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Macrophage sphingolipids are essential for the entry of mycobacteria

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ABSTRACT

Mycobacteria are intracellular pathogens that can invade and survive within host macrophages. Mycobacterial infections remain a major cause of mortality and morbidity worldwide, with serious concerns of emergence of multi and extensively drug-resistant tuberculosis. While significant advances have been made in identifying mycobacterial virulence determinants, the detailed molecular mechanism of internalization of mycobacteria into host cells remains poorly understood. Although several studies have highlighted the crucial role of sphingolipids in mycobacterial growth, persistence and establishment of infection, the role of sphingolipids in the entry of mycobacteria into host cells is not known. In this work, we explored the role of host membrane sphingolipids in the entry of *Mycobacterium smegmatis* into J774A.1 macrophages. Our results show that metabolic depletion of sphingolipids in host macrophages results in a significant reduction in the entry of *M. smegmatis*. Importantly, the entry of *Escherichia coli* into host macrophages under similar conditions remained invariant, implying the specificity of the requirement of sphingolipids in mycobacterial entry. To the best of our knowledge, our results constitute the first report demonstrating the role of host macrophage sphingolipids in the entry of mycobacteria. Our results could help in the development of novel therapeutic strategies targeting sphingolipid-mediated entry of mycobacteria into host cells.

1. Introduction

Membrane lipids play a crucial role in membrane organization, dynamics, trafficking and cellular signaling. Intracellular pathogens have evolved diverse mechanisms to exploit host cell lipids for their survival (Toledo and Benach, 2015; van der Meer-Janssen et al., 2010; Vromman and Subtil, 2014). In particular, *Mycobacterium tuberculosis*, the causative organism of tuberculosis (TB) in humans, utilizes a large part of its coding capacity to produce enzymes for lipogenesis and lipolysis. It exploits host lipids as an energy source for growth and survival inside the host cells (Lovewell et al., 2016; Toledo and Benach, 2015; Vromman and Subtil, 2014; Wipperman et al., 2014). TB remains a major cause of mortality and morbidity worldwide with an estimated 10.4 million new cases and 1.4 million deaths reported in 2015 alone (World Health Organization, 2016). This is further worsened by opportunistic TB infection among HIV positive patients (Pawlowski et al., 2012; World Health Organization, 2016). In addition, the emergence of multi and extensively drug-resistant TB is a serious concern and has necessitated research into novel therapeutic strategies to combat this disease (Dye, 2009; Gandhi et al., 2006; Keshavjee and Farmer, 2012).

Mycobacteria infect their hosts *via* inhalation and after entering the lungs, the bacilli are internalized primarily by alveolar macrophages (Russell, 2007). Work from several laboratories has shown that cellular plasma membranes serve as entry portals for intracellular pathogens (Cossart and Roy, 2010; Kumar et al., 2016a; Rosenberger et al., 2000; Shin and Abraham, 2001; Toledo and Benach, 2015; van der Goot and Harder, 2001; van der Meer-Janssen et al., 2010; Vromman and Subtil, 2014). In order to achieve entry into host cells, intracellular pathogens such as mycobacteria interact with the host cell plasma membrane as a prerequisite to gain access to the cellular interior (Kumar et al., 2016a). However, the detailed molecular mechanism for the entry of mycobacteria into host cells is not clear. A number of candidate receptors have been identified on the cell surface of host macrophages that are important for mycobacterial recognition and entry (Ernst, 1998; Killick et al., 2013). Macrophage receptors such as mannose receptor, scavenger receptors, CD-14, dectin-1, DC-SIGN, and complement receptors

Abbreviations: BCA, bicinchoninic acid; CFU, colony forming unit; DAPI, 4′,6-diamidino-2-phenylindole; DIC, differential interference contrast; DMSO, dimethyl sulfoxide; dsRed2, red fluorescent protein; FB_1, fumonisin B_1; GPCR, G protein-coupled receptor; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; SBD, sphingolipid binding domain; TB, tuberculosis

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have been found to be important for *Mycobacterium* to gain access to the interior of host cells (Ernst, 1998; Tailleux et al., 2003; Yadav and Schorey, 2006). The wide variety of receptors responsible for mycobacterial entry into host macrophages highlights the redundancy in the entry process, and therefore no panacea targeting this critical step has been developed for the treatment of TB.

