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TISSUE-RESIDENT INNATE IMMUNITY IN THE LUNG

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Abbreviations

AMs	alveolar macrophages
BAL	bronchoalveolar lavage fluid
cDCs	conventional dendritic cells
COPD	chronic obstructive pulmonary disease
DCs	dendritic cells
ILCs	innate lymphoid cells
IMs	Interstitial macrophages
MARCO	macrophage receptor with collagenous structure
moDCs	monocyte derived dendritic cells
Mtb	*Mycobacterium tuberculosis*

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NK	natural killer
NSCLC	non-small cell lung cancer
PAMP	pathogen-associated molecular pattern
pDCs	plasmacytoid dendritic cells
PAF	platelet activating factor
SeV	Sendai virus
Tregs	regulatory T-cells
Trm	tissue-resident T-cells

SUMMARY <250 words

The lung is a unique organ which must protect against inhaled pathogens and toxins, without mounting a disproportionate response against harmless particulate matter and without compromising its vital function. Tissue-resident immune cells within lung provide local immunity and protection from infection but are also responsible for causing disease when dysregulated. There is a growing appreciation of the importance of tissue-resident memory T-cells to lung immunity, but non-recirculating, tissue-resident, innate immune cells also exist. These cells provide the first line of defence against pulmonary infection and are essential for co-ordinating the subsequent adaptive response. In this review we discuss the main lung-resident innate immune subsets and their functions in common pulmonary diseases, such as influenza, bacterial pneumonia, asthma and inflammatory disorders.

INTRODUCTION

Human lungs are remarkable organs, which consume up to 11, 500L of air each day. This air contains small particulate matter such as dust, smoke, dirt, pollen and aerosols that the lung must tolerate, in addition to the mechanical forces of spontaneous respiration (1), whilst at the same time providing protection from inhaled viruses, bacteria and other respiratory pathogens. Clearly, proper lung function is essential for good health, and so immune cells in the lung must strike a delicate balance between immunity and tolerance. Studies within the last decade have shown this relies on a complex network of non-recirculating immune subsets that reside within lung tissue.

Tissue-resident cells span across innate and adaptive immunity and provide localized, tissue-specific immunity. Many studies have focused on tissue-resident T-cells (Trm) within lung tissues, and these adaptive immune cells have proved to play an important part in host defence against a variety of pulmonary infections (2) (3,4). Indeed, recent work suggests that tissue-resident B-cells also form an important part of the adaptive immune defence in the lung (5). However, it is important to remember that innate immune cells can also be tissue-resident and are vital in providing the first line of defence against inhaled allergens and pathogens (6). Indeed, alveolar macrophages have long been recognized as a unique and non-recirculating subset, which establishes during early

development (7), and might be considered the prototypic lung-resident immune subset. In this review we focus on lung-resident innate immune subsets of hematopoietic origin that are long-lived and often maintained long-term via self-renewal. Other innate immune cells play vital roles in lung immunity, including eosinophils and neutrophils, and may be morphologically and phenotypically distinct from cells in the circulation (8,9). However, as these cells are very short lived (1-2 days) and must be continually replenished from blood, they will not be discussed in this review (10,11).

Macrophages

In mice, lung development starts as early as 9 days after conception, but lungs remain sterile until birth (1). Within the developing embryo, lineage tracing, parabiosis and fate-mapping experiments show that lung-resident macrophages arise through at least two distinct developmental programs (12,13). In the first stage, primitive macrophages develop from the fetal yolk sac, bypassing the formation of monocyte intermediates (13,14). These primitive macrophages predominantly give rise to microglia in the brain, but also go on to seed a variety of tissues, including the lung, where they contribute to a fraction of the tissue-resident macrophage population (13). The majority of tissue-resident macrophage populations, however, are derived from fetal monocyte precursors that colonize the liver during development(13). Following the first breath, alveologenesis begins and these macrophages differentiate into long-lived alveolar macrophages (AMs) (1,15) and interstitial macrophages (IMs) (12). At steady-state, the majority of lung-resident macrophages are AMs, residing exclusively within the alveolar space, whereas a smaller population of IMs reside with the lung parenchyma (16).

