Division of labor between lung dendritic cells and macrophages in the defense against pulmonary infections

M Guilliams[1,2], BN Lambrecht[1,2] and H Hammad[1,2]

The lung is highly exposed to the external environment. For this reason, the lung needs to handle a number of potential threats present in inhaled air such as viruses or bacteria. Dendritic cells (DCs) and macrophages (MFs) play an important role in orchestrating the immune responses to these challenges. The severe lung inflammation caused by some pathogens poses a unique challenge to the immune system: the potential insult must be eliminated rapidly whereas tissue inflammation must be controlled in order to avoid collateral damages that can lead to acute respiratory failure. Immune responses to infectious agents are initiated and controlled by various populations of antigen-presenting cells with specialized functions, which include conventional DCs (cDCs), monocyte-derived DCs (moDCs), plasmacytoid DCs (pDCs), and alveolar MFs (AMFs). This review will discuss the role of these different cells in responses to pulmonary infections, with a focus on influenza virus and *Mycobacterium tuberculosis*.

LUNG DENDRITIC CELL AND MACROPHAGE SUBSETS

The lung is constantly exposed to the environment and to a wide variety of microbes, dusts, and pollutants. It is therefore not a surprise that the lung contains a rather developed network of cells, such as dendritic cells (DCs) and macrophages (MFs), which are well equipped with various pathogen-associated molecular pattern receptors or damage-associated molecular pattern receptors to recognize these threats. It has become obvious during these past years that lung DCs and MFs are made of different subsets with specialized functions. In the absence of inflammation, lung conventional DCs (cDCs) can be subdivided into three distinct subsets based on the expression of a combination of specific cell surface markers and on their distinct ontogeny: CD103^{+} cDCs that belong to the CD8α-type cDCs, CD11b^{+} cDCs that belong to the CD11b-type cDCs, and plasmacytoid DCs (pDCs).[1,2] In addition, monocyte-derived DCs (moDCs) have been described to populate the lungs upon inflammation. Lung MFs consist of well-defined alveolar MFs (AMFs) and much less defined so-called interstitial MFs, which can either be CD11b^{+}MHCII[+3] (and unrelated to AMFs[4]) or CD11c^{+} CD11b^{-}MHCII^{lo} cells.[5,6] In this review, we will only focus on

CD103^{+} cDCs, CD11b^{+} cDCs, pDCs, moDCs, and AMFs. The role of interstitial MFs will not be discussed as we feel that further work is required to properly identify these cells in the steady state and upon inflammation before we can attribute them specific functions.

PHENOTYPIC IDENTIFICATION OF LUNG DCS AND MFS

It is increasingly clear that all peripheral tissues contain two families of cDCs, namely CD8α-type cDCs and CD11b-type cDCs, that are developmentally linked and share major specialized functions.[1,2,7] In the lungs, both subsets of cDCs were historically distinguished based on their exclusive high expression of either CD103 (the E-cadherin–binding integrin $\alpha_E\beta_7$) or CD11b. We will thus refer to these cells as CD103^{+} cDCs and CD11b^{+} cDCs, respectively. The CD103^{+} cDCs form a highly developed network in the epithelial layer of the conducting airways and this particular subset of DCs displays long cellular protrusions in between the basolateral space made up by basal epithelial cells (**Figure 1**). These DCs also express CD207 (Langerin) and, as in other tissues, they express high levels of lymphotactin receptor XCR1 and Clec9a/DNGR-1 but do not express the CX3CR1 fractalkine
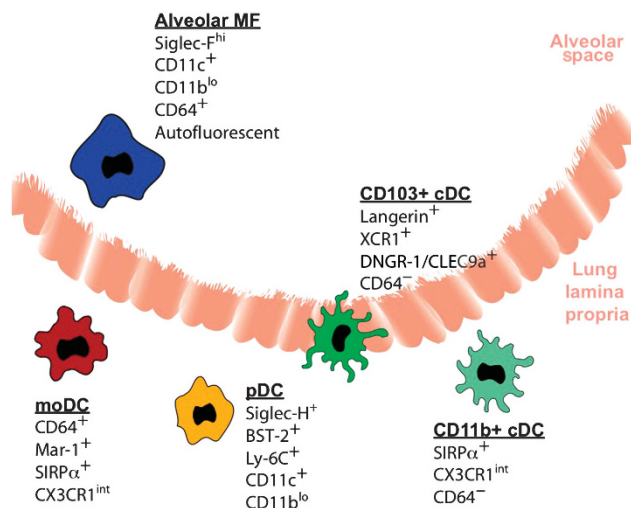
Figure 1 Lung dendritic cell and macrophage phenotype. cDC, conventional dendritic cell; DC, dendritic cell; MF, macrophage; moDC, monocyte-derived DC; pDC, plasmacytoid DC.

