Alpha1-antitrypsin-beyond emphysema

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Human plasma contains 7-8% of soluble proteins

- IgG: 8-17 g/L
- Albumin: 35 - 50 g/L
- Alpha1-antitrypsin: 1.3-2 g/L
- Haptoglobin
- Alpha1-acid glycoprotein
- Apolipoprotein A1
- Apolipoprotein A2
- Complement C3
- Apolipoprotein B
- Less abundant proteins (5-10%)

Other proteins include:
- IgM
- IgA
- Alpha2-macroglobulin
- Fibrinogen
- Transferrin
Alpha1-antitrypsin is structurally complexed and functionally sophisticated molecule

The structure and chemistry of proteins has been developed over billions of years of evolutionary history.

Proteins are so precisely built that the change of even one amino acid can disrupt the structure of the whole molecule so severely that function is lost or new function is gained.

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Alpha-1-antitrypsin-Pittsburgh (Met358Arg) has greatly diminished anti-elastase activity but markedly increased antithrombin activity.
Alpha1-Antitrypsin is an acute phase protein

**The liver plays a central role in the sensing of and the response to systemic inflammation**

**Inflammation**
(recruitment of neutrophils and macrophages, release of pro-inflammatory cytokines-IL-6)

**Acute Phase Proteins**
(Resolution of inflammation)

limit tissue damage caused by the inflammatory process and enhance the repair

Janciauskiene S, Acute Phase Proteins: structure and function relationship, Book chapter, 2012
Inherited PiZZ (Glu342Lys) deficiency of alpha1-antitrypsin

Low levels of alpha1-antitrypsin (10-15% of normal)

Loss-of-function effect

Gain-of-function toxic effect

High levels of aggregated intracellular alpha1-antitrypsin

Janciauskiene et al. Res Med 2010
Alpha1-Antitrypsin Deficiency

variability in clinical manifestations
variability in symptom onset and severity

No clinical symptoms

This wide variation in the incidence and severity of liver and lung disease among individuals makes this condition one of the most challenging of the rare genetic disorders to diagnose and treat.
The wide variation in the incidence and severity of liver and lung disease among individuals with inherited alpha1-antitrypsin deficiency suggests a role for:

**Environmental factors** or ecological factors that influence our organisms include: climate, air pollutants, and ect.

**Social factors** include: family, physical status, economic status, location, life partners and political systems

**Mutations in other genes** that alter lipid metabolism, protein synthesis, response to inflammation, oxidative stress and ect.

**Developmental abnormality of the lung** that increases risk for both childhood pneumonia and lung disease in adults, especially smokers

**Function of alpha1-antitrypsin** that is determined not only by the levels but also by the biological function of the protein
Alpha1-Antitrypsin: an acute phase protein, serine protease inhibitor (serpin) and biological product

**Alpha1-Antitrypsin**

- **Molecularly complexed**
  - Protein alterations: Latent, Polymeric, Cleaved, Peptide, Oxidized, Nitrosylated, Variably glycosylated

- **Functionally sophisticated**
  - Host defence, Inflammation, Lipid metabolism:
    - Anti-protease
    - Immunomodulator
    - Anti-apoptotic
    - Anti-microbial
    - Anti-inflammatory
    - Anti-oxidant
    - Tumor suppressor/activator?
    - Cell growth, tissue repair regulator
A single amino acid variation in alpha1-antitrypsin can change its interaction with lipids and increase risk for large artery stroke.

Previously we thought- M1(A213) and M1(V213) variants have the same anti-elastase activity.

New data show- a stronger interaction of M1 (V213) with plasma lipids enhances anti-elastase activity.

A stronger interaction with lipoproteins and reduced flexibility of the M1 (Val213) reduces the extent of proteolytic inactivation by other proteases.

