Macrophages and the Pathogenesis of COPD*

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Macrophages are long-lived effector cells within the lung. They are reactive, responding to endogenous and exogenous stimuli, as well as proactive, producing mediators that modulate the behavior of surrounding cells. In addition, they play a critical role in the clearance of apoptotic neutrophils. Their role in COPD probably reflects a number of functional properties. However, if the link between increased proteinase burden and tissue destruction and injury in patients with COPD is correct, then macrophages must be very significant. Even though other cells, including epithelial cells and fibroblasts, have been shown to express higher matrix metalloproteinase (MMP) levels in lung tissue from subjects with COPD and emphysema, the numbers of resident cells do not appear to increase by the same factor as that of sequestered macrophages. The combination of a 5- to 10-fold increase in macrophage numbers, the up-regulation of MMPs, and their corelease with other classes of stored proteinases must be highly significant in terms of an increase in proteinase potential in the small airways and respiratory units. This may account for increased tissue destruction and inflammatory mediator activation leading to the pathology that occurs during COPD. Since only about 15% of smokers develop clinically significant disease, it seems likely, in smokers without COPD, that these processes either are strictly controlled or that lung repair mechanisms are more effective.

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Abbreviation: MMP = matrix metalloproteinase

An early response to inhaled toxins, organic material, and inorganic material is the recruitment of macrophages to the lung, where they undergo phagocytosis and, if possible, destroy unwanted irritants. Examination of the lungs of young smokers has revealed an accumulation of macrophages in the bronchiolar region and bronchiolitis.1,2 Phagocytosis of cigarette smoke-derived particles is obviously an important defense mechanism with which to neutralize and clear the toxic particulate fraction. Particle-laden macrophages would be expected to exit the lung via the mucociliary escalator or lymphatic system, but there is little evidence in smokers that macrophage clearance matches pulmonary macrophage accumulation, partly reflecting the continuous elevated sequestration triggered by the effects of cigarette smoke. Macrophages in BAL fluid from the lungs of smokers and those patients with COPD are elevated many fold. They are phenotypically heterogeneous, a proportion having normal appearances, while others are enlarged and/or are full of engulfed particles. Yet another subset of macrophages in lung lavage appear smaller than the normal pulmonary macrophage and may be immature, perhaps due to the premature release of monocyte precursors from the bone marrow and homing to the lungs in response to cigarette smoke. Macrophages have a long tissue life span, up to many months under normal circumstances. This life span increases in smokers, and some studies have suggested that pulmonary macrophages may exist for more than 2 years before clearance or cell death. There are a number of possible explanations for this. For example, mucociliary clearance is known to be less effective in smokers. Alternatively, lymphatic clearance may be reduced due to tissue injury and/or loss of lung lymph structure, or the pulmonary lymphatic system may simply be unable to deal with the increased pulmonary influx.

Macrophages and COPD

Whatever the situation, macrophages are elevated in the lungs of smokers and those patients with COPD, where they accumulate in the alveoli, bronchioli, and small airways. Furthermore, there is a positive association between macrophage numbers in the alveolar walls and the presence of mild-to-moderate emphysema as well as the degree of small airways disease in patients with COPD.1,2 The slow progression and chronicity of COPD parallels the chronic increase of macrophages that is seen at sites of tissue damage/injury and supports the concept that macrophages are, at least in part, responsible for the pathologic consequences. In addition to the clearance of deposited debris, they have many other diverse roles in maintaining pulmonary homeostasis, including antigen presentation, and in the production of multiple mediators such as cytokines and growth factors. Despite the colocation of macrophages at sites of pathology, the role of the macrophage in COPD is hotly debated, particularly in the face of the early clinical and scientific studies, which implicate the neutrophil.