receptor or SIRPα (CD172α, a ligand for CD47) and are CD11b^{lo}.[8–13] Underneath the basement membrane, the lamina propria contains a population of CD11b-type cDCs characterized by the high expression of CD11b and SIRPα and intermediate levels of CX3CR1.[8,14,15] These CD11b^{+} cDCs do not express XCR1, Clec9a/DNGR-1, or CD207, and are mostly CD103^{−}, although upon inflammation some of these cells can acquire a CD103^{+}CD11b^{+} phenotype as observed in the intestine (Guilliams and Plantinga, unpublished results). Recently, both cDC subsets were shown to specifically express zbtb46, a transcription factor that seems highly specific for cDCs and which in the future should be helpful for the proper identification of cDCs.[16,17] It should be noted that zbtb46 does not seem required for the development of cDCs but rather represents a central negative regulator of DC activation that is downregulated upon Toll-like receptor (TLR) stimulation.[18] The conducting airways also contain pDCs expressing Siglec-H and bone marrow stromal antigen-2 (BST-2) as well as Ly-6C but not CD11b or SIRPα—which allows to distinguish them from moDCs (Guilliams and Plantinga, unpublished results and ref. 19). In addition, the lung parenchyma (where gas exchange is occurring) also contains CD11b^{+} cDCs, as well as pDCs.[20] When the lung is exposed to any foreign proinflammatory substance, such as microbes, TLR ligands, allergens, or environmental pollutants, an additional population of CD11b^{+} moDCs is massively recruited to the conducting airways and lung parenchyma. moDCs have long been identified by their MHCIICD11cCD11bLy-6C phenotype.[10,21] However, monocytes downregulate Ly-6C upon differentiation into moDCs in vitro[22] and in vivo,[23] making this marker highly specific but poorly sensitive. Indeed, using adoptive transfer of monocytes, we have recently demonstrated that monocytes rapidly downregulated Ly-6C upon differentiation into moDCs within the lungs.[24] Other markers have been proposed to be specifically expressed by moDCs,

including E-cadherin[25] and DC-SIGN (Dendritic Cell–Specific Intercellular adhesion molecule-3–Grabbing Non-integrin).[22] However, E-cadherin expression was found to be low on moDCs in the lungs and the skin (Guilliams et al., unpublished results). Moreover, DC-SIGN[+] DCs recruited following lipopolysaccharide injection in vivo and originally defined as moDCs were recently shown to depend on Flt3L (Fms-related tyrosine kinase 3 ligand) for their development and to express zbtb46,[16] identifying these cells as cDCs.[26] We have recently reported that CD64 expression could discriminate moDCs and MFs from cDCs in the inflamed muscle and intestine.[27,28] We have recently confirmed this observation in the lungs of mice with allergic airway inflammation.[24] We have found that CD64^{+} moDCs were already present in the steady-state lungs, where they accounted for ∼25% of the CD11b^{+}CD11c^{+}MHCII^{+} lung DCs. In addition to CD64, we and others have recently found that the MAR-1 antibody directed against the high-affinity IgE α chain receptor (FcεRIα) could be used as a marker specifically expressed by moDCs recruited to the lung following allergen or viral challenge.[29,30] The reason why this marker is induced only on moDCs and not on cDCs remains to be addressed but could involve the cytokines interleukin-3 (IL-3) and granulocyte macrophage colony-stimulating factor, which are produced in the lung as part of the inflammatory response.[30] As such, we recommend the combination of both CD64 and MAR-1 expression as the most reliable method to unequivocally identify moDCs in the lungs and the lung-draining mediastinal lymph node (MLN).