Membrane lipids such as cholesterol and sphingolipids play an important role in host-pathogen interaction (Chattopadhyay and Jafurulla, 2012; Dumas and Haanappel, 2017; Goluszko and Nowicki, 2005; Hanada, 2005; Kumar et al., 2016a; Lovewell et al., 2016; Pucadyil and Chattopadhyay, 2007; Riethmüller et al., 2006; Simons and Ehehalt, 2002; Toledo and Benach, 2015; van der Meer-Janssen et al., 2010; Vromman and Subtil, 2014; Wipperman et al., 2014). Cholesterol and sphingolipids are essential components of eukaryotic cell membranes (Bartke and Hannun, 2009; Holthuis et al., 2001; Kumar and Chattopadhyay, 2016; Mouritsen and Zuckermann, 2004; Simons and Ikonen, 2000) and are together known to exhibit non-random distribution into domains in biological and model membranes (Brown, 1998; Chaudhuri and Chattopadhyay, 2011; Lingwood and Simons, 2010; Masserini and Ravasi, 2001; Mukherjee and Maxfield, 2004; Ramstedt and Slotte, 2006; Xu and London, 2000). Mycobacteria are known to utilize host cholesterol and sphingolipids as nutrient source for their growth and survival (Lovewell et al., 2016; Speer et al., 2015; Toledo and Benach, 2015; Vromman and Subtil, 2014; Wipperman et al., 2014). Although the role of host membrane cholesterol in the entry of *Mycobacterium* has previously been addressed (Gatfield and Pieters, 2000; Viswanathan et al., 2015), the role of host membrane sphingolipids in mycobacterial entry has not been explored.

Sphingolipids are essential components of eukaryotic cell membranes and constitute ~10–20% of membrane lipids (Holthuis et al., 2001). They are relatively abundant in plasma membranes compared to intracellular membranes and are known to mediate several cellular processes such as cellular signaling, growth and differentiation (Bartke and Hannun, 2009; Holthuis et al., 2001). In addition, sphingolipids have been shown to modulate the function and organization of important classes of membrane proteins such as G protein-coupled receptors (GPCRs) (Jafurulla and Chattopadhyay, 2013, 2015), which are major drug targets (Chattopadhyay, 2014). Importantly, sphingolipids have been identified as key players in various infectious diseases (Hanada, 2005; Heung et al., 2006). It is interesting to note that mycobacteria have been reported to utilize sphingomyelin, a predominant sphingolipid in mammals, as a nutrient source and sphingomyelin has been shown to promote mycobacterial growth *in vitro* (Speer et al., 2015). Importantly, the ceramide/sphingolipid components generated during mycobacterial infection have been shown to be crucial in establishing bacterial persistence (Gutierrez et al., 2009; Speer et al., 2015), which has been suggested to contribute to their observed drug resistance (Sharma and Prakash, 2017).

Although sphingolipids have been identified as key players in mycobacterial growth, survival and establishing infection, their possible role in the entry of mycobacteria into host cells remains unexplored. In this work, we explored the role of host membrane sphingolipids in the entry of *Mycobacterium smegmatis*. *M. smegmatis* serves as a surrogate model for mycobacterial internalization since it is believed that the entry mechanisms are conserved in pathogenic and non-pathogenic mycobacteria (Zhang, 2013). Our results show that metabolic depletion of sphingolipids in host macrophages results in a significant reduction in the entry of *M. smegmatis*. To the best of our knowledge, our results constitute the first report demonstrating the role of host macrophage sphingolipids in the entry of mycobacteria.

2. Materials and methods

2.1. Materials

Penicillin, streptomycin, gentamicin sulfate, fumonisin B_1, sphingomyelinase (EC 3.1.4.12 from *Bacillus cereus*), 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide (MTT), Triton X-100, DMSO, NaCl and Tween 80 were obtained from Sigma Chemical Co. (St. Louis, MO). Middlebrook 7H9 broth and 7H10 agar were obtained from Becton, Dickinson Difco (Sparks, MD). Amplex Red sphingomyelinase assay kit was from Molecular Probes/Invitrogen (Eugene, OR). Bicinchoninic acid (BCA) assay reagent was obtained from Pierce (Rockford, IL). RPMI-1640 medium and fetal bovine serum were obtained from Gibco/Life Technologies (Grand Island, NY). All other chemicals used were of the highest available purity. Water was purified through a Millipore (Bedford, MA) Milli-Q system and used throughout.

