



LUND UNIVERSITY

Polymerized alpha-antitrypsin is present on lung vascular endothelium. New insights into the biological significance of alpha-antitrypsin polymerization.

Aldonyte, Ruta; Jansson, L; Ljungberg, Otto; Larsson, S; Janciauskiene, S

Published in:
Histopathology

DOI:
[10.1111/j.1365-2559.2004.02021.x](https://doi.org/10.1111/j.1365-2559.2004.02021.x)

2004

[Link to publication](#)

Citation for published version (APA):

Aldonyte, R., Jansson, L., Ljungberg, O., Larsson, S., & Janciauskiene, S. (2004). Polymerized alpha-antitrypsin is present on lung vascular endothelium. New insights into the biological significance of alpha-antitrypsin polymerization. *Histopathology*, 45(6), 587-592. <https://doi.org/10.1111/j.1365-2559.2004.02021.x>

Total number of authors:
5

General rights

Unless other specific re-use rights are stated the following general rights apply:

Copyright and moral rights for the publications made accessible in the public portal are retained by the authors and/or other copyright owners and it is a condition of accessing publications that users recognise and abide by the legal requirements associated with these rights.

- Users may download and print one copy of any publication from the public portal for the purpose of private study or research.
- You may not further distribute the material or use it for any profit-making activity or commercial gain
- You may freely distribute the URL identifying the publication in the public portal

Read more about Creative commons licenses: <https://creativecommons.org/licenses/>

Take down policy

If you believe that this document breaches copyright please contact us providing details, and we will remove access to the work immediately and investigate your claim.

LUND UNIVERSITY

PO Box 117
221 00 Lund
+46 46-222 00 00

Polymerized α_1 -antitrypsin is present on lung vascular endothelium. New insights into the biological significance of α_1 -antitrypsin polymerization

R Aldonyte, L Jansson,² O Ljungberg,¹ S Larsson & S Janciauskiene

Department of Medicine and ¹Department of Pathology, University Hospital Malmö, Malmö, and ²R&D, AstraZeneca, Lund, Sweden

Date of submission 11 November 2003

Accepted for publication 5 March 2004

Aldonyte R, Jansson L, Ljungberg O, Larsson S & Janciauskiene S

(2004) *Histopathology* 45, 587–592

Polymerized α_1 -antitrypsin is present on lung vascular endothelium. New insights into the biological significance of α_1 -antitrypsin polymerization

Aims: The damage to lung tissue in chronic obstructive pulmonary disease (COPD) may involve the progressive loss of pulmonary vascular endothelial cells. Endothelial binding of α_1 -antitrypsin (α_1 -AT) derived from plasma has been identified, and α_1 -AT deficiency is a known genetic risk factor associated with α_1 -AT polymerization and COPD development. Therefore, in the present study we aimed to investigate if α_1 -AT is present on the lung vascular endothelium, and if it is in a polymeric form.

Methods and results: Postmortem paraffin-embedded tissue specimens from 15 COPD (chronic bronchitis and emphysema) cases with and without Z α_1 -AT (Glu342Lys) deficiency and from 10 cases without

signs of COPD were studied. Immunohistochemistry was performed using the streptavidin–biotin method with a monoclonal ATZ11 antibody specific for polymeric α_1 -AT, and polyclonal antibodies against human α_1 -AT and neutrophil elastase. Vascular endothelium showed intense staining for α_1 -AT with the ATZ11 antibody in all cases; however, intensity of staining in patients with α_1 -AT deficiency was greater. No endothelial staining was observed with the anti-elastase antibody.

Conclusions: This is the first demonstration that α_1 -AT bound to the vascular endothelium of lungs is in a polymeric form, which also suggests a possible previously unknown role for polymeric α_1 -AT *in vivo*.

