Chapter

Physiological Role of Alveolar Macrophage in Acute Lower Respiratory Tract Infection: Phagocytosis and Aging

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Abstract

Acute lower respiratory tract infections (LRTIs) are the deadliest communicable diseases. Inhaled pathogens that reach the alveoli are eliminated by lung-resident alveolar macrophages. Bacteria and fungi are detected and phagocytosed by specific pattern recognition receptors (PRRs) that are highly expressed in alveolar macrophages. In addition, early pro-inflammatory responses assist alveolar macrophages in the efficient phagocytosis of these pathogens. Viruses are also directly or indirectly endocytosed by pinocytosis or opsonization, respectively, whereas alveolar macrophages contribute to the prevention of pneumonia by removing endogenous dead cells through an alternate type of phagocytosis, efferocytosis. Macrophage phagocytosis and efferocytosis require not only sufficient expression of the relevant PRRs but also the coordinated interplay of intracellular factors that regulate engulfment. Given the current situation in which emerging infectious diseases spread worldwide, this chapter summarizes the physiological roles of alveolar macrophages in acute LRTIs, focusing on phagocytosis, pro-inflammatory responses, efferocytosis, and their regulatory machinery. This chapter also reviews recent insights into age-associated dysfunction of alveolar macrophages and discusses their relevance to vulnerability to acute LRTIs in the elderly population.

Keywords: alveolar macrophage, acute lower respiratory tract infection, pneumonia, phagocytosis, pro-inflammatory response, Efferocytosis, pattern recognition receptor, intracellular signaling, aging

1. Introduction

Lung-resident alveolar macrophages play a pivotal role in maintaining lung homeostasis by eliminating airborne pathogenic microorganisms. The process by which cells ingest particles >0.5 μm in diameter, such as bacteria (0.5 to 2 μm) and fungi (3 to 10 μm), is defined as phagocytosis, which is composed of recognition, engulfment, and subsequent steps of the digestion process [1, 2]. Pathogen recognition occurs by directly detecting microbe-specific molecular signatures, known as

pathogen-associated molecular patterns (PAMPs), using the corresponding pattern recognition receptors (PRRs), which activate downstream intracellular signaling that regulates cytoskeletal rearrangement and cell motility, leading to engulfment of pathogens [2–4]. As a result, efficient pathogen clearance necessitates sufficient expression of scavenger receptors as well as the continued concerted action of downstream signaling molecules. In addition to triggering phagocytosis, PAMPs induce the production of pro-inflammatory cytokines and chemokines via interactions with another family of PRRs, toll-like receptors (TLRs), resulting in the recruitment and activation of circulating phagocytes in the foci of infection and assisting the enhancement of macrophage phagocytosis [5–7].

However, unbridled inflammation is detrimental to tissue homeostasis, leading to organ failure if not properly treated. A typical example is the coronavirus disease 2019 (COVID-19), wherein critically ill patients are characterized by manifesting cytokine storm syndrome, resulting in respiratory failure and multiple organ failure [8, 9]. During viral infection, alveolar macrophages have been suggested to contribute to the alleviation of pneumonia by removing apoptotic epithelial cells and neutrophils from fighting viruses rather than by endocytosing viruses via pinocytosis and/or opsonization [10, 11]. Indeed, critically ill patients with COVID-19 are depleted of alveolar macrophages, which is accompanied by a remarkable increase in the proportion of pro-inflammatory monocyte-derived macrophages in bronchoalveolar lavage fluid [12]. Since the alternative type of phagocytosis, termed efferocytosis, is indispensable for preventing excessive inflammation during host defense against viral infection, failure of this protective action leads to the exacerbation of pneumonia from mild to life-threatening.

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), the virus that causes COVID-19, has received much attention from researchers since its outbreak owing to its highly virulent and transmissible nature; notably, COVID-19 is not the only threat to people. Acute lower respiratory tract infections (LRTIs), caused predominantly by *Streptococcus pneumoniae* and influenza viruses, remain the deadliest epidemics [13–15] because the older population is particularly liable to develop pneumonia and thereby respiratory failure [16, 17]. The vulnerability of the elderly to acute LRTIs has been suggested to be associated with immune senescence. In line with this trend, age-associated declines in immune cell functions and their mechanisms have been discussed [18–20]. Moreover, age-related alterations in the tissue microenvironment deeply influence immune cell senescence [21–23], and recent progress has enabled the analysis of the reality of alveolar microenvironment degeneration with aging and its adverse effects on alveolar macrophages.

In this chapter, we summarized the physiological roles of alveolar macrophages in acute LRTIs, focusing on phagocytosis, pro-inflammatory responses, efferocytosis, and their regulatory mechanisms. This chapter then reviewed recent insights into age-associated dysfunction of alveolar macrophages and discussed their relevance to the vulnerability of the elderly population to acute LRTIs.

