C-TYPE LECTINS WITH A SWEET SPOT FOR *MYCOBACTERIUM TUBERCULOSIS*

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The pattern of receptors sensing pathogens onto host cells is a key factor that can determine the outcome of the infection. This is particularly true when such receptors belong to the family of pattern recognition receptors involved in immunity. *Mycobacterium tuberculosis*, the etiologic agent of tuberculosis interacts with a wide range of pattern-recognition receptors present on phagocytes and belonging to the Toll-like, Nod-like, scavenger and C-type lectin receptor families. A complex scenario where those receptors can establish cross-talks in recognizing pathogens or microbial determinants including mycobacterial components in different spatial and temporal context starts to emerge as a key event in the outcome of the immune response, and thus, the control of the infection. In this review, we will focus our attention on the family of calcium-dependent carbohydrate receptors, the C-type lectin receptors, that is of growing importance in the context of microbial infections. Members of this family appear to be key innate immune receptors of mycobacteria, capable of cross-talk with other pattern recognition receptors to induce or modulate the inflammatory context upon mycobacterial infection.

Keywords: mycobacteria, tuberculosis, pattern recognition receptor, C-type lectin, macrophage, dendritic cell

Introduction

Recent WHO (World Health Organization) reports on tuberculosis (TB) indicate that this disease is still one of the leading causes of death due to a single infectious agent, *Mycobacterium tuberculosis* (Mtb), with 1.7 million deaths and 9.4 million new cases in 2009 [1]. It is generally considered that one third of the human population may be latently infected with Mtb. Active TB may occur directly after infection or through the reactivation of latent infection, which happens in about 5% of infected individuals. During latent TB, the infection is confined in a non pathological and non-contagious state, within a specific, dynamic structure called the granuloma. The elaboration and maintenance of this structure depends on a dedicated immune response, which is not fully understood. It is believed that the efficiency of the immune response both during the early and late phases of infection is key to controlling the outcome of the disease. This may be initially determined by the interactions between Mtb and the various pattern recognition receptors (PRRs) expressed in cells of the innate immune system but also in non-immune cells, such as lung epithelial cells [2, 3]. Although several studies have identified single nucleotide polymorphisms (SNP) associated with differential susceptibility to TB in Mtb receptors of the Toll-like receptor (TLR) [4–10] or Nod-like receptor (NLR)

families [11], the strongest links between genetic polymorphisms and TB susceptibility usually do not show up at the level of PRR [12]. In addition, inactivation of one gene encoding PRR of the TLR or NLR families does not usually exhibit major phenotypes in mouse models of Mtb infection [13–16]. This observation can be interpreted as a modest role played by individual receptors or redundancy between them, and it suggests that understanding the cross-talks and signal integration by PRR in combination rather than isolated is crucial to decoding the dialog between receptors for mycobacteria and their ligands [2]. This is well illustrated by the example of TLR2 which has been shown to cooperate with other TLR [17], TLR-related molecules like RP105 [18], or with non-TLR receptors such as C-type lectin receptors (CLRs) [19], to recognize mycobacteria. Furthermore, it has recently been shown that cooperation between PRRs can modulate immune response to single mycobacterial antigens such as the trehalose dimycolate (TDM, also known as cord factor). Indeed, in addition to being recognized by the CLR Mincle (macrophage-inducible C-type lectin, see below), TDM can be recognized by the scavenger receptor MARCO, in a TLR-dependent [20] and FcγR-dependent [21] manner to modulate immune response. Yet, another level of complexity is a certain level of redundancy between receptors making it difficult to isolate the contribution of individual receptor to anti-TB im-

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munity [16]. All-in-all, Mtb-PRR interactions may contribute to the persistence of the bacillus within host phagocytes or may favor the host, by inducing immune defense mechanisms, such as autophagy, phagosome maturation, apoptosis, pyroptosis and various bactericidal mechanisms.

Here, we will not cover the full list of receptors such as receptors of the TLR, NLR and scavenger families involved in recognition of Mtb [2, 14, 15, 22–26]. Rather we will focus on the group of CLRs, that usually recognize diverse carbohydrate containing molecules in a calcium (Ca^{2+})-dependent manner and employ a conserved Syk/Erk pathway for their intracellular signalling activities [27–29], and will discuss their diversity, their ligand binding properties, and their impact on human tuberculosis as well as animal models and their functions.

Recognition of *M. tuberculosis* by C-type lectins

CLRs are Ca^{2+} -dependent glycan-binding proteins displaying similarities in the primary and secondary structures of their carbohydrate-recognition domain (CRD). These proteins have in common a C-type lectin fold, a structure with a highly variable primary protein sequence that is also found in many proteins that do not bind carbohydrates [C-type lectin domain (CTLD)-containing proteins]. CLR and CTLD-containing proteins are widely expressed in all organisms [30]. The CLR family contains a large number of members, including collectins, selectins, endocytic and phagocytic receptors, and proteoglycans. Some of these proteins are soluble and secreted, whereas others are anchored in the plasma (or sometimes internal) membrane of cells. They often oligomerize into homodimers, homotrimers, and higher-order oligomers, which may have a higher avidity for multivalent ligands and lead to significant differences in the types of glycans that they recognize with high affinity. From a functional point of view, CLR can act as adhesion molecules, endocytic, phagocytic and/or signalling receptors with many immune functions, including inflammation and immunity to tumors and microbes [31]. Thus, CLRs are key receptors of the innate immune response and have been strongly conserved throughout evolution.

The carbohydrates expressed on the surfaces of host cells and pathogens (i.e. their respective “glycome”) are often markedly different, making segregation based on sugar composition an effective way for the innate immune system to recognize foreign organisms [32]. As a very good example, the Mtb cell envelope is particularly rich in specific and complex carbohydrate-containing molecules, such as glycolipids (e.g. phosphatidyl-myo-inositol mannosides (PIMs), and TDM), lipoglycans (e.g. lipomannan (LM) and lipoarabinomannan (LAM)), polysaccharides (the α -glucan) and glycoproteins (including the 19 kDa, 45 kDa and 38 kDa antigens) [31, 33, 34]. In recent years, a lot has been learned regarding the interactions of mycobacterial ligands with members of the CLR family such as soluble CLR of the collectin family and membrane-anchored CLR of the

myeloid transmembrane lectins family. Below are presented the members of the CLRs for which a role in recognition of mycobacterial ligands is the most compelling.

