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Lipid metabolism and its implication in mycobacteria-host interaction

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Abstract

The complex lipids present in the cell wall of *Mycobacterium tuberculosis* (*Mtb*) act as major effector molecules that actively interact with the host, modulating its metabolism and stimulating the immune response, which in turn affects the physiology of both, the host cell and the bacilli. Lipids from the host are also nutrient sources for the pathogen and define the fate of the infection by modulating lipid homeostasis. Although new technologies and experimental models of infection have greatly helped understanding the different aspects of the host-pathogen interactions at the lipid level, the impact of this interaction in the *Mtb* lipid regulation is still incipient, mainly because of the low background knowledge in this area of research.

Introduction

Tuberculosis (TB) is a lung infection disease caused by *Mtb* that has afflicted humans for thousands of years, and still remains a major health emergency provoking more than a million deaths each year (World Health Organization, 2016). The fact that approximately one third of the world's population is infected with *Mtb* demonstrates a remarkable well-adapted long-term interaction between this pathogen and its host. *Mtb* is transmitted to a new individual via inhalation and within the lung bacteria are ingested by alveolar macrophages, the first line of the innate immune system [1]. However, due to the effectiveness of this pathogen at subverting many of the host immune defenses, instead of being cleared by the immune system, *Mtb* often resist degradation by arresting phagosome maturation creating a permissive niche, in which it can persist or replicate to ultimately trigger the formation of a granuloma [2,3]. Furthermore, it is well established now that a fraction of the *Mtb* population can produce phagosomal rupture and escape to the cytosol allowing bacterial replication and inducing host cell death involving necrosis [4–6]. In order for *Mtb* to succeed and actively replicate causing an acute TB, or to survive within the granuloma for long periods of time, in an asymptomatic state, *Mtb* has evolved a wide array

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of specific lipids and related metabolisms that actively interact with the immune response and the lipid metabolism in the host [7,8]. This review will focus in the latest understanding of the adaptation and regulation of the different lipid related pathways in *Mtb* and in the interactions between the bacteria and the host lipid metabolisms during the infection process.

Lipid Metabolism and its regulation in the fate of *Mtb* infection

The complexity of lipid biosynthesis in Mtb

The composition and complexity of the mycobacterial cell envelope is the most distinctive feature of the *Mycobacterium* genus. Cryotransmission electron microscopy (EM) data provided direct evidence for a multilayered cell wall organization in mycobacteria and confirmed that the outermost layer of mycobacteria is an outer membrane with a bilayer structure [9,10]. The most recent model divides the mycobacterial cell envelope in three entities: an outermost layer, also called capsule in the case of pathogenic species, a cell wall core and a conventional plasma membrane [11]. The cell wall core consists of the mycomembrane and peptidoglycan (PG) covalently linked to arabinogalactan (AG). The mycomembrane exhibits an asimetric bilayer organization: the inner leaflet is made of α-alkyl, β-hydroxy long-chain mycolic acids linked to AG and the outer leaflet is composed of free, non covalently bound to the cell, lipids and glycolipids like trehalose monomycolates (TMM), trehalose dimycolates (TDM), glycerol monomycolates, glucose monomycolates, phthiocerol dimycocerosates (PDIM), poly-acylated threaloses (PAT), sulfolipids (SL), phosphatidylinositol mannosides (PIM), phenolic glycolipids (PGL) and mannose-capped lipoarabinomannans (Man-LAM). Genomics, bioinformatics, proteomics as well as advances in genetic manipulation of mycobacteria have resulted in a thorough understanding of the enzymes involved in the biosynthesis of the main cell envelope constituents. A remarkable characteristic of mycobacteria is the use of a multifunctional fatty acid synthase (FAS) system, FAS I, for *de novo* synthesis of medium and long chain acyl-CoAs [12,13]. The extraordinary diversity of lipids synthesized by *Mtb* is directly related with the unusual level of complexity regarding the fate of fatty acids, as they are substrates of all the different lipid biosynthetic pathways. The complex interplay that exists between these pathways is schematized in Figure 1.

