

Alveolar macrophages in pulmonary host defence – the unrecognized role of apoptosis as a mechanism of intracellular bacterial killing

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Summary

Alveolar macrophages play an essential role in clearing bacteria from the lower airway, as the resident phagocyte alveolar macrophages must both phagocytose and kill bacteria, and if unable to do this completely must co-ordinate an inflammatory response. The decision to escalate the inflammatory response represents the transition between subclinical infection and the development of pneumonia. Alveolar macrophages are well equipped to phagocytose bacteria and have a large phagolysosomal capacity in which ingested bacteria are killed. The rate-limiting step in control of extracellular bacteria, such as *Streptococcus pneumoniae*, is the capacity of alveolar macrophages to kill ingested bacteria. Therefore, alveolar macrophages complement canonical microbicidal strategies with an additional level of apoptosis-associated killing to help kill ingested bacteria.

Keywords: bacteria, killing, macrophage, *Streptococcus pneumoniae*

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Macrophage adaptation to function

Alveolar macrophages are the resident phagocytes in the lung [1]. They are mononuclear phagocytes that, potentially, can originate from either the replication of resident post-mitotic macrophages or through the migration of bone marrow progenitors and peripheral blood monocytes that are recruited into tissue, where they develop further in response to environmental imprinting [2]. Macrophage transcriptional profiles are highly responsive to the prevailing stimuli and a range of phenotypes are possible that are often grouped as M1, M2a, M2b and M2c types but which, in reality, are likely to result in multiple variations whose real importance lies in adaption of the macrophage to a range of specialized functions [3]. Inflammatory macrophages are adapted to anti-microbial host defence producing proinflammatory cytokines such as tumour necrosis factor (TNF)- α , interleukin (IL)-1 β , IL-6 and IL-12 as well as reactive oxygen species (ROS) and nitric oxide (NO), but have the potential to induce tissue injury. The M2 alternatively activated macrophages are characterized by high levels of IL-10 expression, and although some subsets contribute to defence against parasites, subsets also play roles in tissue homeostasis through tissue remodelling and repair and exert anti-inflammatory and immunoregulatory roles [4].

In the lung the alveolar macrophage is the resident phagocyte in the air space, and these have been viewed traditionally as a cell that results from the recruitment of peripheral blood monocytes into the lung, which undergoes differentiation through intermediate lung phenotypes to become a highly specialized macrophage [5]. However, studies suggesting a predominantly monocyte origin for alveolar macrophages have involved the use of irradiation, which has depleted pulmonary macrophage populations capable of replication. When radiosensitive blood monocyte precursors are depleted using methods whose effect is localized to the bone marrow, but that do not alter proliferation of macrophage populations at remote anatomical sites, the numbers of resident alveolar macrophages and indeed pulmonary proliferation of macrophages are unaltered [6]. Murine models employing conditional cell ablation and adoptive cell transfer have refined our understanding by demonstrating that alveolar macrophage numbers are maintained through recruitment of peripheral blood CX3CR1^{hi}/CCR2⁻ monocytes, which differentiate through a parenchymal intermediate to become alveolar macrophages [7,8]. Both the parenchymal intermediate-stage macrophage and the alveolar macrophage have the capacity for local replication and represent the source of alveolar macrophages in the steady state, while during increased turnover, such as may occur during inflammatory states, the

recruitment and differentiation of monocytes via the lung intermediate is probably accelerated to allow maintenance of alveolar macrophage numbers. Although not tested formally in the lung, evidence supports the concept that M1 polarized macrophages may arise preferentially from recruitment of monocyte populations, while replication of tissue macrophages may be the predominant source of M2 polarized macrophages during T helper type 2 (Th2) biased inflammation [9].

Alveolar macrophages are very long-lived cells that may live for very prolonged periods in the steady state [10]. In murine models there was no detectable turnover and little local replication observed over 8 months, so in a steady state there may be only a limited requirement for *in-situ* replication because of intrinsic macrophage persistence. This reflects, in part, the resistance of differentiated tissue macrophage to apoptosis and their expression of high levels of anti-apoptotic molecules, including the Bcl-2 family members myeloid cell leukaemia sequence (Mcl)-1 and A1 or the inhibitor of death receptor signalling FLICE (Fas-associated death domain-like IL-1 β -converting enzyme)-inhibitory protein (FLIP) [11–13].

