Airways inflammation in chronic bronchitis: the effects of smoking and α_1-antitrypsin deficiency

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Airways inflammation in chronic bronchitis: the effects of smoking and α_1-antitrypsin deficiency. A.T. Hill, D.L. Bayley, E.J. Campbell, S.L. Hill, R.A. Stockley. ©ERS Journals Ltd 2000.

ABSTRACT: Airways inflammation in chronic bronchitis is thought predominantly to be a direct consequence of neutrophil recruitment and release of elastase in response to factors such as cigarette smoke. The aims of this study were to assess the role of smoking and determine whether the serum elastase inhibitor α_1-antitrypsin (α_1AT) influenced the process.

Airways inflammation was compared between patients with chronic obstructive bronchitis with (n=39) and without (n=42) severe α_1AT deficiency. The authors assessed the sputum concentration of the neutrophil chemoattractants interleukin-8 (IL-8) and leukotriene (LT)B$_4$, myeloperoxidase (MPO) as a marker of neutrophil influx, neutrophil elastase activity and its natural inhibitors, α_1AT and secretory leukoprotease inhibitor (SLPI). Finally serum α_1AT was measured to determine the degree of protein leakage (sputum sol serum α_1AT ratio).

Compared to current smokers, the exsmokers had a lower concentration of the chemoattractant IL-8 ($p<0.05$) and a lower MPO concentration, although this failed to reach conventional statistical significance ($p=0.06$). Patients with α_1AT deficiency had greater inflammation in the larger airways with increased LTB$_4$ ($p<0.005$), MPO ($p<0.001$), neutrophil elastase activity ($p<0.01$), protein leak ($p<0.001$), and were found to have a lower anti-proteinase screen with both reduced sputum α_1AT ($p<0.001$) and SLPI concentrations ($p<0.05$).

The reduction in sputum interleukin-8 levels in exsmokers may decrease neutrophil influx and thus explain the slower rate of neutrophil mediated progression of lung disease compared to subjects who continue to smoke. Patients with α_1-antitrypsin deficiency had greater inflammation suggesting that α_1-antitrypsin plays an important role in protecting the larger airways from the inflammatory effects of elastase activity and may explain their more rapid progression of disease.
Eur Respir J 2000; 15: 886–890.

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Chronic bronchitis was first recognized as a disabling disorder in 1808 [1]. It is often a feature of chronic obstructive pulmonary disease (COPD), and recent studies have shown that airway neutrophils are increased in patients with chronic bronchitis [2] and that the degree of neutrophil recruitment is related to the severity of airflow obstruction [3, 4]. Furthermore, increased neutrophils in the airways are related to the rate of progression of airflow obstruction [5]. Smoking (the major risk factor in COPD) increases neutrophil recruitment to the lung [3, 6] possibly by inducing the bronchial epithelium to secrete the important neutrophil chemoattractant interleukin (IL)-8 [7].

Neutrophils contain a serine elastase that has been shown to reproduce many of the features of COPD including emphysema [8], epithelial damage [9], impaired ciliary function [10], mucus gland hyperplasia [11], mucus secretion [12], and also to inactivate many of the critical lung host defences [13, 14]. These latter effects may facilitate bacterial colonization, which is present in patients with COPD [15]. However, for neutrophil elastase to have these effects, it has to overcome the antielastases that protect the tissues. Secretory leukoprotease inhibitor (SLPI) is thought to be the most critical antielastase protecting the airways [16], whereas α_1-antitrypsin (α_1AT) is thought to be less important at this site, although critical at the alveolar level protecting against the development of emphysema [17].

Subjects with severe α_1AT deficiency (PiZ phenotype; serum α_1 AT concentration <11 μM, likely to be 22 homozygotes) have decreased (~15–20% normal) circulating [18], and alveolar [17] concentrations of α_1AT, which facilitates the development of early onset and rapidly progressive emphysema [19]. Around 30–40% [19] of patients also have chronic bronchitis [20], although the nature of the inflammation in the larger airways has not been studied in these individuals.

