Diversity of Macrophages in Lung Homeostasis and Diseases

Fei Hou¹,²†, Kun Xiao¹†, Li Tang³* and Lixin Xie¹*

¹ College of Pulmonary and Critical Care Medicine, Chinese PLA General Hospital, Beijing, China, ² Medical School of Chinese PLA, Beijing, China, ³ State Key Laboratory of Proteomics, Beijing Proteome Research Center, National Center for Protein Sciences-Beijing, Beijing Institute of Lifeomics, Beijing, China

Lung macrophages play important roles in the maintenance of homeostasis, pathogen clearance and immune regulation. The different types of pulmonary macrophages and their roles in lung diseases have attracted attention in recent years. Alveolar macrophages (AMs), including tissue-resident alveolar macrophages (TR-AMs) and monocyte-derived alveolar macrophages (Mo-AMs), as well as interstitial macrophages (IMs) are the major macrophage populations in the lung and have unique characteristics in both steady-state conditions and disease states. The different characteristics of these three types of macrophages determine the different roles they play in the development of disease. Therefore, it is important to fully understand the similarities and differences among these three types of macrophages for the study of lung diseases. In this review, we will discuss the physiological characteristics and unique functions of these three types of macrophages in acute and chronic lung diseases. We will also discuss possible methods to target macrophages in lung diseases.

Keywords: macrophage, inflammation, infection, fibrosis, COVID-19

INTRODUCTION

In recent years, our understanding of lung immune cell heterogeneity has advanced significantly with the emergence of new technologies, such as single-cell RNA sequencing (scRNA-seq). The COVID-19 pandemic also made us painfully aware of the impact respiratory viral infections can have and sparked interest in the cellular and molecular mechanisms of lung immunity. Lung macrophages, including AMs and IMs, are important innate immune cells involved in the normal physiological functions of lung tissue and some acute and chronic diseases, such as infections and fibrosis (1–3). The microenvironment of the alveoli is different from that of other sites, making TR-AMs different from IMs and other tissue macrophages. Due to their different origins, AMs can be subdivided into TR-AMs and Mo-AMs. Unique growth factors and receptors in the steady state restrict the plasticity of TR-AMs, rendering them hyporesponsive to inhaled particles and dust (4). Following insults, newly recruited Mo-AMs move to the alveoli, join the AM pool and develop their own features, while the monocyte lineage is more plastic than mature AMs (5, 6). Early inflammatory Mo-AMs may be associated with immune disorders such as cytokine storms in some infectious diseases, and late profibrotic Mo-AMs are associated with lung fibrosis (6–9). By summarizing recent studies of lung macrophages, we concluded that TR-AMs function as sentinels that maintain immune balance while the characteristics and functions of Mo-AMs are mainly dependent on the lung microenvironment. Homeostatic maintenance, immune surveillance,
phagocytosis and inflammatory resolution may be performed by these three types of macrophages together or separately and these cells may communicate to maintain the immune balance (10–13). Defining the exact role of different lung macrophages in pathology and diseases may help to identify the cause and find appropriate therapeutic strategies.

**MACROPHAGE SUBSETS IN THE LUNG**

The characteristics and functions of different types of macrophages in various organs are gradually being discovered (14–18). Location and origin are the two main factors that determine the characteristics of lung macrophages (19). The lung contains the following two different macrophage subsets based on anatomical position and function: AMs and IMs. AMs exist in the alveolar cavity, while IMs exist in the interstitium (20, 21). When infection or injury occurs, monocytes enter the alveolar cavity and develop into Mo-AMs (Figure 1) and they differ substantially from TR-AMs in both phenotypic and metabolic characteristics in the first few days (9, 22–24). Thus, AMs contain two distinct subpopulations as follows: TR-AMs and Mo-AMs. TR-AMs seem to have lower responsiveness and limited plasticity, while Mo-AMs are more likely to be remodeled by the microenvironment (5, 6). The different characteristics of these two types of macrophages determine their distinct functions in lung diseases (19, 25, 26). Due to the widespread use of single-cell and tracer technologies, the subpopulations and physiological characteristics of IMs are gradually being elucidated, and their roles in lung diseases such as fibrosis and infections are also beginning to be discovered. IMs can also be divided into TR-IMs and Mo-IMs according to their origins. However, little is known about the differences in the functions and characteristics of these two types of IMs, so the present review will discuss TR-IMs and Mo-IMs together.