Keywords: α_1 -antitrypsin, COPD, endothelial cells, polymers

Abbreviations: α_1 -AT, α_1 -antitrypsin; COPD, chronic obstructive pulmonary disease

Introduction

It is now well recognized that endothelial cell functions are altered in sites of acute and chronic inflammation¹ and it has been proposed that the disappearance of lung tissue may involve the progressive loss of pulmonary vascular endothelial cells.^{2,3} Endothelial dysfunction and intimal thickening have also been observed in the

pulmonary arteries of patients with chronic obstructive pulmonary disease (COPD).^{4,5} The surface structure and composition of the endothelium play a major role in determining the metabolic status of endothelial cells, endothelial permeability to water and solutes, leucocyte adhesion and emigration, and microvascular resistance to pressure. Proteins adsorbed from the blood plasma and directly bound to the plasma membrane of endothelial cells are known to affect the expression of multiple endothelial cell markers.^{6–9} Most of the proteins at the endothelial surface are glycoproteins and proteoglycans. However, the biological role of each endothelial-bound protein is poorly understood.

Address for correspondence: Sabina Janciauskiene, Department of Medicine, Wallenberg Laboratory, Plan 2, University Hospital Malmö, S-20502 Malmö, Sweden.
e-mail: sabina.janciauskiene@medforsk.mas.lu.se

Endothelial binding of α_1 -antitrypsin (α_1 -AT) derived from plasma has been identified.^{10,11} Findings that neutrophil-derived proteinases are capable of damaging endothelial-bound α_1 -AT led to speculation that preventing depletion of endothelial-bound proteinase inhibitors may help to minimize vascular damage during inflammation.¹¹ α_1 -AT is an archetypal member of the serine proteinase inhibitor system in humans, and is a potent inhibitor of neutrophil elastase.^{12–14} α_1 -AT is found in most tissues and body fluids; it is an acute-phase reactant whose plasma concentration can rise by three- to four-fold above normal (average 1.34 mg/ml) during inflammation, infection and malignant diseases. The local balance between proteinases and endogenous inhibitors, such as α_1 -AT, is an important factor in determining whether inflammation results in connective tissue damage.^{15–18} When the concentration of α_1 -AT in plasma falls below 0.7 mg/ml the individual is considered to have α_1 -AT deficiency.^{19–21} The lack of circulating α_1 -AT results in uncontrolled proteolytic attack and early-onset panacinar emphysema.^{22,23} A single amino acid change in certain domains of the α_1 -AT can lead to polymerization of the mutant α_1 -AT into intracellular aggregates. The retention of Z- α_1 -AT polymers in the endoplasmic reticulum of hepatocytes can cause liver damage.^{24,25} Recent studies provide evidence that the polymeric form of α_1 -AT is present in the lungs of COPD patients with Z- α_1 -AT deficiency,^{26,27} and we have found that plasma from patients with Z- α_1 -AT deficiency contains a significant amount of circulating α_1 -AT polymers.¹² These may have important implications for the pathogenesis of the disease, since polymerization obscures the reactive centre loop of α_1 -AT, rendering the protein inactive as an inhibitor of proteolytic enzymes.²⁸

Because α_1 -AT deficiency is known genetic risk factor for the development of COPD and because it has been proposed that the damage to lung tissue may involve the progressive loss of pulmonary vascular endothelial cells, we aimed to determine whether α_1 -AT is present on lung microvascular endothelium and if it is in a polymeric form, and to compare endothelial-bound α_1 -AT between COPD and normal lung tissues with and without Z- α_1 -AT deficiency.

Materials and methods

TISSUE SPECIMENS

Lung tissue samples for immunohistochemical examination were obtained from the tissue bank at the Department of Pathology and Cytology, Malmö

University Hospital. The tissues were prepared at autopsies performed between 1980 and 1990, fixed in formalin, embedded in paraffin wax and stored as paraffin blocks. The specimens for this study were selected via the autopsy diagnosis registry and classified as COPD (chronic bronchitis and emphysema) ($n = 10$) and without signs of COPD ($n = 10$). A microscopic examination of the liver was performed in all autopsies in order to identify cases with α_1 -AT-inclusion bodies in liver cells. This examination enabled us to identify five cases with liver histology indicative of a PiZ gene carrier and also to exclude PiZ gene carriers from COPD and control cases. However, it did not allow us to distinguish between hetero- and homozygous PiZ gene carriers. The lung microscopy in five PiZ carriers exhibited changes compatible with COPD (chronic bronchitis and emphysema). The study protocol was approved by the Ethics Committee of Lund University.

