Factors influencing airway inflammation in chronic obstructive pulmonary disease

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Airway inflammation in chronic obstructive pulmonary disease (COPD) is currently the subject of rapidly increasing research interest, investigating both the nature of the inflammatory cells and the cytokines present. The data are being used to define and assess the severity, cause, prognosis, and response to treatment of this disease. The collection of spontaneous sputum or the induction of sputum has been used to study inflammation in the larger airways. It remains unclear, however, how these data relate to the pathological processes at the site of airflow obstruction (the small airways and alveolar region). Nevertheless, even the interpretation of the results from the large airways depends on the many factors that will influence the inflammation.

The initial process of sputum collection, sample processing, and the performance of assays in the biological fluids being tested may all influence the results. In addition, airways inflammation may be altered by the patient’s clinical state, current treatment, and the nature of the disease (asthma, COPD, or bronchiectasis). Other factors including smoking, α1-antitrypsin (AAT) deficiency, and bacterial colonisation may also affect airways inflammation. Despite these many factors that may influence airways inflammation, many papers currently in the literature do not address them. The purpose of this review is to indicate the influence of these factors on airways inflammation to assist interpretation of the data currently in the literature.

Pathophysiology

EOSINOPHIL

Airway inflammation in asthma is classically related to the eosinophil. Eosinophil granules contain a variety of mediators capable of causing inflammatory damage to the airway epithelium including eosinophil peroxidase (EPO), major basic protein (MBP), eosinophil cationic protein (ECP), metalloproteinases, platelet activating factor, and cysteinyl leukotrienes.1 In addition, the immunological basis of asthma is distinct and reflected in the nature of the cytokines released into the airway.

However, there is some overlap with COPD which may reflect the fact that asthma can lead to fixed airflow obstruction or that patients with COPD may have coincidental asthma.2 Indeed, some recent studies have indicated that sputum eosinophilia is a predictor of steroid responsive airflow obstruction3,4 and thus may identify the chronic asthma component or a subtype of the COPD syndrome.

NEUTROPHIL

In contrast, however, the neutrophil is thought to be the major cell involved in the airways of patients with COPD and those with bronchiectasis, although it has also been implicated in the later irreversible phase of asthma. In neutrophilic inflammation in the bronchial tree there is a complex interaction of many mediators including chemoattractants and cytokines that regulate gene transcription, adhesion molecule expression, the processes of cell migration, their activation and degranulation.

PROTEASES

The recruited neutrophil releases proteolytic enzymes including neutrophil elastase, cathepsin G, and matrix metalloproteinases. The importance of these enzymes—and, specifically, neutrophil elastase—has been demonstrated by both in vitro and in vivo studies. Neutrophil elastase can produce most of the pathological features of chronic bronchitis and emphysema, and promote bacterial colonisation as seen in patients with bronchiectasis. More specifically, neutrophil elastase can cause epithelial damage,5 reduce ciliary beat frequency,6 produce mucous gland hyperplasia,7 stimulate mucus secretion,8 inactivate many critical lung host defences,9,10 and damage interstitial tissue11 leading to pathological changes that cause human alveolar destruction.

ANTI-PROTEINASES

In order for neutrophil elastase to have these effects, the elastase released from the activated neutrophil has to overcome the naturally occurring inhibitors such as secretary leukocyte protease inhibitor (SLPI) and AAT. SLPI is generally thought to be the main inhibitor in the airways12 and hence protects against bronchial disease, whereas AAT is thought to be of importance predominantly in the lower airways13 and is thus more relevant to alveolar disease.