**RESIDENT ALVEOLAR MACROPHAGES: TERMINALLY DIFFERENTIATED SENTINELS**

**Physiological Characteristics**

TR-AMs reside in the alveolar cavity and have important functions in the turnover of pulmonary surfactant and the removal of dead cells from the alveoli (27–29). TR-AMs originate from yolk sac-derived erythromyeloid progenitors and fetal liver monocytes (30–32). Many factors are involved in AM maturation and self-maintenance (32–35), among which GM-CSF and TGF-β are the most important (Figure 1). Defective production of GM-CSF may affect the process of AM maturation, thereby rendering mice more susceptible to pathogens (36). Several transcription factors, such as Bhlhe40 and Bhlhe41, also regulate alveolar macrophage self-renewal and identity. Decreased proliferation is observed in Bhlhe40/Bhlhe41-deficient alveolar macrophages (37). AM development, maturation, and regeneration are also regulated by epigenetic factors, such as histone deacetylase 3 (38). Basophils can also regulate AM development by promoting the transition of naive macrophages toward the AM signature (39).

Studies have shown that TR-AMs can maintain self-renewal independent of monocytes at the steady state via proliferation (40, 41). However, in some states, such as injury or infection, TR-AMs are depleted, and Mo-AMs help restore the AM pool. These
recruited Mo-AMs can acquire a resident AM phenotype a few weeks later, but some transcriptional characteristics are still different because of their monocyte origin (5, 24, 42). It remains unclear why Mo-AMs do not fully acquire the characteristics of TR-AMs in the same environment, and the functional differences between such Mo-AMs and TR-AMs remain unclear. Other studies have demonstrated that AMs can refill their niche independent of blood monocytes even after insult. Hashimoto et al. (40) found that TR-AMs repopulate through local proliferation after nongenotoxic ablation and genotoxic insult. Lai et al. (43) also found that TR-AMs restore their numbers through self-renewal during blood-stage malaria, which was distinct from splenic red pulp macrophages and hepatic Kupffer cells. The different results of these studies may be due to the degree of AM depletion and the severity of inflammation (6). When AMs are not severely depleted, they can be restored through proliferation with minimal contribution from circulating monocytes. In some conditions where AMs are substantially depleted, however, the remaining AMs are too few to repopulate in a short time, resulting in the recruitment of monocytes to help restore their numbers.

Because most experimental mice live in a specific pathogen-free (SPF) environment while humans live in an environment with bacteria and dust, the situation becomes more complicated in regard to human lung macrophages. The AM pool in adults may contain multiple types of macrophages, especially after they experience multiple infections and lung diseases in their early life. The origin and self-renewal capability of human AMs remain unclear due to the lack of reliable markers and limitations in sampling and study methods. Using humanized mice, Evren et al. (44) found that blood CD14+ monocytes give rise to CD206+ resident AMs and IMs. Using single-cell technology, Byrne et al. (45) found that macrophages in transplanted lungs are predominantly recipient-derived, implying that AM cannot fully self-renew in the transplanted state. Thus, in addition to self-renewal, resident AMs may be replenished by peripheral monocytes in humans, and TR-AMs and Mo-AMs may exist at the same time (46).