The purposes of this study were to assess inflammation in the larger airways using sputum from patients with chronic bronchitis and airflow obstruction, to determine

the effect of continued smoking and, in particular, to investigate the role of α_1AT in the larger airways by studying subjects with α_1AT deficiency (α_1ATD).

Patients and methods

Patients

Forty-two patients with chronic obstructive bronchitis (COB) (forced expiratory volume in one second (FEV1) <70% predicted) referred to as COB with normal α_1AT concentrations (PiMM phenotype, likely to be MM homocygotes) and 39 patients with a similar degree of airflow obstruction who had severe α_1ATD of the PiZ phenotype. All patients had chronic bronchitis defined on clinical grounds [21] and emphysema confirmed by high resolution computed tomography of the thorax. They were studied in the stable clinical state at least 8 weeks after a clinical exacerbation and none had received oral steroids or antibiotics within that time period. More patients with α_1ATD [26] received regular inhaled steroids than the COB group (18, p<0.05) although all other therapy was similar.

Sputum and serum processing

Sputum was collected over a 4 h period, from rising, into sterile containers from all patients on a single occasion. A portion was removed for quantitative bacterial culture to obtain the number of viable organisms present [22] and the remainder was ultracentrifuged at $50,000 \times g$ for 90 min at $4°C$, the soluble (sol) phase was removed and stored at -70°C until analysed. Venous blood was collected at the same time into a plain vacutainer tube and the serum was harvested and also stored at -70°C until analysed.

Assay analysis

Sputum myeloperoxidase (MPO) and elastase activity was used to assess neutrophil influx and enzyme release, respectively, as described previously [23]. The lower limit of detection for elastase was 0.01 µM and samples below this level were classified as zero for statistical purposes.

Sputum IL-8, leukotriene (LT)B$_4$, and SLPI were measured by enzyme linked immunosorbent assay (ELISA) using commercially available kits. These assays and their characteristics have been described in detail elsewhere [24].

α_1AT concentration in sputum sol phase was measured by ELISA relative to a commercially available serum standard (The Binding Site Ltd., Birmingham, UK) as described previously [16] and α_1AT in serum was measured by radial immunodiffusion (The Binding Site Ltd.). The ratio of sol/serum α_1AT was calculated and expressed as a percentage to assess the degree of protein leakage from serum as described previously [25].

Statistical analysis

The Mann Whitney U-test was used throughout to compare different groups as most of the data were not normally distributed. Values are reported as mean±SEM and as median (interquartile range (IQR)) where appropriate. A p-value of <0.05 was considered to be statistically significant.

Results

The mean age for the patients with COB was 67.1 yrs (SEM±1.2), range 44–79 yrs and 15 were female. The patients with α_1ATD were younger with a mean age of 50 (±1.5 yrs), range 33–66 yrs (p<0.005) and 9 were female (p>0.1). Average lung function for both groups is summarized in table 1. The mean post-bronchodilator (nebulized salbutamol 5 mg) FEV1 increase was 65±36 mL (6.7±2.0% improvement from baseline) in the COB group and 120±20 mL (14.8±1.9% improvement from baseline) in the α_1ATD group. Twenty of the patients in the COB group were exsmokers and the remaining 22 were current smokers, whereas the majority of patients in the α_1ATD group [34] were exsmokers with five current smokers.

Table 2 summarizes the average sputum values for all the patients in each group, as well as data for the current and exsmokers in the COB group. The current smokers had slightly better lung function than the exsmokers (p<0.05) with an average FEV1 (% pred) for the patients age and height [26] of 35.1±3.4% and 25.0±1.8% respectively, although the ages were similar for both groups (current smokers 66.0±1.9 yrs and 68.8±1.5 yrs for exsmokers).

All samples from the COB group contained measurable quantities of all proteins with the exception of elastase activity which was present in only 16 of the 42 patients. Subgroup analysis showed that the IL–8 and MPO concentrations were lower in the exsmokers although the latter just failed to reach the accepted level of significance (p= 0.06; table 2).