Malik R et al. PNAS March 2017
Alpha1-Antitrypsin associates with fatty acids

<table>
<thead>
<tr>
<th>Samples</th>
<th>α-linoleic acid (pM)</th>
<th>Oleic acid (pM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A1AT</td>
<td>Mean</td>
<td>Mean</td>
</tr>
<tr>
<td>M</td>
<td>2.8</td>
<td>nd</td>
</tr>
<tr>
<td>Z</td>
<td>35.6</td>
<td>63.9</td>
</tr>
<tr>
<td>M+FA</td>
<td>92.7</td>
<td>17.0</td>
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</table>
An interaction with essential fatty acids affects immunomodulatory properties of alpha1-antitrypsin.
## Effects of alpha1-antitrypsin in animal models of human diseases

<table>
<thead>
<tr>
<th>Human disease animal models</th>
<th>Effect of A1AT</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Graft-versus-host-disease</td>
<td>Promotes expansion of DCs, Tregs, and NK cells, decreases pro- and enhances ant-inflammatory cytokines (IL-10 and IL-1Ra) increases survival.</td>
<td>Marcondes AM et al., Blood 2011; Marcondes et al, Blood 2014; Tawara I et al, PNAS 2012</td>
</tr>
<tr>
<td>Islet allograft survival</td>
<td>Prolongs graft survival, immune cell infiltration reduces, intragraft VEGF transcript elevates, immune tolerance improves, IL-1Ra elevates</td>
<td>Song S et al Gene Ther 2004; Lewis E et al, PNAS, 2005; Pileggi A et al., Transplant Proc 2008; Shahaf G et al., Mol med 2011</td>
</tr>
<tr>
<td>Inflammatory bowel disease</td>
<td>Reverses intestinal lesions, improves epithelial barrier function, attenuates inflammatory cell infiltration, and reduces tissue injury</td>
<td>Rivera-Nieves et al . Gastroenterology 2003; Collins, C. B et al., 2013</td>
</tr>
<tr>
<td>Gouty arthritis</td>
<td>Increases IL-1Ra levels, reduces inflammatory cell infiltration and joint swelling, inhibits IL-1β</td>
<td>Joosten LA et al., Ann Rheum, 2015</td>
</tr>
<tr>
<td>Acute liver failure</td>
<td>Inhibits caspases, reduces activity of ADAM17, and prolongs survival.</td>
<td>Jedicke N et al., Hepatology 2014</td>
</tr>
<tr>
<td>Autoimmune encephalomyelitis</td>
<td>Lowers disease incidence, lowers disease score, increased Treg in lymphoid compartments</td>
<td>Subramanian S, et al. Metab Brain (2011)</td>
</tr>
</tbody>
</table>
## Effects of alpha1-antitrypsin in animal models of human diseases

<table>
<thead>
<tr>
<th>Condition</th>
<th>Effects</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Systemic LPS challenge</td>
<td>Reduced pro-inflammatory cytokines, platelet-activating factor, inhibits matrix degradation and neutrophil influx</td>
<td>Libert C et al., 1996, Dhami R et al., 2000; Churg A et al., 2001</td>
</tr>
<tr>
<td>Skin transplantation</td>
<td>Turns DC with reduced MHC class II, CD40, CD86, IL-6, but increased IL-10 and maintained inducible CCR7</td>
<td>Ozeri E et al., J Immunology 2012</td>
</tr>
<tr>
<td>Cancer (B6 melanoma model)</td>
<td>Induces NK cell degranulation and cancer cell killing</td>
<td>Guttman et al, Immunology, 2014</td>
</tr>
<tr>
<td>Peritonitis and sepsis</td>
<td>improves survival, reduces bacterial load</td>
<td>Kaner et al J Infect Dis. 2015</td>
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</table>
Therapy with α1-antitrypsin for conditions other than pulmonary emphysema