**Infections and Lung Injury**

TR-AMs are the first line defense against pathogens. Phagocytosis is an important mechanism by which TR-AMs defend against bacterial infections. Aging, smoking and severe systemic diseases, such as trauma and sepsis, impair the phagocytosis of TR-AMs, thereby reducing their ability to fight pathogens (47–49). Neupane et al. (50) found that TR-AMs move between alveoli to phagocytose inhaled bacteria and TR-AM migration is crucial for bacterial clearance, which is impaired during viral infection, rendering the lung susceptible to secondary bacterial infections. Additional studies need to confirm whether TR-AMs move within the alveolar lumen, but at least one group of AMs is sessile and attached to the alveolar wall (13). It is also unknown whether the TR-AMs move toward the bacteria or whether the bacteria encounter the TR-AMs and are engulfed. Equipped with several pathogen-recognition receptors, TR-AMs are quickly activated and release several cytokines and chemokines after the onset of infections. However, TR-AMs are less inflammatory than monocytes and neutrophils (9). Depleting TR-AMs can reduce cytokines in the early phase but have less impact on subsequent cytokine release, such as IL-6 and TNF-α, but it has less effect on subsequent proinflammatory processes (51, 52). TR-AMs are less plastic, and proinflammatory TR-AMs and anti-inflammatory TR-AMs may exist at the same time and can be distinguished by the expression of CXCL2 (53). Despite their function in the initiation of inflammation, TR-AMs also play an important role in inflammatory recovery and injury repair by clearing apoptotic cells, also called efferocytosis, and releasing resolving mediators (54–56). Apoptotic cells, such as neutrophils, express “eat me” signals, and macrophages recognize these signals through the Murky kinase (MeRTK) receptor, integrins, scavenger receptors and complement receptors (54). Efferocytosis reduces NO production and tissue injury through the PPAR-δ and PPAR-γ pathways (57). Various lipid mediators secreted by macrophages during efferocytosis, such as lipoxin, resolvins and protectins, also promote inflammation resolution by the 15-lipoxygenase pathway (58). TR-AMs also promote inflammation resolution and tissue repair by secreting a series of factors, such as TGF-β and IL-10, as well as promoting the secretion of GM-CSF by alveolar epithelial cells in LPS-induced lung injury (11, 60, 61). During pathological states, the process of efferocytosis may be impaired. In acute respiratory distress syndrome (ARDS) mice, apoptotic neutrophil clearance is impaired and can be reversed by activating AMP-activated protein kinase (AMPK) or neutralizing high-mobility group box 1 (HMGB1) (62). Some pathogens, such as Klebsiella pneumoniae and Staphylococcus aureus, can also increase tissue damage by inhibiting the efferocytosis of AMs (63, 64).

TR-AMs can protect bodies from the damaging effect of bacteria and viruses, such as Staphylococcus aureus, Klebsiella pneumoniae and influenza (65–67). Depleting AMs significantly reduces the survival rate and increases lung injury severity. However, some other pathogens, such as Pseudomonas aeruginosa and respiratory syncytial virus (RSV), can induce AM pyroptosis or necroptosis, thereby causing severe inflammation and increasing lung injury severity (68, 69). Some intracellular bacteria, such as Mycobacterium tuberculosis, can infect TR-AMs and, thus, mediate the spread of M. tuberculosis from the alveoli to the lungs (70). TR-AMs may also be related to nonpathogen-induced lung injuries, such as ventilator-induced lung injury, and a reduction in TR-AMs reduce the severity of ventilator-induced lung injury (71). Therefore, the role of TR-AMs in infections is complex and difficult to define in terms of good or bad, and their role depends on specific pathogens.

**Type II Inflammation**

Atopic diseases, such as allergies and asthma, as well as certain infections caused by parasitic helminths, can induce type II immune responses (72). The type II immune response is mainly regulated by T helper 2 (TH2) cells. TH2 cells mainly secrete the IL-4, IL-5 and IL-13 cytokines and stimulate type II immunity (72). Although airway type II immune responses are mainly mediated by cells like eosinophils, mast cells and basophils, lung macrophages can participate in type II inflammation and affect disease progression (73, 74). Unlike
many other macrophages, TR-AMs are hyporesponsive to the canonical type 2 cytokine, IL-4, and parasitic worm infection (75). The reason may be due to the unique lung microenvironment that limits the plasticity of TR-AMs and restricts TR-AMs to a “M2”-like phenotype. Removal of TR-AMs from the alveolar niche restores their ability to respond to type 2 cytokine stimulation (75). Despite their hyporesponsiveness to the canonical type 2 cytokine in vivo, TR-AMs can suppress asthmatic lung inflammation caused by house dust mites in mice (25, 76). Depleting TR-AMs decreased IL-27 levels and exacerbated type II inflammation caused by IL-13 and house dust mite allergens (77). TR-AMs can alleviate type II inflammation in many ways. Antigen-bearing TR-AMs can promote the development of Foxp3+ regulatory T cells, which contribute to airway tolerance and prevent the development of asthmatic lung inflammation (78). Apoptotic cell engulfment in TR-AMs promotes the production of the regulatory T cell-inducing molecule retinoic acid, which impacts the development of allergen-induced asthmatic airway inflammation (76). Intrinsic TGF-β1 in TR-AMs is essential for TR-AM maturation and is also involved in the control of allergic reactions (59). TGF-β1-deficient AMs expressed enhanced levels of monocyte-attractant chemokines and displayed augmented type II inflammation to house dust mite (59). Lung macrophage mannose receptor (MRC1/CD206) also have functions in protection against allergen-induced lung inflammation and Mrc1-/- mice had an exacerbated lung inflammation that caused by allergen (79).

