Neutrophils and the Pathogenesis of COPD*

Robert A. Stockley, MD, DSc

(CHEST 2002; 121:151S–155S)

Key words: α₁-antitrypsin deficiency; chemoattractant; COPD; elastase; emphysema; interleukin-8; leukotriene B₄; neutrophil

Abbreviations: α₁-AT = α₁-antitrypsin; IL = interleukin; LT = leukotriene; NE = neutrophil elastase

The polymorphonuclear leukocyte is a key effector cell of the secondary host defense system. This cell, however, also has been implicated in the pathogenesis of chronic lung disease for >30 years. The association dates to an observation in 1963 that severe early-onset emphysema was associated with deficiency in α₁-antitrypsin (α₁-AT).¹ The recognition that α₁-AT was a serum protein that functioned as an endogenous inhibitor of serine proteinases suggested that an enzyme or enzymes, the activity of which normally was regulated by α₁-AT, was responsible for the lung damage leading to emphysema. This chance observation led to the proteinase/antiproteinase theory of the pathogenesis of emphysema that is now accepted widely as a key mechanism of disease development.

Broadyly, the theory states that the release of proteolytic enzymes within the lungs in healthy subjects is normally prevented from causing lung damage by a protective “screen” of antiproteinases. When the protective screen is deficient or when the enzyme load exceeds the capacity of the antiproteinases to protect the tissues, proteolytic lung destruction ensues.

The Neutrophil

α₁-AT is a serum inhibitor of serine proteinases, although it also provides an effective antiproteinase screen in the alveolar region² and, by inference, in the lung interstitium. Studies in vivo have shown that two serine proteinases, elastase and proteinase-3, which are released by neutrophils,³,⁴ can induce in animals pathologic changes that resemble human emphysema. Furthermore, neutrophil sequestration in the pulmonary circulation also will lead to the development of emphysema in dogs.⁵ These studies, therefore, confirm that the neutrophil and its proteinases have the potential to produce human emphysema.

More recently, the neutrophil also has been implicated in other manifestations of COPD since experimental application of neutrophil elastase (NE) can reproduce many of the features of patients with this syndrome. For instance, the aerosol administration of NE to guinea pigs within 20 min of contact.⁶ The damage and subsequent repair is consistent with the loss of ciliated epithelium and squamous metaplasia seen in the major bronchi in human patients with COPD. Furthermore, NE reduces the ciliary beat frequency of the human respiratory epithelium in vitro,⁷ which is consistent with the reduced mucociliary clearance that is seen in COPD patients.⁸ The instillation of NE into the airways leads to mucous gland hyperplasia in hamsters,⁹ and the enzyme is a major mucus secretagogue in bovine airway serous gland cells.¹⁰ Such observations suggest important roles for NE in the bronchitis pathology and mucus expectoration of some COPD patients. Indeed, clinical studies have shown a direct relationship between the volume of sputum expectorated daily and the NE content of the secretions (Fig 1). Finally, NE can impair many other important host defenses that may, in part, be responsible for bacterial colonization in a proportion of patients. Despite these theoretical mechanisms, however, only a few patients have α₁-AT deficiency, and, thus, it has been difficult to explain why the lung is not normally protected in the majority of COPD patients.

Neutrophil Differentiation and Migration

The neutrophil originates in the bone marrow where it differentiates over a period of 7 to 10 days from promyeloblast to a mature cell. During this period, the neutrophil manufactures its full complement of NE and proteinase-3, and stores the enzymes within the primary or azurophil granules. The enzyme genes are “switched on” early during differentiation at the promyelocyte stage and are “switched off” at the myelocyte stage.¹¹ Thereafter, there is no further production of these enzymes. The mature neutrophil is released into the circulation where it has a short half-life during which it is either recruited to sites of inflammation or it becomes senescent and is cleared. The recruitment of neutrophils is a complex sequence of events that relates to the release of various chemokines, small chemoattractant proteins, at relevant sites. These chemokines stimulate the up-regulation of vascular adhesion molecules, the local arrest of neutrophils as they become adherent to the vascular endothelium, and cell migration into the tissues. This process of migration is associated with neutrophil activation, which leads to the mobilization of the azurophil granules and to their exocytosis with the release of enzyme content, some of which becomes adherent to the neutrophil cell membrane.¹²

Neutrophils in COPD

The neutrophil is a short-lived and transient cell. In the lung it is usually (in the absence of pneumonia or interstitial lung disease) recruited from the circulation to the airways. Its passage through the interstitial space is a rapid event, and neutrophils are usually found in the circulation or the airways. Nevertheless, pathology studies¹³ have identified an increased number of neutrophils in the bronchial tissue of some patients with COPD and have shown that this relates to the severity of airflow obstruction. Furthermore, neutrophils are increased in the airways of smokers¹⁴ and patients with COPD,¹⁵ especially those with chronic bronchitis (Fig 2).

