Published in final edited form as:

Expert Rev Respir Med. 2008 December ; 2(6): 753–765. doi:10.1586/17476348.2.6.753.

Phagocytic clearance of apoptotic cells: role in lung disease

Jeong H Yun, MD, MPH,

Program in Translational Lung Research, Division of Pulmonary Sciences and Critical Care Medicine, Department of Medicine, University of Colorado at Denver, School of Medicine, 12700 East 19th Avenue, Aurora, CO 80045, USA, Tel.: +1 303 724 6049, jeong.yun@uchsc.edu

Peter M Henson, PhD, and

Department of Pediatrics, National Jewish Medical and Research Center, Denver, CO, USA, Tel.: +1 303 398 1380, Fax: +1 303 398 1381, hensonp@njc.org

Rubin M Tudor, MD[†]

Program in Translational Lung Research, Division of Pulmonary Sciences and Critical Care Medicine, Department of Medicine, University of Colorado at Denver, School of Medicine, 12700 East 19th Avenue, Aurora, CO 80045, USA, Tel.: +1 303 724 6062, rubin.tudor@ucdenver.edu

Abstract

Apoptosis and apoptotic clearance are matched processes that are centered in the maintenance of homeostasis. Similar to apoptosis, apoptotic cell clearance is a conserved mechanism that is highly efficient and redundant, highlighting its overall functional importance in homeostasis. Increasing evidence suggests that the mismatch between apoptosis and apoptotic cell clearance underlies pathologic conditions including inflammatory lung diseases, such as chronic obstructive pulmonary disease, cystic fibrosis, asthma, acute lung injury/acute respiratory distress syndrome and cancer immunity. Although direct causality has yet to be established, this paradigm opens novel approaches towards the understanding and treatment of lung diseases. Glucocorticoids, statins and macrolide antibiotics, which are already in use for treating lung conditions, have a positive effect on apoptotic clearance and are among novel agents that are potential candidates for treatment of these disorders.

Keywords

acute lung injury; apoptosis; apoptotic cell clearance; asthma; chronic obstructive Pulmonary disease; cystic fibrosis; efferocytosis; lung cancer; phagocytosis

Phagocytosis, the process of taking up large particles ($>0.5\mu\text{m}$) into cells, is a phylogenetically conserved mechanism that is essential for self protection and organismal maintenance. It mediates the removal of foreign pathogens and senescent cells. Furthermore, it participates in immune responses, inflammation, tissue remodeling and development.

Apoptosis, which represents one form of programmed cell death along with autophagic cell death and autonomous necrosis [1,2], is a mechanism for removing excessively damaged or potentially harmful cells in order to maintain normal organ and cellular homeostasis. Although apoptosis is a clean form of cell death that maintains some degree of cell integrity, apoptotic cells that are not promptly removed will eventually undergo secondary necrosis,

[†]Author for correspondence: Program in Translational Lung Research, Division of Pulmonary Sciences and Critical Care Medicine, Department of Medicine, University of Colorado at Denver, School of Medicine, 12700 East 19th Avenue, Aurora, CO 80045, USA, Tel.: +1 303 724 6062 rubin.tudor@ucdenver.edu.

resulting in leakage of cellular content and inflammation. It follows that rapid clearance of apoptotic cells by phagocytic cells is crucial for protecting tissues from damage due to inflammation or even autoimmune injury while maintaining constant cell turnover [3]. It is becoming apparent that phagocytic removal of apoptotic cells is different from phagocytosis of pathogens, in that the former induces active antiinflammatory and immunosuppressive responses. We have suggested the term 'efferocytosis' (taken from 'effero', meaning to take to the grave or to bury) [4] to distinguish this unique engulfment process of apoptotic cells (Figure 1).

Detection of apoptotic cells in a tissue provides a snapshot that reflects the balance between proliferation, apoptosis and clearance of apoptotic cells. Since efferocytosis is a highly efficient process, normally complete in minutes, the finding of increased numbers of apoptotic cells leads one to suspect underlying defects in apoptotic clearance or a massive induction of apoptosis in vast excess of the clearance process. Defects in the clearance could further be attributed to abnormalities in recognizing apoptotic cells due to a loss of receptor or recognition molecules and/or alterations in the engulfment process itself. Accordingly, impaired efferocytosis has been linked to autoimmunity and various pulmonary diseases. In this review, the basic mechanism of apoptotic cell removal and its implications in lung diseases will be discussed.

Mechanism of apoptotic cell clearance

The process of efferocytosis can be divided into three stages:

- Recognition of apoptotic cells by phagocytes
- The actual engulfment steps
- Postengulfment digestion and processing

In addition, there are a number of critically important responses that are initiated by the interaction of apoptotic cells with the responding potential phagocyte, leading to suppression of inflammation, immunosuppression and activation of healing mechanisms contributing to normal tissue repair. Various signaling molecules, receptors and ligands that are involved in each step of the process will be discussed here briefly (Figure 2).

Recognition of apoptotic cells

To recognize apoptotic cells vis-à-vis viable cells, professional phagocytes (macrophages) or nonprofessional phagocytic cells utilize many ligands and receptors. Phosphatidylserine (PS) was an early characterized 'eat me' signal expressed on apoptotic cells [5]. In normal viable cells, PS is localized and retained in the inner leaflet of the plasma membrane phospholipid bilayer by the action of aminophospholipid translocases, which flip back inside any PS exteriorized to the outer leaflet of the membrane. Low rates of flip/flop (also known as scrambling) in resting cells lead to PS exposure and the resulting phospholipid symmetrization is rectified by the ATP-dependent translocases. Many cell types increase their membrane flip/flop during the course of cellular activation, leading to exposure of PS on the outer leaflet; however, in the presence of active translocases; this effect is transient. By contrast, during apoptosis, flip/flop is enhanced while the translocases are inactivated, thus leading to permanently exposed PS in the outer leaflet [6,7]. PS can now be recognized by soluble bridging molecules and/or receptors on phagocytes. Oxidized forms of PS are also found on apoptotic cells and appear to play an important role in their recognition [8]. Calreticulin is a protein that is increased and/or redistributed on apoptotic cells and serves as another ligand for their recognition [9]. It is normally found in the endoplasmic reticulum, where it serves to chaperone proteins and/or to bind calcium. Although it is also found on the surface of most cells in low amounts, calreticulin expression is substantially higher in

apoptotic cells, where it appears to colocalize with PS [10]. In addition to these potential mediators of efferocytosis, glycosylation of cell surface proteins of apoptotic cells [11] and/or changes in surface charge have also been implicated as apoptotic ligands and signaling molecules [12].

Elucidation of receptors on the phagocytes that recognize PS and other apoptotic ligands has been an active area of research. Importantly, the apoptotic cell recognition by phagocytes is a highly redundant system. There is no single universal receptor for apoptotic ligands; rather, one ligand can be recognized by different receptors. Therefore, not all apoptotic signals nor all receptors for recognition are required for efficient apoptotic cell removal. The PS on apoptotic cells is recognized by a number of candidate receptors as well as groups of bridging molecules that bind to the PS and then to secondary receptors on the phagocytic cell. Among the former are T-cell immunoglobulin- and mucin domain-containing molecules (TIM4 and TIM1), brain-specific angiogenesis inhibitor (BAI)-1 and stabilin-2 [13–16]. A previously described ‘PS receptor’ (PSR) [17] now appears to serve a different function [18] and may or may not participate in apoptotic cell clearance [19]. CD36 is a class B scavenger receptor and has been shown to recognize oxidized PS on apoptotic cells [8,20]. PS-binding bridging molecules include milk fat globule EGF 8 (MFG-E8) [21] and growth arrest-specific 6 (Gas6) [22]. They interact with integrins and Mer receptor tyrosine kinases α_v on the phagocytic cell, respectively. Other potential receptors for apoptotic cells include CD14 [23], class A scavenger receptor [24] and CD68 [25], although their respective ligands are less well characterized. The collectin family molecules of the innate immune system bind to the apoptotic cell surface (possibly to altered phospholipid, carbohydrates and/or DNA) and function as bridging molecules between the apoptotic cell and the phagocyte, facilitating efficient engulfment. This family of molecules includes mannose-binding lectin (MBL), C1q, surfactant protein (SP)-D, -A and adiponectin. These bridging molecules bind to calreticulin on the phagocytes, which in turn mediates downstream signaling through CD91 [9,26,27]. Some of the earlier mentioned ‘receptors’ may not only play a greater role in tethering the apoptotic cell to the phagocyte, but also may actively signal for uptake; some serve both functions [4]. This apparent redundancy further supports the complex nature of apoptotic cell removal.

Contrary to the ‘eat me’ signals, apoptotic cells may lose ‘do not eat me’ signals that normally protect viable cells from inappropriate uptake into phagocytes. Cell types that express CD31 on their surface (e.g., endothelial cells) can engage in a homophilic interaction between phagocyte CD31 and that on viable cells, resulting in detachment without engulfment. In the case of apoptotic cells, the detachment signal is lost, thereby promoting tight binding and engulfment [28]. CD47 on viable cells interacting with the signal inhibitory regulatory protein (SIRP)- α on phagocytes is another set of such signals to prevent efferocytosis, mediated by downstream signaling through tyrosine phosphatases, such as SHP-1 [29].