The α_1ATD group had a greater degree of inflammation in the larger airways compared to the COB group. The LTB$_4$ and MPO concentrations were higher and elastase activity was detected in most samples (31/39) but the concentrations of its inhibitors SLPI and α_1AT were lower despite increased protein leakage of α_1AT. These differences were maintained even when the α_1ATD group was compared to the subgroups of patients with COB who were current or exsmokers (table 2).

Table 1. – Pulmonary function tests

	COB		PiZ α_1-ATD	
	Observed	% Predicted	Observed	% Predicted
FEV1 L	0.8±0.1	30.1±3.1	0.9±0.1	27.4±3.0
FVC L	2.0±0.2	64.3±4.4	2.9±0.2	70.7±4.6
FEV1/FVC %	36.4±2.2	46.3±2.8	29.9±2.1	36.5±2.5
RV L	4.2±0.3	187.3±8.6	3.2±0.2	159.2±11.5
TLC L	6.9±0.4	116.8±3.8	7.9±0.3	120.8±3.2
RV/TLC %	61.3±1.7	159.8±3.9	41.6±2.4	130.8±7.4
KCO mmol·m^{-1}·kPa·L^{-1}	0.8±0.1	51.4±5.1	1.0±0.1	53.3±6.2

Lung function data for patients with chronic obstructive bronchitis (COB) and patients with PiZ phenotype (serum α_1-antitrypsin concentration <11 µM, likely to be 22 homocygotes) α_1-antitrypsin deficiency (ATD). Data is presented as mean± SEM. Values are also expressed as a percentage of that predicted for the patients age, height and sex. FEV1: forced expiratory volume in one second; FVC: forced vital capacity; RV: residual volume; TLC: total lung capacity; KCO: carbon monoxide transfer coefficient.

Table 2. – Airways inflammation in patients with chronic obstructive bronchitis and patients with PiZ α_1-antitrypsin deficiency (ATD)

	COB	COB		PiZ α_1ATD
		Current	Exsmokers	
n	42	22	20	39
MPO units·mL^{-1}	0.2 (0.1–0.4)****	0.3 (0.1–0.5)****	0.1 (0.1–0.3)****	0.6 (0.3–2.2)
Elastase μM	0 (0–0.04)**	0 (0–0.04)*	0 (0–0.04)*	0.02 (0.01–0.04)
IL-8 nM	4.7 (1.3–12.2)	8.8 (3.5–15.5)$^{\#}$	2.0 (1.0–9.5)	4.6 (1.8–13.9)
LTB$_4$ nM	6.0 (2.4–10.0)***	5.9 (2.1–9.8)*	6.6 (2.4–11.1)*	10.4 (5.2–27.7)
SLPI μM	2.4 (1.6–5.3)*	2.0 (1.0–2.8)	2.7 (1.5–5.9)*	2.0 (0.6–3.8)
α_1AT ratio %	0.7 (0.5–1.1)****	0.9 (0.5–2.9)****	0.6 (0.5–0.7)*	2.0 (1.0–3.1)
α_1AT μM	0.17 (0.13–0.34)****	0.24 (0.13–0.86)****	0.17 (0.13–0.24)****	0.11 (0.04–0.14)

Data for all COB patients, and subset into COB current and exsmokers, and those with PiZ α_1ATD. Values were presented in sputum and are presented as medians with the (interquartile range in parentheses). Significant differences between the COB group and the group with PiZ phenotype (serum α_1-antitrypsin (AT) concentration <11 μM likely to be 22 homozygotes) α_1ATD are indicated: *: p<0.05; **: p<0.01; ***: p<0.005; ****: p<0.001. Significant difference between the COB current and exsmokers ($^{\#}$: p<0.05). MPO: myeloperoxidase; IL-8 interleukin-8; LTB$_4$: leukotriene B$_4$; SLPI: secretory leukoprotease inhibitor.

