Chapter

Macrophages in the Pathogenesis of Leprosy

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Abstract

Leprosy is a chronic infectious disease caused by the intracellular pathogen *Mycobacterium leprae*. The disease may present different clinical forms depending on the immunological status of the host. *M. leprae* may infect macrophages and Schwann cells, and recent studies have demonstrated that macrophages are fundamental cells for determining the outcome of the disease. Skin lesions from patients with the paucibacillary form of the disease present a predominance of macrophages with a pro-inflammatory phenotype (M1), whereas skin lesions of multibacillary patients present a predominance of anti-inflammatory macrophages (M2). More recently, it was shown that autophagy is responsible for the control of bacillary load in paucibacillary macrophages and that the blockade of autophagy is involved in the onset of acute inflammatory reactional episodes in multibacillary cells. So, strategies that aim to induce autophagy in infected macrophages are promising not only to improve the efficacy of multidrug therapy (MDT) but also to avoid the occurrence of reactional episodes that are responsible for the disabilities observed in leprosy patients.

Keywords: macrophages, leprosy, innate immunity, scavenger receptors, autophagy

1. Introduction

Macrophages are highly plastic and heterogeneous in several aspects, presenting a spectrum of distinct phenotypes according to the microenvironment [1–3]. During mycobacterial infection, its membrane components have the ability to induce polarization and interaction with this type of cell [4]. The cell wall of *M. leprae* consists of lipids and contains large amounts of phthiocerol dimycocerosate and phenolic glycolipid-1 (PGL-1) [5, 6]. PGL-1 has been identified as an important antigen and virulence factor, which has also been shown to be a promising diagnostic molecule by inducing the production of IgM class antibodies [7, 8]. Interestingly, the presence of lipids and sugars in the cell wall also induces an increase in phagocytosis [9], both by macrophages and by other cell types. Besides that, the presence of *M. leprae*-PGL-1 interacting with resident macrophages is able to lead to the

production of nitric oxide, thus causing peripheral nerve damage characteristic of patients with leprosy [10]. Other studies have shown the ability of *M. leprae* to induce the production of oxidative mediators and their products, peroxynitrite and nitrotyrosine [11–14].

Studies have demonstrated the ability of *M. leprae* to interact with a range of scavenger receptors of macrophages culminating in a tolerogenic response profile. The scavenger receptors are membrane receptors whose main function is the removal of molecules and cellular debris from the body, binding through a variety of polyanions, leading to phagocytosis of the target, being found in several cell types such as macrophages [15]. The ability of *M. leprae* to interact with the CD163 receptor, a scavenger receptor, which, during this interaction, can act as a co-receptor for *M. leprae* entry in macrophages, has been described [16]. It is known that activation of this receptor is related to the activation of the transcription factor nuclear factor erythroid 2-related factor 2 (NRF2), leading to the synthesis and increase of the activity of the enzyme heme oxygenase-1 (HO-1), which, through anti-inflammatory and antioxidant pathways, releases interleukin (IL)-10 and generates carbon monoxide, contributing to the polarization of these cells [17–19]. Bonilla and colleagues [20] demonstrated that autophagy, a mechanism of metabolic control, regulates the expression of scavenger receptors macrophage receptor with collagenous structure (MARCO) and scavenger receptor type A (SRA-I) that increase phagocytosis and NRF2 activity during *Bacillus Calmette-Guérin* (BCG) or *M. tuberculosis* (H37Rv) infection.

M. leprae is able to induce macrophage SRA-I and CD36 expression [6] that contributes to the uptake of lipids, culminating in an increase in the uptake and accumulation of oxidized lipids within the macrophages, leading to a foamy cell phenotype, associated with an inhibition of the pro-inflammatory response with downregulation of major histocompatibility complex (MHC) II and toll-like receptor (TLR) 2 [21, 22]. Dendritic cell-specific intercellular adhesion molecule-3-grabbing non-integrin (DC-SIGN or CD209) is another scavenger receptor present in macrophages that interacts with *M. leprae*, and it is involved in the phagocytosis of the bacilli [23, 24]. Other receptors have been described with great importance in the initial interaction and polarization of the response of macrophages to bacteria. It has recently been observed that *M. leprae* is able to activate innate receptors such as TLR4 [25], through PGL-1 that induces the irregular production of interferon (IFN)-β, chemokine (C-X-C motif) ligand (CXCL)-10/interferon gamma-induced protein 10 (IP-10), and inducible nitric oxide synthase (iNOS), thus decreasing the production and activation via tumor necrosis factor (TNF) [26].

The persistence of *M. leprae* infection depends on the type of the host immune response. Macrophages are crucial modulators of innate and adaptive immune responses are the main cell types directly infected by the bacillus, and can lead to different immune responses. The initial interaction of the macrophage with *M. leprae* is essential for the polarization of the response toward a susceptible phenotype, favoring the survival of the bacilli. In this way, studies that elucidate this contact may favor the protective response against infection, thus contributing to strategies of control of the disease.

2. Macrophage polarization and *M. leprae* infection

Macrophages are specialized cell types present in most mammalian tissue. Recently, many studies have been highlighting the "general" and "tissue-specific" functions of macrophages, including their roles in systemic metabolism, fibrosis, development, cancer, and tissue homeostasis [27]. However, these cells are best

known for their role in the innate immunity, which was first addressed by Ilya Metchnikoff in 1884 in his work describing the "phagocytes" [28]. Several subsets of macrophages were described in different pathological conditions and tissues of humans and mice based on their phenotype and biological functions [1, 29–31]. Despite their high plasticity, macrophages are classically described in two main functionally distinct phenotypes—classically activated or inflammatory macrophages (M1) and alternatively activated or healing macrophages (M2)—reflecting the T helper type (Th) 1 and Th2 response profiles [2, 3, 30].