2. Global epidemiology of acute LRTIs

2.1 Top causes of death

The World Health Organization (WHO) estimated that 55.4 million people died worldwide in 2019, with the top 10 leading causes accounting for 55% of deaths [13]. Further,

seven of these causes are non-communicable diseases (NCDs), with the first, second, and third leading causes being ischemic heart disease, stroke, and chronic obstructive pulmonary disease. The total number of deaths caused by all NCDs accounts for 74% of the total deaths in the world. However, among communicable diseases, acute LRTIs kill 2.6 million people worldwide, making them the fourth leading cause of death.

2.2 Morbidity and mortality of acute LRTIs in children

According to the analysis results of the Global Burden of Disease Study (GBD) in 2016, acute LRTIs caused 336 million episodes and 2.4 million deaths in 2016 [14]. The rates of episodes and deaths attributable to acute LRTIs in children under the age of 5 were 2.4 and 3.2 times higher, respectively, compared with those in people of all ages; in particular, the mortality rates in children were the highest in developing countries in sub-Saharan Africa and South Asia. However, worldwide deaths from acute LRTIs in children decreased by 36.4% between 2007 and 2017 [16]. The substantial improvement in mortality in children is suggested to be primarily due to the implementation of vaccines against *S. pneumoniae* and *Haemophilus influenzae*, antibiotic therapy, and continuous improvements in education, nutrition, water, sanitation, and hygiene [24].

2.3 Morbidity and mortality of acute LRTIs in the elderly

Notably, the rates of episodes and deaths attributable to acute LRTIs in the elderly over the age of 70 were also 3.4 and 8.3 times higher, respectively, compared with those in people of all ages, but the mortality rates in older adults were globally higher than those in people of all ages [14]. Worldwide deaths from acute LRTIs in the elderly increased by 33.6% between 2007 and 2017 compared with those in children [16]. The deterioration of mortality in the elderly is likely associated with the extended longevity of the frail older population, chronic diseases, comorbidities, multiple medication use, and functional disability in high-income countries; further, it is associated with the adverse effects of air pollution, smoking, and alcohol consumption in low-income countries [24].

2.4 Most common causative agent of pneumonia

Acute LRTIs are responsible for inflammation of either the mucous membranes that line the bronchi or the lung tissue in one or both lungs, accompanied by infiltration and inflammation of the alveoli, leading to bronchitis or pneumonia, respectively [25]. Of the two conditions, pneumonia is the major cause of death, as it causes respiratory failure by filling the alveoli with fluid and pus resulting from inflammation [26]. Notably, pneumonia is caused by various pathogens, including bacteria, fungi, and viruses. *S. pneumoniae*, a Gram-positive bacterium, is the most common bacterial cause of pneumonia. In fact, across generations, *S. pneumoniae* accounted for approximately half of the pathogens that caused deaths in 2016, contributing to a higher number of deaths compared with all other major etiologies combined (respiratory syncytial virus, *H. influenzae* type b, and influenza) [14].

2.5 Seasonal influenza

Seasonal influenza epidemics occur every winter, annually resulting in 3–5 million cases of severe illness and 290,000–650,000 deaths from respiratory illness [15].

According to the analysis results of the GBD 2017, acute LRTIs attributable to influenza were estimated to have caused 55.5 million episodes, 9.5 million hospitalizations, and 145,000 deaths in 2017, and the highest mortality rates were observed, especially among adults over the age of 70 [17]. Of the influenza A and B viruses that cause seasonal epidemics, influenza A viruses, in particular, have a high mutagenic capacity to generate new strains that can escape from acquired immunity, which causes a pandemic every few decades. Further, the influenza A(N1H1)pdm09 strain emerged in April 2009 and caused a pandemic, globally resulting in 200,000 respiratory and 80,000 cardiovascular deaths that year [27].

2.6 COVID-19

The ongoing pandemic is COVID-19, which is caused by SARS-CoV-2. Since the first case of COVID-19 was reported in Wuhan, China, in December 2019, the infection has rapidly spread worldwide and continues to be a global epidemic, regardless of the season. According to the WHO, as of January 2023, the confirmed cases of infected patients had reached approximately 750 million worldwide, and deaths had risen to >6.8 million [28]. As with other acute LRTIs, older adults are at a higher risk of severe illness or death from COVID-19, even after the Delta-virulent strain was replaced by the Omicron-attenuated strain [29–33].