IMMUNOHISTOCHEMISTRY

Monospecific antisera against human α_1 -AT and neutrophil elastase, negative control mouse IgG2b, and all secondary, peroxidase-labelled antibodies were obtained from Dako (Glostrup, Denmark). Monoclonal antibody ATZ11, specifically reacting with polymerized and elastase-complexed α_1 -AT, is raised against the Z- α_1 -AT isolated from liver tissue and is available in our laboratory.²⁹ The embedded tissues were sectioned at 4 μ m and dried at 60°C for 1 h. The sections were deparaffinized, rehydrated, and microwaved twice for 5 min in 10 mM citrate buffer at pH 6. After cooling, the sections were washed in distilled water for 20 min. Immunohistochemical analyses were performed by an indirect, streptavidin–biotin method with an automated TechMate 500 Plus apparatus (Dako A/S). Blocking antibody was introduced and left to react for 20 min at room temperature and the primary antibody, monoclonal ATZ11 (1 : 50), polyclonal anti- α_1 -AT (1 : 20 000) and polyclonal antineutrophil elastase (1 : 100) antibody were added and allowed to react for 90 min at room temperature. The specificity of the ATZ11 antibody was tested by ELISA methods.¹² The antibody was diluted with phosphate-buffered saline (PBS). Controls were performed in which the primary antibody was omitted or replaced with non-immunized mouse IgG (1 : 1000). The biotinylated secondary antibody and solutions supplied in a ChemMate Detection kit (Dako) were added and incubated for 30 min at room temperature. In this method, a biotinylated secondary antibody is detected with horseradish peroxidase-conjugated streptavidin, and peroxidase activity is detected with 3,3-diam-

inobenzidine tetrahydrochloride (DAB). The tissues were counterstained with haematoxylin. The specimens were analysed by microscopy, using an Olympus Bx41 (Olympus Optical Co., Hamburg, Germany). Images were taken with an Olympus camera DP50 (Olympus Optical Co.) at an original magnification of $\times 100$ and $\times 400$.

Results

Our results demonstrated the positive immunoreactivity of vascular endothelial-bound α_1 -AT in all 25 cases investigated. Examples of positively stained endothelial cells with ATZ11 antibody in cases with morphologically and histochemically normal lung tissue are shown in Figure 1A,B. Positive staining was seen only on the endothelial layer, and not in the background or neighbouring alveolar epithelium. The polyclonal anti- α_1 -AT antibody manifested a nearly identical staining pattern of endothelial cell layers compared with the monoclonal ATZ11 antibody, but in addition the polyclonal anti- α_1 -AT showed background immunoreactivity (data not shown). It is important to emphasize that the ATZ11 antibody has no affinity to native, oxidized, latent and cleaved forms of α_1 -AT, but is known to cover a neoepitope on both α_1 -AT complexed with enzyme and polymeric α_1 -AT,¹² which means that this antibody cannot discriminate between these two forms of α_1 -AT. To investigate whether endothelial-bound α_1 -AT occurs both in a polymeric and in complex with elastase, we also stained with a polyclonal antibody to human neutrophil elastase. No endothelial staining was seen in any specimens with this antibody. The only positive staining was intracellular (Figure 1C), arguing against any significant contribution of α_1 -AT–elastase complexes to the endothelial staining pattern. Tsuji and coworkers have also shown that circulating α_1 -AT–elastase complex can pass through the endothelial cells without inducing any degenerative changes, and they did not detect α_1 -AT–elastase complexes on the endothelial layer.³⁰ These findings still do not eliminate the possibility that other endothelial-bound α_1 -AT–enzyme complexes can be recognized by ATZ11 antibody, although in a Western blot analysis ATZ11 antibody did not react with α_1 -AT–cathepsin G or α_1 -AT–proteinase 3 complexes (data not shown).

Figures 2 and 3 show lung tissue with histological features of COPD, including focal fibrosis (Figures 2A,B and 3A), squamous metaplasia of bronchial epithelium (Figure 2A,C) and inflammation (Figures 2 and 3A,C). Immunostaining with ATZ11 antibody resulted in positive endothelial immunoreactivity in all cases.

