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Innate immune responses in the ageing lung

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Summary

The world is undergoing an unprecedented shift in demographics, with the number of individuals over the age of 60 years projected to reach 2 billion or more by 2050, representing 22% of the global population. Elderly people are at a higher risk for chronic disease and more susceptible to infection, due in part to age-related dysfunction of the immune system resulting from low-grade chronic inflammation known as 'inflamm-ageing'. The innate immune system of older individuals exhibits a diminished ability to respond to microbial threats and clear infections, resulting in a greater occurrence of many infectious diseases in elderly people. In particular, the incidence of and mortality from lung infections increase sharply with age, with such infections often leading to worse outcomes, prolonged hospital stays and life-threatening complications, such as sepsis or acute respiratory distress syndrome. In this review, we highlight research on bacterial pneumonias and pulmonary viral infections and discuss age-related changes in innate immunity that contribute to the higher rate of these infections in older populations. By understanding more clearly the innate immune defects in elderly individuals, we can design age-specific therapies to address lung infections in such a vulnerable population.

Keywords: aging, inflammation, infection, lung, macrophages, neutrophils, pneumonia

Introduction

In elderly people, the environment of the lung is characterized by chronic low-grade inflammation, an aspect of a systemic inflammatory state associated with ageing often referred to as 'inflamm-ageing' [1–3]. Many studies have found higher baseline levels of proinflammatory mediators, such as C-reactive protein, tumour necrosis factor (TNF)-α, interleukin (IL)−1β and IL-6 in elderly individuals, and elevated levels of these mediators correlate with disease-associated mortality in this population [4–10]. Moreover, the heightened basal levels of proinflammatory mediators present in older subjects probably contributes to decreased pulmonary function and blunted immune responses to respiratory tract infections [11–13]. Seniors, defined as those greater than 65 years of age, are at higher risk for developing lung infections and, once acquired, have more complications, longer hospital stays [14] and increased mortality [15]. While seniors have higher rates of co-morbidities that may worsen clinical outcomes after infection, baseline immune dysfunction plays a central role in their susceptibility to respiratory infections and higher mortality rates [16].

It is known that advanced age affects multiple aspects of pulmonary immunity, including the structure and function of the lung itself, and both the innate and adaptive arms of the immune system [11]. Immunosenescence is thus one of the major factors underlying the increased incidence and severity of respiratory tract infections in elderly people. In this review, we will discuss studies examining age-related changes in the

response to lung infections, with a particular focus on cellular dysfunction and altered signalling in the innate immune system. By understanding the effects of ageing on the cells of the innate immune system in the context of respiratory infections, we can gain insight into the common deficits in innate immunity that predispose elderly people to these illnesses.

Pulmonary infections in elderly people

Pneumonia is a primary cause of morbidity, mortality and socioeconomic cost leading to >50 000 deaths [17] and costing more than $7 billion in medical costs annually in the United States alone [18]. The incidence of pneumonia has been increasing in elderly people in recent years [19]; in 2014, more than 83% of pneumonia deaths in the United States occurred in seniors [17]. The leading cause of community-acquired pneumonia among elderly individuals is streptococcal respiratory infections [20]. Fewer studies have examined the ageing immune system in the context of Gram-negative pneumonias, yet these infections also contribute to the overall increase in pneumonia-related deaths in this population [20]. Furthermore, rates of hospitalization, requirements for intensive care and mortality rates from respiratory tract infections increase drastically as seniors continue to age [17]. Nosocomial pneumonias are common after hospitalization [21], particularly in geriatric trauma patients [22,23], with bacterial infections as the most frequent cause of ventilator-associated [24] and hospital-acquired pneumonias [25].