Alveolar Macrophages

AMs are adaptable cells that can adopt different functions depending on their microenvironment and differentiation state, but they are considered anti-inflammatory (**Figure 1**). Their primary location within the alveolar lumen means that they are continuously exposed to environmental stimulants; and their historical name of “dust cells”, reflects their role in phagocytosis of particulate matter, dying cells and cellular debris. The physical removal of this material is itself essential to limiting lung inflammation, and in order to prevent overt inflammatory responses to this process, under

homeostatic conditions AMs are largely kept in a quiescent state, through a variety of mechanisms (17-19). Over the last decade, fate-mapping, parabiosis and adoptive transfer experiments have established that AM are a largely self-renewing population that doesn't rely on replenishment from the bone marrow (15,20). Hashimoto *et al.* (2013) developed three different fate-mapping models, all of which showed that lung-resident macrophages originate independently from monocytes and hematopoietic precursors. These findings are supported by parabiotic mouse experiments in which lung macrophages display negligible chimerism after one year, despite proliferation occurring. When host macrophages are depleted by a diphtheria-toxin or Toxoplasma infection, repopulation occurs primarily through local proliferation and is largely independent of circulating monocytes (20). However, new AMs can develop from recruited blood monocytes, which then persist long term and gradually transition to more closely resemble tissue resident AMs (21). Thus, irrespective of their origin, AMs appear to be a truly unique lung resident population.

In the absence of inflammation, AMs are critical for maintaining immune homeostasis of other alveolar subsets, including alveolar epithelial cells, dendritic cells and T-cells (16,22-24), through the production of anti-inflammatory molecules including TGF-β. This molecule has broad anti-inflammatory effects and also limits AM activation through an autocrine loop (17). In addition, together with retinoic acid, TGF-β from AMs is responsible for converting naïve or activated T-cells into $FoxP3^+$ expressing regulatory T-cells (Tregs) (25), which are important for limiting immune responses in many pulmonary diseases. AMs can also directly mediate suppressive immunity through continual contact-mediated crosstalk with the lung epithelium (26) and through secretion of vesicles that supress cytokine secretion in these cells (27).

Under inflammatory conditions however, the immunosuppressive signals in AMs can be overridden, through a variety of pathogen associated molecular pattern (PAMP) receptors (16). Their position in the alveolar space makes them ideally suited as a first line of defence against many bacterial and fungal pathogens; and their phagocytic capacity is generally increased during infection (28,29). The importance of this process is suggested by the link between and the reduced phagocytic capacity of the AMs of patients with

chronic obstructive pulmonary disease (COPD), and their failure to clear lung infections (30). This is associated with decreased expression of phagocytic molecules such as the mannose receptor, and maybe restored by treatment with Azithromycin which increases phagocytic capacity of AMs (31), and may improve patient outcomes (32). In mice, other phagocytic receptors, such as the macrophage receptor with collagenous structure (MARCO), are important for clearance of respiratory pathogens including *Streptococcus pneumonia*e and *Mycobacterium tuberculosis* (*Mtb*) (33,34). In humans, MARCO polymorphisms that reduce phagocytic capacity in monocyte-derived macrophages are linked to TB susceptibility (33), and lower MARCO expression on AMs from diabetic mice reduces their ability to phagocytose *Mtb* (35). In addition to phagocytosis, AMs secrete numerous cytokines and chemokines, including IL-6, TNFα, MCP-1, RANTES and G-CSF, which recruit other inflammatory cells (36-38).

During infection, AMs still play an important role in limiting inflammation, which could otherwise be fatal. Critically, the phagocytosis of apoptotic cells by AM, prior to lysis, prevents the release of their inflammatory intracellular contents and is accompanied by production of anti-inflammatory cytokines such as TGFβ1, prostaglandin-E2 and platelet-activating factor (PAF). Defective AM phagocytosis is associated with increased inflammation, as observed in children with poorly controlled asthma (39). AMs can also mediate JAK-STAT signalling via the secretions of SOCS proteins 1 and 3, which inhibit STAT activation (27). Indeed, the anti-inflammatory properties of AMs during infection have been exploited by several pulmonary pathogens to enhance their persistence, as has been reviewed extensively elsewhere (40). In addition, AMs play an important role in promoting tissue repair after resolution of infection through a variety of mechanisms (41).