Lung AMFs express high levels of CD11c but do not express CD11b, and can therefore easily be confused with CD11b^{low} cDCs. However, AMFs, unlike DCs, are highly autofluorescent and are F4/80^{hi}, CD11b^{low}, MHCII^{low}, SIRPα^{hi}, CD64^{hi}, Siglec-F^{hi}, and Ly-6C^{low}.[24,31,32] Although AMFs are mostly considered to be lung-resident cells, they have recently been proposed to migrate to the MLN.[33] However, using 12-color flow cytometry to ensure the proper identification of AMFs, we found little evidence for such migration in the steady state and upon exposure to house dust mite or during influenza infection (Guilliams and Plantinga, unpublished data). Therefore, although we do not exclude the possibility that in some particular cases AMFs may migrate to the MLN, we believe these cells to be mainly pulmonary alveoli-resident cells.

In summary, although many markers have been proposed to be specific for distinct subsets of lung DCs and MFs, these cells have often been mischaracterized, therefore inducing a lot of confusion on their exact role. This can mainly be explained by the fact that: (i) AMFs express high levels of CD11c and often contaminate the DC gate in flow cytometry, (ii) moDCs contaminate the CD11b^{+} cDC population because the most commonly used markers (F4/80, CD68, and Ly-6C) do not allow to properly distinguish cDCs from moDCs and MFs,[32] and (iii) "DC lineage-specific" markers overlap between different DC subsets upon inflammation. For example, the pDC "specific" marker BST-2 (recognized by moAb 120G8) is strongly induced on moDCs and B cells under the

Table 1 Markers of lung DC and MF subsets

	CD103+ cDCs	CD11b+ cDCs	pDCs	moDCs	AMFs
Reliable markers					
XCR1	+ + +	−	−	−	−
Clec9a/DNGR-1	+ + +	−	−	−	−
SIRPα (CD172α)	−	+ + +	−	+	+
CD11b	Low	+ + +	Low	High	Low
Siglec-H	−	−	+ + +	−	−
CD64	−	−	−	+ + +[a]	+
MAR-1	−	−	−	+ + +[a]	+
Siglec-F	−	−	−	−	+ + +
Other markers					
CD103	+	+/−[b]	−	−	−
CD207 (Langerin)	+	−	−	−	−
CD24	+	+/−	−	−	−
CX3CR1	−	Int	−	Int	−
BST-2	−	−	+	+/−	−
Ly-6C	−	−	+	+/−	−
CD11c	+	+	+	+	+
F4-80	Low	Int	Low	High	High

AMF, alveolar macrophage; cDC, conventional dendritic cell; DC, dendritic cell; Int, intermediate; MF, macrophage; moDC, monocyte-derived DC; pDC, plasmacytoid DC.
[a]The moDCs can downregulate CD64 expression and upregulate MAR-1 expression upon migration to the mediastinal lymph node (MLN). Hence, we found that the combination of both markers yields the best method for proper identification of moDCs.[24]
[b]A small fraction of the CD11b+ cDCs express CD103 upon inflammation (Guilliams et al., unpublished results).

influence of type I interferons (IFNs).[34] However, more markers have emerged and we show in **Table 1** which markers in our hands are the most reliable to identify DC and MF populations.

DCS AND MFS DURING PULMONARY VIRAL INFECTIONS
Role of cDCs in antiviral immunity
Respiratory viral infections represent a good example of lung inflammatory responses where the specialized functions of DC subsets can be appreciated. DCs have been shown to play an important role in the initiation of antiviral CD8 cytotoxic T-cell responses that lead to viral clearance. DCs can also control the degree of inflammatory responses and in this way contribute significantly to the severity of disease.[35] In extreme cases, these events initiated by DCs will lead to death from acute lung injury. This is particularly relevant in the case of influenza A virus (IAV) infection, where the strength of the immune response can dramatically affect host survival. Of note, DCs can also contribute to immunopathology associated with vaccine-exacerbated viral infections such as seen formerly when formaline-inactivated respiratory syncytial virus (RSV) vaccine was used to protect from subsequent infection in mice.[36]