Engulfment of apoptotic cells

Following recognition of apoptotic cells, their internalization occurs by the cytoskeletal rearrangement of the engulfing cell. Intriguingly, the signal pathways leading to engulfment are remarkably conserved, with striking similarities between mammals and *Caenorhabditis elegans*. In much simplified terms, two partially redundant genetic pathways involve (with the mammalian homolog in parentheses):

- ced-1(CD91 or LRP1), ced-6 (GULP) and ced-7 (ATP-binding cassette transporter [ABC])

- ced-2 (CT 10 regulator of kinase II, CrkII), ced-5 (DOCK180) and ced-12 (engulfment and cell motility [ELMO]) [30]

There is evidence in *C. elegans* that the pathways merge in their use of ced-10 (Rac) and this small GTPase has also been shown to exert essential roles in the uptake in mammalian cells. In the first pathway, ELMO is an adaptor protein, which works with DOCK180 to activate Rac. In addition to Rac, other Rho family GTPases have been studied for their role in the cytoskeletal rearrangement needed for efferocytosis. Cdc42 has similar functions to Rac [31] and potentially increases engulfment of apoptotic cells [32]. On the other hand, active Rho A blocks the uptake of apoptotic cells, while inhibition of Rho A results in its enhancement. This negative effect seems to be mediated by Rho kinase, which controls myosin light chain phosphorylation leading to increased cell contractility [33]. Accordingly, inhibition of Rho kinase enhances clearance of apoptotic cells *in vitro* and *in vivo* and appropriate concentrations of statins (which can block Rho A by altering its prenylation) have a similar beneficial effect (see later) [34].

Consequence of apoptotic cell clearance

Clearance of apoptotic cells is distinct from most other forms of phagocytosis in that it induces anti-inflammatory responses. Apoptotic cells can inhibit inflammatory cytokines induced by lipopolysaccharide (LPS) *in vivo* and *in vitro* [35,36]. Upon apoptotic cell recognition and ingestion, immunomodulatory cytokines, such as IL-10 [37] and TGF- β 1 [38], are secreted by macrophages themselves, while production of proinflammatory chemokines, cytokines and eicosanoids is reduced [36,39].

Recent studies provide evidence that the recognition step of apoptotic cells is necessary and sufficient to induce anti-inflammatory effects, since engulfment is not required for these responses. Exteriorization of PS is required for induction of TGF- β and suppression of IL-12 by the engulfing cell [35,40]. PS-coated liposomes alone can stimulate TGF- β , albeit to a lesser degree than when macrophages are stimulated with apoptotic cells. In line with this evidence, stimulation by apoptotic cell membranes is sufficient to inhibit IL-12 in macrophages [41] and immediate-early inhibition of proinflammatory cytokine genes occurs via apoptotic cell binding by macrophages [42]. The autocrine/paracrine functions of these cytokine responses apply specifically to macrophages that have bound to apoptotic cells [43].

Although professional phagocytes (e.g., macrophages and dendritic cells) are most commonly studied for apoptotic cell clearance, various nonprofessional phagocytes, including endothelial cells, epithelial cells, vascular smooth muscle cells and fibroblasts, can engulf apoptotic cells [29,44–47]. Furthermore, the nonprofessional phagocytes not only inhibit inflammation via TGF- β production [48], but also generate VEGF [49] and HGF [50] upon apoptotic cell clearance. Secretion of growth/survival factors suggests important roles for apoptotic cell recognition in providing tissue cell replenishment signals to help maintain tissue repair and homeostasis.

Impaired apoptotic cell clearance is increasingly accepted as a participant in the development of autoimmune diseases. Secondary necrosis from uncleared apoptotic cells can be the source of autoantigen and the proinflammatory effect from impaired efferocytosis can create an environment susceptible to developing autoimmune disease [51]. Supporting this hypothesis, decreased efferocytosis is observed in systemic lupus erythematosus macrophages [52] and impaired islet β -cell clearance may contribute to development of autoimmune diabetes [53]. Furthermore, genetic manipulation of the receptors or molecules involved in efferocytosis in animals often leads to development of autoimmune responses [23,54].

Apoptotic clearance in the lung

An increased number of detectable apoptotic cells is associated with major lung diseases, including emphysema, cystic fibrosis (CF), acute lung injury (ALI) and asthma. As efferocytosis is a highly efficient process, few apoptotic cells are detected in normal lung tissue [55]. Thus, increased apoptotic cells can be explained by increased levels or rates of apoptosis, decreased apoptotic cell clearance (Table 1), or both.

Chronic obstructive pulmonary disease

Chronic obstructive pulmonary disease (COPD) is a chronic respiratory disease (chronic bronchitis and emphysema) caused mainly by cigarette smoke, characterized by persistent inflammation and destruction of the lung, leading to obstructive airflow limitation that is not completely reversible. The pathogenesis of COPD is conventionally explained as the imbalance between protease and antiprotease led by enhanced inflammation or a genetic defect in the α 1-antitrypsin gene causing its reduction in the serum, all resulting in parenchymal destruction. Recently, the role of apoptosis in COPD has gained attraction as a novel paradigm [56]. This concept is supported by both animal models and human data, and suggests that increased apoptosis and/or decreased apoptotic cell clearance can contribute to defective restoration of the structural cells and participate in emphysematous and perhaps even airway changes in this condition. In animal models, induction of alveolar cell and/or endothelial cell apoptosis seems to be sufficient to cause emphysema [57]. VEGF [58] or VEGF receptor blockade [59,60], caspase-3 [61], IFN- γ [62] and ceramide [63] have been shown to induce apoptosis in the murine lung, leading to emphysematous changes. However, one should note with caution that increased airspace size in rodents may or may not reflect the changes observed in human emphysema. Human data also demonstrate increased endothelial and epithelial cell apoptosis in COPD patients when compared with smokers or nonsmokers [64–66]. Increased caspase-3, apoptosis-associated proteins (Bcl-2, Bax and Bad), ceramide levels and decreased VEGF and VEGF receptor-2 expression in emphysema patients' lungs further support that apoptosis occurs with emphysema [55].

As mentioned earlier, the few apoptotic cells observed in a given time in normal tissues in part may reflect rapid and efficient removal of apoptotic cells. Detection of increased numbers of apoptotic cells in COPD likewise reflects a defect in clearance mechanisms [56]. Thus, Hodge *et al.* reported phagocytic defects of alveolar macrophages that were specific to the uptake of apoptotic cells from COPD patients and that COPD patients have greater deficits than healthy smokers [67]. However, the efferocytosis defect improved with cessation of smoking, suggesting a direct role of cigarette smoke in the pathogenesis of defective apoptotic cell clearance [68]. This is also supported by *in vitro* data that cigarette smoke can suppress apoptotic cell clearance by activating RhoA [69].

Patients with COPD seem to have lower SP-D levels independent of cigarette-smoking status [70]. In animal models, SP-D-knockout mice, which have an apoptotic cell clearance defect [9], develop emphysematous changes and increased pulmonary inflammation. Although there is no direct evidence that the clearance defect causes the structural changes, reduction of apoptotic cells and chemokines by treatment with SP-D supports its potential role in the pathogenesis of destructive airspace enlargement [71].

Cystic fibrosis

Cystic fibrosis is characterized by massive and persistent neutrophilic inflammation in the lung with abnormally high numbers of detectable apoptotic and necrotic cells in the airways. Indeed, phagocytosis of apoptotic cells appears to be impaired in CF [72], which may exacerbate the pronounced airway inflammation. The defect may be explained by multiple

facets of the CF disease process, including the influx of inflammatory cells, increased levels of proteases, decreased SP-A and SP-D, apoptotic cells entrapped within the thick mucus and the commonly encountered *Pseudomonas aeruginosa* infection, all creating a vicious cycle of inflammation and destruction.

Granule proteases released from inflammatory cells are responsible for airway destruction and impaired host defense [73]. Furthermore, Vandivier *et al.* have shown that neutrophil elastase cleaves apoptotic cell-recognition receptors on macrophages, leading to impaired efferocytosis [73]. *P. aeruginosa* infection, a major cause of morbidity and mortality in susceptible CF patients [74], also contributes to the impaired apoptotic clearance mechanism by producing pyocyanin. Pyocyanin is a toxic metabolite of *P. aeruginosa* that induces neutrophil apoptosis [75]. It also generates oxidative stress in macrophages, which in turn can inhibit efferocytosis by activation of Rho GTPase [75–77].

Asthma

Clinical features of asthma include reversible airway obstruction, airway hyper-responsiveness and inflammation. Eosinophils are effector cells that play a central role in the pathogenesis of asthma as increased eosinophil levels correlate with disease severity in asthmatic patients [78]. Release of major basic proteins, lipid mediators, such as leukotrienes and platelet-activating factor, and reactive oxygen species by eosinophils mediate epithelial damage, airway remodeling and hyper-responsiveness. Therefore, removal of eosinophils is an important mechanism to protect the tissue from persistent allergic inflammation. Apoptosis and subsequent efferocytosis are suggested to be a major mechanism of eosinophil removal from the tissues [79]. However, cytokines that are increased in asthmatic airways (IL-3, IL-5 and granulocyte-macrophage colony-stimulating factor [GM-CSF]) promote eosinophil survival [80–82]. Eosinophils isolated from asthmatic patients also survive longer than controls [80].