3. Physiological roles of alveolar macrophage phagocytosis in acute LRTIs

3.1 Development and maintenance of alveolar macrophages

Lung-resident alveolar macrophages play a leading role in the clearance of airborne microorganisms that enter the alveoli during inspiration. Murine alveolar macrophages originate from fetal monocytes [34]. The development of alveolar macrophages from fetal monocytes is regulated by granulocyte-macrophage colony-stimulating factor (GM-CSF) and the downstream transcription factor peroxisome proliferator-activated receptor γ (PPARγ) [35]. After birth, however, alveolar macrophages are essentially not replenished by bone marrow-derived monocytes but are self-maintained by the paracrine action of GM-CSF secreted by epithelial cells [35]. Moreover, further maturation of alveolar macrophages requires transforming growth factor (TGF)-β1, which is secreted in an autocrine manner and upregulates PPARγ expression [36]. A similar developmental pathway is presumed to occur in humans since immunostaining of lung sections from stillborn infants revealed that interstitial macrophages were abundant in the interstitium, whereas mature alveolar macrophages were completely absent in the alveoli [37]. The acquisition of specific functions by alveolar macrophages, including advanced phagocytic capacity, is partly due to the unique maturation processes in the alveolar microenvironment, where GM-CSF acts as a key regulator.

3.2 Phagocytic receptors expressed on alveolar macrophages

3.2.1 Scavenger receptors and their functions

Among the PRRs, two members of the scavenger receptor superfamily proteins, macrophage scavenger receptor 1 (MSR1) and macrophage receptor with collagenous

structure (MARCO), recognize both Gram-positive and Gram-negative bacteria by detecting their pyrogenic cell wall components, lipoteichoic acid (LTA) and lipopolysaccharide (LPS), respectively [38–40]. Alveolar macrophages constitutively express MSR1 and MARCO, which are essential to eliminate airborne pathogenic bacteria. Knockout mice lacking MSR1 or MARCO displayed an impaired ability to remove live bacteria, exacerbated pneumonia, and reduced survival after intranasal inoculation with *S. pneumoniae* [41, 42]. The expression and function of MSR1 and MARCO are conserved in human alveolar macrophages [43]. Further, mice lacking another scavenger receptor, CD36, exhibited similar phenotypes during pulmonary infection caused by the Gram-positive bacterium *Staphylococcus aureus* [44]. In addition, alveolar macrophages are characterized by higher expression of scavenger receptors with one or more C-type lectin-like domains, such as β-1,3/1,6-D-glucan receptor dectin-1 [45, 46] and the mannose receptor CD206 [37, 47], which pivotally contribute to the removal of fungi and bacteria from the alveoli by detecting their respective target carbohydrates that cover the cell wall surface.

3.2.2 Opsonin receptors and their functions

Murine alveolar macrophages highly express Fcγ receptors FcγRI/II/III and further enhance their phagocytic activity when Gram-negative bacteria, *Pseudomonas aeruginosa*, are opsonized with IgG, whereas they hardly express complement receptors CR1/2/3, and their ability is not affected by complement opsonization [48]. Further, the other subset of complement receptor CRIg is expressed in murine and human alveolar macrophages [49], but its ability to directly recognize Gram-positive bacteria by detecting LTA suggests that it can act as a PRR in the lungs [50]. Notably, alveolar macrophages isolated from GM-CSF-knockout mice were deficient in Fcγ receptors and had impaired phagocytic activity against both IgG-opsonized and non-opsonized latex beads and their phenotypes were restored by epithelial cell-specific expression of GM-CSF [51]. A recent study reported that human alveolar macrophages express FcγRI/II/III at higher levels than other systemic counterparts, such as macrophages in the bone marrow, spleen, and liver [52]. Moreover, peripheral blood monocyte-derived macrophages that differentiated in GM-CSF-containing culture exhibited properties that were partially similar to those of alveolar macrophages, expressing a larger amount of FcγRI/II compared with that of their counterparts [52].

3.3 Regulation of engulfment in alveolar macrophages

3.3.1 Roles of small-GTP binding proteins in engulfment

Pathogen recognition by scavenger and opsonin receptors initiates cytoskeleton remodeling, leading to pathogen engulfment. The regulatory signaling pathways rely on each receptor ligated to the particles, but all forms of engulfment require the recruitment of filamentous (F)-actin beneath tethered particles and subsequent rearrangement of F-actin. F-actin is primarily controlled by three small-GTP binding proteins, including Ras homolog (Rho) family member A (RhoA), Ras-related C3 botulinus toxin substrate 1 (Rac1), and cell division control protein 42 homolog (Cdc42), both of which are members of Rho family [2, 4]. The binding of particles to receptors causes RhoA, Rac1, and Cdc42 to be converted from the GDP-bound inactive form to active form and then recruited from the cytosol to the cell membrane under tethered particles, where they regulate F-actin rearrangement and subsequent

cell motility by triggering the formation of stress fibers, lamellipodia, and filopodia, respectively [2].

3.3.2 Receptor-dependent roles of small-GTP binding proteins

The roles of these small-GTP binding proteins have been systematically studied after the ligation of Fcγ receptors. FcγRIIA-transfected COS fibroblasts treated with IgG-opsonized particles facilitated recruitment of all the small-GTP binding proteins to the nascent F-actin phagocytic cup, whereas blocking Rac1 and Cdc42 suppressed engulfment by preventing the formation of membrane ruffles and filopodia, respectively; however, blocking RhoA had no effects on the engulfment [53]. In contrast, when CR3-transfected COS fibroblasts were treated with complement-opsonized particles, only RhoA colocalized with F-actin, and blocking RhoA compromised CR3-mediated phagocytosis [53]. Dectin-1 has downstream signaling cascades that are highly similar to those of Fcγ receptors [54]. Although the downstream pathways of MSR1 and CD36 have not yet been reported, a recent study indicated that the Gram-negative bacterium *Escherichia coli* interacts with MARCO, which activates Rac1 to initiate F-actin polymerization, filopodia formation, and subsequent engulfment in murine alveolar macrophages [55].