Individuals aged 65 years and older also have higher mortality rates due to viral infections in the lung, the two most prevalent being influenza virus and respiratory syncytial virus (RSV) infections [26]. Infection with respiratory syncytial virus (RSV) is a major cause of morbidity and mortality in individuals over the age of 65, with rates just below that of influenza virus infection [26–32]. RSV, like influenza virus, infects cells of the respiratory tract [33]. However, less is known about the host immune response to RSV compared to influenza virus, due mainly to a lack of decent animal models that recapitulate the response to infection in humans [34]. Studies examining the impact of advanced age on the immune response to RSV infection have demonstrated that viral titres are higher and the virus persists longer in aged compared to younger hosts. Interestingly, some studies have shown an early delay in viral replication in older hosts, which the authors hypothesize is due possibly to changes in the pulmonary epithelium due to ageing [35–39]. Contributing to the enhanced mortality is that elderly individuals do not respond to influenza virus vaccinations as well as younger individuals, and a Food and Drug Administration (FDA)-approved RSV vaccination does not currently exist [40,41]. Underlying poor vaccination responses and higher prevalence of and mortality due to infection in older individuals is the reduced responsive capacity of the immune system of this population [42]. However, this discussion will focus upon the innate arm of the immune system in the lungs, although a diverse body of detailed literature exists on changes in adaptive immunity with ageing. Furthermore, it should be noted that while similar responses to vaccination and natural infection by bacterial and viral pathogens are useful to examine general age-related dysfunction in leucocytes and pulmonary immunity, such disparate models are not directly comparable and may yield different conclusions based on the pathogen and type of immunological challenge.

Changes in the ageing lung environment

There are many alterations in the ageing lung environment that impact innate immune function and host defences against lung infections. For example, the mucociliary barrier is an important defence against pathogens in the upper respiratory tract and bronchioles of the lung, both providing a physical barrier as well as sweeping microbes and debris upwards out of the airways. It has been shown that elderly individuals exhibit reduced mucociliary clearance [43,44], contributing to microbial invasion of the lower airways and alveoli. The chronic inflammation present in aged mice also causes an up-regulation of two proteins implicit in the attachment and infiltration of bacteria in the lung in epithelial cells: polymeric immunoglobulin receptor and platelet-activating factor receptor (PAFr) [45,46]. Shivshankar *et al.* demonstrated the importance of these findings for host survival, in that increased expression of bacterial adhesion ligands in the lungs, including PAFr, correlated with mortality after pulmonary infection. Furthermore, up-regulation of such proteins and other markers of cellular senescence were identified in the lung tissue from both elderly humans and aged mice, demonstrating that immunosenescence probably plays an important role in the increased susceptibility to infection in ageing populations [47]. Other factors in the immune environment of the lung are also susceptible to age-related changes. Pulmonary levels of complement proteins and surfactant proteins, important anti-microbial factors in the lung, have been found to increase with age [48]. While antibodies from aged individuals opsonize bacteria adequately, research suggests that serum levels in seniors are insufficient to facilitate antibody-mediated phagocytosis of microbes by innate immune cells [49,50]. As such, host defence in the ageing lung is impaired not only by leucocyte dysfunction, but also by other changes in innate immunity and the local tissue environment resulting from inflamm-ageing.

There is recent evidence that the age-related changes in resident gut and lung microbiota may also be involved in regulating overall immunity to respiratory infections. Microbiome studies have revealed an age-related shift in the composition and diversity of the respiratory tract microbiome [51]. Endogenous bacteria of the murine gut

microbiome are protective against both *Pseudomonas aeruginosa* and *Staphylococcus aureus* pneumonia [52,53], and alterations in the lung microbiome of aged mice may also play a role during the host response to these lung infections. For example, dysbiosis of the respiratory tract is observed in elderly pneumonia patients [54] as well as aged mice colonized with *Streptococcus pneumoniae* [55–57]. Although these results hint that age-related changes in microbiota correspond with alterations in immune function, there is currently no evidence directly connecting age-related alterations in respiratory microbiome with innate immunity.

