Biochemistry and Biophysics Reports 40 (2024) 101826

Contents lists available at ScienceDirect

Biochemistry and Biophysics Reports

journal homepage: www.elsevier.com/locate/bbrep

The role of alveolar macrophages in viral respiratory infections and their therapeutic implications

Atefe Panahipoor Javaherdehi [a], Somayyeh Ghanbari [b], Pooya Mahdavi [c], Alireza Zafarani [d,e], Mohammad Hossein Razizadeh [f,g,*]

[a] Department of Biotechnology, Tehran Medical Sciences, Islamic Azad University, Tehran, Iran
[b] Faculty of Medicine, Qom Branch, Islamic Azad University, Qom, Iran
[c] Department of Immunology, Pasteur Institute of Iran, Tehran, Iran
[d] Cellular and Molecular Research Center, Iran University of Medical Sciences, Tehran, Iran
[e] Department of Hematology & Blood Banking, School of Allied Medicine, Iran University of Medical Sciences, Tehran, Iran
[f] Department of Virology, School of Medicine, Iran University of Medical Sciences, Tehran, Iran
[g] Antimicrobial Resistance Research Center, Institute of Immunology and Infectious Diseases, Iran University of Medical Sciences, Tehran, Iran

ARTICLE INFO

Keywords:
Innate immunity
Viral infections
Alveolar Macrophages
Viral Immunology

ABSTRACT

Alveolar macrophages are pivotal components of the lung's innate immune defense against respiratory virus infections. Their multifaceted role spans from viral clearance to modulation of immune responses, making them essential players in shaping disease outcomes. In this comprehensive review collection, we look into the intricate interplay between Alveolar macrophages and various respiratory viruses, shedding light on their dynamic contributions to immune resilience. From influenza to respiratory syncytial virus, Alveolar macrophages emerge as sentinels of the airways, actively participating in viral detection and initiating rapid antiviral responses. Their ability to recognize viral pathogens triggers a cascade of events, including cytokine and chemokine production that guides the recruitment and activation of immune effectors. Furthermore, Alveolar macrophages impact the fate of adaptive immune responses by modulating the activation of T lymphocytes and the secretion of key cytokines. These reviews encompass a range of insights, including the regulation of inflammasome activation, the influence of Alveolar macrophages on cytokine dysregulation, and their role in preventing secondary bacterial pneumonia post-infection. Collectively, they highlight the significance of Alveolar macrophages in preserving pulmonary integrity and immune homeostasis during viral challenges.

1. Introduction

Macrophages are a main group of innate immune cells that dwell in many tissues and are important in maintaining the immune function in the host tissue. By possessing a set of different receptors, they can be stimulated by pathogens and commence immune response. Also, they are responsible for homeostatic activities including releasing tissue repair and remodeling, clearing dead cells, angiogenesis, and metabolism [1,2]. Classically, macrophages are classified into 2 groups: M1 macrophages, which are a part of inflammatory cells and help body fight microorganisms; and the second group, M2 macrophages, that are responsible for immune tolerance and tissue repair [3]. However, this classification has recently been challenged by studies that identified presence of both M1 and M2 features within the same macrophages [4].

It has been found that various groups of tissue-resident macrophages differ in terms of developmental pathway and cellular function. These differences stem from environmental factors within tissues, signaling pathways, and transcription factors. In airways and lungs, alveolar macrophages (AMs) are the primary immune cells. In mice, AMs can be identified by specific markers, including high levels of integrin CD11c and sialic acid–binding immunoglobulin-like lectins (Siglec)-F, which distinguish them from transient monocyte-derived cells. In humans, AMs are characterized by the expression of markers such as CD206, CD169, CD11c, CD163, and the macrophage receptor with collagenous (MARCO) [5]. Their lack of proper function results in the development of various diseases such as acute lung injury and pulmonary fibrosis [6].

* Corresponding author. Department of Virology, Faculty of Medicine, Iran University of Medical Sciences, Antimicrobial Resistance Research Center, Institute of Immunology and Infectious Diseases, Iran University of Medical Sciences, Tehran, Iran.
E-mail addresses: Razizadeh.mh@gmail.com, razizadeh.mh@iums.ac.ir (M.H. Razizadeh).

https://doi.org/10.1016/j.bbrep.2024.101826
Received 9 July 2024; Received in revised form 9 September 2024; Accepted 13 September 2024

Respiratory infections pose a serious threat to the fragile respiratory environment since they are frequently caused by viral pathogens. The largest surface area of the human body in contact with the environment is the lungs' epithelium. Each day, enormous volumes of air and aerosols flow through these cells, exposing the lung tissue and other parts of the respiratory tract to microorganisms found in the air. Viruses penetrate the respiratory epithelium when inhaled, with the intention of seizing control of the cellular machinery for their own replication. A series of actions may result from this incursion, such as the activation of immunological responses [7]. Therefore, in the early stages of respiratory infections, the interaction between viruses and immune cells becomes a critical battlefield that determines the course of later immune responses and the infection's ultimate fate. This review discusses the pivotal role of AMs in host defense against respiratory viruses.

2. AM biology

2.1. Development of AMs

Recent studies indicate that AMs originate from embryonic monocyte progenitors, initially produced in the yolk sac or fetal liver during embryonic development [8,9]. Subsequently, these ancestors go to the lung and differentiate into AMs. Furthermore, monocyte-derived macrophages and interstitial macrophages from circulating monocytes may act as progenitors to AMs in situations such as injury or depletion [10,11].

The development AMs is complexly regulated by various signaling pathways such as Granulocyte-macrophage colony-stimulating factor (GM–CSF)–peroxisome proliferator-activated receptor gamma (PPAR-γ) axis and transforming growth factor beta (TGF-β) [6,12,13]. Traditionally, Macrophage colony-stimulating factor (M-CSF) has been recognized as a key regulator of monocyte/macrophage lineage cells. However, it does not have a wide role in the development of AMs [14]. Instead, GM-CSF has gained attention for its critical contribution to AM maturation. Investigation of GM–CSF–deficient mice have revealed pathological lung alterations reminiscent of human pulmonary alveolar proteinosis (PAP), which is characterized by the accumulation of eosinophilic materials in alveolar spaces due to impaired surfactant production by AMs [12]. Furthermore, those mice have less number of AMs precursors at different embryonic stages, which indicates the importance of GM-CSF in the developmental pathway of AMs [15].

During embryonic lung development, the levels of GM-CSF surge, which coincides with the differentiation of AM precursors into mature AMs [12]. This cytokine acts as a potent inducer of PPAR-γ expression and activity, which serves as a major transcriptional regulator of AM development [15]. Noteworthy, Phenotypic similarities between GM–CSF–deficient, GM-CSFR β-chain (GM-CSFR βc)-deficient, and PPAR-γ-deficient mice show the significance of the GM-CSF–PPAR-γ axis in AM biology [6]. PPAR-γ is a critical transcription factor for the differentiation and functional identity of AMs. Using conditional knockout models have shown that the absence of PPAR-γ in macrophage populations, particularly in CD11c + cells, results in a profound defect in AM development. These mice exhibit a significant reduction in the number of mature AMs, which are instead replaced by immature, CD11b-high macrophage-like cells. Furthermore, PPARG-deficient AMs accumulate lipid droplets, resembling foam cells, and fail to effectively clear surfactant from the lungs. This is consistent with the role of PPAR-γ in regulating lipid metabolism, immune responses, and maintaining lung homeostasis. Importantly, PPAR-γ operates upstream of other transcription factors, such as EGR2, and is essential for the cytokine GM–CSF–driven pathways that promote AM terminal differentiation. Without PPAR-γ, AMs cannot fully mature or perform their key functions in tissue repair and pathogen clearance, highlighting its indispensable role in the immune and metabolic programming of AMs [15]. The transcription factor EGR2 has been identified as a distinct characteristic of AMs in both mice and humans, and it is essential for establishing the phenotypic identity of these cells. In mice, the deletion of EGR2 in myeloid cells disrupted the effective clearance of bacteria from the respiratory tract and hindered tissue repair following a sterile injury. This process depends on monocyte-driven AMs repopulation. The expression of EGR2 was triggered by the cytokines TGF-β and GM-CSF through a PPAR-γ–dependent pathway, indicating that EGR2 functions downstream of other critical signals that collectively regulate the AMs functional program [16]. The immune function of AMs relies on the activation of the transcription factor PU.1 by GM-CSF. In $GM^{-/-}$ mice, PU.1 levels were significantly reduced in AMs, but selective expression of GM-CSF in the lungs of SPC-GM/$GM^{-/-}$ transgenic mice restored PU.1. Additionally, introducing PU.1 through a retrovirus in AMs from $GM^{-/-}$ mice restored their host defense functions and the ability to break down surfactant. PU.1 plays a crucial role in GM–CSF–dependent terminal differentiation of AMs, which is essential for their innate immune functions and surfactant metabolism [17,18]. Recent research has highlighted the role of the transcription factor Bach1 in regulating oxidative stress and its impact on AMs. Bach1 plays a role in modulating antioxidant protein levels, such as heme oxygenase-1 (HO-1). Elevated levels of Bach1 have been associated with decreased expression of antioxidant proteins in conditions such as pulmonary emphysema. This shows it contribution in oxidative stress and impaired AM function [19]. Additionally, Bach1 has shown to work with Bach2 to repress inflammatory response genes in AMs, as demonstrated by studies using Bach1/Bach2 double knockout (DKO) mice. While Bach2 deficiency alone leads to impaired lipid handling and a PAP-like phenotype, Bach1 and Bach2 DKO mice display more severe abnormalities, suggesting their complementary roles in maintaining lung homeostasis [20]. These findings imply that Bach1 may influence AM function by regulating oxidative stress responses and surfactant metabolism, similar to the pathways mediated by EGR2 and PU.1.

Although GM-CSF is indispensable for AM development, it should be noted that excessive signaling can lead to aberrant outcomes. The Ssu72 protein is a crucial regulator in fine-tuning GM-CSFR signaling intensity. AMs upregulate Ssu72 expression in response to GM-CSF stimulation, which dephosphorylates GM-CSFR βc and attenuates downstream signaling. Developmental defects in AM are caused by dysregulation of this fine-tuning mechanism, which can be rectified by adjusting the strength of GM-CSF signaling [21].

In addition to GM-CSF, TGF-β plays a pivotal role in AM development [13]. Fetal liver (FL) monocytes continuously produce this cytokine, which causes autocrine and paracrine processes that drive their differentiation into AMs. The disruption of homeostasis caused by the selective deletion of TGFβ receptor 2 (TGFβR2) in AMs emphasizes the need for ongoing TGFβ signaling to maintain AMs. PPAR-γ expression and activity in AMs are probably regulated by TGF-β via intricate signaling cascades that involve the SMAD3 and MAPK pathways [22]. TGFβ1 triggers an elevation of DNA methyltransferase 1 (DNMT1) expression in human trabecular meshwork cells. It has been shown that this event can in turn promote M1 AMs polarization through microRNA (miR)-124/-Pellino 1 (PELI1)/interferon regulatory factor 5 (IRF5) Axis [23]. Moreover, TGFβ can increase the expression of Mast-cell expressed membrane protein-1 (MCEMP1) in AMs and monocytes, which promotes cell chemotaxis, adhesion, and migration in these cells [24].

Using a humanized mouse model, MISTRG mice, to delineate the ontogeny of AMs, a study [25] pinpointed AMs progenitors from human fetal liver by characterizing the expression of CD116, the transcription factor MYB, and GM-CSF receptor. Within the lungs of MISTRG mice, transplantation tests revealed a precursor-product link between mature human AMs and $CD34^{-}CD116+$ fetal liver cells. Interestingly, precursors of circulating macrophages expressing CD116, $CD64^{-}$, and CD115+ were observed to migrate from the liver to the lung. These precursors are identical to those reported in human fetal lung tissue and express the CX3CR1 chemokine receptor. With a proliferative gene profile, these fetal macrophage precursors can outcompete their adult counterparts and adapt well to the perinatal alveolar niche. Eventually,

they develop into functional AMs [25]. This showed not only a developmental route of AMs but also underscored their role in early immune response in the lung microenvironment.

Epigenetic analysis revealed the importance of histone deacetylase 3 (HDAC3) in AM development and homeostasis. In early embryonic development, a defect of HDAC3 negatively affects AM development, which initiates at E14.5. Additionally, postnatal loss if HDAC3 impedes proper homeostasis and maturation of these cells. Single-cell RNA sequencing analyses showed that loss of HDAC3 can also cause mitochondrial oxidative dysfunction, which is probably via the disruption of HDAC3-PPAR-γ axis [26]. This shows the importance of HDAC3 as an essential developmentary regulator of AMs.

In conclusion, the development and function of AMs are intricately regulated by a network of signaling pathways and molecular factors, with GM-CSF, PPAR-γ, TGF-β, and HDAC3 playing pivotal roles. AMs originate from embryonic monocyte progenitors and undergo complex differentiation processes influenced by the lung microenvironment. The GM-CSF–PPAR-γ axis is critical for AM maturation, while TGF-β signaling sustains their development and function. Epigenetic regulation, mainly through HDAC3, further underscores the importance of precise molecular control in AM ontogeny. Disruptions in these pathways can impair AM function, highlighting the need for a balanced regulatory environment to maintain lung immunity. Understanding these mechanisms provides insights into potential therapeutic strategies for lung-related diseases and immune responses.