Quantitative bacterial culture indicated that some of the patients in both the COB and α_1ATD groups were colonized by bacteria despite being in a stable clinical state. Nineteen of the patients with COB had either no bacterial growth in their sputum or low numbers (<10^5 colony forming units (cfu)·mL^{-1}) of mixed normal flora. However, for the remainder a single organism was isolated and the median bacterial load was 3×10^8 cfu·mL^{-1} (range 1×10^7 – 5×10^9). Of these patients 17 cultured *Haemophilus* sp., four *Branhamella catarrhalis* and two *Streptococcus* sp. Sputum from 13 patients with α_1ATD grew no recognized organism or few mixed normal flora. Twelve patients had <10^7 cfu·mL^{-1} of a single viable organisms in their sputum (all were *Haemophilus* sp.). The remaining 14 patients had organisms at $\geq10^7$ cfu·mL^{-1} in their sputum with a median bacterial load of 8×10^8 cfu·mL^{-1} (range $2\times10^7 – 2\times10^9$). Eleven of the organisms were *Haemophilus* sp., one *Staphylococcus aureus*, one a coliform, and one *Pseudomonas aeruginosa*.

The presence of bacterial colonization may influence the degree of inflammation in the larger airways [27]. In view of this, both the COB and α_1ATD groups were subdivided according to the results of bacterial culture. However, in the COB group the identified pathogen load was always >10^7 cfu·mL^{-1}, whereas the patients with α_1ATD had a wider concentration range of colonizing organisms (10^5–10^9). Therefore the groups were only compared for those subjects where either no pathogen was present or where the bacterial load was >10^7 cfu·mL^{-1}. There was no significant difference in FEV1 (% pred) between colonized and noncolonized patients in either group.

Mixed normal flora

The sputum MPO (fig. 1) and LTB$_4$ concentration (fig. 2) were greater in subjects with α_1ATD. Samples from nine of the 13 patients with α_1ATD had low but detectable concentrations of elastase activity (median=0.02 μM; IQR=0–0.04 μM) whereas 18 of the 19 samples from the COB patients had no detectable elastase activity (remaining subject=0.03 μM, p<0.0005 for group comparison). The median protein leakage in the α_1ATD group (1.7%; IQR=0.4–3.0) was higher (p<0.05) than the COB

group (0.6%; IQR=0.5–0.8), whereas sputum median α_1AT concentration was lower in the α_1ATD group (0.09 μM; IQR=0.04–0.13, p<0.005) than the COB group (0.16 μM; IQR=0.11–0.25) which is consistent with the serum deficiency of this inhibitor. The concentrations of SLPI, however, were similar in both the α_1ATD (3.9 μM; IQR=2.3–4.3) and COB patients (3.9 μM; IQR=2.4–5.4).

More than 10^7 colony forming units per millilitre

The sputum MPO, elastase activity, LTB$_4$ and IL-8 concentrations were increased in both the COB and α_1ATD group colonized by >10^7 cfu·mL^{-1} compared to those not colonized (mixed normal flora) as shown in figures 1–3. Elastase activity was detectable in 15 of 23 samples from COB and 13 of 14 for α_1ATD patients (median=0.04 μM; IQR=0–0.04, and 0.08 μM; IQR=0.02–0.49 respectively). In addition protein leakage (COB 0.9%; IQR= 0.6–3.4 and α_1ATD 2.8%; IQR=1.8–4.3) was greater in both
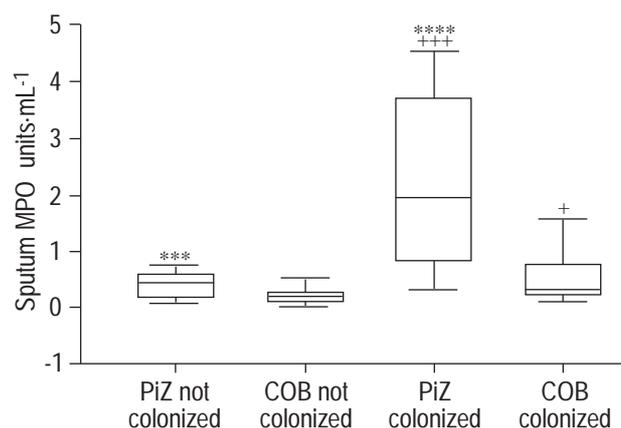