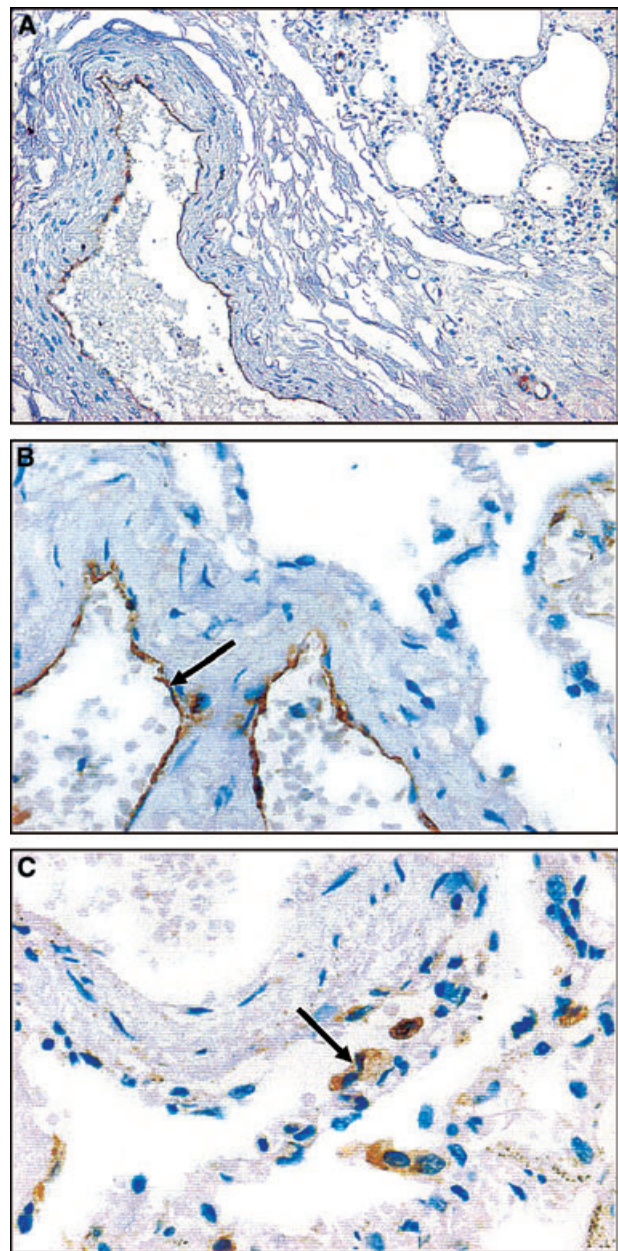


Figure 1. Immunostaining of endothelial-bound polymeric α_1 -anti-trypsin in lung autopsy specimens from controls (with microscopically and histochemically normal lung tissue). Representative pictures are shown. A, Specific endothelial layer immunostaining with monoclonal ATZ11 antibody (1 : 50) with no background and alveolar cell immunoreactivity. B, Higher magnification of endothelial immunostaining with ATZ11 antibody. Arrow indicates endothelial staining. C, The primary ATZ11 antibody was replaced by polyclonal antihuman neutrophil elastase antibody (1 : 100), which shows no endothelial immunoreactivity, but positive in alveolar inflammatory cells. Arrow indicates cell staining.

However, it must be pointed out that the intensity of staining in patients with α_1 -AT deficiency was much greater (Figure 3A,B). The alveolar inflammatory cells

