



Defective Phagocytosis in Airways Disease

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Maintaining an airway clear of inhaled particles, pathogens, and cellular debris is paramount for lung homeostasis. In healthy individuals, the phagocytes of the innate immune system act as sentinels to patrol the airway and ensure sterility. However, in airways diseases, including asthma, COPD, and cystic fibrosis, there is a propensity for bacterial colonization that may contribute to disease worsening. Evidence suggests that this may be due to dysfunctional phagocytosis. In patients with COPD, phagocytosis of several bacterial species and removal of apoptotic cells (efferocytosis) by alveolar macrophages are significantly reduced; however, these cells can remove inert beads normally. Attenuated phagocytosis is also apparent in monocyte-derived macrophages from the same patients, suggesting an inherent defect in these cells. Reduced expression of cell surface recognition receptors has been suggested as one mechanism for these observations; however, the literature is currently contradictory and requires further clarification. In cystic fibrosis, a similar defect is also observed in both airway neutrophils and macrophages, leading to ineffective bacterial uptake and subsequent killing. In asthma and other airways diseases, there are also reports of defective phagocytosis of bacterial pathogens, although the relevance to disease pathophysiology is not understood. Oxidative stress is emerging as a common mechanism that may be altering both macrophage and neutrophil functions that can be reversed by various antioxidant strategies. The identification of this and other mechanisms underlying phagocyte dysfunction may present novel therapeutic opportunities for the treatment of many of these intractable diseases and improve patient morbidity and mortality.

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Abbreviations: CD = cluster of differentiation; CF = cystic fibrosis; CR = complement receptor; HLA-DR = human leukocyte antigen-DR; MARCO = macrophage receptor with collagenous structure; SR = scavenger receptor; TLR = toll-like receptor

Clearance of inhaled particles and bacteria is of utmost importance in maintaining the sterility of the lung. This is achieved through a number of mechanisms, including mucociliary clearance and secretion of antimicrobial proteins from epithelial cells and leukocytes in the airways.^{1–3} Other essential mechanisms include phagocytosis of particulate matter and invading organisms, with the macrophage being the most prominent phagocyte in the lung,⁴ although,

during a specific insult or infection, neutrophils are also recruited rapidly and contribute to this clearance process.⁵ Neutrophils are released by the bone marrow into the circulation and are relatively short lived but will respond to specific signals that recruit them to sites of invasion or damage. Macrophages are longer-lived cells derived from circulating monocytes that migrate into lung tissue from the bloodstream and subsequently differentiate with little, if any, local proliferation.⁶ Together, these phagocytic cells act with soluble proteins that bind microbial products to maintain the sterility of the airway tract and remove detrimental invading pathogens and particles.

Bacterial infections contribute to the pathophysiology of a number of pulmonary conditions, including COPD, asthma, and cystic fibrosis (CF). There is now accumulating evidence that defects in the ability of the innate immune system to counteract bacterial pathogens in these conditions appear to be associated

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with macrophage dysfunction, leading to persistence of bacteria in the airways and resultant colonization.^{7,8} In addition, engulfment of bacteria by macrophages may be associated with increased release of inflammatory mediators via activation and recruitment of toll-like receptors (TLR) to the phagosome.⁹ Macrophages also play a significant role in the removal of apoptotic cells (efferocytosis).¹⁰ This pathway shares a number of similarities with phagocytosis, and failure of this process leads to increased necrotic material damaging the surrounding area and contributing to pathology.¹¹ There is evidence that pyocyanin released from *Pseudomonas aeruginosa* compromises macrophage efferocytosis of apoptotic neutrophils,¹² thus demonstrating that the microbial environment could further suppress phagocytic functions. Taken together, phagocyte dysfunction could be significant in the perpetuation of bacterial colonization and destruction of the lung. Thus, better understanding of pulmonary phagocytic pathways and their contribution to pathophysiology is warranted.

