Innate Immunity

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Introduction

Mycobacterium leprae is transmitted from an infected to a healthy person through aerosols containing bacteria. The most common route is likely to be the upper airways, indicating that interaction between *M. leprae* and the human host begins in the nasal mucosa. It is possible to detect *M. leprae* DNA in nasal swabs from healthy individuals in endemic areas (1, 2). A follow-up of asymptomatic qPCR positive household contacts of leprosy patients using nasal swabs indicates that the air route is an important point of entry for *M. leprae* and, therefore, that these contacts have a higher chance of developing leprosy (3).

In the infection sites, resident macrophages and dendritic cells participate in the initial interaction between pathogen and host. Curiously, *M. leprae* has an exclusive ability to infect Schwann cells in peripheral nerves. All of these cells have a prominent role in the host immune response against infection with *M. leprae*, participating in processing and antigen presentation, cytokine secretion, and elimination of the pathogen.

Macrophages are capable of inducing inflammatory mediators that may activate specific lymphocyte subpopulations (see Chapter 6.2). This process establishes a profile of innate/adaptive immune responses that can restrict or permit the growth of infectious agents. Therefore, the

initial interaction between *M. leprae* and macrophage processes is decisive for the outcome of the disease.

The immune response is dependent on genetic, environmental, and other factors that will ultimately induce macrophage activation that, in turn, may or may not commit the host cell towards effective antimicrobial activities. For example, a high bacilli count during infection can create a favorable niche for multiplication within macrophages (4). In fact, *in vitro* assays have shown that *M. leprae* is able to maintain a stable metabolic state for a few weeks inside macrophages with a permissive phenotype when IL-10 is added to culture media and maintained at 33°C (5). So, it is likely that a permissive program can be activated under certain conditions such as high bacillary numbers during infection and the presence of specific cytokines such as IL-10 (6). Other environmental factors can also impact disease outcomes, such as nutrition and pregnancy (7, 8, 9). The emergence of this type of permissive response is also dependent on genetic factors influencing the outcome of leprosy (see Chapter 8.1). It is worth reiterating that several genes encoding proteins involved in innate immunity have been associated with leprosy (10). In this regard, well-known innate pathways can play a role in disease outcome, including the development of inflammatory reactions. These latter forms are immune-mediated altered clinical states (see Chapter 2.2).

In several aspects, our knowledge regarding potential mechanisms of *M. leprae*-induced innate immune activation comes from studies using other pathogenic mycobacteria. But, in recent years, advances in methodological approaches contributed to the understanding of immunopathogenic processes related to *M. leprae* infection. In the first part of this chapter, we describe the non-specific early mechanisms of host-pathogen interaction for *M. leprae* uptake by Schwann cells or macrophages. We also explore the regulation of cellular responses such as autophagy that culminate in a restrictive status that favors the killing of *M. leprae*. In addition, the subversion of these antimicrobial responses that induces a permissive environment for the bacteria is discussed. The second half of this chapter discusses the events of innate response and iron metabolism that regulate the secretion of cytokines, macrophage differentiation, activation of T cells, and adaptive immunity.

Early Events of Innate Immunity

M. LEPRAE–HOST CELLS INTERACTION

Macrophages are present in almost all tissues, where they coordinate developmental, metabolic, and immunological functions and thus contribute to the maintenance of homeostasis. Macrophages provide a critical systemic network of cells to the innate immune system. They are major cytokine producers and are often placed to orchestrate not only innate but also adaptive immune responses (11). Tissue macrophages are one of the main host cells for *M. leprae*, although *in vitro*

experiments have shown that *M. leprae* is able to interact with keratinocytes promoting gene expression profile changes, increases in inflammatory cytokine, and cathelecidin production. Accordingly, the role of keratinocytes, in addition to macrophages, has been considered when evaluating the pathogenesis of leprosy (12, 13, 14). Indeed, a comprehensive understanding of macrophage biology is crucial for grasping *M. leprae* pathogenesis.

M1 and M2 activated macrophages co-exist in adult tissue-based studies focused on primary macrophages and cell lines that are exposed *in vitro* to single, strongly polarized ligands such as lipopolysaccharide (LPS), interferon γ (IFN-γ), and interleukin 4 (IL-4). The term 'classical activation', which originally referred to macrophages stimulated with IFN-γ, is now used interchangeably to describe macrophages stimulated with IFN-γ and with TLR (15, 16). In contrast, IL-4 is now described as inducing 'alternative activation', as compared to the effects of IFN-γ.

The initial phagocytosis of *M. leprae* is mediated by complement receptors CR1, CR3, and CR4 (17). At the same time, *M. leprae*-macrophage interactions are mediated by pattern recognition receptors (PRRs) that recognize common molecular structures associated with pathogens (PAMPs). The interaction involves PRRs such as toll-like receptors (TLRs) and nucleotide-binding oligomerization domain-containing protein 2 (NOD2). In this context, TLR2/1 heterodimer activation has been demonstrated to be crucial for *M. leprae* elimination. The mycobacterial synthetic lipoproteins (19 kDa and 33 kDa) increase pro-inflammatory cytokine responses in monocytes and dendritic cells mediated by TLR2/1 heterodimer (18). This activation leads to an increase in both IL-12 and tumor necrosis factor (TNF) release. The latter of these is responsible for driving protective Th1-type responses, as an M1 macrophage phenotype is predominantly observed. It has been shown that the expression of TLR1 and TLR2 is up-regulated in skin biopsies of tuberculoid (TT) patients as compared to lepromatous (LL) patients (18). Therefore, the activation of PRRs is key for stimulating antimicrobial responses. However, multiple TLRs participate during *M. leprae* infection that later trigger miRNA up-regulation as a feedback control. It is likely that TLR4 also participates in the *M. leprae* interaction, since miRNA-146a is one of the most highly induced miRNA by live *M. leprae* (19). The up-regulation of miRNA-146a could be interpreted as a mechanism that the pathogen uses to circumvent the antimicrobial response (20). In macrophage infection by other mycobacteria (BCG), miRNA-146a inhibits the activation of pro-inflammatory cytokines via down-regulation of IRAK1 and TRAF6 and induces the intracellular proliferation of bacteria (21).