Alveolar macrophages are highly adapted to the unique environment of the lung, and at steady state their ability to generate inflammatory responses is regulated tightly to ensure that lung injury is kept to a minimum, thus preserving precarious alveolar physiology and gas exchange [14]. Alveolar macrophages with M2 polarization play a key role in lung development [15]. M2 polarized alveolar macrophages are thought to play important roles in lung homeostasis ensuring tissue remodelling and repair, but emerging data suggest that alveolar macrophages may demonstrate simultaneously both M1 and M2 characteristics during acute inflammation and disease [16]. Differentiated macrophages such as alveolar macrophages have a large surface area with a dynamic cell membrane facilitating active phagocytosis or endocytosis of inhaled particles [17,18]. A large range of surface receptors enable ingestion of a diverse range of particles [19]. Alveolar macrophages have a large complement of both secondary lysosomes, containing the enzymes with which they degrade ingested particles, and mitochondria, ensuring that the energy requirements of the macrophage are supplied adequately [20,21]. Rodent studies suggest that the secondary lysosomes, which fuse with endocytic vacuoles and are a key characteristic of alveolar macrophages, are a specific feature of adaptation to the air breathing environment [22]. Differentiated macrophages of human or rodent origin lack the capacity to generate certain potent ROS species via myeloperoxidase, used by other phagocytes such as neutrophils. However, they can generate hydrogen peroxide through the nicotinamide adenine dinucleotide phosphate (NADPH)-oxidase system [23,24] and ROS via their mitochondria [25]. They can form more potent microbicidal factors though the reaction of ROS with NO generated

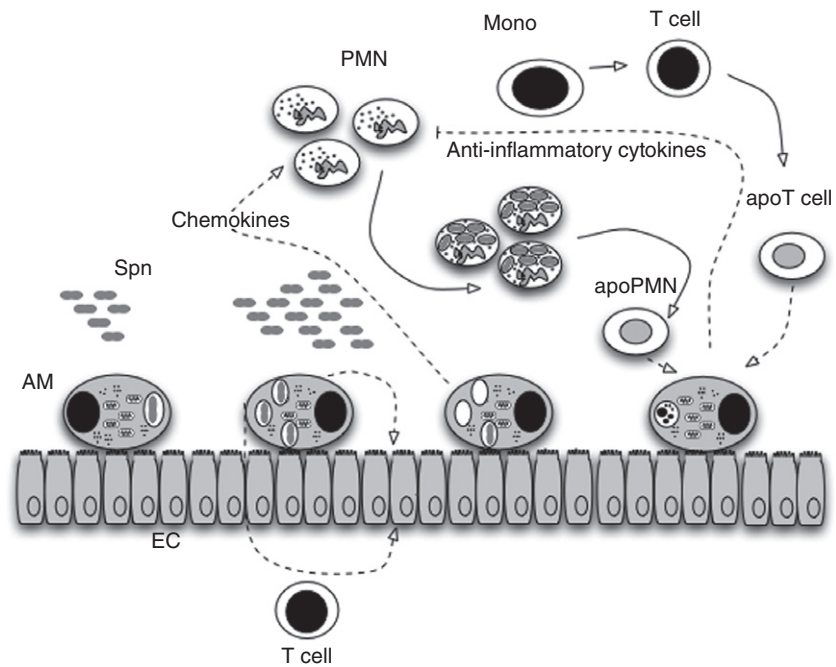
through the inducible nitric oxide synthase (NOS2/iNOS) system [26]. They also utilize proteases such as lysozyme activated at low pH in lysosomes to degrade ingested bacteria [27]. Macrophage degradation of bacteria contributes to the activation of pattern recognition receptors and resultant proinflammatory signalling, although the extent depends upon the capacity of bacteria to resist degradation with individual enzymes [28].

