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Mini Review: Alpha 1-antitrypsin and Its Involvement In Rheumatoid Arthritis

Khushtar A. Salman[1]*, Akif Ahsan and Sarah Ashraf[1]

[1]Department of Biochemistry,
J.N Medical College
Aligarh Muslim University, Aligarh (U.P), India.

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Abstract. Rheumatoid arthritis is a common autoimmune disorder, most often affecting women from 40 to 60 years old. The major symptom is chronic inflammation of the joints, although the hematologic, cardiovascular, and respiratory systems are also frequently affected. Many individuals with rheumatoid arthritis produce a group of auto-antibodies called rheumatoid factors that are reactive with determinants in the Fc region of IgG. Human Alpha1-antitrypsin (AAT) is well known as a serine proteinase inhibitor (SERPIN) which inhibits proteinase 3, neutrophil elastase, and cathepsin G. These serine proteases are released by joint invading neutrophils following inflammatory stimuli and have shown to be involved in arthritis development. A lack of Alpha1-antitrypsin in patients with rheumatoid arthritis could allow inflammation to increase because of uninhibited lysosomal enzymes. Future studies will focus on improvement of the therapeutic effect by optimizing the dose and timing of hAAT or rAAV8 vector delivery, and by combination therapy with other anti-arthritic drugs.

Keywords: Rheumatoid arthritis, Rheumatoid arthritis, inflammation, protease inhibitor

INTRODUCTION

Alpha1-antitrypsin is a glycoprotein serving as the most important protease inhibitor in human sera which is synthesised primarily in the liver (Michalski *et al.,* 1986). It belongs to the SERPIN (serine protease inhibitor) superfamily of proteins and is found in majority among all the SERPINS circulating the human serum (Rajpara *et al.,* 2010; Ahmad *et al.,* 2004). Different types of protease inhibitors are found in the human serum, which includes alpha1-antitrypsin, alpha-2-antitrypsin, alpha1-antichymotrypsin, alpha-2-macroglobulin, inter-alpha-trypsin inhibitor etc as listed in table 1 (Almonte and Sweatt , 2011). However, the main protease enzyme inhibitor among these is alpha1-antitrypsin as it inhibits trypsin, chymotrypsin, thrombin, plasmin, elastase and proteolytic enzymes from granulocytes (Ahmad *et al.,* 2004; Swedlund *et al.,* 1974).

Table 1. Serine proteases and their inhibitors.

Serine protease	Serine protease inhibitor
Thrombin	Protease-nexin-1 (PN-1), antithrombin III collagen, phosphatidylethanolamine-binding protein
Tissue plasminogen activator (tPA)	Plasminogen activator inhibitor-1 (PAI-1), neuroserpin, PN-1
Plasmin	α_2-antiplasmin, PN-1
Trypsin	PN-1, α_1-antitrypsin
Neuropsin	Serine protease inhibitor 3, murinoglobin I

* Author for correspondence: Dr. (Mrs) Khushtar A Salman,
Associate Professor
Department of Biochemistry,
J.N Medical College, AMU, Aligarh 202002 (U.P), India.
Email : ka_slmn@yahoo.co.in.

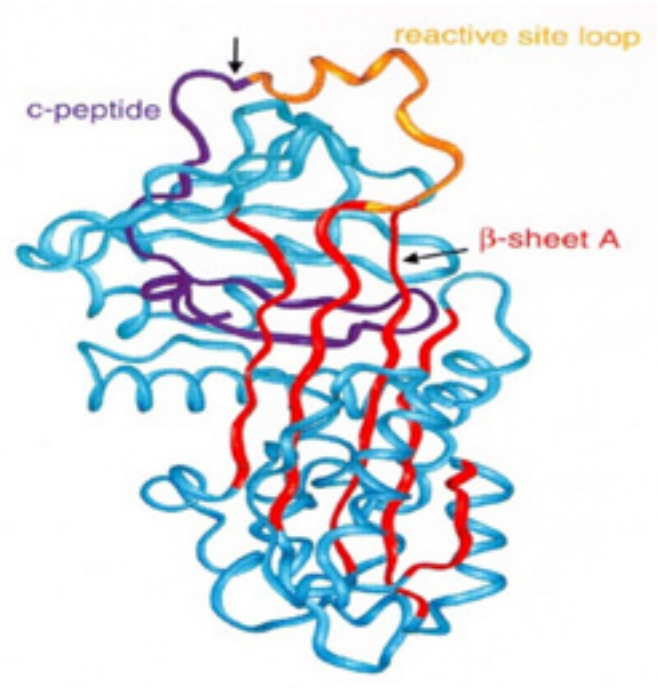

Figure 1. Structure of Alpha 1-antitrypsin (Janciauskiene, 2001).

Alpha1-antitrypsin is a 52 kDa molecular weight protein which consists of 394 amino acid residues and also 3 aspargine linked carbohydrate side chains. Fig 1 shows the secondary structure of Alpha 1-antitrypsin which contains 3 β-sheets and 8 α-helixes. The reactive loop site contains the neutrophil elastase binding site with a methionine residue (Janciauskiene, 2001).