Another important group of PRRs includes the nucleotide-binding oligomerization domain-like receptors called NLRs (or NOD-like receptors) that have been associated with the significant participation of the innate immune response during *M. leprae* infection (22). In this context, NOD2 is a cytoplasmic sensor responsible for recognizing peptidoglycan, including mycobacterial derivatives, in which the main ligand is muramyl dipeptide (MDP) (23, 24). NOD2 recognizes MDP and activates the transcription factor NF-kB through the adapter molecule RIP2, starting the production of pro-inflammatory mediators. Moreover, MDP can activate inflammation by caspase-1 recruitment, which is responsible for IL-1β cleavage and activation (25, 26). NOD2 activation increases the autophagy pathway in dendritic cells and mediates antigen processing and presentation (27).

REGULATION OF AUTOPHAGY AND THE TYPE I IFN PATHWAY

Functional genomics and epidemiological studies have identified new pathways and candidate genes involved in leprosy susceptibility. One of these studies demonstrated that the E3 ubiquitin ligase, PARK2 (parkin), is a key player in trafficking mycobacteria to the autophagy pathway. Of potential relevance, PARK2 is a gene that has polymorphisms associated with leprosy in different populations (28) (see Chapter 8.1). Parkin has also been described as one of the proteins regulating autophagy. There is a great similarity between the process by which intracellular bacteria and mitochondria are marked for autophagy or mitophagy (29).

Autophagy is a critical cellular process for controlling cytosol cleansing and can also direct pathogens for phagolysosomal degradation. Indeed, parkin knockdown reduces autophagy and increases *M. tuberculosis* viability (30). It is clear that autophagy plays a central role in the control of mycobacteria replication and spread. Several studies have dissected the pathways leading to activation of autophagy. *M. tuberculosis* induces selective autophagy mediated by ubiquitination in macrophage, resulting in the relocation of the bacilli to phagolysosomes. Other ubiquitin ligase proteins have been observed to cooperate with parkin in the formation of an autophagic complex in order to eliminate the mycobacteria (31). Autophagy induced by *M. tuberculosis* is dependent on the ESX-1 secretion system and host cytosolic receptor STING (Stimulator of interferon genes) (30, 32, 33) that is triggered by type-I interferons.

Type I IFN-induced pathways are classically associated with antiviral responses, but they can also be up-regulated using DNA microarrays during live *M. leprae* infection (34, 35). Although rare, leprosy and tuberculosis (TB) have been observed as side effects after treatment of hepatitis C with IFN-alpha (36, 37). Moreover, other global gene expression studies point to the importance of this pathway in TB progression (38). Indeed, an important family member of interferon-stimulated genes is 2'–5' oligoadenylate synthetase like (OASL), which has also been described as induced during progression from latent to active TB (39). OASL was strongly induced in Schwann cells and human macrophages during *M. leprae* infection, suggesting that this gene is an early sign of infection with virulent mycobacteria (35). The pathway that induces type I IFN is also dependent on the ESX-1/ESAT-6 mycobacterial secretion system and the cGAS/STING pathway (32). In addition, type I IFN induces OASL expression (35) and, when it is knocked down, a large decrease in the viability of mycobacteria and increased autophagy, thereby suggesting that the OASL participates in the regulation of an immune response that diverts antimicrobial responses. One of the readouts of this kind of response is CCL2/MCP-1, which is secreted in high amounts when a permissive phenotype is observed and is down-regulated when OASL is silenced (35). Curiously, parkin knockdown also decreased CCL2/MCP-1 and IL-6 production in cells stimulated with live *M. leprae* (40).

Thus, mechanisms that trigger Type I IFN and parkin are related to pathways induced upon *M. leprae* infection. But it remains unclear as to how the interaction of macrophages with *M. leprae* leads to autophagy, or to a permissive state that allows *M. leprae* a safe intracellular niche.

Another study has provided evidence that the permissive phenotype, largely observed in disseminated LL skin lesions, is indeed due to reduction in the activation of autophagy, decreasing macrophage antimicrobial activity (41). These data suggest that the inhibition of autophagy activation mediated by mycobacteria, and possibly involving down-regulation of parkin, constitutes a crucial immunological mechanism associated with control of disease and polarization towards disseminated forms of the disease.

In fact, a number of other simultaneous signaling processes involving pattern recognition receptors, such as NOD2 and TLR1/2, also participate in the response of inflammatory macrophages against infection by virulent mycobacteria. Genetic variations associated with susceptibility to leprosy suggest subtle changes in expression levels of genes in the type I IFN pathway, lipid metabolism, energy metabolism, and autophagy that could impact the disease outcome (see Chapter 8.1). In this context, lipid bodies (LBs), which are considered lipid-rich organelles, may also play an important role in regulating autophagy (42). The location of ubiquitination system components inside these lipid bodies (LB) suggests that these processes are actually interconnected in the regulation of innate immune responses to *M. leprae*.