M. tuberculosis recognition by CLR of the transmembrane myeloid lectin family

Transmembrane CLR of the type 1 and type 2 (i.e. harbouring an extracellular CRD at their N-terminal and C-terminal end, respectively) have been frequently involved in immune responses to pathogens. The specificity of a given CLR for a given carbohydrate ligand is determined by both its primary amino-acid sequence and its number of CRDs. Myeloid CLRs are believed to mainly act as endocytic/phagocytic receptors. Their ligands are internalized in a clathrin-dependent manner and delivered to early then late endosomes. The receptors themselves may be recycled or degraded, depending on the receptor and the type of ligand. At acidic pH (< 5), Ca^{2+} is released from CLRs, shifting the equilibrium toward ligand dissociation. The cytoplasmic portion of these receptors dictate their trafficking along the endocytic pathway and contributes to intracellular signalling events (some CLRs display signalling activities). For instance, the stimulation of Dectin-1 in myeloid cells leads to activation of the mitogen-activated protein kinase (MAPK) and NF κ B (nuclear factor κ B) pathways, resulting in the up-regulation of genes encoding effectors or modulators of the innate immune response (see [35] for a review). Among the many myeloid CLRs, those reported to interact with Mtb or mycobacterial components include the mannose receptor (MR) [36–38], Dectin-1, Dectin-2 [39, 40], Mincle [41, 42], and DC-SIGN [43, 44] and its mouse putative analogs, SIGNR3 [19] and SIGNR1 [19, 45, 46].

DC-SIGN and its murine homologs

Human DC-SIGN (hDC-SIGN, CD209) is a type II transmembrane CLR that possesses one single extracellular CRD capable of recognizing mannose-containing molecules. hDC-SIGN assembles as a tetramer, which is the structure required for efficient ligand binding [47]. Its N-terminal, intracellular region contains three different motifs: a tyrosine-based internalization motif, a di-leucine motif also involved in internalization and a triacidic amino acid cluster involved in the sequestration of the receptor in intracellular components in certain conditions. Together, these motifs are thought to contribute to endocytosis/phagocytosis, through interaction with clathrin, as well as in the intracellular trafficking of ligand particles ultimately reaching the phagolysosomes [48], but they can also be involved in signalling. The structure of the transmembrane domain seems to play a role in the stabilisation of multimeric structure through coiled–coiled interactions and thus indirectly contributing to ligand binding. The C-terminal, extracellular part of hDC-SIGN contains the CRD Ca^{2+} binding site and ligand binding site. hDC-SIGN was initially identified as the counter-receptor for ICAM-3 [49] and as a receptor for the human immunodeficiency virus (HIV) glycoprotein-120 (GP120) [50], and was thought to

be specifically expressed in dendritic cells (DC) [49, 51]. More recently, hDC-SIGN has also been shown to be the receptor for sialylated immunoglobulins and to mediate the therapeutic effects of intravenous immunoglobulins through this binding property [52]. With time, the expression of DC-SIGN was found to be not restricted to DC, but to extend to other cell types, such as macrophages, including alveolar macrophages [53–55], and some lymphocyte populations [56]. It was recently shown that alternative transcripts or proteolytic cleavage can lead to the natural expression of a soluble form hDC-SIGN [57], which might be relevant to the response to infectious agents [58] and could behave more analogous to collectins.

Studies by us and others clearly demonstrated that hDC-SIGN was a key receptor for Mtb in human DC [43, 44], and that it acted through the recognition of mannose-containing motifs, such as those present in LAM and the 19 kDa antigen molecules [43, 59, 60]. Since then, hDC-SIGN binding to eight different purified carbohydrate-containing ligands of the mycobacterial envelope has been reported: Man-LAM (mannose-capped LAM), LM, arabinomannan, glycoproteins (19 kDa, 38 kDa, 45 kDa), PIM-6 and α -glucan [43, 44, 59–62]. More recently, additional Mtb ligands have been reported for hDC-SIGN: the 70 and 60 kDa chaperone/heat shock proteins DnaK/Hsp70/Rv0350 and GroEL/Hsp60/Rv3417c, respectively, the glyceraldehyde-3-phosphate deshydrogenase (Gap/Rv1436), and the lipoprotein LprG/Rv1411c [63]. Of these, only the latter seems to bind hDC-SIGN using the conventional carbohydrate-binding domain. Our group has also reported that hDC-SIGN expression is actually induced in alveolar macrophages of TB patients, again suggesting a major role as a receptor for the bacillus in these cells [54].

Recent epidemiological and population genetics studies have shown that SNPs located in the promoter region of the hDC-SIGN gene may be differentially associated with susceptibility to the disease [64, 65], further supporting a possible role for this CLR in immunity to TB. The results of these two reports seem to conflict and could not be reproduced in two other studies [66, 67], which could reflect differences in the origin and genetic background of the populations that was studied. With the aim to elucidate the role of hDC-SIGN/CD209 in TB *in vivo*, Ehlers et al. [68, 69] produced a mouse model where the hDC-SIGN gene was expressed under the control of the CD11c promoter, which drives the expression mostly on DC and some macrophages subpopulations like alveolar macrophages, for instance. These humanized mice survived significantly longer as compared to their wild-type counterparts and exhibited fewer physiopathological signs in their lungs after infection with Mtb [69]. Thus, in this humanized mouse model, hDC-SIGN seems to protect the host, which was found to depend on modulation of the intensity of the immune response to Mtb [69].

An alternative *in vivo* approach to study the role of DC-SIGN was to analyze the susceptibility of mice deficient in mouse homologs of hDC-SIGN to Mtb infection. This approach is complicated by the fact that eight putative DC-SIGN homologs were found in the mouse genome, named

Signr1-8 [70, 71]. One of these genes, *Signr-6*, is a pseudogene and yet another, *Signr-2*, encodes a secreted protein thus lacking the characteristic of transmembrane CLR. They all display a unique CRD at their C-terminus. The sequences of the CRD of the mouse homologs SIGNR1, SIGNR3 and SIGNR4 most closely match that of the CRD of hDC-SIGN [19, 71] but only SIGNR1 and SIGNR3 harbour the three motifs found in the intracellular domain of hDC-SIGN. Functional analysis of the interactions between these CLRs and various sugar motifs demonstrated that SIGNR1 and SIGNR3, like hDC-SIGN, interacted with mannosylated motifs, whereas SIGNR4 had no lectin activity, probably due to inactivation of one of the Ca^{2+} -binding sites. Only SIGNR3 was found to interact with both fucosylated and mannosylated complexes, an unusual feature common to hDC-SIGN, and from this point of view, SIGNR3 can thus be considered the closest functional homolog of hDC-SIGN [71]. However, with regard to other properties of hDC-SIGN such as binding of sialylated immunoglobulins for example, SIGNR1 was reported to efficiently substitutes hDC-SIGN [52]. Wide expression, markedly different ligand binding properties and limited sequence homologies are also characteristics of SIGNR7 and 8, which might not be appropriate analogs of hDC-SIGN on these bases [71]. It is unlikely that SIGNR2 plays similar roles as soluble hDC-SIGN [57] as it is mainly expressed in testis and binds different sets of glycans [70, 71]. In addition, it is not known if soluble forms similar to hDC-SIGN exist in the mouse. All-in-all, although it is not clear which murine molecule is the best analog of hDC-SIGN, SIGNR3 and possibly SIGNR1 could play these roles.