CHEMOATTRACTANTS

The major neutrophil chemoattractants in patients with COPD and bronchiectasis are believed to be interleukin 8 (IL-8) and leuko-
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Neutrophil elastase is thought to be of major importance in the pathogenesis of airways disease in COPD and bronchiectasis. Studies from spontaneous sputum have shown relationships between neutrophil elastase activity and several features of airways disease including the following: neutrophil influx; chemotactic activity of lung secretions in patients with COPD and bronchiectasis can be attributed to IL-8 and LTB₄. Studies by Mikami et al. have shown that up to 43% of the chemotactic activity was dependent on IL-8 and 27% on LTB₄. It was further shown that the chemoattractants worked in an additive way for neutrophil recruitment. However, these workers also emphasised that some of the chemotactic activity of the lung secretion was due to a protein or proteins that remain to be identified. This is particularly true in patients with bronchiectasis who usually have marked bronchial inflammation associated with significant neutrophil influx. Other potential chemoattractants listed above may play a role and cells migrating into tissues may thus encounter multiple chemoattractant signals. Foxman et al. have shown that neutrophils can even migrate “down” a local chemoattractant gradient in response to a distal gradient of a different chemoattractant. In addition, cells can migrate effectively to a secondary distant agonist after migrating “up” a primary gradient into a saturating non-orienting concentration of the initial attractant. These mechanisms of navigation from one gradient to another in complex chemoattractant fields are likely to be necessary for successful localisation and phagocytosis within sites of inflammation.

The relationship between chemoattractants and neutrophils may be further complicated since neutrophil activation can lead to the release of both IL-8 and LTB₄ that may enhance further neutrophil recruitment. Release of elastase from the neutrophil may also stimulate epithelial cells to produce more IL-8 and macrophages to release LTB₄ while at the same time reducing production of its own natural inhibitor, SLPI, thereby perpetuating its function. Furthermore, impairment of host defences by elastase could facilitate bacterial colonisation and endotoxin release from bacteria can also provide a further potential mechanism for neutrophil recruitment by stimulating production of IL-8 by epithelial cells. The understanding of these complex interrelationships and the role of each component awaits in vivo studies with specific agonists and antagonists.

RELATIONSHIP BETWEEN ELASTASE ACTIVITY AND OTHER INFLAMMATORY MARKERS

Neutrophil elastase is thought to be of major importance in the pathogenesis of airways disease in COPD and bronchiectasis. Studies from spontaneous sputum have shown relationships between neutrophil elastase activity and several features of airways disease including the following: neutrophil influx; chemoattractants (IL-8 and LTB₄); SLPI; and protein leakage (as determined by sputum/serum albumin ratio). The close correlation between neutrophil elastase and neutrophil influx would be expected as it is stored within the primary granules of the neutrophil and would be released simultaneously with other granule

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triene B₄ (LTB₄). IL-8 is a 16 kDa protein that is a member of the C-X-C family of cytokines. It is made and released by bronchial epithelial cells, monocytes/macrophages, and even the neutrophils themselves. Regulation of IL-8 production in the lung is complex but recent studies have started to determine the processes involved. It is known that tumour necrosis factor-α (TNF-α) can increase the expression of the IL-8 gene, and lung secretions from patients with COPD and bronchiectasis contain TNF-α. Furthermore, recent work has indicated that cigarette smoke can stimulate epithelial cells to release IL-8 via the transcription factor Nkx2.2 and this may be the mechanism that leads to neutrophil recruitment to the lungs of smokers. In addition, bacteria can also induce IL-8 expression by epithelial cells18-21 but at present it is uncertain whether the effect of bacterial products on IL-8 expression is direct or the result of initial TNF-α secretion. IL-8 levels are raised in patients with COPD and patients with bronchiectasis26-29 and correlate with myeloperoxidase (MPO) released from activated neutrophils. This relationship between IL-8 and a neutrophil marker supports the concept that IL-8 is an important lung chemoattractant for neutrophils.

LTB₄ is a biologically active fatty acid synthesised in myeloid cells from arachidonic acid. It was isolated and purified from neutrophils in 1978 but was subsequently chemically synthesised and its ability to activate neutrophils was shown in 1980. LTB₄ is now known to be a major chemotactic product of activated neutrophils and macrophages. LTB₄ induces recruitment and activation of neutrophils as well as monocytes, eosinophils, and lymphocytes. It promotes chemotaxis by increasing neutrophil adherence to venule walls via the integrin Mac-1 and its receptor ICAM-1.

The cytokine TNF-α has been found to be increased in the airways of patients with COPD and bronchiectasis. It is produced by activated macrophages, mast cells, and other inflammatory cells as well as by airway epithelial cells. TNF-α induces airway hyperresponsiveness, may promote IL-8 production by epithelial cells and neutrophils, is chemotactic for neutrophils and eosinophils both directly and by activation of adhesion molecules on endothelial cells, and promotes vascular leakage. However, in vivo it has been shown to inhibit neutrophil chemotaxis and therefore its role in lung inflammation is complex and requires further study.