Similar to apoptotic neutrophils, apoptotic eosinophils can be recognized and engulfed by alveolar macrophages and resident cells in the lung through receptor-dependent mechanisms [47,80–82]. A study by Hyunh *et al.* demonstrated that alveolar macrophages from severe asthmatic patients had decreased phagocytic bodies within phagosomes *in vivo* and defective apoptotic clearance *in vitro*, and that this was associated with failure to induce production of prostaglandin (PG)E₂ and 15-hydroxyicosatetraenoic acid (HETE) [83]. PGE₂ inhibits fibroblast proliferation and 15-lipoxygenase products exert anti-inflammatory actions by activating PPAR γ and generating downstream lipoxins (lipoxygenase interaction products [LX]), LXA₄ and LXB₄ [84]. The lipoxins contribute to resolution of inflammation by suppressing neutrophil and eosinophil actions. However, the observed phenomenon does not establish a cause and effect relationship between asthma and phagocytic clearance of apoptotic cells. Contribution of delayed apoptosis versus impaired apoptotic clearance, involvement of eosinophils or other inflammatory cells, and causal relationship all need to be clarified in the setting of asthma pathogenesis.

Acute lung injury

Direct and indirect injury to the lung causes the clinical syndromes ALI or its advanced form, acute respiratory distress syndrome, resulting in impaired oxygenation [85]. Various pathways of cell death, including apoptosis, have been shown to be involved in the pathogenesis of ALI, including an emerging role for the Fas–Fas ligand system [86]. Neutrophils play an important role in the pathogenesis of ALI, releasing reactive oxygen species, cationic proteins and proinflammatory cytokines [34,87]. In the normal lung, neutrophils recruited after an inflammatory episode will undergo apoptosis and are then cleared very efficiently. Even after *Streptococcus pneumoniae* lobar pneumonia where the

lung is replete with neutrophils, they are cleared completely and there is minimal evidence of detectable apoptotic cells [88]. In ALI, decreased neutrophil apoptosis [89] as well as increased neutrophil number have been observed [90].

Many lines of evidence suggest the important role of apoptotic cell clearance in the resolution of inflammation in ALI. In CD44-knockout mice, characterized by defective apoptotic neutrophil clearance, the lung injury model induced by bleomycin instillation had resulted in increased inflammation, impaired activation of TGF- β and higher mortality [91]. Restoration of CD44 by bone marrow transfer into CD44-knockout mice corrected impaired efferocytosis, inflammation and survival. CD44 is a transmembrane adhesion receptor for hyaluronan, fibrin, fibronectin and collagen, all of which have been shown to increase apoptotic neutrophil engulfment *in vitro* [92]. It is unclear whether the improvement of bleomycin-induced lung injury is solely due to correction of efferocytosis, given that CD44 also mediates removal of hyaluronan and proinflammatory cytokines [93]. However, the dramatic improvement in efferocytosis defect after CD44 restoration suggests that apoptotic clearance is at least an important mechanism for resolving acute inflammation.

Further supporting this notion, resolution of ALI has been shown to occur with concomitant clearance of apoptotic cells. Administration of oleic acid causes a rapid influx of neutrophils and ALI. Evidence of macrophages ingesting apoptotic neutrophils parallels histologic demonstration of resolving inflammation after oleic acid-induced lung injury [94]. A similar process of synchronous resolution of lung inflammation and apoptotic cell clearance was observed in a vanadium-induced lung injury model [95] and LPS-mediated lung inflammation [96]. A limitation of these studies is their correlative nature between apoptotic clearance and the outcome of ALI models. More direct evidence comes from a study by Medan *et al.* where mice were treated with PS-containing liposomes aimed at inhibiting phagocytic uptake of apoptotic cells. Apoptosis and necrosis were increased after LPS injury, suggesting apoptotic clearance contributes to the acute inflammatory process [97].

Cancer

Defects in apoptosis are major mechanisms underlying tumorigenesis and resistance to therapies in cancer cells. Lung cancers, especially non-small-cell lung cancers, are resistant to apoptosis, as demonstrated by increased expression of inhibitor-of-apoptosis protein family [98,99], whereas most chemo/radiation therapies induce apoptotic cell death. Although few studies have focused on the role of apoptosis in lung cancer [100], no articles have been published on apoptotic lung cancer cell clearance to our knowledge. As in non-neoplastic lung disease, the understanding of the roles of cancer cell or bystander cell efferocytosis may open novel areas of investigation in a highly relevant field of lung diseases.

In contrast to the impaired efferocytosis contributing to the pathology of inflammatory lung diseases reviewed earlier, some of the physiological impacts of efferocytosis may rather enhance cancer growth and dissemination. The anti-inflammatory environment induced by apoptotic cell recognition and ingestion may suppress the host immune response against tumors, promoting immune escape and progression of the disease [101]. Uptake of apoptotic cells and release of the anti-inflammatory cytokine TGF- β suppress dendritic cell maturation and alter the phenotype of the tumor-associated macrophages. As a result, antigen presentation of phagocytosed tumor cells by dendritic cells to T cells is prevented [102]. Furthermore, secretion of prosurvival factors, such as VEGF, by macrophages upon apoptotic tumor cell phagocytosis may also promote tumor cell growth and angiogenesis [49]. In support of this evidence, blocking the efferocytosis process by PS binding annexin V enhanced tumor immunogenicity [103]. Another molecule, sulfatide, can act as phagocytic ligand on apoptotic colon and renal cancer cells, therefore facilitating

efferocytosis. Consequently, this leads to TGF- β secretion by macrophages [104], suggesting that the worse prognosis found in sulfatide-overexpressing colon cancer may be related to immune escape [105]. Sulfatide may also play a similar role in lung cancer, since it is expressed in lung adenocarcinoma [104].

Regulation of apoptotic clearance & therapeutic approaches

Recent advances in apoptosis research have opened various opportunities for therapeutic modulation of apoptotic processes aimed at controlling inflammation. Correction of the rate of apoptosis should therefore be matched with maintaining or restoring efficient apoptotic cell clearance [80,106]. Novel approaches, for instance, increasing CD36 (a scavenger receptor that participates in uptake of apoptotic cells) expression by gene transfer, have been investigated [107]. Endogenous and exogenous regulators of apoptotic cell phagocytosis are presented here as potential therapeutic targets.

Glucocorticoids

Glucocorticoids have a potent anti-inflammatory action and are widely used to treat inflammatory conditions, including the lung diseases reviewed earlier. It affects both apoptosis and phagocytosis of apoptotic cells.

Glucocorticoids accelerate eosinophil apoptosis and delay neutrophil apoptosis, both of which can be blocked by a glucocorticoid receptor antagonist [108].

Liu *et al.* reported that incubating macrophages with glucocorticoids for 24 h increases the uptake of apoptotic leukocytes via the glucocorticoid receptor without releasing proinflammatory cytokines IL-8 and monocyte chemoattractant protein-1 [109]. The same group has demonstrated that long-term exposure of monocytes to dexamethasone enhances phagocytosis of apoptotic cells by monocytes or monocyte-derived macrophages. Monocytes/macrophages undergo cytoskeletal reorganization and Rac activation, leading to enhanced phagocytosis. Decreased tyrosine phosphorylation of adhesion contacts, paxillin and pyk2, and downregulation of p130Cas, involved in signaling through DOCK180/CrkII, may account for this change in phenotype [110].

In keeping with these observations, the apoptotic clearance defect observed in asthmatic alveolar macrophages was corrected after treatment with dexamethasone [83], suggesting facilitation of apoptotic cell clearance is another mechanism for glucocorticoid-mediated suppression of inflammation.

Lipoxins

Lipoxins are arachidonic acid-derived lipid mediators that are formed by cell-cell interaction in conditions such as inflammation and hypersensitivity [111]. Similar to some actions of PGE₂, lipoxins are anti-inflammatory mediators, suppressing polymorphonuclear neutrophil chemotaxis, degranulation and generation of reactive oxygen species [112,113]. In a murine asthma model involving oval-blocked bumin challenge, LXA₄ airway hyperresponsiveness and production of proinflammatory cytokines [114]. Lipoxin levels were found to be lower in CF patients, and administration of lipoxin in a *P. aeruginosa* infection model suppressed neutrophilic inflammation and decreased bacterial burden [115].

Interestingly, LXA₄ also increases phagocytosis of apoptotic neutrophils by macrophages *in vitro* [116]. Furthermore, when mice with thioglycollate-induced peritonitis were challenged with apoptotic neutrophils intraperitoneally, LXA₄, LXB₄ and aspirin-induced LX enhanced uptake of exogenous apoptotic cells [117]. The observed facilitation of apoptotic clearance can be explained by recent findings of cytoskeletal organization induced by LX. RhoA and

Rac activation are triggered by treatment of macrophages with LXA₄, which primes macrophages to rearrange actin filaments and form pseudopodia [118]. Similar studies demonstrated that stimulation of THP-1 cells with LXA₄ causes polarized redistribution of myosin IIA and Cdc42 [119]. Myosin IIA is a nonmuscle myosin filament important for phagocytosis; for instance, localization of myosin is observed during complement receptor- or FcγR-mediated phagocytosis [120]. As noted earlier, activation of both RhoA and Rac seem to have opposing effects on efferocytosis. However, the observed increase in apoptotic cell clearance and Cdc42 activation imply that Rac and/or Cdc42 activation drive the overall direction to enhancing efferocytosis by LX [4].

