**Mycobacterium Lepraemurium** uses TLR-6 and MR, But Not Lipid Rafts or DC-SIGN, to Gain Access into Mouse Macrophages

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**Abstract**

Objective/Background: *Mycobacterium lepraemurium* (MLM), the etiologic agent of murine leprosy, is an intracellular parasite of macrophages; the mechanism used by this bacterium to enter macrophages is not known. The fate of the MLM phagosome inside macrophages is also unknown. This study was conducted to investigate how MLM enters macrophages and to define the maturation process of MLM phagosome inside macrophages. **Materials and Methods:** Peritoneal macrophages were incubated in the presence of mannan–bovine serum albumin (BSA), and antibodies to known macrophage receptors, including, anti-FcγRIII/RII (anti-CD16/32), anti-CD35 (anti-CR1), anti-TLR2, anti-TLR4, anti-TLR6, anti-CD14, and anti-dendritic cell-specific intercellular adhesion molecule-3-grabbing nonintegrin (DC-SIGN). Then, macrophages were challenged with Iris Fuchsia-stained MLM, at a multiplicity of infection of 50:1. The blocking effect of the antibodies (and mannan–BSA) used was analyzed using direct microscopy and flow cytometry. The maturation process of MLM phagosomes was visualized by their interaction with antibodies to Rab5, Rab7, proton ATPase, and cathepsin D, by confocal microscopy. **Results:** Only mannan–BSA and anti-TLR6 antibody significantly blocked the entry of MLM into macrophages. None of the other antibodies, including that for DC-SIGN, meaningfully inhibited the endocytic process. We also found that MLM is a fusiogenic mycobacterium. This was deduced from the orderly association of MLM phagosomes with Rab5, Rab7, Proton ATPase, and lysosomes (cathepsin D). **Conclusion:** Fusion of MLM phagosomes with lysosomes seems to be a necessary event for the intracellular multiplication of MLM; similar to *Mycobacterium leprae*, this microorganism hardly grows on artificial, synthetic, bacteriologic media.

**Keywords:** Macrophages, mannose receptor, *Mycobacterium lepraemurium*, toll-like receptor-6

**INTRODUCTION**

Murine leprosy is a disease of rats and mice caused by *Mycobacterium lepraemurium* (MLM). Murine leprosy was first described in 1902 by Stefansky, who found the disease in rats while working on a rat eradication campaign in the old city of Odessa in Ukraine, Russia. The following year, Dean reported the disease in England, and within a few years, cases were reported from other parts of the world. The first description of the disease by Stefansky and Dean recognized a striking similarity between murine and human leprosy, both in terms of the causative organisms and the clinical and histopathological manifestations of the disease. These findings led to the belief that human and murine leprosy were identical or very closely related diseases, and for several years, this was the major motivation for studying murine leprosy. As a result, considerable attention was given to murine leprosy, both in clinical journals and in public health reports. The similarity between the two...
mycobacterioses was supported by (a) studies showing serological cross-reactivity between *Mycobacterium leprae* and MLM, the causative agent of murine leprosy;[11] (b) reports that human leprosy had been transferred from humans to mice;[4] (c) reports on murine leprosy in humans;[13] and (d) microbiological reports claiming that *M. leprae* and MLM were identical or almost identical.[9] However, as knowledge about diseases and microorganisms grew, the idea that mice serve as a reservoir for the human leprosy agent was gradually abandoned. The most recent studies regarding a possible causative relationship between human and murine leprosy agents were published around 1930.[8,12]

Currently, murine leprosy, which is a natural disease of the mouse, is rarely referred to in literature. Despite this, the mouse model of leprosy offers unique opportunities to study the basic mechanisms of this host–parasite interaction. This endeavor clearly has scientific value of its own, regardless of whether it can be used directly as a model for human diseases, such as leprosy.[13]