2.2. Methods

2.2.1. Cell culture

J774A.1 murine macrophages (American Type Culture Collection) were cultured as described previously (Kumar et al., 2016b) with some modifications. In short, cells were maintained in RPMI-1640 medium supplemented with 2.4 g/l sodium bicarbonate, 10% fetal bovine serum, 60 μg/ml penicillin, 50 μg/ml streptomycin and 30 μg/ml gentamicin sulfate in a humidified atmosphere at 37 °C with 5% CO_2.

2.2.2. Bacterial culture

Mycobacterium smegmatis mc^26 was cultured as described previously (Viswanathan et al., 2015). In brief, *M. smegmatis* was grown at 37 °C under shaking conditions in Middlebrook 7H9 broth and 7H10 agar supplemented with albumin dextrose complex (5 g/l BSA, 2 g/l glucose and 0.85 g/l NaCl), 0.5% (v/v) glycerol and 0.05% (v/v) Tween 80. *E. coli* DH5α was cultured in Luria Bertani broth at 37 °C under similar conditions.

2.2.3. Depletion of cellular sphingolipids and quantitation of sphingomyelin

Sphingolipid depletion in J774A.1 cells was carried out using fumonisin B_1 as described earlier (Paila et al., 2010). For this, J774A.1 cells were seeded in 6 well plates at a density of $\sim 2 \times 10^5$/well and incubated for 24 h prior to treatment. A stock solution (10 mM) of FB_1 was prepared in DMSO and macrophages were treated with final concentrations of 0.5 and 1 μM FB_1 in RPMI-1640 medium with 5% serum for 60 h at 37 °C. After treatment, cells were washed with PBS and used for further experiments. Total cellular sphingomyelin was estimated from cell lysates using the Amplex Red sphingomyelinase assay kit. Sphingomyelin content was normalized to total cellular protein quantified using the bicinchoninic acid assay (Smith et al., 1985).

2.2.4. MTT viability assay

Macrophage viability upon treatment with FB_1 was assessed using MTT assay as described previously (Viswanathan et al., 2015). Briefly, macrophages were plated at an initial density of $\sim 2 \times 10^4$ in 24 well plates and treated with FB_1 as described in Section 2.2.3. Cells were then washed and incubated with MTT dissolved in serum-free RPMI-1640 medium at a final concentration of 0.4 mg/ml at 37 °C for 1 h. The color obtained upon dissolving formazan crystals, formed on reduction of MTT salt by mitochondrial enzymes in live cells (Vistica et al., 1991) in DMSO, was measured by absorbance at 540 nm in a PowerWave XS2 microplate spectrophotometer (BioTek, Winooski, VT).

2.2.5. Quantitation of bacterial entry into macrophages

Entry of *M. smegmatis* and *E. coli* DH5α into J774A.1 macrophages was quantitated by scoring colony forming units (CFU), as described previously (Viswanathan et al., 2015). Exponentially growing *M. smegmatis* and *E. coli* strains at a multiplicity of infection (MOI) of 100:1 (bacteria to macrophage) were used to perform infections. Prior to infection, single cell suspension of *M. smegmatis* was obtained by passing the culture through a 26 1/2 gauge needle 5–6 times. Macrophages were incubated with bacteria for 2 h and washed with PBS. These macrophages were then treated with gentamycin (50 μg/ml) in serum-

free RPMI-1640 medium for 30 min at 37 °C to kill extracellular bacteria, followed by 3 washes with antibiotic free media. The efficacy of gentamycin treatment was verified by plating the wash fractions on Middlebrook 7H10 agar to enumerate CFU counts. No colonies appeared on these plates, indicating that all extracellular bacteria had indeed been killed by this treatment. Macrophages were lysed using 0.1% (v/v) Triton X-100 and appropriate dilutions of *M. smegmatis* were made in Middlebrook 7H9 media and were plated on Middlebrook 7H10 agar. In case of *E. coli,* appropriate dilutions were made in Luria Bertani broth and were plated on Luria Bertani agar. Plates were incubated at 37 °C till visible colonies appeared for counting CFUs.

