Granulocyte apoptosis in the pathogenesis and resolution of lung disease

Stephen M. Bianchi†, David H. Dockrell†, Stephen A. Renshaw†, Ian Sabroe* and Moira K. B. Whyte*

*Academic Unit of Respiratory Medicine, Division of Genomic Medicine, University of Sheffield, M Floor, Royal Hallamshire Hospital, Sheffield S10 2JF, U.K., and †Academic Unit of Infectious Diseases, Division of Genomic Medicine, University of Sheffield, M Floor, Royal Hallamshire Hospital, Sheffield S10 2JF, U.K.

ABSTRACT

Apoptosis, programmed cell death, of neutrophil and eosinophil granulocytes is a potential control point in the physiological resolution of innate immune responses. There is also increasing evidence that cellular processes of apoptosis can be dysregulated by pathogens as a mechanism of immune evasion and that delayed apoptosis, resulting in prolonged inflammatory cell survival, is important in persistence of tissue inflammation. The identification of cell-type specific pathways to apoptosis may allow the design of novel anti-inflammatory therapies or agents to augment the innate immune responses to infection. This review will explore the physiological roles of granulocyte apoptosis and their importance in infectious and non-infectious lung disease.

INTRODUCTION

Apoptosis (programmed cell death) is essential for tissue homeostasis and for normal development in multicellular organisms. It is an energy-dependent process in which cells execute an active suicide programme, allowing removal of senescent cells or those whose continued survival is contrary to the overall survival strategy of the organism, e.g. cells undergoing malignant transformation. Apoptosis is classically held to be a ‘silent’ process [1], where removal of the target cells is without adverse consequences and proceeds unnoticed by the immune system, although it is becoming apparent that apoptosis is often far from silent. The induction of apoptosis is tightly choreographed by the cell and results in a series of cell- or stimulus-specific biochemical and morphological changes. This careful regulation of apoptosis emphasizes the considerable capacity for pathological perturbation of this process, and an ever-increasing number of medical conditions are identified where dysregulated apoptosis contributes to disease pathogenesis. In general, alterations in apoptosis result either from failure of apoptosis induction, leading to inappropriate accumulation of a specific cell population, e.g. in malignancy, or from enhanced susceptibility to apoptosis, resulting in inappropriate cell loss, e.g. neurodegenerative diseases and HIV infection [2].

The key role of apoptosis in the resolution of tissue inflammation was first appreciated by Savill et al. [3]. They described the ability of the shortest-lived leucocyte, the neutrophil, to undergo a constitutive programme of apoptosis [3] and postulated this might represent a

Key words: apoptosis, eosinophil, granulocyte, inflammation, lung disease, neutrophil.

Abbreviations: BALF, bronchoalveolar lavage fluid; BEC, bronchial epithelial cell; DISC, death-induced signalling complex; ERK, extracellular-signal-regulated kinase; FADD, Fas-associated death domain; FasL, Fas ligand; G-CSF, granulocyte colony-stimulating factor; GM-CSF, granulocyte/macrophage colony-stimulating factor; IAP, inhibitor of apoptosis protein; IFN, interferon; IL, interleukin; IκB, inhibitory κB; LPS, lipopolysaccharide; MAPK, mitogen-activated protein kinase; MOMP, mitochondrial outer membrane permeabilization; Mst, mammalian sterile 20-like; NF-κB, nuclear factor κB; NO, nitric oxide; PI3K, phosphoinositide 3-kinase; PKA, protein kinase A; PKC, protein kinase C; PS, phosphatidylserine; RDS, respiratory distress syndrome; ARDS, acute RDS; ROS, reactive oxygen species; SH2, Src homology domain 2; TLR, Toll-like receptor; TNF, tumour necrosis factor; TRAIL, TNF-related apoptosis-inducing ligand; XIAP, X-linked IAP.