Upon lung IAV infection, adaptive cytotoxic CD8 T-cell responses to the internal viral proteins nucleoprotein and acid polymerase are generated in the draining MLNs. Such responses, at least in murine models of IAV infection, are associated with the clearance of the virus from the lung and with the subsequent protection from reinfection with hetero-subtypic virus. Moreover, the induction of neutralizing antibodies to highly variable surface neuraminidase and hemaglutinin are necessary for prevention of reinfection with the same influenza strain. Early studies have focused on trying to identify the cell population that is able to induce such protective immune responses to IAV. IAV was shown to infect and to activate MHCII+CD11c+ cDCs and pDCs,[37,38] although the degree of maturation induced depends on the type of DCs, the viral dose, and the strain used. In this respect, a recent study showed that specific DC subsets were differentially susceptible to IAV infection. This susceptibility is related to the level of major histocompatibility complex class II (MHCII) expression by the DC subsets. Thus, CD103+ cDCs and CD11b+ cDCs that express the highest levels of MHCII were efficiently infected by IAV, whereas MHCIIlo pDCs were not susceptible to infection following exposure to the virus.[39] Virus-infected DCs then migrate to the lung-draining lymph nodes where they will ensure the interaction of rare virus-specific naive and memory T cells with their cognate antigens. It is important to note that IAV does not flow freely to the MLN and is strictly dependent on migratory lung DCs to reach the MLN.[40] The migration of infected migratory DCs not only requires chemotactic cues but also IL-1 and IL-18 production through NLRP3 inflammasome activation.[41] Although a transfer of viral antigens from lung migratory DCs to a lymph node-resident CD8α+-resident DCs has been shown in early studies,[42] several recent reports have shown that the antigen presentation to CD4+ and CD8+ T cells was done mainly by the antigen-bearing migratory CD103+ cDCs.[10,43] Geurtsvankessel et al.[10] confirmed antigen presentation by lymph node-resident CD8α+ DCs, but this population of DCs was able to present virus-encoded antigen only to naive CD8+ and not to naive CD4+ T cells. More importantly, the depletion of the CD103+CD11b− cDC subset using Langerin–diphtheria toxin receptor (DTR) mice at the time of primo-infection led to a severe defect in antiviral immunity and in viral clearance, resulting in severe weight loss.[10] This can be explained by the fact that the CD103+ cDC subset was shown to carry infectious virus to the draining lymph nodes.[44] These data were crucial in identifying DCs as the main driver of antiviral immunity. We believe that the reason why CD103+ DCs are so efficient at inducing a protective response to IAV could be because they are the only DC subset able to capture and cross-present apoptotic cells that died from viral infection. A very elegant study from the Desch et al.[9] concluded that CD103+ cDCs, unlike CD11b+ cDCs, expressed receptors for apoptotic cells along with the machinery to cross-present phagocytosed dead cells. Another explanation came from a recent study where CD103+ cDCs were shown to be better than CD11b+ cDCs in driving CD8 responses because of their

enhanced capacity to process and load viral antigens in MHCI molecules.[45] More recently, Moltedo *et al.*[44] found that CD103^{+} cDCs were less sensitive to type I IFNs. This attenuated response to type I IFNs not only allowed a stronger viral replication in CD103^{+} cDCs and better antigen presentation to CD8+ T cells, but also designated the CD103^{+} cDCs as the main DC subset carrying infectious particles to the MLN, which they proposed may help the spread of the virus within the body.[44] In contrast, Helft *et al.*[40] have recently proposed that CD103^{+} cDCs were in fact not productively infected by the IAV and uniquely preserved viral proteins in their endosomal compartments. Moreover, they identified type I IFNs as the major mediator of protection of CD103^{+} cDCs against infection. Additional studies will be required to settle the issue. The dominant role for CD103^{+} DCs in stimulating CD8 T-cell immunity was not restricted to IAV infection but was also seen in response to a modified vaccinia poxvirus carrying the ovalbumin MHCI-restricted epitope.[46] The absence of CD103^{+} cDCs in Batf3^{−/−} mice also led to a defective CD8 T-cell priming in response to Sendai virus.[47] However, whether infected lung CD103^{+} cDCs are better at inducing CD8^{+} T-cell responses than CD103^{+} cDCs that acquired viral antigens from a dying cell is currently unknown.

At later time points coinciding with the peak of viral infection, CD11b^{+} DCs accumulate in the lung-draining lymph nodes and then represent the predominant DC subset stimulating CD8 T cells via expression of the costimulatory molecule CD70.[48] However, whether these DCs represent mainly moDCs or mainly CD11b^{+} cDCs is still unclear. This later wave of antigen presentation is likely to ensure the expansion of activated effector CD8^{+} T cells in the draining lymph node.