Age-related defects in innate immune receptors

Infection by microbial pathogens activates multiple pathogen recognition receptors (PRRs) in both respiratory epithelial cells and haematopoietic innate immune cells, including Toll-like receptors (TLRs), retinoic acid inducible gene (RIG)-I-like receptors (RLRs) and nuclear oligomerization domain-like receptors (NLRs) [58,59]. Triggering of these receptors leads to the induction of cytokine and chemokine production and maturation of some cell types, such as dendritic cells (DCs) [60–64]. Several studies have shown that ageing leads to reductions in TLR expression (both mRNA and protein), signalling and downstream cytokine production in some cell types and models [59,65,66]. Unfortunately, there is a paucity of information regarding changes due to advanced age in PRR-mediated signalling after influenza virus infection. However, a recent study of influenza virus infection in mice suggests that monocytes from aged animals have diminished anti-viral interferon production but intact inflammasome responses [67]. RSV is thought to be detected by various TLRs, RLRs and NLRs, but the exact role of each type of receptor in host immunity to this virus has not been studied extensively [33]. Activation of these PRRs in lung epithelial cells and other innate immune cells initiates a signalling cascade that results in the secretion of important proinflammatory cytokines, such as IL-1β and IL-6 [33,68]. In response to RSV infection, advanced age alters cytokine production such that there are decreased levels of type I and II interferons (IFNs) and TNF-α, but elevated levels of IL-1β and IL-4 [35,37,38,69–71]. As a result, older animals exhibit increased bronchopulmonary inflammation after RSV infection compared to younger animals. Infiltrating cells are comprised of granulocytes, with the large majority being neutrophils [36,37,71]. Tissue damage caused by immune cells may contribute to the elevated rate of RSV-induced mortality in elderly people.

Similarly, peripheral blood mononuclear cells isolated from elderly individuals exhibit reduced and delayed production of TNF-α, IL-6, IL-1b, IFN-α, IFNc, C-C motif chemokine ligand (CCL)2 and CCL7 after stimulation with TLR-4, TLR-7/8 and RIG-1 agonists, subsequently hindering the ability of stimulated cells to induce T cell proliferation *in vitro* [72]. Hinojosa and colleagues demonstrated that the chronic, low-grade inflammation in the lungs of aged mice up-regulates regulators of immune signalling such as A20, a de-ubiquitinase that inhibits TLR signalling and downstream nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) activation, showing that not only is TLR signalling itself dampened by ageing, but negative feedback loops associated with TLR signalling are up-regulated with advanced age [45,73]. Constitutive expression of such negative regulators impede the ability of epithelial and immune cells to sense and respond to microbes, decreasing the host's ability to mount an immune response to microbial challenge. Thus, age-mediated deficiency in proper TLR (and probably other PRR) function by epithelial cells and leucocytes probably contributes to dysregulated inflammation and worsened outcomes in elderly people infected with respiratory infections.

Ageing and alveolar macrophages

Alveolar macrophages, the resident innate immune cells of the airways, stand as the first line of defence against microbes, including those that cause pneumonia, and play central roles in the initiation and resolution of inflammation (Fig. 1). The initial response of macrophages to microbes and other inflammatory stimuli is reduced in aged hosts, which has been attributed to inflamm-ageing [45,72,74–77]. This reduction in pathogen detection is probably a result of changes in TLR signalling pathways and constitutively elevated negative feedback signalling due to chronic inflammation present in older individuals [73]. Specifically, studies of macrophages isolated from aged mice show a diminished response to TLR-1, TLR-2 or TLR-4 stimulation with peptidoglycans, zymosan or lipopolysaccharide (LPS), respectively. Specifically, these cells produced less TNF-α and IL-6 due to attenuated activation of proinflammatory signal transduction in the NF-κB, p38 and c-jun NH_2 terminal kinase (JNK) pathways [45,75–79]. Furthermore, the same decreases in proinflammatory cytokine (TNF-α, IL-1β and IL-6) production by macrophages from aged mice have been observed after pulmonary infection with *S. pneumoniae*, suggesting that the reduction in TLR signalling occurs *in vivo* during an active infection [45,73,77]. These alterations in TLR signalling and associated downstream events are highlighted in Fig. 2. Furthermore, the ability of macrophages to activate $CD4^+$ T cells is also probably impaired due to ageing, as macrophages from aged mice do not express the same levels of major histocompatibility (MHC) class II molecules (required for antigen presentation to $CD4^+$ T cells) as macrophages from younger animals [80].