4. Physiological roles of alveolar macrophage pro-inflammatory responses in acute LRTIs

4.1 PAMPs closely associated with activation of alveolar macrophages

In addition to phagocytosis, alveolar macrophages induce pro-inflammatory responses by detecting PAMPs using a wide variety of PRRs, including TLRs, to facilitate the immediate mobilization and activation of phagocytes such as neutrophils and monocytes. For instance, during pulmonary infection with *S. pneumoniae*, the cell wall components of Gram-positive bacteria, lipoproteins [56], LTA [57], peptidoglycan [58], and the structural ancillary pilus protein, RrgA oligomer [59], are detected by TLR2, while the pneumococcal virulence factor pneumolysin is detected by TLR4 [60, 61]. Endopeptidase O, a new pneumococcal virulence protein, induces pro-inflammatory responses in macrophages by activating both TLR2 and TLR4 signaling [62]. For Gram-negative bacteria such as *H. influenzae* type b, the cell wall components, LPS and porin proteins, are detected by TLR4 [63] and TLR2 [64], respectively. Further, TLR9 detects bacterial DNA [65]. Thus, bacterial infection stimulates multiple TLRs simultaneously, rather than singly, resulting in complex signal activation.

4.2 Downstream signaling of TLRs and their outcomes

Detailed figures illustrating downstream signaling by TLRs are available in a highly specialized review article [5]. When TLR4 is activated by its agonists, it engages two distinct adaptor proteins in the signaling process: myeloid differentiation factor 88 (MyD88) and toll/interleukin (IL)-1 receptor domain-containing adapter-inducing interferon (IFN)-β (TRIF). The MyD88-dependent pathway recruits IL-1 receptor-associated kinases 1 and 4, which phosphorylate tumor necrosis factor (TNF) receptor-associated factor 6 (TRAF6), leading to the

activation of nuclear factor-κB (NF-κB), p44/42 mitogen-activated protein kinase (MAPK), p38 MAPK, and c-Jun N-terminal kinase (JNK). However, the TRIF-dependent pathway facilitates the formation of a complex consisting of TRAF3, TRAF family member-associated NF-κB activator (TANK), TANK-binding kinase 1, and inhibitor of NF-κB kinase subunit ε, which phosphorylates IFN regulatory factor 3, resulting in the activation of dimers to translocate from the cytoplasm into the nucleus. The MyD88-dependent pathway elicits the production of pro-inflammatory cytokines (TNF-α, IL-6, and IL-1β), chemokines (IL-8 and monocyte chemoattractant protein 1), and anti-microbial proteins (inducible nitric oxide synthase), whereas the TRIF-dependent pathway triggers the production of type I IFNs (IFN-α/β). Unlike TLR4, TLR2 and TLR9 only initiate the MyD88-dependent pathway.

4.3 Roles of TLRs in pneumococcal infection

Studies using TLR2-, TLR4-, or TLR9-knockout or mutant mice suggested the protective role of TLR2, TLR4, and TLR9 against pneumococcal infection. However, TLRs are ubiquitously expressed in cells other than immune cells. Therefore, the phenotypes observed in these studies are attributable to the lack of TLR signaling not only in alveolar macrophages but also in alveolar structural cells.

4.3.1 Roles of TLR2 in pneumococcal infection

The comparison of TLR2-knockout mice with wild-type mice indicated only a partial reduction in pro-inflammatory cytokine production after intranasal *S. pneumoniae* inoculation, with no significant difference in survival rate or bacterial clearance, suggesting that TLR2 signaling plays a minor role in eliciting local inflammation and bactericidal activity against *S. pneumoniae* [66]. Further, no differences were observed between TLR2-knockout and wild-type mice in bacterial growth, lung inflammation, or pro-inflammatory cytokine and chemokine production in post-influenza pneumococcal pneumonia [67]. Similar results were obtained in splenectomized mice [68].