Alveolar macrophages role in host defence against pulmonary bacteria

We have used the Gram-positive bacterium *Streptococcus pneumoniae* (the pneumococcus) to probe the macrophage role in pulmonary host defence. *S. pneumoniae* is the most common cause of pneumonia and may spread beyond the lung, resulting in invasive disease such as meningitis [29]. In murine models of pulmonary infection alveolar macrophages clear bacteria up to a defined threshold without overt features of pneumonia, but when alveolar macrophages fail to control these subclinical infections recruitment of inflammatory cells, predominantly neutrophils, is required to control infection [30,31]. Using defined inocula of *S. pneumoniae*, depletion of alveolar macrophages with liposomes containing clodronate or mice with genetic modifications that alter intracellular killing of bacteria (as discussed below), we have demonstrated that mice with decreased numbers of alveolar macrophages, or with alveolar macrophages of reduced microbicidal capacity, are more susceptible to development of pneumonia and that the threshold inoculum required to generate pneumonia is reduced significantly [30,32–34]. The capacity of macrophages to clear bacteria is finite, and even in wild-type mice the inoculum can be increased to a ‘tipping-point’ beyond which alveolar macrophages no longer control the bacteria in the airway [30,31]. At these higher inocula alveolar macrophage depletion does not alter bacterial clearance because the macrophage capacity for bacterial clearance is already saturated. Hence neutrophils become critical for bacterial clearance [31]. The finite capacity for macrophages to clear bacteria is a feature of both human and murine cells *in vitro*.

Once the capacity of alveolar macrophages to control infection directly has been overwhelmed, the macrophage plays alternative roles in regulating the inflammatory response (Fig. 1). Macrophages are key orchestrators of inflammatory responses in the lung. They produce key regulatory cytokines, such as IL-1 β , which helps to prime release of the neutrophil chemokine CXCL8 from epithelial cells [35]. They also help to induce apoptosis in target cells such as monocytes, and efferocytosis of these apoptotic cells helps to down-regulate the proinflammatory cytokine network [36,37]. When the threshold at which macrophages can no longer control bacterial numbers in the lung is exceeded, T cells play an important role in enhancing bacte-

Fig. 1. Macrophages play critical roles in host defence against *Streptococcus pneumoniae*. *S. pneumoniae* (Spn) are ingested and killed by alveolar macrophages (AM) in the distal airway. When the bacterial inoculum increases above the level at which AM can contain bacteria without recruitment of further phagocytes cross-talk between AM, epithelial cells (EC) and T cells results in the release of chemokines and recruitment of further inflammatory cells, in particular polymorphonuclear leucocytes (PMN). PMN become critical effectors of bacterial clearance. Ultimately, PMN undergo apoptosis. Monocytes may play an important role in regulating inflammatory cells and may induce apoptosis in activated T cells. Ingestion of apoptotic cells (apoPMN and apoT cells) as well as apoptotic monocytes/macrophages by macrophages, a process termed efferocytosis, helps to down-regulate the inflammatory response and results in the generation of anti-inflammatory cytokines and other factors that help to down-regulate the inflammatory response.



rial clearance [38]. Achieving complete clearance of *S. pneumoniae* while minimizing lung damage requires tight regulation of neutrophilic inflammation. To achieve this, different T cell populations interact closely with phagocyte populations to enhance clearance capacity for *S. pneumoniae* but with variable effects on the extent of the inflammatory response [39]. T helper type 1 (Th1) and Th17 CD4⁺ T cell responses play important roles in clearance of *S. pneumoniae*, contributing to the anti-microbial host response through activation of phagocyte-driven microbial killing, while CD8⁺ T cells may have important roles regulating the extent of Th17 responses and neutrophilic inflammation [40–43]. Although adaptive immune responses contribute to these responses, innate immunity can amplify responses in the form of monocyte-induced stimulation of memory T cells, and innate responses generated by cells such as invariant natural killer T cells (iNKT) can prime phagocyte bacterial killing [41,43–45]. Dysregulated T cell activation can influence bacterial clearance adversely [38]. Fas ligand, which plays a role in the pathogenesis of a diverse range of infections [46], has an important role in regulating the extent of T cell activation through the induction of T cell apoptosis in susceptible activated cells [38]. This helps to optimize bacterial clearance. Monocytes induce Fas ligand-mediated T cell apoptosis (including CD4⁺ T cells, CD8⁺ T cells and CD4⁺ Th17 T cells) during pneumococcal infection, limiting excessive T cell activation and preventing bacteria-induced proinflammatory necrotic cell death [47]. As macrophages also induce Fas-mediated apoptosis in susceptible T cells [46,48] and induce apoptosis of Fas-susceptible cells during