METABOLISM MODULATION

Cholesterol is among the lipids with increased abundance in *M. leprae*-infected cells, but its essential role for *M. leprae* intracellular survival does not rely on its utilization as a nutritional source (43, 44). Even though they do not use cholesterol as a nutrient, macrophages infected with *M. leprae* have a greater capacity to synthesize and sequester exogenous cholesterol, contributing to bacterial intracellular survival. The analysis of LL macrophages has revealed an increased expression of host lipid metabolism genes (45). In this context, lipid body formation induced by *M. leprae* in the host cell has been associated with the pathogenesis of leprosy contributing to persistent infection (46). *M. leprae* decreases hormone sensitive lipase (HSL), facilitating the maintenance of a lipid-rich environment suitable for intracellular survival of *M. leprae*. Studies *in vitro* have demonstrated that live *M. leprae* has the ability to actively induce and support adipophilin/adipose differentiation related protein/peripilin (ADRP) expression to facilitate the accumulation of lipids within the phagosome (47). LBs are transported to lysosomes via autophagic complex formation, where LB hydrolysis occurs to generate free cholesterol efflux mainly dependent on ABCA1 in foamy macrophages. This process mobilizes cholesterol associated with LB for reverse cholesterol transport (48). Clofazimine, used in multibacillary (MB) multi-drug therapy (MDT), attenuates ADRP in *M. leprae*-infected cells and increases HSL (49, 50). Finally, statins, which are drugs that inhibit cholesterol synthesis, have demonstrated efficacy as an adjuvant to controlling the infection. The statins enhanced the antimicrobial effect of rifampin in *M. leprae*-infected mice, demonstrating that cholesterol metabolism modulation may play an important role in the pathogenesis of leprosy (51).

M. leprae has experienced reductive evolution at the genome level (see Chapter 8.2), which resulted in the loss of essential genes of bacterial metabolism associated with energy metabolism. Thus the bacteria became highly dependent upon host cell resources for its survival. This strategy

is demonstrated when *M. leprae* enhances G6PDH enzyme activity, and increases glucose uptake in Schwann cells, redirecting cell metabolism towards the pentose phosphate pathway (52). This strategy switches cellular responses to lipid synthesis and glutathione antioxidant system maintenance, suggesting a Warburg-like phenomenon. Also, a decrease in lactate release in a culture medium of *M. leprae*-infected Schwann cells can lead to lactate restriction for the axon. This lactate restriction may be involved in neurodegenerative and demyelination processes, both of which are observed in leprosy (50).

Metabolic pathway regulation has been shown to play a crucial role in leprosy pathogenesis. Metabolomic analysis shows that LL patients have altered metabolism when compared to healthy subjects (51). In fact, the patient's metabolic profile is restored after treatment. The altered profile mainly consists of polyunsaturated fatty acids with pro-inflammatory resolvins and higher levels of lipids derived from lipase activity. Modulation of polyunsaturated lipids as omega-3 and -6 has been observed during the course of *M. leprae* infection. Also, high levels of PGD_2, PGE_2, lipoxin A_4, and resolvins in skin lesions of LL patients have been reported (53).

Indeed, energetic metabolism can be orchestrated by growth factors. *M. leprae* infection is able to increase the expression and secretion of insulin-like growth factor-I (IGF-I), a classic hormone that exhibits anti-apoptotic and proliferative activities in Schwann cells and macrophages. Macrophages from dermal lesions of LL patients may show a higher expression of IGF-I than those from the self-limited borderline tuberculoid (BT) leprosy form. *In vitro* studies have demonstrated that the dampening of IGF-I signaling reverts the capacity of *M. leprae*-infected human and murine macrophages to produce antimicrobial molecules and promotes bacterial killing (54, 55, 56). The inhibition of antimicrobial activity in LL patients is in accordance with *in vivo* observations in which a higher SOCS3 expression and lower phosphorylation of STAT1 levels were found in LL versus BT dermal lesions (54, 55). An overview of the most important mediators and processes involved in the early events of the immune response after *M. leprae* infection is shown in Figure 1.

Late Events of Innate Immunity

IRON METABOLISM: REGULATION OF INNATE RESPONSES AND *M. LEPRAE* SURVIVAL

Mycobacteria require iron for growth and virulence. Iron is an essential nutrient for the survival of organisms, and pathogenic bacteria have developed sophisticated pathways to satisfy their iron requirements. With some exceptions like *Borrelia burgdorferi*, which uses manganese in place of iron, most pathogenic bacteria require iron to maintain essential pathways like electron transport, antioxidant defense, synthesis of aromatic amino acids, and other functions (65). Free iron is toxic to host cells. Therefore, both host and bacteria tightly regulate iron and heme release.