There are a number of other chemoattractants that could play a role in the airways including C5a generated from complement activation; modified AAT; leucocyte elastase-inhibitor complexes; protein and peptide components of damaged extracellular matrix such as collagen, elastin, and laminin; antigen-antibody complexes; bacterial factors including the formyl peptides such as fMLP and lipopolysaccharide or endotoxin which can prime neutrophil functions including the chemotactic responses.

A significant proportion, however, of the chemotactic activity of lung secretions in patients with COPD and bronchiectasis can be attributed to IL-8 and LTB₄. Studies by Mikami et al. have shown that up to 43% of the chemotactic activity was dependent on IL-8 and 27% on LTB₄. It was further shown that the chemoattractants worked in an additive way for neutrophil recruitment. However, these workers also emphasised that some of the chemotactic activity of the lung secretion was due to a protein or proteins that remain to be identified. This is particularly true in patients with bronchiectasis who usually have marked bronchial inflammation associated with significant neutrophil influx. Other potential chemoattractants listed above may play a role and cells migrating into tissues may thus encounter multiple chemoattractant signals. Foxman et al. have shown that neutrophils can even migrate “down” a local chemoattractant gradient in response to a distal gradient of a different chemoattractant. In addition, cells can migrate effectively to a secondary distant agonist after migrating “up” a primary gradient into a saturating non-orienting concentration of the initial attractant. These mechanisms of navigation from one gradient to another in complex chemoattractant fields are likely to be necessary for successful localisation and phagocytosis within sites of inflammation.

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RELATIONSHIP BETWEEN ELASTASE ACTIVITY AND OTHER INFLAMMATORY MARKERS

Neutrophil elastase is thought to be of major importance in the pathogenesis of airways disease in COPD and bronchiectasis.
Figure 1 Concentrations of IL-8 (nM) in patients with COPD with (PiZ) and without (PiM) α1-antitrypsin deficiency, and in patients with bronchiectasis in the stable state (S) and during an exacerbation (E). Both inpatient and outpatient exacerbations are shown. The histograms represent mean (SE) values derived from Crooks et al., Hill et al., and Gompertz et al. *p<0.05 versus patients in the stable clinical state.

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Patients with bronchiectasis are characterised by major airways inflammation thought to be driven by persistent bacterial colonisation. Such patients have been shown to have significantly neutrophil influx with raised MPO and readily detectable elastase activity. 

Patients with COPD also have airways inflammation, but to a lesser degree. This is illustrated in fig 1 which compares IL-8 levels in patients with COPD and without AAT deficiency, and in patients with bronchiectasis in the stable state and during an exacerbation. 

Technical factors

Induced sputum samples have confirmed the presence of increased concentrations of products derived from neutrophil granules (MPO, human neutrophil lipocalin (HNLI)) and from eosinophils (EPO, ECP) in patients with asthma or COPD compared with normal individuals. Although MPO and HNLI were significantly higher in patients with COPD than in those with asthma, the concentrations of ECP and EPO were similar in the two groups of patients indicating a degree of eosinophil influx in addition to neutrophil influx in COPD. The cytokines TNF-α and IL-8 were also increased in both conditions, although only IL-8 was significantly higher in COPD than in asthma. Other inflammatory mediators have also been found to be increased in induced sputum, including the tachykinin substance P in individuals with asthma and chronic bronchitis.

Disease process

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Comparison of induced and spontaneous sputum samples in patients with COPD and asthma has demonstrated higher cell viability in induced samples, less squamous cell contamination, and better quality cytospins.
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Many other workers use the reduction of untreated whole spontaneous sputum samples obtained by ultracentrifugation of untreated whole spontaneous sputum from the same patients. Whether this relates to lysis of less viable cells as a result of hypertonic saline required to induce expectoration remains to be determined. Total and differential cell counts are similar in both types of sample but other measurements can be altered—for example, lower fibrinogen levels have been found in induced samples. Bayley et al found that sputum induction (whole sample) produced approximately a 50% dilution in a number of inflammatory parameters including MPO, elastase activity, IL-8, LTB₄, and albumin leakage compared with levels in spontaneous sputum from the same patients.