Correspondence: Professor Moira K. B. Whyte (email m.k.whyte@sheffield.ac.uk).
physiological mechanism for clearance of inflammatory cells from tissues. Further work has shown that apoptosis leads to down-regulation of neutrophil pro-inflammatory functions [4] and that the balance between neutrophil survival and death by apoptosis is exquisitely regulated by a range of factors, including host-derived cytokines and pathogen-derived molecules [5,6]. In the context of acute inflammation, leucocyte apoptosis must be delayed until essential host functions, such as pathogen clearance, are completed, but then proceed promptly to abrogate inflammation and avoid tissue damage, which could arise from a perpetuated response [7].

**MECHANISMS OF GRANULOCYTE APOPTOSIS**

Apoptosis can be viewed as resulting from activation of either an ‘extrinsic’ or an ‘intrinsic’ pathway to cell death [8] (Figure 1). The ‘extrinsic’ pathway is mediated through ligation of cell-surface death receptors, with formation of a DISC (death-induced signalling complex), involving adaptor proteins such as FADD (Fas-associated death domain) and cleavage of caspase 8 [9]. In contrast, the ‘intrinsic’ or ‘stress’ pathway is mediated through oxidant damage, cytotoxic agents or ultraviolet radiation, leading to MOMP (mitochondrial outer membrane permeabilization), release of small intermembrane proteins, such as cytochrome c and apoptosis-inducing factor, and subsequent activation of caspase 9 or caspase-independent pathways [8,10,11]. Induction of apoptosis via the intrinsic pathway is regulated by members of the Bcl-2 family of proteins. Anti-apoptotic family members, including Bcl-2 itself, inhibit induction of MOMP [11]. In contrast, pro-apoptotic family members can induce MOMP. The latter include both multi-domain family members, such as Bax and Bak, or proteins such as Bid that only contain the BH3 domain [12].

The death receptor and mitochondrial pathways interact to enhance apoptosis in certain cell types; for example, the pro-apoptotic Bcl-2 protein Bid mediates cross-talk by actions of a Bid caspase-cleavage product on MOMP [13]. Both pathways typically culminate in a common pathway with activation of executioner caspases, such as caspase 3, and cleavage of DNA and chromatin [14]. Caspase activation can in turn be inhibited by IAPs (inhibitor of apoptosis proteins) [15].

The resulting apoptotic cell contains one or more apoptotic bodies, comprising condensed nuclear material, and has an intact cell membrane that prevents release of harmful intracellular proteases. Exposure of PS (phosphatidylserine) and other glycosylated aminophospholipids on the outer membrane of the apoptotic cell label it for clearance by phagocytes, particularly, but not exclusively, macrophages [16]. In a further layer of anti-inflammatory regulation, ingestion of apoptotic cells by macrophages results in re-programming of the macrophage to a ‘resolution’ phenotype, down-regulating production of pro-inflammatory cytokines, e.g. TNF (tumour necrosis factor-)α, and up-regulating cytokines that initiate remodelling, e.g. TGF-β (transforming growth factor-β) [17]. In contrast, cells that have not died through the apoptotic pathway are actively pro-inflammatory [18]. The efficiency and capacity of apoptotic cell clearance can be observed in vivo, for example in Streptococcal pneumonia, where tissue architecture is returned to a pre-inflammatory state by induction of apoptosis and clearance of recruited inflammatory cells [19]. However, the consequences of apoptotic cell ingestion are context dependent and, if microbial products are present when the apoptotic cell is ingested by the macrophage, there may be a regulated pro-inflammatory response [20]. Moreover, a previous study [21] suggests macrophages can discriminate between apoptotic cells that have undergone a ‘constitutive’ death programme (thought to correspond to normal senescence in the peripheral blood or bone marrow) and those where apoptosis has been pathogen-induced, and generate a pro-inflammatory response to pathogen-killed neutrophils.

**BIOCHEMICAL FEATURES OF GRANULOCYTE APOPTOSIS**

**Mitochondria and Bcl-2 proteins**

The paucity of mitochondria in neutrophils, together with their dependence upon glycolysis rather than mitochondrial respiration for ATP generation, led some investigators to question the importance of the mitochondrial pathway of apoptosis in neutrophils. However, studies [22,23] have shown that the loss of mitochondrial inner transmembrane potential (Δψm) is an early feature of neutrophil apoptosis. Although neutrophils also contain low levels of cytochrome c, they require only small amounts of this molecule to activate caspases and undergo a mitochondrial-mediated pathway of apoptosis [24].

