 pmol/mL), whereas in the children group there was a decrease (-0.19 pmol/mL) . Especially 72 hours after the infusion of 90 mg/kg, AAT concentration in most subjects had fallen below 2.0 g/L; inhibiting expression of gene that relate to NF-κB required an AAT concentration of 2.5-5.0 mg/mL. This suggests the weekly dose of 90 mg/kg may be insufficient to maintain effective concentrations. A higher dose is required [11].

The limitations of two trials both include absence of a placebo-controlled group and a small sample size. In addition, no long-term follow-up in Rachmiel et al. [8], making the efficacy of AAT needs to have further proofs. However, certain conclusions can still be drawn. AAT

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demonstrated favorable efficacy in both pediatric and adult patients with new-onset T1D. In children, AAT can significantly reduce HbA1c levels and partially stabilizes C-peptide; patients with shorter disease duration may benefit more readily. In adults, C-peptide remains relatively stable, whereas in children it declines more rapidly. Based on RETAIN Phase I trial, higher dose need to be explored. Both trials demonstrated that AAT exhibits a certain degree of efficacy in treating new-onset T1D.

3.2.2 Perioperative application of islet transplantation: TPIAT randomized trial

TPIAT is effective for chronic pancreatitis, but early islet loss due to the instant blood-mediated inflammatory reaction (IBMIR) limits outcomes [9]. AAT has been attempted for the perioperative management of TPIAT due to its anti-inflammatory and immunomodulatory properties [9]. Despite both randomized controlled trials (RCTs) failed to prove that AAT has the ability to bring continuous pancreatic islet function benefits, they revealed significant associations between endogenous AAT levels and its compensatory elevation as well as gender differences [9,10,12]. To study whether AAT could relieve pancreatic islet injury and improve transplantation outcomes, Abdel-Karim et al. [9,10] randomized 43 TPIAT patients to AAT (90 mg/kg×6 doses), etanercept, or the standard care. Acute insulin response at 3 months in the etanercept group (AIRglu) and acute c-peptide response (ACRglu) best but the differences between groups disappeared at 1 year. Notably, at 1 year, women who got AAT had noticeably higher insulin secretion ($p=0.05$ and 0.06), suggesting that the drug may work differently in females [9]. At 2 years, no differences in outcomes were observed. However, endogenous AAT significantly rose in all groups ($p<0.001$), peaking on day 7 and staying high for 28 days. This potentially made exogenous efficacy not correct. Adverse events were consistent with TPIAT, with no treatment-related serious adverse events [10].

Another TPIAT trial tested 60 mg/kg AAT (weekly×4) against a placebo. The main goal was to see if it improved the 12-month C-peptide AUC, but there was no real difference between the two groups ($p=0.43$) [12]. Nevertheless, the AAT group showed some biological signals compared to placebo ($p < 0.05$), even though the primary endpoint was not met. Adverse events were similar between the groups, with no treatment-related deaths reported. The authors concluded that the 60 mg/kg was safe but did not work for the main outcome, so higher doses or starting therapy earlier might be needed [12].

In conclusion, AAT shows favorable safety profiles and preliminary efficacy signals in children with new-onset T1D. However, during perioperative administration in the TPIAT trial, neither of the two dosage regimens yielded sustained improvements in pancreatic islet function. The compensatory elevation of endogenous AAT is a key factor explaining the negative results. It means that in further research people need to pay more attention to dose optimization and find earlier or longer intervention time windows.

3.3 Novel Delivery Strategy and Optimization of Dosing Regimen

In animals, AAT works well at protecting β-cells, but the results from clinical trials have been mixed. One key problem is that the current dosing regimens cannot reach or maintain high enough AAT in the body [11]. In addition, AAT breaks down quickly, so patients need frequent infusions. It is hard to stick with over the long term. That is why researchers are exploring ways to improve things from several angles.