Fig. 1. – Sputum myeloperoxidase (MPO) is shown for patients with chronic obstructive bronchitis (COB) and α_1-antitrypsin deficiency (α_1ATD). The data is separated into patients colonized (>10^7 colony forming units·mL^{-1}) or not colonized (mixed normal flora only) with bacteria in the stable clinical state. The boxplots represent the median results, interquartile range, with minimum and maximum values. ***: p<0.005; ****: p<0.001, COB compared to α_1ATD patients. $^+$: p<0.05; $^{+++}$: p<0.005, differences within the COB and α_1ATD patients in relation to bacterial load.
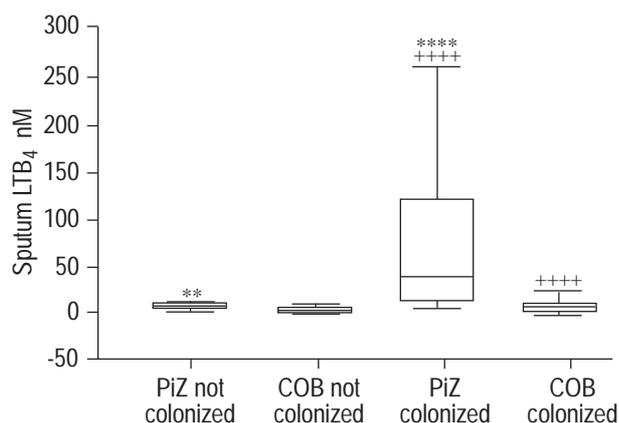
Fig. 2. – The sputum concentrations of leukotriene (LT)B$_4$ are shown for patients with chronic obstructive bronchitis (COB) and α_1-antitrypsin deficiency (α_1ATD), divided into those with and without bacterial colonization. The boxplots represent the median results, interquartile range, with minimum and maximum values. **: $p<0.01$; ****: $p<0.001$, COB compared to α_1ATD patients. ++++: $p<0.001$, differences within the COB and α_1ATD patients in relation to bacterial load.

patient groups (both $p<0.05$) and sputum α_1AT concentration (COB 0.24 μM; IQR=0.15–0.81, and α_1-ATD 0.13 μM; IQR=0.08–0.16) were increased ($p=0.08$, and $p<0.05$ respectively). On the other hand SLPI concentration (COB 1.9 μM; IQR=1.4–2.1 and α_1ATD 0.4 μM; IQR=0.2–1.6) were reduced ($p<0.05$, and $p<0.0005$ respectively).

Comparisons between patient groups colonized with $>10^7$ cfu·mL^{-1} showed that the α_1ATD group had greater concentrations of MPO (fig. 1), elastase activity ($p<0.05$), LTB$_4$ (fig. 2), IL-8 (fig. 3) and protein leakage ($p<0.05$) but lower concentrations of α_1AT ($p<0.01$) and SLPI ($p<0.05$).

Discussion

The present data shows evidence of inflammation in the larger airways in patients with COB with a wide range of
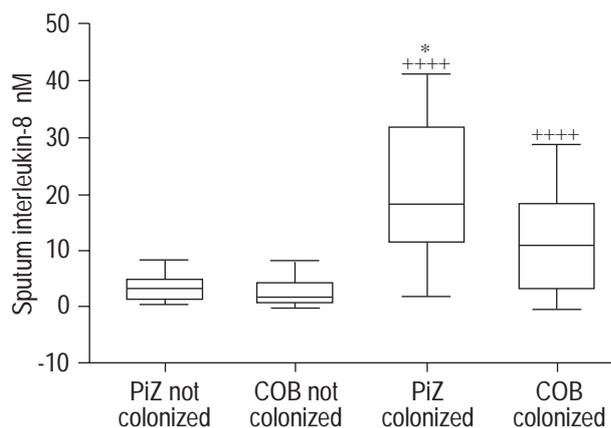

