Efferocytosis and Lung Disease

Alexandra L. McCubbrey, BS; and Jeffrey L. Curtis, MD

In healthy individuals, billions of cells die by apoptosis each day. Clearance of these apoptotic cells, termed “efferocytosis,” must be efficient to prevent secondary necrosis and the release of proinflammatory cell contents that disrupt tissue homeostasis and potentially foster autoimmunity. During inflammation, most apoptotic cells are cleared by macrophages; the efferocytic process actively induces a macrophage phenotype that favors tissue repair and suppression of inflammation. Several chronic lung diseases, particularly airways diseases such as chronic obstructive lung disease, asthma, and cystic fibrosis, are characterized by an increased lung burden of uningested apoptotic cells. Alveolar macrophages from individuals with these chronic airways diseases have decreased efferocytosis relative to alveolar macrophages from healthy subjects. These two findings have led to the hypothesis that impaired apoptotic cell clearance may contribute causally to sustained lung inflammation and that therapies to enhance efferocytosis might be beneficial. This review of the English-language scientific literature (2006 to mid-2012) explains how such existing therapies as corticosteroids, statins, and macrolides may act in part by augmenting apoptotic cell clearance. However, efferocytosis can also impede host defenses against lung infection. Thus, determining whether novel therapies to augment efferocytosis should be developed and in whom they should be used lies at the heart of efforts to differentiate specific phenotypes within complex chronic lung diseases to provide appropriately personalized therapies.

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Abbreviations: ALI = acute lung injury; AM ϕ = alveolar macrophage; CF = cystic fibrosis; DC = dendritic cell; IPF = idiopathic pulmonary fibrosis; MAMP = microbe-associated molecular pattern; MFG-E8 = milk fat globule epidermal growth factor-VIII; M ϕ = macrophage; NKT = natural killer T; PS = phosphatidylserine; SP = surfactant protein; TAMs = a family of receptor tyrosine kinases, consisting of Tyro3, Axl, and Mertk; TNF = tumor necrosis factor; TRAIL = tumor necrosis factor-related apoptosis-inducing ligand

Ingestion of apoptotic cells, “efferocytosis,” is vital to development, homeostatic cell turnover, and tissue injury.¹ Apoptotic cells contain potential autoantigens plus alarmins such as adenosine, heat-shock proteins, and high-mobility group box-1. Release of these factors during secondary necrosis contributes to mortality in a relevant model of sepsis² and, if chronically sustained, induces autoimmunity. Apoptotic cells are engulfed by macrophages (M ϕ s), den-

dritic cells (DCs), and some types of epithelial cells. In health, uptake is so efficient that very few apoptotic cells can be detected without a coexisting efferocytic defect. Efferocytosis also actively induces an M ϕ phenotype that favors tissue repair and suppression of inflammation.³

Defective efferocytosis observed in cystic fibrosis (CF), COPD, and asthma⁴ has led to the proposal that enhancing efferocytosis pharmacologically might arrest disease progression.⁵ The goal of this article is to highlight advances published in the English language since this field was last reviewed^{4,5} and to point out

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Affiliations: From the Graduate Program in Immunology (Ms McCubbrey and Dr Curtis), and the Division of Pulmonary and Critical Care Medicine (Dr Curtis), Department of Internal Medicine, University of Michigan Health System; and the Pulmonary and Critical Care Medicine Section (Dr Curtis), VA Ann Arbor Healthcare System, Ann Arbor, MI.

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Correspondence to: Jeffrey L. Curtis, MD, Pulmonary and Critical Care Medicine Section (506/111G), VA Ann Arbor Healthcare System, 2215 Fuller Rd, Ann Arbor, MI 48105-2303; e-mail: jlc Curtis@umich.edu

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areas of continued uncertainty. We focus successively on mechanisms and immune consequences of efferocytosis, evidence for defective efferocytosis in human lung diseases and selected experimental models, and pharmacologic means to enhance efferocytosis. We discuss why improved efferocytosis may increase the risk of pneumonia and so requires thorough study before novel proefferocytic therapies can be used clinically.