In addition to initiating and sustaining the innate immune response to bacterial infection, macrophages promote resolution of inflammation caused by infection by removing extracellular debris and clearing apoptotic cells

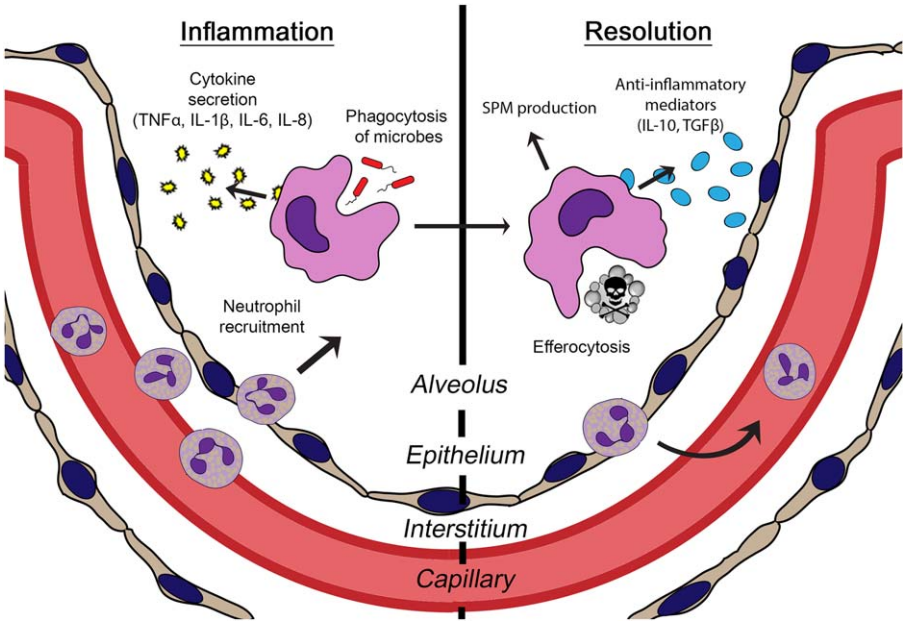

Fig. 1. Innate immune functions of alveolar macrophages. As the resident innate immune cell of the pulmonary airspace, alveolar macrophages stand at the forefront of host defence against microbial invaders in the lung. Along with their role in effecting and propagating the inflammatory response by phagocytosing microbes and secreting proinflammatory mediators, alveolar macrophages also facilitate resolution by clearing away dead cells (efferocytosis) and producing anti-inflammatory mediators.

from the airways, a process known as efferocytosis. Advanced age disrupts these functions, reducing the ability of macrophages to remove apoptotic cells and resulting in prolonged inflammation after infection, even after the pathogen is cleared [81,82]. Monocytes and macrophages from aged individuals and mice also exhibit reduced phagocytic capacity [83–85], impairing their ability to remove microbes from the host in the inflammatory response to infection. Macrophages further play an essential role in controlling inflammation and restoring tissue homeostasis after infection by producing signalling molecules such as the anti-inflammatory cytokines IL-10 and transforming growth factor beta (TGF-β), and pro-resolving lipid mediators [86]. In response to pneumococcal pneumonia, aged mice produce less IL-10 but higher levels of chemokines chemokine (C-X-C motif) ligand (CXCL)9, CXCL12, chemokine (C-C motif) ligand 3 (CCL3), CCL4, CCL5, CCL11 and CCL17, suggesting a defect in anti-inflammatory cytokine production by immune cells in the lung [87]. Similarly, others have found a decline in IL-10-producing macrophages with ageing in a murine model of spinal cord injury [88].