Role of Lipids in Mtb entry and phagocytosis mechanisms

Several studies have demonstrated the relevance of *Mtb* and the host lipids in their interaction and in the early events of phagocytosis (Figure 2) [7,8,14]. The entry of *Mtb* into the macrophage occurs through receptor mediated phagocytosis and in this process the most relevant components are the pathogen-associated molecular patterns (PAMPs) [15], which are recognized by macrophages and dendritic cells (DCs) through pattern recognition receptors (PRRs). This recognition triggers the internalization of bacilli and the lipid specific signaling cascades that differentially modulate the host immune response [reviewed in 15,16] (Figure 2). A recent publication in this area demonstrated that sulfoglycolipids contribute to *Mtb* virulence by inhibiting the human innate immune responses acting as competitive antagonists of TLR2 receptors [17].

Lipids and modulation of phagosome-maturation

Once mycobacteria is delivered into a phagosome, it could be eliminated by the cellular lysosomal machinery; however, virulent mycobacteria have evolved unique strategies to modulate host endocytic pathways, including immune evasion [18,19]. Cell wall lipids may have multiple, overlapping functions [8] and this is the case for Man-LAM, TDM and PDIM, which besides their role in phagocytosis they also mediate the intracellular trafficking and the vacuole maturation arrest induced by *Mtb*. While most wild type *Mtb* are found in non-acidic phagosomes, PDIM-deficient mutants are principally found in acidic phagosomes, demonstrating the relevance of PDIM in the arrest of phagosomal acidification [20]. Furthermore, the characterization of mutants attenuated for intracellular survival revealed that ESX-1 and PDIM deficient mutants shared similar characteristics of attenuation (Figure 2). Building upon the shared phenotype of these mutants, it was found that PDIM production and export are required for phagosomal permeabilization and for production of the macrophage type I interferon response [21,22]. The characterization of an *Mtb* mutant in the transcriptional repressor Rv3167c demonstrated that increased PDIM levels correlated well not only with an increased capacity of *Mtb* to escape from the phagosome but also to induce host cell necrosis; attributing a new role to PDIM in intracellular host-cell modulation [23]. TDM also modulate phagosome maturation but by not fully understood mechanisms [24]. Only recently, it was shown that IgG-opsonized TDM-coated particles could recruit Mincle and FcγRIIB to induce signals that delay FcγR-mediated phagosome maturation [25]. The contribution of SL, DAT and PAT to bacterial survival, is relevant only in a PDIM mutant strain [8].

Lipids and granuloma biogenesis

The vacuole that contains *Mycobacterium* (MCV) encloses lipids moieties of the mycobacterial cell wall [26], which are trafficked out of MCV to associate with several intracellular organelles, representing one possible mechanism by which *Mycobacterium* influences the environment within the infection foci [27]. These lipids were also found extracellularly in small vesicles where they can elicit proinflammatory cytokines from macrophages, contributing to the granulomatous response [28]. Although *Mtb* survives and grow, albeit slowly, inside vacuoles, this bacterium can also escape from the MCV to the cytosol and induce a non-apoptotic mechanism [4–6], supporting mycobacterial growth and the recruitment of additional host phagocytes which leads to infection spread. *Mtb* aggregates can also be internalized by macrophages directly into the cytosol, where they actively replicate and kill the host cell. Dead cells full of active bacteria can then be phagocytosed by other macrophages triggering a cell death cascade, which might be the dynamics of necrosis and bacterial proliferation in lung granulomas of active TB [29].

Lipid metabolism and regulation within the host

Mtb resides or replicates in a very nutritionally-defined environment, either a macrophage, a DC, or a granuloma, relying on specific metabolic pathways to use host-derived nutrients [30]. The different carbon and nitrogen sources, as well as the varying oxygen tension that *Mtb* encounters during infection are known to impact the lipid composition of the envelope, i.e. the changes observed in the typical acid-fast staining of *Mtb* during infection [31].