2.2. Immune surveillance

The airways are always exposed to non-sterile air, which poses a challenge for airways immune surveillance. AMs play a significant role in airways immune surveillance by constantly patrolling the alveoli, sensing, chemotaxing, and phagocytosing inhaled pathogens. Understanding the role of AMs in immune surveillance is crucial, especially in the context of respiratory infections. Intravital imaging has revealed the dynamic behavior of AMs, crawling within and between alveoli to intercept pathogens. Impairment of AM chemotaxis leads to excessive neutrophil recruitment, which leads to imperfect inflammation and tissue injury, as observed in secondary bacterial co-infections following influenza A virus infection [27]. Pathogens have evolved strategies to evade immune surveillance and exploit the immunosuppressive properties of AMs [28], benefiting from the anti-inflammatory effects of prostaglandin E2 (PGE2) produced by these cells, which can suppress immune response by decreasing Natural Killer (NK) cell activity and Major Histocompatibility Complex (MHC) II expression [29,30]. Additionally, MTB can manipulate AMs metabolism by decreasing its oxygen consumption rate and suppressing its glycolytic pathway [31]. Inflammation can also dampen the phagocytic capability of AMs. In fact, Secondary immunosuppressive signals can develop tolerance in AMs. Signal-regulatory protein α (SIRPα), which regulates tyrosine kinase-coupled signaling processes such as phagocytosis, released after the resolution of inflammation induced by Influenza A virus can influence the lung microenvironment by epigenetically inducing tolerance in AMs via downregulating Setdb2, which regulates chemokine responses during viral pneumonia by encoding a methyltransferase [32]. Age is another factor that influences AMs immune surveillance. Infecting mice of different age groups with Group B Streptococcus (GBS) resulted in higher mortality among neonate mice in comparison with young and adult mice. AMs phagocyte highly sialylated GBS capsule by the cell surface Siglec receptors. Since neonate mice express less Singlec receptors than older ones, it can be comprehended that a developmental impairment in Singlec gene expression can impede efficient phagocytosis of pathogens [33].

3. Modulation of inflammation

3.1. Balancing pro-inflammatory and anti-inflammatory responses

The lung microbiome plays a crucial role in determining the activation state of AMs. A harmonious microbiome supports immune tolerance, whereas an imbalance in the microbiota can trigger catabolic pathways and change the functions of AMs [34,35]. For example, a study [36] has shown that the microbiota enhances respiratory defenses by activating GM-CSF signaling, which improves the ability of AMs to eliminate pathogens through extracellular signal-regulated kinase signaling. The microbiota stimulates an elevation in pulmonary GM-CSF synthesis in response to infection, facilitated by interleukin (IL)-17A [36]. AMs have a reduced tendency to absorb apoptotic cells when inactive, but this changes during acute inflammation. Surfactant proteins A (SP-A) and D (SP-D) have a substantial impact on macrophage function by blocking the clearance of dying cells through the activation of the transmembrane receptor called SIRPα [37]. AMs in the lungs are essential for identifying and reacting to inhaled infections. When stimulated by these pathogens, AMs release proinflammatory cytokines that activate receptors on alveolar epithelial cells [38]. This stimulation results in the mobilization of neutrophils, which aid in phagocytosing and eradicating the infections. AMs positioned on the alveolar wall create channels with connexin 43 (Cx43) that enable direct communication with epithelial cells. During lipopolysaccharide-induced inflammation, AMs stay attached to the alveoli and interact by releasing coordinated waves of calcium ions (Ca2+) [38,39]. Cx43 facilitates communication leading to immunosuppression by activating Akt through calcium signaling. Deletion of Cx43 in AMs boosts neutrophil recruitment to the alveoli and elevates the release of proinflammatory cytokines in the bronchoalveolar lavage fluid [38,39]. Studies have revealed that when compared to macrophages in other regions of the body, AMs express more of the negative regulator CD200 receptor (CD200R). The presence of CD200 on the airway epithelium regulates AM activity. Mice lacking CD200 had higher macrophage activity and were more vulnerable to influenza infection, which caused inflammation to resolve more slowly and eventually resulted in death [40]. Previous studies have demonstrated that AMs do not upregulate the production of B7-1 and B7-2 when stimulated with interferons (IFNs)-γ. Research has shown that AMs can cause antigen-specific T-cell inactivation and reduce the responsiveness of CD4 T cells upon future stimulation. To offset this induced inactivity, CD28 co-stimulation or IL-2 might be added [41,42]. Studies have shown that AMs can cause anergy in $CD4^+$ T lymphocytes through the involvement of nitric oxide, prostaglandins, and leukotrienes in this tolerance mechanism. Studies have demonstrated that both AMs and conditioned media from AMs (AM-CM) from mice and humans may induce the production of FoxP3 in naive $CD4^+$ T cells in vitro. Part of this impact was undone by preventing retinoic acid (RA) from attaching to its receptor (RAR) or by obstructing TGF-β1 signaling. The RAR antagonist administered nasally decreased the presence of CD4+FoxP3+ T cells in the lungs of mice after being exposed to *Bordetella pertussis* by aerosol [43]. An interesting feature of how AMs regulate the immune system relates to asthma and bacterial infections. Research has demonstrated that murid herpesvirus 4 (MuHV-4) can inhibit the development of experimental asthma triggered by house dust mite (HDM) through its impact on lung innate immune cells. Specifically, MuHV-4 infection led to the replacement of local AMs by monocytes with regulatory functions. The monocyte-derived AMs inhibited the ability of dendritic cells to trigger a house dust mite-specific response by T helper 2 (TH2) cells [44]. Another Research has demonstrated that mice display enhanced defense against *Streptococcus pneumoniae* infection one month after recovering from influenza. This improved protection is due to a specific group of AMs produced from monocytes that secrete increased amounts of IL-6. AMs originating from monocytes after influenza infection exhibit a surface phenotype resembling resident AMs, although they have unique functional,

transcriptional, and epigenetic characteristics compared to resident AMs [45]. A recent study indicates that exposing innate immune cells, including tissue-resident macrophages, to damaging stimuli multiple times can strengthen the body's defense systems [46]. It has been shown that lung-resident AMs become more resistant to cell death from infections when pre-exposed to bacterial endotoxin or *Pseudomonas aeruginosa*. These trained AMs also show enhanced capacity to absorb cellular debris and facilitate tissue repair. Reprogrammed AMs exhibit increased amounts of the efferocytosis receptor myeloid epithelial reproductive proto-oncogene tyrosine kinase (MERTK), which is controlled by the transcription factor KLF4. Transferring these trained antimicrobial agents into mice has been proven to reduce inflammatory lung damage when exposed to fatal *P. aeruginosa* infection [46]. AMs are critical in maintaining lung immunity and are heavily influenced by the lung microbiome, cytokine signaling, and environmental stimuli. The lung microbiome supports immune tolerance and enhances AM function through pathways like GM-CSF signaling, which is vital for pathogen clearance. AMs exhibit a range of functional responses, from coordinating with epithelial cells during inflammation to modulating T-cell activity and promoting immune tolerance. Moreover, AMs can undergo reprogramming in response to repeated exposure to pathogens, enhancing their resilience and capacity for tissue repair.

Research findings indicate that depleting AMs either 3 or 5 days after infection with a lethal influenza virus didn't notably impact the disease outcome. However, eliminating these cells before a sublethal infection resulted in unchecked virus proliferation and mortality in mice [47]. This suggests that AMs play a protective role in the initial stages of infection before the virus gains dominance [47–49]. Interestingly, it has been shown that after depletion of AMs, monocyte derived macrophage replace them and cause to a more severe influenza infection [50,51]. For instance, research has demonstrated that H5N1 influenza has a greater capacity to stimulate AMs to produce higher levels of proinflammatory cytokines compared to the H1N1 strain. This characteristic renders H5N1 more pathogenic than H1N1 influenza [52,53]. Interestingly, the H5N1 virus was able to replicate remarkably well in DCs and AMs, even though these cells exhibited a strong antiviral interferon response. This ability to spread efficiently in immune cells might help explain the severe pathogenicity of H5N1 virus infections in humans [54]. Research indicates that IFN-β released by AMs is a critical factor in damaging alveolar epithelial cells in influenza infection. In extreme cases of influenza infection, IFN-β triggers an autocrine pathway that leads to the synthesis of the pro-apoptotic factor tumor necrosis factor (TNF)-related apoptosis-inducing ligand (TRAIL) Consistent with earlier studies on the harmful impact of recruited macrophages on alveolar epithelial cells due to the release of TRAIL [55,56]. Influenza infection can cause lung damage and acute respiratory distress syndrome (ARDS), which is marked by an abnormal buildup of fluid in the tiny air sacs of the lungs, leading to low oxygen levels and perhaps lethal consequences if not addressed. The removal of excess edema fluid depends mostly on the activity of the alveolar epithelial Na, K-ATPase, which is crucial for the survival of patients with ARDS. Researchers have discovered a communication network between infected and non-infected alveolar epithelial cells and AMs that results in a decrease in alveolar epithelial Na, K-ATPase function, and plasma membrane abundance, ultimately hindering alveolar fluid clearance. The decrease in Na, K-ATPase caused by influenza is controlled by a host signaling cascade that includes epithelial type I IFN and an IFN-dependent rise in macrophage TRAIL [57]. A study has demonstrated that the transcription factor PPAR-γ decreases in AMs during influenza infection as a result of type I IFN-dependent signaling. PPAR-γ is essential for regulating excessive antiviral and inflammatory reactions of AMs during influenza and respiratory syncytial virus (RSV) infections. PPAR-γ deficiency in myeloid cells results in heightened host morbidity and lung inflammation after influenza and RSV infections, underscoring its role in restricting the advancement of severe illness. PPAR-γ expression in resident AMs is crucial for controlling the development of host diseases and is important for the activation of genes related to wound healing. Therefore, the absence of myeloid PPAR-γ leads to reduced ability to resolve inflammation and inadequate tissue repair after influenza infection [58,59]. Administering the PPARγ agonist Troglitazone reduce the secretion of proinflammatory cytokines induced by the virus, suggesting it as a potential therapeutic option [59]. In general, the replacement of depleted AMs by monocyte-derived macrophages can exacerbate the severity of influenza, highlighting the unique protective functions of resident AMs. The heightened pathogenicity of viruses such as H5N1 underscores the importance of AMs in controlling viral spread and modulating inflammatory responses. Additionally, the balance of antiviral and inflammatory responses is critically regulated by factors such as IFN-β and PPAR-γ within AMs. Disruptions in these pathways can lead to excessive inflammation, impaired fluid clearance, and severe lung damage, as seen in conditions such as ARDS.

3.2. Tissue repair and resolution

Macrophages continuously remove apoptotic cells to avoid necrosis and facilitate the healing of injuries. The processes behind this process are not completely known. A study showed that efficient efferocytosis is improved by linking the metabolism of absorbed material during early clearance to later stages. It has been discovered that the continuous process of efferocytosis is enhanced by the conversion of arginine and ornithine from apoptotic cells into putrescine by macrophage enzymes arginase 1 and ornithine decarboxylase (ODC) [60]. Furthermore, it has been noticed that methionine from apoptotic cells contributes to resolution by epigenetically suppressing the extracellular signal-regulated kinase 1/2 (ERK1/2) phosphatase Dusp4. Another research shows that efferocytosis triggers the activation of prostaglandin-endoperoxide synthase 2/cyclooxygenase 2 (Ptgs2/COX2), leading to PGE2 and the stimulation of TGF-β1. ERK1/2 phosphorylation/activation by apoptotic cells-activated CD36 is necessary for Ptgs2 induction, but it is limited by an ERK-DUSP4 negative feedback mechanism that decreases phospho-ERK levels. After apoptotic cells are engulfed and degraded, Dusp4 is repressed, leading to increased p-ERK levels and activation of the Ptgs2-PGE2-TGF-β1 pathway. Apoptotic cell-derived methionine is converted to S-adenosylmethionine, which is used by DNMT3A to methylate Dusp4 [61]. There are also other ways that AMs aid resolution of inflammation and tissue repair for example, through the secretion of immunomodulatory cytokines such as IL-10 and CCL22, as well as a variety of growth factors including TGF-β, VEGF, trefoil factor 2, and PDGF, AMs actively contribute to the resolution of inflammation and the repair of damaged airways [51]. Specifically, it has been demonstrated that the production of TNF-α by AMs can induce the release of GM-CSF by alveolar epithelial cells, hence contributing to the process of alveolar epithelial healing [62]. IL-4 and IL-13, in addition to apoptotic cells, were responsible for inducing the activation of anti-inflammatory and tissue-repair genes within AMs in a model of helminth infection [51,63,64]. Recent studies have found that AMs have elevated expression of Placenta-expressed transcript 1 (Plet1). The study revealed that Plet1, produced by AMs, acts as a key mediator in the interaction between macrophages and epithelial cells in lung healing. It promotes the growth of alveolar epithelial cells and helps repair the epithelial barrier. Injecting recombinant Plet1 directly into the lungs at an early stage of the disease has been proven to reduce viral lung damage and save mice from a potentially deadly condition, showing its potential as a treatment [65].

It is necessary to strike a delicate balance between the resolution of inflammation, the restoration of the epithelial barrier, and the avoidance of aberrant remodeling to successfully repair and restore damaged tissue, which failing in this process can lead to fibrosis. Interestingly, Research indicates that monocyte-driven AMs are the primary cells involved in this process, as they express genes associated with fibrosis [66]. Chronic herpesvirus infection in the lung caused AMs to be attracted to regions exhibiting epithelial hyperplasia and fibrosis. The

AMs that were attracted showed elevated levels of proteins such Ym1/2, FIZZ1, insulin-like growth factor-1, and arginase I, as well as enhanced fibronectin transcription, suggesting activation through an alternate pathway. Arginase I was found in interstitial fibroblasts, and increased arginase activity was discovered in the lungs of infected mice. In individuals with idiopathic pulmonary fibrosis (IPF), lung tissue displayed increased levels of arginase I expression in epithelial cells, fibroblast foci, and AMs compared to healthy lung tissue. The results indicate that the increase in arginase I caused by the virus may have a role in the development of lung fibrosis [67]. Patients with IPF showed significantly decreased efferocytosis by AMs in comparison to patients with other types of interstitial pneumonia. This disruption in the process of efferocytosis could be a contributing factor in the progression of IPF [68].