Bcl-2 family proteins play an important role in determining the susceptibility or resistance of neutrophils to apoptosis. Neutrophils do not express Bcl-2 at the protein level [25,26]. Low levels of Bcl-x are detected in unstimulated neutrophils [26,27], which are increased by GM-CSF (granulocyte/macrophage colony-stimulating factor) stimulation [27]. Mcl-1 appears to be important in maintaining neutrophil viability in response to cytokines [28]. Another anti-apoptotic Bcl-2 protein, A1, may also play a role in maintaining granulocyte viability, since neutrophils from mice lacking A1-a have accelerated constitutive apoptosis and a reduced survival effect of specific pro-survival stimuli and trans-endothelial migration [29,30]. Mcl-1 and A1 have relatively short half-lives and therefore may be particularly important in regulating inducible neutrophil survival in response to pro-inflammatory stimuli [26]. In contrast, pro-apoptotic Bcl-2
Figure 1  Biochemical pathways of granulocyte apoptosis

Granulocyte apoptosis proceeds through two 'major' death pathways: the 'extrinsic' or death-receptor-mediated pathway or the 'intrinsic' or 'stress' pathway. Regulation occurs at the level of the DISC, at the level of Bcl-2 protein insertion into the mitochondrial outer membrane (Mcl-1 and A1) and through inhibition of caspases by IAPs and survivin.
family members, such as Bax, Bak, Bad and Bik, show stable expression over time in neutrophils, reflecting slow rates of turnover [26]. Bax is involved in regulation of constitutive neutrophil apoptosis [31], whereas deletion of the BH3-only protein Bim [32] results in neutrophil accumulation at inflammatory sites and delayed apoptosis.

**Death receptors**

Death receptor signalling can also regulate granulocyte apoptosis. Apoptosis of neutrophils [33,34] and eosinophils [35] can be induced by the archetypal death receptor ligand Fas and macrophage expression of FasL (Fas ligand) can induce bystander cell death in neutrophils [36,37]. However, Fas signalling in the lung is also associated with CXC-chemokine production and thus with neutrophil recruitment as well as induction of apoptosis [38]. TNF-α, also a death receptor ligand, induces neutrophil apoptosis at early time points, but a pro-survival NF-κB (nuclear factor κB)-dependent signal predominates later [39]. TNF-α-induced apoptosis is caspase-dependent, and previous reports which suggested this pathway is caspase-independent [40] are explained by additional actions of the caspase inhibitor employed [41]. Neutrophils express TRAIL (TNF-related apoptosis-inducing ligand) receptors and are sensitive to TRAIL-induced apoptosis [42]. TRAIL-induced neutrophil apoptosis, mediated by autologous T-cells, is a likely mechanism of neutropenia in SLE (systemic lupus erythematosus) [43]. Interestingly, TRAIL lacks the chemotactic effects of FasL, which might imply it is a ‘cleaner’ death ligand and thus a potential therapeutic target [42].

Although there have been contrasting reports on expression of death ligands by granulocytes [32,34,36], the majority of studies have not detected FasL expression on unstimulated neutrophils and neither lpr (Fas-deficient) nor gld (FasL-defective) mice show altered granulocyte apoptosis, arguing against a role for the Fas system in constitutive death [44]. Neutrophil FasL expression can, however, be detected in specific situations, such as influenza infection [45]. Similarly, TRAIL is not expressed extracellularly on unstimulated neutrophils [42], although they can release soluble TRAIL following IFN (interferon) stimulation [46].