Interstitial Macrophages

Macrophages located within the lung tissue parenchyma (IMs) have previously been considered an interim state between recruited macrophages and true tissue-resident alveolar macrophages (41,42). However, recent studies now show that stable IM populations exist in the lung and have important immunoregulatory properties (43,44), although the origin and ontogeny of these cells is complex and less well documented than that of AMs. Runx1 lineage tracing experiments show that primitive IMs initially

derive from the fetal yolk sac during embryogenesis, followed by a second wave of definitive IMs arising from bone marrow precursors. A small portion of these IMs persist through to adulthood and are localized in the perivascular mesothelium. However, mouse parabiosis experiments suggest that this pool is mostly maintained by circulating monocyte precursors (12). Adoptive transfer experiments demonstrate continual monocyte migration into lung tissue regardless of inflammation (45), and transcriptomic analyses confirm the expression of genes which repress self-renewal (*Maf* and *Mafb)*, as well as monocyte-related genes *CD14*, *CD163* and *Csfr1* (46). Thus, in contrast to AMs, IMs appear to rely on replenishment by blood monocytes at the steady state.

Recent work suggests that multiple IM subsets exist in the lung that that differ in their location, function and longevity. Gibbings et al. (2017) described three resident IM populations within the murine lung parenchyma, although their interrelatedness and roles in immunoregulation or disease were not clear (46). Subsequently, Chakarov *et al.* (2019) confirmed two genetically and phenotypically distinct monocyte-derived IM populations in mice, distinguished by their expression of MHCII and CX3CR1 (Lyve1^{lo}MHCII^{hi}CX3CR1^{hi} and Lyve1^{hi}MHCII^{lo}CX3CR1^{lo}). Fate-mapping experiments show these cells derived from independent lineages, and differed in their function and localization within the lung (47). Importantly, similar populations were identified in human lung tissue samples by transcriptomics. These distinct lung IMs have been independently confirmed by single cell transcriptomics and can be further distinguished by expression of the mannose receptor CD206 (48). IMs expressing CD206+ (MHCII^{lo}CX3CR1^{lo}), are peribronchial, involved in immunoregulation, wound healing and repair, and are self-sustaining over an extended period. The CD206-MHCII^{hi}CX3CR1^{lo} IMs, on the other hand, are involved in antigen presentation, associate with the alveolar interstitium, and, although also long lived in the lung, appear to be continually replenished for extravasated blood monocytes (48). More work is needed to understand the different IM populations present in the lung, which maybe further complicated by the fact that IMs appear to be highly plastic and adapt their phenotype and function in response to the unique disease environment (49).

Irrespective of their origin, IMs play a key role in immunoregulation within the lung, and they are important sources of immunoregulatory cytokines at steady state (43,50-52). IMs

express IL-10 both constitutively (47) and after exposure to environmental stimuli, such as unmethylated CpG DNA and LPS from bacteria (43,44,49) (**Figure 1**). Indeed, this latter property may be central to the reduced risk of asthma development in microbe-rich environments (44). IM secreted IL-10 has also been implicated in limiting Th2 allergic inflammation (43) and neutrophilic asthma (51). Aside from their production of immunoregulatory cytokines, lung Lyve1^{hi}MCHII^{lo}CX3CR1^{lo} IMs express genes linked with wound healing and repair. Depletion of this subset was associated with increased fibrosis, highlighting an important antifibrotic role, probably by preventing excessive immune cell infiltration (47). Although not as phagocytic as AM, a number of studies have demonstrated the ability of IMs to phagocytose small particles (43,53), which is enhanced upon exposure to LPS along with their chemotaxis and ability to produce reactive oxygen species (50). The surface expression of MHC-II in mice (44,46,47,51) and HLA-DR in humans (52) by IM also suggests a role in antigen-presentation; and in co-culture experiments, IMs possess superior antigen-presentation capacities than AMs, and drive both T-cell proliferation and Treg differentiation (47).

Overall, our understanding of IM is not as advanced as AM, and more work is needed to unpick this complex and highly heterogenous tissue cell type, particularly the importance of the tissue resident fraction.