All these pieces of evidence show that AMs are vital for lung homeostasis, clearing apoptotic cells, and promoting tissue repair through metabolizing absorbed materials and secreting immunomodulatory factors. While they aid in resolving inflammation and healing, disruptions in these processes can lead to conditions like fibrosis, especially when monocyte-derived AMs drive abnormal tissue remodeling.

3.3. Anti-tumor properties of AM

AMs' ability to modulate immune responses makes them critical in the context of lung cancer [69]. In patients with various subtypes of lung cancer, including small cell carcinoma, squamous cell carcinoma, and large cell undifferentiated carcinoma, AMs exhibit altered phagocytic functions and cytokine secretion profiles. Specifically, these AMs show a reduced uptake of particles and decreased secretion of pro-inflammatory cytokines such as TNF-α, IL-1, and IL-6, which potentially depicts an impaired anti-tumor immune response. Additionally, phenotypic changes in AMs, such as decreased expression of MHC class II, ICAM-1, and CD83, have been observed, particularly in patients with small cell carcinoma. These alterations suggest that AMs in lung cancer patients may have a diminished capacity to stimulate an effective anti-tumor immune response, which could vary depending on the type of lung cancer [69]. However, AMs can be "trained" by prior immune challenges, such as respiratory viral infections, to develop enhanced anti-tumor capabilities [70]. For instance, exposure to influenza virus has been shown to reprogram AMs, endowing them with long-lasting, tissue-specific antitumor immunity. These trained AMs infiltrate tumor lesions and demonstrate enhanced phagocytic activity and cytotoxic functions against tumor cells. This training is associated with epigenetic, transcriptional, and metabolic changes that enable AMs to resist tumor-induced immune suppression. The process of generating this trained immunity in AMs is dependent on IFN-γ and NK cells. Furthermore, the presence of AMs with trained immunity traits in human non-small cell lung cancer tissue correlates with a favorable immune microenvironment, highlighting their potential role in tumor surveillance and control [70]. These findings suggest that the functional state of AMs, whether impaired in certain lung cancer subtypes or enhanced through trained immunity, is crucial in determining their effectiveness in anti-tumor responses. Understanding the mechanisms that govern AM training can show researchers new ways for therapeutic strategies aimed at enhancing the anti-tumor potential of these immune cells.

4. AM in viral respiratory infections

4.1. AMs in RSV infection

Findings indicate that AMs are the primary producers of Type I IFNs in response to viral infections [71]. A study conducted on mice infected with Newcastle disease virus (NDV) revealed that targeted depletion of AMs resulted in a significant impairment in the initial clearance of the virus from the lungs, confirming the crucial role of these cells in secreting IFNs [72]. Moreover, Study has shown that AMs are the main source of IFNs when mice are infected with RSV. AMs detect RSV through the use of mitochondrial antiviral signaling protein (MAVS)--coupled retinoic acid-inducible gene 1 (RIG-I)-like receptors (RLRs). MAVS deficiency heavily impairs the innate immune response to RSV [73]. Research shows that C fibers (the nociceptive sensory nerves) are essential for the host's resistance against RSV infection. Blocking transient receptor potential vanilloid 1 (TRPV1) to inhibit C fiber activity after RSV infection in mice results in lower viral reproduction and decreased lung inflammation compared to control mice. A large increase in AMs has been seen in mice with deteriorated C fibers following RSV infection, accompanied by elevated levels of IFN-α/β production in bronchoalveolar lavage fluid (BALF) samples. C fiber degradation results in the release of vasoactive intestinal peptide (VIP), which helps regulate AM and IFN-α/β levels, offering defense against RSV infection. The results emphasize the important role of C fibers in controlling antiviral responses against RSV infection through VIP, which shows novel possible treatment strategies for RSV infection [74].

4.2. AMs in influenza infection

Investigations indicate that AMs are depleted along with disrupted phagocytic activity during influenza infection, which prepares the environment for virus progression and subsequent bacterial infection. However, following recovery from the disease or treatment with GM-CSF therapy, the pool of AMs is successfully replenished [75–77]. Another study demonstrates that the presence of bacteria in the normal microbiota helps mitigate influenza infection by promoting the activation of AMs [78]. It was discovered that blocking the entry of macrophages into the lungs in a mouse model of influenza-induced pneumonitis worsened the damage and programmed cell death of alveolar epithelial cells. Hepatocyte growth factor (HGF), released by macrophages in response to influenza, helps in the repair of alveolar epithelial tissue [79]. It has been shown that IFN-I not only makes the host more susceptible to secondary bacterial infections after influenza but also hinders the ability of human macrophages to engulf bacteria through phagocytosis [80]. In the case of AMs, an investigation revealed that influenza infection activates IFN-γR signaling, impairing the ability of AMs to eliminate microorganisms without lowering immunological responses or neutrophil function across the body. Reduced ability of AMs to eliminate microorganisms results in increased vulnerability to pneumococcal infection; it is shown that the highest vulnerability to pneumococcal superinfection corresponds with the production of IFN-γ; this opens a way for future studies to evaluate the exact role of IFN-γ in AMs defense against viral infections [81]. In other viral infections, it has been demonstrated that neonatal rhinovirus hampers the immune response of AMs, making them more susceptible to *S. pneumoniae* infection by upregulating Toll-Like Receptor (TLR)-3 signaling, which impairs the phagocytosis ability via poly I:C (polyinosinic:polycytidylic acid) [82]. A study revealed that mice with lung-expressed GM-CSF displayed strong resistance against influenza virus infection. This resistance was attributed to AMs, which exhibited heightened resistance to apoptosis [83]. Another research emphasizes the crucial function of AMs in the survival of mice infected with influenza and vaccinia viruses. AMs prevent asphyxiation by preserving lung function, which is a major factor in preventing death. When AMs were absent in mice without GM-CSF or in those with selectively depleted AMs, it caused poor gas exchange, leading to lethal hypoxia and severe illness during influenza virus infection [84]. However, the ability to clear the virus was only slightly impacted. Reintroducing AMs formation in mice lacking GM-CSF or its receptor avoided severe sickness and mortality, highlighting the crucial role of AMs in disease outcomes [84]. Fig. 1 illustrates interactions between viruses and AMs. Interestingly, during influenza infection, there is a considerable release of IL-1β, which is strongly associated with the infiltration of neutrophils and is stopped when neutrophils are removed. AMs are identified as the main contributors to IL-1β production during influenza infection and the virus

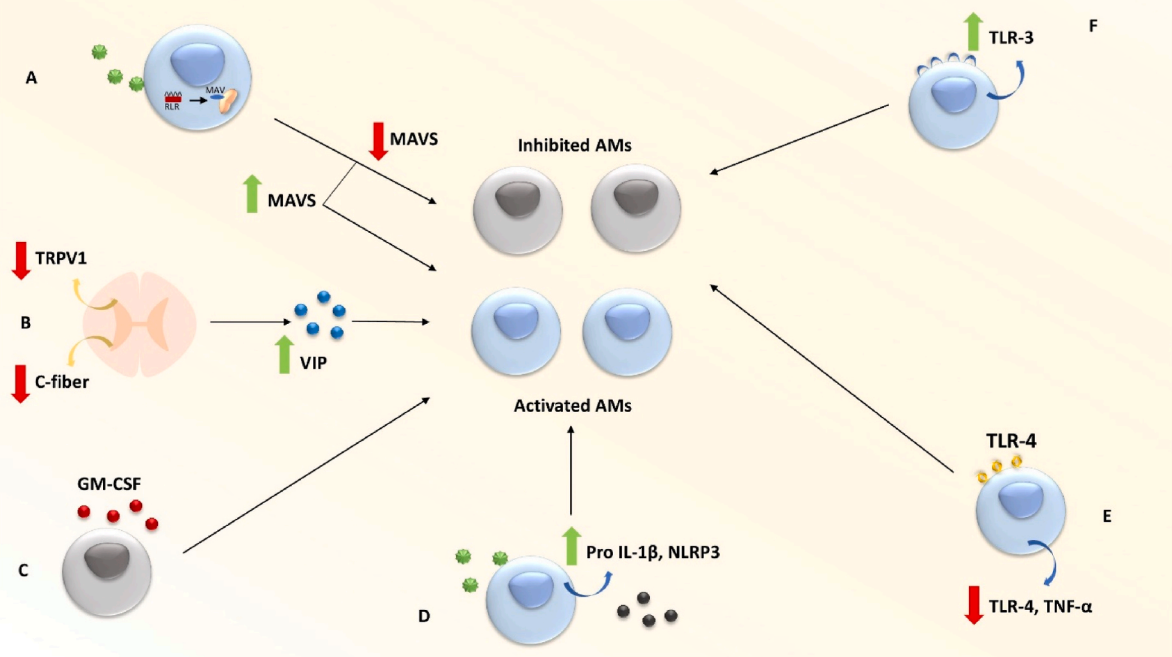

Fig. 1. Schematic of virus-alveolar macrophages (AMs) interactions in vial respiratory infections. A) AMs use mitochondrial antiviral signaling protein (MAVS)-coupled retinoic acid-inducible gene 1 (RIG-I)-like receptors (RLRs) to detect viruses. A deficiency in MAVS results in the lack of capacity in AMs to be activated. B) vasoactive intestinal peptide (VIP) regulated AMs and its level is controlled by Unmyelinated (C-fiber) nociceptors. Blocking transient receptor potential vanilloid 1 (TRPV1) causes a decrease in C-fiber, which eventually helps virus clearance by increasing AMs number. C) Granulocyte macrophage colony-stimulating factor (GM-CSF) is an important cytokine in the developmet of AMs. Its presence during viral infections can help the immune system by increasing AMs. D) viral infection stimulates the production of NLRP3 and pro-interleukin-1β in AMs, which are two activating factors for AMs, leading to a robust antiviral response. E) TLR-4 has an inhibitory role in the inhibition of AMs by decreasing tumor necrosis factor-α (TNF-α) release. Therefore, Downregulation of TLR-4 results in the inhibition of AMs F) Toll-Like Receptor 3 (TLR3) can initiate an inhibitory signaling pathway and AMs, which is utilized by viruses to inhibit AMs.

stimulates the expression of NLRP3 inflammasome and pro-IL-1β in these macrophages [71]. Mice lacking in AMs had severe widespread alveolar damage following infection with influenza A virus [48]. This damage ultimately resulted in deadly respiratory impairment and finally death. The lethal injury that occurred in those mice was caused by an increased infection of their Type-1 Alveolar Epithelial Cells (T1AECs), which was then followed by the elimination of the infected T1AECs by the adaptive immunological T-cell response. After further examination, it was discovered that AMs inhibited the expression of genes that were implicated in the cysteinyl leukotriene (cysLT) pathway in T1AECs, both in vivo and in vitro. The sensitivity of T1AECs to influenza A virus infection was reduced when the enzymes of the cysLT pathway were inhibited in a T1AECs cell line. This finding suggests that the inhibition of this pathway by AMs is a contributing factor to the resistance of T1AECs to influenza A virus infection. Furthermore, inhibiting the enzymes that are part of the cysLT pathway and blocking the cysteinyl leukotriene receptors in mice that were lacking in AMs resulted in a reduction in the sensitivity of their T1AECs to influenza A virus infection, which in turn protected these mice from a potentially fatal case of infection [48]. One of the most intriguing findings was the capacity of various influenza virus strains to infect murine airway macrophages varies dramatically from strain to strain. For instance, the BJx109 strain (H3N2) was capable of infecting macrophages with great efficiency and was linked to a mild illness in mice that had been infected through the nasal passages. On the other hand, the PR8 strain (H1N1) demonstrated a low level of infectiousness for macrophages, but it was quite virulent in laboratory mice. The depletion of airway macrophages led to the development of severe viral pneumonia in mice infected with BJx109, whereas it had no effect on the severity of the disease in mice infected with PR8. There was a correlation between the severe illness that was observed in macrophage-depleted mice that were infected with BJx109 and enhanced virus replication in the airways, which ultimately led to severe inflammation of the airways [85]. Conversely, another study with mice lacking AMs found that they had higher amounts of PR8 influenza virus in their lungs after being infected with a normally non-lethal dosage of the virus. As a consequence, there was significant inflammation in the airways, pulmonary edema, and blood vessel leaking, leading to the death of the affected animals [86]. These findings show that AMs are essential in defending against influenza infection by modulating immune responses, clearing pathogens, and aiding in tissue repair. When AMs are depleted, it results in increased viral replication, severe lung damage, and greater susceptibility to secondary infections. AMs help to reduce the severity of influenza through the inhibition of harmful pathways and support recovery, as evidenced by the effectiveness of GM-CSF therapy. The differing ability of influenza virus strains to infect AMs highlights the complexity of their role, making them a vital focus for better understanding and treating influenza-induced lung injury.