Macrophages and Neutrophils in the Pathogenesis of COPD

Historically, the neutrophil and neutrophil proteinases have been thought to play a major role in the development of emphysema, based on the relationship between α1-antitrypsin deficiency and the predisposition of those affected to develop the disease.3 α1-Antitrypsin (ie, α1-proteinase inhibitor) inhibits neutrophil serine proteinases, especially elastase, and, in turn, neutrophil-derived serine proteinases cleave elastin, the disruption of which may be a significant feature of emphysema. Furthermore, the intratracheal instillation of neutrophil elastase into the lungs induces emphysema in experimental animals.4 Neutrophils store high levels of serine proteinases (ie, neutrophil elastase, cathepsin G, and proteinase-3) in primary
azurophilic granules. In addition, they store at least two matrix metalloproteinases (MMPs) in secondary and tertiary granules, neutrophil collagenase (MMP8), and gelatinase B (MMP9). All these enzymes work optimally at neutral pH and, in combination, can degrade most components of the extracellular matrix. They are released on neutrophil activation, rather than synthesized as required, and as such release is rapid and unregulated. In addition, unlike lung macrophages, neutrophils have a short tissue life span of 1 to 2 days, and any long-term effect of neutrophils therefore might involve continuous neutrophil recruitment, most likely in response to local lung mediator production, and/or a prolonged life span, possibly as a result of reduced apoptosis or inadequate clearance by macrophages.

Neutrophil numbers increase both proportionally (from 30 to 70% of total cells) and in total (> 10-fold higher) in sputum and induced sputum from COPD subjects. Furthermore, the levels of neutrophil chemoattractants (eg, interleukin-8) and of neutrophils and their products in sputum correlate with reduced lung function in COPD subjects. Thus, neutrophils may mediate some of the pathologic responses in the conducting airways of COPD subjects, particularly during exacerbations. Circulating neutrophils from subjects with emphysema are primed, and microvasculature sequestration of neutrophils increases acutely during an exacerbation of COPD and when smoking cigarettes, partly due to reduced deformability. However, in the peripheral lung, the sequestration of neutrophils (when not smoking cigarettes) and neutrophil numbers are inversely related to the severity of emphysema. The studies have suggested that the neutrophil is a minor player in the later stages of emphysema, although it is possible that neutrophil accumulation in the peripheral lung may be an important acute early event in smokers who go on to develop emphysema and COPD. It seems likely that other cell types contribute to the pathology of COPD, especially emphysema and small airways disease.

**Proteinases in COPD**

If the proteinase hypothesis for the development of emphysema is true, one might hypothesize that the macrophage is responsible. For > 20 years, studies have demonstrated the release of metalloproteinase activity by macrophages, although the lack of adequate technology long delayed a full understanding of the exact nature of this activity and its contribution to disease processes. More recently, however, a class of proteinases, the MMPs, has been described that accounts for some of the observations. The MMPs, a large, growing family of structurally related Zn\(^{2+}\)-dependent and Ca\(^{2+}\)-dependent proteinases, degrade extracellular matrix proteins. When they are released into the extracellular environment, or when they are membrane-bound and in contact with the pericellular zone, these enzymes degrade a broad range of connective tissue proteins and are important during the development of healthy tissue as well as in remodeling damaged tissue. Unlike neutrophil proteinases, which are rapidly released from the storage granules on activation, the synthesis and secretion of macrophage MMPs are strictly regulated in response to the cellular environment, where their expression is modified by factors such as cytokines, endotoxin, phagocytosis, and growth factors. Once released, MMPs can modulate the activity of cytokines, for example by the proteolytic activation of transforming growth factor-\(\beta\) and membrane-bound tumor necrosis factor-\(\alpha\) and by the degradative inactivation of interleukin-1\(\beta\). MMPs are usually secreted as latent, inactive proenzymes, which need to be activated (Fig 1), and/or are bound to their native inhibitors, the tissue inhibitors of metalloproteinases. Activation may involve proteolysis and/or oxidation of the latent enzyme. Proteolytic activation of the proenzymes cleaves the propeptide sequence, which masks the active site of the latent enzyme. A variety of proteinases may be responsible, including other MMPs, urokinase, plasmin, neutrophil elastase, and cathepsin G. Some MMPs are

![Diagram](https://example.com/diagram.png)