**Caspases and other proteases**

As in most cell types, downstream caspases play key roles in granulocyte apoptosis [47]. Caspase 8 is activated by death receptor signalling and caspase 3 is activated as an executioner of further downstream effects. In certain settings caspase 9 may contribute to neutrophil, but not eosinophil, apoptosis, emphasizing further a role for mitochondrial pathways [48]. MOMP requires Bax translocation to the mitochondria prior to caspase 3 activation in neutrophils, as in many other cells [49]. In neutrophils, caspase 3 activation in turn activates PKC (protein kinase C) δ during spontaneous apoptosis [50]. Caspase substrates may differ between granulocytes, for example the Mst (mammalian sterile 20-like) 1/Mst2 kinases are caspase substrates in eosinophils, but not neutrophils [51]. Caspase activation is suppressed by ROS (reactive oxygen species) generation during neutrophil activation [52] but, conversely, patients who have genetic defects in NADPH oxidase and cannot generate ROS have delayed spontaneous neutrophil apoptosis [53], suggesting ROS effects are context specific. IAPs inhibit activated caspases, but their role in granulocytes is uncertain as several, such as cIAP1 (cellular IAP1), XIAP (X-linked IAP) and survivin [24,54], are expressed at very low levels. G-CSF (granulocyte colony-stimulating factor) may up-regulate cIAP2 (cellular IAP2) to promote neutrophil survival [55]. Another family of proteases, the calpains, may play a role in apoptosis induction by degrading XIAP, but the importance of this, in view of the low levels of XIAP in neutrophils, requires clarification [56]. Calpain-1 also plays a direct role in neutrophil apoptosis by enhancing cleavage of Bax to an 18 kDa form that no longer interacts with Bcl-xL upstream of MOMP [57].

**Kinase-regulated signalling pathways**

Multiple signalling pathways are implicated in regulation of granulocyte apoptosis, with studies focused mainly on neutrophils. NF-κB signalling delays constitutive neutrophil apoptosis, but enhances LPS (lipopolysaccharide)-delayed apoptosis [58,59]. Regulation of NF-κB shows unique features in the neutrophil, with a prominent role for nuclear IκB (inhibitory κB) inhibition of NF-κB. Following LPS and TNF-α stimulation, degradation of IκB may allow NF-κB to inhibit apoptosis and prolong pro-inflammatory cytokine production [60]. Prostaglandin D2 selectively induces eosinophil (but not neutrophil) apoptosis and this involves inhibition of IκB degradation [61]. The anti-apoptotic PI3K (phosphoinositide 3-kinase)/Akt pathway is stimulated by pro-survival factors such as GM-CSF, leading to up-regulation of Mcl-1 [28] and down-regulation of the pro-apoptotic functions of Bad, both transcriptionally and by phosphorylation [62], and Bax via phosphorylation [27]. JAK/STAT (signal transducer and activator of transcription) pathways may co-operate with PI3K in this pathway [63]. Activation of the p38 MAPK (mitogen-activated protein kinase) pathway contributes to spontaneous neutrophil apoptosis in some studies [64,65], but in others transient MAPK inhibition was associated with both spontaneous apoptosis and Fas-mediated apoptosis, suggesting it also has pro-survival functions [66]. Cleavage of p38 MAPK following pro-apoptotic stimuli such as TNF-α may explain these findings and may represent a mechanism by which responsiveness to inflammatory cytokines is down-regulated [64]. Pro-survival functions of p38 MAPK include phosphorylation and, therefore, inhibition of
calcium also have a pro-survival effect in neutrophils [73]. Neutrophils [72,23]. Transient increases in intracellular (protein kinase B) may trigger elevation of intracellular β1, which is required for IL-1β important in tissue [80], although mice lacking caspase (interleukin)-1β and is an important pro-survival factor in blood [79]. IL-1β is released by activated endothelial cells be exogenous environmental or infectious stimuli. Of many extracellular factors have been identified that influence granulocyte survival. These may be host-derived or be exogenous environmental or infectious stimuli. Of host-derived factors, the roles of cytokines are perhaps best characterized. G-CSF and GM-CSF are potent pro-survival stimuli, in part through down-regulation of Bax [78]. GM-CSF is released by activated endothelial cells and is an important pro-survival factor in blood [79]. IL-1 (interleukin)-1β inhibition of neutrophil apoptosis may be important in tissue [80], although mice lacking caspase 1, which is required for IL-1β processing, paradoxically demonstrate delayed neutrophil apoptosis following LPS challenge in the lung, perhaps reflecting effects of caspase 1 upon other, pro-apoptotic substrates [81]. IL-15 is also anti-apoptotic for neutrophils [82]. TNF-α can be pro-apoptotic to neutrophils and in vitro this effect is enhanced during phagocytosis [83]. In contrast, TNF-α has a pro-survival effect on neutrophils at later time points, an effect mediated via IL-8 release, IκBα degradation and NF-κB activation [84]. IFN-γ and IL-6 combine to enhance neutrophil apoptosis in animal models of inflammation [85].