The effect of advanced age on the production of lipid mediators by alveolar macrophage has not been studied. However, evidence from infections at other sites suggests that this probably also occurs in the lung. For example, in a model of self-resolving peritonitis, macrophages from aged mice produced more proinflammatory eicosanoids and less specialized pro-resolving mediators (SPMs), contributing to delayed resolution of acute inflammation [82]. Moreover, SPMs may serve as potential therapeutics in the context of prolonged inflammation due to respiratory infection, as nanoparticles loaded with leucocyte-derived SPMs (resolvins D1 and D3) were able to correct age-related decline in efferocytosis by macrophages. Although there is still much to be learned about the resolution of acute inflammation in the lung, these data hint at a significant impairment in the ability of macrophages from aged subjects to promote resolution, further adding to the inability of the aged immune system to properly clear pulmonary infections with excessive inflammation and tissue damage.

DCs in the ageing lung

DCs are another immune cell subset residing in the lungs that are affected by the detrimental effects of inflammageing (reviewed in [89]). Macrophages and DCs both carry influenza virus antigen, and upon activation of PRRs they can traffic to the draining lymph node to present antigen and activate virus-specific T cells [90]. Similarly, upon detection of RSV by PRRs and in response to proinflammatory cytokines, DCs traffic to the lung draining lymph nodes, where they activate $CD4^+$ and $CD8^+$ T cells [68,91]. DCs from aged subjects have impaired phagocytosis and pinocytosis *in vitro* [92], and the migratory capacity of DCs is reduced in aged mice, decreasing the number available to stimulate T cells in the lymph node after influenza or RSV infection [93,94]. Ageing reduces the up-regulation of co-stimulatory molecules critical for T cell priming and diminishes cytokine production by alveolar macrophages and DCs after exposure to influenza virus [94–96]. These age-mediated alterations in macrophages and DCs are sufficient to cause a reduction in the ability of these antigen-presenting cells (APCs) to activate $CD8^+$ T cells [93,95]. There is mounting evidence that these defects in APC function, in combination with intrinsic changes in T cells, are responsible for the blunted adaptive immune responses that occur in elderly people [97–99]. The