4.3.2 Roles of TLR4 in pneumococcal infection

On inoculation with a non-lethal dose of *S. pneumoniae*, TLR4 mutant mice exhibited decreased survival rates, accompanied by increased bacterial growth, monocyte and lymphocyte infiltration, and interstitial inflammation in the lungs [69]. Notably, a recent study demonstrated that although mice lacking TLR4 also displayed lower viability and augmented colonization in the lung after intranasal *S. pneumoniae* inoculation compared with that of wild-type mice, this exacerbation of infection was accompanied by an attenuated pro-inflammatory profile, reduced live alveolar macrophages, diminished infiltration of neutrophils and monocytes, and inhibition of monocyte differentiation into macrophages [70]. In addition, MyD88 deletion was not able to completely reproduce these phenotypes, implying that pro-inflammatory responses via both MyD88- and TRIF-dependent TLR4 signaling are necessary for the mobilization and activation of phagocytes [70]. Therefore, TLR4 signaling could have led to the sufficient elimination of bacteria and subsequent protection of alveolar macrophages from pneumococcal cytotoxicity.

4.3.3 Roles of TLR9 in pneumococcal infection

Both TLR9-knockout and wild-type mice developed pulmonary inflammation during *S. pneumoniae* infection, but TLR9-knockout mice exhibited worse survival and more bacterial invasion from the bronchoalveolar fluids into the lung tissue and blood stream, with abrogated upregulation of phagocytic activity in alveolar macrophages [71]. This early finding indicates that the activation of TLR9 signaling is indispensable for maximizing phagocytosis in alveolar macrophages during pneumococcal infection. The priming effects of TLR agonists have also been investigated. A prior inhalational challenge with the TLR9 agonist ODN2395 in combination with the TLR2 agonist Pam2CSK4 protected mice from death due to *S. pneumoniae* infection, although administration of agonists of any individual TLR had no protective effect [72]. However, ODN2395/Pam2CSK4 stimulation enhanced intracellular bacterial death in isolated tracheal epithelial cells, but not in alveolar macrophages. Taken together, maintaining basal levels of TLR9 expression and signaling in alveolar macrophages is likely to be critical for defensing the host from pneumococcal infection.

5. Physiological roles of alveolar macrophage efferocytosis in acute LRTIs

5.1 Roles of alveolar macrophages in viral infection

Alveolar macrophages can directly or indirectly endocytose viruses via pinocytosis or opsonization, respectively. In the case of SARS-CoV-2, alveolar macrophages also recognize viral components such as envelop protein [73], spike protein [74–77], and single-stranded RNA [78, 79] using TLR2, TLR4, and TLR3/7, respectively, which trigger pro-inflammatory responses. However, the phagocytic and pro-inflammatory responses of alveolar macrophages against viruses appear to be dispensable for protecting the host from viral infection. Indeed, the absence of mature alveolar macrophages in GM-CSF-deficient mice resulted in severe respiratory failure and increased mortality after pulmonary infection with a non-lethal dose of influenza A virus, and these conditions were improved by neonatal transplantation of alveolar macrophage progenitor cells from wild-type mice [11]; however, alveolar macrophage-depleted mice exhibited severe manifestations, with viral clearance not being largely impaired and the functions of antibody-producing B lymphocytes and cytotoxic CD8-positive T-lymphocytes being normally activated [11]. Similarly, critically ill patients with COVID-19 have been characterized by a depletion of alveolar macrophages and a remarkably increased proportion of recruited pro-inflammatory monocyte-derived macrophages in bronchoalveolar lavage fluid [12]. These suggest that alveolar macrophages contribute to host survival by suppressing excessive pulmonary inflammation, which is caused by removing endogenous apoptotic cells rather than by phagocytosing the exogenous virus itself during infection.

5.2 Regulation and roles of efferocytosis in alveolar macrophages

Notably, clearance of apoptotic cells, termed efferocytosis, is an essential process for maintaining tissue homeostasis under both healthy and diseased conditions. Efferocytosis differs morphologically and mechanistically from the classical form of phagocytosis against pathogens and requires the expression of

receptors that recognize "eat me" signatures such as phosphatidylserine (Ptd-L-Ser) exposed on the membrane surface of apoptotic cells [80]. Macrophages perform efferocytosis primarily using tyrosine receptor kinases as Ptd-L-Ser receptors, including Tyro 3, Axl, and proto-oncogene c-mer tyrosine kinase (MerTK) (collectively abbreviated as TAM) [81]. In a recent study, transcriptome and flow-cytometric analyses revealed that murine alveolar macrophages highly express Axl and MerTK, but little or no expression was found in lung-mobilized monocytes after the LPS challenge [82]. Moreover, human alveolar macrophages predominantly express Axl, and peripheral monocytes do not express either Axl or MerTK [83]. Although Axl-knockout mice did not manifest inflammatory disorders under healthy conditions, they exhibited exaggerated severity during pulmonary infection with influenza A virus, accompanied by increased accumulation of apoptotic cells, elevated infiltration of neutrophils and T-lymphocytes, and increased secretion of pro-inflammatory cytokines and chemokines, without compromising virus clearance [10]. In addition, during acute lung injury after LPS challenge in mice, alveolar macrophages engulfed Pst-L-Ser-exposed microparticles but not lung-mobilized monocytes, and deletion of MerTK abrogated efferocytosis activity in both in vivo and in vitro experiments [82]. Therefore, alveolar macrophages prevent excessive pulmonary inflammation via efferocytosis using Axl and MerTK in lung injuries caused by viruses and bacteria; notably, lung-mobilized pro-inflammatory monocytes do not contribute to efferocytosis, at least at the early stage of infection.