Within the lung, both intracellular and extracellular environments are critical regulators of neutrophil survival. The lung is uniquely exposed to oxidant-induced injury through occupational exposures, cigarette smoking and iatrogenic ventilator-induced hyperoxia. Generation of ROS is associated with induction of apoptosis, and production of ROS is preserved until the late stages of neutrophil apoptosis [4]. Patients with chronic granulomatous disease, who cannot produce ROS, show decreased spontaneous and Fas-mediated apoptosis [53]. ROS enhance death receptor signalling by allowing ceramide generation and clustering of Fas in lipid rafts, permitting formation of the DISC and caspase 8 activation in the absence of FasL engagement [86]. The antioxidant glutathione inhibits Fas-mediated apoptosis and its intracellular levels are enhanced by pro-survival stimuli [87]. Oxidants may activate p38 MAPK pathways that inhibit the pro-survival effects of ERK (extracellular-signal-regulated kinase) in maintaining levels of XIAP in neutrophils [88]. The β2 integrin CD11b/CD18 may contribute to neutrophil apoptosis, since phagocytosis of opsonized particles via this receptor induces ROS-dependent apoptosis [89], but CD18-deficient mice have elevated neutrophil counts and reduced apoptosis [90]. NO (nitric oxide) has both pro- and anti-apoptotic effects depending on concentration and flux of NO. NO induces neutrophil apoptosis but may enhance or inhibit eosinophil apoptosis [91].

Intracellular acidification is an early feature of apoptosis and stimulation of the vacuolar (H+) ATPase membrane pump inhibits both apoptosis and intracellular acidification [92]. During phagocytosis of bacteria, intracellular alkalinization delays neutrophil apoptosis, but internalization of larger numbers of bacteria leads to intracellular acidification and increased apoptosis [93]. In contrast, a reduced extracellular pH promotes death by necrosis [93]. Hypoxia promotes neutrophil survival via HIF-1α (hypoxia-inducible factor-1α) and NF-κB activation [94]. Finally, temperature elevation can modify neutrophil apoptosis, notably abrogating TNF-α-mediated delay of apoptosis [95].

Regulation of eosinophil apoptosis

Eosinophil apoptosis is delayed by IL-3, IL-5 and GM-CSF via activation of Lyn and Syk tyrosine kinases [96,97]. Although eosinophils are sensitive to Fas ligation [35,98], Fas does not regulate spontaneous eosinophil apoptosis in purified cell populations [97]. In contrast with neutrophils, ligation of TRAIL receptors can prolong survival in eosinophils [99]. Mitochondria appear to contribute to eosinophil apoptosis and caspase 9 activation is a feature [97]. Spontaneous eosinophil apoptosis is regulated by oxidant-induced mitochondrial injury in a
similar manner to spontaneous neutrophil apoptosis [97]. Spontaneous eosinophil apoptosis is inhibited by hypoxia or following treatment with SOD (superoxide dismutase) mimetics [100].

Eosinophil expression of Bcl-2 family proteins differs from neutrophils. Eosinophils down-regulate Bcl-xL expression during spontaneous apoptosis, but do not demonstrate Bax down-regulation during exposure to pro-survival cytokines [101]. Unstimulated eosinophils, like neutrophils, do not express Bcl-2, but up-regulate expression following IL-5 stimulation [102].