Fig. 3. – The sputum concentrations of interleukin-8 are shown for patients with chronic obstructive bronchitis (COB) and α_1-antitrypsin deficiency (α_1ATD), divided into those with and without bacterial colonization. The boxplots represent the median results, interquartile range, with minimum and maximum values. *: $p<0.05$, COB compared to α_1ATD patients. ++++: $p<0.001$, differences within the COB and α_1ATD patients in relation to bacterial load.

neutrophil influx (as reflected in the MPO concentration) in response to the chemoattractants IL-8 and LTB$_4$ which are thought to play a key role in neutrophil recruitment [28]. Elastase activity was absent or low even though these samples are known to contain the enzyme [29] probably due to inhibition by the natural inhibitors, especially SLPI. Comparisons between current and ex-smokers showed that the only differences were lower IL-8 and MPO concentrations in the latter group. This did not reflect differences in therapy and the exsmokers had worse lung function which would, if anything, increase neutrophil numbers [3, 4]. IL-8 is thought to be a major airways neutrophil chemoattractant [30] and is increased in healthy smokers [3] and subjects with COPD [3] although the effect of smoking cessation has not been assessed previously. The current data indicate that cessation of smoking in chronic bronchitis is related to a reduction in airways IL-8 concentration that in turn would reduce neutrophil recruitment, thereby explaining the beneficial effect of smoking cessation on progression of lung disease. The reasons for this effect are not clear at present but may be due to loss of cigarette smoke induced epithelial production of IL-8 [7].

The current data shows that inflammation in the larger airways is increased in the α_1ATD subjects despite more using inhaled steroids (which would be expected to have a beneficial effect) [23] and the subjects being younger (which would also be associated with less inflammation in the larger airways) [31, 32]. MPO concentrations were higher, as was the chemoattractant LTB$_4$. This suggests that LTB$_4$ may be the major chemoattractant responsible for the increased neutrophil migration and is consistent with previous findings in bronchoalveolar lavage [33]. The source of the LTB$_4$ is uncertain although HUBBARD *et al.* [33] suggested that it was released from alveolar macrophages as a direct effect of uninhibited elastase due to α_1ATD. Indeed, in the current study elastase activity was more readily detected in the α_1ATD patients irrespective of bacterial colonization and hence would support the suggestion that this may be responsible [33]. The elastase activity was more readily detected in the larger airways in α_1ATD probably due to the combined effect of the lower concentrations of both α_1AT and SLPI. The mechanisms involved may be complex but elastase can reduce the secretion of SLPI [34] as well as the increase in the permeability of airway cells [35]. Despite the latter effect which accounts for an increase in α_1AT "leak" into the lung, the low α_1AT concentrations in these subjects may be critical in determining the overall changes, suggesting that this inhibitor also has a major role in the larger airways where SLPI has been conventionally thought of as the important inhibitor.

Bacterial colonization is clearly related to the degree of airways inflammation, suggesting that this may not always be a benign state even though the patients were "clinically" stable. However, even when controlled for airways colonization, the α_1ATD group still had increased evidence of inflammation that is likely to be responsible for or to reflect the development of severe airflow limitation at an earlier age. It is of importance to note that these changes were present in exsmokers with α_1ATD and would suggest that measures other than smoking cessation may be critical in stabilizing lung disease in these patients. Although by convention this could include α_1AT augmentation therapy,

antibiotics may also be of value in patients that are colonized in the stable clinical state to reduce the degree of inflammation in the larger airways.

In conclusion, patients with chronic bronchitis have evidence of inflammation in the larger airways. The chemoattractant interleukin-8 plays a key role in neutrophil recruitment and the beneficial effects of smoking cessation may be mediated through reduction of the concentration of this cytokine. Patients with α_1-antitrypsin deficiency have greater inflammation (probably related to increased leukotriene B_4 production) and this suggests that α_1-antitrypsin has an important role in protecting the larger airways from the inflammatory effects of elastase. Bacterial colonization may play a major role in determining the degree of airways inflammation and hence progression of airflow obstruction even in the stable state.