*From the Lung Resource Center, Queen Elizabeth Hospital, Edgbaston, Birmingham, UK.
Correspondence to: R. A. Stockley, MD, DSc, Lung Resource Center, Queen Elizabeth Hospital, Edgbaston, Birmingham, B15 2TH, UK; e-mail: r.a.stockley@bham.ac.uk

www.chestjournal.org
The factors influencing neutrophil migration into the airways are now becoming clear. Studies have shown that interleukin (IL)-8, a CXC chemokine, is detectable in BAL fluid from current smokers. Overall, the concentrations of this chemoattractant are similar to those seen in nonsmokers. However, in a small subpopulation, the concentration of IL-8 in BAL fluid was increased, and this was associated with increased chemotactic activity. The increase in IL-8 in this small subgroup of smokers may explain the susceptibility of some individuals to the development of emphysema. In addition, studies in \( \alpha_1 \)-AT deficiency have suggested that the major chemoattractant in the peripheral airways of these patients is leukotriene B4. In patients with \( \alpha_1 \)-AT deficiency, the mechanism thought to be important is the failure to inhibit NE that is released within the airway. Studies have shown that NE activity can stimulate macrophages to release LTB4, which leads to an amplification of neutrophil recruitment that is believed to be the cause of the development of early emphysema in these patients. Furthermore, recent preliminary studies indicate that IL-8 levels also are increased in the BAL fluid samples obtained from patients with \( \alpha_1 \)-AT deficiency early in the development of the disease. This suggests that more than one chemoattractant may be involved in neutrophil migration in patients with \( \alpha_1 \)-AT deficiency.

Secretions obtained more proximally in the bronchial tree also have been shown to contain LTB4 and IL-8, and, moreover, secretions from this site also have higher concentrations of LTB4 in patients with \( \alpha_1 \)-AT deficiency than in patients with COPD that is not due to \( \alpha_1 \)-AT deficiency. This suggests further that a failure to inhibit bronchial NE activity leads to stimulation of the release of macrophage LTB4, which may be a pivotal mediator of neutrophil migration in vivo (Fig 3).

The contribution of these chemoattractants to cell migration remains uncertain. There is no doubt that airway secretions are chemotactic, but few studies have been carried out to identify the contributions of individual chemoattractant components. Mikami et al. determined that there was a significant contribution of both chemotactants to the overall chemotactic activity of secretions in sputum from patients with bronchiectasis. However, the data showed that some of this chemotactic activity could not be accounted for solely by IL-8 and LTB4. The nature of the remaining chemoattractant activity remains to be determined.

**Figure 1.** The active concentration of NE using a synthetic substrate, maapvpna, is shown on the horizontal axis for individual sputum samples from patients with bronchiectasis. The results are plotted against the sputum volume produced (vertical axis). The correlation coefficient of the relationship is shown (\( p < 0.002 \)).

**Figure 2.** The proportion of neutrophils identified in induced sputum samples is shown for healthy control subjects and subjects with COPD. In addition, the proportion of neutrophils is shown for COPD patients who have chronic cough and sputum expectoration (bronchitis).