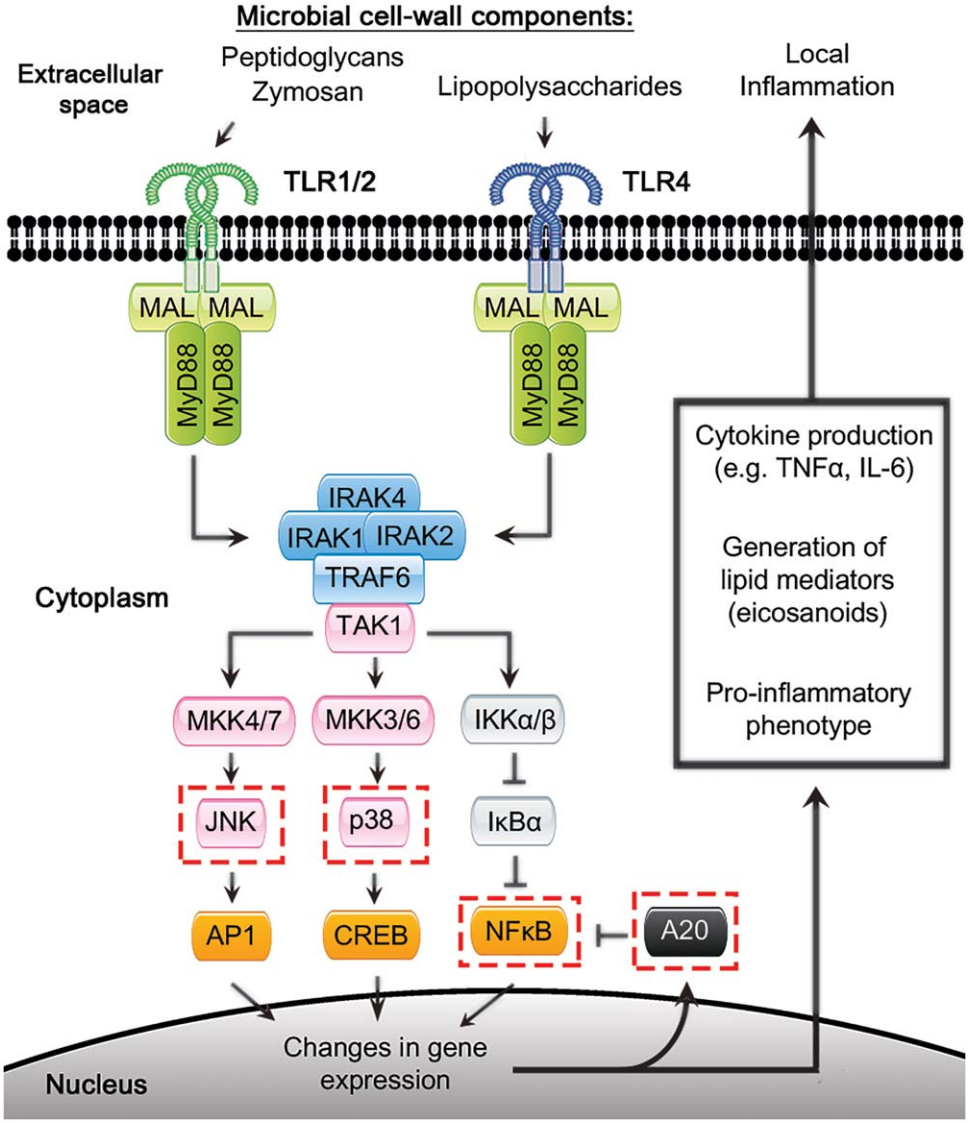

Fig. 2. Dysregulated Toll-like receptor signalling associated with advanced age. This figure depicts signalling pathways downstream of Toll-like receptor signalling, some of which have been shown to be disrupted or altered with age. Dashed red boxes indicate specific pathway components known to be affected by ageing, as discussed in this review [29–34].

reduction in DC and macrophage function due to ageing leads to poor viral clearance, ultimately causing increased mortality after influenza virus infection [93,94,100]. While the exact underlying mechanism by which ageing alters DC function has not been determined, one study suggests that age-mediated changes in histone modifications might contribute [96]. Others have implicated age-related mitochondrial dysfunction as resulting in impaired phagocytosis and antigen presentation by DCs [101]. However, further work is necessary to elucidate the intrinsic and extrinsic factors that drive aberrant DC function in ageing, particularly in the context of bacterial infections.

Age-related changes in neutrophil function

Neutrophils are another key effector cell in the innate immune response to pathogens, employing a wide range of microbicidal functions to clear pathogens from tissues in the early stages of lung infections. Neutrophils migrate into infected tissues soon after a pathogen is detected, and they work together with macrophages to contain and clear infections [102,103]. However, neutrophil functions decline with age in many different models, as summarized in Table 1. As such, these granulocytes are impaired in their ability to

Table 1. Anti-microbial neutrophil functions altered by ageing

Function	Species	Reference
Phagocytosis	Human, mouse	22,104,125–129
Cytokine production	Human	130
ROS generation	Human, mouse, rat	104,126–128,130–133
Chemotaxis	Human	114,126,127,131,134
NET formation	Human, mouse	105,135
Degranulation	Human	134

ROS = reactive oxygen species; NET = neutrophil extracellular trap.

eliminate bacteria and other microbes. When considering the sometimes conflicting results of *in-vitro* and *in-vivo* studies on neutrophil function in elderly people, it is important to note that many of the studies cited in this review used different stimuli to examine neutrophil function, e.g. particles *versus* microbes for experiments on phagocytosis, with resulting discrepancies in results. For instance, neutrophils from aged individuals exhibit reduced ROS generation in response to *S. aureus* but not *Escherichia coli* [104]. Such a disparity in the response between Gram-positive and Gram-negative organisms illustrates the different ways in which ageing affects neutrophil responses to distinct stimuli. Additionally, no reports to date have examined the impact of ageing on the response of neutrophils during viral infection. None the less, the impaired functions observed in neutrophils from aged subjects can be taken collectively as a consensus acknowledging general decline in cell-based immunity, often with severe ramifications. One such example comes from research by Tseng *et al.*, suggesting that impaired neutrophil extracellular trap (NET) formation permits the systemic dissemination of bacteria from the lungs of aged mice, demonstrating how such age-related defects in neutrophil function may lead to dire outcomes [105].