Although it has been shown in different models that *Mtb* uses different carbon sources at different stages of the infection process [32–35], it is generally accepted that host lipids are the primary carbon source for *Mtb in vivo*. Furthermore, *Mtb* infected macrophages induce the formation of foamy macrophages (FM) by the accumulation of lipid bodies (LB) which mainly contain cholesterol esters and TAG (Figure 2). The formation of FM is a clear consequence of a bacilli manipulation of the host metabolism promoting the accumulation of neutral lipids [36,37]. Within the FM, *Mtb*-containing phagosomes progressively surround and engulf the LB, which then serve as nutrient for the microorganism [38]. Under these conditions *Mtb* faces important physiological changes that result in the accumulation of TAG within intracytosolic lipid inclusions (ILI), reduced growth and lower metabolic activity and resistance to antibiotics; which represent a hallmark of persistent and non-dividing bacilli [34]. Interestingly, TAG accumulation can be prevented by transporting them out of the cell through the putative efflux pump LprG-Rv1410 which is essential for *Mtb* growth in mouse [39].

The characterization of several mutant strains, demonstrated that genes involved in metabolizing the products of FA oxidation through the glyoxylate shunt and the gluconeogenesis had a strong impact in the *Mtb* life cycle in different infection models [40–42]. Mutants in the cholesterol transport system Mce4 provided the first evidences that this lipid was not required for establishing infection but was essential for persistence in the lungs of chronically infected animals and for growth within the IFN-γ-activated macrophages [43]. Later on, mutants unable to metabolize cholesterol also showed important defects in intracellular growth or survival [44]. Furthermore, a whole cell-based drug screen against *Mtb* in macrophages found that a large fraction of hit compounds inhibited cholesterol related processes, indicating that cholesterol plays a central role for *Mtb* during infection [45]. However, it has been demonstrated that *Mtb* co-metabolizes simple carbon substrates *in vitro* [46], suggesting that this bacterium uses both cholesterol and FAs during infection. Supporting the simultaneous use of FAs and cholesterol, it was shown that the metabolic pressure experienced by *Mtb* inside the host macrophage by the use of cholesterol can be balanced by the utilization of host FAs which increase the acetyl-CoA pool and allows the utilization of propionyl-CoA in the synthesis of methyl-branched lipids [30]. Furthermore, a protein named LucA was found to facilitate the simultaneous uptake of FA and cholesterol by stabilizing protein subunits of the Mce1 and Mce4 transporters. These studies also revealed that the Mce1 complex transports fatty acids and that LucA is essential for full virulence *in vivo* [47]. Altogether, these results suggest that although FAs cannot substitute for cholesterol during intra-cellular growth, they are needed in order to prevent or relieve the toxicity of propionyl-CoA. It will be interesting to obtain mutants unable to β-oxidize FAs to rigorously test the essentiality of this catabolic pathway. Likewise, it is still not known if exogenous FAs could overcome the absence of *de novo* FA biosynthesis.

As shown in Figure 1, FAS I provides the substrates for several other lipids biosynthesis pathways in *Mtb,* suggesting that a highly complex network of regulation between all these pathways should exist in order to maintain lipid homeostasis under control. However, the components and the molecular mechanisms involved in the regulation and interaction of all these pathways, is only starting to emerge. Regulatory proteins and transcriptional regulators, that directly control the transcription of lipids biosynthesis genes, have been