2.2.6. Fluorescence confocal microscopy of the entry of M. smegmatis into macrophages

J774A.1 macrophages were seeded on glass cover slips at a density of $\sim 2 \times 10^4$. Upon treatment with FB_1, macrophages were infected with *M. smegmatis* stably expressing the fluorescent protein dsRed2 (strain transformed with pMSP12:*dsRed2* (Cosma et al., 2004)) as described in Section 2.2.5. Cells were subsequently washed with PBS, fixed with 4% (v/v) formaldehyde and mounted in media containing DAPI. Images were acquired on a Leica SP8 confocal microscope (Wetzlar, Germany) with a 63 × /1.4 NA oil immersion objective. Maximum intensity projections were generated upon merging confocal z-sections of infected macrophages.

2.2.7. Statistical analysis

Significance levels were estimated using the Student's two-tailed paired *t*-test using Graphpad Prism software version 4.0 (San Diego, CA). Plots were generated using OriginPro software, version 8.0 (OriginLab, Northampton, MA).

3. Results

3.1. Treatment of macrophages with fumonisin B_1 leads to a reduction in total cellular sphingomyelin

Sphingolipid biosynthesis is initiated by condensation of serine with palmitoyl CoA to form 3-ketosphinganine (see Fig. 1a). This is further converted to sphinganine, the basic building block of sphingolipids. Sphinganine (or sphingosine) is acylated by ceramide synthase (*N*-acetyltransferase) to form dihydroceramide (or ceramide). Ceramide is converted to sphingomyelin or glycosphingolipids. Sphingolipids exhibit enormous diversity with different headgroups and fatty acids attached to sphingoid base and ceramide (Fantini and Barrantes, 2009; Slotte, 2013). Cellular sphingolipid levels can be conveniently modulated using fumonisin B_1 (FB_1), and this approach has been extensively used to decipher the role of sphingolipids in various cellular processes (Jafurulla and Chattopadhyay, 2015; Merrill et al., 1996; Paila et al., 2010; Soriano et al., 2005). FB_1 is a mycotoxin produced by *Fusarium* species and is a potent inhibitor of sphingolipid biosynthesis. Owing to its structural similarity with sphingoid bases (sphingosine and sphinganine), FB_1 competitively inhibits ceramide synthase, the enzyme responsible for acylation of sphingoid bases during the biosynthesis of sphingolipids (see Fig. 1a).

In order to study the role of sphingolipids in the entry of mycobacteria into host cells, we metabolically depleted sphingolipids from J774A.1 macrophages using FB_1. Upon treatment of macrophages with FB_1, we estimated the levels of sphingomyelin, which constitutes a major fraction (~80%) of total cellular sphingolipids (Bartke and Hannun, 2009; Holthuis et al., 2001) as a representative indicator of total macrophage sphingolipids. As shown in Fig. 1b, treatment with FB_1 resulted in a concentration-dependent reduction in sphingomyelin content of macrophages. We observed a reduction of ~23% and ~52% sphingomyelin in macrophages upon treatment with 0.5 and 1 μM FB_1, respectively.