S. pneumoniae infection [34,47], it is likely that a variety of monocyte/macrophage cell types play an important role in regulating the inflammatory response through the regulation of T cell activation via the induction of Fas ligand-mediated apoptosis. This represents an emerging but important aspect of macrophage control over neutrophilic inflammation in the lung [46,48].

Microbicidal mechanisms by which macrophages kill *S. pneumoniae*

Although ROS generated by the NADPH oxidase system contributes to the killing of many bacteria, it is not required for killing of *S. pneumoniae* by alveolar macrophages [33,49]. Neutrophils, which have a greater reliance on NADPH oxidase-generated ROS for bacterial killing in general than macrophages, also do not utilize this microbicidal strategy to control *S. pneumoniae*, instead requiring the neutrophil granule serine proteases, such as cathepsin G and neutrophil elastase, for the effective killing of ingested *S. pneumoniae* [50,51]. These proteases are not expressed in macrophages. Analysis of *S. pneumoniae*, which is a catalase-negative bacterium, reveals that it has a variety of adaptations to withstand oxidative stress (Fig. 2), and that it can use the production of hydrogen peroxide to inhibit other bacteria with which it must compete for the environmental niche it exploits in the upper airway. Three enzymes are involved primarily in the production of hydrogen peroxide during pneumococcal metabolism; pyruvate oxidase encoded by *spxB*, lactate oxidase encoded by *lox*, which converts lactate to pyruvate and carbamoyl