described. The first transcriptional regulator of the main *fasII* operon (*fabD-acpM-kasA-kasB*) that was characterized is MabR [48]. Genetic studies showed that *mabR* is essential for *M. smegmatis* survival and biochemical analysis carried out in a *mabR* conditional mutant strain showed alterations in mycolic acid and in *de novo* FA biosynthesis, demonstrating for the first time the existence of a crosstalk between the two FAS systems and confirming MabR as one of the key modulator of lipid homeostasis in *Mycobacterium* [48,49]. A second non-essential transcription factor, FadR, that represses the *fasII* operon expression has also been described [50]. FadR is induced upon starvation, leading to reduced *fasII* expression under those conditions [51]. The *hadABC* genes, coding for the dehydratase complexes of the FAS II system, are part of a seven-gene operon together with four genes involved in translation, and they all respond to the alarmone (p)ppGpp which leads to dramatic reprogramming of cell transcription [52]. This suggests that the coordination of the expression of the complete set of FAS II genes is really complex and indicates that an eventual interplay exists between different regulatory pathways. The transcription of the *fas-acpS* operon (encoding for the multidomain FAS I and the phosphopantetheinyl transferase AcpS) is regulated by FasR [53]. Analysis of a conditional mutant in *M. smegmatis* showed that FasR is a transcriptional activator and proved its essential role in mycobacteria viability. Interestingly, the activity of all these transcription factors (MabR, FadR and FasR) is modulated by long chain acyl-CoAs, the products of the FAS I system, highlighting a key role for these molecules in the modulation of lipid biosynthesis in mycobacteria [49,50,53]. An important difference between MabR and FasR with other bacteria transcriptional regulators of FA biosynthesis is that these two proteins are essential for bacterial survival while all the others are not. This suggests that the coordination and modulation of the two FAS systems is highly relevant in order to maintain lipid homeostasis in mycobacteria. Different approaches also revealed that genes involved in complex lipid biosynthesis (SL, DAT/PAT and PDIM) are up regulated upon infection [54,55]. However, only some global transcriptional regulators of those pathways have been described. EspR, a nucleoid-associated protein with architectural and regulatory roles, impact cell wall functions and pathogenesis through the transcriptional regulation of multiple genes, particularly those involved in PDIM biosynthesis [56]. The global transcriptional regulator PhoP has also been involved in the EspR mediated response [57]. Disruption of PhoP leads to the absence of SL, DAT, and PAT [58] and global transcription assays demonstrated that PhoP is an activator of the genes required for SL and DAT/PAT biosynthesis [59,60]. More recently, Quigley *et al* (2017) analyzed the regulon of the transcription factor Rv3167c [23], previously characterized as a repressor of *Mtb* virulence [61], and found that it is enriched for genes involved in the synthesis and transport of PDIM. However, this regulation appears to be indirect and the ligands and regulators involved in the molecular mechanism of this response remains to be elucidated.

The impact of having reduced levels of *de novo* FA biosynthesis was studied in a *fas* conditional mutant in *M. smegmatis* [62]. As expected, *fas* was essential for survival and in the non-permissive conditions the reduction of FAS I activity led to the accumulation of the FAS I substrates (acetyl-CoA and malonyl-CoA) and to a strong reduction of $C_{12–18}$ acyl-CoAs. Unexpectedly, even when *de novo* FA biosynthesis was impaired, the *fas* mutant was still able to synthesize mycolic acids at the expense of TAG, suggesting that storage lipids

could be an intracellular reservoir of FAs for the biosynthesis of complex lipids in mycobacteria. Understanding the interaction between FAS I and the metabolic pathways that rely on FAS I products is a key step to better understand how lipid homeostasis is regulated in *Mtb* and how this regulation could play a role during infection in pathogenic mycobacteria.

Conclusions

The extensive exchange of information between *Mtb* and its environment during infection induces a reciprocal modulation of both, host and bacteria metabolisms. Many of these interactions are mediated by lipids, opening the question about how different lipids or lipids derive molecules interact and modulate host and pathogen metabolisms for the outcome of an *Mtb* infection. On this regard, identifying and characterizing the proteins, the signal molecules and the mechanisms involved in the complex network that regulate lipid homeostasis in *Mtb,* in the context of the host environment, could help us identify essential regulatory elements for maintaining an efficient and successful interaction of the pathogen with the host. These key regulatory components could be potential drug-targets for the development of conceptually new anti-mycobacterial agents.

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◆◆ of outstanding interest

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Lipids from *Mycobacterium tuberculosis* and the host play essential roles in almost every step of the infection cycle of this pathogen.

Mtb lipid metabolism is highly complex and the regulatory network that preserves lipid homeostasis in this organism is now starting to be understood.

Host lipid metabolism has strong impact in *Mtb* lipid homeostasis

The regulatory proteins involved in the regulation of *Mtb* lipid metabolism are potential therapeutic targets.