3.3.1 Dose optimization

The RETAIN trial (Weir et al. [11]) gave key data. 72 hours after infusion of 90 mg/kg AAT, plasma concentrations in most patients fell below 2.0 μg/L, and at 120 hours, almost all patients had values below this level. It indicates that a weekly dose of 90 mg/kg is insufficient to meet long-term treatment needs, as AAT concentrations of 2.5-5.0 mg/mL are needed to inhibit genes related to the NF-κB pathway. However, 180 mg/kg could raise the 72-hour trough concentration to approximately 2.5 mg/mL, while 270 mg/kg could maintain higher concentrations for a longer period [11]. Future studies may focus on higher-dose concentrations.

3.3.2 Engineered cell delivery

Combining AAT with MSCs to make AAT-overexpressing MSCs (AAT-MSCs) is another approach. For NOD mice, a single infusion of AAT-MSCs can reverse newly onset diabetes and delay its progression. AAT-MSCs can enhance the function of regulatory T cells, reduce Th1 cells and $CD8^+$ cytotoxic T cells, and induce $CD8^+$ T cell exhaustion [5]. Song and colleagues found that overexpression of AAT itself can enhance the function of MSCs, improving their differentiation potential into adipocytes, osteocytes, and chondrocytes. The expression of 23 genes related to stem cell characteristics, migration, and survival was upregulated [13,14]. Results show that AAT can not only protect β-cells but also optimize the role of MSCs as delivery vehicles, so they can enhance their therapeutic efficacy [14].

3.3.3 Gene delivery

To achieve continuous production of AAT, Shahaf and colleagues used gene delivery techniques to introduce

plasmids encoding AAT into mice. The results showed that AAT gene delivery significantly prolonged the survival time of allogeneic islet grafts (>100 days), whereas the control group experienced rapid rejection; the levels of pro-inflammatory factors (MCP-1, TNF-α, iNOS) in the grafts were significantly reduced, while the level of the anti-inflammatory factor IL-1Ra was increased; in addition, the proportion of regulatory T cells in the spleen increased by 1.67 times; the serum from these mice could also inhibit the release of inflammatory factors mediated by macrophages [13]. This study provides theoretical validation for "single-dose, long-acting" AAT gene therapy.

In summary, future studies can prioritize higher AAT doses and extend the treatment duration, and use of engineered AAT-MSC cells has double benefits. Animal studies have demonstrated these effects, indicating a highly promising approach. Finally, gene delivery systems are able to sustain AAT production in body, protect transplanted pancreatic islets and promot regulatory T-cell generation. Although clinical application remains distant, this represents a promising long-term research direction.

4. Conclusion

AAT protects islet β-cells through multiple immunoregulatory mechanisms. Firstly, AAT can inhibit NK cell-mediated β-cell killing and downregulate surface NKp46 ligands; then, AAT reduces the number of Th1 and $CD8^+$ cytotoxic T cells by enhancing Treg cell function and inducing $CD8^+$ T cell exhaustion; additionally, AAT can inhibit the NF-κB/IL-1β inflammatory pathway, upregulate IL-1Ra expression, alleviate β-cell apoptosis, and promote regeneration. In newly diagnosed children and adults with type 1 diabetes, AAT is well tolerated. HbA1c significantly decreased in pediatric patients, and some patients maintained relatively stable C-peptide levels; however, due to the lack of a placebo control, its efficacy still needs verification. In the perioperative period of TPIAT, two randomized controlled trials did not reach the primary endpoint, but showed a compensatory increase in endogenous AAT levels ($p<0.001$), with a gender difference—female patients responded better. A dose of 90 mg/kg/week only maintained effective concentrations for a few days, while in vitro experiments indicate that inhibiting NF-κB requires concentrations of 2.5-5.0 mg/mL.

AAT protects β-cells through multiple immune-regulating mechanisms, indicating that it is a potential therapeutic candidate for T1D and may provide new treatment options. However, existing clinical data are insufficient to support the use of AAT as a new therapy. Future optimization of dosing regimens, improved experimental design, or the development of new delivery strategies could be used to enhance its clinical application value.

The limitations of this review include open-label designs, small sample sizes and differences in dosing regimens of included clinical studies, limiting comparability. Some novel delivery strategies remain at the animal-study stage and require clinical validation. High-quality research are expected to further refine these findings.

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