Innate Lymphoid Cells (ILCs)

Innate lymphoid cells (ILCs) are another innate immune subset that is important for maintaining tissue homeostasis within the lung (**Figure 2**). These diverse lymphoid cells share many functional characteristics of their T-cell counterparts, but lack antigen-specific receptors and respond primarily to locally secreted cytokines. ILCs mediate protective immunity from pathogens and parasites and promote tissue repair and homeostasis following infections. However, when their functions become dysregulated ILCs may also play roles in pathogenesis (54-56). Based on functional characteristics, ILCs have been designated into three subsets (ILC1s, ILC2s, and ILC3s), which are roughly analogous to Th1, Th2 and Th17/22 cells (57). However, ILCs remain somewhat plastic and can alter their phenotype and function in response to signals from their surrounding tissue microenvironments (58,59). Importantly, although ILCs do circulate in peripheral blood,

they are considered to have an extreme "sedentary" lifestyle, and are maintained by self-renewal in broadly different tissue microenvironments and physiological settings; consistent with their proposed roles as sentinels and local keepers of tissue function (60,61). Most ILC pathways and functions have been elucidated in the mouse model, however two studies have characterized pulmonary ILCs in humans (62,63).

ILC1s

ILC1s and cytotoxic natural killer (NK) cells have shared developmental pathways and are considered to fall under the same immunological lineage (57); however as NK cells have been long described and ILC1s are a relatively new player, here we will review cytotoxic ILC1s ("NK cells") and non-cytotoxic ILC1s (referred to as "ILC1s") separately. Despite only representing a minor fraction of total ILCs in the lung (58,64), there is evidence that ILC1s play a role in immunosurveillance and infection control. In mice infected with H1N1 influenza, ILC1s become activated and produce IFN-γ and TNF-α as early as day 3, suggesting a role in initiating the early response to infection (65); and transfer of ILC1s to lymphocyte deficient mice ($Rag2^{-/-}\gamma C^{-/-}$), reduces viral titres in the lung. The concept of ILC1s as important early producers of IFN-γ in tissue is supported by a recent study showing depletion of ILC1s from T-cell deficient mice increased titres of another respiratory virus, Sendai virus (SeV) in the lung following nasal challenge. Of all the known IFN-γ-producing lymphocyte subsets, including NK cells and Trm CD8s, only ILC1s in the lung, were found to produce significant amounts of this cytokine early in infection with SeV or PR8 influenza (66). In both studies, ILC1s are probably responding to IL-12 or IL-18 produced by lung resident DC subsets. In addition, these cytokines have been shown to drive transition of ILC2 into "ILC1-like" during influenza infection, and other canonical triggers of COPD including bacterial infection and cigarette smoke (59). Interestingly, the frequency of "ILC1-like" cells, is increased in COPD patients and inversely correlates with disease severity and lung function, suggesting a pathological role for these cells in human COPD. Finally, INF-γ-producing ILCs, either from the ILC1 lineage or transitional ILC2 subsets, also play a role in shaping vaccine responses in mice (67), suggesting this subset may be leveraged during vaccination. The precise mechanisms are unclear, but ILC1s may mediate crosstalk between DCs and $CD8^{+}$T-

cells, as co-culturing with ILC1 leads to DC maturation/activation and increased IFN-γ production by T-cells during *in vitro* influenza challenge (65).

ILC2s

As the name might suggest, ILC2s phenotypically and functionally mirror Th2 cells, and produce type 2 cytokines IL-13, IL-5 and IL-4 in response to IL-25, IL-33 and TSLP (57). Consequently, like their Th2 counterparts, they are important in allergic responses, asthma and the clearance of helminth infections from the lung. ILC2s in the lung constitutively express IL-5 and are induced to secrete IL-13 under inflammatory conditions, resulting in eotaxin production and thereby controlling local eosinophil accumulation (68), a key immune subset in allergy, asthma and response to multicellular pathogens. In the mouse model of allergic asthma, increases in ILC2s leads to increased IL-5 and IL-13 in the lung, which in turn exacerbates allergic inflammation and smooth muscle tissue hyperreactivity (69), and mucus hyperproduction (70). Although Th2 cells produce the same cytokines, ILC2s appear to be important, as $Rag2^{-/-}\gamma^{-/-}$ mice experience reduced allergic inflammation compared to $Rag2^{-/-}$ mice (which retain ILCs) (71). Indeed, as with ILC1s, there is probably important crosstalk between ILC2s and other immune subsets during airway inflammation. The Th2 response to inhaled allergens, for example, is enhanced by the presence of ILC2s (71). Unsurprisingly, data from humans is more sparse, but ILC2s are enriched in sputum from asthmatic patients (72), and have been shown produce large amounts of type 2 cytokines (73). Moreover, the frequency of ILC2s in bronchoalveolar lavage (BAL) fluid was found to correlate inversely with lung function (74).