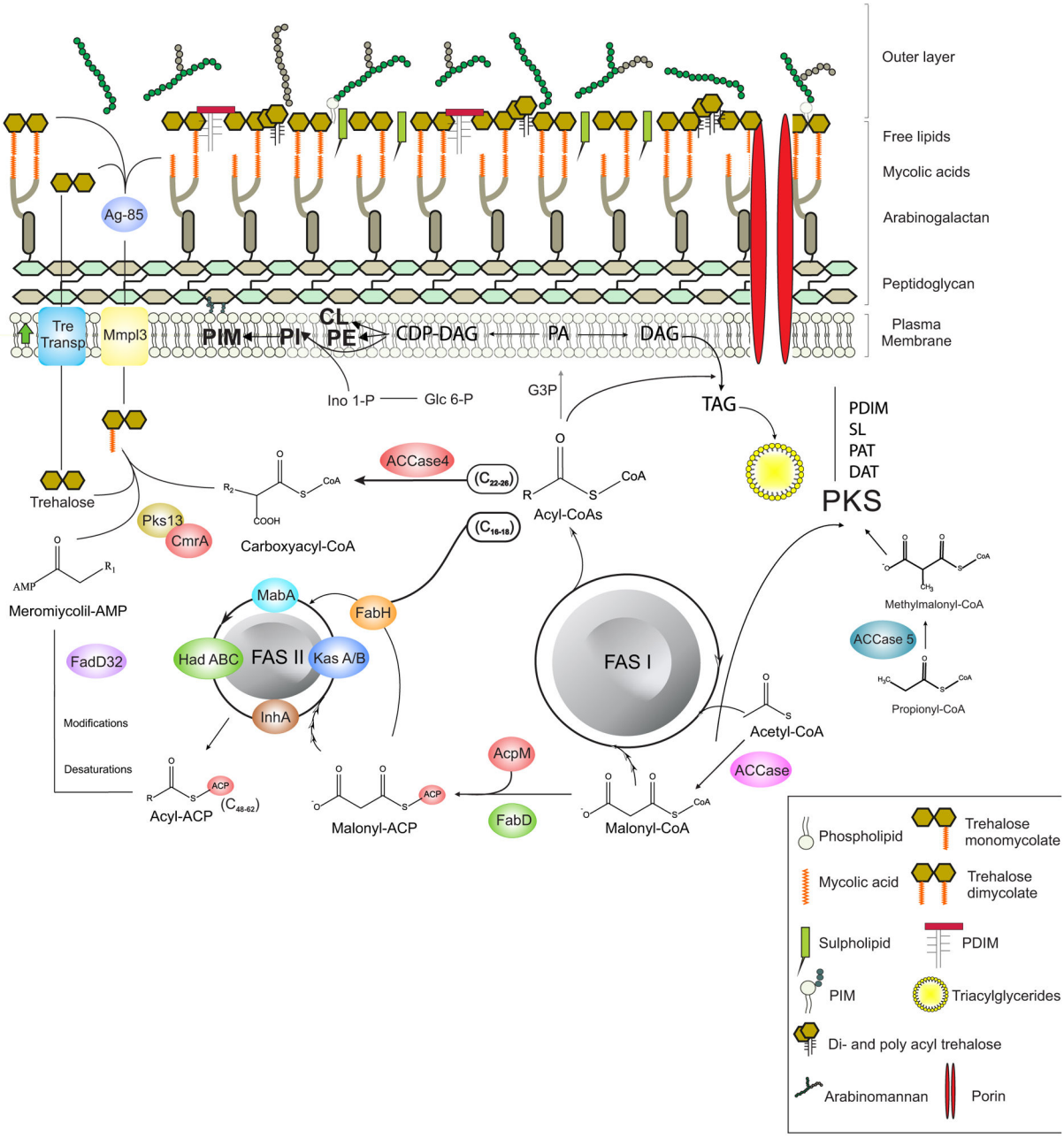

Figure 1.