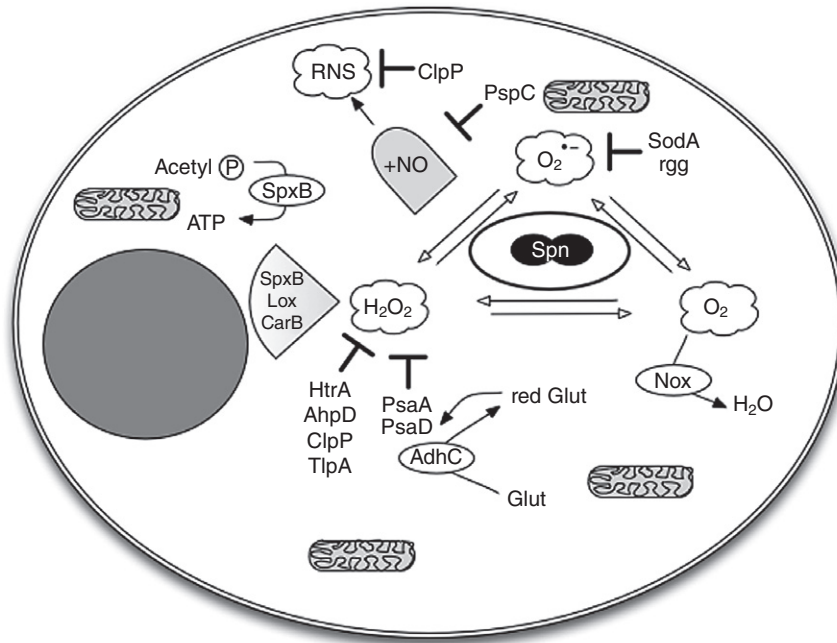


Fig. 2. *Streptococcus pneumoniae* resists anti-microbial killing. *Streptococcus pneumoniae* (Spn) express several genes, which help them resist reactive oxygen species, generated within phagocytes. Spn express several enzymes that generate hydrogen peroxide (H_2O_2); *SpxB* encodes a pyruvate oxidase, *Lox* a lactate oxidase and *CarB* a carbamoyl phosphate synthase. Pneumococcal proteins which help resist H_2O_2 include high temperature requirement A (*HtrA*), a heat shock-stimulated serine protease, the alkylhydroperoxidase D (*AhpD*), the caseinolytic peptidase P (*ClpP*), another heat shock-stimulated serine protease, the thioredoxin-like protein A (*TlpA*), the pneumococcal surface antigen A (*PsaA*) a manganese permease, *PsaD* a putative glutathione peroxidase and an alcohol dehydrogenase (*AdhC*) that generates the reduced glutathione required by *PsaD*. Resistance to superoxide ($O_2^{\cdot-}$) involves the superoxide dismutase A (*SodA*) and the transcriptional regulator *Rgg*. *Nox* is a nicotinamide adenine dinucleotide (NADH) oxidase that converts O_2 to H_2O , limiting the availability of O_2 to form $O_2^{\cdot-}$. *Spx* allows acetyl phosphate (Acetyl P) to provide a source of adenosine triphosphate (ATP) during oxidative stress. In addition, pneumococcal surface protein C (*PspC*) helps Spn to withstand nitric oxide (NO) and *ClpP*-reactive nitrogen species (RNS), produced by macrophages.

phosphate synthase encoded by *carB* [52–54]. *S. pneumoniae* has evolved to cope with the resultant oxidative stress in several ways; *sodA* encodes the manganese superoxide dismutase (MnSOD) that removes superoxide, the NADH oxidase encoded by *nox* removes O_2 to H_2O , preventing its conversion to superoxide, *SpxB* enables acetyl phosphate to be converted to adenosine triphosphate (ATP), preventing ATP depletion during oxidative stress, *psaA* encodes a manganese permease and *psaD* a putative glutathione reductase, which together alter redox status and limit hydrogen peroxide production via *SpxB*, while *AdhC* is an alcohol dehydrogenase that generates the reduced glutathione required for *PsaD* [55–60]. Other factors contributing to resistance to oxidative stress include the heat shock-induced protease *HtrA*, a putative alkylhydroperoxidase *AhpD*, the *ClpP* protease, a putative transcriptional regulator *Rgg* and a putative thioredoxin-like protein *TlpA* [61–65]. Although arising primarily as a mechanism by which *S. pneumoniae* can withstand the ROS generated by their own metabolism, the range of strategies employed to withstand oxidative stress suggest that the bacteria should be relatively more resistant to the ROS, produced by macrophages after ingestion of bacteria, than bacteria that lack these adaptations.

Macrophages have evolved additional anti-microbial strategies, including generation of NO via NOS2, and NO can react to form S-nitrosothiols, such as S-nitrosoglutathione (GSNO) or can react with ROS to generate reactive nitrogen species (RNS), such as peroxynitrite formed between NO and superoxide, which are potent anti-microbicidal molecules [26]. Therefore, although ROS may of itself be dispensable as an anti-microbicidal, it can still contribute to pneumococcal killing through formation of RNS. Microbial components, including the toxin pneumolysin and pneumococcal cell wall, stimulate macrophage NO production and NO-mediated bacterial killing [66–68]. In keeping with this, we and others have shown that mice that lack NOS2 are less able to clear *S. pneumoniae* from the lung [33,69]. Nevertheless, there are also factors which restrict the capacity of NO or RNS to kill ingested bacteria. First, *S. pneumoniae* also has genes that allow it to adapt to nitrosative stress; pneumococcal surface protein C (*PspC*) reduces NO production, *AdhC* is an S-nitrosoglutathione reductase, while *ClpP* has been implicated in resistance to RNS [70–72]. To compound these challenges imposed by the bacterial genome, human macrophages appear to generate lower levels of NO than

murine macrophages and levels of production are lower in differentiated tissue macrophages [47,73]. Much less is known about the capacity of proteases to contribute to pneumococcal killing in macrophages. Although RNS are likely to make a significant contribution to early phagolysosomal killing of *S. pneumoniae* and proteases have the potential to add additional killing capacity, it is clear that the macrophage is likely to have a finite capacity for bacterial killing and needs to employ an additional microbicidal strategy to control *S. pneumoniae*. The fact that bacteria such as *S. pneumoniae* are not believed to demonstrate intracellular persistence suggests this additional mechanism must be effective.