Role of pDCs in antiviral immunity

The precise role of pDCs in pulmonary IAV infection is very controversial. There is no evidence for a major role for pDCs in controlling IAV infection *in vivo*. pDCs were found to be recruited to the lungs and to the draining lymph nodes of IAV-infected mice.[10] Although pDCs have been shown to prime strong IAV-specific CD8 responses *in vitro*,[37,49] they were not able to present acquired viral antigens to CD8 T cells *in vivo* or *ex vivo*.[10,43] Moreover, the depletion of pDC using specific antibodies did not affect the strength of the antiviral CD8 T-cell response, nor did it affect the speed by which the virus was cleared from the lungs, and others have confirmed these findings.[50] Instead, pDCs might play a role in antibody production as the deletion of pDCs at the time of IAV infection induced decreased levels of neutralizing antibodies.[10] Others have found that pDCs can have a deleterious role during lethal IAV infection, by eliminating virus-specific CD8 T cells in a process involving Fas ligand.[51] Although pDCs are an early source of antiviral type I IFN via recognition of viral RNA, it was striking to observe that they are dispensable during IAV infection. However, in the lung, several other cell types such as AMFs and airway epithelial cells have been shown to also produce this cytokine. This could be an explanation as to why early viral titers or production of type I IFN in the lung-lining fluid of IAV-infected mice were not affected in the absence of pDCs. Although pDCs seem to be dispensable in IAV infection, they have been shown to be important in responses to other viruses such as pneumonia virus where pDCs, through TLR7 triggering, were shown to be critical in the antiviral responses.[52] Models of specific conditional depletion of pDCs employing the DTR technology should address the precise contribution of pDCs to antiviral responses.

Role of moDCs in antiviral immunity

Monocytes are massively recruited to the lungs and differentiate rapidly into moDCs upon IAV infection,[53] and this process has been shown to depend on type I IFN signaling[54] and CCR2 (C-C chemokine receptor type 2).[35] Monocytes have been shown to be particularly sensitive to IAV infection. In fact, human monocyte infection with IAV was shown to be sufficient to differentiate monocytes into type I IFN-producing moDCs capable of effectively limiting viral replication *in vitro*.[55,56] Whether moDCs participate directly in viral clearance *in vivo* is unclear. However, mice with reduced numbers of monocytes such as CCR2^{−/−} mice or mice treated with a CCR2 antagonist did not show increased viral loads.[35,57] Whether moDCs of IAV-infected mice are capable of migrating to the MLN and actively participate in the induction of effector CD8 T-cell responses is also a matter of debate. On one hand, the Randall group suggested that CD11b^{+} DCs that massively migrated to the MLN upon IAV infection were moDCs.[48] On the other hand, Kim and Braciale[43] proposed that, because of their low Ly-6C expression level, this wave of CD11b^{+} DCs contained only cDCs, again showing the need for better markers to identify moDCs.

The effector functions of T cells require T cells to encounter their cognate antigen in the peripheral tissues.[58] The depletion of DCs early after infection was accompanied by a defective viral clearance and by a reduced effector T-cell expansion.[59] Moreover, the cytotoxic activity of primed CD8 T cells was shown to be elicited after their interaction by lung DCs.[60] During Herpes simplex virus infection, moDCs were shown to be crucial for the local reactivation of Th1 (T helper type 1) cells in the infected tissues but dispensable for the priming of naive T cells and their conversion into Th1 T cells.[61] Similarly, CCR2^{−/−} mice, which have impaired moDC recruitment, displayed significantly less effector CD8 T cells in the lungs during influenza infection.[62] Moreover, it was recently reported that moDCs may also play a major role in the reactivation of CD8 memory T cells and natural killer cells, although this was proposed to happen through IL-15 and IL-18 secretion in a T cell receptor–nonspecific manner.[63] Interestingly, IL-15 production by CD11b^{+} moDCs was recently found to be controlled by thymic stromal lymphopoietin.[64] Importantly, these CD8 memory T cells in term license moDCs for effective pathogen killing function through the secretion of CCL3 (chemokine (C-C motif) ligand 3).[65,66] It is therefore very likely that moDCs would be crucial in the interaction with

effector T cells locally in the infected tissues, rather than in the induction of effector T cells in the lymph nodes. In addition, moDCs have also been proposed to be the predominant cause in immune pathology caused by IAV infection. Indeed, compared with wild-type animals, $CCR2^{-/-}$ mice showed less weight loss and mortality.[35] The proinflammatory role of moDCs in IAV infection has recently been challenged by a study in which mice that lack the negative regulator A20 (also known as TNFAIP3) in Lysozyme M^+ myeloid cells using the Cre/lox technology displayed an enhanced production of inflammatory mediators but were more resistant to IAV infection.[67] Why these mice are more resistant to IAV infection as well as the specific contribution of moDCs still remains to be addressed.