Others and we have found that both SIGNR1 and SIGNR3 indeed interact with various glycosylated Mtb ligands, such as ManLAM, LM and the 19 kDa antigen LpqH [19, 45, 46, 72]. Yet, they differ in their fine specificity for selected ligands [73]. Mouse strains in which SIGNR1 or SIGNR5 are inactivated display no particular phenotype upon Mtb infection in terms of survival, and bacterial load in the lung and spleen [19], although a stronger Th1 response against Mtb in SIGNR1-deficient mice was reported by Wieland et al. [46]. This finding can be explained by the inability of SIGNR5 to recognize mycobacterial ligands, or by the apparent lack of expression of SIGNR1 in the lungs. By contrast, SIGNR3-deficient mice were found to have significantly higher bacteria loads in their lungs 21 and 42 days after infection, whereas the number of bacteria in their spleen was comparable to that of their wild-type counterparts [19]. This result suggests that SIGNR3 does not contribute to early mycobacterial dissemination, but plays a role in controlling the immune response during the early phases of infection. Finally, SIGNR3-deficient animals managed to control the infection and did not display more severe physiopathological lesions or die earlier than the wild-type controls. SIGNR3 function may thus be restricted to the early immune response to the pathogen. In uninfected mice, SIGNR3 is expressed in the spleen and lymph nodes, and is undetectable or present at only low levels in other organs [70]. However, recalling the observation made with

hDC-SIGN in TB patients, we found that SIGNR3 expression was induced soon after Mtb infection in cells displaying a macrophage-like morphology, along with macrophage markers such as iNOS and F4/80. More recently, using novel anti-SIGNR3 antibodies, SIGNR3 protein expression was observed on subsets of macrophages, DC and monocytes [74], a property that could also apply to more distant murine analogs of hDC-SIGN such as SIGNR5.

Collectively, these studies in various mouse models tend to suggest that DC-SIGN is involved in protection against TB, using mechanisms that still remain to be fully understood [68, 69, 72]. It is also still unclear how these results compare with those obtained *in vitro* in human cells, which suggest that DC-SIGN engagement by Mtb results in escape mechanisms [43].

The mannose receptor

MR (CD207) is a transmembrane CLR and the prototypic marker of alternatively activated M2 macrophages. It is a 175 kDa type I protein with an N-terminal cysteine-rich domain, a single fibronectin type II domain, 8 CRDs, a transmembrane region and a short cytoplasmic tail containing a tyrosine-based motif thought to be important for ligand-receptor internalization [75]. Only one of its CRDs seems to recognize terminal glycosylated motifs, such as D-mannose, L-fucose and N-acetyl-D-glucosamine [76–78]. MR is widely expressed on tissue macrophages, such as alveolar macrophages, and on DC subsets mediating antigen uptake, enhancing the presentation of antigens to T cells [79]. MR recognizes various endogenous epitopes [80], as well as numerous pathogens, including *Candida albicans*, *Pneumocystis jiroveci*, *Klebsiella pneumoniae*, *Shistosoma mansoni*, *Cryptococcus neoformans* and the Dengue virus [81–83]. Yet, no impairment of host responses to infections with several of these pathogens is observed in mice lacking MR [84–86].

MR recognizes various glycosylated ligands in the Mtb envelope, including mannose-capped LAM, phosphatidylinositol mannosides 5 and 6 (PIM5–6), arabinomannan, mannan and mannose-containing proteins [36, 37]. Cross-talk with additional receptors including TLR-2 has been well documented [40]. Recently, recognition of Mtb by MR has been shown to regulate the immunomodulatory transcription factor PPAR γ (Peroxisome Proliferator-Activated Receptor γ) in human macrophages [87]. This transcription factor mediates immunosuppression by repressing NF κ B and providing Th2 cytokines secretion. PPAR γ silencing in macrophages prevents the MR-dependent immunosuppressive effect and improves the control of mycobacteria by infected macrophages [87]. However, the role of MR in immunity to TB has not yet been completely elucidated, and again MR-deficient mice display no particular phenotype upon Mtb infection [16].

The Dectin-2 family: Dectin-2 and Mincle

Dectin-2 and Mincle are members of the Dectin-2 cluster and have a common structure, consisting of a single extracellular CRD, a stalk region of variable length, a transmembrane re-

gion, and a cytoplasmic domain [88]. Dectin-2 and Mincle have been reported to bind carbohydrate-containing ligands. Yet, there are several non carbohydrate-containing ligands for these lectins, such as SAP130, an endogenous protein recognized by Mincle. Dectin-2 is a CLR expressed principally in DC and some macrophages. It recognizes mannosylated ligands in a Ca²⁺-dependent manner. Dectin-2 has no known intracytoplasmic motif involved in signal transduction but can associate with FcR γ through a charged residue and thus propagate intracellular signals [89, 90]. One study reported that a soluble form of the Dectin-2 CRD recognized Mtb envelope polysaccharides [91]. However, the role of Dectin-2 in the control of Mtb infection is unknown.