**Figure 1.** Top, A: inactivation of serine protease inhibitors, such as \(\alpha_1\)-antitrypsin, by MMPs, leading to increased levels of uninhibited serine proteinases. Bottom, B: activation of latent MMPs and inactivation of tissue inhibitors of MMPs (TIMPs), leading to elevated MMP activity. This in turn enables the autoactivation of MMPs. PMN = polymorphonuclear neutrophil.
believed to be optimally functional pericellularly; thus, some membrane-bound MMPs (eg, MMP14) can facilitate this by the pericellular activation of secreted MMPs (eg, the activation of MMP2). Once activation takes place, some MMPs undergo autoactivation and also can inactivate inhibitors of other types of proteinases (eg, α1-antitrypsin; Fig 1). This can lead to a cascade of proteinase activation, especially where more than one class of uninhibited proteinase is present. Thus, in addition to the rigid control of cellular MMP production by factors in the surrounding environment, there can be a complex sequence of events to ensure that, once released, MMPs are activated at an appropriate time, in the right compartment of the tissue and to maximum effect. In addition to degrading the extracellular matrix, the proteolytic modification of the activity of other mediators, some of which are membrane-bound, will modulate, indirectly, the inflammatory process.

Evidence is emerging that supports the roles of elevated macrophage numbers and MMPs in the etiology of COPD. There are a number of animal models that illustrate the potential significance of macrophages in the development of emphysema. For example, in guinea pigs that were exposed to cigarette smoke for up to 8 weeks, emphysematous changes were associated with a loss of collagen and increased collagenase messenger RNA expression, which was particularly evident in the macrophages. A subsequent study also supported the role of the macrophage and their metalloproteinases in emphysema. Thus, mice that are genetically deficient in MMP12 did not exhibit macrophage infiltration of the lungs following exposure to cigarette smoke. In addition, the MMP12-deficient animals, in contrast to wild-type controls that were exposed to cigarette smoke, did not develop emphysema.

In humans, a comparison of proteinase production in vitro by macrophages that were isolated from BAL fluid from subjects with emphysema and from healthy volunteers showed elevated messenger RNA expression of MMP1 (ie, interstitial collagenase 1) and MMP9 (gelatinase B) in emphysema. There was also increased secretion into the culture media of active collagenase and MMP9; much of the MMP9 appeared to be of neutrophil origin and, thus, internalized by macrophages. In this study, there was no change in MMP2 (gelatinase A) production. However, another investigation using the immunolocalization of MMP1, MMP2, MMP9, and MMP14 (MMP14 is a membrane-bound MMP) showed elevated levels of MMP2 and MMP14 in macrophages, as well as in the type II cells and fibroblasts, in emphysematous, compared to nonemphysematous, tissue. Since membrane-bound MMP14 activates MMP2, this suggests the presence of a coordinated expression to enhance pericellular MMP2 activity. A similar approach, using tissue from subjects with COPD, immunolocalized MMP2 and MMP9, and MMPs 1, 8, and 13 (ie, interstitial collagenases 1, 2, and 3), and it illustrated increased MMP1 and MMP2 levels in luminal and interstitial macrophages, and also in bronchial epithelial cells when compared to healthy tissue. More recently, macrophages and alveolar type II cells in emphysematous tissue have been demonstrated to contain MMP1. Thus, these studies illustrate elevated levels of MMP1, MMP2, MMP9, and MMP14 in macrophages from subjects with emphysema and/or COPD. Although elevated MMP12 has been described to be present in macrophages present in specimens from smokers' lungs, the results of the investigation of MMP12 in the studies that were described above were negative. Other types of MMPs, such as MMP12, may be important at an earlier stage of the disease process.

Variations between studies in the presence or level of specific MMPs could be due to a number of factors, including different types of experimental analysis (eg, isolated cell culture, tissue sections, messenger RNA, or protein levels/location), or could be the result of differences between patients (eg, the presence of early or late disease, current smoker or ex-smoker status, and the presence of emphysema or small airways disease). Regardless, these studies all clearly show that elevated macrophage-derived MMPs could contribute to the pathogenesis of COPD. In combination, these MMPs degrade collagen and elastin, the major components of the extracellular matrix, thereby implicating the macrophage as a major contributor to excessive connective tissue breakdown and tissue injury in COPD.