In summary, M1 macrophages are induced by lipopolysaccharide and IFN-γ in a pro-inflammatory environment promoting a microbicidal and inflammatory phenotype, while polarization to M2 macrophages, induced in response to IL-4 (M2a), immune complexes (M2b) or IL-13 and IL-10 (M2c), is rather anti-inflammatory and associated with healing and tumor progression. In addition, granulocyte and macrophage colony-stimulating factor (GM-CSF) and macrophage colony-stimulating factor (M-CSF) induce the differentiation of macrophages into, respectively, M1 and M2 phenotypes [2, 3, 32, 33]. Previously, it was demonstrated that macrophages differentiated with GM-CSF or M-CSF were able to phagocytose *M. leprae* [34]. Despite this, only GM-CSF-differentiated M1 cells were able to stimulate T cells to produce IFN-γ, after treatment of the macrophages with IFN-γ and CD40 ligand; furthermore, this treatment induced expression of major membrane protein (MMP)-II on the macrophage cell surface, suggesting its ability to process the phagocytosed bacteria [34]. In addition, *M. leprae* was able to induce IL-10 production in M-CSF-differentiated M2 cells, but not in GM-CSF-differentiated M1 macrophages.

In 2016, the protein jagged 1 (JAG1) was identified as a potential regulator of macrophage polarization in leprosy [35]. While unstimulated endothelial cells lead to M2 macrophage polarization, in the presence of IFN-γ, endothelial cells induce the differentiation to M1 macrophages. JAG1 is preferentially expressed in the vascular endothelium in skin lesions of paucibacillary tuberculoid patients, stimulating the differentiation of M1 antimicrobial macrophages by the IFN-γ-JAG1 axis [35].

Due to increased systemic pro-inflammatory mediators, a higher frequency of apoptosis was described in paucibacillary tuberculoid patients [36]. Curiously, the phagocytosis of apoptotic cells in the presence of *M. leprae* induces a shift from M1 to M2 phenotype in GM-CSF-differentiated macrophages with increased expression of scavenger receptors as SRA-I, production of IL-10 and transforming growth factor beta (TGF-β) anti-inflammatory cytokines, and decreased levels of pro-inflammatory IL-15 and IL-6 by a mechanism mediated by arginase [37] (**Figure 1**). Based on those results, it was suggested that in paucibacillary tuberculoid skin lesions, the phagocytosis of apoptotic cells would induce an M2 phenotype in some macrophages, explaining the persistence of the disease besides the ability to mount an effective cellular immune response to *M. leprae* infection [37].

Analysis of paucibacillary tuberculoid and reversal reaction (an acute inflammatory clinical condition associated with increased levels of IFN-γ in leprosy patients) patients' skin lesions demonstrated that macrophage subtypes with microbicidal and homeostatic functions are spatially distributed in tuberculoid granulomas according to the specific microenvironments [38]. The center of the tuberculoid granulomas appears to be populated by pro-inflammatory $CD68^+$ $CD163^-$ M1 macrophages, responsible for containing the infection, while the periphery is composed of anti-inflammatory $CD68^+$ $CD163^+$ M2 macrophages, tasked with limiting tissue damage caused by the M1 macrophage antimicrobial activity [39]. Accordingly, Montoya and colleagues [22] proposed two different macrophage functional programs for the polar clinical forms of leprosy. They suggested that in tuberculoid paucibacillary patients, IL-15 induces the vitamin D-mediated antimicrobial program in the macrophages, resulting in killing of the