MECHANISMS OF PHAGOCYTOSIS

The process whereby a phagocyte recognizes and removes a foreign particle is termed “phagocytosis.” There are a number of mechanisms whereby a particle can be engulfed and removed by a phagocyte, but first the particle must be recognized as foreign. Most commonly, invading particles and organisms are coated by soluble proteins found in serum. This process, known as opsonization, usually involves complement or immunoglobulins, which are recognized by specific complement receptors (CR) such as C3b (cluster of differentiation [CD]11b) or C3bi, or, alternatively, by immunoglobulin receptors such as FcγRIII (CD16).¹³ Activation of these receptors generates cytoskeletal rearrangements, leading to particle engulfment. CR-mediated engulfment leads to “sinking” phagocytosis, in which the particle sinks into the cell membrane.^{14,15} This contrasts with immunoglobulin receptor-mediated phagocytosis, in which pseudopods extend out from the cell surface and capture the particle.¹⁴ Both processes ultimately lead to formation of a phagosome, which then fuses with lysosomes, forming a phagolysosome. Acidification of the phagolysosome occurs and the invading organism is destroyed. This process has been the most studied mechanism of phagocytosis to date.^{16,17} However, the airway is not considered a serum-rich environment; therefore, phagocytosis via nonopsonin mechanisms is thought to predominate.

Nonopsonin phagocytosis can occur via a plethora of scavenger receptors (SRs); however, the major SR for alveolar macrophages has been identified as macrophage receptor with collagenous structure (MARCO).^{18,19} Other SRs implicated in the clear-

ance of particles by alveolar macrophages include the mannose receptor (CD206), CD136, CD163, and CD36.^{20,21} However, a number of other recognition molecules also contribute and are required to drive the phagocytic process, including TLRs and integrins.²² The precise signaling processes, driving internalization of particles following recognition by nonopsonin receptors occurs, have not yet been determined.

PHAGOCYTOSIS IN AIRWAYS DISEASE

The persistence of bacteria in the airways of patients with pulmonary diseases suggests a failure to remove the invading organism. The prevalence of specific bacterial species in airways diseases suggests some selectivity in these responses. For example, patients with COPD are most commonly colonized with *Haemophilus influenzae*, *Streptococcus pneumoniae*, and *Moxarella catarrhalis*,²³ whereas patients with CF are often colonized with *Pseudomonas aeruginosa*.²⁴ The role of bacteria in asthma is not as clear; however, *Mycoplasma pneumoniae* and *Chlamydia pneumoniae* have both been reported to be associated with severe asthma and with asthma exacerbations.²⁵ In some instances, colonization is due to the inherent defense mechanisms of the bacterial species. For example, *Mycobacterium* species avoid removal and killing. In particular *Mycobacterium tuberculosis* avoids destruction of intracellular pathogens by subverting phagosome maturation and fusion with the lysosome.²⁶ This enables the bacteria to survive within the macrophage and continue to colonize the respiratory tract. Although such mechanisms may explain the propensity of certain species to colonize the airway, there is now emerging evidence that innate immune mechanisms, including those mediating phagocytosis and efferocytosis, are failing. This allows persistent bacterial colonization and accumulation of necrotic material, increasing exacerbation frequency and contributing to underlying inflammatory processes and pathophysiology.

COPD

Approximately one-half of COPD exacerbations are associated with bacterial infection with *H influenzae*, *S pneumoniae*, *M catarrhalis*, *H parainfluenzae*, and *P aeruginosa* reported to be present in sputum samples obtained from patients with COPD.²³ However, more recent microbiome studies have implicated the presence of a number of other bacterial species,²⁷ although their contribution to exacerbations and disease progression is unclear. The numbers of alveolar macrophages in the lungs of patients with COPD are increased at least 20 fold compared with healthy subjects.²⁸ Similarly, airway neutrophilia is a key

characteristic of COPD,²⁹ yet despite the presence of phagocytes, bacteria persist within the airways. This suggests that the ability of phagocytes from patients with COPD to remove bacterial pathogens is attenuated.