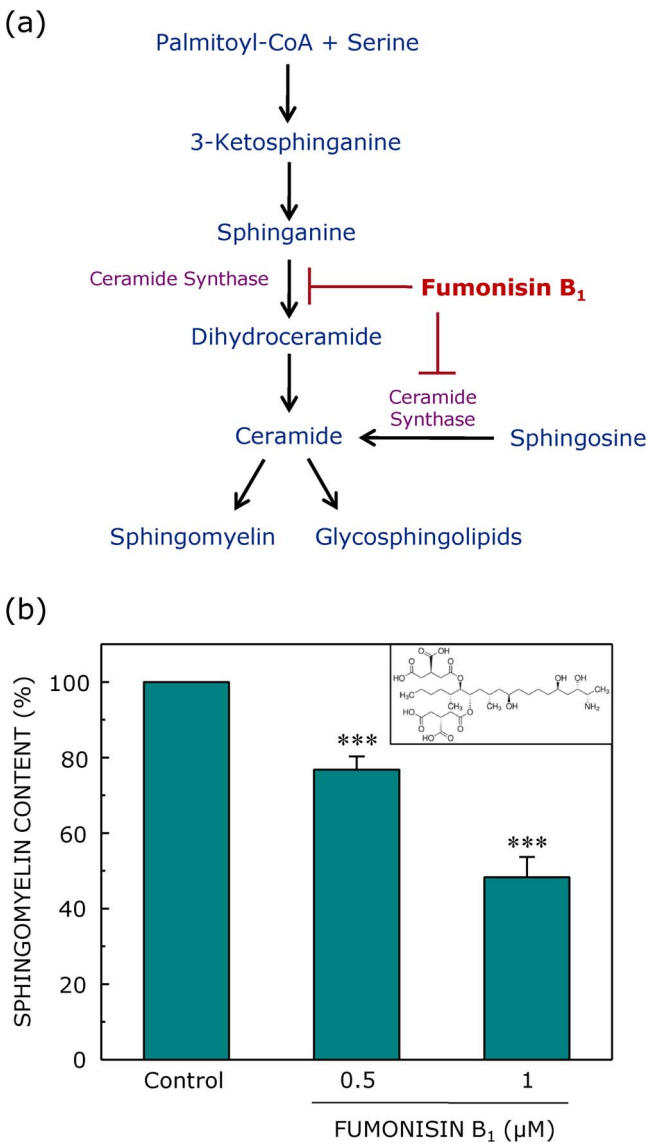

Fig. 1. Biosynthetic pathway of sphingolipids and inhibition of sphingolipid biosynthesis in macrophages utilizing fumonisin B_1. (a) Sphingolipid biosynthetic pathway highlighting specific steps at which fumonisin B_1 (FB_1) acts. FB_1 is a competitive inhibitor of ceramide synthase (*N*-acetyltransferase), the enzyme that catalyzes the acylation of sphinganine in *de novo* biosynthesis of sphingolipids and reutilization of sphingosine derived from sphingolipid turnover. Cellular sphingolipids in J774A.1 macrophages were depleted by treatment with FB_1 (as described in Section 2). (b) Sphingomyelin content of macrophages upon treatment with FB_1 was quantitated using Amplex Red assay. Values are normalized to sphingomyelin levels of untreated (control) cells. Data represent means ± S.E. of three independent experiments (*** corresponds to significant ($p < 0.001$) differences in sphingomyelin content of FB_1-treated cells relative to control cells). The chemical structure of FB_1 is shown in the inset. See Section 2 for more details.

3.2. Treatment of macrophages with FB_1 does not compromise macrophage viability

It has been previously shown that FB_1, apart from inhibiting sphingolipid biosynthesis, could be cytotoxic by inducing oxidative stress at high concentrations (Cetin and Bullerman, 2005; Kouadio et al., 2005; Sjögren and Svenningsson, 2007; Yu et al., 2001). We therefore assessed viability of macrophages upon treatment with FB_1 under our experimental conditions, utilizing MTT viability assay. Fig. 2 shows that treatment with 0.5 and 1 μM FB_1 does not compromise macrophage viability.

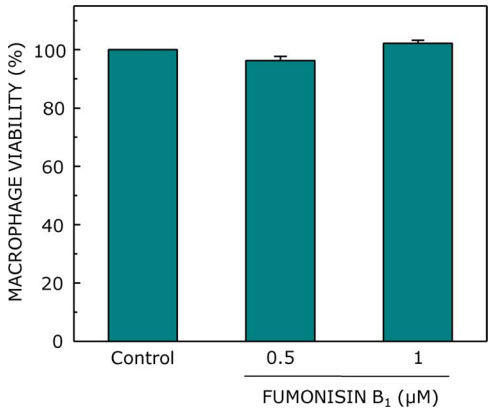

Fig. 2. Macrophage viability is not affected by FB_1 treatment. J774A.1 macrophages were treated with FB_1 and assayed for viability using MTT. Values are expressed as percentages of viability of treated cells normalized to control cells. Data represent means ± S.E. of six independent measurements. See Section 2 for more details.

3.3. Depletion of cellular sphingolipids inhibits mycobacterial entry into host cells

In order to assess the role of cellular sphingolipids in mycobacterial entry into host cells, macrophages treated with FB_1 were infected with *M. smegmatis* and bacterial colony forming units were scored. As shown in Fig. 3, we observed ~12% decrease in mycobacterial entry upon treatment with 0.5 μM FB_1. Upon treatment with 1 μM FB_1, which resulted in reduction of sphingomyelin content by ~52%, we observed ~35% reduction in the entry of *M. smegmatis*.