Role of AMFs in antiviral immunity

AMFs have been shown to be directly infected by influenza viruses but, compared with *in vitro*–generated monocyte-derived macrophages, murine AMFs are less susceptible to infection, produce less viral particles, and secrete less tumor necrosis factor (TNF).[68] Similarly, human AMFs infected with IAV produced high levels of type I IFN, CCL2, and CCL4 (which attract monocytes and T cells), and CCL5 (which attracts eosinophils and T cells) but produced very little viral particles.[69,70] Of note, AMFs are more susceptible to infection than cDCs upon *in vivo* infection, but this may be because AMFs have a more exposed localization in the lungs.[40] AMFs seem to actively participate in viral clearance because depletion of AMFs before infection resulted in higher viral load, increased mortality, and decreased type I IFN production.[71] Depletion of AMFs before RSV infection also resulted in higher viral loads in mice, confirming the central role of AMFs in the defense against viruses.[72] Importantly, depletion of AMFs in pigs, a natural host for IAV, increased the mortality and the weight loss during IAV infection.[73] The precise mechanism by which AMFs control IAV infection is unclear, but AMFs may also phagocytose virus-infected apoptotic cells, thereby contributing to viral clearance even when they are not themselves infected. Moreover, AMFs have been shown to represent the main producers of type I IFN upon pulmonary virus infection,[74] and AMFs were shown to produce significantly more type I IFNs than cDCs during IAV infection.[40] This type I IFN production was reported to have a direct antiviral role through the induction of the STAT-1 (signal transducer and activator of transcription)–dependent production of antiviral proteins[75] and has been shown to be crucial for the defense against Lassa virus[76] and Measles virus,[77] but may also participate in the anti-inflammatory function of AMFs. Indeed, type I IFN limits inflammasome activation and IL-1 production while increasing IL-10 production by monocyte-derived cells.[78] Absence of type I IFN signaling was also associated with decreased IL-10 production and increased IL-1α, IL-1β, TNF, and nitric oxide (NO) production by moDCs.[79] As such, production of high levels of type I IFN by AMFs may confer antiviral activity to these cells and at the same time inhibit inflammation induced by moDCs. It should

be mentioned that during IAV infection, type I IFN clearly controlled the inflammation induced by moDCs, as mice deficient in type I IFN signaling showed similar influenza viral loads than wild-type mice, but displayed increased inflammation and more severe immune pathology.[80,81] Therefore, because influenza viral disease is often largely caused by the host response rather than by direct cytopathology, the protective role of AMFs has been proposed to be primarily mediated through the suppression of inflammation.[82] Importantly, this type I IFN-mediated suppression of inflammation during IAV infection renders mice more susceptible to secondary bacterial infections due to impaired bacterial clearance.[83] This is clinically relevant as IAV-related deaths are sometimes not attributable to the primary viral infection but to secondary bacterial infections. However, note that as primary source of type I IFN during viral infections, AMFs may also stimulate memory CD8 T cells in a T cell receptor–independent way. Indeed, type I IFN has been shown to induce the cytolytic activity of memory $CD8^+$ T cells in the lungs during respiratory virus challenge.[84] As such, although AMFs may not participate directly in the conversion of naive T cells into effector/memory T cells in the MLN because of their lung-resident phenotype, and although they are considered to be poor antigen-presenting cells, they may act directly on memory T cells within the lungs through the production of type I IFN, thereby enabling memory T cells to rapidly destroy infected host cells once they enter infected tissues.