References

1. Badham C. Observations on the inflammatory affections of the mucous membrane of the bronchiae. London, UK, Callow, 1808.
2. Riise GC, Ahlstedt S, Larsson S, *et al*. Bronchial inflammation in chronic bronchitis assessed by measurement of cell products in bronchial lavage fluid. *Thorax* 1995; 50: 360–365.
3. Keatings VM, Barnes PJ. Comparison of inflammatory cytokines in chronic obstructive pulmonary disease, asthma and controls. *Eur Respir Rev* 1997; 7: 146–150.
4. Di Stefano A, Capelli A, Lusuardi M, *et al*. Severity of airflow limitation is associated with severity of airway inflammation in smokers. *Am J Respir Crit Care Med* 1998; 158: 1277–1285.
5. Stanescu D, Sanna A, Veriter C, *et al*. Airways obstruction, chronic expectoration, and rapid decline of FEV1 in smokers are associated with increased levels of sputum neutrophils. *Thorax* 1996; 51: 267–271.
6. Hunninghake GW, Crystal RG. Cigarette smoking and lung destruction. Accumulation of neutrophils in the lungs of cigarette smokers. *Am Rev Respir Dis* 1983; 128: 833–838.
7. Mio T, Romberger DJ, Thompson AB, Robbins RA, Heires A, Rennard SI. Cigarette smoke induces interleukin-8 release from human bronchial epithelial cells. *Am J Respir Crit Care Med* 1997; 155: 1770–1776.
8. Snider GL, Lucey EC, Christensen TG, *et al*. Emphysema and bronchial secretory cell metaplasia induced in hamsters by human neutrophil products. *Am Rev Respir Dis* 1984; 129: 155–160.
9. Amitani R, Wilson R, Rutman A, *et al*. Effects of human neutrophil elastase and *Pseudomonas aeruginosa* proteinases on human respiratory epithelium. *Am J Respir Cell Mol Biol* 1991; 4: 26–32.
10. Smallman LA, Hill SL, Stockley RA. Reduction of ciliary beat frequency *in vitro* by sputum from patients with bronchiectasis: a serine proteinase effect. *Thorax* 1984; 39: 663–667.
11. Snider GL, Stone PJ, Lucey EC, *et al*. Eglin C, a polypeptide derived from the medicinal leech, prevents human neutrophil elastase-induced emphysema and bronchial secretory cell metaplasia. *Am Rev Respir Dis* 1985; 132: 1155–1161.
12. Sommerhoff CP, Nadel JA, Basbaum CB, Caughey GH. Neutrophil elastase and cathepsin G stimulate secretion from cultured bovine airway gland serous cells. *J Clin Invest* 1990; 85: 682–689.
13. Solomon A. Possible role of PMN proteinases in immunoglobulin degradation and amyloid formation. Baltimore, USA, Urban and Schwarzenberg, 1978.
14. Tosi MF, Zakem H, Berger M. Neutrophil elastase cleaves C3bi on opsonized pseudomonas as well as CR1 on neutrophils to create a functionally important opsonin receptor mismatch. *J Clin Invest* 1990; 86: 300–308.
15. Monso E, Ruiz J, Rosell A, *et al*. Bacterial infection in chronic obstructive pulmonary disease. A study of stable and exacerbated outpatients using the protected specimen brush. *Am J Respir Crit Care Med* 1995; 152: 1316–1320.
16. Morrison HM, Kramps JA, Afford SC, Burnett D, Dijkman JH, Stockley RA. Elastase inhibitors in sputum from bronchitic patients with and without alpha-1-proteinase inhibitor deficiency: partial characterization of a hitherto unquantified inhibitor of neutrophil elastase. *Clin Sci* 1987; 73: 19–28.