4.3. AMs in coronavirus infection

Severe acute respiratory syndrome coronavirus (SARS-CoV)-2 infection causes a cytokine storm by infecting the lungs through the Angiotensin-converting enzyme 2 (ACE2) receptor [87]. Studies have shown that IL-10 induces the upregulation of ACE2 in healthy AMs, converting them into vehicles for SARS-CoV-2. Hamster models showed decreased pathogenicity of SARS-CoV-2 when this mechanism was inhibited. Scientists discovered a transcript called Coronavirus disease 2019 (COVID-19) infectivity-enhancing dual receptor (CiDRE) through genome-wide association and quantitative trait locus analyses. CiDRE is created by the readthrough of the interferon-α receptor 2 (IFNAR2)--IL10RB and is found in high levels in patients with COVID-19 risk alleles at the IFNAR2 locus. CiDRE was shown to synergistically affect AMs through the IL-10-ACE2 pathway and function as a decoy receptor for type I IFNs [87]. AMs in the M1 state promote virus propagation,

including SARS-CoV-2, but those in the M2 state limit their spread [88]. M1 macrophages use their more pliable cellular structure to effectively ingest SARS-CoV-2 particles. Upon entry, the viruses take control of the endo-lysosomal system to avoid being detected. M1 AMs uphold a reduced pH in their endosomes, facilitating the liberation of viral RNA into the cytoplasm for reproduction. On the other hand, M2 AMs have elevated endosomal pH and reduced lysosomal pH, resulting in the breakdown of viruses. The process includes the endosomal protease cathepsin L (CTSL), which breaks down the SARS-CoV-2 spike protein in acidic conditions. This cleavage aids in the merging of viral and endosomal membranes, allowing the viral RNA to be released into the cytoplasm of macrophages. The Delta variant of the virus can overcome the endosomal restriction in M2 macrophages by using spike protein alterations to neutralize the inhibitory effects of the increased endosomal pH on CTSL [88,89]. A further study revealed that the invasion of AMs by SARS-CoV-2 may lead to the initiation of the cytokine storm, which could result in damage to pulmonary tissues, as well as the heart and lungs, ultimately resulting in multi-organ failure [90]. SARS-CoV-2 invades AMs, which is followed by the attraction of T cells by AMs. Attracted T cells produce IFN-γ, which stimulates AMs to release inflammatory cytokines, enhancing T-cell activation. The research suggests that SARS-CoV-2 triggers a slowly developing, confined inflammation in the alveoli, where AMs, the virus and T cells create a cycle that maintains continuous alveolar inflammation [91]. Researchers studied if SARS-CoV-2 remains in the body and the mechanisms that cause its persistence using a macaque model of viral infection. Macaques with persistent virus showed reduced IFN-γ production in NK cells. IFN-γ was found to enhance the expression of human leukocyte antigen E (HLA-E) on AMs, which can impede NK cell-mediated killing. Macaques with reduced amounts of persistent virus generated adaptive NK cells that circumvented the inhibition induced by MHC-E. The results emphasize the interaction between NK cells and macrophages in controlling the long-term presence of SARS-CoV-2 [92]. Researchers studied the involvement of AMs in the development of Middle East respiratory syndrome coronavirus (MERS-CoV) using a mouse model [93]. Their study showed that reducing AMs using clodronate (CL) liposomes notably increased both morbidity and mortality in human dipeptidyl peptidase 4 knock-in (hDPP4-KI) mice. Examination of lungs from animals lacking AM and control mice at different time points after infection revealed increased neutrophil activity but a reduced MERS-CoV-specific CD4 T-cell response in AM-deficient lungs in the latter stages of illness. Moreover, heightened severity of MERS in mice lacking AMs was associated with lung inflammation and lesions. The results highlight the crucial function of AMs in promoting a potent virus-specific T-cell response and in controlling excessive inflammation in MERS-CoV infection [93]. AMs are pivotal in the immune response to SARS-CoV-2 and MERS-CoV. Depending on their activation state, AMs can either facilitate or hinder viral propagation. The interaction between AMs and other immune cells, such as T cells and NK cells, is crucial in determining the severity

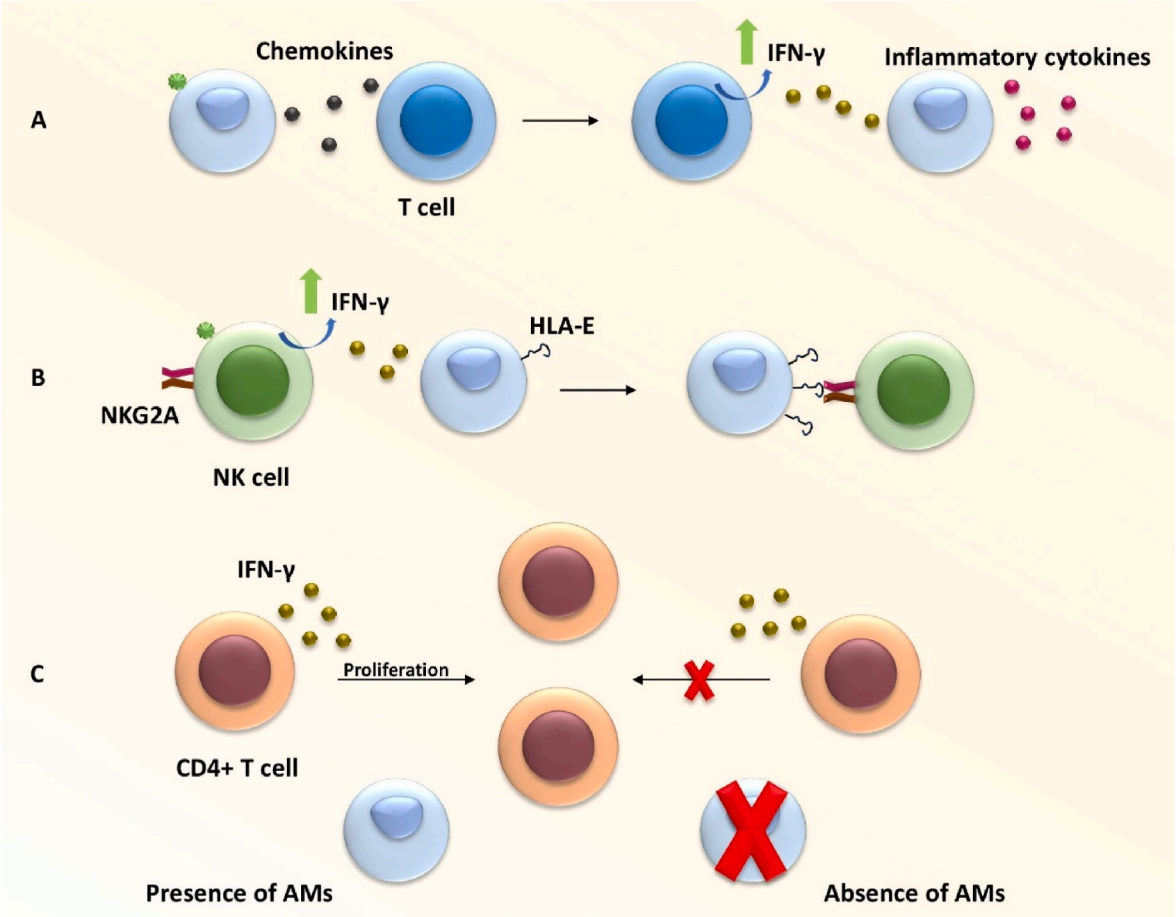

Fig. 2. AMs' interaction with other immune cells in viral infections. A) Upon infection of AMs with viruses, these cells release chemokines that attract T cells. In turn, T cells produce IFN-γ, which triggers the release of inflammatory cytokines by AMs. B) Infecting Natural Killer cells (NK cells) by viruses makes these cells produce IFN-γ, which increases the expression of inhibitory ligand human leukocyte antigen E (HLA-E) on AMs' surface. This can in turn inhibit NK cells, which are crucial sentinels of antiviral response. C) IFN-γ-producing $CD4^+$ T cells are important players in immune response against viral infections. Their proliferation in virus infected bodies is associated with the presence of AMs. Therefore, AM depletion obstructs proper virus-specific $CD4^+$ propagation, paralyzing efficient adaptive immune response.

and outcome of infections. As seen in severe COVID-19 and MERS cases, disruptions in these interactions can lead to excessive inflammation, cytokine storms, and multi-organ failure. Understanding the mechanisms of AMs in viral infections is essential for developing targeted therapies to mitigate disease severity and improve patient outcomes. Fig. 2 exhibits interactions of AMs and other immune cells in the course of viral infections.

4.4. AMs in HIV-1 infection

Human Immunodeficiency Virus (HIV)-1 infection reduces the immunological capacities of AMs, making patients more prone to pneumonia. It has been shown that HIV-1 transgenic expression decreases the expression of GM-CSFRβ in AMs, impairing their ability to phagocytose germs in vitro. The study suggests that a lack of zinc in the lungs, together with a decrease in GM-CSFRβ, may be a mechanism by which long-term HIV-1 infection weakens the function of AMs, making individuals more vulnerable to severe lung infections [94]. HIV-positive macrophages have a reduced capacity to release TNF-α due to impaired TLR-4-mediated signaling [95,96]. HIV infection is associated with impaired TLR-4-mediated signaling in macrophages, specifically impacting the MyD88-dependent pathway of TLR-4 signaling. These results indicate that long-term HIV infection causes specific and targeted interference with important TLR-4 signaling in macrophages, possibly contributing to the onset of bacterial pneumonia linked to the illness [95,96]. Therefore, it can be concluded that HIV-1 infection impairs AMs, reducing their ability to fight lung infections. This is due to decreased GM-CSFRβ expression and disrupted TLR-4 signaling, making patients more vulnerable to pneumonia and other respiratory infections.

5. Age-related changes in AM

AMs are believed to undergo age-related changes that impair their defense capabilities. Research has shown that bacteria trigger cytoskeletal remodeling in AMs by interacting with structure MARCO. This remodeling enhances the cell surface expression of MARCO and facilitates bacterial phagocytosis [97]. Further research revealed that Rac1-GTP mediates MARCO signaling, activating the actin-related protein-2/3 complex, an F-actin nucleator, which leads to F-actin polymerization, filopodia formation, and increased MARCO expression on the cell surface, which are all crucial steps for effective phagocytosis. However, AMs isolated from older mice show reduced Rac1 mRNA and protein expression, leading to lower Rac1-GTP levels, decreased activation of the actin-related protein-2/3 complex, and a subsequent reduction in F-actin polymerization, filopodia formation, and MARCO expression on the cell surface. Consequently, phagocytosis is diminished in aging AMs [97]. AMs from aged mice displayed markers of cellular senescence and exhibited downregulation of genes related to growth and cell cycle pathways compared to those from younger mice [98]. Additionally, AMs from older mice showed a reduced transcriptional response to distal injury, in contrast to the more robust response seen in AMs from younger mice. Many of these changes involved glucocorticoid-regulated genes, and when primary AMs from aged animals were treated with corticosteroids ex vivo, their transcriptional response was diminished. These findings reveal a complex, age-related AM phenotype characterized by disrupted stress hormone signaling, which may hinder AM responses to physiological stress and contribute to their dysfunction, leading to a decline in pulmonary immunity during the aging process [98].

While aging, there is a reduction in the quantity of AMs, which play a vital role in preserving lung function [99]. It has been noticed that these macrophages are crucial in influenza-related deaths in older people. Aging drastically changes the gene expression profile of AMs, specifically by inhibiting cell cycling mechanisms. Age-related changes hinder AMs' capacity to safeguard the lungs from harm caused by influenza infection. In addition, aging diminishes the capacity of AMs to engulf dying neutrophils, reduces the presence of the scavenger receptor CD204, and causes the accumulation of neutrophils during influenza infection [99]. In older mice, there was an increase in PGE2 levels in their BALF compared to younger mice. Externally administered PGE2 inhibited AM proliferation through an E prostanoid receptor 2 (EP2)-cyclic AMP-dependent pathways. Moreover, EP2 knockout (EP2 KO) mice had higher baseline AM numbers, and their AMs were resistant to the inhibitory effects of PGE2 and BALF from aged mice on proliferation [100]. As mice ages, naive mice undergo a gradual transition where AMs are replaced by those derived from bone marrow monocytes (BMo)-AM. This transition significantly contributes to the severity of influenza infection in a manner inherent to the cells themselves. The presence of both naive and experienced BMo-AM heightened the severity of influenza infection, which correlated with an inflammatory phenotype [50]. Single-cell RNA-Seq analysis in both mice and humans indicated that the observed changes were not due to the emergence of a new AMs subpopulation during aging [101]. Surprisingly, age-related transcriptomic alterations in tissue-resident AMs were largely reversed by heterochronic adoptive transfer but remained unaffected by heterochronic parabiosis. Additionally, the transcriptomic profiles of tissue-resident AMs and BMo were similar during aging and in response to influenza A infection [101]. These observations suggest that the transcriptomic changes in AMs with age are predominantly influenced by the lung microenvironment. Analysis of ligand-receptor interactions between genes that altered with age in alveolar type 2 cells and AMs suggested that changes in the extracellular matrix contribute to an age-related hyporesponsiveness to GM-CSF in AMs. Supporting this idea, levels of hyaluronan, a ligand for CD44 on AMs, were found to be higher in the epithelial lining fluid of older mice compared to younger ones. Hyaluronan was also shown to inhibit the proliferation of bone marrow-derived macrophages when cultured on matrix proteins from the epithelial lining fluid. These findings show the role of age-related changes in the alveolar environment, including alterations in the extracellular lining fluid, and the reduced number and altered function of AMs during aging [101]. Aging significantly impairs the function and efficiency of AMs. Age-related changes, such as reduced cell cycling, diminished phagocytic capacity, and altered gene expression, weaken AMs' ability to protect the lungs, leading to increased susceptibility to severe respiratory infections in the elderly. Additionally, the aging lung environment, including alterations in the extracellular matrix and stress hormone signaling, further contributes to AM dysfunction, highlighting the need for targeted interventions to enhance pulmonary immunity in older individuals. A schematic of the impacts of ageing on AMs' antiviral response is shown in Fig. 3.