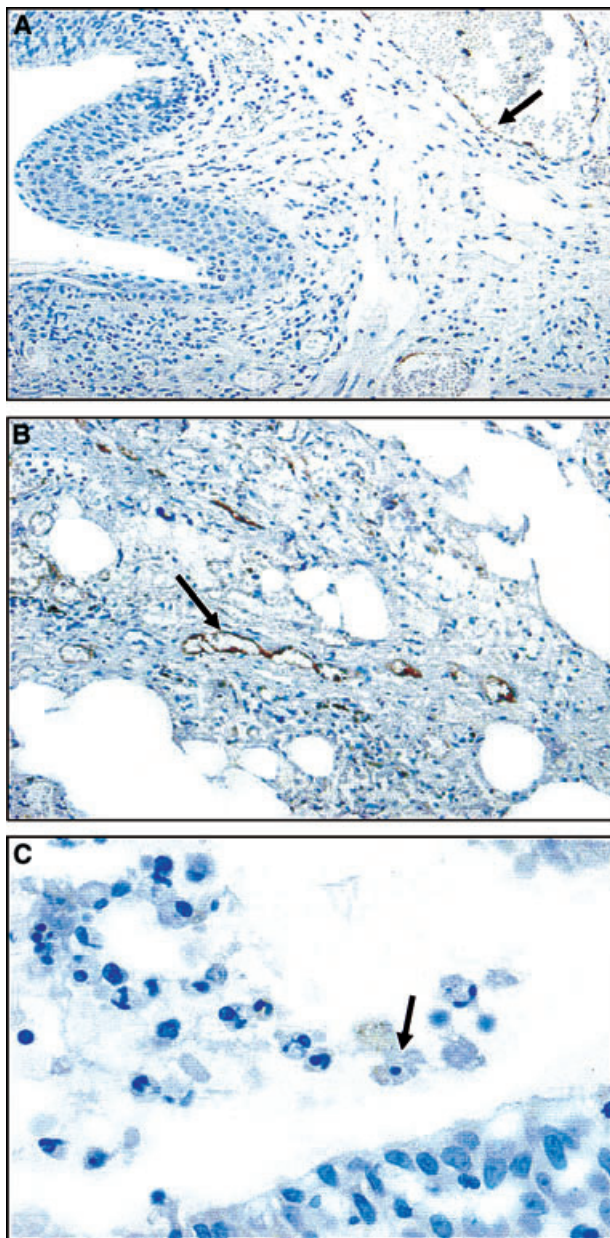


Figure 2. Immunohistochemical analysis of endothelial-bound α_1 -antitrypsin (α_1 -AT) in lung tissue autopsy sections from chronic obstructive pulmonary disease cases with wild-type α_1 -AT. A,B, Lung fibrosis and chronic inflammation. Squamous metaplasia of the bronchial epithelium is seen. Positive α_1 -AT endothelial staining with monoclonal ATZ11 antibody (1 : 50) is indicated by arrows. C, Only weak immunostaining with ATZ11 antibody of inflammatory cells in the bronchial lumen can be seen. Arrow indicates cell staining.

were mainly negative, although faint immunoreactivity could be detected with the ATZ11 antibody (Figure 2C). Both anti-neutrophil elastase and ATZ11 antibody reacted more intensely with inflammatory