MLM is among the most evolved parasites of macrophages; it enters these cells inconspicuously, as it does not fully activate the oxygen-dependent microbicidal response.[13] It also does not fully activate the signaling cascades that lead to tumor necrosis factor α [TNFα] and inducible nitric oxide synthase gene expression; therefore, nitric oxide (NO) production remains low to nonexistent.[14] On comparison, macrophages infected *in vitro* with *Mycobacterium bovis* bacillus Calmette–Guérin (BCG) produce large amounts of reactive oxygen intermediaries, TNFα, and NO, which impedes the survival of this mycobacterium.[13,14]

Phagocytosis of mycobacteria by macrophages is a widely studied field. It is known, for instance, that *Mycobacterium tuberculosis* penetrates into macrophages through mannose receptor (MR), complement receptors (CR1, CR3, CR4), scavenger receptor, and toll-like receptors (TLR2 and TLR4).[15-21] MR was unable to discriminate *Mycobacterium kansasi* and *Mycobacterium smegmatis* from *Mycobacterium phlei*, and *M. leprae* used CR1 and CR3[13,22] as well as TLR1 and TLR2 to penetrate into macrophages.[23-25]

However, nothing is known on the manner by which MLM enters macrophages; there is no information on the macrophage receptors (pattern recognition receptors [PRRs]) or on the bacterial components (microorganism-associated molecular patterns [MAMPs]) that are involved in the process. Consequently, nothing is known about the signaling cascades triggered upon the entrance of MLM or on the maturation process of MLM-containing phagosomes. However, it is known that MLM is a fusogenic microorganism, that is, MLM-phagosomes fuse with lysosomes successfully.[26,27] To understand these processes more clearly, we investigated the participation of several PRRs in the phagocytosis of MLM by murine peritoneal macrophages as well as the fate of MLM-phagosomes within these cells.

**Materials and Methods**

**Reagents**

If not specified, chemicals were obtained from Sigma Chemical Co. (St. Louis, MO, USA).

*Mycobacterium lepraemurium*

MLM is a microorganism that does not usually grow on standard bacteriological media. Therefore, MLM was purified from the spleens of mice that were infected for 4–5 months. The complete Prabhakaran *et al.*[28] protocol and the Percoll step of Draper’s[29] method, in that order, were used to purify the bacilli. The process for isolation of the bacilli has been reported elsewhere.[30,31] Isolated, washed bacilli were suspended in Middlebrook 7H9 broth medium (Becton Dickinson Co., MD, USA) supplemented with 10% OADC (Becton Dickinson Co.), and the number of bacilli was calculated in reference to a standardized nephelometer curve at 580 nm. Bacterial viability was assessed by the technique of Jarnagin and Luchsinger[32] with fluorescein diacetate and ethidium bromide. Finally, the bacterial suspension, which was divided into aliquots, was stored at −20°C until used.

**Macrophages**

Female Balb/c mice, weighing 24–25 g, were used as the source of macrophages; the Balb/c strain is highly susceptible to leprosy. All animal experiments were conducted with the approval of the local Institutional Animal Care and Management Committee, which surveys the appropriate housing, feeding, and handling of animals. Macrophages were collected from the abdominal cavity of mice by two washes with 5-mL portions of Alsever’s solution as described previously.[13,14] The collected cell suspension was adjusted to contain 20 million cells per milliliter of standard Dulbecco’s modified Eagle’s culture medium (DMEM) supplemented with 10% fetal calf serum (FCS), amino acids, and antibiotics. Two milliliters of the suspension was placed per dish into 3-cm plastic Petri dishes (NUNC, Roskilde, Denmark); each dish contained a 2.5 cm × 2.5 cm sterile glass cover slide, which was thoroughly washed and degreased.