Early studies examining the phagocytic capacity of circulating neutrophils and monocytes from patients with chronic bronchitis gave conflicting results. Some showed no impairment of phagocytosis of *Candida* species by circulating phagocytes,^{30,31} whereas others reported attenuated phagocytic and killing responses of circulating phagocytes to *Candida* species³²⁻³⁵ that were substantiated by studies using alveolar macrophages from patients with COPD.³⁶⁻³⁸ Both circulating neutrophils and monocytes have also been shown to exhibit decreased phagocytic responses to *Escherichia coli*³⁹; a similar defect has been reported in alveolar macrophages from patients with COPD.⁴⁰ There are no clear mechanisms for these defects, but exposure to particulates is known to impair macrophage phagocytosis^{41,42} and, therefore, alveolar macrophages from smokers and patients with COPD could be replete, but this is unlikely to account for reduced phagocytosis in circulating cells. More recently, studies have focused on the phagocytic response of macrophages to pathogenically relevant bacterial species. Alveolar macrophages isolated from patients with COPD exhibited reduced phagocytosis of *H influenzae*.^{7,43} This effect has also been observed in monocyte-derived macrophages and using *S pneumoniae* as prey,⁴⁰ further supporting an inherent defect in phagocytic cells from patients with COPD that is not due to local differentiation or environmental exposure of macrophages in the lung. Of note, alveolar macrophages from patients with COPD have a normal capacity to phagocytose latex beads,^{11,42,43} indicating that macrophages have the capacity to phagocytose, but when confronted with bacteria their response is blunted.

Alveolar macrophages from patients with COPD also exhibit decreased efferocytosis.¹¹ These defects in the removal of pathogens and apoptotic materials are thought to be due to reduction of cell surface recognition receptors. Reduced efferocytosis by macrophages from patients with COPD and smokers correlated with distinct alterations in CD31, CD91, CD44, and CD71,⁴⁴ suggesting that cigarette smoking per se alters macrophage function. However, other studies have not seen differences in expression of CD11b (CR3a), CD14, CD58, CD80, CD7, or human leukocyte antigen-DR (HLA-DR) but have reported reduced expression of CD86 and CD11a,⁴⁵ whereas others have reported no difference in expression of CD44, CD36, CD61, CD14, CD86, or CD40 but did observe reduced expression of HLA-DR and CD80.⁴⁶ One putative mechanism has been proposed invoking oxidative stress as the driver behind these alterations of macrophage function.⁴⁷ It has been demonstrated

that exposure of the extracellular matrix to cigarette smoke extract causes modification of proteins, which, when in contact with macrophages, causes down-regulation of the receptors required for efferocytosis.⁴⁸ In a murine model, cigarette smoke also suppressed efferocytosis via activation of the RhoA-Rho kinase pathway, and this effect could be attenuated by antioxidants.⁴⁹

The mannose receptor, which may be involved in pathogen phagocytosis, has also been reported to be reduced in alveolar macrophages from patients with COPD compared with that of nonsmokers.⁵⁰ Recognition of gram-negative bacteria requires TLR4, whereas gram-positive bacteria use TLR2. At present, there are no reports of altered TLR4 expression in COPD. However, TLR2 is reduced on COPD alveolar macrophages⁵¹ but upregulated on circulating monocytes in these patients⁵²; therefore, their role remains unclear. One study has shown that increasing expression of the SR MARCO improved phagocytosis of *H influenzae*,⁵³ although decreased expression of this receptor in macrophages from patients with COPD has not been reported. However, in monocyte-derived macrophages in which defects in bacterial phagocytosis were reported, there are no differences in the number of cell surface recognition molecules,⁴⁰ indicating that more work is required to understand the discrepancies in the literature in order to understand whether failure to express specific molecules important in the recognition of foreign material is the driver of defective phagocytosis in COPD. The fact that different bacteria are recognized by different arrays of SR suggests that a defect in a single receptor is unlikely to be critical because phagocytosis of several bacterial species is defective.