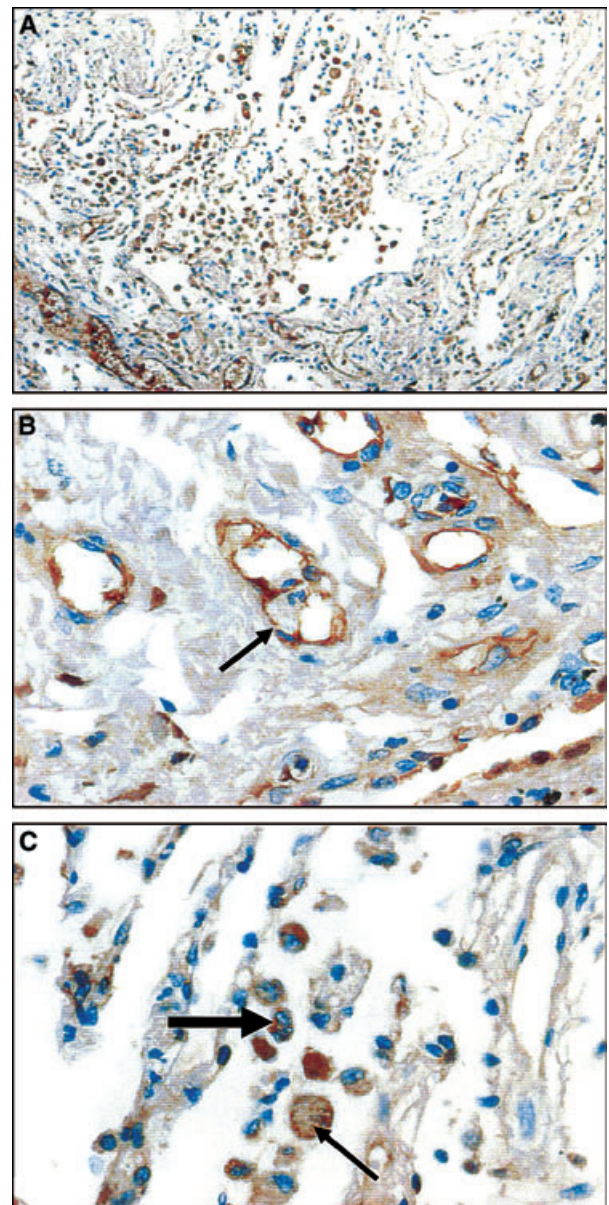


Figure 3. Immunohistochemical analysis of endothelial-bound α_1 -antitrypsin (α_1 -AT) in autopsy sections of lung tissue from PiZ gene carrier with chronic obstructive pulmonary disease (COPD)-compatible pulmonary changes. A, Lung tissue changes characteristic of COPD. Both the endothelial layer and the inflammatory cells are immunoreactive with ATZ11. B, Close-up view of the endothelial staining with ATZ11 (1 : 50) of a small blood vessel. Background staining is also visible. Arrow indicates endothelial immunoreactivity. C, Immunostaining with ATZ11 antibody (1 : 50) of inflammatory cells in the alveolar lumina (indicated by arrows). These cells are also positive for immunostaining with a polyclonal antihuman α_1 -AT and anti-neutrophil elastase antibody (data not shown).

cells in Z- α_1 -AT deficiency cases (Figure 3C), thereby making it impossible to discriminate between the molecular forms of α_1 -AT in these cells.

Discussion

Multiple forms of α_1 -AT have been identified in biological fluids, including native, inhibitory, and non-inhibitory forms, the latter including oxidized, cleaved and polymeric forms.¹⁸ Dependent on its molecular form, α_1 -AT has been reported to influence cell function and behaviour via both direct and indirect mechanisms.^{13,18} The studies described here are an extension of our previous work, which showed that polymeric α_1 -AT is bound to the endothelium of temporal arteries.¹² This indicated to us that polymerization of endothelial-bound α_1 -AT may be a general phenomenon, and we sought to test this hypothesis by examining whether endothelial-bound α_1 -AT in the lungs is in a polymeric form. The current study is the first convincing demonstration of endothelial-bound polymeric α_1 -AT in normal and diseased lung tissue. Moreover, the presence of polymeric α_1 -AT was independent of Z- α_1 -AT deficiency.

The flexibility of the reactive centre loop of α_1 -AT allows insertion of the reactive centre loop of one molecule into the A β -sheet of a second α_1 -AT molecule which then extends to form chains of polymers.²⁸ It is this polymerization that occurs spontaneously in Z- α_1 -AT, underlies the formation of hepatic inclusions, and is associated with plasma deficiency of α_1 -AT.^{21,23} The propensity for the wild type α_1 -AT to undergo loop-sheet polymerization is much less pronounced. To date, *in vivo*, wild-type α_1 -AT polymers have been described only in bile, where the interaction of α_1 -AT with a denaturing milieu is thought to enhance polymer formation.³¹ Other conditions that are known to induce α_1 -AT polymerization *in vitro* include high protein concentration, low pH and increased temperature.²⁸ The potential cytotoxic effect of Z- α_1 -AT polymers on hepatocytes and their occurrence in the circulation have prompted investigators to discuss the potential biological role of these polymers in extrahepatic tissues. Since polymerization abolishes α_1 -AT inhibitory activity, it is proposed that polymer formation will exacerbate the already reduced antiproteinase screen and increase the susceptibility of the tissues to proteolytic attack. It has even been suggested by Parmar and coworkers that polymers of α_1 -AT are proinflammatory and chemotactic for human neutrophils *in vitro*.³² In our experimental models, however, neither native nor polymeric α_1 -AT at concentrations up to 0.5 mg/ml stimulated neutrophil chemotaxis, adhesion, superoxide generation or proteinase release.³³ Our findings that polymeric α_1 -AT is present on the vascular endothelium in

both normal and diseased lungs do not support a proinflammatory role for polymeric α_1 -AT, *in vivo*.