17. Gadek JE, Fells GA, Zimmerman RL, Rennard SI, Crystal RG. Antielastases of the human alveolar structures. Implications for the protease-antiprotease theory of emphysema. *J Clin Invest* 1981; 68: 889–898.
18. Lieberman J, Gaidulis L, Garoutte B, Mittman C. Identification and characteristics of the common alpha-1-antitrypsin phenotypes. *Chest* 1972; 62: 557–564.
19. Brantly ML, Paul LD, Miller BH, Falk RT, Wu M, Crystal RG. Clinical features and history of the destructive lung disease associated with alpha-1-antitrypsin deficiency of adults with pulmonary symptoms. *Am Rev Respir Dis* 1988; 138: 327–336.
20. Eriksson S. Studies in alpha-1-antitrypsin deficiency. *Acta Med Scand* 1965; 432: 1–85.
21. Medical Research Council. Definition and classification of chronic bronchitis for clinical and epidemiological purposes. A report to the Medical Research Council by their Committee on the Aetiology of Chronic Bronchitis. *Lancet* 1965; 1: 775–779.
22. Pye A, Stockley RA, Hill SL. Simple method for quantifying viable bacterial numbers in sputum. *J Clin Pathol* 1995; 48: 719–724.
23. Llewellyn-Jones CG, Harris TA, Stockley RA. Effect of fluticasone propionate on sputum of patients with chronic bronchitis and emphysema. *Am J Respir Crit Care Med* 1996; 153: 616–621.
24. Hill AT, Bayley D, Stockley RA. The interrelationship of sputum inflammatory markers in patients with chronic bronchitis. *Am J Respir Crit Care Med* 1999; 160: 893–898.
25. Stockley RA. Measurement of soluble proteins in lung secretions. *Thorax* 1984; 39: 241–247.
26. British Thoracic Society. Guidelines for the measurement of respiratory function. *Respir Med* 1994; 88: 165–194.
27. Hill A, Bayley D, Crooks S, Hill S, Campbell E, Stockley R. The relationship of bacteria to bronchial inflammation. *Eur Respir J* 1998; 12: Suppl. 28, 230s.
28. Mikami M, Llewellyn-Jones CG, Bayley D, Hill SL, Stockley RA. The chemotactic activity of sputum from patients with bronchiectasis. *Am J Respir Crit Care Med* 1998; 157: 723–728.
29. Stockley RA, Burnett D. Alpha-1-antitrypsin and leukocyte elastase in infected and noninfected sputum. *Am Rev Respir Dis* 1979; 120: 1081–1086.
30. Richman-Eisenstat JB, Jorens PG, Hebert CA, Ueki I, Nadel JA. Interleukin-8: an important chemoattractant in sputum of patients with chronic inflammatory airway diseases. *Am J Physiol* 1993; 264: L413–L418.
31. Meyer KC, Ershler W, Rosenthal NS, Lu XG, Peterson K. Immune dysregulation in the aging human lung. *Am J Respir Crit Care Med* 1996; 153: 1072–1079.
32. Meyer KC, Rosenthal NS, Soergel P, Peterson K. Neutrophils and low-grade inflammation in the seemingly normal aging human lung. *Mech Ageing Dev* 1998; 104: 169–181.
33. Hubbard RC, Fells G, Gadek J, Pacholok S, Humes J, Crystal RG. Neutrophil accumulation in the lung in alpha-1-antitrypsin deficiency. Spontaneous release of leukotriene B_4 by alveolar macrophages. *J Clin Invest* 1991; 88: 891–897.
34. Sallenave JM, Shulmann J, Crossley J, Jordana M, Gauldie J. Regulation of secretory leukocyte proteinase inhibitor (SLPI) and elastase-specific inhibitor (ESI/elafin) in human airway epithelial cells by cytokines and neutrophilic enzymes. *Am J Respir Cell Mol Biol* 1994; 11: 733–741.
35. Peterson MW, Walter ME, Nygaard SD. Effect of neutrophil mediators on epithelial permeability. *Am J Respir Cell Mol Biol* 1995; 13: 719–727.