Like Dectin-2, Mincle has been shown to associate with the FcR γ chain [92]. This interaction involves the positively charged arginine residue in the transmembrane region of Mincle and is essential for signalling through the receptor [92]. Initial microarray analyses of Mincle gene expression suggested that this gene was upregulated in bone marrow-derived macrophages exposed to *C. albicans*. Two independent groups recently identified Mincle as a key CLR involved in Mtb recognition [42, 93]. These two studies identified Mincle as the receptor for a key immunomodulatory component of the mycobacterial envelope, TDM [94]. TDM is also recognized by the scavenger receptor MARCO which can co-signal with other PRR such as TLR [20] or FcR γ [21]. Ishikawa et al. [42] shown that Mincle recognizes mycobacteria, in a CRD-dependent manner, and that such recognition, together with FcR γ recruitment, may modulate the transcription program of activated cells in a TLR-independent manner. In parallel, Schoenen et al. [93] also demonstrated the involvement of this CLR in innate immunity to Mtb, by showing that Mincle regulates the immunomodulatory function of TDM in a FcR γ -, Syk-, and CARD9-dependent manner. Mincle-deficient mice developed no granulomatous response to TDM injection, and produced smaller amounts of cytokines in response to mycobacteria, demonstrating that Mincle is a key PRR regulating the anti-mycobacterial immune response, in a TLR-independent manner [42]. Yet, the susceptibility of the Mincle-deficient mice to Mtb remains to be evaluated.

Additional myeloid CLR involved in M. tuberculosis recognition: Dectin-1 and the complement receptor Type 3 (CR3)

Additional CLRs, such as Dectin-1 (CLEC7A) and CR3 have been shown to recognize Mtb components. Dectin-1 is a type II transmembrane receptor with a single extracellular CRD and an intracellular portion containing an ITAM motif, which, upon phosphorylation by Src family kinases can recruit Syk family kinases and transduce signals of activation. Dectin-1 is mainly expressed on macrophages, DCs, neutrophils and microglial cells, and, to a lesser extent, in subsets of T cells, B cells and epithelial cells [95]. Dectin-1 is overexpressed on the surface of alveolar epithelial cells infected with mycobacteria [96]. These cells are not professional phagocytes, but are targeted by the TB bacillus *in vivo*, in the lungs [96]. Dectin-1 is an atypical CLR, because

it can interact with $\beta(1-3)$ and $\beta(1-6)$ glucans in a Ca^{2+} -independent manner. Dectin-1 may also recognize unidentified components of the mycobacterial envelope, α -glucan being a potential good candidate ligand for Dectin-1 [39, 40, 97]. Dectin-1-deficient animals display a slightly reduced bacterial burdens in their lungs, but do not show significant changes in pulmonary pathology, cytokine levels nor ability to survive the infection [98], thus far suggesting a minor or redundant role of Dectin-1 in anti-TB immunity.

CR3 (integrin $\alpha\beta 2$, Mac 1), a heterodimeric receptor (CD11b/CD18) of the integrin family [99, 100], is mainly expressed on neutrophils, monocytes, natural killer cells and macrophages, including alveolar macrophages [99]. Although CR3 can recognize C3b-opsonized Mtb *in vitro*, it is unlikely that this interaction occurs *in vivo* given the very low concentration of complement factors in the lungs [101]. Alternatively, CR3 can directly interact with bacterial components, such as the 85C antigen (FbpC/Mpt45/Rv0129c) of Mtb through its integrin domain [101]; recognize the bacilli through its CLR domain, which can bind mycobacterial oligosaccharides, such as LAM [102–104]; and bind to mycobacterial PIMs [105]. Taken together, these observations indicate that CR3 directly binds Mtb, but its relevance has not yet been fully evaluated *in vivo*. However, a study using CD11b-deficient mice reported no relevant phenotype on Mtb infection [106], suggesting that CR3 may have a redundant function in interactions between the host and Mtb [107].

M. tuberculosis recognition by collectins

Collectins are CLRs displaying a collagen-like domain that usually assemble into large, oligomeric structures of 9 to 27 subunits [108]. Among the nine different members of the collectin family that have been identified so far, three have been shown to be involved in Mtb recognition and immunity to TB: surfactant protein A (SP-A), surfactant protein D (SP-D) and mannose binding lectin (MBL). MBL and SP-A are soluble, secreted collectins organized into a “bouquet”, whereas SP-D adopts a “cruciform” shape. Collectins are well known for their contribution to innate immunity in response to many different microbial pathogens [31].

Surfactant proteins A and D

SP-A and SP-D are collectins produced by the respiratory epithelium [109] but are also expressed in the intestine [31]. Surfactant proteins play an important role in lung physiology. SP-A and SP-D are also known to be important PRRs involved in the innate immunity-mediated maintenance of lung integrity [110]. Their role ranges from clearance of bacteria, viruses and fungi as well as of apoptotic and necrotic bodies to the modulation of allergy and inflammation [111, 112]. Surfactant proteins consist of multimers of a basic trimeric structure. Each trimer is formed of three disulfide-linked monomers (each of which being composed of one CRD), an α -helical coiled-coil neck domain, a collagen-like domain, and a N-terminal domain. Multimerization of the 32 kDa SP-A and 43 kDa SP-D subunits results in the gen-

eration of decaoctameric and dodecameric pattern molecules, respectively. The CRD domains of collectins bind glycoconjugates on the surface of pathogens in a Ca^{2+} -dependent manner [113, 114]. Several studies have shown that SP-A and SP-D modulate the interaction between the host and mycobacteria upon infection in the lung [115–122]. Both SP-A and SP-D bind to lipoarabinomannans in the mycobacterial cell wall [48, 123]. A critical residue at position 343 of SP-D was shown to be responsible for the selective binding to LAM, LM and PILAM [124]. SP-A also interacts with the 45 kDa Apa/Mpt32/Rv1860 protein, a serine- and proline-rich glycoprotein of the Mtb cell envelope [125]. It also enhances the expression of other innate immune receptors involved in *M. tuberculosis* recognition, including the scavenger receptor SR-A [126] and the complement receptor CR3 [127]. Although the phenotype of mice lacking SP-A and SP-D in the context of Mtb infection has yet to be reported, GM-CSF-deficient mice, in which surfactant metabolism is highly impaired [128], are much more susceptible to Mtb infection than wild-type mice [129], suggesting a contribution of surfactant proteins in the control of Mtb infection. As a matter of fact, polymorphisms in SP-A genes have been associated in susceptibility to TB in independent human genetics studies in an Ethiopian [130] and Indian populations [131], confirming the important role of this CLR to TB etiology.