Although early studies indicated similar defects in neutrophils, mostly in relation to *Candida* species,³²⁻³⁵ there has been little further investigation. Cigarette smoke extract suppresses neutrophil phagocytosis of *E coli*, thereby suggesting that there might be a common mechanism for phagocyte dysfunction in COPD.⁵⁴ Taken together, failure of phagocytes to clear bacterial pathogens and remove necrotic material from the lung in COPD could contribute to the persistence of the inflammation characteristic of this disease (Fig 1). Normalization of phagocytosis in COPD would provide a novel therapeutic pathway that could break the cycle of colonization and inflammation and halt the relentless progression of COPD.

Cystic Fibrosis

CF is characterized by various genetic mutations of the CF transmembrane regulator protein, which lead to reduced chloride ion uptake by the airway epithelium, resulting in dehydration of the periciliary liquid

layer of the airway. This contributes to reduced mucociliary transport, and together with production of mucus, provides an environment conducive for bacterial colonization.⁵⁵ Colonization of the CF airway leads to neutrophil recruitment, which contributes to the progressive deterioration of lung structure, decline in lung function, and respiratory failure in 90% of patients with CF. During infancy, the lungs of patients with CF are colonized with *H influenzae* and *Staphylococcus aureus*; however, this switches during adolescence to predominately *P aeruginosa*.²⁴ However, other pathogens are also present, including *Klebsiella* species and *Burkholderia cepacia* and fungi such as *Aspergillus* and *Candida* species,⁵⁶ all of which are associated with an increased frequency of acute exacerbations and increased morbidity.⁵⁷ Numerous mechanisms have been proposed to explain colonization of CF airways; however, macrophage phagocytosis is defective in patients with CF.⁵⁸ Phagosome acidification is significantly reduced in macrophages isolated from mice deficient in the CF transmembrane regulator gene.⁵⁹ Furthermore, glycolipids derived from *P aeruginosa* reduce phagocytosis of both *P aeruginosa* and *Saccharomyces cerevisiae* by mouse peritoneal macrophages⁸ and are associated with defective lysosome/phagosome fusion. These mechanisms suggest that persistence of infection in CF is partly due to ineffective uptake and killing of pathogens due to a defective macrophage innate response. Other studies have shown that sputum macrophages from patients with CF express reduced levels of MARCO and the mannose receptor.⁶⁰ Efferocytosis by alveolar macrophages is also impaired in CF⁶¹ and is associated by cleavage of the cell surface phosphatidylserine receptor by neutrophil elastase.⁶² This suggests that perpetuation of inflammation in the CF airway may be driven by defective macrophage clearance mechanisms.

Asthma

Bacterial colonization is not characteristic of asthma; however, stable asthmatics have a propensity to develop lower respiratory tract infections of *M pneumoniae* and *C pneumoniae*, although their contribution to disease pathophysiology is unknown.^{25,63} The role of the alveolar macrophage in asthma has been largely overlooked because numbers are similar to those found in healthy individuals. Nevertheless, there have been some studies in mild asthmatics showing reduced phagocytic responses to IgG-opsonized yeast particles, which is associated with decreased CD64 (FcγRI) expression and the presence of airway eosinophils,⁶⁴ but uptake of colloid particles and opsonized zymosan has been shown to be increased both in vivo and ex vivo in mild asthmatics compared with healthy volunteers.⁶⁵ Macrophages from children