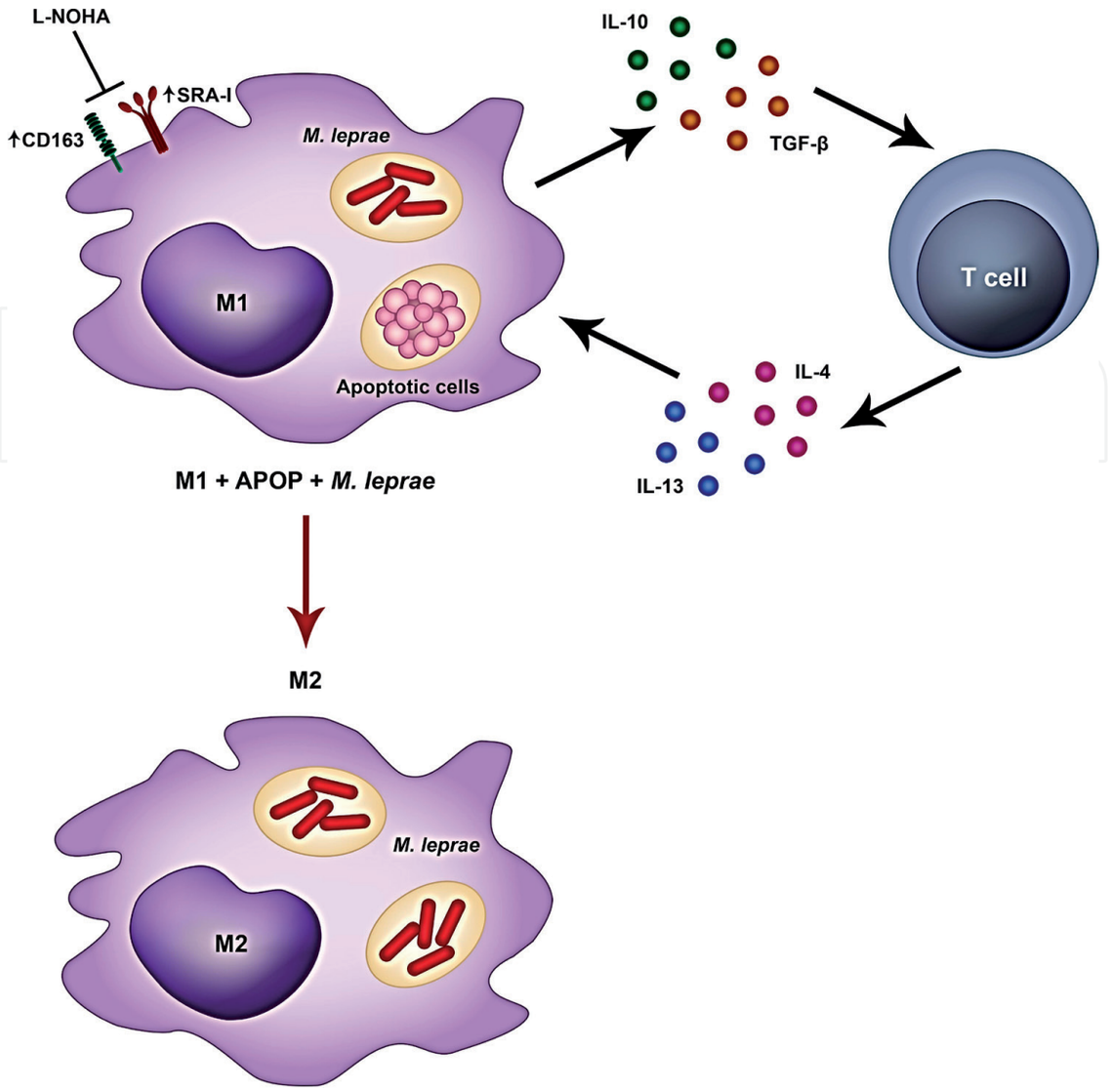

Figure 1.
Macrophage plasticity in tuberculoid skin cells. M1 phenotype prevails in tuberculoid cells. The pro-inflammatory cytokines present in the tissue may contribute to increased host cell apoptosis, and the removal of the apoptotic cells may contribute to changes in macrophage plasticity. M1 macrophages that uptake M. leprae *and apoptotic cells have an increase in the percentage of M2 markers CD163 and SRA-I that is dependent on arginase production, since the arginase inhibitor N-hydroxy-nor-L-arginine (nor-NOHA) blocks these phenotype changes. In addition, the stimuli with* M. leprae *and apoptotic cells induce an increase in the production of IL-10 and TGF-β that contributes for inducing the secretion of IL-4 and IL-13 by Th2 cells that sustain some M2 cells in tuberculoid lesions.*

mycobacteria, while in multibacillary lepromatous patients, the higher levels of IL-10 would induce the phagocytic pathway by increasing the expression of CD209 and scavenger receptors as CD163 in the macrophage cell surface, resulting in phagocytosis of *M. leprae* and oxidized low-density lipoproteins (LDL) favoring the formation of foam cells and persistence of the infection [22]. In addition, antimicrobial M1 macrophages differentiated with IL-15 could be repolarized into the phagocytic M2 phenotype after treatment with IL-10, while phagocytic IL-10-differentiated M2 macrophages could only be repolarized into the M1 phenotype after co-stimulation with TLR2/1 ligand and IFN-γ or TLR2/1 ligand and anti-IL-10-neutralizing antibodies, but not IL-15 or IFN-γ alone, suggesting that production IL-10 by M2 macrophages might create a barrier for M1 reprogramming [39].

M. leprae infection of IL-10-differentiated M2 cells results in induction of type I IFN and suppression of the vitamin D directed pathway, suggesting that *M. leprae* evades the intrinsic capacity of human cells to activate the vitamin

D-mediated antimicrobial pathway via the induction of type I IFN [40]. Although previous studies have demonstrated the activation of antimicrobial pathways in IL-15-differentiated macrophages, there is no study demonstrating how vitamin D status modulates IL-15-differentiated macrophage phenotype and function. More recently, it was demonstrated that the presence of vitamin D during macrophage differentiation bestows the capacity of human macrophages to mount an antimicrobial response against *M. leprae* [41]. However, more studies are needed to evaluate if the plasma levels of vitamin D could be a predictor of the outcome of the disease.