The role of ILC2s in the immune response to multicellular parasites, is best studied in *Nippostrongylus brasiliensis* infection (55). In response to this parasite, secretion of IL-25 and IL-33 by epithelial cells triggers a robust expansion of lung ILC2s and production of IL-13; which alone is sufficient to mediate worm clearance (75). More recently, it was found that IL-33 specifically upregulates OX40L on ILC2s, which is essential for licensing the required Th2 and Treg response (76). Ablation of OX40L on ILC2s alone prevented the development of effective Th2 immunity and pathogen clearance.

As mentioned, ILC2s are important mediators of lung tissue repair after infection. Monticelli *et al.* (2011) were the first to demonstrate this in a mouse model of influenza in which depletion of ILC2s was associated with poorer lung function and failure to restore epithelial integrity. Like AMs, the tissue restorative functions of ILC2s were attributed to amphiregulin (55); and; again like AMs, its production by ILC2s was found to be increased in the lungs of mice with helminth infection (77). ILC2s may also limit tissue damage via secretion of IL-9, which protects endothelial cell death, limits lung inflammation during sepsis (78), and stimulates ILC2s to secrete IL-13 and IL-5 in an autocrine loop (79). ILC2s are the main secretor of IL-9 in the lung, and IL-9 receptor expression on ILC2s is required for their engagement in tissue repair following lung damage, including secretion of amphiregulin (77). However, the role of ILC2s in lung tissue repair is clearly context dependant, because, ILC2 secretion of IL-13 can directly reduce bronchial epithelial barrier integrity by impairing tight junction formation (80).

ILC3s

ILC3s are defined by their expression of the RORγt transcription factor, and consequently are functionally similar to Th17/Th22 cells. They appear to be the most abundant ILC group in the human lung (62), but studies highlighting the role of ILC3s in the lung are limited. However, they rapidly secrete both IL-17 and IL-22, key molecules in pulmonary immunity, they are thought to be important to lung health (81). ILC3s are in fact the major producers of IL-22 in the lung (82,83), and consequently are probably important for tissue repair following viral infection. Genetic IL-22 knock-out mice, for example, had impaired lung epithelial regeneration following influenza infection, which could be restored through adoptive transfer of "ILC3-like" $CD3^{-}NCR1^{+}NK1.1^{+}$ cells (84). IL-22-producing ILC3s are also important for clearance of bacterial infections, such as *S. pneumoniae*. Indeed, boosting of IL-22 production by exogenous administration of flagellin, allowed mice to clear an otherwise lethal infection (82). Both IL-22 and IL-17 are both important in protecting mice from hypervirulent strains of *Mtb* (85,86), and IL-22 from $CD3^{-}CD56^{+}$ ILC3s-like cells inhibits *Mtb* growth through enhanced phagolysosomal fusion (87). Consistent with this, we recently showed that ILC3s play an important role in the immune response to *Mtb* infection. As with many of the examples cited above, timing is probably a key factor in their importance, as ILCs are the earliest responders to infection and appear

to help orchestrate the subsequent immune response (88). Various ILC3 knock-out mouse models display reduced control of early bacteraemia, which could be restored by adoptive transfer of purified ILCs.

IL-17 production by ILC3s may also play an important role in protection from extracellular bacteria and fungi (81). In mice infected with *Klebsiella pneumoniae* IL-17 secreting ILC3s were essential for bacterial clearance (89), and ILC3-derived IL-17 is required for survival in mice infected with *Pseudomonas aeruginosa*, a pulmonary pathogen commonly associated with cystic fibrosis and COPD (90). ILC3s can also drive inflammatory responses, as IL-17^{+}ILC3s mediate airway hyperresponsiveness in both obesity-linked asthma (91) and allergic asthma induced by house dust mite challenge (92). In humans with severe asthma, populations of IL17^{+}ILC3s are enriched in BAL samples (91) and ILC3 gene signatures are enriched in nasal brushings from patients with adult onset asthma (93).