**Figure 3.** The pathogenic process hypothesized by Hubbard et al. to explain the amplification of neutrophil recruitment in patients with \( \alpha_1 \)-AT deficiency. The failure to inhibit elastase in the airway because of the deficiency of \( \alpha_1 \)-AT enables the enzyme to activate macrophages to release the neutrophil chemoattractant LTB4. This in turn recruits more neutrophils, releasing further elastase into the airway, thereby facilitating an amplification circle.
Susceptibility

Only a proportion of smokers develop significant airflow obstruction, suggesting an interaction between genetic and environmental factors. It is clearly possible to explain the development of emphysema in patients with α₁-AT deficiency because of a defective protective antiproteinase screen within the lung. However, as intimated previously, the majority of patients with COPD and emphysema appear to have normal circulating concentrations, and therefore lung concentrations of, α₁-AT. Previous studies²³ have suggested that the inactivation of α₁-AT within the lung secretions, possibly by oxidation of its active site by oxidants in cigarette smoke, may explain the development of proteinase-mediated lung damage. However, this concept remains unable to explain susceptibility because the inactivation of α₁-AT by this mechanism is a simple biochemical process and, therefore, would be expected to occur in all smokers.

Studies have helped to explain how neutrophils cause connective tissue damage and, potentially, all the elastase-mediated pathologic changes. The NE is stored within neutrophil azurophil granules, where its concentration has been estimated at approximately 5 mM. Following neutrophil activation, the granules undergo exocytosis, and the enzyme diffuses away from the granule, its concentration falling as it does so. Inhibitors such as α₁-AT and secretory leukoproteinase inhibitor inactivate NE on a 1:1 mol/L basis. The concentrations of these inhibitors within the lung interstitium are unknown, however, α₁-AT, for instance, is a freely diffusible molecule, and the serum protein albumin (which is the same molecular size as α₁-AT) is thought to be present in the interstitium at approximately 80% of the concentration in the serum.²⁵ Since the serum concentration of α₁-AT in healthy subjects is approximately 30 μM, the predicted concentration in the interstitium should be approximately 24 μM, which is some 200 times lower than the concentration of elastase in the azurophil granule. Thus, NE has to diffuse away from the granule until the concentration has fallen sufficiently for the enzyme to be completely inactivated by the local concentration of α₁-AT. The relationship between the concentration of elastase and the distance away from the azurophil granule has been predicted to be exponential.²⁶ There is a rapid fall in concentration until it reaches approximately 11 μM, and, thereafter, the concentration falls more slowly.

This theoretical relationship may explain not only the tissue destruction that occurs in the absence of α₁-AT deficiency, but also the extensive destruction seen in deficient subjects. First, the release of NE in such high concentrations means that some tissue destruction will always occur at the site of release. Indeed, this may well be an important factor in facilitating cell migration through the tight connective tissue matrix. Normal concentrations of α₁-AT restrict the area of damage, whereas the serum concentration is 5 μM in patients with α₁-AT deficiency (by inference, the interstitial concentration would be 4 μM), a level that is below the threshold on the exponential curve of NE concentration to distance diffused away from the azurophil granule (Fig 4).

It can be predicted, therefore, that in subjects who do not have α₁-AT deficiency a degree of tissue damage will always occur in the immediate vicinity of a degranulating neutrophil. When the α₁-AT concentrations are significantly reduced (as in patients with α₁-AT deficiency), the area of damage that will occur can be predicted to be far greater. This would explain not only the increased susceptibility of patients with α₁-AT deficiency to tissue damage, but it also provides an explanation for the development of similar but limited damage in non-α₁-AT-deficient subjects. Thus, it is likely that the factors of key importance in

![Figure 4: Diagrammatic representation of the mechanism involved in NE release from a neutrophil azurophil granule. Once the granule undergoes exocytosis from the neutrophil, elastase diffuses away. The high intrinsic concentration rapidly decreases initially and subsequently decreases more slowly. The individual arrows on the figure indicate representative concentrations at given distances from the granule. Note: in patients with normal levels of α₁-AT, the enzyme activity would be completely blocked at a distance away from the granule when the concentration falls to < 30 μM. On the other hand, in patients with α₁-AT deficiency, the enzyme activity would not be inhibited until it is diffused far enough away for the concentration to fall to 5 μM.](http://journal.publications.chestnet.org/pdfaccess.ashx?url=/data/journals/chest/21978/ on 06/26/2017)
the susceptibility of individuals to the development of the pathologic changes of COPD in cases of emphysema are the size of the neutrophil traffic and the period of time over which it occurs.

With this concept in mind, there are several factors that have been identified that could influence the process. First, as indicated earlier, McCrea and colleagues demonstrated that the lung concentration of IL-8 was increased above the normal range in a small number of healthy smokers. It would, therefore, be predicted that in these individuals a greater amount of neutrophil traffic would occur. The mechanisms have yet to be elucidated, but studies have indicated that cigarette smoke can lead to the release of IL-8 from airway epithelial cells, and it is possible that, in the susceptible individual, an increased response to such a stimulus takes place.