The mannose-binding lectin

MBL, like SP-A and SP-D, belongs to the collectin family. MBL is primarily synthesized in the liver and circulates in the blood although it also has been detected in the synovial and amniotic fluids [132]. It has also been localized to specific subcellular compartments, namely in the endoplasmic reticulum (ER) and in COPII vesicles [133]. MBL adopts a trimeric helical structure through its collagenous tails, stabilized by disulfide bonds in the cysteine-rich amino-terminal region [132]. The trimer serves as a basic subunit and forms higher oligomers, which are able to active complement on microbial surfaces [134, 135]. MBL requires Ca^{2+} in binding to the terminal sugars D-mannose, L-fucose and N-acetyl-D-glucosamine, but not to D-galactose or sialic acid [136, 137]. The binding of MBL to a target results in the activation of complement via the lectin pathway [138] and generates opsonic and iC3b fragments that coat pathogens, targeting them for phagocytosis [132]. The role of MBL in immunity to microbial infections has been established with the observation that individuals with mutations within exon 1, which disrupt MBL multimerization, have increased risks of microbial infections. This clinical observation was confirmed for certain pathogens by animal model studies using MBL knockout mice [138]. Furthermore, several polymorphisms within the promoter region of MBL were associated with greater susceptibility or protection against several pathogens [132].

With regards to Mtb recognition, MBL has been shown to bind to the LAMs of Mtb, *M. leprae* and *M. avium* [139, 140]. Several genetic association studies have evaluated the relationship between certain MBL2 genotypes and suscep-

tibility to TB. However, firm conclusions cannot be drawn concerning the role of MBL in TB [141–144]. Indeed, the role of MBL in innate immunity remains a matter of debate, because MBL2 deletion genotypes are present at high frequency in the population, and this is not consistent with strong selection operating on an essential gene [145]. The susceptibility of MBL-KO mice and of mice carrying MBL-deficient genotypes [146, 147] to Mtb remains to be studied, to provide insight into the possible role of MBL in Mtb infection *in vivo*.

Biological consequences of CLR stimulation by *M. tuberculosis*

One important feature for PRRs, once a pathogen is recognized, is to be able to translate this signal into a series of downstream events to induce innate and adaptive immunity. In general terms, CLR activation results in downstream events such as endocytosis, oligomerization, intracellular trafficking and signal transduction. These downstream events are mediated specifically by the cytoplasmic tail of CLRs that contains various amino acid clusters, including tyrosine-based motif such as immunoreceptor tyrosine-based activation/inhibition motifs (ITAM/ITIM). For instance, the tyrosine-based motif in the cytoplasmic domain of the MR promotes ligand delivery to early endosomes and receptor recycling to the cell surface [75]. In addition to a tyrosine-based, coated pit sequence-uptake motif similar to that in the macrophage mannose receptor, hDC-SIGN carries a dileucine motif essential for internalization, and a triacidic cluster essential for targeting to endosomes/lysosomes. However, it is the CLR-induced gene expression through the cytoplasmic tail that perhaps has the most profound effect in the immune response. In this regard, the characterization of signaling pathways used by membrane-bound CLRs involved in Mtb recognition has improved substantially over the past few years, and these pathways seem to converge on a limited set of interaction mechanisms, which include both synergistic and antagonistic interactions [148, 149]. This phenomenon is now known as signalling crosstalk and emerging evidence now supports its important role in the immune system [150]. A couple of characteristic examples in the literature include the synergistic interaction between TLR2 and Dectin-1 to amplify the antifungal immunity [151], and the antagonistic interaction between the TLR-induced pro-inflammatory response and the suppressive effects of glucocorticoid and adenosine receptors [152, 153]. For this section of the review, we will focus on the biological consequences of CLR signalling crosstalk stimulated by Mtb.

The pro-inflammatory response

The activation of CLRs is composed by a pro-inflammatory insult against an invading pathogen accompanied by an anti-inflammatory response thought to prevent tissue immunopathology [27]. Along with NLRs, the pro-inflam-

matory response by CLRs forms part of the TLR/MyD88-independent immune response to Mtb, and one that amplifies the innate arm of immunity that mediates host defense. Recognition of Mtb by CLRs has been shown to induce gene expression of pro-inflammatory cytokines such as TNF, IL-6 and IL-12, that are essential for the establishment of Th1/IFN γ -mediated responses in mycobacterial infection [27, 148]. However, the contribution by most of the CLRs to the immune response against Mtb seems to be redundant. Mouse models harbouring single deficiencies in MBL, MR, Dectin-1, CR3/CD11b, SIGNR1 do not have an impairment in the control of Mtb *in vivo* [16, 98, 106, 154], implying that a deficiency of a single CLR might be compensated by the other receptors that Mtb is likely to engage simultaneously. Nonetheless, there is evidence that indicates that the Syk and CARD9 signaling pathways play a key non-redundant role in anti-mycobacterial immunity, and there are two CLRs known to use these pathways that seem to play a pivotal role in early immunity against Mtb: SIGNR3 and Mincle. Indeed, we have shown that signaling via the intracellular hemITAM motif of SIGNR3 plays an essential role in the production of IL-6 and TNF by macrophages, probably accounting for the significantly increased burden of bacilli observed in the lungs of SIGNR3-deficient mice [19]. Using Syk-specific inhibitors, piceatannol and R406, we demonstrated that it abolishes in a dose-dependent manner the SIGNR3 signaling along with the cytokine production, allowing us to conclude that SIGNR3-mediated production of IL-6 and TNF is Syk-dependent [19, 72]. As aforementioned, Mincle was identified as the CLR responsible for recognizing the mycobacterial cord factor TDM, and its triggering leads to production of inflammatory cytokines and nitric oxide [42, 93]. In fact, Mincle deficiency results in the absence of a granulomatous response to TDM injection, and in a significant reduction of inflammatory cytokines in response to Mtb [42]. Given the crucial importance of CARD9 for TB control [155], and that Mincle is dependent on the Syk-CARD9 signaling pathway [93], it is possible that this receptor plays a non-redundant role in translating the signal from the carbohydrate-based moieties in Mtb into an immune response. Nevertheless, the final production of pro-inflammatory cytokines via CLRs, such as SIGNR-3 and Mincle, and other receptor families, is one example of the signalling crosstalk between synergistic pathways that greatly increase the sensitivity of detection, by integrating several individual weak stimuli to elicit a vigorous induction of innate immune gene expression.