Apoptosis-associated killing

Induction of apoptosis has been demonstrated to aid bacterial killing for intracellular pathogens such as *Mycobacterium tuberculosis* [74,75]. We have demonstrated that macrophage apoptosis is a feature of *in-vitro* challenge of macrophages to *S. pneumoniae* and also occurs *in vivo* [30,36]. It is observed with both human and murine macrophages. The induction of apoptosis is linked to the burden of intracellular bacteria, suggesting that it is activated when the intrinsic capacity for phagolysosomal killing is exhausted [76]. Apoptosis induction is delayed, occurring typically 16–20 h after bacterial ingestion *in vitro*, and the apoptosis-associated killing occurs after the initial phase of phagolysosomal killing associated with microbicidal molecules in the phagolysosome. Apoptosis-associated killing of *S. pneumoniae* requires NO generation [68]. Although induced following phagocytosis, apoptosis is not the result of phagocytosis *per se* nor linked to uptake by any specific receptor that recognizes *S. pneumoniae*. It is enhanced in response to opsonized bacteria and in response to less virulent unencapsulated strains, but this seems to be because these circumstances enhance numbers of intracellular bacteria [76,77]. It is also facilitated by responses associated with pathogen recognition; Toll-like receptor (TLR)-4 plays a role in enhancing the response [78]. Apoptosis-associated killing is observed with a range of serotypes of *S. pneumoniae*, but as the efficiency of ingestion of different serotypes can vary and apoptosis induction is influenced by the number of intracellular bacteria it is seen to greater extents for strains that are phagocytosed more efficiently and is seen to the greatest extent for unencapsulated strains [76].

Inhibition of elements of the apoptotic response reduces bacterial killing *in vitro* and does not result in cell survival, but rather results in pathogen-driven death by necrosis with impaired bacterial killing [32,34,68]. Analysis of the macrophage proteome demonstrates that apoptosis results from a concerted programme that not only induces apoptosis but also inhibits alternative death paradigms, including necroptosis, a form of programmed necrosis, and

the endoplasmic reticulum (ER) stress pathway [79]. We have found no evidence that the death process we observe involves pyroptosis, an alternative form of programmed cell death that is caspase-1-dependent, but which is also associated with anti-microbial responses, although with greater degrees of inflammatory response. Although pyroptosis can be induced in monocytes to a range of bacteria we have found that it is observed predominantly with infections associated with greater levels of internalized bacteria and greater levels of ATP depletion than we observe with *S. pneumoniae*, and in this model *S. pneumoniae* did not engage pyroptosis [80]. The delayed apoptotic death process seems to be enhanced in the more differentiated tissue macrophage phenotype [76]. Our group also has unpublished evidence that mice that lack caspase-1 do not show any reduction in cell death in response to *S. pneumoniae*, when rates of cell death are examined in bronchoalveolar lavage 24 h after intratracheal challenge with *S. pneumoniae*, a finding which appears to suggest that the death process observed does not represent pyroptosis (Marriott and Dockrell unpublished observations). Recent observations provide further insight into why apoptosis may be induced in macrophages exposed to *S. pneumoniae* in preference to pyroptosis. Caspase-1-dependent pyroptosis following challenge with *S. pneumoniae* is inhibited by induction of chitinase 3-like-1, a host response to *S. pneumoniae* which seems to have a conserved role in evolution, allowing bacterial clearance with a death process that results in lower levels of inflammation than occur with pyroptosis [81].

Murine models illustrate that impairment of apoptosis results in decreased bacterial killing in the lung and enhanced tissue invasion, as reflected by greater levels of bacteraemia [30,32,34]. Ultimately, apoptotic macrophages are ingested by lung macrophages and help to down-regulate proinflammatory cytokine expression, in particular TNF- α -dependent signalling, and this is associated with an overall reduction in neutrophil recruitment to the lung and lung inflammation [33,37].

