Research and new discoveries about alpha1-antitrypsin-related diseases

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Alpha 1-antitrypsin

- Alpha1-antitrypsin or α1-antitrypsin (AAT) is a glycoprotein, mainly produced in liver.
- The correct name, however, is alpha-1 protease inhibitor (α1-PI) because it is inhibiting a wide variety of proteases.
- It protects tissues from enzymes of inflammatory cells, especially neutrophil elastase.
- It is present in human blood at 1.5 - 3.5 gr/liter, and the concentration can rise many folds upon acute inflammation.
In 1980, was identified a new protein superfamily containing ovalbumin, antithrombin III, and α1-antitrypsin.

Robin Carrell and James Travis utilized the acronym serpins to describe this superfamily, as the members were predominately serine proteinase inhibitors.

Inspection of the nucleotide repositories provides evidence for over 2000 family members, including 37 in humans.
Inhibitory mechanism of alpha1-antitrypsin

Huntington J, Read R and Carrell R,
Nature 2000; 407: 923-926
The protease inhibitory activity of human plasma was recognised in 1894 by Fermi and Pernossi.

Arne Wilhelm Kaurin Tiselius Uppsala University Uppsala, Sweden received the Nobel Prize in 1948 for discovering how to separate proteins by electrophoresis.

Herman Schultze Behring Werke in Marburg, Germany 1955 have shown that the major inhibitor for trypsin is located in the α1-globulin fraction and was named α1-antitrypsin.
The introduction of plasma protein electrophoresis for clinical investigations:

1952 paper electrophoresis

1961 agarose electrophoresis
Components of plasma on agarose electrophoresis

C-B Laurell received 4th annual Edwin Ullman Award to recognize outstanding contributions that advance the technology of clinical laboratory science.

Slide from the presentation at Oak Ridge Conference May 4, 2001 Seattle, USA.
It was expected that patients with COPD may have some abnormalities in their immunoglobulins. C-B Laurell observed two patient samples with similar paper electrophoretic patterns but both missing the normal α1-band.

The discovery of alpha-1-antitrypsin deficiency appeared at the right time!

Association between alpha1-antitrypsin deficiency and emphysema
1962

Alpha1-antitrypsin 1958 Emphysema 1958
The birth of the concept to explain emphysema: protease-antiprotease imbalance hypothesis

**Neutrophil elastase**
- Cathepsins
- MMP-1, MMP-9, MMP-12
- Granzymes, Perforins

**Alpha-1-antitrypsin**
- SLPI, elafin, TIMPs
- Alpha1-antichymotrypsin

**Proteases**

**Anti-Proteases**

**Emphysema**
1964 the experimental emphysema after intratracheal instillation of papain

1969 an association between $\alpha_{1}$-antitrypsin deficiency and neonatal cirrhosis
Low serum alpha1-antitrypsin concentrations arise not from a lack of synthesis but from a blockage of its processing and secretion.

Z (GLU$^{342}$-LYS$^{342}$)
M Malton (PHE 52 deleted)
Siyyama (SER 53-PHE)

Severe alpha1-antitrypsin Deficiency

Z mutation $\text{Gly}^{342} \rightarrow \text{Lys}^{342}$

Polymerization of AAT

INTRACELLULAR ACCUMULATION
neonatal hepatitis, hepatocellular carcinoma, liver cirrhosis

PLASMA DEFICIENCY
10-15% of normal
early onset COPD
Diseases associated with alpha1-antitrypsin deficiency

- COPD
- Liver cirrhosis
- Hepatocellular carcinoma
- Diabetes
- Bronchial asthma
- Cystic Fibrosis
- Vasculitis
- Panniculitis
- Rheumatoid arthritis
- Psoriasis
- Chronic urticaria
- Glomerulonephritis
- Pancreatitis and pancreatic tumours
- Multiple sclerosis
- Fibromyalgia
An association between type 2 diabetes and alpha1-antitrypsin deficiency

Control group (n=158)

A

PiMM (n=151)

PiMZ (n=6)

PiFZ (n=1)

Caroline S. Sandström et al., Diabetes Medicine, 2008, in press
Alpha1-antitrypsin deficiency worldwide

116 million carriers of MZ and MS
3.4 million carriers of SZ, SS and ZZ

The total country database of 373 control cohorts has been combined to estimate the numbers of carriers and deficiency allele combinations for PiS and PiZ in 11 geographic regions and worldwide

Frederick J. de Serres, Chest 2002
Frequency of the most common genotypes of alpha1-antitrypsin

- MM (93/100)
- MZ (4.6/100)
- SS (1/1600)
- SZ (1/750)
- ZZ (1/1600)
Map of Z alpha1-antitrypsin frequency in Europe
Alpha1-antitrypsin deficiency is under-recognized and under-diagnosed

COPD is the 4th leading cause of death worldwide, claiming **2.75 million** lives annually

**19.3 million** COPD patients in USA
**9.5% (1.8 million patients)** are estimated to have the SZ, MZ or ZZ genotype

~5% of cases have been diagnosed

The protease-antiprotease hypothesis has provided many new directions in emphysema research