**Secondary Infection and Trained Immunity**

TR-AMs may also be related to secondary infection and trained immunity. After primary infection, the body may become more susceptible to bacterial infections. RSV infection stimulates the local production of growth arrest–specific 6 (Gas6), thereby converting TR-AMs into the M2 type, which impacts their ability to defend against secondary bacterial infections (80). After sepsis and severe trauma, TR-AMs are induced by secondary immunosuppressive signals of signal-regulatory protein α, and they are epigenetically altered, leading to long-term lung immunoparalysis (49). However, Yao et al. (81) found that respiratory viral infection induces long-lasting memory AMs that are programmed to express high MHC II levels, increase glycolytic metabolism and produce more neutrophil chemokines. These memory AMs are induced by CD8+ T cells (81). After influenza infection, some TR-AMs were replaced by Mo-AMs, which can protect the body from Streptococcus pneumoniae infection (42). This phenomenon can also be observed in a model where the pathogen of the first infection is bacteria. After recovery from Streptococcus pneumoniae infection, TR-AMs were reprogrammed and protected against another pneumococcal serotype (82). It is unclear why TR-AMs exhibit a completely different phenotype and function after the initial infection. The severity of the initial infection may be one of the reasons. Severe systematic infections such as sepsis can cause AMs to develop immune paralysis states where the phagocytosis and proinflammatory ability of AMs are impaired (83, 84). While some local mild infections can make AMs more likely to be activated in subsequent infections (81, 82) (**Figure 2**). However, both the protective effect and dysfunction of TR-AMs may be restricted to a particular model. Further investigations are required to determine how different pathogens induce unique microenvironments and how these microenvironments differentially affect AMs and lead to long-term changes in AM.

**MONOCYTE-DERIVED ALVEOLAR MACROPHAGES: FANNING THE FLAMES**

In homeostasis, two types of monocytes exist in the lung as follows: classical monocytes (Ly6C$^{hi}$CX3CR1$^{lo}$–midCCR2$^+$) and
non-classical/patrolling monocytes (Ly6C\textsuperscript{lo}CX3CR1\textsuperscript{hi}CCR2\textsuperscript{-}) (85). Non-classical monocytes primarily function to scavenge damaged cells and debris from the luminal side of the vascular endothelium as well as the parenchyma of tissues, while classical monocytes are the main recruited monocytes in inflammation (85, 86). During inflammation or injury, TR-AMs, epithelial cells and other innate cells release cytokines and chemokines, and classical monocytes are then recruited to the lungs where they differentiate into macrophages (Figure 1). Mo-AMs have a limited ability of self-maintenance (40, 87) and may undergo apoptosis at the late stage of inflammation (26). The remaining Mo-AMs can acquire the phenotype of AMs and exist for a long time, while some expressed genes still show changes. In addition to inflammation and injury states, Mo-AMs may also contribute to the AM pool in some steady states. Fate-mapping studies have shown that there is a substantial contribution of monocytes to the AM compartment in older mice (30, 88). This might be due to age-related depletion of TR-AMs (45, 47).

Local microenvironmental cues generated by tissue cells are increasingly recognized as critical determinants of resident macrophage identity. Newly recruited Mo-AMs are more plastic than TR-AMs and can be more easily instructed by the local microenvironment. Due to their monocyte origin, Mo-AMs are more likely to be remodelled by the microenvironment than TR-AMs. Newly arrived Mo-AMs may quickly acquire an inflammatory phenotype in an inflamed lung and further promote the development of inflammation. In the late stage of lung injury, a resolving environment can also instruct Mo-AMs to a pro-resolving phenotype and promote the resolution of inflammation. Similarly, the profibrotic effect of Mo-AMs may be due to the early changes of the lung microenvironment, which imprint a profibrotic phenotype on Mo-AMs.