The pattern of caspase activation in eosinophils appears different from the neutrophil. Caspase 8 and 3, although present, do not play a role in spontaneous or Fas-mediated apoptosis [48]. Eosinophils employ caspase-mediated cleavage of several kinases such as Mst1 in the execution of apoptosis, events that do not occur in neutrophils [51]. This variation in downstream signalling between cell types may explain the differential effects of some agonists. For example, glucocorticoids accelerate eosinophil apoptosis while delaying cell death in neutrophils [103]. Furthermore, although macrophages can phagocytose apoptotic eosinophils [104], apoptotic eosinophil clearance may differ from neutrophils, since airway epithelial cells efficiently clear apoptotic eosinophils but not neutrophils [105].

**GRANULOCYTE APOPTOSIS IN LUNG DISEASE**

**Eosinophil apoptosis in allergic inflammation**

Eosinophil apoptosis is a mechanism of prolonged survival in tissues and thus tissue eosinophilia [106]. Eosinophil apoptosis has been described in the resolution of acute episodes of asthma [107] occurring as a consequence of spontaneous resolution of allergic inflammation and/or induced by corticosteroid treatment [103]. Reduced levels of eosinophil apoptosis in sputum correlate with asthma severity [108]. The concentration of BALF (bronchoalveolar lavage fluid) GM-CSF correlates with eosinophilia, suggesting eosinophil lifespan may be directly regulated by GM-CSF [109,110]. Derangements of eosinophil apoptosis in asthma are suggested by a number of studies. Eosinophils from patients with steroid-resistant asthma may have defects of caspase-induced apoptosis [111]. Fas expression on eosinophils of healthy and asthmatic subjects appears similar [112], but studies of patients with ragweed-allergic asthma have demonstrated increased concentrations of TRAIL 24 h after allergen challenge in asthmatic subjects as compared with controls, which correlates with airway eosinophilia [113]. There is evidence of increased expression of the anti-apoptotic protein Bcl-2 in eosinophils from sputum of patients with severe asthma [114]. Eosinophil apoptosis regulates clearance of these cells from the airway; BECs (bronchial epithelial cells) phagocytose apoptotic eosinophils [115] and treatment with glucocorticoids increases the clearance of these cells by both BECs and alveolar macrophages [113].

Finally, delay of eosinophil apoptosis may also contribute to the pathogenesis of Churg-Strauss syndrome, with elevated serum levels of soluble Fas delaying eosinophil apoptosis [116].

**Neutrophil apoptosis in pulmonary infection**

The efficiency of ‘physiological’ neutrophil apoptosis and macrophage clearance is demonstrated by the response to *Streptococcus pneumoniae* pneumonia, where bacterial clearance and complete resolution of the neutrophil inflammatory infiltrate are typically seen [19]. Reduced apoptosis of pulmonary neutrophils is observed in patients with community-acquired pneumonia, supporting the concept of delayed neutrophil death in a pro-inflammatory environment [117]. Reduced apoptosis of peripheral blood neutrophils is also observed with neutrophils isolated from patients with community-acquired pneumonia with or without concomitant chronic bronchitis [118]. These alterations in neutrophil lifespan can be influenced, both directly and indirectly, by pathogen-derived, as well as host-derived, signals.

TLRs (Toll-like receptors) are important pathogen recognition molecules that can activate pathways regulating apoptosis. Neutrophils express TLR2 and 4, whereas eosinophils do not [119]. Purified LPS delays constitutive neutrophil apoptosis in a NF-kB- and MAPK-dependent fashion [120]. Interestingly, however, small numbers of co-cultured monocytes appear to be required for prolonged LPS-mediated inhibition of neutrophil apoptosis [120] and also explain the apparent ability of LPS to cause eosinophil survival [121]. TLR2 agonists, such as purified lipoteichoic acid, can also prolong neutrophil survival [122] as can CpG motifs in bacterial DNA, which bind TLR9 [123]. Thus ligation of TLRs 2, 4 or 9 by pathogen-derived molecules can delay neutrophil apoptosis, whereas the effects of other TLRs, notably those mediating responses to viral structures, have yet to be studied (for a review on TLRs in lung disease, see [123a]).