NK cells

NK cells are classified as members of the ILC1 family based on shared transcription factor requirements and IFN-γ production, but perform important cytolytic functions via their secretion of perforin and granzyme B (94). Initial mapping of NK cells from healthy human tissues demonstrates that the lung contains several distinct populations based on CD56 and NKp46 expression levels (95). Later, Marquardt et al. (2017) described lung-resident NK cells in humans as hyperdifferentiated with a CD56^{dim}CD16^{+} phenotype and hyporesponsive to target cell stimulation (96,97). Interestingly, most of these NK cells lack the tissue-resident marker CD69, which might suggest they are predominantly recirculating rather than resident within the lung. Indeed, detailed mouse parabiotic experiments found that, unlike other ILC subsets in the lung, NK cells readily recirculate (60). However, other studies have found evidence of a functional and potentially resident NK subset in human lung explants that are CD49a^{+}CD103^{+}CD69^{+}, present in parenchyma and degranulate in response to viral infection (98). Additionally, blocking recruitment of circulating NK cells into the lung has no effect on the ability of this cell type to control tumour growth, suggesting both the existence and importance of lung resident NK cells (99). Lung NK cells from Influenza-naïve Indian rhesus macaques infected with

seasonal H1N1 increase early in infection, and upregulate CD107a and IFN-γ (100). Similarly, populations of $CD107a^{+}IFN\text{-}\gamma^{+}$ NK cells were also increased in lungs of influenza-infected mice (101). As with ILC1s, in this setting NKs are important early producers of IFN-γ that limit initial disease severity, although they are ultimately not required for viral clearance (102). Indeed, NK cells have also been observed to exacerbate pathology during high dose influenza infection (103), and may themselves be susceptible to influenza infection, causing reduced cytotoxicity and production of pro-inflammatory cytokines (104). In humans, lung-associated NK cells respond to influenza A infection by upregulating degranulation/cytotoxic activation marker CD107a and produce granzyme B and IFN-γ which mediates killing of infected macrophages (98).

Like ILC1s, Lung NK cells may contribute to chronic inflammatory disorders, such as COPD and asthma through production of inflammatory cytokines. $CD56^{+}CD16^{+}$ NK cells isolated from airways of COPD patients have increased natural cytotoxicity in comparison to those from controls (93), and demonstrate increased killing of autologous lung cells in co-culture experiments (105). Recently, Finch *et al.* (2018) showed increased killing of lung epithelial cells by lung, but not blood NKs from COPD patients, was due to DC-NK cell interactions and IL-15a transpriming (106). In asthmatics, NKs cells in BAL fluid are skewed to a cytolytic phenotype and express higher levels of granzyme A (107). However, the importance of lung resident NKs, compared to recruited NKs overall remains unclear.

Dendritic Cells

Dendritic cells (DCs) are professional antigen-presenting cells, whose primary role in the respiratory tract is to sample inhaled pathogens before migration to lymph nodes where they present processed peptides to antigen-specific T-cells (**Figure 3**). DCs must be continually replaced by new recruits from the bone marrow and are not a self-replenishing tissue-resident population in the strictest sense. However experiments with parabiosis and cell tracking demonstrate that the turnover of lung DCs is considerably lower than that of other non-lymphoid tissues (16,108); and lung DCs have been observed presenting antigen for up to 8 weeks after exposure (109). In addition, there is evidence of bone marrow derived preDCs in the lung which give rise to $CD103^{+}$DCs in

other non-lymphoid tissues, although not specifically shown in the lung (108). Given the longevity and the potential existence of DC precursors, it is probably helpful to think of lung DCs as distinctive subsets, even if they not display the same “extreme sedentary” lifestyle as lung ILCs or macrophages.