DCS AND MFS DURING PULMONARY BACTERIAL INFECTIONS
Role of cDCs in bacterial infection

Mycobacterium tuberculosis (Mtb) infection is a major international public health problem. It is considered that approximately one-third of the population worldwide has latent tuberculosis. In these individuals, Mtb is contained in the lungs, within structures called granulomas. This condition is rather serious because it puts these individuals at risk for reactivation if their immune system fails. Early after inhalation of Mtb, bacilli are phagocytosed by AMFs and DCs. These cells then produce high levels of inflammatory mediators such as TNF-α, IL-6, IL-12p80, IL-1α, and IL-1β, which have microbicidal activities, ensure the control of Mtb growth, and the formation of granulomas.[85] The recognition of Mtb by DCs requires TLR9 and leads to the production of IL-12 by DCs.[86] Once infected by Mtb, DCs migrate to the MLNs. IL-12p40 was shown to mediate this DC migration to the MLN and was necessary for the optimal activation of $CD4^+$ T-cell responses.[87] Interestingly, mostly $CD11b^+$ DCs were found to be infected by Mtb in the lung and this subset was the exclusive one transporting the bacteria to the MLN.[88] However, whether these $CD11b^+$ DCs belong to the cDC and/or the moDC subset remains to be addressed. One intriguing characteristic of Mtb is its potential to attenuate CD4 and CD8 T-cell responses. One way to achieve this is through the induction of regulatory T cells early after Mtb infection. Regulatory T cells underwent a massive proliferation

after DCs have transported Mtb to the MLNs, and this proliferation was driven by the recognition of Mtb antigens.[89] Regulatory T cells were shown to accumulate at sites of infection in mice and humans.[90,91] Ultimately, effector T cells were delayed in their entry to the lung.[89] These data suggest that the direct presentation of Mtb antigens to regulatory T cells by DCs is a way to increase bacterial load in the lung of infected individuals. Clearly, more work is required to address (i) which cDC subset is affected by Mtb infection and (ii) the functional role of cDCs in response to Mtb.

Role of moDCs in Mtb infection

Once in the lung parenchyma, Mtb sets off a slow inflammatory process where infected MFs or moDCs recruit new monocytes that in turn differentiate into MFs and moDCs to ultimately form granulomas.[92,93] Although granuloma formation is believed to be essential for the containment of Mtb infection, during the early phase of infection, it may actually favor Mtb expansion.[94] This may be because of the fact that moDCs have been shown to migrate in and out of granulomas.[95] Although this could favor Mtb dissemination, an alternative possibility is that this will participate in the induction of Mtb-specific T-cell responses,[95] as they do in case of fungal infections.[96] Moreover, $CCR2^{-/-}$ mice display a delayed Th1 priming during Mtb infection.[97] This suggests that moDCs might play an active role in Th1 induction, which is believed to be crucial for Mtb elimination. However, proof of direct moDC involvement in the Th1 induction in the MLN during Mtb infection is lacking. So far, the Mtb-associated Th1 response was shown to be induced by $CD11b^{+}$ DCs. moDCs within the granulomas could also participate directly to Mtb elimination through TNF and NO production and through production of IL-1α and IL-1β. Indeed, IL-1R signaling has been shown to be crucial for Mtb control and moDCs were recently shown to produce high levels of IL-1α and IL-1β during Mtb infection.[98]

Role of AMFs in bacterial infection

AMFs represent ideal sentinels against bacterial infections because of their exposed position in the alveolar lumen, their strong phagocytosis capacity, and their expression of many pattern recognition receptors. In fact, it has been estimated that AMFs can handle up to 10 intratracheally injected bacteria[9] before there is "spillover" of bacteria to lung DCs.[99] Indeed, although Brucella infection within the lungs was mainly confined to AMFs with only minor uptake of Brucella by cDCs, depletion of AMFs before the infection greatly increased the uptake of Brucella bacteria by cDCs.[100] Importantly, absence of AMFs before Brucella infection was associated with exacerbated inflammation. This could be explained by (i) an increase in bacterial load as AMFs have been proposed to play a direct role in pathogen clearance, and (ii) an increased uptake of bacteria by moDCs and cDCs that may indirectly lead to higher inflammation. Indeed, although in the presence of AMFs, Brucella infection was not associated with moDC recruitment, infection in the absence of AMFs led to a massive recruitment of monocytes and their development into inflammatory TNF- and inducible nitric oxide synthase–producing DCs