As mentioned earlier, elastinolysis is thought to be an essential component of emphysema. This is supported by both pathophysiologic and biochemical investigations, which show reduced lung elastic recoil, disrupted elastic tissue structure, higher circulating levels of elastin peptide breakdown products, and higher levels of desmosine in urine and lung secretions. Of the macrophage-derived MMPs identified in emphysematous and COPD tissue, the gelatinases MMP2 and MMP9 are elastinolytic. In addition to MMPs, macrophages release elastinolytic cysteine proteinases (ie, cathepsins K, L, and S), which are also capable of degrading elastin. Indeed, at an acid pH, these cathepsins are more effective in degrading elastin than is neutrophil elastase. Cathepsin K is particularly potent and, as cathepsins may be released into an acidic pocket at the cell membrane-extracellular matrix interface via endosomal and lysosomal mechanisms, these enzymes may be important contributors to elevated proteinase activities in COPD. Furthermore, cathepsin S is very effective at neutral pH. Studies in vitro suggest that the pericellular release of cathepsins K, L, and S results in elastin degradation. The cathepsins also degrade collagen and gelatin. Although such cathepsin activity may be more important in terms of the macrophage clearance of connective tissue debris by internalization and phagocytosis, it is possible that these enzymes also have an extracellular role and contribute to tissue remodeling.

Alveolar macrophages have been shown to release neutrophil elastase over a number of days in vitro. Although they do not synthesize neutrophil elastase, macrophages can internalize the enzyme by receptor-mediated processes. Macrophages, therefore, may represent a reservoir for neutrophil elastase in vitro. The coreleasing of neutrophil elastase with inactive MMPs may be particularly significant in the extracellular compartment, where neutrophil elastase could activate MMPs and trigger an inappropriate cascade of proteinase activity, thereby precipitating injury and triggering abnormal re-
pair processes. Thus, the macrophage has enormous proteolytic potential, spanning many classes of enzymes with broad ranges of substrate specificity. The secretion of proteinases by other inflammatory cells, as well as resident structural cells into the same extracellular environment may provide a potent proteinase cocktail that overwhelms the native inhibitors, particularly in pockets of concentrated enzyme release. If such proteinase activity remains unopposed, it is likely that the connective tissue matrix is continually abraded in the vicinity of these inflammatory cells.

Extracellular proteinases of known macrophage origin have been difficult to identify at high levels in lung secretions from subjects with COPD and/or emphysema. Although elevated levels of MMP9 in these samples may be both neutrophil-derived and macrophage-derived, evidence favors neutrophil MMP9. Indeed, neutrophil proteinases are consistently detected at high levels in such secretions, possibly reflecting their rapid release once the luminal neutrophil is stimulated. Perhaps the lung lumen is one of the primary sites of action of neutrophil proteinases, either as proinflammatory or antimicrobial agents. Alternatively, there may be inadequate clearance of neutrophils by macrophage apoptosis and the release of neutrophil proteinases during necrotic neutrophil death. In contrast, the release and activity of macrophage proteinases are likely to be rigidly controlled in the local milieu of the macrophage or adjacent cells, and in the lumen of the lung, in the absence of connective tissue substrates, they may be more important in the proteolytic regulation of the activity of inflammatory mediators and growth factors at the apical epithelial surface. In addition, since macrophages at this site internalize and degrade foreign material (i.e., cellular debris and apoptotic neutrophils), it is logical for intracellular proteinases to be preserved.

Of particular relevance in COPD, macrophage numbers are elevated within the walls of the lung in areas of known pathology. Some of these cells contain particles and are presumably in transit from the airway lumen to the lymphatic system. Others may not have migrated as far as the airspaces. It is unclear whether these cells have been sequestered to facilitate lung repair or whether the normal transmigration process has failed. These cells will be in close contact with lung connective tissue and under the influence of mediators released by surrounding cells. Such factors could stimulate macrophage MMP production, which in turn could result in the degradation of extracellular matrix and MMP activation of proinflammatory cytokines and growth factors, thus perpetuating the process. Although it is tempting to suggest that alveolar macrophages from smokers also regulate pulmonary inflammation by the production of elevated levels of proinflammatory cytokines, the data show that cytokine and chemokine production by BAL fluid-derived macrophages is lower. Low cytokine production by smokers’ macrophages may be due to the presence of a subpopulation of “burnt out” macrophages. There is, however, no evidence of a subpopulation of macrophages that contain lower MMP levels, suggesting that the up-regulation of macrophage MMPs in COPD is a significant long-lasting component of the disease process. This is particularly important in the context of the longevity of the macrophage in the lung, and the chronic nature and slow progression of emphysema and COPD.

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