Vasculitis

Asthma

Fibromyalgia


<table>
<thead>
<tr>
<th>Year</th>
<th>Selected contributions</th>
</tr>
</thead>
<tbody>
<tr>
<td>1987</td>
<td>Smith et al, in Rochester, Minnesota (USA), treat with success two Pi*ZZ patients with severe panniculitis with intravenous infusions of A1AT.</td>
</tr>
<tr>
<td>1996</td>
<td>Furey et al (Chicago, USA) successfully treat a Pi*ZZ patient with severe panniculitis with Prolastin®.</td>
</tr>
<tr>
<td>1997</td>
<td>O’Riordan et al (Chicago, USA) successfully treat a Pi*ZZ case with severe panniculitis with Prolastin®.</td>
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<tr>
<td>2002</td>
<td>Chowdhury et al (Cardiff, UK) successfully treat a Pi*ZZ patient with severe panniculitis with Prolastin®.</td>
</tr>
<tr>
<td>2003</td>
<td>Kjus et al (Oslo, Norway) successfully treat a Pi*ZZ patient with severe panniculitis with Prolastin®.</td>
</tr>
<tr>
<td>2009</td>
<td>Gross et al (Frankfurt, Germany) report the favorable outcome of treatment with Prolastin® in a Pi*ZZ patient with panniculitis.</td>
</tr>
<tr>
<td>2011</td>
<td>Al-Niaimi et al (York, UK) successfully treat a Pi*ZZ patient with severe panniculitis with Prolastin®.</td>
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<tr>
<td>2012</td>
<td>Olson et al (Seattle, USA) successfully treat a Pi*ZZ patient with severe panniculitis with Prolastin®.</td>
</tr>
<tr>
<td>2014</td>
<td>Elsensohn et al (Salt Lake City, USA) successfully treat a Pi*ZZ patient with severe panniculitis with Prolastin®.</td>
</tr>
<tr>
<td>2015</td>
<td>Cathomas et al (Bern, Switzerland) successfully treat with A1AT a Pi*ZZ patient with severe wound healing disturbance caused by a neutrophil panniculitis.</td>
</tr>
<tr>
<td>2015</td>
<td>Rasool et al (Leicester, UK) successfully treat a Pi*ZZ patient with severe panniculitis with Prolastin®.</td>
</tr>
</tbody>
</table>
Therapy with alpha1-antitrypsin in non-deficient patients

In accordance with the safety profile and anti-inflammatory properties of human plasma alpha1-antitrypsin preparations, new protocols are evaluated for the treatment of persons without inherited alpha1-antitrypsin deficiency

**Cystic fibrosis** (Griese M, et al. Eur Respir J 2007)

**Recent onset Type 1 diabetes** (Gottlieb PA, et al. J Clin Endocrinol Metab 2014)


**Ongoing clinical trials to study A1AT therapy in children with type 1 diabetes**

Sponsored by the Kamada Ltd, Israel (NCT01304537); by the University of Colorado, Denver, together with Omni Bio Pharmaceutical, Inc., USA, (NCT01319331) and by the National Institute of Allergy and Infectious Diseases (NIAID), in collaboration with Immune Tolerance Network (ITN) and Juvenile Diabetes Research Foundation (NCT01183468).

**Ongoing Phase 2 clinical trial for the treatment of steroid refractory graft-versus-host-disease**

Sponsored by the University of Michigan Cancer Center, in collaboration with CSL Behring, and the Leukemia and Lymphoma Society (NCT01700036).
Effect of augmentation therapy on Pi ZZ alpha1-antitrypsin expression in the patient lungs

Unpublished data, 2017

N=10 PiZZ patients with augmentation therapy
N=4 PiZZ patients without therapy
Conclusions

- An impaired biological activity of alpha1-antitrypsin as a result of inherited or acquired (functional) deficiencies may lead to faster senescence, persistent inflammation and development of chronic diseases.

- We need to learn better about alpha1-antitrypsin.

- We need to learn better when and how to use alpha1-antitrypsin as a drug.
Our research is dedicated to all Alpha1 patients: you are the force that drives our work and our most important teachers.

Thanks to all collaborators and friends!
A1AT levels are 6-fold higher in human atherosclerotic lesions compared with healthy arteries

Inouje M et al, Plos Genetics 2012

A1AT associates with low-density lipoproteins (LDL)

Diffenderfer MR1, Schaefer E, J Curr Opin Lipidol. 2014 (review)

A1AT-LDL serves as a marker of smoking-specific oxidative stress in cardiovascular diseases

Bristow CL et al., Discov Med. 2013, Mashiba et al., Atherosclerosis Vasc Biol. 2001,

A1AT-high-density lipoproteins (HDL) complex makes more effective A1AT therapy in emphysema model, in vivo.

Moreno et al., AJRCMB Articles 2014

Statin therapy enhances A1AT-HDL association and anti-elastase activity

Gordon Ms et al. Mol Cell Proteomics, 2015