An alternative explanation has been suggested by studies from Burnett et al who demonstrated that neutrophils from patients with chronic bronchitis and airflow obstruction were more sensitive to chemotactic stimuli and, when activated, had greater potential to cause connective tissue damage. If this phenomenon is a primary event, the release of normal concentrations of chemotactic neutrophils in the lungs of such individuals can be postulated to lead to an amplified neutrophil response and to more extensive tissue damage, which could explain their susceptibility to the development of COPD. The exact mechanisms of the neutrophil response have yet to be determined, although subsequent studies by the same group have indicated that the neutrophils overexpressed cell surface chemokine receptors.

Understanding the mechanisms that lead to increased susceptibility should permit the development of new therapeutic strategies. It may be possible to down-regulate the chemotactants if they are being overexpressed or, alternatively, to deactivate or desensitize the neutrophil to normal chemotactants.

**ROLE OF NEUTROPHILS IN EXACERBATIONS**

Exacerbations of COPD are episodes in which the patient’s symptoms worsen for several days, requiring therapeutic intervention. Although neutrophils respond to bacterial infections in all tissues, and represent an important component of secondary host defenses, their role during such episodes is uncertain. Some exacerbations of COPD are undoubtedly related to bacteria, and careful controlled studies of antibiotic therapy have indicated an advantage to such interventions, confirming that bacteria are responsible for some exacerbations. However, the role of bacterial infection in exacerbations of COPD remains controversial. This relates in part to observations that bacteria may be cultured from the airway secretions of patients with COPD even in a clinically stable condition and, in addition, that many patients studied during exacerbations do not produce positive bacterial cultures. The possibility that bacteria may not be responsible for all exacerbations is supported by the observation that therapy with corticosteroids or antioxidants is effective in some such episodes.

Studies, however, indicate that colonization of the airways per se is not a major stimulus to neutrophil recruitment. Indeed, because of effective local host defense, it is possible to retain sterility of the airways and to ensure that bacterial numbers remain low. There is, however, a clear relationship between the size of the colonizing microbial load in the airway and the neutrophilic response. Furthermore, once the neutrophilic response has occurred, there is an obvious change in the sputum, in that its color changes from clear or white to yellow or green, and this is related directly to the myeloperoxidase released from the recruited neutrophils. Indeed, if samples from patients are assessed by their purulent nature alone, the incidence of a positive bacterial culture rises dramatically to 80 to 100%.

During these exacerbations related to bacterial infection, there is an increase in the concentrations of chemotactic neutrophils. In mild episodes, it is likely that LTB4 is the major neutrophil chemoattractant. However, as an exacerbation increases in severity, there is also a dramatic increase in IL-8 levels. The recruitment of neutrophils during these episodes results in an increased level of free elastase activity in the airways, and this has the potential to cause the manifestations of bronchial damage that were discussed previously. This concept has been confirmed, in that exacerbations of COPD associated with clear or mucoid sputum are not associated with a change in bacteriology or neutrophil recruitment, whereas exacerbations that are clearly associated with increased purulence are associated with an increased incidence of bacterial isolation, and a two- to three-order of magnitude increase in the numbers of bacteria.

Isolated as well as recurrent exacerbations may be associated with a deterioration in health status. At present, however, it is unknown whether the exacerbations associated with neutrophil recruitment alone are responsible, or whether declining health status is a feature of all exacerbations. However, of more importance, neutrophilic exacerbations are associated with increased free elastase activity and, hence, with the potential for further bronchial damage. It remains possible that recurrent purulent exacerbations may be responsible for the progression of lung disease, and this possibility is worthy of further study.

**Summary**

In conclusion, the neutrophil can play a central role in many of the features of COPD. The neutrophil contains the only cell products that have been shown directly to cause all of the pathologic features of COPD. It is likely, therefore, that even in the absence of α1-AT deficiency, the size and extent of neutrophil traffic is of major pathogenic importance. Understanding the mechanisms involved should lead to the design of appropriate therapeutic strategies. The site of neutrophil recruitment may determine the individual pathologic features of COPD.

**References**