**Red fluorescent staining of mycobacteria**

Five microliters of Iris Fuchsia (Cyane Technologies, Torino, Italy) prepared in dimethyl sulfoxide (1 mg/mL) was incubated with about 10⁹ bacteria suspended in 0.5 mL of phosphate-buffered saline (PBS) for 30 min in dark. Iris Fuchsia is a fluorescent lipophilic dye with a maximal absorption at 570 nm (ethanol) and a maximal emission at 588 nm (ethanol). Iris Fuchsia-stained bacteria emit a red fluorescence at 588 nm; from here on, they will be referred to as red-MLM.

Stained bacteria were washed 3 times with PBS (2700 g for 5 min) and then suspended in DMEM–FCS to 500 × 10⁶ bacilli/mL for use in *in vitro* infection experiments.

**Transmission electron microscopy**

Control and infected murine macrophages were collected, washed with PBS with glucose, and fixed with glutaraldehyde
and osmium tetroxide. Infected macrophages were then dehydrated gradually in increasing concentrations of ethanol, embedded in lipophilic Spurr resin, and processed for electron microscopy according to standard procedures. A Carl Zeiss EM-910 electronic microscope was used for this analysis.

**Washing procedure**

For the washing steps, we used PBS (0.15M NaCl, 0.01M phosphate salts, pH 7.4) or culture medium, depending on the experiment. Washing of cell suspensions was done by centrifugation at 300 g for 3 min, 3 times, whereas washing of fixed or unfixed cell monolayers was done by dipping the slides or cover slides in PBS for 5 min, 3 times.

**Blockage of macrophage receptors**

Murine macrophages were placed in Nalgene, low cell-adherence tubes, at a concentration of 1 × 10⁶ cells per milliliter of DMEM–FCS. Each cell suspension was then mixed with 1 µg of one of the following monoclonal antibodies: Anti-FcγRIII/RII (anti-CD16/32), anti-CD35 (anti-CR1), anti-TLR2, anti-TLR4, anti-TLR6, anti-CD14, anti-dendritic cell-specific intercellular adhesion molecule-3-grabbing nonintegrin (DC-SIGN), or bovine serum albumin (BSA)-coupled mannan–fluorescein isothiocyanate (these reagents were obtained from Santa Cruz Biotechnology, Pharmingen, eBioscience, or Sigma). Washed MLM preincubated for 30 min at 37°C in fresh serum from a mouse bearing a 6-month infection with MLM was used to evaluate the participation of CD16/32, whereas MLM preincubated in fresh guinea pig serum (complement) was used to evaluate the participation of CD35 in the entry of immunoglobulin G-or C3b-opsonized bacteria into macrophages (MLM can activate the alternative complement pathway).[35] A home-made rabbit antiserum to whole macrophage membranes at a dilution of 1:500 was also used to block the entry of MLM. The mixtures were incubated for 60 min at 4°C, and then washed with cold DMEM–FCS prior to being challenged with red (opsonized or nonopsonized)-MLM at the optimal concentration established empirically (usually 1:50:1) for 15, 30, and 60 min. Some monolayers were pretreated with 10 mM methyl-alpha-cyclodextrin (Sigma) for 60 min prior to infection. Cyclodextrin is a molecule that removes cholesterol on the cell membrane, thus arresting the redistribution of lipid rafts.[34,35] At the end of each infection time point, triplicate cultures were removed, washed with PBS, fixed with 2% paraformaldehyde in PBS for 30 min, washed again with PBS, incubated for 30 min with 2% mouse serum in PBS (blocking agent), washed with PBS, and then incubated with the β-fraction of fluoresceinated cholera toxin (Sigma) at a concentration of 0.5 µg/mL for 1 h at RT. The β-fraction of cholera toxin interacts with the oligosaccharide chain of the ganglioside G₀, on lipid rafts.[16] Finally, the cell preparations were washed with PBS, mounted with Vectashield (Vector Labs., Inc.), and analyzed by ultraviolet (UV) and confocal microscopy.