MECHANISMS OF EFFEROCYTOSIS

Efferocytosis is a regulated, evolutionarily conserved process requiring phagocyte migration, adhesion, and ingestion. Apoptotic cells actively recruit mononuclear phagocytes by secreting chemotaxins and further identify themselves by shifting both surface glycoprotein composition and the basal asymmetry of their membrane lipids, displaying phosphatidylserine (PS) and other molecules.⁶ Mø and DCs bind PS either directly, using receptors such as TIM-4 and brain angiogenesis inhibitor 1, or through PS-binding bridge molecules, including complement components, milk fat globule epidermal growth factor-VIII (MFG-E8), Gas6, and protein S. The latter two serum proteins are recognized by the receptor tyrosine kinases Tyro3, Axl, and MerTK, collectively known as TAMs, which are essential for efferocytosis by mononuclear phagocytes.^{7,8} Integrins also contribute to Mø binding of apoptotic cells. Engulfment requires cytoskeletal rearrangement triggered via signaling from these recognition receptors along two partially redundant pathways³ culminating in Rac activation.

Phagocytes digest apoptotic bodies by a process that uses autophagy genes such as LC3⁹ and induces specific signal transduction. Ingested apoptotic cell products, particularly lipids, activate the liver X receptor and peroxisome proliferator-activated receptors δ and γ ,¹⁰⁻¹² creating a positive-feedback loop that upregulates efferocytic receptors and opsonins.³

IMMUNE CONSEQUENCES OF EFFEROCYTOSIS

Besides preventing release of alarmins during secondary necrosis, efferocytosis profoundly impacts phagocyte function.¹³ Efferocytosis was initially believed to be invariably antiinflammatory or tolerogenic but is now recognized to induce more complex responses. Strong evolutionary pressure has assured that efferocytosis permits maintenance of tolerance to host molecules while allowing generation of immune responses to infected apoptotic cells. How phagocytes achieve these potentially conflicting goals is not fully understood. Efferocytosis can be immunogenic when it occurs in the presence of microbe-associated molecular patterns (MAMPs), contributing to Mø cytokine

production and to DC maturation and T-cell activation.¹⁴ Host defense activity of tissue Mø can also be increased during inflammation by acquisition of neutrophil microbicidal molecules,¹⁵ which occurs in part by efferocytosis. During sepsis, however, apoptotic cells induce immune unresponsiveness,¹⁶ so it is unlikely that stimulation by MAMPs is the only determining factor.

Aspects of the apoptotic cell (type, location, and timing of detection relative to microbe clearance), the phagocyte (type and concurrent sensing of infection), and probably their ratios influence the immunologic consequence of efferocytosis.^{14,17} For example, several recent studies contest the view that failed apoptotic cell clearance is invariably proinflammatory.^{18,19} Both apoptotic and necrotic human neutrophils (but not other cell types) inhibit Mø inflammatory response through release of α -defensins; the absence of this effect in mice, in which neutrophils lack α -defensins, is an important species difference that should be considered in extrapolating murine models.¹⁸

The dominant Mø response to efferocytosis is anti-inflammatory due to release of transforming growth factor- β and PGE₂,^{3,20} activation of Mø adenosine receptors,²¹ and production by both apoptotic neutrophils and ingesting Mø of specialized proresolving lipid mediators, including resolvins and lipoxins.²² Additionally, the inhibitory intracellular proteins SOCS1, SOCS3, and Twist, upregulated by signaling through TAM receptors, dampen Toll-like receptor signaling and reduce proinflammatory cytokine release.^{23,24} Collectively, these changes accelerate resolution of inflammation following successfully combated infections. However, in a murine model, massive intrapulmonary efferocytosis led to decreased bacterial uptake and killing, increased bacterial dissemination, and death.²⁵ This key study demonstrates the potential for efferocytosis to block crucial phagocyte defensive functions. This potential assumes increased importance based on the discovery that the human lower respiratory tract contains appreciable numbers of potentially pathogenic bacteria in health or mild disease.²⁶ Even this issue appears to be pathogen-specific, as efferocytosis facilitates clearance of mycobacteria-infected Mø.²⁷