Several studies demonstrated the predominance of M2 markers like CD68, CD209, CD163, SRA-I, HO-1, arginase-1, IL-10, IL-13, TGF-β, and basic fibroblast growth factor in multibacillary lepromatous patients' skin lesion macrophages [16, 22, 37, 42–44]. In the same way, CD163, the hemoglobin (Hb) scavenger receptor, might contribute to the polarization of multibacillary lepromatous macrophages to an anti-inflammatory profile by increasing the expression of indoleamine 2,3-dioxygenase (IDO) and IL-10, in addition to increasing the internalization of *M. leprae* and iron, contributing to the mycobacterial persistence [16, 45]. The increase in the internalization of Hb-haptoglobin (Hp) complex by CD163 contributes to the activation of the enzyme HO-1 via IL-10 [46]. de Mattos Barbosa and colleagues [42] proposed that *M. lepra*e-infected skin macrophages would increase the acquisition of iron both by transferrin and heme-bound, via transferrin receptor 1 and CD163, activating the enzyme HO-1 that catalyzes heme into carbon monoxide, biliverdin, and free iron, increasing the intracellular iron pool and the iron storage in the protein ferritin (Ft), due to a reduction in expression of the sole iron exporter, ferroportin 1 (Fpn-1) [42] **(Figure 2)**. Iron retention via Ft and reduced secretion of iron by Fpn-1 are classical traits of microbicidal inflammatory M1 macrophages, while tissue repair-associated M2 are characterized by enhanced HO-1-mediated heme catalysis and increased iron exportation via Fpn-1 [46]. Even though there is a prevalence of M1 or M2 markers in the polar clinical forms of leprosy, skin lesion macrophages present themselves in a spectrum of heterogeneous phenotypes sharing characteristics of both subtypes, and more than one specific population can be present at the same time [38, 42, 47].

A different subset of macrophages, known as M4, was described in skin lesions from lepromatous patients. M4 macrophages in lepromatous skin lesions were described as CD68-positive cells that express myeloid-related protein 8 (MRP8) and matrix metalloproteinase (MMP)-7 [48]. This particular subset of macrophages is differentiated with the platelet chemokine CXCL4 and is mostly related to the formation of foamy cells present on atherosclerotic lesions due to increased expression of LDL receptors. Macrophages differentiated with this chemokine present a functionally distinct phenotype characterized by increased expression of CD206, CD68, IL-6, TNF, MRP8, MMP7, and MMP12, suppressed phagocytic capacity, and the complete lack of CD163 accompanied by the inability to induce HO-1 in response to Hb-Hp complexes, which is irreversible even after removal of CXCL4 and stimulation with M-CSF or IL-10 [32, 48, 49]. Expression of IL-6 and TNF, cytokines associated with the promotion of microbicidal M1 macrophages responses, was increased on skin lesions of paucibacillary tuberculoid patients [48]. Additionally, in vitro exposure to *M. leprae* or PGL-1 impairs the capacity of healthy donor's monocytes to differentiate to M1 macrophages, reducing the cell surface expression of M1 markers and the production of M1-associated chemokines and cytokines [50]. It was hypothesized that previous contact with *M. leprae* might limit the functional capacity of monocytes, reducing the ability to mount an effective immune response in a secondary contact [50]. Together, these data support the idea that an anti-inflammatory regenerative environment restrictive of microbicidal response is promoted in lepromatous patient's skin lesions, leading to