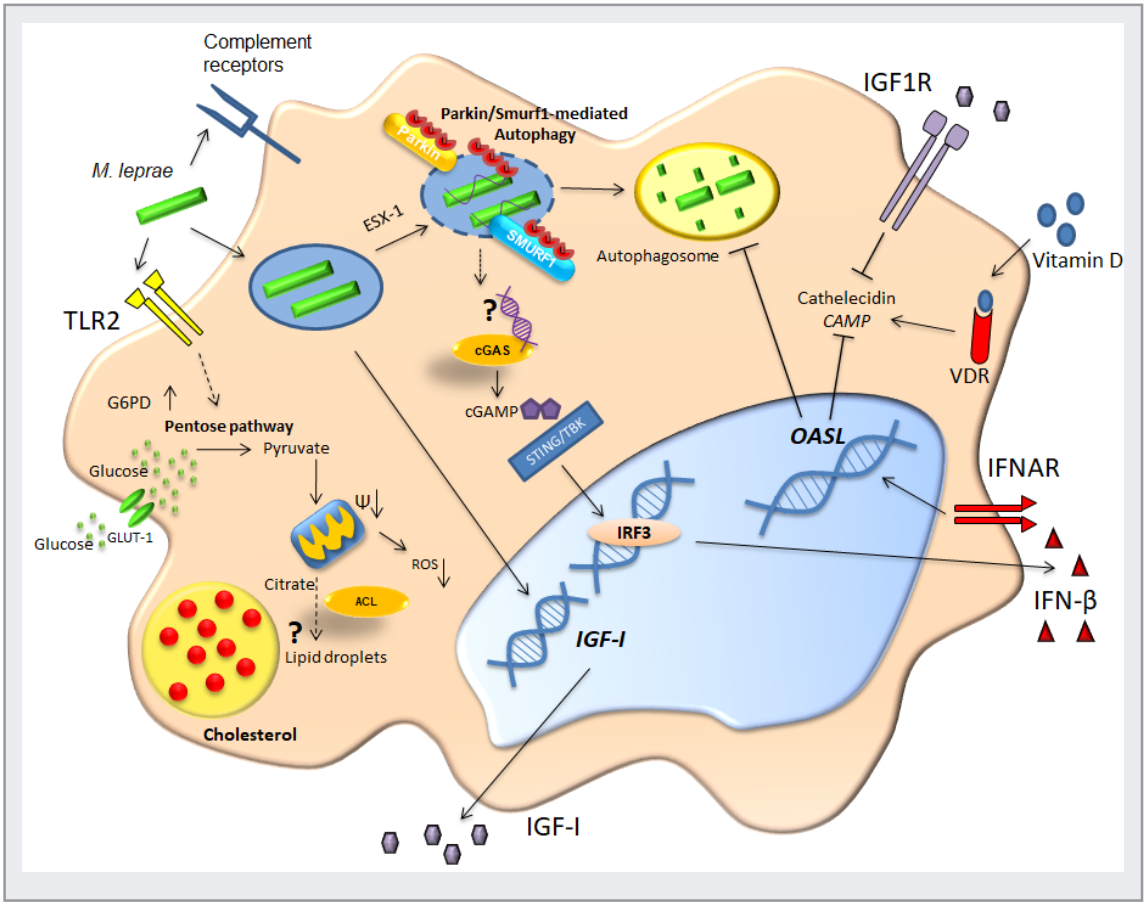

FIG 1 Early events to inhibit or induce innate antimicrobial immune response to *M. leprae* during macrophage infection.

In a first step of host-bacteria interaction, *M. leprae* is phagocytosed by macrophages using complement receptors and then the mycobacteria cell wall is recognized by TLR2, inducing lipid body formation (17, 57). The phagocytosed *M. leprae* can breach the phagosome membrane in a bacterial ESX-1 secretion system-dependent manner.

This event leads to bacterial DNA release into the cytosol, activating the cGAS/STING/IRF3 pathway and increasing type I IFN secretion-inhibiting autophagy by a mechanism dependent on OASL activation (35, 58). Also, *M. leprae*-infected macrophages increase glucose uptake, IGF-I expression, and G6PD expression (52) that, along with OASL, favor mycobacterial survival (35, 54). All of these events characterize a permissive program that culminates with a successful infection (52).

In contrast, antimicrobial routes can be induced in order to clear bacteria. In this case, the cell activates the ubiquitin ligases Parkin and Smurf1 that are responsible for ubiquitination of the bacilli (or an intermediary host protein that ligates the bacteria), targeting the bacteria for degradation in the autophagosome. Also, vitamin D activates the VDR transcription factor that is responsible for increased levels of the antimicrobial peptide cathelicidin (CAMP) (6).

Schwann cell invasion by *M. leprae*

The interaction of *M. leprae* with Schwann cells (SC) is mediated by adhesins present in the cell wall of the bacillus that interact with the host cell extracellular matrix. The *M. leprae* HLP (histone-like protein) protein, PGL-I, and other cell wall components have been described as ligands of the *M. leprae* wall that are capable of interacting with the G domain of the α-2 laminin chain extracellular matrix protein of the host. Indeed, the α-2 laminin chain functions as a cellular receptor for the bacillus, promoting its interaction with the α-dystroglican present in the basal lamina that surrounds the SC, leading to internalization of the pathogen (59, 60, 61). Furthermore, GD3 ganglioside is also involved in adhesion and internalization during *M. leprae* infection (62).

SC-*M. leprae* interaction is mediated by several pattern recognition receptors as observed in macrophages. In SCs, TLR6 is crucial to the induction of lipid body formation and PGE_2 production favoring intracellular bacterial survival. Indeed, SC foamy morphology in lepromatous leprosy nerves is due to the regulation of lipid body biogenesis and altered intracellular lipid distribution after *M. leprae* uptake, as described earlier for macrophages (52, 57).

M. leprae infection promotes SC demyelination mediated by direct bacterial binding and ErbB2 receptor activation (63). It has been demonstrated that during *M. leprae* infection, human primary SC undergo a cellular reprogramming that leads to an immature progenitor/stem cell-like cell (pSLC) phenotype. Before de-differentiation, SCs are highly infected, and right after the reprogramming step, pSLCs lose bacilli retention capacity, acquiring a high bacterial transfer capacity to other cells, such as fibroblasts. pSLCs facilitate the spread of infection to other tissues, creating a bacilli flow that enables a sustained infection (64).

Regulation of iron metabolism involves sensing intracellular iron concentrations and modulating uptake and storage accordingly. Iron storage proteins are important to protect against oxidative stress by sequestering ferrous iron and consequently limiting toxic hydroxyl radical (OH^-) formation by Fenton chemistry (66, 67).