**Maturation of Mycobacterium lepraemurium-containing phagosomes**

Cultured murine macrophages were infected with red-MLM at MOI 50:1 and incubated for several time points (5, 15, 30, 45, 60, and 120 min). At the end of each time point, macrophage monolayers were washed once with PBS, fixed with 2% paraformaldehyde in PBS, washed with PBS, incubated for 10 min with 0.02% Triton X-100 (Sigma) or 0.3% sodium dodecyl sulfate in PBS, washed again with PBS, treated with a blocking solution of 3% BSA in PBS for 30 min, and then incubated for 1 h with goat antibodies to Rab5, Rab7, cathepsin D, and vacuolar ATPase, all from Santa Cruz Biotechnology, at the optimal concentration established empirically (usually diluted 1:100 in PBS). The monolayers were then washed twice gently with PBS prior to being incubated for 45 min with a fluoresceinated rabbit anti-goat polyclonal antibody (also from Santa Cruz Biotechnology) and diluted at 1:200 in PBS. Finally, the macrophage monolayers were washed 3 times with PBS and mounted with Vectashield (Vector Labs., Inc.); then, the preparations were examined under UV and laser (confocal) microscopy.

**Results**

**Phagocytosis of Mycobacterium lepraemurium**

Contact of MLM with macrophages occurred at the apical regions of the cytoplasmic projections and filaments [Figure 1]; currently, we do not know the nature or structure of the molecules present in these regions. Although it may occur immediately upon contact, endocytosis was evident after 15 min of infection. MLM penetrated the cells in an orderly...
manner and moved centripetally as if they were sliding on tracks in the cytoskeleton, probably transported by microtubules, to eventually locate in a cytoplasmic region adjacent to the nucleus [Figure 2]. At an MOI of 50:1, the highest MOI used in this study, phagocytosis continued over several hours.

**Fate of macrophages and bacilli**

Once within macrophages, MLM multiplied with a doubling time of about 72 h, and multiplication continued up to the saturation of macrophages. Figure 3 depicts macrophages infected for 12 days with MLM; it can be observed that despite the vast amount of bacilli, macrophages retained a healthy, although rounded morphology, with no evidence of necrosis or apoptosis as deduced by the integrity of both the whole cell and the nucleus. Macrophages were eventually disrupted, releasing their bacillary cargo. *In vivo*, these bacilli are then taken up by newly arrived macrophages, which then extend the infection.

**Transmission electron microscopy**

With transmission electron microscopy, it was observed that MLM penetrated the macrophages and were localized within isolated vesicles that then fused with each other to produce giant phagosomes. MLM-infected macrophages did not show any evidence of activation (ruffling of the membrane) or damage of any kind, which suggests a largely inert phenotype within these cells. MLM itself did not show obvious signs of damage, even after many days after ingestion. It is probable that the lipid envelope of MLM (electron-transparent zone) protects the bacteria from any possible deleterious, if any, activity of macrophages [Figure 4].

**Blocking of macrophage surface receptors**

Although several macrophage receptors may participate variably in the recognition and entry of MLM, only the blockage of TLR6 and MR resulted in a significant diminution (~18% and 13%, respectively) in the ingestion of MLM [Figure 5]. Blocking of other receptors, including DC-SIGN, did not have a significant effect on the phagocytosis of MLM (in these cases, alteration in the degree of phagocytosis varied from 0% to 5%, at most). Blockage with the home-made rabbit antiserum to whole macrophage membranes showed, as expected, the strongest blocking effect [~24%; Figure 6].

**Colocalization studies**

Assessment of contact between MLM and mouse macrophages showed colocalization with TLR6 and the MR, which are the receptors most apparently involved in the entry of MLM into murine macrophages. This is clear from the colocalization lines in Figure 7.

**Lipid rafts**

Infection of macrophages with MLM did not induce the redistribution of lipid rafts in the cell membrane. Cyclodextrin treatment of macrophages prior to infection did not prevent the contact or entry of MLM into these cells. In contrast, infection of macrophages with *Brucella abortus* induced redistribution of lipid rafts, and lipid rafts colocalized with *Brucella*. Furthermore, treatment of macrophages with cyclodextrin deterred the ingestion of *Brucella* by macrophages.