6. Therapeutic implications

6.1. Immunomodulation strategies

6.1.1. Targeting c-Myc and SIRPα

Regarding the importance of c-Myc in EBV infection, it can be used as a target in therapeutic approaches to avoid cancers associated with that virus. After the primary pneumonia was resolved, mouse AMs showed decreased phagocytic capacity for several weeks such as decreased phagocytic activity after Rhinovirus and influenza A virus infection [32,102]. The impairment was caused by resident AMs undergoing an epigenetic process known as tolerogenic training. SIRPα greatly influences the creation of this state by generating a milieu suitable for tolerogenic training [32]. In humans with systemic inflammation, both AMs and circulating monocytes displayed indications of reprogramming even six months after the inflammation had subsided [32]. Experiments conducted in a controlled environment showed that inhibiting SIRPα using antibodies improved the ability of monocytes to engulf pathogens in very unwell patients, indicating a possible strategy to reduce the risk of hospital-acquired pneumonia [32].

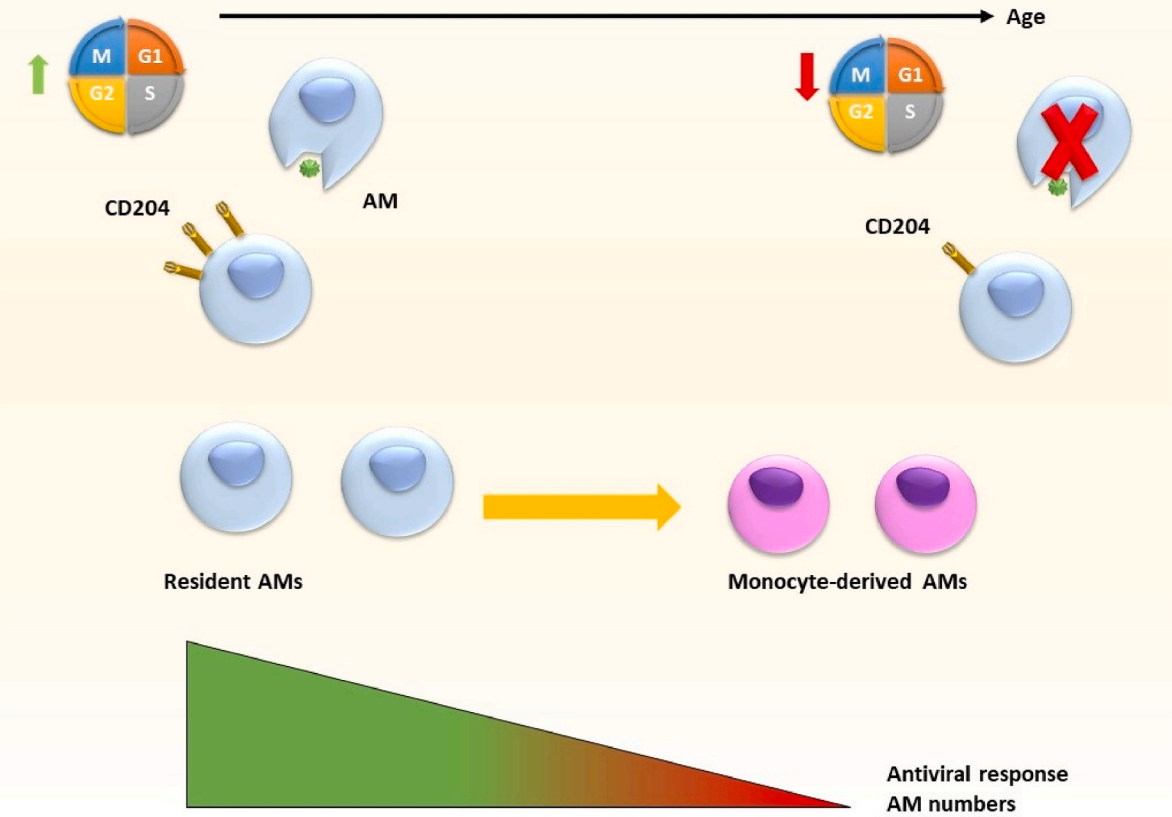

Fig. 3. Ageing and its effects on antiviral activity of AMs. Ageing hampers cell proliferation by inhibiting cell cycle, and also hinders efficient antiviral response by reducing CD204 expression and AM's phagocytosis capacity. Furthermore, lung-resident AMs will be replaced by monocyte-derived AMs, which is related to disease severity as monocyte-derived AMs are less efficient in antiviral response in comparison with lung-resident AMs.

6.1.2. Targeting TGF-β1

Exposure of AMs to TGF-β1 decreased their ability to fight viruses and inflammation, and also hindered many aspects of mitochondrial function such as basal and maximal respiration, and mitochondrial ATP generation. Exposure to RSV following TGF-β1 therapy exacerbated mitochondrial dysfunction [103]. AMs treated with TGF-β1, either alone or with RSV, exhibited higher rates of cell death and decreased capacity to ingest pathogens, possibly caused by mitochondrial stress. New treatment approaches could involve disrupting TGF-β1 signaling or restoring mitochondrial respiration to improve AMs function and prevent subsequent bacterial infections that commonly accompany viral respiratory illnesses [103].

6.1.3. Targeting necroptosis

Targeting TNF and/or the necroptotic machinery could be effective therapeutic approaches to reduce respiratory issues caused by RSV infection in young children [104]. Research has shown that RSV infection triggers necroptosis in primary mouse macrophages and human monocytes via a process that involves RIPK1, RIPK3, and MLKL. Moreover, triggering necroptosis pathways hinders the removal of RSV from AMs, worsening lung damage. Autocrine TNF triggers RSV-induced macrophage necroptosis, involving necroptosis pathways in TNF release, exacerbating lung injury during RSV infection. Tnfr1-deficient mice showed decreased expression of Ripk3 and Mlkl genes and a significant reduction in the number of necrotic AMs in the lungs [104].

6.1.4. GM-CSF therapy and AM maturation

GM-CSF's role in the formation and function of AMs has been well studied. However, further research is needed to understand the impact of GM-CSF treatment on AMs because of its various biological activities. In a study, mice were first infected with influenza through intranasal injection, then given aerosolized recombinant mouse GM-CSF, and later exposed to a secondary infection with *S. pneumoniae* [105]. Administering GM-CSF through inhalation significantly increased the survival rate of mice when exposed to a secondary challenge with *S. pneumoniae*. Inhaled GM-CSF did not reduce bacterial growth in the airways or lung tissues, but it did dramatically decrease the incidence of *S. pneumoniae* bacteremia [105]. During COVID-19, researchers have noticed that the disease is marked by a deficiency in GM–CSF–dependent guidance of AMs and the buildup of pro-inflammatory macrophages. It has been shown that inhaling GM-CSF did not result in notable enhancements in average oxygenation parameters when compared to normal therapy alone [106]. A greater percentage of patients who received GM-CSF treatment exhibited a positive clinical response. GM-CSF inhalation led to an increase in virus-specific CD8 effector lymphocytes and class-switched B cells, without exacerbating systemic hyper-inflammation. This study proposes the exploration of inhaled GM-CSF as a non-invasive treatment to improve alveolar gas exchange and boost antiviral immunity in COVID-19, acting as a preliminary demonstration of its potential [106]. After allergic airway inflammation, the lungs exhibited decreased levels of GM-CSF, a cytokine important for the maturation of AMs. Administering GM-CSF directly into the airways of mice with post-allergic airway inflammation facilitated the final maturation of AMs, thereby preventing the heightened susceptibility to hyperreactivity and inflammation induced by RSV [107]. The findings revealed that M1 macrophages exhibited greater expression of CTSL compared to M2 macrophages. Treatment of AMs with dexamethasone (Dex) and alendronate (ALN) was shown to decrease CTSL expression and increase endosomal pH in AMs, respectively. As a result, the viral load, indicated by the expression of viral NP and ORF1ab, was diminished in AMs treated with either ALN alone, Dex alone, or a combination of both treatments [108].

6.1.5. Microparticle-based therapy

Extracellular vesicles (EVs) have emerged as a significant mode of paracrine intercellular communication, gaining increasing recognition over the past decade [109]. These small membrane-bound structures, typically less than 1 μm, originate from endosomal or plasma membranes in various cell types and organisms. Cells can generate different extracellular vesicles, including exosomes and microparticles (MPs), also known as microvesicles or ectosomes. Exosomes are smaller, ranging from 30 to 100 nm, and are released from endosomes, whereas MPs, which range in size from 100 to 1000 nm, are released from the plasma membrane in response to various stimuli [109]. It has been shown that AMs continuously secrete EVs with paracrine activity that inhibits influenza infection in epithelial cells both in vitro and in vivo. The susceptibility of different influenza strains to inhibition by AM-EVs varied. Strains that escape early from endosomes and have a high fusion pH were inhibited due to a reduction in endosomal pH. In contrast, strains with later endosomal escape and lower fusion pH were resistant to this inhibition [110]. A study has shown that MPs produced from A549 cells with increased ACE2 expression (AO-MPs) have potential as a treatment for SARS-CoV-2 infection [111]. When given intranasally, AO-MPs efficiently travel through the lungs' structural and biological characteristics to reach the alveoli, where they are absorbed by AMs. AO-MPs in AMs increase endosomal pH and decrease lysosomal pH, aiding in the movement of SARS-CoV-2 from phago-endosomes to lysosomes for breakdown. The change in pH is caused by oxidized cholesterol found in AO-MPs, which moves to endosomal membranes, blocking proton pumps and preventing endosomal acidification. AO-MPs help degrade viruses and reduce inflammation in AMs, leading to improved treatment of SARS-CoV-2 in mice without causing harm [111]. The results highlight the effectiveness of AO-MPs in treating SARS-CoV-2 infections and show that microparticle-based therapeutics can be used to combat other respiratory viruses in the future [111]. These findings suggest that EV-based therapies, particularly those leveraging MPs, hold promise for treating respiratory viruses like SARS-CoV-2. Future studies should explore the broader applicability of these therapies across various viral infections, optimizing delivery methods and ensuring safety in clinical use.

6.1.6. Gene therapy using fetal liver monocytes for AM restoration

A recent study has shown that fetal liver monocytes, when cultivated with GM-CSF (CSF2-cFLiMo), quickly differentiate into a consistent population that resembles AMs in vitro [112]. The CSF2-cFLiMo cells retain their capacity to differentiate into genuine AMs when transferred into Csf2ra−/− newborn mice, successfully halting the progression of alveolar proteinosis and the buildup of apoptotic cells for a period of one year in vivo. CSF2-cFLiMo cells show better integration into vacant AMs spaces in the lungs and provide improved defense against severe pathology caused by viral respiratory infections compared to transplanting macrophages from bone marrow cells cultured with M-CSF, regardless of GM-CSF presence. By utilizing this method for gene therapy, scientists have effectively repaired a malfunctioning Csf2ra gene in fetal liver monocytes, demonstrating their ability to transform into AMs in vivo [112].

6.1.7. Immunobiotics and post-immunobiotics

Immunobiotics and post-immunobiotics, which are beneficial microorganisms with immune-modulating capabilities, have potential in formulations designed to prevent respiratory virus infections. Research shows that post-immunobiotics from specific strains such as *Lactobacillus gasseri* TMT36, TMT39, and TMT40 (known as HK36, HK39, or HK40) can regulate the innate antiviral response of AMs and reduce lung inflammation caused by TLR-3 activation in vivo [113].

In summary, targeting specific pathways and mechanisms in AMs offers promising therapeutic strategies to combat viral infections and associated complications. From inhibiting SIRPα to enhance phagocytosis to leveraging GM-CSF for better AM function and utilizing immunobiotics to modulate immune responses, these approaches hold the potential to improve outcomes in diseases like COVID-19, RSV, and influenza. Further research into these strategies could lead to more effective treatments for respiratory viral infections and their complications.

6.2. Vaccine development

Candidate universal influenza vaccines aim to provide broad protection against both influenza A and B viruses. Studies indicate that widely reactive antibodies depend on interactions between Fc regions and Fc gamma receptors for successful protection [114]. It has been demonstrated that AMs play a vital role in the protective effects provided by both widely neutralizing and non-neutralizing antibodies in mice. The research also reveals the potential ways in which AMs contribute to protection in living organisms, such as through antibody-induced inflammation and antibody-dependent cellular phagocytosis [114]. Furthermore, it has been demonstrated that the transfer of wild-type AMs into mice lacking FcγRI and FcγRIII receptors reinstates the protective effects of passively transferred anti-The ectodomain of matrix protein 2 (M2e) IgG antibodies [115]. Another study demonstrates that utilizing a recombinant influenza virus containing the Lymphocytic choriomeningitis (LCMV) GP33-41 epitope does not effectively provide cross-subtype protective immunity through influenza virus-specific $CD8^+$ T cells or virus-specific non-neutralizing antibodies alone. When these two components are joined, they cooperate to provide robust protective immunity. This boost in immunity is achieved through the collaboration of AMs [116]. Universal influenza vaccines depend on AMs for effective protection. AMs enhance the impact of antibodies through mechanisms like phagocytosis and inflammation. Restoring AM function can boost vaccine efficacy, demonstrating their crucial role in broad influenza immunity.