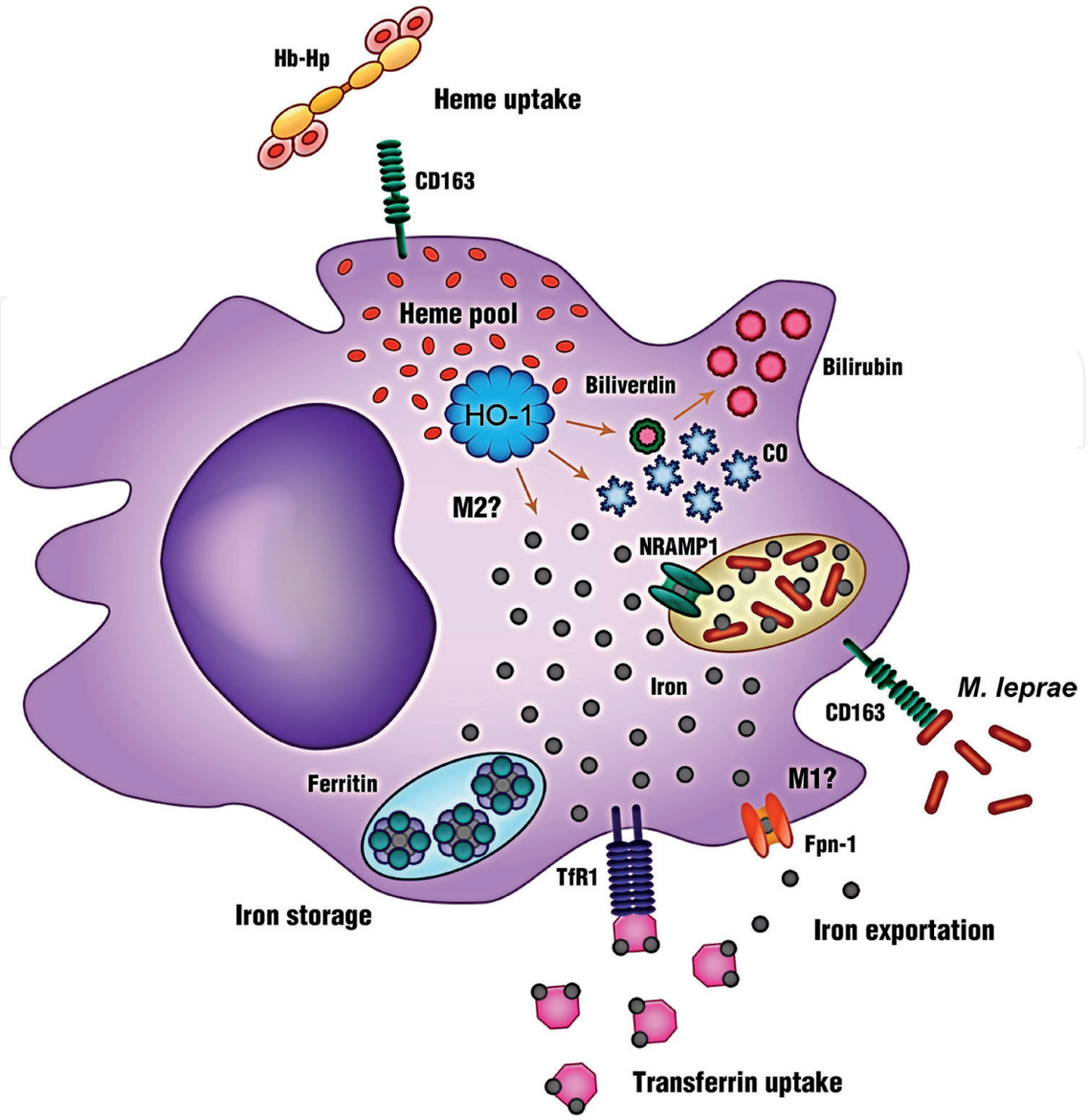

Figure 2.
Lepromatous leprosy macrophage iron metabolism. Skin lesion macrophages of lepromatous patients present high expression of M2 markers as CD163, a scavenger receptor that recognizes hemoglobin-haptoglobin (Hb-Hp) complex and was previously implied in M. leprae *internalization. The heme molecules are degraded by heme oxygenase-1 (HO-1) that catalyzes heme in free iron, carbon monoxide (CO), and biliverdin that is converted to bilirubin by the enzyme biliverdin reductase; these are classically upregulated in M2 macrophages. Transferrin receptor 1 (TfR1) is also increased in lepromatous macrophages. This receptor recognizes iron-bound transferrin, which is endocytosed, and the iron is later liberated to the cytoplasm. Lepromatous macrophages also present a lower expression of ferroportin 1, the iron cellular exporter, characteristic from M1 macrophages, contributing to increasing the cellular iron pool. The free iron present in the cytoplasm is quickly stored in the form of ferritin but can also be available for* M. leprae *use and increased growth in the phagosomes, as observed in this clinical form. Natural resistance-associated macrophage protein 1 (NRAMP1) is also increased in lepromatous macrophages, but its role in* M. leprae*-infected cells is still to be determined.*

differentiation of a heterogeneous subset of highly phagocytic iron and lipid-loaded foamy macrophages that create an ideal environment for survival and vast propagation of the *M. leprae* infection and consequently the increase in the number of skin lesion in this pole of the disease [16, 22, 37, 42–44, 48].

3. The role of macrophages in the immune response to *M. leprae*

One of the most crucial steps in a human innate immune response is how the host cells recognize a microbial pathogen. The TLR family has a vital role in the

mycobacterial recognition and subsequently induction of antimicrobial defenses and adaptive immune response [51]. Recognition of *M. leprae* pathogen-associated molecular patterns (PAMPs) occurs through the TLR2/1 heterodimer to the tri-acylated lipopeptides, leading to the differentiation of monocytes into macrophages and dendritic cells and triggering the production of TNF as part of an acute inflammatory response [52]. The tissue expression of TLRs correlated with the immunological spectrum of the disease, once both TLR1 and TLR2 were prominently observed in the self-limited tuberculoid lesions when compared to the disseminated lepromatous lesions [53]. Another pattern recognition receptor (PRR) involved in *M. leprae* detection is nucleotide-binding oligomerization domain-containing 2 (NOD2). Human NOD2 receptor recognizes structurally unique muramyl dipeptides from *M. leprae*, triggering an IL-32-mediated innate immune response that induces the differentiation of monocytes into dendritic cells [54, 55]. Interestingly, activation of monocytes via NOD2 agonist was more efficient in the induction of dendritic cell differentiation than TLR2/1 ligand treatment [54].

The activation of PRR can induce the antimicrobial autophagy pathway, a biological process regulated by multiple specialized proteins known as autophagy-related proteins (ATG), and can be started in response to various cellular stresses and signals such as nutrient withdrawal, growth factor deprivation, and cytokine stimulation and also by pathogen infection [47]. In addition to the role of autophagy in the elimination of potentially toxic protein aggregates and in the prevention of neurodegeneration [56], autophagy plays a key role in the host's response to mycobacterial infection, because it is able to reverse the blockade of phagosome maturation, inhibiting the intracellular survival of the pathogen [57]. It has been shown that autophagy is an important innate mechanism associated with leprosy immunopathogenesis [58]. Recently, it was demonstrated that autophagy enhances the ability of *M. leprae*-infected Langerhans cells to present antigens to CD1a T cells [59].