Statins

Well-known for their pleiotropic effects, statins lower cholesterol levels by inhibiting HMG-CoA reductase, exerting anti-inflammatory action through the prenylation of signaling molecules and inducing apoptosis of certain cells, including eosinophils and vascular endothelial cells [121,122]. The anti-inflammatory effect of statins includes attenuation of LPS-induced lung injury [123], allergic bronchial asthma [124,125], cigarette smoke-induced emphysema and pulmonary hypertension [126], all of which were demonstrated to have decreased inflammatory cell infiltrate after statin treatment. Lovastatin increases efferocytosis in the murine lung *in vivo* as well as in alveolar macrophages from COPD patients *ex vivo*, by inhibiting RhoA, affecting actin polymerization and chemotaxis [34]. Recent clinical studies suggest that statins may reduce COPD morbidity/mortality [127,128] and slow progression of pulmonary hypertension, suggesting a wide range of benefits in the treatment for COPD.

PPARγ agonists

PPARs are members of the nuclear hormone receptor superfamily that form a heterodimer with retinoid X receptor. PPARγ, one of the three isoforms of PPAR, is highly expressed in adipose tissue and regulates adipogenesis and metabolism. PPARγ is the pharmacologic target of the insulin sensitizers thiazolidinediones. It is also present in macrophages, lymphocytes and dendritic cells, implying that PPARγ has an immunomodulatory role [129]. Indeed, the anti-inflammatory effects of PPARγ activation have been studied in various lung disease models, including asthma, ALI and COPD [129–132]. The main mechanism of PPARγ-mediated anti-inflammatory effects is thought to be the downregulation of proinflammatory genes and upregulation of anti-inflammatory genes (see [133] for a detailed review). Furthermore, PPARγ agonists increase expression of CD36 in alveolar macrophages, resulting in enhanced efferocytosis and resolution of inflammation [134]. Conversely, treatment with a PPARγ antagonist or macrophage-specific deletion of PPARγ leads to decreased phagocytosis of apoptotic cells [135]. Although no large-scale clinical studies so far support use of PPARγ, its multiple effects leading to anti-inflammatory actions, including enhancement of efferocytosis, place PPARγ agonists as potential novel therapeutic tools for inflammatory lung disease.

Macrolide antibiotics

In addition to their antibacterial activity, macrolides possess anti-inflammatory effects, including suppression of proinflammatory cytokines, mucus secretion, neutrophil chemotaxis in airways and facilitation of efferocytosis. Alveolar macrophages from COPD patients who had lower baseline efferocytosis and healthy nonsmokers demonstrated increased efferocytosis *ex vivo* after treatment with azithromycin and erythromycin, respectively [136,137]. Recently, Hodge *et al.* reported increased efferocytosis by alveolar macrophages after a 12-week treatment with a low dose of azithromycin in COPD patients [138]. They attributed this effect to an upregulation of the mannose receptor by the

azithromycin treatment, as COPD patients had significantly lower mannose receptor expression on macrophages (although evidence for a major role for this receptor in efferocytosis is weak) and reduced SP-D and MBL levels. Although it was an open-labeled study, this study provided the first evidence of an *in vivo* effect of macrolides on efferocytosis, implying a therapeutic role of macrolides in the treatment of COPD.

Granulocyte–macrophage colony-stimulating factor

Granulocyte–macrophage colony-stimulating factor belongs to a group of anti-inflammatory cytokines that can increase uptake of apoptotic cells by enhancing thrombospondin-dependent recognition mechanisms [139] and PS-binding MFG-E8 [140]. GM-CSF-deficient mice develop systemic lupus erythematosus and their macrophages demonstrate defects in apoptotic clearance [141]. A recent study by Jinushi *et al.* suggests that GM-CSF is required for the expression of the bridging molecule MFG-E8 [140]. MFG-E8 modulates the role of GM-CSF in the balance of immune tolerance versus immunity, in that increased MFG-E8 results in immune tolerance. Expression of MFG-E8 inhibits GM-CSF-secreting tumor vaccination activity (possibly by the immunosuppressant effect of efferocytosis), while MFG-E8 mutant enhances GM-CSF-mediated tumor destruction [140]. Although GM-CSF exhibits a number of other biologic activities, including inhibition of apoptosis of mature leukocytes, *in vivo* administration of GM-CSF to cancer patients increases phagocytosis of apoptotic cells [142], suggesting therapeutic targeting directed at apoptotic cell clearance.

Expert commentary

In this review, we have discussed the basic mechanisms of efferocytosis (phagocytic uptake of apoptotic cells) and how defects in this process may be related to inflammatory or even structural outcomes in specific pulmonary diseases. Possible therapeutic targets that can modulate efferocytosis process have also been highlighted.

It is important to consider apoptotic cell clearance in the context of the balance between apoptosis and proliferation. Inflammatory diseases in the lung are often associated with detection of increased apoptotic cells, which may indicate massive proliferation, massive apoptosis induction or, as often seems to be the case, impaired efferocytosis. Increasing evidence suggests defective clearance of apoptotic cells may account for inflammation and impaired maintenance of homeostasis. However, the redundancy among the molecular effectors of efferocytosis and the ensuing antiinflammatory mechanisms makes it difficult to establish clear mechanistic and causal relationships related to lung diseases. On the other hand, highly redundant and conserved characteristics of efferocytosis highlight the importance of its function. Furthering the knowledge of these fundamental processes will enable us to translate this novel paradigm in a better understanding and treatment of several lung diseases. These include diseases not discussed in this review given the scarcity of studies regarding pulmonary hypertension and granulomatous lung diseases.

Five-year view

Research on apoptosis has been evolving rapidly. New discoveries of ligands and receptors on apoptotic cells and phagocytes as well as a greater knowledge of the execution mechanisms of efferocytosis will advance our understanding of disease pathogenesis and provide novel insights into therapeutic strategies. Although animal models and clinical data suggest that defective apoptotic clearance underlies many types of inflammatory lung diseases, our challenge lies in establishing direct evidence of causality between defective efferocytosis and disease etiology. Relevant *in vivo* systems for studying disease models and

therapeutic approaches need to be further developed, along with human studies, to provide practical and directly applicable information.

Key issues

- Apoptotic cell clearance, or efferocytosis, is important in maintaining tissue homeostasis and resolution of inflammation.
- Efferocytosis occurs in three main steps:
 - Recognition of apoptotic cells by phagocytes
 - Engulfment
 - Postengulfment processing
- Efferocytosis is normally so efficient that detection of apoptotic cells may suggest defects in efferocytosis or overwhelming levels of apoptosis.
- Defects in efferocytosis may be associated with autoimmunity and pulmonary diseases, including asthma, chronic obstructive pulmonary disease, cystic fibrosis and acute lung injury/acute respiratory distress syndrome.
- Immunosuppressive consequences of efferocytosis may alter the host immune response against tumors.
- Glucocorticoids, lipoxins, statins, PPAR- γ agonists, macrolide antibiotics and granulocyte-macrophage colony-stimulating factor can enhance efferocytosis and, therefore, may have therapeutic potential to correct such defects.

Acknowledgments

Financial & competing interests disclosure

This work was supported by the NIH HL81151, HL68864, HL88138, GM61031 (to PM Henson), HL66554, P50 HL084945 (Project 1) and Alpha 1 Foundation research grants (to RM Tudor). The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.

No writing assistance was utilized in the production of this manuscript.

References

Papers of special note have been highlighted as:

•of interest

••of considerable interest

1. Blank M, Shiloh Y. Programs for cell death: apoptosis is only one way to go. *Cell Cycle*. 2007; 6(6): 686–695. [PubMed: 17361099]
2. Henson PM, Tudor RM. Apoptosis in the lung: induction, clearance and detection. *Am J Physiol Lung Cell Mol Physiol*. 2008; 294(4):L601–L611. [PubMed: 18178675]
3. Maderna P, Godson C. Phagocytosis of apoptotic cells and the resolution of inflammation. *Biochim Biophys Acta*. 2003; 1639(3):141–151. [PubMed: 14636945]
- 4••. Vandivier RW, Henson PM, Douglas IS. Burying the dead: the impact of failed apoptotic cell removal (efferocytosis) on chronic inflammatory lung disease. *Chest*. 2006; 129(6):1673–1682. Excellent review on efferocytosis and lung diseases. [PubMed: 16778289]