Since little unchelated iron exists within the host, invading bacteria must obtain their iron from circulating iron-containing proteins, such as transferrin or lactoferrin, or from the iron-storage protein ferritin. Mycobacteria synthesize low molecular mass molecules called siderophores. Due to higher affinity, siderophores may remove iron from these molecules (68) and provide a means for iron acquisition, leading to mycobacteria growing *in vitro* and *in vivo*. Mycobacteria produce two classes of siderophores: mycobactins and exochelins (69, 70, 71, 72). Mycobactins are found in two forms that differ in polarity and solubility. The less polar form remains cell-associated (mycobactin), and the more polar one (carboxymycobactin) is secreted into the medium. Intraphagosomal mycobacteria must induce the synthesis of siderophores in order to acquire iron from transferrin but, when sufficient iron has been acquired, regulatory pathways may down-regulate iron uptake in response to the iron-sufficient conditions that they may now face (73).

Microbial iron uptake seems to occur as follows. Soluble siderophores are secreted to obtain iron from the environment or from the host and are transformed into ferric siderophores. These ferric siderophores are then transported into cells by receptors located in the cell envelope. Next, iron ions are delivered to bacterioferritin to satisfy the physiological requirements. Both exochelins and carboxymycobactins can seize the extracellular iron and transfer iron to the intracellular siderophores (74).

M. leprae does not contain mycobactin (see Chapter 8.2). This lack suggests that *M. leprae* might be considered a mycobactin-dependent microbe and that other mycobacteria present in humans might provide sufficient mycobactin for *in vivo* multiplication of *M. leprae*. *M. leprae* does produce several proteins involved in iron transport, storage, and regulation in the absence of recognizable genes encoding iron scavengers (75).

The complete annotation of the *M. leprae* genome includes two bacterioferritin genes (76). One of these genes (ML2038) encodes the 18 kDa BfrA, which corresponds to the previously identified *M. leprae* bacterioferritin (77). The second is a pseudogene (ML0075) which encodes the ferritin-like protein BfrB. MDP-1 from *M. leprae* (Mycobacterial DNA-binding protein 1: ML1683; ML-LBP) has been described as a histone-like protein that is a ferroxidase-iron storage protein outside of the ferritin superfamily proteins (78).

IRON REQUIREMENTS IN *M. LEPRAE*-INFECTED HOST CELLS

The major pathway for eukaryotic cells to avoid toxicity from intracellular iron accumulation is the translocation of Fe^{+2} into the cytosol. There, it is safely stored in the oxidized Fe^{+3} form bound to ferritin, a function that is in large part performed by the divalent cation efflux pump Nramp2 (slc11a2/DMT1). Macrophages express an additional metal transporter, Nramp1 (slc11a1), in their late endosomes/lysosomes. Nramp1 is expressed in the lysosomal compartment, whereas Nramp2 is not detectable in the lysosomes but is expressed primarily in recycling endosomes and also, to a lesser extent, at the plasma membrane, colocalizing with transferrin. Nramp2 transports free Fe^{2+} across the endosomal membrane and into the cytoplasm (79, 80). Authors have hypothesized that the phagocyte-specific Nramp1 protein may regulate the intraphagosomal replication of mycobacteria by controlling divalent cation concentrations at that site (81).

Iron-mediated anti-mycobacterial defenses of the host may follow two pathways. The first is to sequester mycobacterial molecules that have already captured iron. For example, siderocalin (Lcn 2), has been shown to bind to soluble siderophores of mycobacteria (82), thus preventing the utilization of the iron by the pathogen.

The second pathway is to make iron unavailable to mycobacteria. Hepcidin is an antimicrobial peptide produced by hepatocytes in response to excess iron or inflammation and is one of the principal iron regulatory hormones (83). Hepcidin orchestrates an innate immune response to

further reduce available iron and to slow or stop the growth of bacteria (84, 85, 86). Hepcidin stimulates iron accumulation inside cells by inducing the internalization and destruction of ferroportin, a plasma membrane-associated iron transporter protein (see Table 1) that regulates the export of iron from duodenal enterocytes and macrophages into plasma in the human host (65, 87, 88). Hepcidin has been considered a link between inflammation and the anemia of chronic diseases. The latter is characterized by reduced levels of iron in the serum and increased iron reserves, as evidenced by elevated ferritin and several inflammatory mediators that may regulate hepcidin expression in host cells, like IL-6 and IL-1β. Bacterial infection of macrophages may also drive hepcidin production via TLR activation (89).

The evaluation of hepcidin in patients with the polar forms of leprosy has demonstrated that hepcidin is elevated in LL lesions, whereas ferroportin is more abundant in TT ones (97). Anemia is a common finding in patients presenting with the MB form of leprosy (98, 99, 100, 101). Indeed, in MB patients, changes in hematologic parameters indicating altered iron metabolism appeared to result from a mixture of anemia from inflammation and iron deficiency (91). Independent of the clinical parameters, the analysis of macrophages present in skin lesions from leprosy patients demonstrated increased iron storage in host cells from LL but not from TT patients, suggesting that iron sequestration inside host cells might provide an optimal environment for the bacillus (102).

The increased iron storage in LL cells (Figure 2) may be due not only to the decreased expression of ferroportin, but also to an increase in CD163 expression. Previous studies demonstrated that the degradation of senescent erythrocytes by macrophages produces heme that may be utilized as a source of iron by *M. tuberculosis* (103, 104). In leprosy, other mechanisms related to iron acquisition for the bacilli have been described. CD163, a receptor of hemoglobin and hemoglobin-haptoglobin (Hb-Hp) complexes, is highly expressed in cells from LL lesions when compared with TT lesions. Because CD163 is related to hemoglobin clearance, it can be speculated that in infected cells, high CD163 expression may function as a pathway for the supply of iron. The increase in CD163 expression in LL macrophages is accompanied by an increase in iron storage (102), which reinforces the hypothesis that iron is important for *M. leprae* survival inside host cells and suggests that heme may be a source of iron for *M. leprae* as well.