As mentioned earlier, the paucibacillary tuberculoid skin macrophages activate the vitamin D pathway and produce antimicrobial peptides that could be involved in autophagy induction. In addition, Silva and colleagues [58] demonstrated that autophagy is differentially regulated between leprosy polar forms. In paucibacillary tuberculoid skin lesion macrophages, IFN-γ/beclin 1-induced autophagy contributes for *M. leprae* control, whereas in lepromatous macrophages B cell lymphoma 2 (BCL2)-mediated blockade of beclin 1 autophagic pathway promotes mycobacterial persistence [58]. Indeed, the *M. leprae* can take advantage of host antiviral protein 2′-5′-oligoadenylate synthetase like (OASL) to inhibit autophagy and promote its own survival through a stimulator of interferon genes (STING)-mediated type I IFN response [60]. Furthermore, the autophagy levels were restored in lepromatous patients undergoing reversal reaction episodes [57]. More recently, de Mattos Barbosa et al. [61] elegantly demonstrated a role for autophagy in the development of reversal reaction. This study showed a downregulation of autophagy associated with inflammasome activation in skin lesion macrophages of multibacillary leprosy patients who developed reversal reaction episodes in the future. Thus, the autophagic pathway is a key factor in multibacillary leprosy patients to avoid exacerbated inflammasome activation and the onset of reversal reaction. A newly published study showed that Th17-derived cytokine IL-26 has a direct bind capacity and antimicrobial effect against mycobacteria in cell-free cultures [62]. In *M. leprae*-infected macrophages, IL-26 treatment was associated with autophagy induction via STING (probably due to its ability to bind DNA) as well targeting of mycobacteria to lysosomal compartments [62]. Curiously, it has been shown in *M. tuberculosis*-infected macrophages that the cytosolic DNA sensor cyclic GMP-AMP synthase (cGAS), an upstream receptor to STING, controls both pro-mycobacterial type I IFN production and the activation of antimycobacterial selective autophagy