5. Ravichandran KS, Lorenz U. Engulfment of apoptotic cells: signals for a good meal. *Nat Rev Immunol.* 2007; 7(12):964–974. [PubMed: 18037898]
6. Verhoven B, Schlegel RA, Williamson P. Mechanisms of phosphatidylserine exposure, a phagocyte recognition signal, on apoptotic T lymphocytes. *J Exp Med.* 1995; 182(5):1597–1601. [PubMed: 7595231]
7. Williamson P, Schlegel RA. Transbilayer phospholipid movement and the clearance of apoptotic cells. *Biochim Biophys Acta.* 2002; 1585(2–3):53–63. [PubMed: 12531537]
8. Greenberg ME, Sun M, Zhang R, Febbraio M, Silverstein R, Hazen SL. Oxidized phosphatidylserine-CD36 interactions play an essential role in macrophage-dependent phagocytosis of apoptotic cells. *J Exp Med.* 2006; 203(12):2613–2625. [PubMed: 17101731]
9. Vandivier RW, Ogden CA, Fadok VA, et al. Role of surfactant proteins A, D, and C1q in the clearance of apoptotic cells *in vivo* and *in vitro*: calreticulin and CD91 as a common collectin receptor complex. *J Immunol.* 2002; 169(7):3978–3986. Study linking apoptotic clearance and its importance in a relevant model system. [PubMed: 12244199]
10. Gardai SJ, McPhillips KA, Frasch SC, et al. Cell-surface calreticulin initiates clearance of viable or apoptotic cells through trans-activation of LRP on the phagocyte. *Cell.* 2005; 123(2):321–334. [PubMed: 16239148]
11. Ezekowitz RA, Sastry K, Bailly P, Warner A. Molecular characterization of the human macrophage mannose receptor: demonstration of multiple carbohydrate recognition-like domains and phagocytosis of yeasts in Cos-1 cells. *J Exp Med.* 1990; 172(6):1785–1794. [PubMed: 2258707]
12. Weihua Z, Tsan R, Schroit AJ, Fidler IJ. Apoptotic cells initiate endothelial cell sprouting via electrostatic signaling. *Cancer Res.* 2005; 65(24):11529–11535. [PubMed: 16357162]
13. Miyanishi M, Tada K, Koike M, Uchiyama Y, Kitamura T, Nagata S. Identification of Tim4 as a phosphatidylserine receptor. *Nature.* 2007; 450(7168):435–439. [PubMed: 17960135]
14. Park D, Tosello-Trampont AC, Elliott MR, et al. BAI1 is an engulfment receptor for apoptotic cells upstream of the ELMO/Dock180/Rac module. *Nature.* 2007; 450(7168):430–434. [PubMed: 17960134]
15. Park SY, Jung MY, Kim HJ, et al. Rapid cell corpse clearance by stabilin-2, a membrane phosphatidylserine receptor. *Cell Death Differ.* 2007; 15(1):192–201. [PubMed: 17962816]
16. Park SY, Kim SY, Jung MY, Bae DJ, Kim IS. EGF-like domain repeat (EGFrp) of stabilin-2 recognizes phosphatidylserine during cell corpse clearance. *Mol Cell Biol.* 2008; 28(17):5288–5298. [PubMed: 18573870]
17. Fadok VA, Bratton DL, Rose DM, Pearson A, Ezekewitz RA, Henson PM. A receptor for phosphatidylserine-specific clearance of apoptotic cells. *Nature.* 2000; 405(6782):85–90. [PubMed: 10811223]
18. Bose J, Gruber A, Helming L, et al. The phosphatidylserine receptor has essential functions during embryogenesis but not in apoptotic cell removal. *J Biol.* 2004; 3(4):15. [PubMed: 15345036]
19. Schlegel RA, Williamson P. PS to PS (phosphatidylserine) – pertinent proteins in apoptotic cell clearance. *Sci STKE.* 2007; (408):E57.
20. Savill J, Hogg N, Ren Y, Haslett C. Thrombospondin cooperates with CD36 and the vitronectin receptor in macrophage recognition of neutrophils undergoing apoptosis. *J Clin Invest.* 1992; 90(4):1513–1522. [PubMed: 1383273]
21. Hanayama R, Tanaka M, Miwa K, Shinohara A, Iwamatsu A, Nagata S. Identification of a factor that links apoptotic cells to phagocytes. *Nature.* 2002; 417(6885):182–187. [PubMed: 12000961]
22. Ishimoto Y, Ohashi K, Mizuno K, Nakano T. Promotion of the uptake of PS liposomes and apoptotic cells by a product of growth arrest-specific gene, *gas6*. *J Biochem.* 2000; 127(3):411–417. [PubMed: 10731712]
23. Devitt A, Moffatt OD, Raykundalia C, Capra JD, Simmons DL, Gregory CD. Human CD14 mediates recognition and phagocytosis of apoptotic cells. *Nature.* 1998; 392(6675):505–509. [PubMed: 9548256]
24. Platt N, Suzuki H, Kurihara Y, Kodama T, Gordon S. Role for the class A macrophage scavenger receptor in the phagocytosis of apoptotic thymocytes *in vitro*. *Proc Natl Acad Sci USA.* 1996; 93(22):12456–12460. [PubMed: 8901603]

25. Ramprasad MP, Fischer W, Witztum JL, Sambrano GR, Quehenberger O, Steinberg D. The 94- to 97-kDa mouse macrophage membrane protein that recognizes oxidized low density lipoprotein and phosphatidylserine-rich liposomes is identical to macrosialin, the mouse homologue of human CD68. *Proc Natl Acad Sci USA*. 1995; 92(21):9580–9584. [PubMed: 7568176]
26. Ogden CA, deCathelineau A, Hoffmann PR, et al. C1q and mannose binding lectin engagement of cell surface calreticulin and CD91 initiates macropinocytosis and uptake of apoptotic cells. *J Exp Med*. 2001; 194(6):781–795. [PubMed: 11560994]
27. Takemura Y, Ouchi N, Shibata R, et al. Adiponectin modulates inflammatory reactions via calreticulin receptor-dependent clearance of early apoptotic bodies. *J Clin Invest*. 2007; 117(2): 375–386. [PubMed: 17256056]
28. Brown S, Heinisch I, Ross E, Shaw K, Buckley CD, Savill J. Apoptosis disables CD31-mediated cell detachment from phagocytes promoting binding and engulfment. *Nature*. 2002; 418(6894): 200–203. [PubMed: 12110892]
29. Tsai RK, Discher DE. Inhibition of ‘self’ engulfment through deactivation of myosin-II at the phagocytic synapse between human cells. *J Cell Biol*. 2008; 180(5):989–1003. [PubMed: 18332220]
30. Krieser RJ, White K. Engulfment mechanism of apoptotic cells. *Curr Opin Cell Biol*. 2002; 14(6): 734–738. [PubMed: 12473347]
31. Nobes CD, Hall A. Rho, Rac, and Cdc42 GTPases regulate the assembly of multimolecular focal complexes associated with actin stress fibers, lamellipodia, and filopodia. *Cell*. 1995; 81(1):53–62. [PubMed: 7536630]
32. Leverrier Y, Ridley AJ. Requirement for Rho GTPases and PI 3-kinases during apoptotic cell phagocytosis by macrophages. *Curr Biol*. 2001; 11(3):195–199. [PubMed: 11231156]
33. Tosello-Tramont AC, Nakada-Tsukui K, Ravichandran KS. Engulfment of apoptotic cells is negatively regulated by Rho-mediated signaling. *J Biol Chem*. 2003; 278(50):49911–49919. [PubMed: 14514696]
34. Morimoto K, Janssen WJ, Fessler MB, et al. Lovastatin enhances clearance of apoptotic cells (efferocytosis) with implications for chronic obstructive pulmonary disease. *J Immunol*. 2006; 176(12):7657–7665. [PubMed: 16751413]
35. Huynh ML, Fadok VA, Henson PM. Phosphatidylserine-dependent ingestion of apoptotic cells promotes TGF- β 1 secretion and the resolution of inflammation. *J Clin Invest*. 2002; 109(1):41–50. [PubMed: 11781349]
36. Voll RE, Herrmann M, Roth EA, Stach C, Kalden JR, Girkontaite I. Immunosuppressive effects of apoptotic cells. *Nature*. 1997; 390(6658):350–351. [PubMed: 9389474]
37. Gao Y, Herndon JM, Zhang H, Griffith TS, Ferguson TA. Antiinflammatory effects of CD95 ligand (FasL)-induced apoptosis. *J Exp Med*. 1998; 188(5):887–896. [PubMed: 9730890]
38. Chen W, Frank ME, Jin W, Wahl SM. TGF- β released by apoptotic T cells contributes to an immunosuppressive milieu. *Immunity*. 2001; 14(6):715–725. [PubMed: 11420042]
39. Fadok VA, Bratton DL, Konowal A, Freed PW, Westcott JY, Henson PM. Macrophages that have ingested apoptotic cells *in vitro* inhibit proinflammatory cytokine production through autocrine/paracrine mechanisms involving TGF- β , PGE2, and PAF. *J Clin Invest*. 1998; 101(4):890–898. Paper describing the anti-inflammatory consequences of apoptotic cell clearance. [PubMed: 9466984]
40. Kim S, Elkon KB, Ma X. Transcriptional suppression of interleukin-12 gene expression following phagocytosis of apoptotic cells. *Immunity*. 2004; 21(5):643–653. [PubMed: 15539151]
41. Kim S, Elkon KB, Ma X. Transcriptional suppression of interleukin-12 gene expression following phagocytosis of apoptotic cells. *Immunity*. 2004; 21(5):643–653. [PubMed: 15539151]
42. Cvetanovic M, Ucker DS. Innate immune discrimination of apoptotic cells: repression of proinflammatory macrophage transcription is coupled directly to specific recognition. *J Immunol*. 2004; 172(2):880–889. [PubMed: 14707059]
43. Lucas M, Stuart LM, Zhang A, et al. Requirements for apoptotic cell contact in regulation of macrophage responses. *J Immunol*. 2006; 177(6):4047–4054. [PubMed: 16951368]
44. Dini L, Lentini A, Diez GD, et al. Phagocytosis of apoptotic bodies by liver endothelial cells. *J Cell Sci*. 1995; 108(Pt 3):967–973. [PubMed: 7622623]