7. Conclusion

AMs play a variety of roles in viral respiratory infections, and they are essential for immunological modulation and viral clearance. By secreting type I interferons and coordinating immune responses against respiratory viruses such as RSV, influenza, and SARS-CoV-2, AMs play a critical role in starting the antiviral response. Depletion or malfunction frequently results in worsening lung damage, reduced ability to clear viruses, and heightened vulnerability to recurrent bacterial infections. Therapeutic strategies targeting AMs offer promising ways for tackling viral respiratory infections. The goal of immunomodulation techniques such as blocking tolerogenic training or reestablishing GM-CSF signaling is to improve AM function and strengthen host defenses. Furthermore, measures that target particular signaling pathways, such as blocking TNF/necroptosis or inhibiting TGF-β1, may be able to lessen the progression of viral pathogenesis and the lung damage that results from it. Cutting-edge approaches to treating viral infections include developing fetal liver monocytes to differentiate into functional AMs or employing microparticle-based therapy. Furthermore, vaccine development strategies underscore the importance of AMs in mediating protective immune responses. Understanding how AMs and antibodies interact during vaccine-induced immunity can help develop more potent universal influenza vaccines and improve cross-subtype protection.

CRediT authorship contribution statement

Atefe Panahipoor Javaherdehi: Writing – review & editing, Writing – original draft, Visualization. **Somayyeh Ghanbari:** Writing – review & editing, Writing – original draft, Visualization, Conceptualization. **Pooya Mahdavi:** Writing – review & editing, Writing – original draft. **Alireza Zafarani:** Writing – review & editing, Writing – original draft. **Mohammad Hossein Razizadeh:** Writing – review & editing, Writing – original draft, Visualization, Supervision, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

The authors received no fundings.

References

[1] S. Chen, A.F. Saeed, Q. Liu, Q. Jiang, H. Xu, G.G. Xiao, L. Rao, Y. Duo, Macrophages in immunoregulation and therapeutics, Signal Transduct. Targeted Ther. 8 (2023) 207.

[2] S. Razi, J. Yaghmoorian Khojini, F. Kargarijam, S. Panahi, Z. Tahershamsi, A. Tajbakhsh, S.M. Gheibihayat, Macrophage efferocytosis in health and disease, Cell Biochem. Funct. 41 (2023) 152–165.

[3] Z. Strizova, I. Benesova, R. Bartolini, R. Novysedlak, E. Cecrdlova, Lily K. Foley, I. Striz, M1/M2 macrophages and their overlaps – myth or reality? Clin. Sci. 137 (2023) 1067–1093, https://doi.org/10.1042/cs20220531.

[4] J. Xue, S.V. Schmidt, J. Sander, A. Draffehn, W. Krebs, I. Quester, D. De Nardo, T. D. Gohel, M. Emde, L. Schmidleithner, Transcriptome-based network analysis reveals a spectrum model of human macrophage activation, Immunity 40 (2014) 274–288.

[5] N. Joshi, J.M. Walter, A.V. Misharin, Alveolar macrophages, Cell. Immunol. 330 (2018) 86–90.

[6] Y.D. Woo, D. Jeong, D.H. Chung, Development and functions of alveolar macrophages, Mol. Cell. 44 (2021) 292–300, https://doi.org/10.14348/molcells.2021.0058.

[7] M. Kikkert, Innate immune evasion by human respiratory RNA viruses, J. Innate Immun. 12 (2019) 4–20, https://doi.org/10.1159/000503030.

[8] L. van de Laar, W. Saelens, S. De Prijck, L. Martens, C.L. Scott, G. Van Isterdael, E. Hoffmann, R. Beyaert, Y. Saeys, B.N. Lambrecht, Yolk sac macrophages, fetal liver, and adult monocytes can colonize an empty niche and develop into functional tissue-resident macrophages, Immunity 44 (2016) 755–768.

[9] G. Hoeffel, Y. Wang, M. Greter, P. See, P. Teo, B. Malleret, M. Leboeuf, D. Low, G. Oller, F. Almeida, Adult Langerhans cells derive predominantly from embryonic fetal liver monocytes with a minor contribution of yolk sac–derived macrophages, J. Exp. Med. 209 (2012) 1167–1181.

[10] S. Libório-Ramos, C. Barbosa-Matos, R. Fernandes, C. Borges-Pereira, S. Costa, Interstitial macrophages lead early stages of bleomycin-induced lung fibrosis and induce fibroblasts activation, Cells 12 (2023) 402.

[11] E. Evren, E. Ringqvist, K.P. Tripathi, N. Sleiers, I.C. Rives, A. Alisjahbana, Y. Gao, D. Sarhan, T. Halle, C. Sorini, Distinct developmental pathways from blood monocytes generate human lung macrophage diversity, Immunity 54 (2021) 259–275. e257.

[12] M. Guilliams, I. De Kleer, S. Henri, S. Post, L. Vanhoutte, S. De Prijck, K. Deswarte, B. Malissen, H. Hammad, B.N. Lambrecht, Alveolar macrophages develop from fetal monocytes that differentiate into long-lived cells in the first week of life via GM-CSF, J. Exp. Med. 210 (2013) 1977–1992.

[13] X. Yu, A. Buttgereit, I. Lelios, S.G. Utz, D. Cansever, B. Becher, M. Greter, The cytokine TGF-β promotes the development and homeostasis of alveolar macrophages, Immunity 47 (2017) 903–912. e904.

[14] C. Draijer, L.R.K. Penke, M. Peters-Golden, Distinctive effects of GM-CSF and M-CSF on proliferation and polarization of two major pulmonary macrophage populations, J. Immunol. 202 (2019) 2700–2709.

[15] C. Schneider, S.P. Nobs, M. Kurrer, H. Rehrauer, C. Thiele, M. Kopf, Induction of the nuclear receptor PPAR-γ by the cytokine GM-CSF is critical for the differentiation of fetal monocytes into alveolar macrophages, Nat. Immunol. 15 (2014) 1026–1037.

[16] J. McCowan, F. Fercoq, P.M. Kirkwood, W. T'jonck, L.M. Hegarty, C.M. Mawer, R. Cunningham, A.S. Mirchandani, A. Hoy, D.C. Humphries, The transcription factor EGR2 is indispensable for tissue-specific imprinting of alveolar macrophages in health and tissue repair, Science immunology 6 (2021) eabj2132.

[17] Y. Shibata, P.-Y. Berclaz, Z.C. Chroneos, M. Yoshida, J.A. Whitsett, B.C. Trapnell, GM-CSF regulates alveolar macrophage differentiation and innate immunity in the lung through PU. 1, Immunity 15 (2001) 557–567.

[18] B.S. Staitieh, X. Fan, W. Neveu, D.M. Guidot, Nrf2 regulates PU. 1 expression and activity in the alveolar macrophage, Am. J. Physiol. Lung Cell Mol. Physiol. 308 (2015) L1086–L1093.

[19] L. Li, F. Sun, L. Han, X. Liu, Y. Xiao, A.D. Gregory, S.D. Shapiro, G. Xiao, Z. Qu, PDLIM2 repression by ROS in alveolar macrophages promotes lung tumorigenesis, JCI insight 6 (2021).

[20] R. Ebina-Shibuya, M. Watanabe-Matsui, M. Matsumoto, A. Itoh-Nakadai, R. Funayama, K. Nakayama, A. Muto, K. Igarashi, The double knockout of Bach1 and Bach2 in mice reveals shared compensatory mechanisms in regulating alveolar macrophage function and lung surfactant homeostasis, J. Biochem. 160 (2016) 333–344.

[21] Y.D. Woo, J. Koh, J.S. Ko, S. Kim, K.C. Jung, Y.K. Jeon, H.Y. Kim, H. Lee, C. W. Lee, D.H. Chung, Ssu72 regulates alveolar macrophage development and allergic airway inflammation by fine-tuning of GM-CSF receptor signaling, J. Allergy Clin. Immunol. 147 (2021) 1242–1260.

[22] J. Han, D.P. Hajjar, J.M. Tauras, J. Feng, A.M. Gotto, A.C. Nicholson, Transforming growth factor-β1 (TGF-β1) and TGF-β2 decrease expression of CD36, the type B scavenger receptor, through mitogen-activated protein kinase phosphorylation of peroxisome proliferator-activated receptor-γ, J. Biol. Chem. 275 (2000) 1241–1246.

[23] Y. Wang, X. Wang, H. Zhang, B. Han, Y. Ye, M. Zhang, Y. Wang, J. Xue, C. a. Wang, Transforming growth factor-β1 promotes M1 alveolar macrophage polarization in acute lung injury by up-regulating DNMT1 to mediate the microRNA-124/PELI1/IRF5 Axis, Front. Cell. Infect. Microbiol. 11 (2021), https://doi.org/10.3389/fcimb.2021.693981.

[24] C.Y. Perrot, T. Karampitsakos, A. Unterman, T. Adams, K. Marlin, A. Arsenault, A. Zhao, N. Kaminski, G. Katlaps, K. Patel, D. Bandyopadhyay, J.D. Herazo-Maya, Mast-cell expressed membrane protein-1 is expressed in classical monocytes and alveolar macrophages in idiopathic pulmonary fibrosis and regulates cell chemotaxis, adhesion, and migration in a TGFβ-dependent manner, Am. J. Physiol. Cell Physiol. 326 (2024) C964–C977, https://doi.org/10.1152/ajpcell.00563.2023.

[25] E. Evren, E. Ringqvist, J.-M. Doisne, A. Thaller, N. Sleiers, R.A. Flavell, J.P. Di Santo, T. Willinger, CD116+ fetal precursors migrate to the perinatal lung and give rise to human alveolar macrophages, J. Exp. Med. 219 (2022), https://doi.org/10.1084/jem.20210987.

[26] Y. Yao, Q. Liu, I. Adrianto, X. Wu, J. Glassbrook, N. Khalasawi, C. Yin, Q. Yi, Z. Dong, F. Geissmann, L. Zhou, Q.-S. Mi, Histone deacetylase 3 controls lung alveolar macrophage development and homeostasis, Nat. Commun. 11 (2020) 3822, https://doi.org/10.1038/s41467-020-17630-6.

[27] A.S. Neupane, M. Willson, A.K. Chojnacki, F. Vargas E Silva Castanheira, C. Morehouse, A. Carestia, A.E. Keller, M. Peiseler, A. DiGiandomenico, M. M. Kelly, M. Amrein, C. Jenne, A. Thanabalasuriar, P. Kubes, Patrolling alveolar macrophages conceal bacteria from the immune system to maintain homeostasis, Cell 183 (2020) 110–125.e111, https://doi.org/10.1016/j.cell.2020.08.020.

[28] A. Lacoma, V. Cano, D. Moranta, V. Regueiro, D. Domínguez-Villanueva, M. Laabei, M. González-Nicolau, V. Ausina, C. Prat, J.A. Bengoechea, Investigating intracellular persistence of Staphylococcus aureus within a murine alveolar macrophage cell line, Virulence 8 (2017) 1761–1775, https://doi.org/10.1080/21505594.2017.1361089.

[29] C. Patterson, K.S. Hazime, S. Zelenay, D.M. Davis, Prostaglandin E_2 impacts multiple stages of the natural killer cell antitumor immune response, Eur. J. Immunol. 54 (2024) 2350635, https://doi.org/10.1002/eji.202350635.

[30] H. Harizi, M. Juzan, C. Grosset, M. Rashedi, N. Gualde, Dendritic cells issued in vitro from bone marrow produce PGE2 that contributes to the immunomodulation induced by antigen-presenting cells, Cell. Immunol. 209 (2001) 19–28, https://doi.org/10.1006/cimm.2001.1785.

[31] L.E. Mendonca, E. Pernet, N. Khan, J. Sanz, E. Kaufmann, J. Downey, A. Grant, M. Orlova, E. Schurr, C. Krawczyk, R.G. Jones, L.B. Barreiro, M. Divangahi, Human alveolar macrophage metabolism is compromised during Mycobacterium tuberculosis infection, Front. Immunol. 13 (2023), https://doi.org/10.3389/fimmu.2022.1044592.

[32] A. Roquilly, C. Jacqueline, M. Davieau, A. Mollé, A. Sadek, C. Fourgeux, P. Rooze, A. Broquet, B. Misme-Aucouturier, T. Chaumette, Alveolar macrophages are epigenetically altered after inflammation, leading to long-term lung immunoparalysis, Nat. Immunol. 21 (2020) 636–648.

[33] S.J. Lund, K.A. Patras, J.M. Kimmey, A. Yamamura, L.D. Butcher, P.G.B. Del Rosario, G.E. Hernandez, A.M. McCoy, O. Lakhdari, V. Nizet, L.S. Prince, Developmental immaturity of Siglec receptor expression on neonatal alveolar macrophages predisposes to severe group B streptococcal infection, iScience 23 (2020) 101207, https://doi.org/10.1016/j.isci.2020.101207.

[34] E. Bernasconi, C. Pattaroni, A. Koutsokera, C. Pison, R. Kessler, C. Benden, P. M. Soccal, A. Magnan, J.-D. Aubert, B.J. Marsland, Airway microbiota determines innate cell inflammatory or tissue remodeling profiles in lung transplantation, Am. J. Respir. Crit. Care Med. 194 (2016) 1252–1263.

[35] S. Das, E. Bernasconi, A. Koutsokera, D.-A. Wurlod, V. Tripathi, G. Bonilla-Rosso, J.-D. Aubert, M.-F. Derkenne, L. Mercier, C. Pattaroni, A prevalent and culturable microbiota links ecological balance to clinical stability of the human lung after transplantation, Nat. Commun. 12 (2021) 2126.

[36] R.L. Brown, R.P. Sequeira, T.B. Clarke, The microbiota protects against respiratory infection via GM-CSF signaling, Nat. Commun. 8 (2017) 1512.