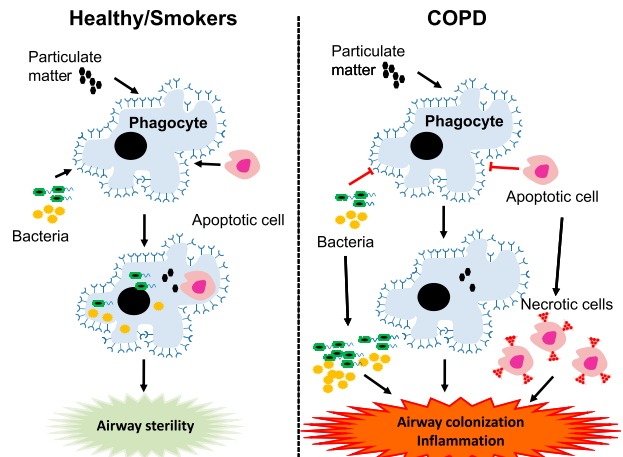


FIGURE 1. Defective phagocytosis in COPD. Phagocytes from healthy individuals and smokers without airways obstruction phagocytose particulate matter, but also apoptotic cells and bacteria in order to maintain airway homeostasis. Removal of invading organisms prevents bacterial colonization from occurring, whereas clearance of apoptotic cells ensures that any necrotic cell contents do not damage the airway. In COPD, particulate matter appears to be phagocytosed normally; however, removal of bacteria and apoptotic cells is attenuated. This allows bacterial species to propagate and colonize the airways, contributing to exacerbations. Failure to clear apoptotic cells results in increased necrotic material being released into the airway, thus contributing to damage and remodeling.

with severe asthma showed decreased phagocytic responses to *S aureus*⁶⁶ possibly due to an imbalance in glutathione homeostasis in the lung.⁶⁷ Other studies, using inhaled lipopolysaccharide, have shown reduced expression of CD11b and phagocytosis of sputum and circulating phagocytes in mild asthmatics.⁶⁸ Similarly, exposure of alveolar macrophages to rhinovirus leads to impaired phagocytosis of bacteria,⁶⁹ suggesting that both viral and bacterial infection can themselves alter macrophage activation states to prevent clearance of bacteria from the airways.

Other Airways Diseases

Defective phagocytosis has been implicated in a number of airways diseases, including chronic rhinosinusitis, in which tissue-derived macrophages were deficient in their capacity to phagocytose *S aureus*, possibly because of switching of the macrophage phenotype.⁷⁰ In patients with ARDS, circulating neutrophils show defective phagocytosis and killing of *S aureus* and *P aeruginosa*⁷¹ and this defect in both neutrophils and monocytes from patients with ARDS is associated with increased expression of CD64 (FcγRI).⁷²

COMMON MECHANISMS OF DEFECTIVE PHAGOCYTOSIS

The role of neutrophil elastase in regulating phagocyte functions has also been explored. This is of particular importance in CF, where the role of this enzyme in

governing the phagocytic response has been investigated. However, because neutrophils and neutrophil elastase levels are also elevated in COPD, the effects of this potent protease on phagocytosis are also relevant. Neutrophil elastase cleaves CR1 on the surface of neutrophils,⁷³ leading to reduced uptake of opsonized bacteria, but the enzyme also cleaves complement fragment, C3bi, on opsonized *P aeruginosa*, thus preventing uptake via CR3.⁷⁴ In phagocytes isolated from the BAL of patients with CF, there is reduced expression of CD14 and HLA-DR, which also appears to be due to the presence of neutrophil elastase.⁷⁵ Neutrophil elastase also cleaves the phosphatidylserine receptor selectively from the macrophage cell surface and hence reduces efferocytosis.⁶² Of note, pulmonary neutrophils phagocytose at a greater rate than do isolated circulating cells, but pulmonary neutrophils from patients with CF show reduced phagocytosis of zymosan opsonized with iC3b, which is not due to altered expression of CR3 but possibly to reduced priming⁷⁶ or a dysfunctional neutrophil phenotype.⁷⁷ Taken together, these studies report defective innate responses in CF that may be unrelated to the underlying genetic defect but are a consequence of the chronic inflammation and neutrophilia associated with this condition and, therefore, could also have functional consequences in other diseases with pronounced airway neutrophilia, such as COPD and severe asthma (Fig 2).