A primary question remains as to how iron is internalized by the mycobacteria inside the phagosome. One possibility is that lipid bodies may contribute to the delivery of iron to the phagosome. As described earlier, *M. leprae* induces several changes in the host cell so that it becomes an advantageous niche for growth. Indeed, the production of lipid bodies could also help with iron uptake (46, 105). Others have demonstrated that, after bacterial infection, LBs within human macrophages concentrate into phagosomes and change the phagosomal contents (106, 107). It is hypothesized that mycobacteria may benefit from this host defense mechanism to deliver iron during infection (108). Mycobactins may diffuse out of mycobacterial-loaded phagosomes within macrophages and become associated with either the intracellular iron pool or migrate to the macrophage plasma membrane for iron chelation. These iron-loaded mycobactins may accumulate within lipid storage and sorting vesicles of the host macrophage. The lipid bodies, carrying iron-loaded mycobactins, may remain in direct contact with mycobacteria-containing phago-

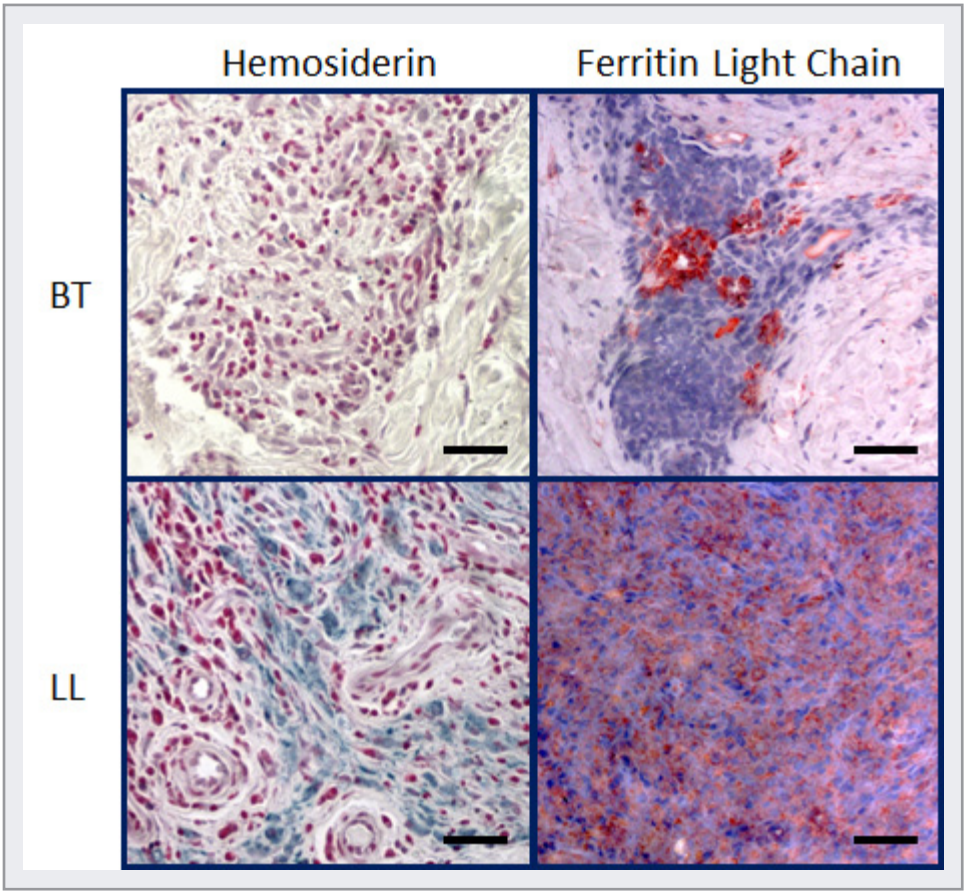

FIG 2 Macrophages of lepromatous (LL) lesions have increased iron storage in the form of ferritin and hemosiderin as compared to borderline tuberculoid patients (BT). Hemosiderin deposition was analyzed by the Prussian Blue method. Expression of the protein ferritin light chain was assessed by immunohistochemical analysis.

somes and are thought to be involved in the delivery of the loaded iron inside the phagosomes, whereby the enriched phagosomal iron pool may be used by the bacteria (108).

MACROPHAGE PROGRAM MODULATION BY *M. LEPRAE* INFECTION

The interaction of *M. leprae* with the macrophage and *M. leprae*'s ability to trigger a genetic, metabolic, and differentiation program will define the cellular commitment towards a permissive or a restrictive phenotype. The very early interaction triggered upon infection described in the beginning of this chapter (Type I IFNs, autophagy, iron uptake) culminates in effector responses such as cytokine secretion and macrophage phenotype differentiation. Generally, the profile of the specific immune response is a factor in distinguishing between the different clinical forms of leprosy. A combination of genetic and environmental factors contribute to the overall cellular

TABLE 1 Iron proteins involved in immune response to *M. leprae*

Protein	Role in Iron Metabolism	Role in Immune Response or Inflammation	Influence on *M. leprae* Infection
Hepcidin (90, 91)	Binds directly to the iron efflux protein ferroportin and contributes to the depletion of circulating iron that would be available to extracellular pathogens.	Upon activation of PPRs, secreted IL-6 induces hepcidin expression and release from the liver. Hepcidin activates the protein kinase Jak2, which phosphorylates the transcription factor Stat3, which may contribute to the modulation of downstream acute cytokine-induced inflammatory responses.	Disruption of the hepcidin/ ferroportin axis has an impact on leprosy immunopathology. Paucibacillary (PB) macrophages have higher expression of ferroportin, whereas MB cells have increased hepcidin and decreased ferroportin expression.
Lactoferrin (92, 93)	A potent chelator of ferric iron that is involved in the modulation of extracellular iron availability.	The exact role of lactoferrin in innate immunity is not fully elucidated, but it is known that TNF may induce the release of lactoferrin from neutrophilic granules at the site of a bacterial infection.	Serum lactoferrin is strongly and inversely associated with increasing BI. It needs to be evaluated, but since increased systemic TNF is observed in patients with leprosy, it can be hypothesized that lactoferrin is released by neutrophils in infection sites to sequester iron.

phenotype that leads to a more disseminated form of the disease, LL, or to a more localized form, TT.