45. Hall SE, Savill JS, Henson PM, Haslett C. Apoptotic neutrophils are phagocytosed by fibroblasts with participation of the fibroblast vitronectin receptor and involvement of a mannose/fucose-specific lectin. *J Immunol.* 1994; 153(7):3218–3227. [PubMed: 7522254]
46. Kolb S, Vranckx R, Huisse MG, Michel JB, Meilhac O. The phosphatidylserine receptor mediates phagocytosis by vascular smooth muscle cells. *J Pathol.* 2007; 212(3):249–259. [PubMed: 17534843]
47. Walsh GM, Sexton DW, Blaylock MG, Convery CM. Resting and cytokine-stimulated human small airway epithelial cells recognize and engulf apoptotic eosinophils. *Blood.* 1999; 94(8):2827–2835. [PubMed: 10515886]
48. Monks J, Rosner D, Jon Geske F, et al. Epithelial cells as phagocytes: apoptotic epithelial cells are engulfed by mammary alveolar epithelial cells and repress inflammatory mediator release. *Cell Death Differ.* 2005; 12(2):107–114. [PubMed: 15647754]
49. Golpon HA, Fadok VA, Taraseviciene-Stewart L, et al. Life after corpse engulfment: phagocytosis of apoptotic cells leads to VEGF secretion and cell growth. *FASEB J.* 2004; 18(14):1716–1718. [PubMed: 15345697]
50. Morimoto K, Amano H, Sonoda F, et al. Alveolar macrophages that phagocytose apoptotic neutrophils produce hepatocyte growth factor during bacterial pneumonia in mice. *Am J Respir Cell Mol Biol.* 2001; 24(5):608–615. [PubMed: 11350831]
51. Michlewska S, McColl A, Rossi AG, Megson IL, Dransfield I. Clearance of dying cells and autoimmunity. *Autoimmunity.* 2007; 40(4):267–273. [PubMed: 17516208]
52. Ren Y, Tang J, Mok MY, Chan AW, Wu A, Lau CS. Increased apoptotic neutrophils and macrophages and impaired macrophage phagocytic clearance of apoptotic neutrophils in systemic lupus erythematosus. *Arthritis Rheum.* 2003; 48(10):2888–2897. [PubMed: 14558095]
53. O'Brien BA, Fieldus WE, Field CJ, Finegood DT. Clearance of apoptotic β -cells is reduced in neonatal autoimmune diabetes-prone rats. *Cell Death Differ.* 2002; 9(4):457–464. [PubMed: 11965499]
54. Botto M, Dell'Agnola C, Bygrave AE, et al. Homozygous C1q deficiency causes glomerulonephritis associated with multiple apoptotic bodies. *Nat Genet.* 1998; 19(1):56–59. [PubMed: 9590289]
55. Kasahara Y, Tudor RM, Cool CD, Lynch DA, Flores SC, Voelkel NF. Endothelial cell death and decreased expression of vascular endothelial growth factor and vascular endothelial growth factor receptor 2 in emphysema. *Am J Respir Crit Care Med.* 2001; 163(3 Pt 1):737–744. [PubMed: 11254533]
56. Tudor RM, Petrache I, Elias JA, Voelkel NF, Henson PM. Apoptosis and emphysema: the missing link. *Am J Respir Cell Mol Biol.* 2003; 28(5):551–554. [PubMed: 12707010]
57. Demedts IK, Demoor T, Bracke KR, Joos GF, Brusselle GG. Role of apoptosis in the pathogenesis of COPD and pulmonary emphysema. *Respir Res.* 2006; 7:53. [PubMed: 16571143]
58. Tang K, Rossiter HB, Wagner PD, Breen EC. Lung-targeted VEGF inactivation leads to an emphysema phenotype in mice. *J Appl Physiol.* 2004; 97(4):1559–1566. [PubMed: 15208295]
59. Kasahara Y, Tudor RM, Taraseviciene-Stewart L, et al. Inhibition of VEGF receptors causes lung cell apoptosis and emphysema. *J Clin Invest.* 2000; 106(11):1311–1319. [PubMed: 11104784]
60. Tudor RM, Zhen L, Cho CY, et al. Oxidative stress and apoptosis interact and cause emphysema due to vascular endothelial growth factor receptor blockade. *Am J Respir Cell Mol Biol.* 2003; 29(1):88–97. [PubMed: 12600822]
61. Aoshiba K, Yokohori N, Nagai A. Alveolar wall apoptosis causes lung destruction and emphysematous changes. *Am J Respir Cell Mol Biol.* 2003; 28(5):555–562. [PubMed: 12707011]
62. Zheng T, Kang MJ, Crothers K, et al. Role of cathepsin S-dependent epithelial cell apoptosis in IFN- γ -induced alveolar remodeling and pulmonary emphysema. *J Immunol.* 2005; 174(12):8106–8115. [PubMed: 15944319]
63. Petrache I, Natarajan V, Zhen L, et al. Ceramide upregulation causes pulmonary cell apoptosis and emphysema-like disease in mice. *Nat Med.* 2005; 11(5):491–498. [PubMed: 15852018]
64. Calabrese F, Giacometti C, Beghe B, et al. Marked alveolar apoptosis/proliferation imbalance in end-stage emphysema. *Respir Res.* 2005; 6(1):14. [PubMed: 15705190]

65. Imai K, Mercer BA, Schulman LL, Sonett JR, D'Armiento JM. Correlation of lung surface area to apoptosis and proliferation in human emphysema. *Eur Respir J*. 2005; 25(2):250–258. [PubMed: 15684288]
66. Yokohori N, Aoshiba K, Nagai A. Increased levels of cell death and proliferation in alveolar wall cells in patients with pulmonary emphysema. *Chest*. 2004; 125(2):626–632. Clinical evidence of impaired apoptotic clearance in chronic obstructive pulmonary disease patients. [PubMed: 14769747]
67. Hodge S, Hodge G, Scicchitano R, Reynolds PN, Holmes M. Alveolar macrophages from subjects with chronic obstructive pulmonary disease are deficient in their ability to phagocytose apoptotic airway epithelial cells. *Immunol Cell Biol*. 2003; 81(4):289–296. [PubMed: 12848850]
68. Hodge S, Hodge G, Ahern J, Jersmann H, Holmes M, Reynolds PN. Smoking alters alveolar macrophage recognition and phagocytic ability: implications in chronic obstructive pulmonary disease. *Am J Respir Cell Mol Biol*. 2007; 37(6):748–755. [PubMed: 17630319]
69. Richens, TR.; Linderman, DJ.; Xiao, YQ., et al. Cigarette smoke impairs removal of apoptotic cells (efferocytosis) through oxidant-dependent activation of RhoA. Presented at: American Thoracic Society International Conference.; Toronto, Canada. 16–21 May 2008;
70. Sims M, Tal-Singer R, Kierstein S, et al. Chronic obstructive pulmonary disease and inhaled steroids alter surfactant protein D (SP-D) levels: a cross-sectional study. *Respir Res*. 2008; 9(1): 13. [PubMed: 18226251]
71. Clark H, Palaniyar N, Strong P, Edmondson J, Hawgood S, Reid KBM. Surfactant protein D reduces alveolar macrophage apoptosis *in vivo*. *J Immunol*. 2002; 169(6):2892–2899. [PubMed: 12218102]
72. Vandivier RW, Fadok VA, Ogden CA, et al. Impaired clearance of apoptotic cells from cystic fibrosis airways. *Chest*. 2002; 121(3 Suppl):S89. Efferocytosis defects in cystic fibrosis.
73. Vandivier RW, Fadok VA, Hoffmann PR, et al. Elastase-mediated phosphatidylserine receptor cleavage impairs apoptotic cell clearance in cystic fibrosis and bronchiectasis. *J Clin Invest*. 2002; 109(5):661–670. [PubMed: 11877474]
74. Gomez MI, Prince A. Opportunistic infections in lung disease: pseudomonas infections in cystic fibrosis. *Curr Opin Pharmacol*. 2007; 7(3):244–251. [PubMed: 17418640]
75. Allen L, Dockrell DH, Pattery T, et al. Pyocyanin production by *Pseudomonas aeruginosa* induces neutrophil apoptosis and impairs neutrophil-mediated host defenses *in vivo*. *J Immunol*. 2005; 174(6):3643–3649. [PubMed: 15749902]
76. Bianchi SM, Prince LR, McPhillips K, et al. Impairment of apoptotic cell engulfment by pyocyanin, a toxic metabolite of *Pseudomonas aeruginosa*. *Am J Respir Crit Care Med*. 2008; 177(1):35–43. [PubMed: 17916805]
77. Ras GJ, Anderson R, Taylor GW, et al. Proinflammatory interactions of pyocyanin and 1-hydroxyphenazine with human neutrophils *in vitro*. *J Infect Dis*. 1990; 162(1):178–185. [PubMed: 2162367]
78. Hogan SP, Rosenberg HF, Moqbel R, et al. Eosinophils: biological properties and role in health and disease. *Clin Exp Allergy*. 2008; 38(5):709–750. [PubMed: 18384431]
79. Woolley KL, Gibson PG, Carty K, Wilson AJ, Twaddell SH, Woolley MJ. Eosinophil apoptosis and the resolution of airway inflammation in asthma. *Am J Respir Crit Care Med*. 1996; 154(1): 237–243. [PubMed: 8680686]
80. Kankaanranta H, Lindsay MA, Gienbycz MA, Zhang X, Moilanen E, Barnes PJ. Delayed eosinophil apoptosis in asthma. *J Allergy Clin Immunol*. 2000; 106(1 Pt 1):77–83. [PubMed: 10887309]
81. Tai PC, Sun L, Spry CJ. Effects of IL-5, granulocyte/macrophage colony-stimulating factor (GM-CSF) and IL-3 on the survival of human blood eosinophils *in vitro*. *Clin Exp Immunol*. 1991; 85(2):312–316. [PubMed: 1864012]
82. Valerius T, Repp R, Kalden JR, Platzer E. Effects of IFN on human eosinophils in comparison with other cytokines. A novel class of eosinophil activators with delayed onset of action. *J Immunol*. 1990; 145(9):2950–2958. [PubMed: 1698867]