It is synthesised by the hepatocytes and is released into the circulating blood by the liver. A few other types of cells also express AAT including macrophages, alveolar macrophages, neutrophils, monocytes, carcinoma cells, intestinal epithelial cells and cells in the cornea. The serum concentration of AAT normally ranges from 1 to 3.5 mg/ml. A 3-4 fold increase in concentration is observed during an inflammation response, suggesting that it is an acute phase protein with an important role in the inflammatory reactions of the human body (Ahmad *et al.,* 2004; Grimstein *et al.,* 2011; Fregonese and Stolk, 2008; Khan *et al.,* 2002; Deserres *et al.,* 2003). Alpha1-antitrypsin plays a crucial role in supporting the protease-antiprotease balance of the body by inhibiting different serine proteases enzymes especially the vital neutrophil elastase. Besides its antiprotease activity, alpha1-antitrypsin also helps in down regulating the inflammatory processes by opposing neutrophil peptide's pro-inflammatory effects and diminishing the expression of pro-inflammatory cytokines. In addition of having a gene that is transcribed continually, alpha1-antitrypsin is an acute phase protein and its transcription is up regulated in situations like inflammation, infection, cancer and pregnancy (Rajpara *et al.,* 2010; Ahmad *et al.,* 2004). Researches and work done in the past decade has made it feasible to subdivide the anti-proteolytic activity of the human serum into numerous individual inhibitors with distinct specificities. Some of these inhibitors fall in the category of acute phase proteins of which alpha1-antitrypsin is an example. They are part of a control mechanism of coagulation, fibrinolysis and the kinin & complement system.

The value and significance of serum proteinase inhibitors can be seen from the fact that inborn deficiencies can cause some characteristic diseases. Chronic obstructive pulmonary disease and severe infantile cirrhosis may be associated with hereditary deficiency of AAT, while a low concentration of the complement esteraseinhibitor or its functional deficiency may lead to hereditary angioneurotic oedema (Brackertz *et al.,* 1975). In 1963 Carl-Bertil Laurell and Sten Eriksson noticed a connection between the symptoms of pulmonary emphysema and decreased serum levels of AAT and were the first to report a case of AAT deficiency. Since then, an awareness and insight of the genetic abnormalities and biochemical mechanisms involved has been developed, and this deficiency is now considered to be among the most common hereditary disorders, almost equivalent in frequency to cystic fibrosis (Fregonese and Stolk, 2008; Stolk *et al.,* 2006).

Rheumatoid arthritis (RA), a chronic articular and systemic inflammatory disease, affects around 1% of the world's adult population and causes significant morbidity as well as increased mortality (Maradit-Kremers *et al.,* 2005). RA is an autoimmune joint disease associated with chronic inflammation of the synovium that causes profound damage to joints. Inflammation results in part from the influx of immune cells secreting inflammatory cytokines (Lin *et al.,* 2012). Onset of the disease generally occurs between the ages of 30 and 55 years, targeting the female population more commonly than the male. Disease hallmarks include synovial inflammation, progressive bone erosion, progressive polyarticularmal-alignment and destruction, and weakness of neighbouring muscles and other tissue. Presentation may range from mild to severe, although the disease in a typical patient follows a progressive course eventually causing functional limitations (Donahue *et al.,* 2008; Mulherin *et al.,* 1996).

Barwell was the first person to describe the involvement of bone in RA in 1865, and since then we know that both peri-articular and generalised osteoporosis are found in RA (Deodhar and Woolf, 1996). Small diarthrodial joints of the feet and hands are mainly involved. With the increased inflammation of synovium, a layer of abnormal fibrovascular granulation tissue known as pannus is formed. The pannus encroaches and damages the local articular structures (Firestein, 2003). Two clinical subtypes of RA have been observed. One type causes acute inflammation and stiffness of the synovial membrane of the joints, quite

possibly leading to ankylosis of the joints. This variety of disease is called the 'stiff' type. The other type mainly involves the synovial membrane of the bursae or tendon sheaths, slowly destroying and eroding the cartilage of the joints, causing instability rather than ankylosis of the joints. This subtype is known as the 'loose' type and is in fact more common than the stiff type (Clayton, 1965).

Although the etiology of RA has not been fully understood, clinical and laboratory findings suggest that pro-inflammatory cytokines have a significant role in its pathogenesis (Moreland *et al.*, 1997). As a result, systemic or local blockers of TNF-α and IL-1β and an anti-IL-6 receptor antibody can be used to neutralise the effector function of IL-6 (McInnes and O'Dell, 2010). A few locally secreted degrading enzymes, such as serine proteases (inhibited by AAT), aggrecanases and metallo-proteinases, destroy the articular structures by digesting the extracellular matrix (Firestein, 2003). Neutrophils release large quantities of elastase at the site of inflammation. Since elastase has a wide ranging substrate-specificity, including proteoglycans and collagen, it can inflict serious damage to the bones and cartilage in the inflamed joints of a RA patient. On the other hand, a major physiological role of the enzyme AAT is the degradation of elastase and thus protection of the connective tissues from its effect. If AAT is deficient the neutrophilic elastase can thus continue damaging the inflamed joint without any inhibition, thus aggravating the severity of the disease.