[37] W.J. Janssen, K.A. McPhillips, M.G. Dickinson, D.J. Linderman, K. Morimoto, Y. Q. Xiao, K.M. Oldham, R.W. Vandivier, P.M. Henson, S.J. Gardai, Surfactant proteins A and D suppress alveolar macrophage phagocytosis via interaction with SIRP alpha, Am. J. Respir. Crit. Care Med. 178 (2008) 158–167, https://doi.org/10.1164/rccm.200711-1661OC.

[38] E.Y. Bissonnette, J.-F. Lauzon-Joset, J.S. Debley, S.F. Ziegler, Cross-talk between alveolar macrophages and lung epithelial cells is essential to maintain lung homeostasis, Front. Immunol. 11 (2020) 583042.

[39] K. Westphalen, G.A. Gusarova, M.N. Islam, M. Subramanian, T.S. Cohen, A. S. Prince, J. Bhattacharya, Sessile alveolar macrophages communicate with alveolar epithelium to modulate immunity, Nature 506 (2014) 503–506.

[40] R.J. Snelgrove, J. Goulding, A.M. Didierlaurent, D. Lyonga, S. Vekaria, L. Edwards, E. Gwyer, J.D. Sedgwick, A.N. Barclay, T. Hussell, A critical function for CD200 in lung immune homeostasis and the severity of influenza infection, Nat. Immunol. 9 (2008) 1074–1083, https://doi.org/10.1038/ni.1637.

[41] R.L. Blumenthal, D.E. Campbell, P. Hwang, R.H. DeKruyff, L.R. Frankel, D. T. Umetsu, Human alveolar macrophages induce functional inactivation in antigen-specific CD4 T cells, J. Allergy Clin. Immunol. 107 (2001) 258–264.

[42] C.J. Chelen, Y. Fang, G.J. Freeman, H. Secrist, J.D. Marshall, P.T. Hwang, L. R. Frankel, R.H. DeKruyff, D.T. Umetsu, Human alveolar macrophages present antigen ineffectively due to defective expression of B7 costimulatory cell surface molecules, J. Clin. Invest. 95 (1995) 1415–1421.
[43] M.M. Coleman, D. Ruane, B. Moran, P.J. Dunne, J. Keane, K.H. Mills, Alveolar macrophages contribute to respiratory tolerance by inducing FoxP3 expression in naive T cells, Am. J. Respir. Cell Mol. Biol. 48 (2013) 773–780.
[44] B. Machiels, M. Dourcy, X. Xiao, J. Javaux, C. Mesnil, C. Sabatel, D. Desmecht, F. Lallemand, P. Martinive, H. Hammad, A gammaherpesvirus provides protection against allergic asthma by inducing the replacement of resident alveolar macrophages with regulatory monocytes, Nat. Immunol. 18 (2017) 1310–1320.
[45] H. Aegerter, J. Kulikauskaite, S. Crotta, H. Patel, G. Kelly, E.M. Hessel, M. Mack, S. Beinke, A. Wack, Influenza-induced monocyte-derived alveolar macrophages confer prolonged antibacterial protection, Nat. Immunol. 21 (2020) 145–157.
[46] S. Chakraborty, A. Singh, L. Wang, X. Wang, M.A. Sanborn, Z. Ye, M. Maienschein-Cline, A. Mukhopadhyay, B.B. Ganesh, A.B. Malik, J. Rehman, Trained immunity of alveolar macrophages enhances injury resolution via KLF4-MERTK-mediated efferocytosis, J. Exp. Med. 220 (2023), https://doi.org/10.1084/jem.20221388.
[47] T.M. Tumpey, A. García-Sastre, J.K. Taubenberger, P. Palese, D.E. Swayne, M. J. Pantin-Jackwood, S. Schultz-Cherry, A. Solórzano, N. Van Rooijen, J.M. Katz, Pathogenicity of influenza viruses with genes from the 1918 pandemic virus: functional roles of alveolar macrophages and neutrophils in limiting virus replication and mortality in mice, J. Virol. 79 (2005) 14933–14944.
[48] A. Cardani, A. Boulton, T.S. Kim, T.J. Braciale, Alveolar macrophages prevent lethal influenza pneumonia by inhibiting infection of type-1 alveolar epithelial cells, PLoS Pathog. 13 (2017) e1006140.
[49] L. Somerville, A. Cardani, T.J. Braciale, Alveolar macrophages in influenza A infection guarding the castle with sleeping dragons, Infectious diseases and therapeutics 1 (2020).
[50] F. Li, F. Piattini, L. Pohlmeier, Q. Feng, H. Rehrauer, M. Kopf, Monocyte-derived alveolar macrophages autonomously determine severe outcome of respiratory viral infection, Science Immunology 7 (2022) eabj5761, https://doi.org/10.1126/sciimmunol.abj5761.
[51] C. Malainou, S.M. Abdin, N. Lachmann, U. Matt, S. Herold, Alveolar macrophages in tissue homeostasis, inflammation, and infection: evolving concepts of therapeutic targeting, J. Clin. Investig. 133 (2023).
[52] W.C. Yu, R.W. Chan, J. Wang, E.A. Travanty, J.M. Nicholls, J.M. Peiris, R. J. Mason, M.C. Chan, Viral replication and innate host responses in primary human alveolar epithelial cells and alveolar macrophages infected with influenza H5N1 and H1N1 viruses, J. Virol. 85 (2011) 6844–6855.
[53] S. Sakabe, K. Iwatsuki-Horimoto, R. Takano, C.A. Nidom, M.T.Q. Le, T. Nagamura-Inoue, T. Horimoto, N. Yamashita, Y. Kawaoka, Cytokine production by primary human macrophages infected with highly pathogenic H5N1 or pandemic H1N1 2009 influenza viruses, Microbiology Society 92 (2011) 1428–1434.
[54] V. Westenius, S.M. Mäkelä, I. Julkunen, P. Österlund, Highly pathogenic H5N1 influenza A virus spreads efficiently in human primary monocyte-derived macrophages and dendritic cells, Front. Immunol. 9 (2018) 1664.
[55] K. Högner, T. Wolff, S. Pleschka, S. Plog, A.D. Gruber, U. Kalinke, H.-D. Walmrath, J. Bodner, S. Gattenlöhner, P. Lewe-Schlosser, Macrophage-expressed IFN-β contributes to apoptotic alveolar epithelial cell injury in severe influenza virus pneumonia, PLoS Pathog. 9 (2013) e1003188.
[56] S. Herold, M. Steinmueller, W. von Wulffen, L. Cakarova, R. Pinto, S. Pleschka, M. Mack, W.A. Kuziel, N. Corazza, T. Brunner, Lung epithelial apoptosis in influenza virus pneumonia: the role of macrophage-expressed TNF-related apoptosis-inducing ligand, J. Exp. Med. 205 (2008) 3065–3077.
[57] C. Peteranderl, L. Morales-Nebreda, B. Selvakumar, E. Lecuona, I. Vadász, R. E. Morty, C. Schmoldt, J. Bespalowa, T. Wolff, S. Pleschka, Macrophage-epithelial paracrine crosstalk inhibits lung edema clearance during influenza infection, J. Clin. Invest. 126 (2016) 1566–1580.
[58] S. Huang, B. Zhu, I.S. Cheon, N.P. Goplen, L. Jiang, R. Zhang, R.S. Peebles, M. Mack, M.H. Kaplan, A.H. Limper, PPAR-γ in macrophages limits pulmonary inflammation and promotes host recovery following respiratory viral infection, J. Virol. 93 (2019), https://doi.org/10.1128/jvi.00030-00019.
[59] H. Zhang, T. Alford, S. Liu, D. Zhou, J. Wang, Influenza virus causes lung immunopathology through down-regulating PPARγ activity in macrophages, Front. Immunol. 13 (2022) 958801.
[60] A. Yurdagul Jr., M. Subramanian, X. Wang, S.B. Crown, O.R. Ilkayeva, L. Darville, G.K. Kolluru, C.C. Rymond, B.D. Gerlach, Z. Zheng, G. Kuriakose, C.G. Kevil, J. M. Koomen, J.L. Cleveland, D.M. Muoio, I. Tabas, Macrophage metabolism of apoptotic cell-derived arginine promotes continual efferocytosis and resolution of injury, Cell Metabol. 31 (2020) 518–533.e510, https://doi.org/10.1016/j.cmet.2020.01.001.
[61] P.B. Ampomah, B. Cai, S.R. Sukka, B.D. Gerlach, A. Yurdagul, X. Wang, G. Kuriakose, L.N.F. Darville, Y. Sun, S. Sidoli, J.M. Koomen, A.R. Tall, I. Tabas, Macrophages use apoptotic cell-derived methionine and DNMT3A during efferocytosis to promote tissue resolution, Nat. Metab. 4 (2022) 444–457, https://doi.org/10.1038/s42255-022-00551-7.
[62] L. Cakarova, L.M. Marsh, J. Wilhelm, K. Mayer, F. Grimminger, W. Seeger, J. Lohmeyer, S. Herold, Macrophage tumor necrosis factor-α induces epithelial expression of granulocyte–macrophage colony-stimulating factor: impact on alveolar epithelial repair, Am. J. Respir. Crit. Care Med. 180 (2009) 521–532.
[63] P.N. Lim, M.M. Cervantes, L.K. Pham, A.C. Rothchild, Alveolar macrophages: novel therapeutic targets for respiratory diseases, Expet Rev. Mol. Med. 23 (2021) e18.
[64] L. Bosurgi, Y.G. Cao, M. Cabeza-Cabrerizo, A. Tucci, L.D. Hughes, Y. Kong, J. S. Weinstein, P. Licona-Limon, E.T. Schmid, F. Pelorosso, Macrophage function in tissue repair and remodeling requires IL-4 or IL-13 with apoptotic cells, Science 356 (2017) 1072–1076.
[65] L. Pervizaj-Oruqaj, B. Selvakumar, M.R. Ferrero, M. Heiner, C. Malainou, R. D. Glaser, J. Wilhelm, M. Bartkuhn, A. Weiss, I. Alexopoulos, B. Witte, S. Gattenlöhner, I. Vadász, R.E. Morty, W. Seeger, R.T. Schermuly, A.I. Vazquez-Armendariz, S. Herold, Alveolar macrophage-expressed Plet1 is a driver of lung epithelial repair after viral pneumonia, Nat. Commun. 15 (2024) 87, https://doi.org/10.1038/s41467-023-44421-6.
[66] A.V. Misharin, L. Morales-Nebreda, P.A. Reyfman, C.M. Cuda, J.M. Walter, A. C. McQuattie-Pimentel, C.-I. Chen, K.R. Anekalla, N. Joshi, K.J. Williams, Monocyte-derived alveolar macrophages drive lung fibrosis and persist in the lung over the life span, J. Exp. Med. 214 (2017) 2387–2404.
[67] A.L. Mora, E. Torres-González, M. Rojas, C. Corredor, J. Ritzenthaler, J. Xu, J. Roman, K. Brigham, A. Stecenko, Activation of alveolar macrophages via the alternative pathway in herpesvirus-induced lung fibrosis, Am. J. Respir. Cell Mol. Biol. 35 (2006) 466–473.
[68] K. Morimoto, W.J. Janssen, M. Terada, Defective efferocytosis by alveolar macrophages in IPF patients, Respir. Med. 106 (2012) 1800–1803.
[69] D. Pouniotis, M. Plebanski, V. Apostolopoulos, C.F. McDonald, Alveolar macrophage function is altered in patients with lung cancer, Clin. Exp. Immunol. 143 (2006) 363–372.
[70] T. Wang, J. Zhang, Y. Wang, Y. Li, L. Wang, Y. Yu, Y. Yao, Influenza-trained mucosal-resident alveolar macrophages confer long-term antitumor immunity in the lungs, Nat. Immunol. 24 (2023) 423–438.
[71] T. Peiró, D.F. Patel, S. Akthar, L.G. Gregory, C.J. Pyle, J.A. Harker, M.A. Birrell, C. M. Lloyd, R.J. Snelgrove, Neutrophils drive alveolar macrophage IL-1β release during respiratory viral infection, Thorax 73 (2018) 546–556, https://doi.org/10.1136/thoraxjnl-2017-210010.
[72] Y. Kumagai, O. Takeuchi, H. Kato, H. Kumar, K. Matsui, E. Morii, K. Aozasa, T. Kawai, S. Akira, Alveolar macrophages are the primary interferon-α producer in pulmonary infection with RNA viruses, Immunity 27 (2007) 240–252.
[73] M. Goritzka, S. Makris, F. Kausar, L.R. Durant, C. Pereira, Y. Kumagai, F.J. Culley, M. Mack, S. Akira, C. Johansson, Alveolar macrophage–derived type I interferons orchestrate innate immunity to RSV through recruitment of antiviral monocytes, J. Exp. Med. 212 (2015) 699–714.
[74] Y. He, C. Li, Z. Wang, Z. Yang, J. Wei, L. Ren, Y. Deng, S. Chen, Z. Ye, N. Zang, C-fiber degeneration enhances alveolar macrophage-mediated IFN-α/β response to respiratory syncytial virus, Microbiol. Spectr. 10 (2022) e02410, 02422.
[75] H.E. Ghoneim, P.G. Thomas, J.A. McCullers, Depletion of alveolar macrophages during influenza infection facilitates bacterial superinfections, J. Immunol. 191 (2013) 1250–1259.
[76] J.R. Coleman, The PB1-F2 protein of Influenza A virus: increasing pathogenicity by disrupting alveolar macrophages, Virol. J. 4 (2007) 1–5.
[77] D. Califano, Y. Furuya, D.W. Metzger, Effects of influenza on alveolar macrophage viability are dependent on mouse genetic strain, J. Immunol. 201 (2018) 134–144.
[78] J. Wang, F. Li, R. Sun, X. Gao, H. Wei, L.-J. Li, Z. Tian, Bacterial colonization dampens influenza-mediated acute lung injury via induction of M2 alveolar macrophages, Nat. Commun. 4 (2013) 2106.
[79] T. Narasaraju, H. Ng, M. Phoon, V.T. Chow, MCP-1 antibody treatment enhances damage and impedes repair of the alveolar epithelium in influenza pneumonitis, Am. J. Respir. Cell Mol. Biol. 42 (2010) 732–743.
[80] G.E. Cooper, Z.C. Pounce, J.C. Wallington, L.Y. Bastidas-Legarda, B. Nicholas, C. Chidomere, E.C. Robinson, K. Martin, A.S. Tocheva, M. Christodoulides, Viral inhibition of bacterial phagocytosis by human macrophages: redundant role of CD36, PLoS One 11 (2016) e0163889.
[81] A.K. Verma, S. Bansal, C. Bauer, A. Muralidharan, K. Sun, Influenza infection induces alveolar macrophage dysfunction and thereby enables noninvasive Streptococcus pneumoniae to cause deadly pneumonia, J. Immunol. 205 (2020) 1601–1607.
[82] H. Wei, H. Zhao, R. Li, F. Yang, Y. Wu, Rhinovirus impairs the immune response of alveolar macrophages to facilitate Streptococcus pneumonia infection, Pathogens and Disease 78 (2020) ftaa020.
[83] F.-F. Huang, P.F. Barnes, Y. Feng, R. Donis, Z.C. Chroneos, S. Idell, T. Allen, D. R. Perez, J.A. Whitsett, K. Dunussi-Joannopoulos, GM-CSF in the lung protects against lethal influenza infection, Am. J. Respir. Crit. Care Med. 184 (2011) 259–268.
[84] C. Schneider, S.P. Nobs, A.K. Heer, M. Kurrer, G. Klinke, N. Van Rooijen, J. Vogel, M. Kopf, Alveolar macrophages are essential for protection from respiratory failure and associated morbidity following influenza virus infection, PLoS Pathog. 10 (2014) e1004053.
[85] M.D. Tate, D.L. Pickett, N. van Rooijen, A.G. Brooks, P.C. Reading, Critical role of airway macrophages in modulating disease severity during influenza virus infection of mice, J. Virol. 84 (2010) 7569–7580.
[86] C. Purnama, S.L. Ng, P. Tetlak, Y.A. Setiagani, M. Kandasamy, S. Baalasubramanian, K. Karjalainen, C. Ruedl, Transient ablation of alveolar macrophages leads to massive pathology of influenza infection without affecting cellular adaptive immunity, Eur. J. Immunol. 44 (2014) 2003–2012.
[87] Y. Mitsui, T. Suzuki, K. Kuniyoshi, J. Inamo, K. Yamaguchi, M. Komuro, J. Watanabe, M. Edamoto, S. Li, T. Kouno, S. Oba, T. Hosoya, K. Masuhiro, Y. Naito, S. Koyama, N. Sakaguchi, D.M. Standley, J.W. Shin, S. Akira, S. Yasuda,