1989

• Augmentation therapy of emphysema with severe α1-antitrypsin deficiency

• The major concept behind augmentation therapy was that a rise in the levels of blood and tissue α1-antitrypsin will protect lungs from continuous destruction by proteases, particularly elastase.
Augmentation therapy: EUROPE

Estimated PiZZ: 124,594

PiZZ with emphysema: 44,854

Augmentation therapy: 1,295

Blanco I et al., Eur Resp J 2006
de Serres FJ et al., Monaldi Arch Chest Dis 2007
Augmentation therapy: USA

• Estimated PiZZ: 59,000

• ~6,000 PiZZ diagnosed (DeSerres, Clin Genet 2003)

• ~3,500 PiZZ emphysema patients treated
Augmentation Therapy: Impact on Mortality

FEV$_1$ < 50% Predicted

- Never (n = 162)
- Partially (n = 285)
- Always (n = 316)

Time (months)

Alpha-1-Antitrypsin Deficiency Registry Study Group. Am J Respir Crit Care Med. 1998
Augmentation therapy: Impact on Infections

![Bar chart showing the impact of augmentation therapy on infections. The x-axis represents the number of infections per year, ranging from 'None' to 'Cnsntnt'. The y-axis represents the number of subjects, ranging from 0 to 35. The chart compares the number of infections before and on Prolastin.](image)

*Source: Lieberman, J. Chest 2000;118:1480-1485*
Augmentation therapy in patients with Cystic Fibrosis

Inhalation of alpha1-antitrypsin decreases:

- sputum taurine
- elastase activity
- neutrophil numbers
- pro-inflammatory cytokines
- numbers of *P. aeruginosa*
- no effect on lung function

Cantin et al., *Clin Invest Med.* 2006

Griese M et al. *Eur Respir J.* 2007
Anti-inflammatory effects of α1-antitrypsin: 
**Animal models**

- Protects mice from TNFα or LPS-induced lethality in galactosamine-sensitized and normal mice  
  *Libert et al., J Immunol 1996*

- Inhibits matrix degradation and PMN influx in acute-cigarette smoke-induced connective tissue breakdown, in C57-BL/6 mice model  
  *Dhami et al., Am J Respir Cell Mol Biol 2000*

- Prevents the PMN inflammatory influx and connective tissue breakdown in a mice model of acute silica-induced inflammation  
  *Churg et al., Laboratory Investigation 2001*

- Ameliorates cigarette smoke-induced emphysema in mice  
  *Churg et al., Am J Res Crit Care Med 2003*
Anti-inflammatory effects of α1-antitrypsin: **Cell culture models**

- **Inhibits the Immune Response**

- **Stimulates Tissue Repair and Matrix Production**
  Qing-Bai She et al., FEBS Letters 2000
  Dabbagh et al., J Cell Physiol 2001

- **Inhibits Neutrophil Activation**
  Janciauskiene et al., BBRC,2004

- **Inhibits Moraxella catarrhalis MID Protein-induced Tonsillar B cell Activation**
  Radinka Hadzic et al., Immunol Letters 2005
α1-antitrypsin inhibits apoptosis

Normal β-cell

AAT

AAT

Normal β-cell treated with α1-antitrypsin (AAT)

Normal β-cell

AAT

AAT

AAT

TNFα

Caspase-3 activation

AAT+

Normal β-cell

AAT

AAT

AAT

AAT+

Caspase-3 No-activation

TNFα

Zhang B et al., Diabetes 2007;56:1316-23
α1-antitrypsin potentiates insulin release from pancreatic beta cells

M Kalis et al., 2008, manuscript
Exogenous alpha1-antitrypsin is fast distributed in the cytoplasm and nuclei

alpha-1antitrypsin alone  alpha-1antitrypsin + endotoxin
Mechanisms of action

Alpha1-antitrypsin regulates endotoxin (LPS)-induced:

• Endotoxin receptor expression (CD14/TLR4)
• Nuclear factor activation (NF-kB)
• Cytokine and chemokine expression and release: TNF, IL-1, IL-6, IL-12, MCP-1, IL-8 and ect.
How alpha1-antitrypsin (AAT) regulates pathways involved in pulmonary innate immunity against pathogens of the lung?
Alpha1-antitrypsin is not just protease inhibitor?

Hypothesis
An impaired biological activity of alpha1-antitrypsin as a result of inherited or acquired (functional) deficiencies may lead to persistent inflammation and organ damage.

Native AAT
- Oxidation
- Nitration
- Cleavage
- Polymerization

Modified AAT

Free radicals, proteases and ect

COPD
Asthma
Dementia
Vasculitis
Panniculitis
Diabetes?
Conclusion

Better understanding of the functional activities of alpha1-antitrypsin, and increased awareness about the deficiency of alpha1-antitrypsin will help:

- to improve our understanding of the pathogenesis of COPD and other diseases
- to explore the therapeutic potential of alpha1-antitrypsin and alpha1-antitrypsin-like molecules
Many Thanks!