Schematic representation of lipid biosynthesis pathways and their interactions in *M. tuberculosis.*

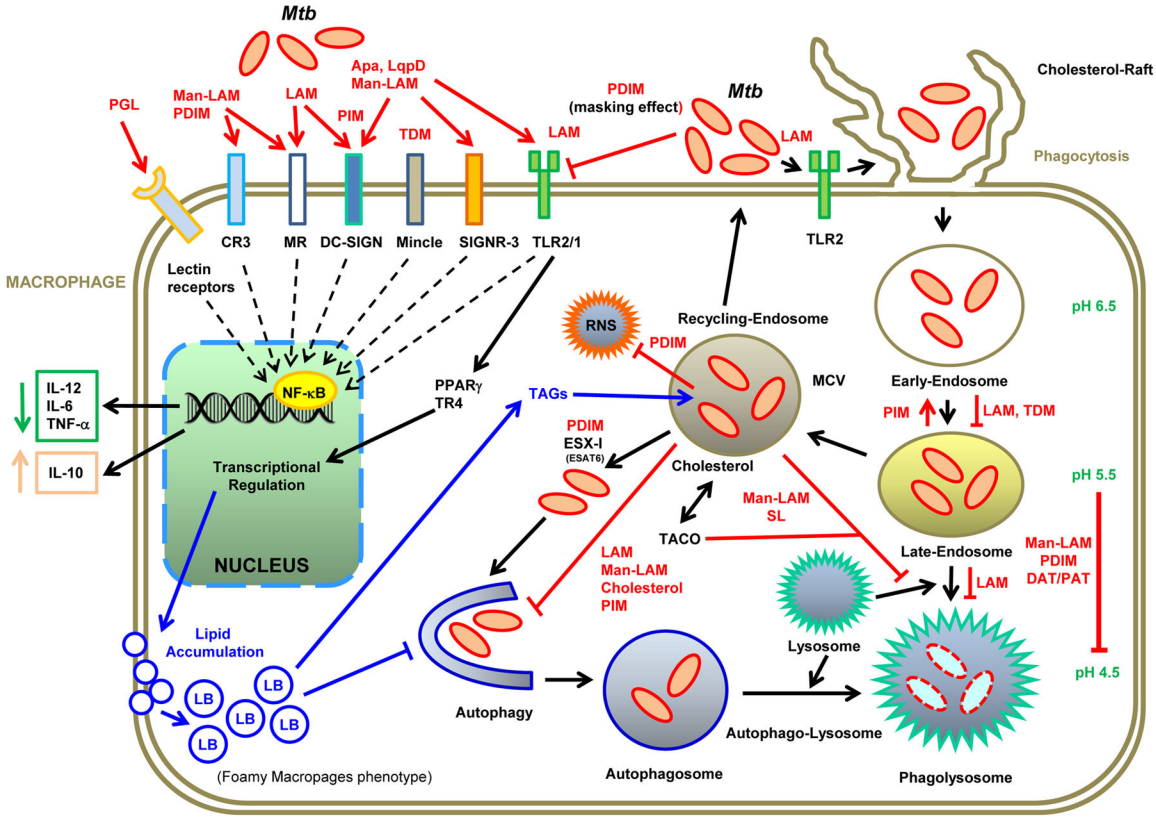

Figure 2.

Role of the host-mycobacterial lipids interactions during macrophages infection.

The cell envelope of mycobacteria comprise a wealth of unique glyco-lipids that act as PAMPs and are recognized by macrophages and DCs through PRRs such as Toll-like receptors (TLRs), Nod-like receptors (NLRs), and CLRs. In this scheme, we highlight the main host-mycobacterial lipid interactions and their consequences. *Mtb* lipids prevent the phagosome maturation, acidification and fusion with lysosomes; they also inhibit autophagy, in order to create a permissive niche that allows bacteria to resist degradation, and eventually replicate within the macrophage. During infection, macrophages accumulate lipids that can be used for *Mtb* as carbon and energy sources. Apa and LpqH: surface glyco- and lipoproteins, TACO/coronin 1: actin-binding host protein, Mincle: macrophage-inducible C-type lectin, RNS: reactive nitrogen species, MR: C-type lectins Mannose Receptor, DC-SIGN: dendritic cell-specific ICAM-grabbing non-integrin (only in DCs), CR3: complement receptor type 3, SIGNR3: DC-SIGN homologue in mice.