**Dendritic cell-specific intercellular adhesion molecule-3-grabbing nonintegrin**

Expression of DC-SIGN in nonstimulated peritoneal mouse macrophages was scarce, but it could be stimulated by interleukin-4 (IL-4), IL-10, TGFβ, or all, together. In this

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**Figure 1:** Peculiar primary contact of MLM with murine peritoneal macrophages. Bacilli get trapped by unknown structures present in the apical cytoplasmic projections of macrophages (arrows) (Ziehl–Neelsen stain, ×100). BCG = bacillus Calmette-Guérin; MLM = *Mycobacterium leprae* murium.

**Figure 2:** Time-lapse microphotography of the ingestion of MLM by murine peritoneal macrophages. (a-e) Bacilli, taken up at the apical region of a cytoplasmic projection, travel centripetally along the projection (white arrows) to eventually localize perinuclearly. (f) Electron microscope image of the oriented, orderly, transport of several bacilli within the cell. MLM = *Mycobacterium leprae* murium.
study, we used IL-10 to induce overexpression of DC-SIGN. From 0.6 to 2.0 ng of IL-10 (Pharmingen) per milliliter was incubated with 10^6 cells for 24 h. After washing with PBS, the monolayers were fixed with 2% paraformaldehyde for 30 min, washed again, blocked with 3% bovine albumin in PBS for 30 min, incubated overnight at RT with a rat anti-mouse DC-SIGN (CIRE, eBioscience) diluted to 1:250, washed with PBS, incubated with a second fluoresceinated anti-rat antibody (1:250) for 2 h at RT, washed with PBS, mounted

Figure 3: Murine peritoneal macrophages at 12 days after infection with MLM at an initial MOI of 50:1. Notice the healthy appearance of the cells despite the enormous numbers of intracellular bacilli. No evidence of apoptosis or necrotic damage is observed (Ziehl–Neelsen, ×60). MLM = *Mycobacterium lepraemurium*; MOI =Multiplicity of infection.

Figure 5: Ingestion of Iris Fuchsia (red)-stained MLM by murine peritoneal macrophages previously incubated with mannosylated-BSA (MR) or anti-TLR6. BSA = Bovine serum albumin; Control+ = MLM-infected macrophages; Control– = Uninfected macrophages (flow cytometry analysis); MLM = *Mycobacterium lepraemurium*; MR = Mannose receptor; TLR6 = Toll-like receptor 6.

Figure 6: Ingestion of red (opsonized or nonopsonized)-MLM by murine macrophages preincubated with antibodies to CD14, CD16, CD35, DC-SIGN, TLR2, TLR4, TLR6, mannosylated-BSA (for MR), or whole macrophage membranes (A-MPH-S). Percent phagocytosis from three experiments is given in relation to phagocytosis in the control system (intact macrophages). Flow cytometry analysis. *P* indicates significant values relative to control unblocked macrophages. A-MPH-S = Antimacrophage serum; BSA = Bovine serum albumin; CD = CD = Cluster of differentiation; MLM = *Mycobacterium lepraemurium*; MR = Mannose receptor; TLR = Toll-like receptor; DC-SIGN = Dendritic cell-specific intercellular adhesion molecule-3-grabbing nonintegrin.
Phagosome maturation
MLM did not interfere with the maturation of its phagosomes as they appeared to be associated to Rab5 as early as 15 min after infection, to Rab7 by 30 min after infection, and to the proton-ATPase by 60 min after infection. At this latest time, most ingested bacteria also colocalized with cathepsin D, a phagolysosomal marker. Colocalization of MLM with several phagosome maturation markers is illustrated in Figure 8.