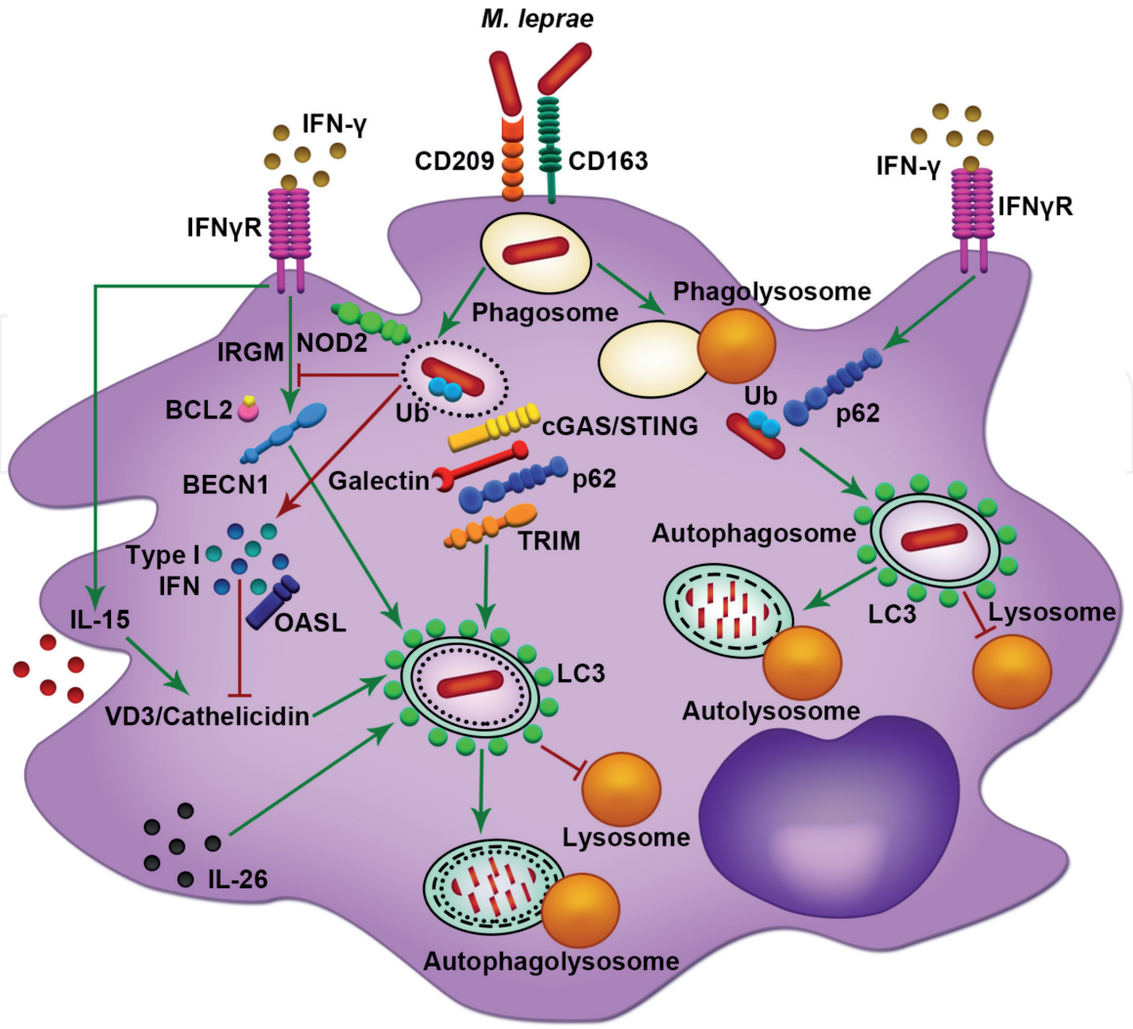

Figure 3.
Targeting M. leprae *to autophagy (xenophagy) in macrophages of leprosy patients. (Left) After phagocytosis,* M. leprae *causes phagosome maturation arrest and phagosomal permeabilization through the bacterial ESX-1 secretion system, which allows the detection of extracellular mycobacterial DNA by the cGAS/STING-sensing cytosolic surveillance pathway that licenses the ubiquitination of mycobacteria and recognition by the ubiquitin-binding autophagy adaptors p62 and NBR1 (and probably NDP52 and OPTN), which finally interact with LC3, allowing the mycobacterial phagosome to become sequestered within an autophagosome. The autophagosome-sequestered phagosome maturates in a degradative autophagolysosome (which also contains antimicrobial peptides) by fusing with a lysosome and leads to the pathogen destruction and antigen presentation. Other molecules such as TBK1, IRF3, DRAM1, UBQLN1, PARKIN, and SMURF1 might be also involved in this process. Microbial invasion can be also detected and targeted to autophagy pathway by galectins that act as a receptor for vacuole-damaging pathogens or by TRIM-mediated precision autophagy, which can directly recognize the bacterial target without required intermediary autophagic tags such as ubiquitin and galectins. TRIMs and galectins also cooperate during selective autophagy. TRIM proteins use galectins and ubiquitins to detect and tag damaged mycobacteria-containing phagosomes and promote the assembly of autophagic machinery via MTOR inhibition and AMPK activation. IFN-γ-mediated autophagy requires IRGM, which interacts with ULK1 and BECN1 and dissociates BCL2 from BECN1-PIK3C3 complex, thus governing the assembly of autophagy initiation complexes that will further promote the incorporation of mycobacterial phagosomes into autophagosomes. Autophagy initiation step is amplified by the detection of* M. leprae*-derived MDP by NOD2, which enhances NOD2-IRGM interaction and induces IRGM ubiquitination. IRGM can also activate BECN1 via AMPK induction. IFN-γ can also induce autophagy through the IL-15/VD3/cathelicidin pathway. IL-26 is reported to activate autophagy via STING.* M. leprae *can dampen autophagy initiation by increasing the BCL2 levels and its interaction with BECN1 or by induction of type I IFN signaling pathway (which includes OASL) that inhibits the VD3-dependent autophagy.* M. leprae *can also hamper the autophagy maturation step by an unknown mechanism, which might involve the BECN1-BCL2 association. Autophagy activating pathways are prominently observed in tuberculoid macrophages, whereas autophagy inhibition processes are predominantly found in lepromatous macrophages. (Right) Another possible pathway is that right after phagocytosis,* M. leprae *is incorporated into phagolysosomes but avoids lysosomal degradation via translocation from the phagolysosomes to the cytosol by using the ESX-1 secretion system. The* M. leprae *cytosolic entry is followed by ubiquitin-mediated autophagy recognition and degradation into mature autolysosomes. Green arrows indicate steps activating autophagy. Red arrows and inhibition bars represent steps inhibiting autophagy.*

pathway, which can be uncoupled from intracellular immune responses mediated by NACHT, LRR, and PYD domains-containing protein 3 (NLRP3) inflammasome activation [63–65].

Galectins are a family of β-galactoside-binding cytosolic lectins that monitors endosomal and lysosomal integrity. These danger receptors can detect bacterial invasion by detecting unusual exposure of glycans to the cytosol and activate antibacterial autophagy [66–68]. Immunohistochemistry analysis of leprosy lesions revealed a higher expression of galectin-3 protein on lepromatous macrophages than tuberculoid cells. The increased galectin-3 expression in lepromatous cells was associated with the reduction of dendritic cell differentiation and T-cell antigen presentation [69]. Interestingly, galectin-3 was associated with both bacterial control and survival, as well as autophagy activation and inhibition [66, 68], whereas galectin-8 was related to antibacterial autophagy activation [67, 68]. The underlying cellular mechanisms of target *M. leprae* as an autophagic cargo destination in human macrophages are still not fully understood; some of them displayed in **Figure 3** are insights from *M. tuberculosis* infection model.

Although the innate activation of macrophages orchestrates antimicrobial responses that contribute to host defense against intracellular pathogens such as *M. leprae*, those responses have been also implicated in the initiation of nerve damage in leprosy. The axonal damage is not directly mediated by *M. leprae* itself, but by *M. leprae*-specific PGL-1 induction of nitric oxide synthase in infected macrophages, which leads to axon damage by injuring their mitochondria and inducing demyelination [10]. Taken together, these findings illustrate the plasticity of human macrophages and how they deploy different strategies to fight against mycobacterial infections. Most of the time, these approaches begin with microbe sensing and culminate in the targeting of the pathogen for destruction in the autolysosomal pathway (**Figure 3**), the tuberculoid leprosy macrophages being more efficient than the lepromatous ones in these processes. Hence macrophages are essential components of mammalian tissues in which they perform a variety of biologic functions; understanding their difference is an essential step toward the development of innate immune countermeasures.

4. Macrophage autophagy as a target for the control of the disease

Leprosy remains a major global problem. Early detection of cases and immediate treatment with multidrug therapy (MDT) remain the main intervention strategies [70]. Despite the effectiveness of MDT in controlling the polar forms of the disease, limitations in terms of persistent activity in paucibacillary patients, in combination with the persistence of live and/or dead bacilli in multibacillary patients, have been observed, which has repercussions on the frequency of relapses and reactional episodes after treatment [71, 72]. Recent studies have demonstrated that autophagy is an important molecular mechanism for controlling the viability of mycobacteria in the host cell and of the bacillary load in patients with leprosy [58–60]. Autophagy can be induced by oxidative stress or by an infectious agent and is closely associated with the immune response and host defense [73, 74]. In addition to its homeostatic role, the autophagic degradation pathway is involved in several human diseases, including metabolic disorders, neurodegenerative diseases, cancer, and infectious diseases. Given these observations, pharmacological approaches to regulate positively or negatively this pathway are receiving considerable attention. For example, positive regulation of autophagy may be of therapeutic benefit in certain neurodegenerative diseases (e.g., Huntington's disease), while inhibition of autophagy is