We have demonstrated that polymeric form of α_1 -AT is bound to lung vascular endothelium in both COPD and control cases independent of α_1 -AT PiZ deficiency, which allows us to propose that the presence of polymerized α_1 -AT on the vascular endothelium is a general phenomenon. The levels and quality of polymeric, endothelial-bound α_1 -AT might be one of the factors determining the susceptibility of individuals to develop vascular damage and related diseases. Further studies are needed to elucidate a biological role of vascular endothelial-bound AAT, *in vivo*.

Acknowledgements

The authors thank Elise Nilsson for expert technical assistance with immunostaining experiments and are grateful to Bengt Johansson for valuable suggestions during manuscript preparation. This work was supported by grants from AstraZeneca R&D Lund, Swedish Research Council and Lund University (Sweden).

References

1. Stevens T, Rosenberg R, Aird W *et al.* NHLBI workshop report: endothelial cell phenotypes in heart, lung, and blood diseases. *Am. J. Physiol. Cell Physiol.* 2001; **281**: C1422–C1433.
2. Chow CK. Cigarette smoking and oxidative damage in the lung. *Ann. NY Acad. Sci.* 1993; **686**: 289–298.
3. Kasahara Y, Tuder RM, Taraseviciene-Stewart L *et al.* Inhibition of VEGF receptors causes lung cell apoptosis and emphysema. *J. Clin. Invest.* 2000; **106**: 1311–1319.
4. Hale KA, Niewoehner DE, Cosio MG. Morphologic changes in the muscular pulmonary arteries: relationship to cigarette smoking, airway disease, and emphysema. *Am. Rev. Respir. Dis.* 1980; **122**: 273–278.
5. Peinado VI, Barbera JA, Ramirez J *et al.* Endothelial dysfunction in pulmonary arteries of patients with mild COPD. *Am. J. Physiol.* 1998; **274**: L908–L913.
6. Squire JM, Chew M, Nneji G *et al.* Quasi-periodic substructure in the microvessel endothelial glycocalyx: a possible explanation for molecular filtering? *J. Struct. Biol.* 2001; **136**: 239–255.
7. Page C, Rose M, Yacoub M *et al.* Antigenic heterogeneity of vascular endothelium. *Am. J. Pathol.* 1992; **141**: 673–683.
8. Cines DB, Pollak ES, Buck CA *et al.* Endothelial cells in physiology and in the pathophysiology of vascular disorders. *Blood* 1998; **91**: 3527–3561.
9. Minshall RD, Tirupathi C, Vogel SM *et al.* Vesicle-formation and trafficking in endothelial cells and regulation of endothelial barrier function. *Histochem. Cell. Biol.* 2002; **117**: 105–112.
10. Fischer HP, Ortiz-Pallardo ME, Ko Y *et al.* Chronic liver disease in heterozygous alpha1-antitrypsin deficiency PiZ. *J. Hepatol.* 2000; **33**: 883–892.
11. Forsyth KD, Talbot V, Beckman I. Endothelial serpins—protectors of the vasculature? *Clin. Exp. Immunol.* 1994; **95**: 277–282.
12. Janciauskiene S, Dominaitiene R, Sternby NH *et al.* Detection of circulating and endothelial cell polymers of Z and wild type alpha