Discussion
Currently, human leprosy is a highly neglected disease, and even more so is murine leprosy. Regardless, they are still excellent disease models with which to conduct relevant pathological, immunological, biochemical, genetic, and bacteriological studies. Present-day inattention to novel research on these diseases can perhaps be attributed to the following: (a) the efficacy of antileprosy chemotherapy in reducing the number of cases worldwide (although some important foci remain active in India, Central Africa, Asia, and the Americas) and (b) the recognition of murine leprosy as a disease essentially different from human leprosy. Many questions remain unanswered regarding both diseases, and murine leprosy offers the opportunity to address some of them. One such question refers to the mechanism(s) that MLM uses to enter the macrophage; nothing is known about this subject. We have regarded MLM as an ideal parasite of the mouse based on the following findings (reviewed by Rojas-Espinosa): (a) Most, if not all, mouse strains are susceptible to murine leprosy, and as few as five bacilli are enough to produce a systemic infection. (b) MLM is a nontoxic bacterium. (c) Murine leprosy evolves parallel to the loss of MLM-specific cell-mediated immunity. (d) During murine leprosy infection, a mechanism that inactivates complement develops. (e) Murine leprosy does not affect the host’s kidneys. (f) MLM does not infect peripheral or central nerve tissue. (g) MLM enters macrophages without triggering an oxidative response. (h) MLM parasitizes macrophages without inducing the production of significant amounts of TNFα or NO. (i) MLM does not induce necrotic or apoptotic death of infected macrophages.

Thus, in this study, we analyzed the participation of several macrophage surface receptors in the entry of MLM and the intracellular changes that occurred upon infection. An interesting observation was the finding that MLM entered macrophages through structures present in the apical regions of the cytoplasmic projections. The nature or structure of these regions is presently unknown. In addition, MLM entered macrophages without modifying their morphology and without inducing their detachment as was the case with BCG (unpublished personal observations).

Once ingested, MLM distributed itself in an orderly manner, around the nucleus and around a cytoplasmic zone in which no structure has been identified by electron microscopy. MLM then began to replicate continuously, living in perfect harmony with its cellular host as deduced from the healthy appearance of both macrophages and bacilli, even at long times of cultivation. From these observations, we deduced that MLM did not induce necrotic or apoptotic death of macrophages, a situation similar to that observed with *M. tuberculosis* strain H37Rv and wild-type *M. bovis.* In general, viable virulent mycobacterial strains avoid or induce less apoptosis than do avirulent strains.

In leprosy, histological evidence of apoptosis, as observed by light microscopy and electron microscopy, has been reported, although apoptosis was not extensive. However, the in vitro studies have been controversial. For example, Hernandez et al. found that *M. leprae* induces apoptosis in monocyte-derived macrophages taken from both leprosy patients and healthy individuals, although at a lower efficiency compared to heat-killed *M. tuberculosis*; in this study, apoptosis was related to the expression of Bax-α, Bak, and TNFα. In contrast, Hasan et al. found that ML does not promote, but
rather inhibits, apoptosis in THP-1 cells by downregulation of Bad and Bak and upregulation of Mcl-1 gene expression; the opposite was observed with BCG, which was a strong inducer of apoptosis in these cells.

Regarding the receptors involved in the uptake of MLM by murine macrophages, only TLR6 and MR, out of the eight receptors tested, appeared to play a significant role. However, this result does not eliminate the participation of other receptors in this process, because MLM surely have a diversity of MAMPs in addition to those tested here.

It is known that TLR6 recognizes lipid compounds, and that these compounds are abundant in the cell walls of MLM and other mycobacteria,[45-47] however, there are no reports of the participation of TLR6 in the uptake of mycobacteria. For example, Nicolle et al.,[49] using mice lacking TLR2, TLR4, and TLR6, found no difference in the infection with *M. bovis* BCG between mutant and wild-type mice. In addition, Sugawara et al.[50] reported that TLR2 and TLR6 did not participate in the infection of murine macrophages by *M. tuberculosis*. So far, TLR6, as with other TLRs, has been recognized as a signaling molecule,[50-52] and its role as an endocytic receptor has not been previously reported. Furthermore, TLR6 might participate in the generation of foamy macrophages in murine leprosy as it has been found in macrophages infected with *M. leprae*.[53]