**Infections and Lung Injury**

In pulmonary viral and bacterial infections, Mo-AMs play an important role in the clearance of pathogens through phagocytosis and inflammatory responses. However, the strong proinflammatory effects of newly recruited Mo-AMs usually exacerbate lung injury. Mo-AMs can cause alveolar epithelial cell apoptosis and lung injury by releasing tumor necrosis factor-related apoptosis-inducing ligand and some inflammatory cytokines, such as TNF-\(\alpha\) (89–92). The level of inflammatory Mo-AMs may also be related to the severity of diseases such as COVID-19 (93–95). The increased number of monocytes and Mo-AMs in COVID-19 patients may lead to cytokine storms, which can cause tissue damage, affect the adaptive immune response and increase mortality (96–99). In the LPS-induced lung injury model, different studies may have obtained opposite results on the role of monocytes and Mo-AMs, with some studies showing that they are important factors in causing injury and others finding that they promote recovery from inflammation, possibly because of the selection of time and choice of experimental method or mouse type (10, 11, 100).

At the later stage of infection, a gradual resolution in inflammation converts proinflammatory Mo-AMs to a pro-repair phenotype, which, in turn, promotes inflammatory resolution. Continuing persistence of inflammatory Mo-AMs may impair the process of recovery (92). However, it remains unknown how inflammatory Mo-AMs gradually disappear from the alveoli and whether apoptosis or migration occurs. The mechanism of how proinflammatory Mo-AMs transfer into a pro-repair phenotype is also under debate. Efferocytosis has been shown to promote macrophages to exhibit a proresolution phenotype. After phagocytosing too many apoptotic cells, the phenotype of macrophages further changes from the M2 type to a nonphagocytic CD11b-low phenotype, which plays a role in promoting inflammation resolution (58, 101). These macrophages have significantly different transcriptional characteristics than M2 macrophages and that they express high levels of IFN\(\beta\)-related genes (102). This type of macrophage promotes the elimination of bacteria by secreting IFN\(\beta\), which promotes the apoptosis of inflammatory neutrophils through the STAT3 pathway, thereby enhancing efferocytosis and further promoting phenotypic changes in other macrophages (102). Because many studies on macrophage efferocytosis do not distinguish their origin, it remains unknown which type of macrophages play the main role of phagocytosis of apoptotic cells, whether their phagocytic ability is the same and which cells mainly promote the repair of damage. After recovery from infections, some Mo-AMs join the AM pool. These Mo-AMs display a unique functional, transcriptional and epigenetic profile, and they produce increased IL-6, which protects the lung from subsequent *Streptococcus pneumoniae* challenge (42). However, influenza-experienced resident AMs remain largely similar to naive AMs (42). Thus, the circumstances under which pathogens induce long-term changes in TR-AMs or substantial replenishment of different functional Mo-AMs remain to be explored.

**Type II Inflammation and Fibrosis**

Studies have found that Mo-AMs can aggravate type II inflammation, which is contrary to TR-AMs. Depleting Mo-AMs can alleviate type II inflammation and fibrosis (25, 103). However, Machiels et al. (104) identified Mo-AMs as having a positive role in type II inflammation after long-term training of lung immunity. The Mo-AMs that replace TR-AMs may block the ability of dendritic cells to trigger an HDM-specific response by the TH2 subset of helper T cells (104). Importantly, the role of Mo-AMs in lung fibrosis has been gradually revealed. Using scRNA-seq, Aran et al. (105) found CX3CR1\textsuperscript{+} SiglecF\textsuperscript{+} macrophages to be a source of Pdgf-aa in the fibrotic niche. These CX3CR1\textsuperscript{+} SiglecF\textsuperscript{+} macrophages may be of monocyte origin and acquire a TR-AM profile (105). Fastré et al. (106) also used scRNA-seq to characterize macrophage/monocyte cell populations in the BALF from dogs with canine idiopathic pulmonary fibrosis (CIPF) (107) and found that monocyte-derived macrophages were enriched in profibrotic genes in CIPF. Misharin et al. (6) found that during lung fibrosis, AMs are partially depleted and replaced by Mo-AMs. These Mo-AMs persist in the lung over the lifespan and drive lung fibrosis. The self-maintenance and persistence of these pathogenic Mo-AMs are controlled by macrophage colony-stimulating factor receptor signaling (7). Selective deletion of Mo-AMs can improve fibrosis (7, 8, 108). These studies indicate that monocyte-derived cells more easily acquire profibrotic phenotypes and, therefore, promote fibrosis. However, it remains unclear how profibrotic...
environments imprint Mo-AMs. Considering that many studies involve monocyte-derived macrophages containing monocyte-derived IMs, it remains unknown whether they act individually or together.