- 1-antitrypsin by a monoclonal antibody. *J. Biol. Chem.* 2002; **277**: 26540–26546.
13. Yao J, Baecher-Allan CM, Sharon J. Serpins identified as cell growth inhibitors in human plasma. *Mol. Cell. Biol. Res. Commun.* 2000; **3**: 76–81.
14. Travis J, Shieh BH, Potempa J. The functional role of acute phase plasma proteinase inhibitors. *Tokai J. Exp. Clin. Med.* 1988; **13**: 313–320.
15. Wawrzos I, Kitagawa Y, Koloczek H. Immunological discrimination of diverse forms of human alpha 1-proteinase inhibitor. *Acta Biochim. Pol.* 1996; **43**: 481–488.
16. Zhu XJ, Chan SK. The use of monoclonal antibodies to distinguish several chemically modified forms of human alpha 1-proteinase inhibitor. *Biochem. J.* 1987; **246**: 19–23.
17. Wong PS, Travis J. Isolation and properties of oxidized alpha-1-proteinase inhibitor from human rheumatoid synovial fluid. *Biochem. Biophys. Res. Commun.* 1980; **96**: 1449–1454.
18. Janciauskiene S. Conformational properties of serine proteinase inhibitors (serpins) confer multiple pathophysiological roles. *Biochim. Biophys. Acta* 2001; **1535**: 221–235.
19. Elliott PR, Pei XY, Dafforn TR *et al.* Topography of a 2.0 Å structure of alpha1-antitrypsin reveals targets for rational drug design to prevent conformational disease. *Protein Sci.* 2000; **9**: 1274–1281.
20. Kalsheker N, Morgan K. Molecular biology and respiratory disease. 7. The alpha 1 antitrypsin gene and chronic lung disease. *Thorax* 1990; **45**: 759–764.
21. Eriksson S. Alpha 1-antitrypsin deficiency. *J. Hepatol.* 1999; **30**: 34–39.
22. Joos L, Pare PD, Sandford AJ. Genetic risk factors of chronic obstructive pulmonary disease. *Swiss Med. Wkly* 2002; **132**: 27–37.
23. Hill AT, Campbell EJ, Bayley DL *et al.* Evidence for excessive bronchial inflammation during an acute exacerbation of chronic obstructive pulmonary disease in patients with alpha(1)-antitrypsin deficiency (PiZ). *Am. J. Respir. Crit. Care Med.* 1999; **160**: 1968–1975.
24. Sveger T. Liver disease in alpha1-antitrypsin deficiency detected by screening of 200,000 infants. *N. Engl. J. Med.* 1976; **294**: 1316–1321.
25. Durr R, Caselmann WH. Carcinogenesis of primary liver malignancies. *Langenbeck's Arch. Surg.* 2000; **385**: 154–161.
26. Reid PT, Sallenave JM. Neutrophil-derived elastases and their inhibitors: potential role in the pathogenesis of lung disease. *Curr. Opin. Invest. Drugs* 2001; **2**: 59–67.
27. Mahadeva R, Lomas DA. Genetics and respiratory disease. 2. Alpha 1-antitrypsin deficiency, cirrhosis and emphysema. *Thorax* 1998; **53**: 501–505.
28. Dafforn TR, Mahadeva R, Elliott PR *et al.* A kinetic mechanism for the polymerization of alpha1-antitrypsin. *J. Biol. Chem.* 1999; **274**: 9548–9555.
29. Wallmark A, Alm R, Eriksson S. Monoclonal antibody specific for the mutant PiZ alpha 1-antitrypsin and its application in an ELISA procedure for identification of PiZ gene carriers. *Proc. Natl Acad. Sci. USA* 1984; **81**: 5690–5693.
30. Tsujii T, Katayama K, Naito I *et al.* The circulating alpha 1-antitrypsin-elastase complex attacks the elastic lamina of blood vessels. An immunohistochemical study. *Histochemistry* 1988; **88**: 443–451.
31. Janciauskiene S, Toth E, Sahlin S *et al.* Immunochemical and functional properties of biliary alpha-1-antitrypsin. *Scand. J. Clin. Lab. Invest.* 1996; **56**: 597–608.
32. Parmar JS, Mahadeva R, Reed BJ *et al.* Polymers of alpha(1)-antitrypsin are chemotactic for human neutrophils: a new paradigm for the pathogenesis of emphysema. *Am. J. Respir. Cell. Mol. Biol.* 2002; **26**: 723–730.
33. Janciauskiene S, Zelvyte I, Jansson L *et al.* Divergent effects of alpha1-antitrypsin on neutrophil activation, *in vitro*. *Biochem. Biophys. Res. Commun.* 2004; **315**: 288–296.