The authors routinely use and have validated assays to measure inflammatory mediators in sputum sol samples obtained by ultracentrifugation of untreated whole spontaneous sputum. Many other workers use the reducing agent DTT to hydrolyse disulphide bonds and to render sputum samples less viscous before processing and cell counting. However, DTT may have effects on the disulphide bonds within inflammatory molecules themselves (such as IL-8) or may alter their release from treated sputum macro-molecules. Efthimiadis et al found that DTT affected both cell numbers and viability and increased the levels of ECP in induced sputum from asthmatic patients but had no effect on the levels of fibrinogen, IL-5, or IL-8. Other authors have found a significant reduction in EPO and MPO levels in DTT treated induced sputum from asthmatic patients but no effect on ECP. It is probably wisest to retain an aliquot of sample without DTT for cytokine assays unless the assays have been formally validated for samples treated with DTT.

Smoking

Prevalence studies in at least 10 countries have confirmed the association between smoking and the development of chronic bronchitis. Cigarette smoke recruits inflammatory cells to the lungs and studies have shown increased numbers of neutrophils in bronchoalveolar lavage fluid from smokers. In addition, the lungs of smokers with airflow obstruction contain more neutrophils than smokers without airflow obstruction and the number of neutrophils recovered is related to the amount smoked.

Continued smoking seems to be the major stimulus to airways inflammation in patients with chronic bronchitis and emphysema, whereas smoking cessation is believed to be beneficial in reducing the rate of lung function decline. In studies of patients with chronic obstructive bronchitis, current smokers had higher sputum (induced and spontaneous) IL-8 concentrations. One hypothesis is that cessation of smoking in patients with COPD leads to a reduction in airways IL-8 concentrations which in turn reduces neutrophil recruitment, thereby providing a mechanism to explain the beneficial effect of smoking cessation on progression of neutrophil mediated lung disease. The reason for this effect is not clear at present but may relate to a reduction in epithelial production of IL-8 which can be induced by cigarette smoke.

AAT deficiency

Subjects with severe AAT deficiency (PiZ phenotype) have decreased (about 15–20% normal) circulating and alveolar concentrations of AAT which facilitates the development of early onset and rapidly progressive emphysema. About 30–40% of these patients have chronic bronchitis, although the nature of the airway inflammation measured in spontaneous sputum has only recently been studied.

The pathogenesis of emphysema in AAT deficient patients is becoming clearer. Initial lavage studies revealed that PiZ patients have increased numbers of neutrophils in the lower airways, suggesting a greater potential elastase burden than in smokers with emphysema who have normal levels of AAT. In addition, patients with PiZ AAT deficiency have reduced concentrations of immunoreactive AAT in both sputum and lavage fluids. The pathogenic mechanism for the development of lung disease is thought to result from the low AAT concentrations in the presence of an increased neutrophil burden, leading to inadequate control of neutrophil elastase activity. In addition, extensive connective tissue destruction by migrating neutrophils may be amplified in AAT deficient patients as the Z type AAT has a slightly lower association rate constant for elastase. Finally, the low AAT levels result in an inability to control elastase activity in a wider area around the activated neutrophil resulting in even more connective tissue destruction.

In the airways, however, SLPI is believed to be the major inhibitor of elastase. Studies with spontaneous sputum have shown that inflammation is increased in the larger airways of patients with chronic obstructive bronchitis and AAT deficiency. Sputum concentrations of MPO and LTB₄ are higher which suggests that LTB₄ is the major chemoattractant responsible for the increased neutrophil migration, as suggested by previous studies using bronchoalveolar lavage. The source of the LTB₄ is uncertain, although Hubbard et al suggested that it was released from alveolar...
Table 1  Airway inflammation at the start of an exacerbation in outpatients with COPD, with and without severe α1-antitrypsin (AAT) deficiency.