While the heterogeneity of DCs in the lung is still being uncovered, they broadly fit into three subsets: Plasmacytoid DCs (pDCs) and myeloid DCs (mDCs), which are further subdivided into cDC1 and cDC2, which all develop under the control of key transcription factors (An updated, detailed review in (110)). cDC1s closely associate with the airway epithelium and are distinguished by expression of CD103, whilst cDC2s lack CD103 but express CD11b and, like pDCs, are mainly found in the lung interstitium (111). cDC1s are specialised to sample antigen from the alveolar space, but all DC subsets, including pDCs, are able to transport antigen to draining lymph nodes (112,113). In addition, DCs can rapidly mature from circulating monocytes recruited during injury and inflammation, and distinguishing monocyte-derived DCs (moDCs) and tissue-resident DCs can be a challenge, particularly in humans (16,114). It is also important to note that the nomenclature can be confusing as definitions have varied overtime and the terms cDC and mDC are used to describe the same cells.

Data from experimental mouse models with house dust mite (HDM) challenge demonstrate the complex roles that DCs play in lung inflammation, and the importance of different DC subsets in determining the subsequent T-cell response. CD11b-expressing cDC2s, for example, readily take up allergens and are responsible for generating robust Th2 and Th17 responses to HDM challenge (115-117), through co-stimulatory molecules and secretion of cytokines and chemokines. $CD103^+$ cDC1s in contrast, are important for limiting allergic inflammation in the context of chronic dust mite exposure, by regulating both Th2 and Th17 immune responses via production of IL-12 (118). This subset is also able to induce Tregs in response HDM via retinoic acid (119). pDCs may also play a tolerogenic role in allergic lung inflammation, at least in part through upregulation of the T-cell inhibitory ligand PDL-1 (120). However, more recently, pDCs have been implicated in driving both and allergen and viral induced asthma in both animal models and humans via potentiation of the Th2 response (121). In humans, both cDC1 and cDC2s are

reported to expanded in the lung of asthmatic subjects following allergen challenge, and promote Th2 and Th9 responses, although this is not always consistent (122-125). Interestingly, asthma in humans has been linked to a lack of exposure to environmental bacteria such as Helicobacter pylori, and sensitization of mice with *H. pylori* extract prevents subsequent allergic inflammation. Importantly, $CD103^+$ accumulate during sensitization are strictly required for its protective effect (126), suggesting a potentially similar role of these cells in humans.

The distinct functional roles of lung DC subsets can also be seen in the context of infections, such as influenza, where $CD103^+$ cDC are responsible for generating effector CD8 T-cells in the lung (127), whilst $CD11b^+$ cDC are establish long lived memory subsets (128). Lung pDCs, which are generally considered poor at priming T-cells, play an important role on the antiviral response by producing copious amount of type I interferon (129). $CD103^+$ DCs alone, appear to orchestrate the appropriate Th17 response to fungal infection through production of IL-2 and IL-23 (130).

Another important aspect of lung health for which DC appear to play a central role is that of cancer. DCs take up necrotic and apoptotic tumour fragments to present to antigen-specific cytotoxic and helper T-cells and are therefore important providers of protective anti-cancer immunity (131,132). Cell-tracking experiments in murine melanoma tumour models show that $CD103^+$DCs are the only intra-tumoural myeloid cell type able to transport and present intact antigen to tumour-specific $CD8^+$T-cells and their activation was protective against tumour re-challenge (133). However, these cells are rare and transcriptomic analysis of lung cancers and peritumoural tissues from mice demonstrate that the majority of infiltrating myeloid cells are actually $CD11b^+$DCs. These cells strongly upregulate expression of PDL-1 and are associated with cancer growth, suggesting that lung tumours can actually exploit DC flexibility to promote an environment supportive of cancer progression (130,131). The importance of DCs in lung cancer is demonstrated by on-going efforts to harness these cells for therapeutic purposes. In a phase III control trial, non-small-cell lung cancer (NSCLC) patients receiving immunotherapy (consisting of adoptive transfer of autologous DCs and activated T cells from the patients' own lymph nodes) in addition to chemotherapy, had increased survival rates in comparison to those

receiving chemotherapy alone (134). In a more recent phase I trial, NSCLC patients receiving autologous genetically-modified DCs (overexpressing lymphoid-tissue organizing chemokine CCL21) had better anti-tumour immunity, CD8 T cell infiltration and increased tumour PD-L1 expression (135). Recently in mice, adoptive transfer of $CD1d^{+}$DCs led to T-cell activation and cytotoxicity, and reduced tumour growth. In humans, CD1d expression has also been associated with better clinical outcomes (136). Thus DC-based immunotherapy is a promising new strategy against lung cancer, however the interactions between lung tumours and DCs are complex and the safe and effective manipulation of this system will require extensive understanding.