Neutrophil apoptosis following phagocytosis of bacteria is a well-recognized phenomenon, first described for *Escherichia coli*, but subsequently for many other bacteria [124,125]. Phagocytosis-induced cell death, which can be induced by latex beads, is regulated by the differential expression of a large number of genes that regulate apoptosis [126] and requires ROS production [124]. Phagocytosis-induced apoptosis is enhanced by TNF-α, which induces ROS, and inhibited by GM-CSF, which activates an ERK-dependent survival pathway [127].
Since extracellular bacterial factors delay apoptosis [5, 6, 122, 123], whereas phagocytosis of bacteria induces apoptosis [124–126], the regulation of neutrophil apoptosis in infection is complex. A biphasic susceptibility can be postulated, with apoptosis delayed until bacterial killing, in association with internalization and ROS generation, is advanced [125]. This model would allow containment of bacterial infection and limit inflammatory lung injury during acute pneumonia. However, induction of neutrophil apoptosis during chronic infection with Mycobacterium tuberculosis may actually enhance pro-inflammatory cytokine production, since phagocytosis of these apoptotic cells can enhance TNF-α production by macrophages [21].

These 'physiological' host responses to infection can, however, be subverted by pathogens as a strategy for immune evasion. Premature neutrophil apoptosis impairs bacterial killing and, if clearance mechanisms are overwhelmed and secondary necrosis of neutrophils results, this leads to lung injury. Pseudomonas aeruginosa employs this strategy to impede pulmonary clearance via the production of the exotoxin pyocyanin [128]. In murine models pyocyanin-associated neutrophil apoptosis results in premature neutrophil apoptosis and decreased clearance of bacteria from the lung [129]. Furthermore, the lungs of individuals with cystic fibrosis and colonization with P. aeruginosa demonstrate increased numbers of apoptotic neutrophils and impaired clearance of apoptotic neutrophil by PS-dependent uptake mechanisms in macrophages [130]. Strep. pyogenes induces premature neutrophil apoptosis by altering the intrinsic apoptosis differentiation programme at the level of gene transcription [131]. Other organisms can induce necrosis as opposed to apoptosis in neutrophils and this may lead to greater lung inflammation; examples are Burkholderia cenocepacia in chronic granulomatous disease (a common pulmonary pathogen in these individuals) [132] and via the E. coli virulence factor haemolysin [133]. B. cepacia also secretes a haemolysin that induces apoptosis in human neutrophils [134]. Gram-negative bacteria, such as E. coli, are common causes of nosocomial pneumonia, so the evidence of a switch from neutrophil apoptosis to necrosis in association with increased lung inflammation in a rat model of E. coli pneumonia is intriguing [133]. Alternatively, pulmonary pathogens may delay neutrophil apoptosis to allow persistence and increase inflammatory lung injury. Examples include the delay in neutrophil apoptosis induced by Chlamydia pneumoniae which allows intracellular replication [135] or the ability of Staphylococcus aureus to survive in neutrophils [136]. Pro-survival effects on neutrophils may also occur indirectly, due to production of G-CSF or GM-CSF in response to pathogens by fibroblasts or epithelial cells, in cystic fibrosis [137].

Viral infections of the lung also affect neutrophil survival with both accelerated and delayed apoptosis described. Influenza A infection is associated with increased neutrophil apoptosis, in part due to ROS production, increased Fas and increased FasL expression [45]. A clinically important observation is that the combination of influenza A virus and Strep. pneumoniae infection results in greater levels of apoptosis than with either agent alone [138]. In contrast, neutrophils isolated from lavage fluid from infants with respiratory syncytial virus bronchiolitis demonstrate prolonged survival in comparison with control neutrophils from healthy adults [139]. A further clinical example of the impact of modulation of neutrophil apoptosis is provided by HIV infection, where pulmonary infections are a leading cause of mortality. The HIV-1 virus increases the level of neutrophil apoptosis ex vivo in HIV-seropositive individuals, an effect that can be reversed in vitro by treatment of neutrophils with G-CSF [140]. Clinical outcomes and rates of pulmonary infection have significantly improved in the era of highly active antiretroviral therapy and one of the classes of antiretrovirals, HIV protease inhibitors, has been shown to inhibit neutrophil apoptosis both in vitro and ex vivo [141].