**LUNG INTERSTITIAL MACROPHAGES: OBSCURITY IN THE PAST**

**Physiological Characteristics**

IMs, which are differentiated from AMs by their localization, remain less studied. Due to new transgenic tools and single-cell technology, the localization and function of IMs has become increasingly clear. Different subpopulations of IMs exist and reside in different anatomical sites. However, the exact locale of IMs is still not clear and requires additional studies. Gibbings et al. (109) identified the following three types of IMs by using flow cytometry in the steady state: CD11c<sup>lo</sup> MHCII<sup>hi</sup> IMs (IM1), CD11c<sup>hi</sup> MHCII<sup>hi</sup> IMs (IM2) and CD11c<sup>−</sup> MHCII<sup>hi</sup> IMs (IM3). All three IMs expressed high levels of CX3CR1 and and Cd11r (109). Compared to IM3 cells, IM1 and IM2 cells express higher levels of CD206, Lyve-1 and CD169 but lower levels of CCR2 and CD11c. BM chimeras and parabiotic mice demonstrate that IM3 more readily replenishes circulating precursor cells than IM1 and IM2 (109). Using CX3CRI-GFP reporter mice and immunostaining for MerTK, they found that IMs are located within the bronchial interstitium and not the alveolar interstitium (109). Chakarov et al. (110) identified two groups of IMs as follows: Lyve<sup>hi</sup> MHCII<sup>hi</sup> and Lyve<sup>hi</sup> MHCII<sup>lo</sup> IMs. Lyve<sup>hi</sup> MHCII<sup>hi</sup> macrophages were mostly found surrounding the nerves and were mainly involved in inflammation and antigen presentation, while Lyve<sup>hi</sup> MHCII<sup>lo</sup> macrophages were often closely associated with blood vessels and were mainly involved in wound healing and tissue repair. Schyns et al. (111) also found two subsets of IMs: CD206<sup>+</sup> and CD206<sup>−</sup> IMs. CD206<sup>−</sup> IMs mainly exist in bronchial interstitium and express high level of chemokines and anti-inflammatory-related genes while CD206<sup>+</sup> IMs are mainly exist in alveolar interstitium and express high level of antigen representation and proinflammation-related genes. Based on the above studies, at least two populations of IMs exist, namely, Lyve<sup>hi</sup> MHCII<sup>hi</sup> CD206<sup>−</sup> IMs and Lyve<sup>hi</sup> MHCII<sup>lo</sup> CD206<sup>+</sup> IMs, which reside in different areas of lung tissue and have distinct characteristics and functions (Table 1).

The origin of IMs is not clarified mainly due to the lack of reliable markers that could be used for lineage tracing experiments. Yolk sac macrophages and fetal liver monocytes may be the main origins of IMs before birth (112). Unlike TR-AMs, IMs can be gradually replaced by circulating monocytes after birth (112). Therefore, adult IMs may constitute a heterogeneous group of cells, comprising embryonically derived TR-IMs and bone marrow monocyte derived Mo-IMs. However, it remains unknown whether TR-IMs and Mo-IMs have different characteristics and functions in steady and pathogenic states similar to TR-AMs and Mo-AMs. Novel methods to distinguish these two types of IMs are needed. It also remains unclear whether there is a group of self-renewing IMs. Schyns et al. (111) found that CD206<sup>+</sup> IMs have a longer life cycle, and CD206<sup>−</sup> IMs may be derived from Ly6C<sup>hi</sup> patrolling monocytes, which are defined as CD64<sup>+</sup> CD16.2<sup>+</sup> monocytes. Ural et al. (113) found a population of tissue-resident interstitial macrophages in the vicinity of sympathetic nerves in the bronchovascular bundle. These nerve- and airway-associated macrophages are derived from the yolk sac, are self-renewing and do not require CCR2<sup>+</sup> monocytes for development or maintenance (113). Keerthivasan et al. (114) also identified a proliferative Ki67<sup>+</sup> IM subpopulation using scRNA-seq. In contrast, using fate mapping and parabiotic mouse models, Chakarov et al. (110) demonstrated that both groups of IMs are replaced by blood monocytes after birth.