For example, IL-10 induces a phagocytic differentiation program resulting in the up-regulation of scavenger receptor CD163, which may function as a co-receptor for *M. leprae* entry in host cells (102). In addition, IL-10 increases the phagocytic capacity of macrophages. In LL lesions, the predominant subset of macrophages exhibits high expression of CD163. CD163 and foamy phe-

TABLE 1 Iron proteins involved in immune response to *M. leprae* (cont'd)

Protein	Role in Iron Metabolism	Role in Immune Response or Inflammation	Influence on *M. leprae* Infection
Siderocalin (94, 95)	Subverts the iron acquisition system through binding to salicylate-derived iron-laden siderophores such as mycobactin secreted by *M. tuberculosis*.	Produced by neutrophil granules and in epithelial cells in response to IL-1β.	It probably limits iron availability to *M. leprae*, as described for *M. tuberculosis*.
Transferrin receptors (96)	Its down-regulation limits the cell's ability to acquire transferrin-bound iron and reduces the endosomal pool of iron accessed by intracellular pathogens.	Production of nitric oxide by LPS/IFN-γ-activated macrophages may down-regulate transferrin receptors and up-regulate the synthesis of ferritin.	Down-regulation during infection reduces endosomal iron to restrict pathogen growth.
Ferroportin	Membrane exporter of iron. It opposes the systemic regulation by hepcidin described above.	Cytokines that regulate hepcidin expression may affect the hepicidin/ferroportin axis, e.g., IL-6 and IL-1β.	Decreased expression in lepromatous macrophages contributes to the retention of iron inside cells and concomitantly aids in pathogen survival.
Nramp1	Reduces intraphagosomal iron.	The mobilization of iron by Nramp1 suppresses the expression of IL-10 and enhances macrophage production of iNOS.	It may confer resistance to *M. leprae*.

notype in LL macrophages are associated with increased iron deposition that contributes to *M. leprae* survival inside host cells (102).

On the other hand, IL-15 leads to the activation of a vitamin D-dependent antimicrobial program, along with a reduced phagocytic capacity. Macrophages with an antimicrobial profile are predominantly found in the lesions of TT patients (6). The vitamin D mechanism involves induction of the CYP27B1 enzyme, which is responsible for converting the 25-hydroxyvitamin D to its biologi-

cally active form, 1,25 hydroxyvitamin D; positive regulation of the vitamin D receptor (VDR); and increased expression of cathelicidin, an important antimicrobial peptide (109, 110, 111). At the same time, VDR activation, along with the production of IL-1β, is capable of inducing defensin B (DEFB4), another peptide required for antimicrobial activity of the host cell (112).

In fact, tissue macrophage analysis from leprosy patients has revealed that in polar LL disease, there is an increase of M2 markers, including CD68, CD163, arginase 1, IL-10, IL-13, TGF-β, and FGF-b (113). Galectin-3 contributes to IL-10 expression and is increased in LL macrophages (114). *In vitro* studies have demonstrated that live *M. leprae* may induce an M2-like macrophage function by decreasing IL-1β, IL-6, TNF, and MHC II expression and increasing both CD163 and IL-10 expression (115). When incubated with naïve T cells, macrophages treated with live *M. leprae* preferentially primed regulatory T (Treg) cell responses with elevated FoxP3 and IL-10 expression, while reducing interferon gamma (IFN-gamma) expression and CD8+ T cell cytotoxicity (115).

Although a predominance of permissive macrophages has been observed in LL lesions, the use of terms such as “suppressive” or “M2 cells” might be an oversimplification, since several lines of evidence suggest the presence of both pro- and anti-inflammatory molecules in LL macrophages. For example, solute carrier family 11a member 1 (Slc11a1) and inducible Nitric Oxide Synthase (iNOS) play an important role in the control of intracellular pathogens, but are positively correlated with the bacillary load in LL macrophages (116), demonstrating that the LL phenotype occurs independently of these “protective” molecules. IDO1 is an enzyme that may have a dual role, acting both in regulatory and pro-inflammatory pathways. Previous studies have demonstrated that IDO1 expression is increased in LL macrophages (117) and, on the other hand, may be increased in an antimicrobial context to control the high bacillary load or, finally, in a negative feedback regulatory axis to increase anti-inflammatory cytokines and contribute to disease persistence (102).

Despite the fact that LL macrophages present classical M2 markers and anti-inflammatory cytokines, iron retention, a marker of M1 cells, is a common finding in LL cells (102). This finding suggests that LL cells may have a phenotype that may not be classified in the paradigmatic M1-M2 profile (Figure 3).

M. leprae is a poor activator of macrophages and dendritic cells *in vitro*. In naïve human monocytes, *M. leprae* induces CCL-2 and IL-1ra while suppressing IL-6 production through PI3K-dependent mechanisms (121). Although *M. leprae* is a weak stimulator of cytokines, it has been shown to prime human monocytes for the increased production of TNF and IL-10 in response to a strongly inducing secondary stimulus (121). IL-10 expression is also correlated with IL-27, a cytokine that inhibits the capacity of IFN-γ to trigger antimicrobial activity against *M. leprae* in infected monocytes (122).