being investigated as a strategy for treatment of some cancers [75, 76]. The molecular regulators interconnecting autophagy and apoptosis, including BCL2, BCL2-associated X protein (BAX), and beclin 1, have been suggested to act as switching points that are critical for the outcome of tumor cells, and lysosomes have been reported to initiate the cell death pathway in autophagic cells [77, 78]. Regarding leprosy, it was observed that in skin cells of patients with the lepromatous form of the disease there is a blockade of the autophagic flux that can be attributed to the increased expression of the antiapoptotic protein BCL2, which inhibits autophagy mediated by beclin 1 [58]. Blockade of the autophagic machinery in lepromatous cells may contribute to the persistence of mycobacteria in host cells. Genetic studies on leprosy have shown that several polymorphisms in genes associated with the control of autophagic pathways such as IFN, immunity-related GTPase family M protein (IRGM), NOD, and TLR play a prominent role in susceptibility to the disease, thus demonstrating the importance of understanding, inducing, and controlling this biological process in leprosy [79–85].

When the initial studies aiming at induction of autophagy were conducted, the only known drug capable of inducing autophagy chronically was rapamycin. However, the adverse effects of rapamycin (which were not associated with the induction of autophagy) made this drug unattractive to use. Several drugs and nutritional supplements can induce autophagy, such as verapamil, statins, metformin, resveratrol, vitamin D, and omega 3 [86]. Although it is not known whether these agents exert their beneficial clinical effects through the induction of autophagy or other pathways, there is a considerable overlap between diseases occurring in an environment of poor autophagy and diseases that respond to drugs that may induce autophagy. With regard to infectious diseases, there are limited data on the usefulness of autophagy-inducing pharmaceutical agents as potential therapeutics against human pathogens. Drug screening studies that aim to identify molecules with pro-autophagic effects have been performed, and promising results demonstrated a pro-autophagic effect of drugs capable of inhibiting the growth of *M. tuberculosis* in human macrophages in vitro [87–89]. In addition, the antibiotics isoniazid and pyrazinamide, two first-line cocktail drugs used to treat tuberculosis, exert their antimycobacterial activity through autophagy [90]. The treatment with statins, drugs that inhibit cholesterol synthesis, reduces the bacillary load of *M. tuberculosis* in human macrophages and mice by increasing autophagy and phagosome maturation [91]. Furthermore, statins also have an antimicrobial effect against *M. leprae* and potentiate the antimycobacterial effect of rifampicin, a first-line cocktail drug used in leprosy treatment [92]. Vitamin D3, which activates autophagy, has been successfully used in the treatment of patients with tuberculosis and could be one of the components of an ideal treatment for leprosy and other chronic infectious diseases in which the cellular immune response is deregulated [93–96].

Activation of autophagy by verapamil has been demonstrated by several groups. Initial studies evaluating the effect of verapamil and its analogs on macrophages infected with *M. tuberculosis* showed an association between the induction of autophagy and inhibition of intracellular replication of mycobacteria, and one of the structural analogs had an additive effect on the inhibitory antimicrobial activity of isoniazid and rifampicin [97, 98]. Metformin is an antidiabetic of the biguanide class. Mechanisms of autophagy induction by metformin are known, but no relationship with infectious processes caused by mycobacteria has been described so far. Similarly, resveratrol has also been studied for its autophagy-inducing role, and no studies in the literature have been found correlating with the mycobactericidal role.

Together, these data show the importance of autophagy in the pathogenesis of leprosy, contributing to a better understanding of the mechanisms of mycobacterial control associated with the lepromatous and tuberculoid leprosy poles, which

may lead to the establishment of new targets and therapeutic strategies to control leprosy. Moreover, the identification of autophagy as an important factor during the establishment of resistant and susceptible forms of the disease opens the door for the development of new therapeutic strategies of disease control through the modulation of autophagy.

5. Conclusion

Considering the aspects observed during the course of this chapter, macrophages have a crucial role in inducing the immune response to *M. leprae*, and their uptake capacity, phagocytosis, and microbicidal activity may depend on the microenvironment. Macrophages, after the interacting with either the bacilli or its wall components, are able to induce oxidative stress [10–14] and to induce various receptors as scavenger receptors [6, 16, 23, 24, 34, 42–44] and PRR [53–55, 69], leading to the polarization of their response. In an anti-inflammatory profile (M2), this cell induces increased uptake of lipids [21, 22] and Hb-haptoglobin [16, 42], which aid the growth of *M. leprae* by the activation of the enzymes IDO [42, 45], HO-1 [42] and arginase [37]. On the other hand, in a pro-inflammatory and microbicidal profile (M1), the macrophage produces TNF [26, 48], IL-6 [37, 48, 49], and IL-15 [22, 37, 39] besides being able to stimulate T cells to produce IFN-γ [34]. In addition, these M1 macrophages induce autophagy [57, 58], an important process of homeostatic regulation recently described with the immunological role [56], which acts on infection control. Several drugs have been described as autophagy inducers and have been studied as treatment for neurodegenerative diseases [76] and to control of *M. tuberculosis* infection [89]. Autophagy-inducing drugs are promising targets as adjuvants to MDT.

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Conflict of interest

None.

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