The molecular regulation of apoptosis-associated killing

Apoptosis following ingestion of *S. pneumoniae* involves an intrinsic mitochondrial-dependent pathway that is independent of death receptors such as Fas ligand [46,68]. The pathway is caspase-dependent [30,36]. Apoptosis is delayed to maximize the time during which the macrophage can kill ingested bacteria, but occurs when intracellular killing by canonical mechanisms becomes exhausted [32]. The onset of apoptosis is carefully scripted. We have shown that the onset of apoptosis is regulated by expression of the anti-apoptotic protein Mcl-1 [32]. The anti-apoptotic protein Mcl-1 is a Bcl-2 family member and has been demonstrated previously to be expressed highly in differentiated macrophages [11]. It has a relatively short half-life,

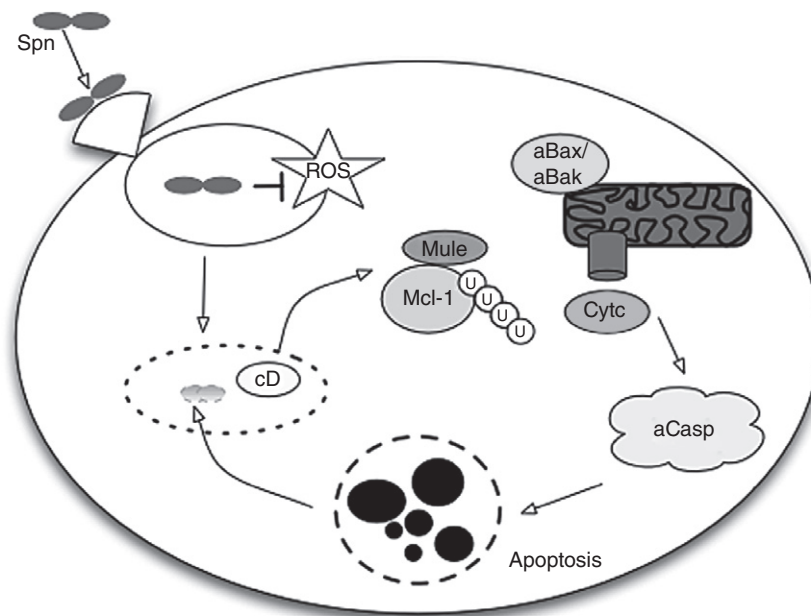


Fig. 3. Induction of apoptosis-associated killing complements bacterial killing in macrophages. *Streptococcus pneumoniae* Spn is internalized following phagocytosis into a phagolysosome, where it can resist killing by reactive oxygen species (ROS). With time, the phagolysosome becomes permeabilized and activation of the lysosomal protease cathepsin D (cD) results in down-regulation of the anti-apoptotic protein myeloid cell leukaemia sequence 1 (Mcl-1). Mcl-1 down-regulation involves ubiquitination mediated by interaction with the Mcl-1 ubiquitin ligase E3 (Mule) and proteasomal degradation. Following Mcl-1 down-regulation, pro-apoptotic Bcl-2 family members result in the ultimate activation of Bcl-2-associated X protein (Bax) and Bcl-2 homologous antagonist/killer (Bak), which interact with the mitochondrion (aBax/aBak) to induce mitochondrial outer membrane permeabilization and cytochrome c (CytC) release. This results in caspase activation (aCasp) and downstream features of apoptosis, illustrated by nuclear fragmentation. Apoptosis results in macrophage cell death but also killing of the residual viable Spn contained in the phagolysosome.

estimated to be 20–30 min, and is well equipped to respond to dynamic changes in the macrophage ingesting and killing bacteria [82]. Initially it is transcriptionally up-regulated, but following prolonged exposure to *S. pneumoniae* protein translation becomes gradually reduced and proteasomal degradation via ubiquitination is increased [32,34,79].

Apoptosis arises in response to *S. pneumoniae* localized in phagolysosomes and we have investigated how this might activate an apoptotic programme that involves Mcl-1 down-regulation. Lysosomal membrane permeabilization can activate a mitochondrial pathway of apoptosis [83]. We have found evidence that lysosomal membrane permeabilization precedes Mcl-1 down-regulation and investigated the role of lysosomal proteases in these events. We have shown, using pharmacological inhibition and genetic manipulation, that the aspartic lysosomal protease cathepsin D plays a key role in the induction of apoptosis [34]; see Fig. 3. Cathepsin D is the most abundant lysosomal protease in differentiated macrophages [84,85]. Activation of cathepsin D allows Mcl-1 to interact preferentially with its E3 ubiquitin ligase, Mcl-1 ubiquitin ligase (Mule, also known as ARF-BP1/HectH9), rather than with heat shock protein 70 with which it can also interact [34]. It also results in down-regulation of eukaryotic elongation factor (eEF) 2, a critical component of the translational machinery required to allow Mcl-1

translation [79]. This results in Mcl-1 down-regulation, induction of apoptosis and enhanced bacterial killing. The role of cathepsin D and Mcl-1 in the regulation of apoptosis-associated bacterial killing are conserved between murine and human macrophages.