Macrophages have evolved a myriad of defense strategies to cope with intracellular bacteria (123), and the understanding of immune mechanisms associated with the paucibacillary (PB) form of the disease may reveal new perspectives in deciphering the crucial pathways related to protection in leprosy. In this sense, it has been demonstrated that the phagocytosis of apoptotic cells (efferocytosis) increases *M. leprae* survival in differentiated M1 cells (human $CD14^+$ cells dif-

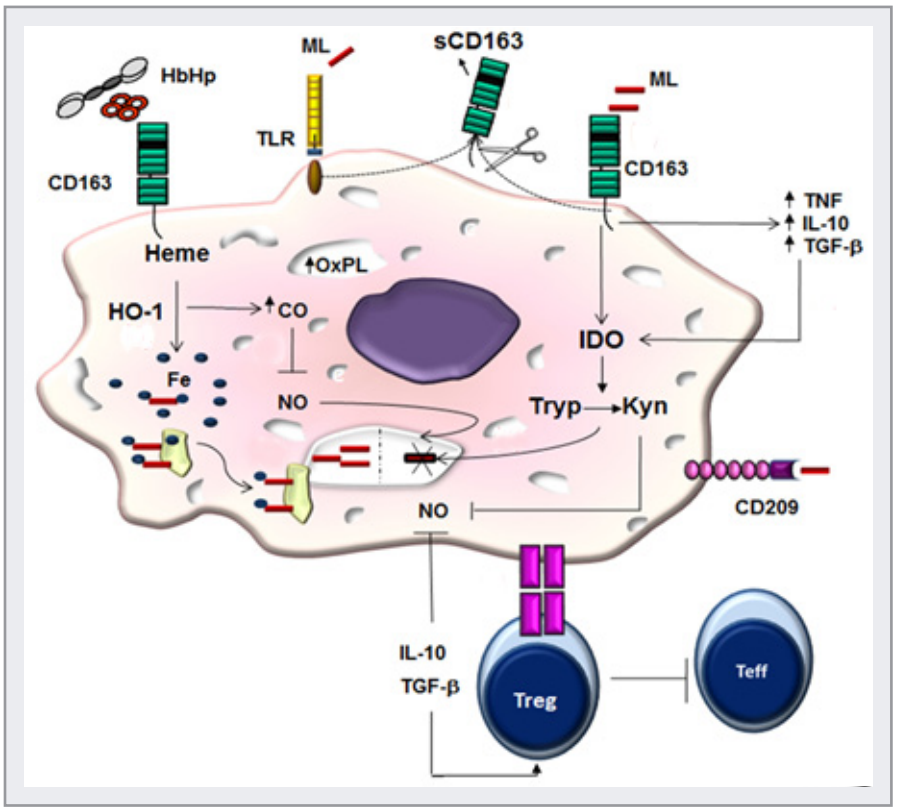

FIG 3 Innate pathways that mediate *M. leprae* survival in LL macrophages. The scavenger receptor CD163 recognizes *M. leprae*, which in turn increases the expression of CD163 in a positive feedback loop. Mycobacterial recognition by CD163 increases the levels of TNF, IL-10, and TGF-β, and likely contributes to IDO expression. It is possible that Hb recognition by CD163 also increases IDO expression. The tryptophan levels are reduced by IDO, which contributes to mycobacteria killing and, in turn, to antigenic spreading and TLR activation by *M. leprae* components, leading to CD163 shedding (118). IDO expression may also increase Treg differentiation and expansion that blocks effector function and contributes to a sustained anti-inflammatory phenotype of LL macrophages. HbHp recognition by CD163 increases HO-1 activity (119). The products of heme degradation involve Fe, biliverdin, and CO. Increased CO levels contribute to NO antimicrobial activity inhibition (120). Fe^{+3} is acquired by mycobacteria that are associated with lipid vesicles, which transport Fe^{+3} to the phagosomal vacuole, thereby contributing to mycobacterial survival.

ferentiated with GM-CSF), accompanied by reduced IL-15 and IL-6 levels and increased TGF-β and IL-10 secretion. GM-CSF-differentiated cells primed with *M. leprae* in the presence of apoptotic cells induced the secretion of Th2 cytokines IL-4 and IL-13 in autologous T cells compared with cultures stimulated with *M. leprae* or apoptotic cells alone. Together, these data suggest that in PB leprosy patients, efferocytosis contributes to mycobacterial persistence by inducing a population of macrophages more susceptible to infection. In this case, GM-CSF-differentiated cells in the presence of *M. leprae* and apoptotic cells displayed an increased CD163 and SRA-I expression as well as a higher phagocytic capacity when compared with non-stimulated cells (124).

Conclusions

Mycobacterium leprae is able to subvert innate immunity, triggering cellular differentiation of macrophages towards a permissive phenotype for mycobacterial growth and survival. This mechanism requires activation of Type I IFNs and a shift in energy metabolism shutting down mitochondria and increasing the pentose phosphate pathway directing lipid synthesis. This phenotype decreases parkin, autophagic mechanisms, and other antimicrobial pathways, resulting in disease progression. The establishment of this permissive phenotype depends on iron sequestration and CD163 as a scavenging receptor, which creates a microenvironment with secretion of IL-10 and CCL2 and decreased production of IL-15 and IL-1-beta. To sum up, in PB clinical forms, macrophages display a microbicidal phenotype, with antimicrobial responses mediated by the vitamin D pathway that contributes to an increase in antimicrobial peptides and also the up-regulation of autophagy. In MB forms, macrophages display a permissive phenotype that contributes to bacterial persistence.

Indeed, early events in *M. leprae* interaction with macrophages and Schwann cells involve several biochemical and immunological pathways. An The increased definition of these pathways may provide novel pharmacological targets that could be use as adjuncts to MDT, thereby improving care for leprosy patients.

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