(TIP-DCs). Uptake of bacteria by migratory cDCs may also explain why absence of AMFs led to strongly increased bacterial dissemination in the body. As such, one mechanism by which AMFs may help to suppress inflammatory responses in the lungs is by limiting the access of cDCs and moDCs to pulmonary pathogens and environmental antigens. Moreover, AMFs have been proposed to directly inhibit the antigen-presenting function of lung DCs.[101] AMFs have also been proposed to actively contribute to the suppression of inflammation because of their production of IL-10 (see ref. 102) and their supposed "alternative activation state."[103–105] The separation of macrophages into "classical" and "alternative" macrophages propose that classically activated macrophages (M1 macrophages) mediate defense of the host against bacteria, protozoa, and viruses, whereas alternatively activated macrophages (M2 macrophages) have anti-inflammatory function and regulate wound healing.[106–108] However, macrophages have been shown to switch from one activation state to another in response to changes in their microenvironment.[109–111] Moreover macrophages in vivo are often found to express M1-associated and M2-associated genes simultaneously (Guilliams et al., unpublished results and refs. 112–114). As such, we believe it is better not to restrict macrophages to a particular M1 or M2 state as this is often an oversimplification of the function of these cells. Moreover, steady-state AMFs are clearly not locked in an M2 state. In fact, although AMFs have been shown to actively participate in pathogen clearance (an M1-associated function), they do so without triggering overt inflammation and by actively suppressing production of proinflammatory cytokines by other cells (an M2-associated function). Nevertheless, some infections can trigger M2-associated functions in AMFs, as was recently illustrated during RSV infection where AMFs expressing typical M2 markers participate actively in tissue repair in an IL-4Rα-dependent manner.[115]

In other bacterial infections such as Mtb infection, AMFs have been proposed to have a dual role. On one hand, AMFs can produce NO and reactive oxygen species, which have been shown to be able to kill Mtb directly. In fact, AMFs isolated from Mtb patients were shown to express high levels of iNOS.[116] Therefore, in theory, AMFs could contribute directly to Mtb elimination even before Mtb can properly infect the host. It is also noteworthy that because NO is suppressive for T cells, its production by AMFs may directly suppress T-cell activation.[117] However, Mtb can subvert MF functions and ensure its survival within these cells.[118,119] Moreover, AMF depletion has been shown to lower the bacterial load and to prolong the survival of Mtb-infected mice.[120] Therefore, although it is clear that the defense against Mtb requires both iNOS and TNF,[121–124] it is likely that the main cells producing these mediators may be moDCs rather than AMFs. As Arginase uses arginine as a substrate and directly competes with iNOS for arginine, the relative expression of these genes affects the ability to kill Mtb. As such, $Arginase^{-/-}$ mice were shown to have an increased production of NO and to be more resistant to Mtb infection.[125] On the other hand, TNF may be crucial for two reasons.

First, infection of AMFs by Mtb induces apoptosis by a TNF-dependent mechanism.[126] This apoptotic response is postulated to be a defense mechanism to limit the growth of this intracellular pathogen, as a virulent Mtb strain was shown to induce substantially less AMF apoptosis than an attenuated Mtb strain.[127] Second, TNF has been shown to be essential for granuloma formation. Therefore, a polarized Th1 immune response that will induce NO and TNF production is essential for the proper elimination of Mtb. AMFs have been shown to be poor inducers of Th1 polarization as compared with DCs. Although they take up more mycobacterium as compared with DCs, they produce less IL-12 p40 and were less able to induce IFN-γ production by T cells.[128] Moreover, Mtb may actively suppress DC function, hence limiting their Th1 polarizing capacity and dampening local Th1 inflammation through the production of IL-10 or type I IFN. In Mtb-infected mice depleted of AMFs, the prolonged survival was associated with an increased Th1 response.[120] Therefore, the anti-inflammatory functions of AMFs that seem crucial to avoid influenza-associated pathology seem at the same time to favor Mtb persistence.

CONCLUSION

Altogether, recent evidence suggests a clear division of labor between DC and MF subsets within the lungs during pulmonary infections and underlines the fact that the particular cell subsets that are beneficial in the fights against bacterial infections may be implicated in the immune pathology in viral infections. Understanding better the role of the distinct cell subsets in the lungs exposed to viral or bacterial threats should pave the way toward immunomodulation strategies that could be used to increase our defenses against specific pathogens while avoiding collateral lung tissue damage.

DISCLOSURE
The authors declared no conflict of interest.

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