APOPTOTIC CELL CLEARANCE IN THE LUNG

Alveolar and Interstitial Lung Mø

Alveolar Mø (AMø) are the most numerous professional phagocyte of the alveolar space and the most commonly studied. In healthy individuals, AMø comprise 90% to 95% of cells recovered from BAL. Although AMø avidly ingest many types of particles, they engulf apoptotic cells poorly when compared with other tissue Mø.⁸ Several factors contribute to reduced efferocytosis by AMø, including reduced

adhesion, very low expression of protein kinase C β II, and inhibition by the lung collectins surfactant protein A (SP-A) and SP-D acting via signal regulatory protein α (SIRP α ; CD172a).¹⁷

Nevertheless, AM ϕ s possess multiple apoptotic cell recognition receptors. Unlike blood monocytes, DCs, and most other tissue M ϕ s,^{28,29} AM ϕ s strongly express all three TAM receptors; blocking or silencing them further decreases apoptotic cell uptake but not binding (Reference 8 and our unpublished data, October 2008). AM ϕ s also express CD36 and α v β 3 integrin, which are important for apoptotic cell uptake by other types of mononuclear phagocytes.³ Although neither molecule is essential for efferocytosis by AM ϕ s in vitro,³⁰ α_v integrins may deliver immunomodulatory signals in response to apoptotic cells or by their ability (shared with the receptor for advanced glycation end products) to bind the alarmin high-mobility group box-1. Importantly, because immunomodulatory effects of efferocytosis do not require ingestion, expression of so many apoptotic cell recognition receptors suggests that AM ϕ s are highly responsive to cell death in the alveolar space.

The lungs also possess a large number of interstitial M ϕ s that appear to be important in emphysema pathogenesis,³¹ but there are no published data on their efferocytic capacity. Defining that capacity will likely be important, particularly for interstitial lung diseases.³² Whether monocytes recruited to the lungs during inflammation develop an avid efferocytic phenotype depends on whether they are exposed to MAMPs.¹⁷

Lung DCs

Similar to other mucosa, the airways possess a rich variety of resident DCs, which in humans have previously been divided into two subsets of “myeloid” DCs (mDC1 [CD11c⁺, CD1c⁺] and mDC2 [CD11c⁺, BDCA-3⁺]) plus the unrelated plasmacytoid DC (CD11c⁻, BDCA-2⁺). Although several other classifications have been proposed, the field appears increasingly to be converging on acceptance that the two myeloid human subsets correspond to the “conventional” DC subsets defined in mice.³³ Tonic efferocytosis has long been believed to keep DCs in a quiescent state essential to maintain peripheral immunologic tolerance. However, it was recently discovered that in mice, efferocytosis is mediated exclusively by the CD103⁺ conventional DC subset,³⁴ which also efficiently cross-presents antigens to CD8⁺ T cells. There are no published studies of efferocytosis by human lung DCs, an important deficit in understanding that merits investigation.

Other Lung Cell Types

Alveolar epithelial cells far outnumber lung leukocytes, and cultured human lung epithelial cells have

been shown to ingest apoptotic eosinophils, but not neutrophils, in vitro (reviewed in Reference 4). Compared with blood-derived M ϕ s, lung epithelial cells exhibit significantly lower efferocytic capacity, but until recently no studies had determined whether numerical advantage might compensate for poor individual ability. However, the Ravichandran laboratory has recently used transgenic murine models to demonstrate the importance of bronchial epithelial cells in the clearance of apoptotic cells and the regulation of airway inflammation.³⁵ Fibroblasts, and indeed probably almost all cell types, can engulf apoptotic cells.^{4,36} Neutrophils can ingest apoptotic neutrophils, which reduces their bacterial killing capacity.³⁷ Defining the contribution of cell types other than mononuclear phagocytes to net clearance of apoptotic cells from the lungs is an unmet research need.