A lack of AAT in patients with RA could allow inflammation to increase because of uninhibited lysosomal enzymes, whereas higher than normal concentrations could help to control tissue damage by effecting a response to these enzymes (Swedlund *et al.,* 1974).

To fully understand the relationship between alpha1-antitrypsin and rheumatoid arthritis, a great deal of work has been done in the past decades by scientists and researchers. Brackertz *et al.* compared the levels of proteinase inhibitors between normal individuals and patients of classical and probable rheumatoid arthritis and found out that there was a significant rise in the levels of alpha1-antitrypsin, inter-alpha trypsin inhibitor and alpha1-antichymotrypsin in serum as well as in synovial fluid of rheumatoid arthritis patients (Brackertz *et al.*, 1975). Keith Chidwick *et al.* studied the proteinase inhibitory ability of alpha1-antitrypsin in the synovial fluid of rheumatoid arthritis patients. The immunochemically determined levels of alpha-1-antitrypsin were found to be raised in the patients but their proteinase inhibitory capacity was surprisingly depressed. This implied to the fact that, though the alpha1-antitrypsin levels were raised in the rheumatoid arthritis patients, they were partially inactivated with a decreased specific elastase inhibitory activity (Moreland *et al.*, 1997). Kimie Iwana *et al.* measured the serum level of IgA-Alpha1-antitrypsin complex using ELISA technique and found that their levels were significantly higher in the rheumatoid arthritis patients as compared to the healthy volunteers (Iwana *et al.*, 1996). In another study, Harry A. Swedlund *et al.* used the quantitative radial immuno diffusion method to study the concentration of alpha1-antitrypsin in the serum and synovial fluid. The levels were much higher in the rheumatoid arthritis patients than the healthy controls (Swedlund *et al.*, 1974). Scott *et al.* studied patients of rheumatoid arthritis and seronegative oligoarthritis. Their IgA-Alpha1-antitrypsin complex was measured using sandwich ELISA, IgA was measured using ELISA and alpha1-antitrypsin levels were measured by radial immuno diffusion techniques. IgA–Alpha1-antitrypsin complex levels in the sera of rheumatoid arthritis patients were significantly higher than the patients of seronegative oligoarthritis and healthy controls. Serum levels of IgA and alpha1-antitrypsin were similar in rheumatoid arthritis and seronegative oligoarthritis patients, but much higher than the healthy controls (Scott *et al.*, 1998).

Recently results from protein and gene therapy showed that human Alpha-1 antitrypsin (hAAT) is effective in delaying arthritis development in mouse model of a collagen-induced arthritis CIA. This indicates that hAAT has immunoregulatory and immunomodulatory effects and has great potential as a new treatment for RA. Studies have also shown that rAAV8 mediated gene therapy resulted in a reduced immune response to the transgene product. The rAAV-CB-hAAT vector construct was produced and packaged (Grimstein *et al.,* 2011). Vector carries hAAT cDNA driven by the cytomegalovirus (CMV) enhancer and chicken b-actin promoter and contains AAV2 inverted terminal repeats (ITRs). It was packaged into AAV serotype 8 capsid by cotransfection of vector plasmid and helper plasmid (XYZ8) into 293 cells.A possible mechanism of hAAT suppressing arthritis development is through inhibition of proteinases to prevent tissue injury and joint destruction. Human AAT is well known as a serine proteinase inhibitor (serpin). It inhibits proteinase 3, neutrophil elastase, and cathepsin G. These serine proteases are released by joint invading neutrophils following inflammatory stimuli and have shown to be involved in arthritis development (Adkison *et al.*, 2002). This protease inhibitor inhibits the attack of lysosomal enzymes on the joint tissues and thus helps to regulate joint inflammation. Human AAT can also reduce ischemia-induced apoptosis, inflammation, and acute phase response in the kidney (Daemen *et al.*, 2000). It has recently been shown that hAAT directly inhibits activity of caspase -3 and protects islet cells from cytokine and chemically-induced apoptosis (Zhang *et al.*, 2007).

CONCLUSION

Alpha1-antitrypsin is a very effective proteolytic enzyme inhibitor, responsible for almost 90% of the total serum trypsin inhibitory capacity. Increased inflammation and lysosomal enzyme activity in rheumatoid arthritis may force the concentration of alpha1-antitrypsin to rise. Now, the higher concentrations of alpha1-antitrypsin can do some damage control by fighting against the proteolytic enzymes, thus controlling the tissue damage and inflammation. A lack of Alpha1-antitrypsin in patients with rheumatoid arthritis could allow inflammation to increase because of uninhibited lysosomal enzymes. Thus most of the studies and data supports the concept that alpha1-antitrypsin inhibits the attack of lysosomal enzymes on the joint tissues and thus helps to regulate joint inflammation. Future studies will focus on improvement of the therapeutic effect by optimizing the dose and timing of hAAT or rAAV8 vector delivery, and by combination therapy with other anti-arthritic drugs.

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