5.3 Anti-inflammatory properties of efferocytosis in alveolar macrophages

Notably, TAM receptor-mediated recognition of Ptd-L-Ser requires soluble cross-linking molecules in the serum (growth arrest-specific gene 6 or protein S) [84]. Similar to pathogen recognition by phagocytic receptors, ligation of TAM receptors results in the activation of Rac1, leading to membrane ruffling to engulf apoptotic bodies [85, 86]. Phagocytic receptors are linked to pro-inflammatory responses [4, 87], whereas TAM receptors activate anti-inflammatory responses in macrophages. For example, TAM receptor ligation activates type I IFN receptor signaling to upregulate the expression of suppressors of cytokine signaling 1 and 3. This induces negative feedback to suppress type I IFN receptor signaling and both MyD88- and TRIF-dependent TLR signaling [88]. Moreover, the detailed molecular mechanisms underlying the promotion of anti-inflammatory IL-10 and TGF-β production during efferocytosis in macrophages have also been elucidated. The coenzyme NAD^+, generated by mitochondrial β-oxidation of apoptotic cell-derived fatty acids, activates sirtuin-1 and downstream transcription factor PBX homeobox 1, producing IL-10 in macrophages [89]. Higher expression of cholesterol 25-hydroxylase, characteristically found in alveolar macrophages, contributes to the biosynthesis of 25-hydroxycholesterol, which stimulates the nuclear receptor liver X receptor to increase transcriptional activity during efferocytosis, leading to the escalation of TGF-β production [90]. Thus, alveolar macrophages have advanced efferocytosis activity, enabling them to promptly and effectively eliminate the apoptotic bodies that prominently appear during viral infection. Furthermore, this property is indispensable for preventing excessive pulmonary inflammation owing to the massive production of viruses and damage-associated molecular patterns (DAMPs) from apoptotic bodies that lose cell membrane integrity.

6. Age-associated dysfunction of alveolar macrophages

As discussed in Section 2, recent epidemiological data indicate that older adults are vulnerable to acute LRTIs that are attributable to either bacteria or viruses, and the globally increasing life expectancy further reinforces this fact. Phagocytosis by alveolar macrophages is responsible for the frontline defense against inhaled bacteria and fungi (Section 3), and the pro-inflammatory responses assist the defense by promoting phagocytosis (Section 4). During viral infection, efferocytosis of alveolar macrophages is indispensable to prevent uncontrolled pneumonia caused by DAMPs that leak from damaged and dead cells (Section 5). Since alveolar macrophages are characterized by advanced phagocytosis and efferocytosis, the decline in their activity is likely associated with the age-dependent exacerbation of acute LRTIs (**Figure 1**). In this section, we discussed the past and recent progress in the findings regarding age-related dysfunction of alveolar macrophages.

6.1 Age-associated decline in alveolar macrophage phagocytosis

A previous study demonstrated that macrophages accounted for approximately 95% of the bronchoalveolar lavage fluid cells in both young and aged mice [91]. The absolute numbers of alveolar macrophages were also similar, but they indicated an age-related decrease when adjusted for lung weight, as discussed later (subsection 6.5). The percentage of alveolar macrophages capable of phagocytosing latex beads was approximately 80% and 60% in young and aged mice, respectively, and the difference was statistically significant. Like bacteria, phagocytosis against non-opsonized latex beads is mediated by MSR1 and CD36 [92]. Thus, these results suggest that aging is associated with reduced expression of scavenger receptors and/or an impaired ability to transduce engulfment signals, leading to an age-dependent decline in alveolar macrophage phagocytosis (**Figure 1A**). This finding is supported by recent evidence from in vivo studies. The phagocytic capacity of each alveolar macrophage for intranasally instilled latex beads was lower in aged mice than in young mice [93]. In this study, aged mice also exhibited decreased cell surface expression levels of MSR1, but not of CD36 and CD206, in alveolar macrophages (**Figure 1A**).