ALTERED EFFEROCYTOSIS IN LUNG DISEASES

COPD

COPD is increasingly recognized to result from several pathologic processes, including loss of airway luminal diameter due to mucus gland hyperplasia and peribronchial fibrosis; pan-alveolar destruction; and, recently, small airways disappearance.³⁸ The clinical phenotype in a given patient with COPD results from variable involvement of these processes, several of which, especially emphysema, are characterized by apoptosis of lung structural cells. Neutrophils, recruited by IL-8 and by generation of the tripeptide proline-glycine-proline from breakdown of extracellular matrix collagen,³⁹ are believed to die almost entirely by apoptosis at the site to which they are recruited. Hence, COPD presents a considerable challenge to sustain high levels of efferocytosis within the lungs.

Evidence that defective efferocytosis contributes to the increased presence of uncleared apoptotic cells is strongest in COPD (Figs 1A, 1B). The laboratory of Sandra and Greg Hodge⁴⁰⁻⁴² has shown impaired in vitro efferocytosis of multiple cell types by AM ϕ s from patients with COPD and associated this impairment with changes in AM ϕ expression of the efferocytic receptors CD31, CD44, and CD91. Lowest among patients with COPD, efferocytosis is most defective in active smokers but remains reduced, relative to healthy nonsmokers, even with sustained smoking cessation.⁴¹ Patients with COPD have altered expression of other proteins involved in efferocytosis, including reduced extracellular pentraxin-3 in small airways.⁴³ Inhibiting efferocytosis exacerbated elastase-induced emphysema in mice.⁴⁴

Asthma

Severe asthma was one of the first lung diseases in which defective efferocytosis was identified (reviewed

FIGURE 1. Interplay between controlling factors and consequences of decreased efferocytosis in the lung. **A**, Processes increasing AC accumulation in inflammatory lung diseases. Although the basal efferocytic capacity of resident AMo is low, oxidant stress and proteolytic events during inflammation can further reduce concentrations of efferocytic opsonins and cleave efferocytic receptors, leading to greater apoptotic cell accumulation. Uncleared apoptotic cells undergo secondary necrosis, which can expose autoantigens. Uningested apoptotic cells can also stimulate NKT cells to activate DCs, driving maturation of T cells, which can be proinflammatory or even autoreactive. The resulting release of inflammatory cytokines can both increase DC activation and further decrease efferocytosis. **B**, Feedback loops resulting from decreased efferocytosis. Oxidant stress, inflammatory cytokines, and autoimmunity can all amplify alveolar destruction, a potential source of ACs. Alveolar destruction itself amplifies inflammatory cytokine release and oxidant stress. Decreased efferocytic opsonins and increased inflammatory cytokines enhance leukocyte recruitment. Evidence linking a specific disease to any of these factors or consequences is noted with colored circles. AC = apoptotic cell; ALI = acute lung injury; AMo = alveolar macrophage; CF = cystic fibrosis; DC = dendritic cell; NKT = natural killer T; TNF = tumor necrosis factor. (Illustration by Haderer & Muller Biomedical Art, LLC.)

in Reference 4), but there has since been scant progress in defining its importance. Murine natural killer T (NKT) cells constitutively express a functional version of the PS receptor TIM-1, and pulmonary NKT cells were activated by induction of apoptosis in airway epithelial cells, resulting in airway hyperreactivity that depended on NKT cells and TIM-1.⁴⁵ These findings complement the previously unexplained observation that although NK and NKT cells do not ingest apoptotic cells, they express other efferocytic receptors such as MerTK. Collectively, these data suggest one mechanism by which uningested apoptotic cells might contribute to asthma severity (Figs 1A, 1B).