Y. Miyazaki, Y. Kochi, A. Kumanogoh, T. Okamoto, T. Satoh, Expression of the readthrough transcript CiDRE in alveolar macrophages boosts SARS-CoV-2 susceptibility and promotes COVID-19 severity, Immunity 56 (2023) 1939–1954. e1912, https://doi.org/10.1016/j.immuni.2023.06.013.

[88] J. Lv, Z. Wang, Y. Qu, H. Zhu, Q. Zhu, W. Tong, L. Bao, Q. Lv, J. Cong, D. Li, Distinct uptake, amplification, and release of SARS-CoV-2 by M1 and M2 alveolar macrophages, Cell discovery 7 (2021) 24.

[89] Z. Wang, Y. Zhou, L. Bao, D. Li, J. Lv, D. Wang, S. Li, W.-M. Tong, J. Liu, C. Qin, Escaping alveolar macrophage endosomal retention explains massive expansion of SARS-CoV-2 delta variant, Signal Transduct. Targeted Ther. 6 (2021) 431.

[90] C. Wang, J. Xie, L. Zhao, X. Fei, H. Zhang, Y. Tan, X. Nie, L. Zhou, Z. Liu, Y. Ren, Alveolar macrophage dysfunction and cytokine storm in the pathogenesis of two severe COVID-19 patients, EBioMedicine 57 (2020).

[91] R.A. Grant, L. Morales-Nebreda, N.S. Markov, S. Swaminathan, M. Querrey, E. R. Guzman, D.A. Abbott, H.K. Donnelly, A. Donayre, I.A. Goldberg, Circuits between infected macrophages and T cells in SARS-CoV-2 pneumonia, Nature 590 (2021) 635–641.

[92] N. Huot, C. Planchais, P. Rosenbaum, V. Contreras, B. Jacquelin, C. Petitdemange, M. Lazzerini, E. Beaumont, A. Orta-Resendiz, F.A. Rey, SARS-CoV-2 viral persistence in lung alveolar macrophages is controlled by IFN-γ and NK cells, Nat. Immunol. 24 (2023) 2068–2079.

[93] R. Channappanavar, M. Selvaraj, S. More, S. Perlman, Alveolar macrophages protect mice from MERS-CoV-induced pneumonia and severe disease, Vet. Pathol. 59 (2022) 627–638.

[94] P.C. Joshi, R. Raynor, X. Fan, D.M. Guidot, HIV-1–transgene expression in rats decreases alveolar macrophage zinc levels and phagocytosis, Am. J. Respir. Cell Mol. Biol. 39 (2008) 218–226.

[95] S.D. Tachado, X. Li, M. Bole, K. Swan, A. Anandaiah, N.R. Patel, H. Koziel, MyD88-dependent TLR4 signaling is selectively impaired in alveolar macrophages from asymptomatic HIV+ persons, Blood, J. Am. Soc. Hematology 115 (2010) 3606–3615.

[96] S.D. Tachado, X. Li, K. Swan, N. Patel, H. Koziel, Constitutive activation of phosphatidylinositol 3-kinase signaling pathway down-regulates TLR4-mediated tumor necrosis factor-alpha release in alveolar macrophages from asymptomatic HIV-positive persons in vitro, J. Biol. Chem. 283 (2008) 33191–33198, https://doi.org/10.1074/jbc.M805067200.

[97] Z. Li, Y. Jiao, E.K. Fan, M.J. Scott, Y. Li, S. Li, T.R. Billiar, M.A. Wilson, X. Shi, J. Fan, Aging-impaired filamentous actin polymerization signaling reduces alveolar macrophage phagocytosis of bacteria, J. Immunol. 199 (2017) 3176–3186.

[98] D.M. Boe, H.J. Hulsebus, K.M. Najarro, J.E. Mullen, H. Kim, A.C. Tan, R. H. McMahan, E.J. Kovacs, Advanced age is associated with changes in alveolar macrophages and their responses to the stress of traumatic injury, J. Leukoc. Biol. 112 (2022) 1371–1386.

[99] C.K. Wong, C.A. Smith, K. Sakamoto, N. Kaminski, J.L. Koff, D.R. Goldstein, Aging impairs alveolar macrophage phagocytosis and increases influenza-induced mortality in mice, J. Immunol. 199 (2017) 1060–1068.

[100] L.R. Penke, J.M. Speth, C. Draijer, Z. Zaslona, J. Chen, P. Mancuso, C.M. Freeman, J.L. Curtis, D.R. Goldstein, M. Peters-Golden, PGE2 accounts for bidirectional changes in alveolar macrophage self-renewal with aging and smoking, Life Sci. Alliance 3 (2020).

[101] A.C. McQuattie-Pimentel, Z. Ren, N. Joshi, S. Watanabe, T. Stoeger, M. Chi, Z. Lu, L. Sichizya, R.P. Aillon, C.-I. Chen, The lung microenvironment shapes a dysfunctional response of alveolar macrophages in aging, J. Clin. Invest. 131 (2021).

[102] B.G. Oliver, S. Lim, P. Wark, V. Laza-Stanca, N.J. King, J.L. Black, J.K. Burgess, M. Roth, S.L. Johnston, Rhinovirus exposure impairs immune responses to bacterial products in human alveolar macrophages, Thorax 63 (2008) 519–525.

[103] J.R. Grunwell, S.M. Yeligar, S. Stephenson, X.D. Ping, T.W. Gauthier, A. M. Fitzpatrick, L.A.S. Brown, TGF-β1 suppresses the type I IFN response and induces mitochondrial dysfunction in alveolar macrophages, J. Immunol. 200 (2018) 2115–2128.

[104] L.D. Santos, K.H. Antunes, S.P. Muraro, G.F. De Souza, A.G. Da Silva, J. de Souza Felipe, L.C. Zanetti, R.S. Czepielewski, K. Magnus, M. Scotta, TNF-mediated alveolar macrophage necroptosis drives disease pathogenesis during respiratory syncytial virus infection, Eur. Respir. J. 57 (2021).

[105] T.M. Umstead, E.K. Hewage, M. Mathewson, S. Beaudoin, Z.C. Chroneos, M. Wang, E.S. Halstead, Lower respiratory tract delivery, airway clearance, and preclinical efficacy of inhaled GM-CSF in a postinfluenza pneumococcal pneumonia model, Am. J. Physiol. Lung Cell Mol. Physiol. 318 (2020) L571–L579.

[106] C. Bosteels, K.F. Van Damme, E. De Leeuw, J. Declercq, B. Maes, V. Bosteels, L. Hoste, L. Naesens, N. Debeuf, J. Deckers, Loss of GM-CSF-dependent instruction of alveolar macrophages in COVID-19 provides a rationale for inhaled GM-CSF treatment, Cell Reports Medicine 3 (2022).

[107] T. Naessens, B. Schepens, M. Smet, C. Pollard, L. Van Hoecke, A. De Beuckelaer, M. Willart, B. Lambrecht, S. De Koker, X. Saelens, GM-CSF treatment prevents respiratory syncytial virus–induced pulmonary exacerbation responses in postallergic mice by stimulating alveolar macrophage maturation, J. Allergy Clin. Immunol. 137 (2016) 700–709. e709.

[108] Z. Wang, Y. Zhou, L. Bao, D. Li, J. Lv, D. Wang, S. Li, J. Liu, C. Qin, W.-M. Tong, Airway administration of bisphosphate and dexamethasone inhibits SARS-CoV-2 variant infection by targeting alveolar macrophages, Signal Transduct. Targeted Ther. 7 (2022) 116.

[109] M.A. Kumar, S.K. Baba, H.Q. Sadida, S.A. Marzooqi, J. Jerobin, F.H. Altemani, N. Algehainy, M.A. Alanazi, A.-B. Abou-Samra, R. Kumar, Extracellular vesicles as tools and targets in therapy for diseases, Signal Transduct. Targeted Ther. 9 (2024) 27.

[110] D.J. Schneider, K.A. Smith, C.E. Latuszek, C.A. Wilke, D.M. Lyons, L.R. Penke, J. M. Speth, M. Marthi, J.A. Swanson, B.B. Moore, Alveolar macrophage-derived extracellular vesicles inhibit endosomal fusion of influenza virus, EMBO J. 39 (2020) e105057.

[111] Z. Wang, J. Lv, P. Yu, Y. Qu, Y. Zhou, L. Zhou, Q. Zhu, S. Li, J. Song, W. Deng, R. Gao, Y. Liu, J. Liu, W.-M. Tong, C. Qin, B. Huang, SARS-CoV-2 treatment effects induced by ACE2-expressing microparticles are explained by the oxidized cholesterol-increased endosomal pH of alveolar macrophages, Cell. Mol. Immunol. 19 (2022) 210–221, https://doi.org/10.1038/s41423-021-00813-6.

[112] F. Li, K.M. Okreglicka, F. Piattini, L.M. Pohlmeier, C. Schneider, M. Kopf, Gene therapy of Csf2ra deficiency in mouse fetal monocyte precursors restores alveolar macrophage development and function, JCI insight 7 (2022).

[113] M. Tomokiyo, F.R. Tonetti, H. Yamamuro, R. Shibata, K. Fukuyama, N. Gobbato, L. Albarracin, M.S.R. Rajoka, A.H. Kober, W. Ikeda-Ohtsubo, Modulation of alveolar macrophages by postimmunobiotics: impact on TLR3-mediated antiviral respiratory immunity, Cells 11 (2022) 2986.

[114] W. He, C.-J. Chen, C.E. Mullarkey, J.R. Hamilton, C.K. Wong, P.E. Leon, M. B. Uccellini, V. Chromikova, C. Henry, K.W. Hoffman, Alveolar macrophages are critical for broadly-reactive antibody-mediated protection against influenza A virus in mice, Nat. Commun. 8 (2017) 846.

[115] K. El Bakkouri, F. Descamps, M. De Filette, A. Smet, E. Festjens, A. Birkett, N. Van Rooijen, S. Verbeek, W. Fiers, X. Saelens, Universal vaccine based on ectodomain of matrix protein 2 of influenza A: Fc receptors and alveolar macrophages mediate protection, J. Immunol. 186 (2011) 1022–1031.

[116] B.J. Laidlaw, V. Decman, M.-A.A. Ali, M.C. Abt, A.I. Wolf, L.A. Monticelli, K. Mozdzanowska, J.M. Angelosanto, D. Artis, J. Erikson, Cooperativity between CD8+ T cells, non-neutralizing antibodies, and alveolar macrophages is important for heterosubtypic influenza virus immunity, PLoS Pathog. 9 (2013) e1003207.