83. Huynh ML, Malcolm KC, Kotaru C, et al. Defective apoptotic cell phagocytosis attenuates prostaglandin E2 and 15-hydroxyeicosatetraenoic acid in severe asthma alveolar macrophages. *Am J Respir Crit Care Med*. 2005; 172(8):972–979. [PubMed: 16040786]
84. Takai D, Nagase T, Shimizu T. New therapeutic key for cystic fibrosis: a role for lipoxins. *Nat Immunol*. 2004; 5(4):357–358. [PubMed: 15052263]
85. Kollef MH, Schuster DP. The acute respiratory distress syndrome. *N Engl J Med*. 1995; 332(1): 27–37. [PubMed: 7646623]
86. Matute-Bello G, Liles WC, Steinberg KP, et al. Soluble Fas ligand induces epithelial cell apoptosis in humans with acute lung injury (ARDS). *J Immunol*. 1999; 163(4):2217–2225. [PubMed: 10438964]
87. Tang PS, Mura M, Seth R, Liu M. Acute lung injury and cell death: how many ways can cells die? *Am J Physiol Lung Cell Mol Physiol*. 2008; 294(4):L632–L641. [PubMed: 18203816]
88. Haslett C. Granulocyte apoptosis and its role in the resolution and control of lung inflammation. *Am J Respir Crit Care Med*. 1999; 160(5 Pt 2):S5–S11. [PubMed: 10556161]
89. Lesur O, Kokis A, Hermans C, Fulop T, Bernard A, Lane D. Interleukin-2 involvement in early acute respiratory distress syndrome: relationship with polymorphonuclear neutrophil apoptosis and patient survival. *Crit Care Med*. 2000; 28(12):3814–3822. [PubMed: 11153619]
90. Fowler AA, Hyers TM, Fisher BJ, et al. The adult respiratory distress syndrome. Cell populations and soluble mediators in the air spaces of patients at high risk. *Am Rev Respir Dis*. 1987; 136(5): 1225–1231. [PubMed: 3674583]
91. Teder P, Vandivier RW, Jiang D, et al. Resolution of lung inflammation by CD44. *Science*. 2002; 296(5565):155–158. [PubMed: 11935029]
92. Hart SP, Dougherty GJ, Haslett C, Dransfield I. CD44 regulates phagocytosis of apoptotic neutrophil granulocytes, but not apoptotic lymphocytes, by human macrophages. *J Immunol*. 1997; 159:919–925. [PubMed: 9218612]
93. McKee CM, Penno MB, Cowman M, et al. Hyaluronan (HA) fragments induce chemokine gene expression in alveolar macrophages. The role of HA size and CD44. *J Clin Invest*. 1996; 98(10): 2403–2413. [PubMed: 8941660]
94. Hussain N, Wu F, Zhu L, Thrall RS, Kresch MJ. Neutrophil apoptosis during the development and resolution of oleic acid-induced acute lung injury in the rat. *Am J Respir Cell Mol Biol*. 1998; 19:867–874. [PubMed: 9843920]
95. Wang L, Medan D, Mercer R, et al. Role of neutrophil apoptosis in vanadium-induced pulmonary inflammation in mice. *J Environ Pathol Toxicol Oncol*. 2002; 21(4):343–350. [PubMed: 12510963]
96. Cox G, Crossley J, Xing Z. Macrophage engulfment of apoptotic neutrophils contributes to the resolution of acute pulmonary inflammation *in vivo*. *Am J Respir Cell Mol Biol*. 1995; 12(2):232–237. [PubMed: 7865221]
97. Medan D, Wang L, Yang X, Dokka S, Castranova V, Rojanasakul Y. Induction of neutrophil apoptosis and secondary necrosis during endotoxin-induced pulmonary inflammation in mice. *J Cell Physiol*. 2002; 191(3):320–326. [PubMed: 12012327]
98. Joseph B, Lewensohn R, Zhivotovsky B. Role of apoptosis in the response of lung carcinomas to anti-cancer treatment. *Ann NY Acad Sci*. 2000; 926:204–216. [PubMed: 11193036]
99. Nachmias B, Ashhab Y, Ben Yehuda D. The inhibitor of apoptosis protein family (IAPs): an emerging therapeutic target in cancer. *Semin Cancer Biol*. 2004; 14(4):231–243. [PubMed: 15219616]
100. Shivapurkar N, Reddy J, Chaudhary PM, Gazdar AF. Apoptosis and lung cancer: a review. *J Cell Biochem*. 2003; 88(5):885–898. [PubMed: 12616528]
101. Krysko DV, Vandenabeele P. From regulation of dying cell engulfment to development of anti-cancer therapy. *Cell Death Differ*. 2008; 15(1):29–38. [PubMed: 18007662]
102. Sauter B, Albert ML, Francisco L, Larsson M, Somersan S, Bhardwaj N. Consequences of cell death: exposure to necrotic tumor cells, but not primary tissue cells or apoptotic cells, induces the maturation of immunostimulatory dendritic cells. *J Exp Med*. 2000; 191(3):423–434. [PubMed: 10662788]

103. Bondanza A, Zimmermann VS, Rovere-Querini P, et al. Inhibition of phosphatidylserine recognition heightens the immunogenicity of irradiated lymphoma cells *in vivo*. *J Exp Med*. 2004; 200(9):1157–1165. [PubMed: 15504819]
104. Popovic ZV, Sandhoff R, Sijmonsma TP, et al. Sulfated glycosphingolipid as mediator of phagocytosis: sm4s enhances apoptotic cell clearance and modulates macrophage activity. *J Immunol*. 2007; 179(10):6770–6782. [PubMed: 17982067]
105. Morichika H, Hamanaka Y, Tai T, Ishizuka I. Sulfatides as a predictive factor of lymph node metastasis in patients with colorectal adenocarcinoma. *Cancer*. 1996; 78(1):43–47. [PubMed: 8646725]
106. Ward C, Dransfield I, Chilvers ER, Haslett C, Rossi AG. Pharmacological manipulation of granulocyte apoptosis: potential therapeutic targets. *Trends Pharmacol Sci*. 1999; 20(12):503–509. [PubMed: 10603493]
107. Ren Y, Silverstein RL, Allen J, Savill J. CD36 gene transfer confers capacity for phagocytosis of cells undergoing apoptosis. *J Exp Med*. 1995; 181(5):1857–1862. [PubMed: 7536797]
108. Meagher LC, Cousin JM, Seckl JR, Haslett C. Opposing effects of glucocorticoids on the rate of apoptosis in neutrophilic and eosinophilic granulocytes. *J Immunol*. 1996; 156(11):4422–4428. [PubMed: 8666816]
109. Liu Y, Cousin JM, Hughes J, et al. Glucocorticoids promote nonphlogistic phagocytosis of apoptotic leukocytes. *J Immunol*. 1999; 162(6):3639–3646. [PubMed: 10092825]
110. Giles KM, Ross K, Rossi AG, Hotchin NA, Haslett C, Dransfield I. Glucocorticoid augmentation of macrophage capacity for phagocytosis of apoptotic cells is associated with reduced p130Cas expression, loss of paxillin/pyk2 phosphorylation, and high levels of active Rac. *J Immunol*. 2001; 167(2):976–986. [PubMed: 11441106]
111. Brady HR, Serhan CN. Lipoxins: putative braking signals in host defense, inflammation and hypersensitivity. *Curr Opin Nephrol Hypertens*. 1996; 5(1):20–27. [PubMed: 8834158]
112. Lee TH, Horton CE, Kyan-Aung U, Haskard D, Crea AE, Spur BW. Lipoxin A4 and lipoxin B4 inhibit chemotactic responses of human neutrophils stimulated by leukotriene B4 and *N*-formyl-L-methionyl-L-leucyl-L-phenylalanine. *Clin Sci (Lond)*. 1989; 77(2):195–203. [PubMed: 2548801]
113. Soyombo O, Spur BW, Lee TH. Effects of lipoxin A4 on chemotaxis and degranulation of human eosinophils stimulated by platelet-activating factor and *N*-formyl-L-methionyl-L-leucyl-L-phenylalanine. *Allergy*. 1994; 49(4):230–234. [PubMed: 8037356]
114. Levy BD, De Sanctis GT, Devchand PR, et al. Multi-pronged inhibition of airway hyper-responsiveness and inflammation by lipoxin A(4). *Nat Med*. 2002; 8(9):1018–1023. [PubMed: 12172542]
115. Karp CL, Flick LM, Park KW, et al. Defective lipoxin-mediated anti-inflammatory activity in the cystic fibrosis airway. *Nat Immunol*. 2004; 5(4):388–392. [PubMed: 15034576]
116. Godson C, Mitchell S, Harvey K, Petasis NA, Hogg N, Brady HR. Cutting edge: lipoxins rapidly stimulate nonphlogistic phagocytosis of apoptotic neutrophils by monocyte-derived macrophages. *J Immunol*. 2000; 164(4):1663–1667. [PubMed: 10657608]
117. Mitchell S, Thomas G, Harvey K, et al. Lipoxins, aspirin-triggered epi-lipoxins, lipoxin stable analogues, and the resolution of inflammation: stimulation of macrophage phagocytosis of apoptotic neutrophils *in vivo*. *J Am Soc Nephrol*. 2002; 13(10):2497–2507. [PubMed: 12239238]
118. Maderna P, Cottell DC, Berlasconi G, Petasis NA, Brady HR, Godson C. Lipoxins induce actin reorganization in monocytes and macrophages but not in neutrophils: differential involvement of Rho GTPases. *Am J Pathol*. 2002; 160(6):2275–2283. [PubMed: 12057930]
119. Reville K, Crean JK, Vivers S, Dransfield I, Godson C. Lipoxin A4 redistributes myosin iia and cdc42 in macrophages: implications for phagocytosis of apoptotic leukocytes. *J Immunol*. 2006; 176(3):1878–1888. [PubMed: 16424219]
120. Olazabal IM, Caron E, May RC, Schilling K, Knecht DA, Machesky LM. Rho-kinase and myosin-II control phagocytic cup formation during CR, but not FcγR, phagocytosis. *Curr Biol*. 2002; 12(16):1413–1418. [PubMed: 12194823]
121. Luo F. Simvastatin induces eosinophil apoptosis *in vitro*. *Chest*. 2004; 126(4):S721.