Cystic Fibrosis

Similarly, despite earlier evidence for increased numbers of apoptotic cells in CF sputa and defective

efferocytosis by AMo from patients with CF due to elastase-mediated destruction of recognition receptors⁴ (Figs 1A, 1B), data on the susceptibility to apoptosis within the lungs of patients with CF are conflicting and may depend on the cell type studied.⁴⁶ In CFTR^{-/-} mice, defective efferocytosis was shown in lung epithelial cells but not AMo.⁴⁷

Idiopathic Pulmonary Fibrosis

Uningested apoptotic cells were significantly higher in BAL fluid from patients with idiopathic pulmonary fibrosis (IPF) compared with other forms of interstitial lung disease.⁴⁸ Expression of tumor necrosis factor (TNF)-related apoptosis-inducing ligand (TRAIL), which accelerates neutrophil apoptosis, is reduced in the serum and lungs of patients with IPF.⁴⁹ TRAIL-knockout mice show reduced lung

neutrophil apoptosis and greater lung collagen in a bleomycin model.⁴⁹ These findings suggest that TRAIL might be both a biomarker and potential therapy in IPF.

Acute Lung Injury

Defective efferocytosis influences prognosis in murine models of acute lung injury (ALI) and ARDS (Figs 1A, 1B). Concentrations of the efferocytic opsonin MFG-E8 decreased during gut ischemia-reperfusion in mice, and intraperitoneal administration of MFG-E8 decreased lung inflammatory cytokines, attenuated lung injury, and increased survival.⁵⁰ In lipopolysaccharide-induced acute ALI, gene-targeted mice lacking MFG-E8 showed increased neutrophil infiltration, increased inflammatory cytokines, and decreased survival.⁵¹ Resolvin E1 enhances apoptosis and uptake of human neutrophils induced by microbe ingestion and reduced lung inflammation in three murine models.⁵²

Conversely, instillation of apoptotic cells into the lungs of mice following bleomycin lung injury reduced inflammatory cytokine production and lung fibrosis via hepatocyte growth factor-dependent mechanisms, resulting in increased survival.⁵³ This last finding suggests that autologous apoptotic cell therapy might be beneficial in selected settings, if issues of timing and dosage could be defined. However, the complexity of that approach is illustrated by the finding that TNF- α reduced net apoptotic cell clearance from the lung and thereby increased lung inflammation as cells progressed to secondary necrosis.⁵⁴

THERAPEUTIC IMPLICATIONS

Common Respiratory Drugs

Efferocytosis by human and murine AM ϕ s is significantly increased by in vitro treatment with clinically

relevant doses of statins, macrolides, or corticosteroids.^{31,40,42,55,56} In mice, this effect of statins has also been demonstrated in vivo; it appears to be mediated by RhoA inhibition (thereby allowing activation of Rac-1, which is necessary for efferocytosis).⁵⁶ Corticosteroids increase AM ϕ efferocytosis by two processes: one rapid and dependent largely on SIRP α down-regulation; the other delayed yet sustained, dependent on new protein synthesis and associated with Mertk upregulation.³¹ The rapid corticosteroid action is nonadditive with statins, as anticipated, because both act on the Rac activation pathway. Whether chronic effects of statins and corticosteroids on efferocytosis are additive requires further study. By contrast, the effect of azithromycin on efferocytosis is additive with corticosteroids,³¹ implying that it results from an unrelated, currently undefined mechanism. The mucoregulatory drug carbocysteine also increases both efferocytosis by murine AM ϕ s and clearance of apoptotic neutrophils from the lungs of mice.⁵⁷ Collectively, these findings suggest that enhanced efferocytosis already occurs in many patients with chronic airways diseases, as one action of widely prescribed medications. However, no research has separated the direct antiinflammatory or antimicrobial effects of agents from their proefferocytic effect. Hence, the contribution of enhanced efferocytosis to improved clinical outcomes in inflammatory lung diseases is uncertain.