**Inflammation and Infections**

IMs may take part in the inflammation process and regulate immune reactions. After intraperitoneal (i.p.) LPS administration, the proportion of IMs gradually increase during the course of inflammation (106). IMs and inflammatory monocytes (iMos) exhibit robust and largely overlapping changes in gene expression (106). In addition to proinflammatory cytokines, IMs are quickly induced to express genes for anti-inflammatory cytokines, active oxygen scavengers and matrix metallopeptidases after i.p. LPS administration (106). In contrast to i.p. delivery, AMs are the most responsive lung macrophages after intranasal (i.n.) LPS administration with few acute changes in gene expression observed in IMs and iMos (106). The lung tissue environment may also have an impact on the immune regulation function of IMs, and IMs may interact with other nearby cells. In response to inflammatory injury, IMs switch to an anti-inflammatory phenotype to maintain lung homeostasis, which is regulated by Rpspondin3 secreted by endothelial cells (115). IMs also have functions in type II inflammation and parasite infections. IMs

| Cell type | Population | Markers | Functions | References |
|-----------|------------|---------|-----------|------------|
| TR-AM     | CD64<sup>+</sup> MerTK<sup>+</sup> F4/80<sup>+</sup> SiglecF<sup>+</sup> CD11c<sup>hi</sup> | homeostasis maintenance, pathogen phagocytosis, inflammation (5, 26, 42) | (5, 26, 42) |
|           | CD11b<sup>+</sup> CD206<sup>+</sup> | initiation, inflammation resolution (49) | (49) |
| Mo-AM     | CD64<sup>+</sup> MerTK<sup>+</sup> F4/80<sup>+</sup> SiglecF<sup>+</sup> CD11c<sup>hi</sup> | pathogen phagocytosis, pro-inflammation, cytokines secretion (5, 26, 42) | (5, 26, 42) |
|           | CD11b<sup>+</sup> CD206<sup>+</sup> | | (49) |
| IM        | Lyve<sup>hi</sup> | CD64<sup>+</sup> MerTK<sup>+</sup> F4/80<sup>+</sup> SiglecF<sup>+</sup> CD11c<sup>hi</sup> | inflammation and antigen presentation (110, 111) | (110, 111) |
|           | MHCII<sup>hi</sup> | CD11b<sup>+</sup> CD206<sup>+</sup> CX3CR1<sup>hi</sup> | wound healing and tissue repair (110, 111) | (110, 111) |
|           | Lyve<sup>hi</sup> | CD64<sup>+</sup> MerTK<sup>+</sup> F4/80<sup>+</sup> SiglecF<sup>+</sup> CD11c<sup>hi</sup> | | (113) |
|           | MHCII<sup>hi</sup> | CD11b<sup>+</sup> CD206<sup>+</sup> CX3CR1<sup>hi</sup> | | (113) |
can expand under the stimulation of bacterial CpG DNA and produce IL-10 to reduce allergic reactions (116). IMs have also been found to produce high levels of IL-10 to inhibit LPS-induced maturation and migration of DCs (117). *Nippostrongylus brasiliensis* infection induces expansion of RELMα lung IMs but not AMs. RELMα, lung interstitial macrophages are necessary for reducing severe lung injury in primary and secondary infection (118).

**Fibrosis**

The role of IMs in lung fibrosis has been gradually discovered, but the exact role is still unclear. During lung fibrosis, both interstitial and alveolar macrophages are detected in clinical and preclinical radiation-induced lung fibrosis (RIF). Depletion of IMs using colony-stimulating factor receptor-1 (CSF1R) neutralizing antibody effectively reduces fibrosis in vivo, while depletion of TR-AMs has no effect on the RIF score (119). The arginase-1 expression level in IMs is significantly higher than that in AMs both in the physiological state and in RIF (119). Other studies on monocyte-derived macrophages indicate a possible role of IMs in promoting fibrosis. Depletion of SiglecFloCD11bhi macrophages, depletion of Cx3cr1⁺ cells or use of CCR2-deficient mice can reduce pulmonary fibrosis (7, 120–122). All these cell depletion methods may cause the loss of IMs. However, Chakarov et al. (110) found that the absence of Lyve1⁻⁰MHCIIP⁻⁰ IMs exacerbates experimental lung and heart fibrosis, demonstrating their protective role in fibrosis. This result suggests the role of IMs in fibrogenesis. Lyve1⁻⁰MHCIIP⁻⁰ IMs that express high levels of CD206 and CD169 are more likely to be resident IMs and may play a positive role in fibrosis. However, monocyte-derived IMs may have a profibrotic role similar to Mo-AMs. It remains unknown whether the origin determines the destiny of IMs.