Along with deficiencies in anti-microbial functions, there is also evidence that neutrophil recruitment and *in-vivo* chemotaxis are dysregulated in the lungs of aged mice and elderly patients. In some infection models neutrophil recruitment is impaired at early time-points, while in others too many neutrophils accumulate at the site of infection and fail to disperse later. For example, in a murine model of pulmonary infection with *Francisella tularensis*, older mice exhibited delayed production of neutrophil-attracting chemokines and diminished neutrophil infiltration in the early stages of infection [106]. Conversely, elderly patients with *S. pneumoniae* respiratory tract infections and aged mice infected with *P. aeruginosa* had increased and prolonged neutrophil accumulation in the lung parenchyma relative to young controls [107–109].

The deregulated recruitment of neutrophils is not infection-specific; studies examining the role of ageing in burn-induced pulmonary inflammation showed dysfunctional neutrophil migration and chemotaxis in the lungs of aged mice due to altered chemokine signalling through CXCR2 [110,111]. Additionally, other pulmonary inflammatory stimuli resulted in heightened neutrophil-attracting chemokine levels and prolonged neutrophilia in aged mice [112,113]. Therefore, inflammatory signalling in the lung is altered markedly due to ageing, contributing to aberrant neutrophil trafficking observed after infection or other pulmonary insults. Few studies have examined the mechanism by which this occurs, but one study suggests that constitutive phosphoinositide-3-kinase (PI3K) signalling contributes to the abnormal chemotaxis by neutrophils from older subjects, finding that inhibition of PI3K γ or δ isoforms restored accuracy to neutrophil migration [114]. Together, these studies show that ageing alters neutrophil recruitment, the direction of which is potentially pathogen- or insult-dependent. Age-related changes in normally tightly regulated neutrophil chemotaxis can result in delayed pathogen clearance [115,116] and contribute to prolonged inflammation and pulmonary tissue damage [114]. Thus, it is evident that neutrophil dysfunction plays an important role in the inability of older individuals to mount an effective response to bacterial pathogens, and to properly resolve neutrophil-mediated pulmonary inflammation.

Ageing and natural killer (NK) cells in the lung

NK cells are responsible for killing infected or transformed cells and they secrete important cytokines for host defence, including IFN-γ [68,117]. Advanced age leads to a reduction in the frequency of NK cells present in the lung after influenza virus infection, and cytokine production by NK cells is diminished in older animals [94,118,119]. Both human and animal studies have shown that ageing also reduces the NK cell cytotoxicity in response to influenza virus or RSV [35,71,118,120]. Exactly how advanced age reduces the frequency and functional capacity of NK cells is not known, although the basal low level of inflammation in older individuals is thought to play a role [119]. Furthermore, one report suggests that age-related alterations in non-haematopoietic cells drive the functional deficits in NK cells in aged mice [121]. In summary, the functional capacity of NK cells is reduced by advanced age, due probably to changes in the pulmonary microenvironment of older individuals.

Outlook and future directions

While a commendable effort has been made to prevent viral and bacterial pneumonias, both community and hospital-acquired infections continue to be a significant burden of morbidity, mortality and socioeconomic cost. Furthermore, few therapeutic strategies or treatments designed specifically for elderly patients with lung infections currently exist, despite the profound, age-dependent changes in innate immune function discussed above. Primary data on elderly individuals' innate immune response during lung infections is still scarce, despite the growing need for more knowledge regarding the physiological changes due to ageing. Therefore, understanding how advanced age alters innate immune cell populations, including the changes in APCs that result in blunted adaptive immune responses and the enhanced pulmonary neutrophilia present in older individuals, is critical in determining why mortality rates due to respiratory infection are higher in elderly people. There have been advances in our understanding of the biology of ageing that give hope for improved care and treatment of elderly people, such as age-specific vaccines and adjuvants [40,122–124]. As a greater proportion of the population become seniors,

it is increasingly important to identify reversible causes of immunosenescence and inflamm-ageing in the lungs in order to develop targeted therapies for this at-risk and quickly growing patient population.

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