The molecular basis of lysosomal permeabilization has not yet been established. It seems to arise due to a host response to ingested *S. pneumoniae* and in particular to the expression of the bacterial toxin pneumolysin [34]. Although the toxin itself causes membrane pores we have no evidence that it causes pores in the lysosomal membrane, as exposure to bacteria expressing toxin lacking pore-forming capability still induce comparable levels of apoptosis to bacteria expressing wild-type toxin (Bewley *et al.*, manuscript submitted). Equally, we have not found any role to date for components of the intracellular recognition systems for pneumolysin, which involve nucleotide-binding oligomerization domain containing protein (Nod)-like receptor (NLR) P3 or the absent in melanoma (AIM) 2 NLR, in the response to pneumolysin that leads to lysosomal membrane permeabilization or to induction of apoptosis [86–88]. The induction of lysosomal membrane permeabilization, cathepsin D activation and induction of apoptosis is not a response limited to *S. pneumoniae*, as it occurs with a range of other extracellular bacteria [80]. Of

some interest, although there are several bacteria in which apoptosis-associated killing appears not to occur, infection with *Staphylococcus aureus* can result in persistent up-regulation of Mcl-1 and an absence of apoptosis-associated killing [89]. We have found that, in contrast to *S. pneumoniae*, *Staphylococcus aureus* fails to induce cathepsin D activation [34]. Furthermore, we have found that *Neisseria meningitidis* does not induce apoptosis in monocytes or macrophages [80,90]. Thus, some bacteria may have developed strategies to limit induction of apoptosis-associated killing to enable their own intracellular persistence.

Recently it has been suggested that macrophage apoptosis-associated killing during *S. pneumoniae* infection can be enhanced by TNF-related apoptosis-inducing ligand (TRAIL) and that neutrophils may be a source of this ligand during established pneumonia [91]. Because macrophages undergo apoptosis-associated killing *in vitro* in the absence of neutrophils, and in murine models in the absence of neutrophil recruitment this mechanism is unlikely to explain the intrinsic mechanism in macrophages, it suggests rather that apoptosis-associated killing can also be engaged by additional pathways involving neutrophils. This may be of relevance during established pneumonia where endogenous activation of apoptosis-associated killing by the macrophage alone is exhausted or inefficient and where additional mechanisms of bacterial killing such as neutrophil-mediated killing are impaired [91]. As cells other than neutrophils also express TRAIL, it remains possible that other cell types such as macrophages, lymphocytes or epithelial cells could contribute to this additional mechanism of apoptosis induction in settings distinct from those described [92].

Concluding remarks

Alveolar macrophages play a key role in host defence, functioning as the resident phagocytes that clear bacteria. They are efficient at phagocytosing bacteria, but the rate-limiting step in bacterial clearance is intracellular killing. Although they utilize a range of anti-microbials to kill ingested bacteria, the restrictions on the microbicidal molecules they generate imposed by their localization in the distal airway and the range of survival strategies employed by a pathogen such as *S. pneumoniae* mean that additional mechanisms are required to kill phagocytosed bacteria. Induction of apoptosis-associated killing is a key component of host defence, which combines bacterial clearance with relatively modest cost to overall pulmonary inflammation. It represents an important and under-recognized component of host defence in the lung. The clearance of intracellular bacteria when initial intraphagolysosomal killing is exhausted is evidence of its efficiency, while the evidence that some bacteria have prevented this host response raises the possibility that selective re-engagement could represent a novel

approach to enhance microbial killing, particularly in the face of bacteria that demonstrate high-levels of antimicrobial resistance.

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Disclosure

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