122. Newton CJ, Ran G, Xie YX, et al. Statin-induced apoptosis of vascular endothelial cells is blocked by dexamethasone. *J Endocrinol.* 2002; 174(1):7–16. [PubMed: 12098658]
123. Jacobson JR, Barnard JW, Grigoryev DN, Ma SF, Tudor RM, Garcia JGN. Simvastatin attenuates vascular leak and inflammation in murine inflammatory lung injury. *Am J Physiol Lung Cell Mol Physiol.* 2005; 288(6):L1026–L1032. [PubMed: 15665042]
124. Chiba Y, Arima J, Sakai H, Misawa M. Lovastatin inhibits bronchial hyperresponsiveness by reducing RhoA signaling in rat allergic asthma. *Am J Physiol Lung Cell Mol Physiol.* 2008; 294(4):L705–L713. [PubMed: 18296496]
125. McKay A, Leung BP, McInnes IB, Thomson NC, Liew FY. A novel anti-inflammatory role of simvastatin in a murine model of allergic asthma. *J Immunol.* 2004; 172(5):2903–2908. [PubMed: 14978092]
126. Lee JH, Lee DS, Kim EK, et al. Simvastatin inhibits cigarette smoking-induced emphysema and pulmonary hypertension in rat lungs. *Am J Respir Crit Care Med.* 2005; 172(8):987–993. [PubMed: 16002570]
127. Mancini GBJ, Etminan M, Zhang B, Levesque LE, FitzGerald JM, Brophy JM. Reduction of morbidity and mortality by statins, angiotensin-converting enzyme inhibitors, and angiotensin receptor blockers in patients with chronic obstructive pulmonary disease. *J Am Coll Cardiol.* 2006; 47(12):2554–2560. [PubMed: 16781387]
128. Soyseth V, Brekke PH, Smith P, Omland T. Statin use is associated with reduced mortality in COPD. *Eur Respir J.* 2007; 29(2):279–283. [PubMed: 17050558]
129. Standiford TJ, Keshamouni VG, Reddy RC. Peroxisome proliferator-activated receptor- γ as a regulator of lung inflammation and repair. *Proc Am Thorac Soc.* 2005; 2(3):226–231. [PubMed: 16222042]
130. Hammad H, de Heer HJ, Soullie T, et al. Activation of peroxisome proliferator-activated receptor- γ in dendritic cells inhibits the development of eosinophilic airway inflammation in a mouse model of asthma. *Am J Pathol.* 2004; 164(1):263–271. [PubMed: 14695339]
131. Liu D, Zeng BX, Zhang SH, Yao SL. Rosiglitazone, an agonist of peroxisome proliferator-activated receptor γ , reduces pulmonary inflammatory response in a rat model of endotoxemia. *Inflamm Res.* 2005; 54(11):464–470. [PubMed: 16307220]
132. Ward JE, Fernandes DJ, Taylor CC, Bonacci JV, Quan L, Stewart AG. The PPAR γ ligand, rosiglitazone, reduces airways hyperresponsiveness in a murine model of allergen-induced inflammation. *Pulm Pharmacol Ther.* 2006; 19(1):39–46. [PubMed: 16286236]
133. Straus DS, Glass CK. Anti-inflammatory actions of PPAR ligands: new insights on cellular and molecular mechanisms. *Trends Immunol.* 2007; 28(12):551–558. [PubMed: 17981503]
134. Asada K, Sasaki S, Suda T, Chida K, Nakamura H. Antiinflammatory roles of peroxisome proliferator-activated receptor- γ in human alveolar macrophages. *Am J Respir Crit Care Med.* 2004; 169(2):195–200. [PubMed: 14563653]
135. Majai G, Sarang Z, Csomos K, Zahuczky G, Fesus L. PPAR γ -dependent regulation of human macrophages in phagocytosis of apoptotic cells. *Eur J Immunol.* 2007; 37(5):1343–1354. [PubMed: 17407194]
136. Hodge S, Hodge G, Brozyna S, Jersmann H, Holmes M, Reynolds PN. Azithromycin increases phagocytosis of apoptotic bronchial epithelial cells by alveolar macrophages. *Eur Respir J.* 2006; 28(3):486–495. [PubMed: 16737992]
137. Yamaryo T, Oishi K, Yoshimine H, Tsuchihashi Y, Matsushima K, Nagatake T. Fourteen-member macrolides promote the phosphatidylserine receptor-dependent phagocytosis of apoptotic neutrophils by alveolar macrophages. *Antimicrob Agents Chemother.* 2003; 47(1):48–53. [PubMed: 12499168]
138. Hodge S, Hodge G, Jersmann H, et al. azithromycin improves macrophage phagocytic function and expression of mannose receptor in chronic obstructive pulmonary disease. *Am J Respir Crit Care Med.* 2008; 178(2):139–148. [PubMed: 18420960]
139. Ren Y, Savill J. Proinflammatory cytokines potentiate thrombospondin-mediated phagocytosis of neutrophils undergoing apoptosis. *J Immunol.* 1995; 154(5):2366–2374. [PubMed: 7532668]

140. Jinushi M, Nakazaki Y, Dougan M, Carrasco DR, Mihm M, Dranoff G. MFG-E8-mediated uptake of apoptotic cells by APCs links the pro- and antiinflammatory activities of GM-CSF. *J Clin Invest*. 2007; 117(7):1902–1913. [PubMed: 17557120]
141. Enzler T, Gillessen S, Manis JP, et al. Deficiencies of GM-CSF and interferon- γ link inflammation and cancer. *J Exp Med*. 2003; 197(9):1213–1219. [PubMed: 12732663]
142. Galati G, Rovere P, Citterio G, et al. *In vivo* administration of GM-CSF promotes the clearance of apoptotic cells: effects on monocytes and polymorphonuclear leukocytes. *J Leukoc Biol*. 2000; 67(2):174–182. [PubMed: 10670577]

Figure 1. Image of efferocytosis in human lung with acute bronchopneumonia
(A) An apoptotic cell is observed attached to an alveolar macrophage (arrow). (B) An alveolar macrophage has nuclear debris, probably reflecting apoptotic debris (arrow).

Figure 2. Efferocytotic process with selected molecules.

(A) Apoptotic cell has 'eat me' signals, such as phosphatidyl serine and calreticulin. CD31, which belongs to the 'do not eat me' signals, is disabled. MFG-E8, $\beta 2$ GPI, MBL, C1q and SP-D are bridging molecules linking the apoptotic cell and phagocyte. Vitronectin, Tim4, Mer, BAI1, $\beta 2$ GPI R, calreticulin, CD91, CD14, CD36, SRA and CD68 are receptors that recognize bridging molecules or ligands on the apoptotic cell. (B) CRKII, DOCK180 and ELMO signaling regulate actin cytoskeleton rearrangements. PS recognition by the receptor activates Rac-1, which induces membrane ruffling for efferocytosis. RhoA is a negative regulator of apoptotic clearance. (C) After apoptotic cell uptake, phagocytic cells secrete anti-inflammatory and immunosuppressive cytokines.

β 2 GPI: β 2 glycoprotein I; β 2 GPI R: β 2 glycoprotein I receptor; ACAMP: Apoptotic cell-associated molecular pattern; BAI: Brain-specific angiogenesis inhibitor; CD: Cluster of differentiation; DOCK: Dedicator of cytokinesis; ELMO: Engulfment and cell motility; Gas: Growth-arrest specific; MBL: Mannose-binding lectin; MFG: Milk fat globule; PS: Phosphatidylserine; SP: Surfactant protein; SRA: Class A scavenger receptor.

Table 1

Efferocytosis defects in inflammatory lung diseases.

Disease	Efferocytosis defects	Ref.
COPD	Apoptotic cell-specific phagocytosis defect	[68]
	Worse defect with current smoking COPD patients	[69]
	Oxidative stress in cigarette smoke activates RhoA	[70]
Cystic fibrosis	PS receptor cleavage by neutrophil elastase	[74]
	Oxidative stress from pyocyanin activates Rho	[77,78]
Asthma	Decreased efferocytosis with improvement with steroid treatment	[84]
Acute lung injury/acute respiratory distress syndrome	Increased bleomycin-induced lung injury in <i>CD44</i> ^{-/-} mice	[94]
	Increased lipopolysaccharide-induced inflammation in PS liposome-treated mice	[98]

COPD: Chronic obstructive pulmonary disease; PS: Phosphatidylserine.