A particularly interesting observation is that DC-SIGN does not seem to participate in the entry of MLM to murine macrophages. DC-SIGN is an important receptor on macrophages and DCs that captures and internalizes intact *M. bovis* BCG[54] and *M. tuberculosis*.[55,56]

In addition, DC-SIGN has been found highly expressed in the lesions of lepromatous but not borderline tuberculoid leprosy, suggesting an association between the expression of DC-SIGN and the prevalence of a Th2 environment.[57] DC-SIGN was present on virtually all *M. leprae*-containing cells, providing evidence for its role as a receptor. In the present communication, we succeeded in overexpressing DC-SIGN in murine macrophages by means of IL-10, but were unable to detect any association between this receptor and MLM.

Very little is known about MLM-phagosomes. Hart et al.[27] were the first to notice that MLM was a fusogenic bacterium, implying that MLM does not arrest the maturation of its phagosome. Phagosome maturation involves interaction with diverse subcellular vesicles prior to coalescing with lysosomes. Our results indicate that MLM-phagosomes interact sequentially with Rab5 (by 15 min), Rab7 (by 30 min), and vacuolar ATPase and cathepsin D (by 60 min). Thus, it is clear that MLM-phagosomes mature to phagolysosomes; it is not clear, however, how MLM survives and multiplies within the harsh phagolysosomal milieu. Some experimental evidence indicates that the thick and complex envelope of this microorganism plays a protective role against the noxious agents within the phagolysosome.[58] MLM neither escapes from phagosomes into the cytoplasm nor arrests the maturation of its phagosomes as it has been documented for *M. tuberculosis* and *M. leprae*.[59-63] Formation of the phagolysosome may, however, be needed to allow the growth of MLM within the cell; the lysosomal acid hydrolases would permeabilize the bacterial cell wall, allowing for the diffusion of nutrients from the phagolysosome into the bacterial cell. Multiplication within phagolysosomes of murine macrophages has also been observed with *M. marinum*.[64]

In relation to lipid rafts, membrane lipid rafts are small (10–200 nm), heterogeneous, highly dynamic, cholesterol-, sphingolipid-, and ganglioside G412M-enriched domains that participate in cellular processes. Small rafts can sometimes
be stabilized to form larger platforms through protein–protein and protein–lipid interactions. Lipid rafts participate in the mobilization of receptors and accessory molecules to the contact sites between cells and microorganisms, thus optimizing the endocytic process. Indeed, lipid rafts serve as entry ports for several microorganisms, such as mycobacteria (Mycobacterium avium\cite{65,66,67} and M. tuberculosis\cite{68,69,70} Brucella\cite{68,70} and others\cite{71-73}). Lipid rafts have not been studied as entry ports for M. leprae or MLM. The present study rules out the participation of lipid rafts in the phagocytosis of MLM, as removal of cholesterol with \( \alpha \)-methyl cyclodextrin did not interfere with the endocytosis of this mycobacterium, and no colocalization was observed between MLM and the fluoresceinated \( \beta \)-subunit of cholera toxin on the macrophage cell surface, which binds to the oligosaccharide chain of ganglioside G\(_{MI}\) of membrane lipids.\cite{74}

**Conclusion**

Four findings on the MLM–macrophage interaction arose from this study. (a) TLR6 and MR participate as macrophage receptors for MLM. (b) MLM does not interfere with the maturation of their phagosomes. (c) DC-SIGN is not involved in the uptake of MLM by mouse macrophages. (d) Lipid rafts do not participate in the endocytosis of MLM by murine macrophages.

The study of signaling cascade(s) triggered by activation of TLR6 and the effect of interference RNAs for TLR6 on the phagocytosis of MLM is currently being researched in our laboratory.

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**Conflicts of interest**

There are no conflicts of interest.

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