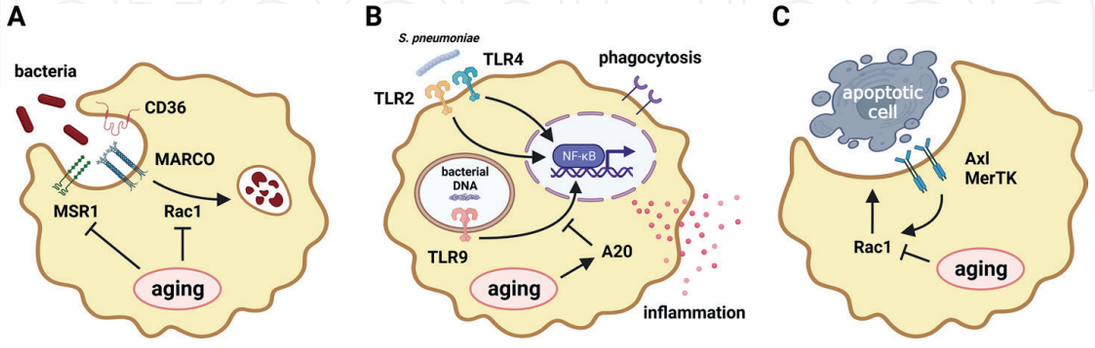

Figure 1.
Intracellular events involved in age-associated dysfunction of phagocytosis (A), pro-inflammatory responses (B), and efferocytosis (C) in alveolar macrophages. (A) Age-associated decline in phagocytosis is mediated by reduced expression levels of MSR1 and Rac1. (B) Age-associated decline in pro-inflammatory responses is due to elevated expression levels of A20, which inactivates TRAF6, an upstream signaling protein of NF-κB, to suppress Streptococcus pneumoniae-stimulated signaling activation of TLRs. (C) Age-associated decline in efferocytosis is possible to be dependent on reduced expression levels of Rac1, which transmits engulfment signal associated with the TAM receptors Axl and MerTK.

Moreover, alveolar macrophages in aged mice exhibited reduced phagocytosis after intratracheal injection of *E. coli*, which could be attributed to the reduced constitutive expression levels of Rac1 and resultant attenuated F-actin polymerization and filopodia formation (**Figure 1A**) [55]. No studies on human alveolar macrophages have been reported; however, unlike animal studies wherein the laboratory environment is maintained, identifying only the pure effects of aging in humans without other confounding factors is challenging. This is because smoking habits [94–96], chronic alcohol abuse [95, 97], and exposure to air pollutants [95] have been found to adversely influence alveolar macrophage phagocytosis.

6.2 Age-associated decline in alveolar macrophage pro-inflammatory responses

Studies indicate that increased susceptibility to pneumococcal infection in elderly people is associated with a compromised initial response to TLR signaling in alveolar macrophages (**Figure 1B**). For instance, alveolar macrophages from aged mice exhibit suppressed responsiveness to in vitro LPS stimulation [98]. Notably, aged mice exhibited reduced survival, impaired bacterial clearance, and attenuated prompt pro-inflammatory cytokine production after intratracheal challenge with *S. pneumoniae*, which was accompanied by attenuated *S. pneumoniae*- or its cell wall-stimulated phosphorylation of NF-κB p65 subunit, p38 MAPK, and JNK, in alveolar macrophages (**Figure 1B**) [99]. Further result was presented as a possible mechanism. In aged mice, the expression of A20 is specifically elevated in alveolar macrophages, which reduces *S. pneumoniae* exposure-induced IL-6 production (**Figure 1B**) [100]. A20 is known to inactivate TRAF6 in the cytosol, resulting in defects in its common downstream NF-κB, p38 MAPK, and JNK signaling cascades [101]. Thus, during pneumococcal infection, TLR9 signaling-mediated upregulation of alveolar macrophage phagocytosis can also be impaired in aged mice or humans (subsection 4.3.3) (**Figure 1B**). Notably, in an in vitro *Mycobacterium tuberculosis* infection model, compared with alveolar macrophages from young mice, those from aged mice constitutively expressed similar levels of TLR2, TLR4, and TLR9. They were able to produce equivalent levels of IL-12 and TNF-α in response to infection, while the contribution of TLR2 signaling to pro-inflammatory cytokine production was distinctly reduced in aged mice [102]. This suggests that phenotypes associated with age-dependent deterioration of TLR signaling differ according to the type of bacteria and possibly the composition of their virulence factors.

6.3 Age-associated decline in alveolar macrophage efferocytosis

Aged mice indicated significant deterioration in survival rate and clinical score after intranasal instillation with influenza A virus, which also caused increased inflammation, accumulation of apoptotic cells in the alveoli, and impaired ability to bind to and engulf apoptotic neutrophils in alveolar macrophages [93]. In this study, alveolar macrophages from aged mice retained normal Axl expression levels but had markedly reduced levels of MSR1, as discussed above (Section 6.1). Further, MSR1 suppresses excessive inflammation by mediating the internalization of DAMPs by macrophages in a mouse model of ischemic stroke brain injury [103]. In addition, MSR1 participates in Tyro 3 signaling in macrophages to mediate efferocytosis in a mouse model of acute aortic dissection [104]. However, since alveolar macrophages express Axl or MerTK, but not Tyro 3 (Section 5.2), whether the age-associated decline in efferocytosis is caused by defects in the MSR1-Tyro 3 signaling axis is

unclear. Engulfment of apoptotic cells via TAM receptors requires Rac1 activation (Section 5.3), and Rac1 expression is depleted in alveolar macrophages from aged mice (Section 6.1), implying that reduced Rac1 expression is involved in the age-associated decline in efferocytosis (**Figure 1C**). In summary, the decreased processing capacity for DAMPs due to suppressed MSR1 expression and decreased efferocytosis activity due to suppressed Rac1 expression in alveolar macrophages can be involved in the exacerbation of viral infection.