The anti-inflammatory response

As important as the amplification of the pro-inflammatory response mediating host defense may be modulated by CLRs, perhaps their most unique role is the establishment of an anti-inflammatory feedback loop to maintain tissue homeostasis or avoid an excessive inflammation detrimental to the host. Indeed, the CLR pro-inflammatory response

is accompanied by induction of anti-inflammatory mediators such as IL-10, retinoic acid-metabolizing enzymes, and TGF- β [27, 156]. Given the inhibitory capacity of these mediators to innate and adaptive immune responses [157], at first glance, their simultaneous production may seem contradictory to the establishment of a strong inflammatory response in order to contain the spread of an invading pathogen and promote its elimination from the host. However, one aspect to be taken into consideration is the context at which activation of CLRs takes place. Usually, myeloid cells such as DCs and macrophages located at the peripheral tissues such as mucosal sites (e.g. lung and gut), express a variety of CLRs that are involved in the performance of various housekeeping functions during steady-state conditions [27, 88, 158]. Since mucosal sites are constantly being repaired and maintained, and they host a multitude of commensal microorganisms (notably in the gut), the housekeeping functions performed by DCs and macrophages are critical to keep a delicate balance between resident cells (e.g. epithelial cells) and commensal microorganisms [88, 158–160]. For instance, IL-10 produced at low levels by resident intestinal macrophages maintains, in a paracrine manner, the Foxp3 expression on regulatory T cells (Treg), whose activity ensures a stable tolerogenic environment benefiting the commensal bacteria in the gut [157, 158]. Furthermore, mice that lack this important cytokine spontaneously develop colitis characterized by massive presence of inflammatory macrophages expressing high amounts of IL-12 and IL-23 [158]. Therefore, the expression of CLRs in myeloid cells during steady states conditions is likely to play a major role in either maintaining mucosal tissues in a hypo-responsive manner, or in relying anti-inflammatory signals by commensal microbiota to establish a cooperation between the innate and adaptive immune systems, in order to ensure a proper host response to maintain mucosa and host integrity.

At the same time, mucosal sites are also constantly exposed to the external environment, and by consequence, to invading pathogens. For this reason, it is also important for mucosal sites to have a rapid and efficient defense system in place in order to recognize and discriminate invading pathogens from symbiotic microorganisms. As discussed above, CLRs help to establish an inflammation response against an invading pathogen that is characterized by the production of soluble factors (e.g. TNF, IL-6, IL-12) and recruitment of effector cells from both innate and adaptive immune systems (e.g. granulocytes and lymphocytes, respectively). However, mucosal sites are also exposed to a myriad of foreign particles (e.g. dust mites and other allergens alike), that may provoke unwanted and unnecessary chronic inflammation responses contributing to disorders such as allergy or asthma, and resulting in permanent damage in local tissue and excessive killing of bystander resident cells and symbiotic microorganisms. Therefore, there needs to be an additional mechanism in place to shut down the pro-inflammatory response. Again, CLRs play an essential role in contributing to the production of anti-inflammatory mediators that helps to protect the integrity of

mucosal tissues. Perhaps the best example illustrating the putative role of CLR in modulating the immune response against Mtb comes from observations in CARD9-deficient mice; these animals succumbed early after aerosol infection displaying a high Mtb burden, pyogranulomatous pneumonia, massive granulocyte infiltration, and high incidence of pro-inflammatory cytokines both in serum and lung [155]. The pronounced susceptibility of CARD9-deficient mice to Mtb infection was expected since CARD9 is an adapter molecule involved in the signaling pathways of various PRRs including TLRs, NODs and CLRs, among others [98]. Yet, the surprising fact is that the excessive inflammation in CARD9-deficient mice arises from defective production of IL-10 [98], and given that CLRs such as Mincle utilize this pathway to produce IL-10, it is tempting to conclude that CLRs indeed participate in the protection against excessive immune responses against Mtb that could result in inflammatory pathology. All things considered, the suppressive nature of CLR-induced IL-10 to dampen the pro-inflammatory response carried by other receptor families is an example of the antagonistic signalling crosstalk to prevent collateral tissue damage.

M. tuberculosis manipulation of CLR signalling crosstalk in innate immunity: the induction of anti-inflammatory mediators

As part of the constant evolutionary process-taking place in all living organisms (e.g. the arms race of host-microbes), microbial pathogens have evolved ingenious ways to evade the host immune response. One of these strategies includes the ability to either prevent an inflammatory response or hijack the anti-inflammatory mechanism in place to protect and maintain the integrity of local tissues. The manipulation of receptor crosstalk in innate immunity is one of these ingenious strategies [149]. Subverting receptor crosstalk from the innate immune response makes sense from the point of view of pathogen survival since it represents the first line of an active defense system in the host, and if successfully done, it can then undermine the overall adaptive immune response [149]. One way for the pathogen to manipulate receptor crosstalk is by the induction of immunosuppressive mediators such as IL-10. As previously described, the IL-10 signaling pathways has an essential role in maintaining homeostasis of the immune system, specifically in peripheral tissues constantly exposed to external environment and that host symbiotic microorganisms such as mucosal sites [88, 158–160]. Excessive production of this anti-inflammatory cytokine results in the impairment of the phagocyte killing capacity, inhibition of upregulation of co-stimulatory molecules (e.g. CD80, CD86) in antigen-presenting cells (APCs), dampening of pro-inflammatory cytokines, preventing of neutrophil recruitment to the site of infection, promoting immune deviation of the T-helper response (Th1 towards Th2) by decreasing the production of Th1-promoting IL-12, that altogether could result in the increased fitness of the invading pathogen [149]. Given that CLR

activation exerts an antagonistic signalling crosstalk through the production of IL-10 to either maintain homeostasis in peripheral tissues or to prevent an excessive inflammation, this family of innate immune receptors represents ideal candidates for an invading pathogen to subvert.

In the case of Mtb, as well as other clinically pathogens (*M. leprae*, *H. pylori*, *C. albicans*, among others), it has evolved the unique ability to influence the crosstalk between TLRs and CLR s in order to modulate the pro-inflammatory being raised against it and tilt the balance in its favor. This is accomplished by targeting hDC-SIGN to induce the immunosuppressive mediator IL-10 and counteract the pro-inflammatory response via TLRs [43, 149]. Indeed, upon the phagocytosis of the bacilli, ManLAM is released into the local environment where it can act to bind hDC-SIGN in an autocrine or paracrine manner, and induce a complex signaling cascade that activates the serine/threonine kinase Raf-1 [43, 149]. The activation of Raf-1 leads to the phosphorylation of the p65 subunit of NFκB on Ser276 and the eventual acetylation of different lysines, enhancing its DNA-binding affinity and transcriptional activity. A direct consequence of prolonged presence of NFκB in the nucleus is the enhanced transcription of the IL-10 gene [43, 149]. The ability of Mtb to hijack the production of IL-10 via DC-SIGN permits it then to modulate the crosstalk with TLRs, resulting in the inhibition of the expression of co-stimulatory molecules and downregulation of IL-12 production by APCs [43, 161], and therefore, shifting the adaptive immune response in its favor (from Th1 towards Th2). Of note, the evidence provided for the crosstalk between DC-SIGN and the TLRs has been in the context of TLR4 stimulation; it remains to be established whether the ability for DC-SIGN to potentiate IL-10 production holds true also with TLR2, which is more relevant than TLR4 in the context of Mtb infection.