**Neutrophil apoptosis in non-infectious lung disease**

Neutrophil apoptosis is modulated in a wide variety of non-infectious lung diseases. In the neonatal lung, neutrophil apoptosis is a feature of resolving neonatal RDS (respiratory distress syndrome) [142], but a delay in apoptosis is observed in CLD (chronic lung disease) of prematurity associated with a persistence of airway neutrophilia [143]. In adults, ARDS (acute RDS) is associated with decreased levels of neutrophil apoptosis [144], and increasing neutrophil numbers in previously neutropenic septic patients [145, 146]. Patients with COPD (chronic obstructive pulmonary disease) display decreased neutrophil apoptosis in peripheral blood, which may contribute to a pro-inflammatory phenotype [147]. When coupled with observations that pulmonary T-lymphocytes have decreased Fas expression and decreased susceptibility to Fas-mediated apoptosis [148], this suggests that decreased apoptosis of specific leucocyte populations can contribute to airway inflammation. Similarly, patients with bronchiectasis experiencing an acute exacerbation have decreased numbers of apoptotic neutrophils in induced sputum samples [149]. In idiopathic pulmonary fibrosis, neutrophils and eosinophils from BALF show marked up-regulation of Bcl-2 family proteins, implicated in the apoptotic machinery of these cells [150]. In addition, Fasl has been identified in serum and bronchial specimens from inflammatory lung disease patients [151], whereas dexamethasone, an occasionally successful treatment modality, has been shown to reduce the extent of fibrosis by interfering with pulmonary inflammatory cell apoptosis [152].
In most of these examples, inflammation is linked to a retardation of neutrophil apoptosis, but accelerated neutrophil apoptosis may contribute to disease pathogenesis. Wegener’s granulomatosis is characterized by the development of antineutrophil cytoplasmic antibodies that react with primary granules and accelerate neutrophil apoptosis in a ROS-dependent fashion [153,154].

GRANULOCYTE APOPTOSIS AS A THERAPEUTIC TARGET IN LUNG DISEASE

The importance of apoptosis in pulmonary disease aetiology and progression is clear, but selection of appropriate targets for therapy will be context specific. For example, depletion of innate immune cells in the early stages of a pneumonic illness would probably prove detrimental, but potentially advantageous later in the subset of patients proceeding to a fibrotic ARDS picture. Similarly, over the course of a lifetime of asthma, induction of eosinophil apoptosis may play beneficial roles in preserving lung function and reducing disease burden, but may divert cellular responses to allergen in as yet uncharacterized ways. Therapeutic strategies may also require enhancement of apoptotic cell clearance by macrophages and other semi-professional phagocytes in addition to pro-apoptotic agents, since excessive apoptosis in the face of impaired or even normal clearance could overburden clearance mechanisms, resulting in tissue damage and further inflammation.

Novel agents, such as non-peptide inhibitors of caspases 3 and 7, are currently being developed [155]. Some drugs already in clinical practice may exert all or part of their effects by modulation of granulocyte apoptosis. Glucocorticoids, the mainstay of asthma management, reverse the delayed eosinophil apoptosis found in poorly controlled asthma [156], even producing complete clearance of sputum eosinophilia with a single dose [157]. Theophyllines increase caspase activation in IL-3-primed eosinophils, suggesting they may deplete reactive inflammatory cells [158]. Theophyllines also increase intracellular cAMP concentrations, which may activate PKA and MAPK [159] and reduce Bcl-2 expression [160], and have other non-PDE (phosphodiesterase)-dependent pro-apoptotic actions [161]. Similarly, leukotriene receptor antagonists have been shown to increase apoptosis in peripheral eosinophils from asthmatic subjects as well as being capable of reversing the pro-survival effects of GM-CSF [162]. The potential of antimicrobials, such as quinolones and macrolides, to modulate apoptosis may also have clinical impact in non-infectious diseases [163,164].

In conclusion, granulocyte apoptosis has proved a fascinating area of research that has advanced our understanding of how inflammation resolves and is poised to deliver novel therapeutic approaches for treatment of infectious and inflammatory lung disease.

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