<table>
<thead>
<tr>
<th>Sputum measurements at start of the exacerbation compared with stable state</th>
<th>COPD</th>
<th>AAT deficiency</th>
<th>AAT deficiency vs COPD at start of the exacerbation</th>
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<tr>
<td>Myeloperoxidase</td>
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<tr>
<td>Elastase activity</td>
<td>↑</td>
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<tr>
<td>Interleukin 8</td>
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<td>Leukotriene B4</td>
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<td>Secretory leukoprotease inhibitor</td>
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<td>Sputum α1-antitrypsin</td>
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<td>Albumin leakage (serum → sputum)</td>
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Macrophages as a direct effect of uninhibited elastase. Sputum elastase activity is also increased in patients with AAT deficiency and could have the same effect in the larger airways as that seen in bronchoalveolar lavage, stimulating LTB4 production by airway macrophages.

The elastase activity detected in the larger airways in patients with AAT deficiency is probably due to the combined effect of lower sputum concentrations of both AAT and SLPI.

Indeed, the low SLPI concentration may be a more important determinant of elastase activity in the larger airway where it is the major inhibitor. The mechanisms involved in disturbing the elastase/anti-elastase balance are likely to be complex in the larger airways. For instance, although elastase has been shown to decrease SLPI secretion from epithelial cells,56 it will also increase epithelial cell permeability, resulting in greater AAT leakage from plasma.

In subjects with normal AAT levels these two effects may balance each other, retaining the anti-elastase screen. However, in patients with AAT deficiency the low serum and hence secretion AAT level may be less effective as SLPI falls, resulting in a continued deficient screen in the larger airways and hence uncontrolled elastase activity and local damage.

Bacteriology

In animal models low numbers of bacteria instilled into the airways are efficiently cleared by the primary host defence mechanisms alone.94 However, as increasing numbers of organisms are instilled, secondary host defences are activated including neutrophil migration.94 95 This is likely to reflect the release of pro-inflammatory mediators resulting in an acute inflammatory response with recruitment of neutrophils.96 97 Some patients with COPD have persistent bacterial colonisation and it is likely that this contributes to the morbidity of the disease. In particular, the chronic bacterial load may stimulate secondary host defences and lead directly to persistent airways inflammation even in the stable clinical state,97 98 although this has received little clinical attention.

In a recent study by Hill et al a wide range of airway bacterial load was found among patients with chronic bronchitis while “clinically stable”. Increasing airway bacterial load was strongly correlated with the following in sputum: (1) increasing numbers of neutrophils (MPO); (2) increasing concentrations of the neutrophil chemoattractants IL-8 (fig 3) and LTB4; (3) increasing concentrations of active leucocyte elastase; and (4) increasing evidence of airway inflammation leading to albumin leakage.9 This has been supported by bronchoalveolar lavage data which showed that patients with stable COPD and potentially pathogenic organisms had a higher proportion of neutrophils and a higher TNF-α concentration.100 Thus, chronic bacterial colonisation can also influence airways inflammation significantly even when patients are “clinically stable” and needs to be taken into account when interpreting secretion data obtained from different patient groups.

Exacerbations

Airway inflammation would be expected to increase during exacerbations of COPD, in particular those that meet Anthonisen’s criteria of increasing sputum volume and sputum purulence.101 Earlier work with spontaneous sputum found an increased proteinase burden during such exacerbations with increased protein leakage.102 Further work by Balbi and colleagues demonstrated a higher number of neutrophils and eosinophils in bronchoalveolar lavage specimens during exacerbations of chronic bronchitis,103 and bronchial biopsy specimens taken during such episodes have a marked eosinophilia and a milder increase in the number of neutrophils, activated CD3+ T lymphocytes, and TNF-α positive cells.104 A recent study by Hill et al used spontaneous sputum to investigate the nature of an exacerbation in patients with chronic bronchitis with and without AAT deficiency.105 The exacerbations filled all three Anthonisen criteria with increased sputum volume, purulence, and worsening breathlessness.106 At the start of the exacerbation there was increased airway inflammation reflected by a high sputum level of MPO, elastase activity, chemoattractant (LTB4), and evidence of protein leakage in patients with chronic obstructive bronchitis without AAT deficiency (table 1). Subjects with AAT deficiency also had increased airways inflammation at the start of their exacerbation and this was greater than in matched patients without AAT deficiency (table 1).107 Acute bacterial exacerbations in patients with chronic obstructive bronchitis that required hospital admission were associated with even greater airways inflammation (fig 1).15
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