6.4 Age-associated change in alveolar macrophage subpopulation

Lung macrophages (a crude fraction containing both alveolar and interstitial macrophages) from aged mice has a high baseline level of dysfunctional expression of IFN-γ target genes, and IFN-γ fails to boost ex vivo *M. tuberculosis* infection-induced phagosome-lysosome fusion and IL-12 production in aged mouse cells [105]. The so-called inflammaging phenotype in alveolar macrophages and lining fluid extends further to a wide variety of pro-inflammatory cytokine and chemokine levels, which was caused by an increased subpopulation of CD11b-positive alveolar macrophages originating from peripheral monocytes [106]. Such inflammaging systemically occurs in humans as well [107]. Although inflammaging of alveolar macrophages has been suggested to increase susceptibility to *M. tuberculosis* in the elderly [105, 106, 108, 109], the relationship between inflammaging and vulnerability to acute LRTIs remains to be elucidated [110]. Further, recruitment of circulating monocytes to the alveoli has been demonstrated in several longitudinal studies using mice in which bone marrow-derived monocytes were labeled with specific reporters [111, 112] and was systematically discussed in a review article [113]. In contrast, another recent genetic lineage-tracing analysis using CD45.1/CD45.2 chimeric mice yielded contradictory observations that the proportion of CD45.1-positive monocyte-derived macrophages and CD45.2-positive tissue-resident macrophages in the alveoli were preserved throughout life [114]. However, when infected with a sublethal dose of the influenza A virus, monocyte-derived macrophages were recruited into the alveoli, and the macrophages persisted for at least 60 days. These results underpin previous findings that alveolar macrophages are not replenished by bone marrow-derived monocytes [35]. Further experimental results and an integrated understanding are required to clarify the age-associated changes in alveolar macrophage subpopulations and their role in susceptibility to acute LRTIs.

6.5 Age-associated change in alveolar macrophage abundance

A previous study reported a significantly reduced proportion of alveolar macrophages in bronchoalveolar lavage fluid cells in the elderly [115]. Likewise, aged mice indicated decreased numbers of alveolar macrophages per unit lung weight in two strains (BALB/c and C57BL/6 J), which was accompanied by the downregulation of gene expression that regulates the cell cycle [93]. These findings suggest that the quantitative decline in alveolar macrophages with age partially contributes to the high vulnerability to acute LRTIs in the elderly. In another recent study, the gene expression profile was reproduced in murine as well as human alveolar macrophages; however, this property could be mediated by the inhibition of GM-CSF signaling in alveolar macrophages due to age-dependent alterations in the alveolar microenvironment (especially, increased hyaluronan levels in the alveolar epithelial lining fluid), but not due to cell-autonomous mechanisms such as alterations in intracellular

signaling protein levels or circulating monocyte migration [114]. Indeed, the transplantation of alveolar macrophages from aged mice into the alveoli of young mice reverted age-related changes in the transcriptome to a state resembling young alveolar macrophages [114]. Although the importance of age-associated changes in the tissue microenvironment has long been proposed [21], recent advances in research methods and techniques have made it possible to elucidate the role of age-related alterations in the alveolar microenvironment. Therefore, the mechanism by which aging reduces phagocytosis, pro-inflammatory responses, and efferocytosis can be primarily explained by the inhibition of the differentiation or maturation of alveolar macrophages through microenvironmental degeneration.

7. Conclusion

Alveolar macrophages acquire heterogeneity with other lineages by receiving unique signals in the alveolar microenvironment. The advanced phagocytosis and efferocytosis activities of alveolar macrophages enable efficient clearance of continuously inhaled pathogens and endogenous dead cells, respectively, which contributes to the prevention of uncontrolled pneumonia. Previous studies have addressed the reasons for the vulnerability of the elderly to acute LRTIs, mainly shedding light on the senescence process of alveolar macrophages from a cell-autonomous aspect. However, in addition to the knowledge gained from such studies, recent progress in experimental methods and techniques is beginning to provide insightful evidence that age-associated alterations in the alveolar microenvironment mediate reversible dysfunction of alveolar macrophages. In other words, to improve age-related dysfunction of alveolar macrophages, an approach that targets the cells is inefficient, whereas exploring methods to recover age-related alterations in the alveolar microenvironment is appropriate. As the average life expectancy is estimated to further increase in the future, exploring health promotion activities (i.e., habitual exercise, healthy diet, and regular sleep cycle) or supplements that influence the alveolar microenvironment and whether such factors can reduce the risk of acute LRTIs in the elderly is essential. We hope that this chapter will help students, trainees, and researchers in their education and research in health and life sciences.

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Conflict of interest

The authors declare no conflict of interest.

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