Caution about more directly manipulating efferocytosis in the lungs comes from the observation in large clinical trials such as TORCH and from administrative data⁵⁸ of an increased incidence of community-acquired pneumonia in patients with COPD receiving potent inhaled corticosteroids, although the significance of this finding has been debated.⁵⁹ We speculate that, especially in emphysema, such an increase could result from glucocorticoid-augmented efferocytosis by AM ϕ s in the presence of a raised lung burden

FIGURE 2. Theoretical positive and negative effects of therapeutic enhancement of efferocytosis. Increasing efferocytosis could accelerate tissue repair and resolution of inflammation, helping to break the destructive cycle of inflammatory lung disease. Conversely, increasing efferocytosis may leave the phagocyte that ingested apoptotic cells less able to recognize and kill pathogenic microbes, as has been demonstrated in a murine model.²⁵ PAMP = pathogen-associated molecular pattern. See Figure 1 legend for expansion of other abbreviation. (Illustration by Haderer & Muller Biomedical Art, LLC.)

FIGURE 3. Surfactant therapy and antioxidants work through the same pathway to enhance efferocytosis. Oxidant stress activates RhoA, an inhibitor of Rac-dependent actin rearrangement that is required for engulfment of ACs; antioxidants suppress RhoA, allowing Rac activation and permitting apoptotic cell engulfment (shown on the left). Surfactant therapy decreases oxidant stress and may favor antioxidant production, shifting the balance toward RhoA inhibition and apoptotic cell ingestion (shown on the right). See Figure 1 legend for expansion of abbreviation. (Illustration by Haderer & Muller Biomedical Art, LLC.)

of apoptotic cells. Aside from effects of inhaled corticosteroids themselves, the combination would be anticipated to upregulate the immunosuppressive molecules SOCS1 and SOCS3,²³ which block nuclear factor- κ B-dependent signaling. On the other hand, PGE₂ was responsible for the majority of immunosuppression in a murine model of pneumococcal pneumonia.²⁵ Because corticosteroids block phospholipase A2 and cyclooxygenase 2, which are necessary for PGE₂ production, whether glucocorticoid-augmented efferocytosis would actually increase the risk for or severity of community-acquired pneumonias is uncertain. Additional study is needed to define the optimal means of therapeutically enhancing efferocytosis to decrease lung inflammation without impairing lung immunity (Fig 2).

Alternate Therapies

Cigarette smoke and other oxidant stresses reduce efferocytosis by activating RhoA (which inhibits Rac),⁶⁰ whereas antioxidant therapy has the converse effects.⁶¹ Because the lung environment activates RhoA in AM ϕ s¹⁷ even in the absence of exogenous oxidants, local antioxidant therapy might be another means of increasing efferocytosis to reduce lung inflammation (Fig 3). Supporting that possibility, antioxidant treatment during lipopolysaccharide-induced lung inflammation increased efferocytosis while decreasing inflammatory mediators and lung damage.⁶¹ Poractant alfa, one type of surfactant therapy effective in neonatal respiratory distress syndrome, also increased in vitro and in vivo efferocytosis by murine AM ϕ s.⁶² Exactly how poractant alfa had this effect is unclear,

but it may relate to the known improvement in antioxidant capacity following treatment.⁶³

CONCLUSIONS

Enhancing the defective efferocytosis seen in multiple lung diseases and active cigarette smoking is a plausible means to arrest ongoing lung inflammation. Improved efferocytosis may contribute to beneficial actions of existing therapies, such as inhaled steroids, macrolides, and statins. Better understanding of the molecular basis for the immunomodulatory effects of efferocytosis could lead to entirely novel therapies. Further investigation is needed to determine the true impact of enhancing efferocytosis on susceptibility to lung infection and the long-term safety of this approach.

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