Inhibition of the differentiation and maturation of antigen-presenting cells

Beyond the known effects that excessive production of IL-10 has on the overall immune response, Mtb might target CLR s to induce IL-10 and modulate the differentiation and maturation of APCs in order to enhance its survival fitness in the host. APCs, such as DCs and macrophages, perform an array functions that includes the constant monitoring and interaction with their local environment, effective detection and capture of invading microbes, proper activation of early defensive mechanisms of inflammation and innate effector cells, a rapid relay of innate information signals to the adaptive immune system resulting in the development of strong immunological memory, and the modulation of peripheral tolerance to avoid excessive or detrimental immunological responses leading to disease [162]. Given the pivotal role APCs play as sentinels and orchestrators of the immune system, and the fact these cells expressed a wide array of CLR s that induce anti-inflammatory mediators [27, 156], they represent ideal cell targets for inhibition by invading pathogens such as Mtb. Indeed, there is evidence that Mtb-induced IL-10 influences the differentiation and maturation

of APCs. It has recently been shown that the Mtb-induced IL-10 inhibits the differentiation of human monocytes into CD1c⁺ DCs *in vitro*, suggesting that Mtb influences the recruitment and replacement of this important APC to the site of infection [163]. In addition, Mtb-induced IL-10 appears to inhibit the maturation of DCs. Dulphy et al. [161] demonstrated that DCs treated with ManLAM displayed an intermediate maturation status characterized by reduced levels of MHC class I and II molecules, CD83 and CD86 co-stimulatory molecules, and chemokine receptor CCR7 that is essential for the DC migration to lymphoid organs. As a consequence of this partial maturation status, ManLAM-treated DCs failed to prime naive T cells [161]. Furthermore, Schreiber et al. demonstrated that Mtb-induced IL-10 in macrophages promotes the differentiation into “alternative” macrophages displaying diminished anti-mycobacterial effector mechanisms compared to pro-inflammatory macrophages [164]. Using macrophage-specific overexpressing IL-10 transgenic mice, the authors demonstrated these animals were highly susceptible to Mtb infection without an obvious effect in Th1 differentiation, displayed a specifically suppressed IL-12 in infected tissues, and were characterized by lung macrophages with an alternative phenotype permissive to Mtb infection [164]. This Mtb-induced deviation into alternative macrophages correlates well with another study where Mtb was shown to promote its survival and ability to cause disease through a MyD88-dependent induction of macrophage arginase 1 (ARG1), which inhibits nitric oxide production by macrophages by competing with iNOS for the common substrate, arginine [149, 165]. The Mtb-induced ARG1 expression modulates macrophage differentiation into an alternative phenotype by decreasing nitric oxide production and become permissive to the Mtb infection. Taken together, these observations suggest the modulation of the differentiation and maturation of APCs by IL-10 might be a novel mechanism of immune escape by persistent pathogens. However, it should be noticed that this phenomenon might also represent a control mechanism of the immune system to preserve the integrity of the organ upon persistent infections. In either case, it is likely that CLR s play a significant role in this phenomenon.

Are effectors and cellular processes for mycobacterial killing modulated by M. tuberculosis to escape immune response and persist?

Another reason to target CLR s for the induction of immunosuppressive mediators by Mtb might be to down modulate effectors with the aim to reduce mycobactericidal mechanisms. Upon recognition of Mtb, macrophages up-regulate the expression of various gene products known to function as effectors for mycobacterial killing [148]. In human macrophages, the induction of *Cyp27b1*, a catalyzer of provitamin D conversion into the bioactive form of vitamin D, leads to the eventual up-regulation of anti-microbial peptides such as cathelicidin and β-defensin HBD-2 that have been shown to contribute to Mtb killing upon phagosome and autophagosome maturation [148, 166, 167].

Interestingly, TLR-2 signaling has been involved in the up-regulation of the expression of HBD-2, indicating this microbial peptide is part of the pro-inflammatory response [148, 168]. In murine macrophages, the induction of *slpi* (secretory leukocyte protease inhibitor) expression is TLR-dependent and involved in the innate immune response against Mtb; SLPI is known to inhibit Mtb growth through disruption of the cell wall structure [148, 169–171]. In fact, mice deficient for *slpi* are highly susceptible to mycobacterial infection, confirming its vital role in anti-mycobacterial immunity [148]. An additional effector for mycobacterial killing is Lcn2 (or siderocalin) whose expression is induced in murine macrophages of LPS-treated mice, and released into alveolar space by alveolar macrophages and epithelial cells during the early stages of Mtb infection [172]. The availability of Lcn2 apparently sequesters iron inside of the cytoplasm of alveolar epithelial cells and macrophages, rendering these cells impermissible for mycobacterial intracellular growth by iron starvation (or deprivation) [148, 172–174]. In the absence of LCN2, mice displayed a pronounced susceptibility to Mtb intratracheal infection, confirming its crucial role as an effector of mycobacterial killing [173]. Although there are no reports demonstrating that Mtb has the ability to modulate down the expression or activity of these genes, over-expression of IL-10 has been correlated with the downregulation HBD-2 expression in atopic dermatitis [175]. It would be interesting to examine if Mtb-induced IL-10, or any other Mtb-immunosuppressive mediator, can actually dampen the activity of these effectors for mycobacterial killing via CLR signaling.