Concluding Remarks

The unique environment of the lung is protected through complex immune interactions. Cells of the innate immune system provide the first lines of defence against inhaled pathogens and toxins through direct mechanisms and orchestrate the adaptive immune system through crosstalk. Innate immune cells respond to non-antigen specific signals from their surrounding environment allowing them to act rapidly in the presence of a threat. However, these prompt responses come at a cost, as they sometimes mount disproportionate responses against harmless particles resulting in excessive inflammation, fibrosis and even tissue damage. Innate immune cells are often described as a double-edged sword, both necessary for host defence, but also drivers of pathogenesis, their exact roles often dependent on context. In this sense understanding the importance of tissue-resident innate immune subset is key and may provide unique opportunities for beneficial modulation of the host immune response. Animal models are valuable tools but are not always translatable to humans, and human studies have often focused on the peripheral blood. Growing efforts to study lung-resident immune populations in humans are providing essential evidence as to how the immune system functions within the tissue environment and will be key to developing novel treatment and disease prevention strategies in future.

Conflict of Interest

The authors have no conflict of interest to report.

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Figure legends

Figure 1. Tissue resident macrophages persist within lung tissues where they provide protection from inhaled pathogens and allergens but simultaneously prevent overt responses to harmless particulate matter. Interstitial macrophages (IM), which populate the lung interstitium, primarily derive from recruited monocytes from blood although a portion may be resident. At least two distinct subsets can be described, which differ in function. IMs produce immunoregulatory cytokines both at steady state, and after exposure to environmental stimuli, which allow them to modulate inflammation. Alveolar macrophages (AM) are a self-renewing population of resident

macrophages within the alveolar space that do not rely on replenishment from the bone marrow. Primarily anti-inflammatory cells they mediate inflammation and promote homeostasis through phagocytosis of apoptotic cells before lysis and the production of anti-inflammatory cytokines such as TGF-β. Conversely, AMs can provide protection from inhaled pathogens through phagocytosis and the recruitment of inflammatory cells which clear infections.

Figure 2. ILCs and NK cells are important tissue-resident cells and mediators of homeostasis within the lung. NK cells are recruited during infection, although potentially resident populations have been described. NK cells are cytolytic cells which produce granzymes that mediate killing of infected macrophages. NK cells themselves are susceptible to direct infection by influenza but remain important producers of IFN-γ, which contributes to inflammation. ILC1s are non-cytotoxic counterparts of NK cells
That produce IFN-γ and are important mediators of inflammation and viral clearance. DC-derived IL-12 and IL-18 can transition ILC2s into "ILC1-like" cells which may play
a pathological role in COPD. ILC2s produce type-2 cytokines IL5 and IL-13 which are important in allergic responses and helminth clearance in the lung. They also facilitate
Tissue-repair through their production of amphiregulin. ILC3s are the most abundant ILC subset in the human lung. IL-22 producing ILC3s are important for bacterial clearance and tissue repair, while IL-17 from ILC3s contributes to inflammation but may also have an important role in protection from extracellular bacteria and fungi.

Figure 3. Dendritic cells (DCs) are professional antigen-presenting cells that sample and present antigen to T-cells within the lamina propria and associated lymph nodes. While lung DCs may not be tissue-resident in the strictest sense (because they do not appear to self-renew), they can arise from precursor cells and persist in the lung for up to 8 weeks. DCs of the lung are heterogenous and different subsets have different immunogenic functions. Epithelial DCs express CD103 and sample antigen from the alveolar space. These cells limit allergic inflammation through IL-12 and prevent fatal inflammatory responses via IL-2. $CD11b^{+}$DCs traffic antigen to the lymph node where they coordinate Th2 responses to house dust mite challenge. In cancer, the balance between $CD103^{+}$DCs and $CD11b^{+}$DCs is important and is being exploited in novel treatment strategies.