THERAPEUTIC IMPLICATIONS

Understanding why phagocytes fail to clear pathogens and apoptotic material could provide new ther-

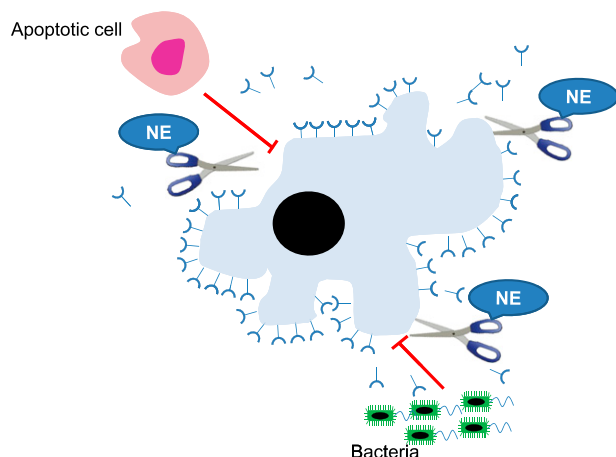


FIGURE 2. Effects of neutrophil elastase on phagocytosis. NE is abundant in the airways of patients with COPD. This enzyme cleaves cell surface complement receptors, thus reducing uptake of opsonized bacteria and also the phosphatidylserine receptor that recognizes apoptotic cells. This results in reduced clearance of bacterial species, thus allowing colonization to occur and apoptotic cells to increase necrotic material, contributing to mucous thickening and airways damage. NE = neutrophil elastase.

apeutic opportunities for the treatment of a number of pulmonary diseases, most notably COPD and CF, in which these defects are apparent. One promising mechanism has been the inhibition of oxidant stress because this appears to be a common mechanism for the suppression of recognition molecules on the cell surface. The antioxidant *N*-acetyl cysteine improved phagocytosis of *Candida* species by monocytes from patients with COPD, yet oral administration failed to improve the antifungal activity of alveolar macrophages.⁷⁸ More recently, the nuclear factor erythroid-2 related factor-2 activator sulforaphane, which increased the expression of endogenous antioxidants, rectified defective phagocytosis of *H influenzae* by COPD macrophages via upregulation of MARCO.⁵³ Similarly, procysteine improves efferocytosis by COPD macrophages,⁷⁹ strongly suggesting that the defective clearance is associated with exposure to oxidative stress. These data implicating the importance of antioxidants are supported by in vivo studies using rat models of macrophage dysfunction, in which oral administration of both procysteine or *N*-acetyl cysteine restored *S aureus* phagocytosis by alveolar macrophages.⁸⁰ Efferocytosis could be improved through inhibition of neutrophil elastase, but other possibilities have been investigated, including improvement of efferocytosis by the coenzyme A reductase inhibitor lovastatin via a RhoA-mediated mechanism.⁸¹ Of interest, the macrolide antibiotic azithromycin improved efferocytosis over 12 weeks in patients with COPD by upregulation of the mannose receptor,⁵⁰ although the precise mechanism of this finding is yet to be elucidated.

CONCLUSIONS

Many chronic lung diseases are exacerbated by infection and this, together with the persistence of necrotic material, contributes to the underlying inflammatory response and ultimately may alter the pathophysiology of the disease process, leading to increased morbidity and mortality. There is now emerging evidence that chronic inflammation may alter macrophage function and/or phenotype and that neutrophil priming may also be altered. Further investigations into these mechanisms may reveal novel therapeutic strategies to boost the innate immune system that could ameliorate many of the detrimental effects of exacerbations in chronic lung diseases such as COPD, CF, and asthma and hence improve the quality of life for patients with these debilitating conditions.

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