**TARGETING MACROPHAGES IN LUNG DISEASES**

Targeting macrophages has been studied in tumor and autoimmune diseases (123–125). Targeting tumor-associated macrophages and related factors has been partially applied in the clinic (126). In various diseases of the lung, targeting macrophages may be a new strategy, especially for infectious diseases such as COVID-19 and pulmonary fibrosis. Macrophage-targeted antibiotics or produgs to the lung are used to treat specific pathogens that infect macrophages (127, 128). Because Mo-AMs are important pathogenic factors in severe infections and pulmonary fibrosis, direct or indirect targeting of Mo-AMs may be a new approach for the future treatment of infectious and fibrotic diseases. Direct clearance of Mo-AMs in mice can effectively reduce infection-induced lung injury and pulmonary fibrosis, and this approach can be optimized for clinical application. Since direct clearance of monocytes or Mo-AMs may have an unexpected impact on the human body, indirect targeting of monocytes and Mo-AMs may be a better option. Researchers can block certain factors or pathways to reduce the proinflammatory nature of macrophages or to promote their conversion to an anti-inflammatory phenotype. For example, in the treatment of COVID-19, targeting GM-CSF can reduce the proinflammatory properties of macrophages (129). Moreover, blocking the CCL2-CCR2 axis may be another method to stop the extensive recruitment of monocytes and Mo-AMs (130). In addition, compared to that of Mo-AMs, the number of TR-AMs is significantly reduced in severe infection and fibrosis. Immunomodulatory effects may be achieved by supplementing TR-AMs or promoting their proliferation, and timely restoration of TR-AMs also prevents the development of fibrosis after Mo-AMs occupy the niche.

**CONCLUDING REMARKS**

Recent findings have revealed different subtypes of lung macrophages that play important roles in both homeostatic and disease states. TR-AMs are long-lived cells shaped by the microenvironment and have immunosuppressive functions in the steady state and less plasticity in the defense state. TR-AMs play an indispensable role in fighting pathogens as they activate the inflammatory response in the early stages and promote the recovery of inflammation in the late stages. However, whether TR-AMs are truly self-renewing and whether TR-AMs have motility properties remain controversial. The differences between mouse AMs and human AMs are unknown, and the origin of human TR-AMs and the composition of the human AM pool remain to be further discovered. It is also unclear whether sustained pathogen and dust exposure leads to a predominantly monocytic origin of human lung macrophages. Derived from monocytes, Mo-AMs are more easily instructed by the environment than TR-AMs, and they are associated with cytokine storms and immune imbalance in severe infections (e.g., COVID-19). Timely regression of inflammatory macrophages and their conversion to an anti-inflammatory phenotype is essential for normal recovery from inflammation. Thus, interfering with excess inflammation, Mo-AMs may be a potential mechanism to correct the immune imbalance. In the recovery period, how Mo-AMs convert to a pro-resolving phenotype and whether they undergo apoptosis or migration are unknown. After primary infection, the function of Mo-AMs in trained immunity are other controversial issues. Considering the pro-fibrotic function of Mo-AMs, it remains to be determined whether Mo-AMs infiltrating the infected lung receive different instructions than the those infiltrating a fibrotic lung, and methods to change the microenvironment to alter Mo-AMs require more research in the future. Studies on IMs are lacking, and these cells may play essential roles in immune regulation, the type II inflammatory response and pulmonary fibrosis. It is important to accurately group IMs and determine their location and to clarify how the location and origin affect their function. It is also important to understand if IMs of different locations and origins have different functions in lung disease as well as how the microenvironment of lung tissue affects the function of IMs. When studying IMs, it is important to consider how to avoid contamination by other cells.
such as Mo-AMs. In future studies, distinguishing different macrophages in lung infections and noninfectious diseases may help better understand macrophages and diseases.

**AUTHOR CONTRIBUTIONS**

All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by FH, KK, LX, and LT. The first draft of the manuscript was written by FH and XK and all authors commented on previous versions of the manuscript. All authors contributed to the article and approved the submitted version.

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