Finally, Mtb is an intracellular pathogen well known for its ability to persist and develop in phagocytes by disrupting normal maturation of the phagosome, or even by possibly escaping in the cytoplasm [176]. In addition to direct and active effects mediated by mycobacterial antigens, it is reasonable to postulate that immunomodulatory effects induced by CLR recognition could mediate the inhibition of important processes favoring the elimination of intracellular pathogens such as phagocytosis and autophagy. Autophagy, a process highly conserved among eukaryotic species, was first described in the 1960s as a lysosomal catabolic process involved in the breakdown, elimination and recycling of cytoplasmic components of the cells. This highly regulated process is a key effector response to starvation. Its functions are related to developmental and physiological processes, lifespan extension, cancer, neurodegeneration and also infectious diseases. Autophagy (also termed macroautophagy or xenophagy) has been demonstrated to be important for the degradation of intracellular pathogens such as Mtb [148, 177, 178]. This cellular process is thought to proceed via the fusion of Golgi- and/or endosomal-derived vacuoles with a subdomain of the endoplasmic reticulum (omegasome). Then specific maturation and recruitment of autophagy effectors allow the elongation of this double isolation membrane to either randomly engulf cytoplasmic components (e.g. peroxysome and mitochondria), or specifically load ubiquitinated protein complexes of large size. The formation of the autophagosome and then of the autophagolysosome

marks the completion of the process. Similar to phagocytosis, the final stage of the maturation process involves acidification together with the acquisition of bactericidal function (e.g. accumulation of the antimicrobial peptides regulated by vitamin D [179]), and thus creating an overwhelming toxic environment for an intracellular pathogen to thrive on. In addition, the autophagosome can fuse with phagosome and therefore these two degradation processes are highly complementary. Autophagy is without a doubt a key cellular process that forms part of a pro-inflammatory response to contain the spread of an intracellular pathogen and ensure its proper elimination from the host [148, 177, 178].

As described above, autophagy is known to contribute to the innate immune response by promoting phagolysosomal maturation in macrophages, a process highly dependent on the recruitment of autophagy effectors. For instance, the IFN γ -induction of *lrg47* (also known as *irgm1*), a member of the immunity-related p47 guanosine triphosphatases (IRG) family, is an essential effector in autophagy. The unequivocal anti-mycobacterial role *in vivo* for *lrg47* was demonstrated by the high susceptibility to Mtb infection displayed by mice that are deficient for this gene [180]. Interestingly, LRG47 expression is thought to be upregulated by LPS stimulation via TLR-4 in macrophages, thus establishing a close link between innate immunity and autophagy [181]. In addition to LRG47, a key regulatory step in both autophagy and phagocytosis is the formation and accumulation of an essential phospholipid (PI3P). PI3P earmarks intracellular organelles destined for degradation for binding and assembly to other effector molecules (e.g. Hrs and ESCRT components), which are required for sequential protein and membrane sorting within the phagosomal system [182]. The regulation of PI3P accumulation has been shown to be essential for autophagy to efficiently act against Mtb [183]. Another important gene for the process of autophagy is *p62* (A170 or SQSTM10). This autophagy effector is implicated in cargo-mediated recognition of cytosol components such as large polyubiquitinated proteins [184]. It regulates the formation of an ubiquitin-dependent sequestosome in order to isolate intracellular bacteria such as *L. monocytogenes* and *Shigella*. However, in the case of Mtb, the p62 sequestosome does not act as a xenophagy-addressing complex but facilitates phagocytosis and autophagy by degrading poly-ubiquitinated proteins and generating new anti-microbial peptides, which limits the spread of Mtb [183, 185]. This p62-dependent anti-mycobacterial effect corroborates a previous finding that mycobacterial killing by ubiquitin-derived peptides is enhanced by autophagy [148, 185, 186]. Given the importance of these effector molecules to the process of autophagy, it is plausible these are perfect targets for inhibition by Mtb.

Different pathogens have been selected for their ability to avoid autophagy. Some intracellular bacteria such as *Shigella* and *Listeria* sp. can escape the normal autophagic process by blocking autophagosome maturation [178]. Some viruses have also been selected for their ability to interfere with autophagosome maturation or to divert it at

their own benefit [178, 187]. Regarding Mtb, it is still unclear how the bacteria can corrupt this process in order to persist in host cells. However, it has recently been demonstrated in a genome wide-screen that Mtb tends to turn-off autophagy in infected macrophages [188]. Different hypotheses can be proposed. First, a mycobacterial factor could directly interfere with the process. Second, by inducing specific immunomodulatory molecules, Mtb could block autophagy by displaying either autocrine or paracrine effects. Indeed, autophagy is a highly regulated process mostly induced by TLRs, NLRs and Th1 cytokines, whereas it is suppressed by Th2 cytokines such as IL-4 and IL-13 [178]. Therefore, one can imagine that Mtb-induced IL-10, or any other Mtb-immunosuppressive mediator, via CLR signaling, can influence the expression or function of the autophagy effectors. There is evidence, as a matter of fact, that shows the inhibitory role of HIV-induced IL-10 in autophagy induction in human macrophages [189]. This inhibition of autophagy by IL-10 was recently corroborated in murine macrophages as well, and this process involved the activation of the mTOR complex 1 (mTORC1), a protein complex known to negatively regulate the initiation of autophagy [190]. This evidence suggests that the inhibition of autophagy by pathogen-derived IL-10 may be an immune evasion mechanism used by invading pathogen, and it is possible that the targeting of CLRs to induce IL-10 might be involved in this process.

Conclusions

Despite the fact that the first lectin was identified and characterized over 30 years ago [191], the characterization of CLRs that signal to induce innate and adaptive immunity has been slow until recently. Indeed, current interest in the development of effective drug targets and in the host immunity for the treatment of emerging diseases such as TB has sparked a rapid advance in our understanding of the biology of CLRs. Yet, much still remains to be done in order to decipher the innate immune response to Mtb as a system, and to determine the integrated roles of all PRRs recognizing Mtb in such a global network of signaling and regulation. In this review, we provided a quick survey of recent literature concerning the CLR diversity, their ligand binding properties, their synergistic and antagonistic signalling crosstalk with other receptor families, and their impact on TB. We envisage that further understanding of CLR biology in Mtb infection will come from the continuing search for novel receptors and their mycobacterial ligands, studying signaling pathways and sequence motifs involved in CLR signalling crosstalk and function upon stimulation with Mtb, and elucidating the CLR impact in innate and adaptive immune responses to acute and latent Mtb infection. Similarly, further advances in the knowledge of the housekeeping functions of CLRs in maintaining the delicate balance in peripheral tissues such as mucosal sites, will also provide great benefits to current efforts to the development of effective therapeutic and prophylactic ap-

proaches for controlling Mtb infection. Special emphasis should be placed to the identification of endogenous ligands to CLRs and their functional response to them. Finally, we believe that a global approach to study CLRs in the context of other PRRs recognizing Mtb should provide further insight into their function as an integrated system, and therefore, shedding some light into understanding the mechanisms of signalling crosstalk manipulation by Mtb, resulting in promising options for controlling infection and immunopathology.

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