3.4. Fluorescence imaging reinforces the requirement of host sphingolipids for the entry of M. smegmatis

In order to validate the bacterial entry phenotype observed upon metabolic depletion of host cell sphingolipids, we carried out confocal microscopy of macrophages infected with *M. smegmatis* (stably expressing dsRed2) under these conditions. Representative confocal images of macrophages treated with 0.5 and 1 μM FB_1 and infected with *M. smegmatis* are shown in Fig. 4. The figure clearly shows concentration-dependent reduction in the entry of *Mycobacterium* into macrophages, in agreement with results shown in Fig. 3. Taken together, these results reinforce the requirement of host cell sphingolipids for the

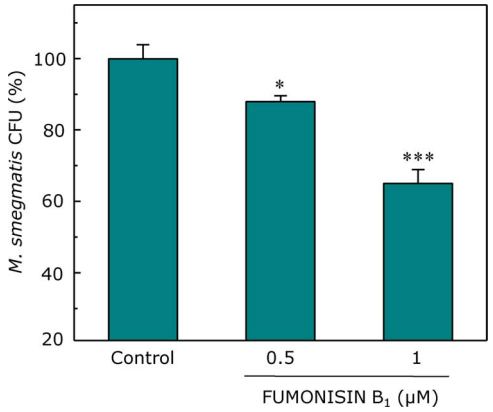

Fig. 3. Metabolic inhibition of sphingolipid biosynthesis inhibits the entry of *M. smegmatis* into macrophages. Macrophages (control and sphingolipid-depleted) were exposed to *M. smegmatis* at a multiplicity of infection of 100:1 (bacteria to macrophage) for 2 h. After lysing macrophages, intracellular bacilli were cultured and the colony forming units (CFUs) were counted. Values are normalized to CFU counts obtained from untreated (control) cells. (* and *** correspond to significant ($p < 0.05$ and $p < 0.001$) difference in CFU counts of sphingolipid-depleted macrophages relative to control macrophages). See Section 2 for more details.

entry of *M. smegmatis*.

3.5. Requirement of host sphingolipids for bacterial entry is specific to Mycobacterium

In order to assess the specificity of cellular sphingolipids in mycobacterial entry into host cells, as a control, we monitored the effect of metabolic sphingolipid depletion on the entry of *E. coli* into host macrophages. As shown in Fig. 5, the entry of *E. coli* into host macrophages displayed no inhibition (remained invariant) with FB_1 treatment, in contrast to *M. smegmatis*, whose entry into macrophages showed significant reduction under similar conditions (see Fig. 3). These results indicate that while the entry of *M. smegmatis* is dependent on membrane sphingolipids, the entry of *E. coli* is not. Our results therefore highlight the specificity of sphingolipid requirement for effective entry of *M. smegmatis* into host cells.

4. Discussion

Sphingolipids are ubiquitous constituents of eukaryotic cell membranes and are recognized as diverse and dynamic regulators of a multitude of cellular processes (Bartke and Hannun, 2009; Holthuis et al., 2001). Besides their physiological functions, sphingolipids are implicated in several pathobiological conditions ranging from cardiovascular, neurodegenerative and metabolic disorders to cancer (Kolter and Sandhoff, 2006; Sonnino and Chigorno, 2000; Truman et al., 2014). Importantly, sphingolipids have been identified as key players in various infectious diseases (Hanada, 2005; Heung et al., 2006). Several pathogens modulate host sphingolipids and their metabolites to manipulate host defense, thereby enabling their survival. In addition, host cell membrane sphingolipids have been exploited as membrane receptors by various pathogens (Hanada, 2005; Slotte, 2013). Microbes including bacteria and viruses utilize host sphingolipids to promote their pathogenicity (Heung et al., 2006). For example, many intracellular pathogens have been shown to use ceramide-rich membrane domains as portals for their entry into macrophages (Mañes et al., 2003), with many others targeting host sphingolipids for promoting their virulence (Heung et al., 2006).

M. tuberculosis is an intracellular pathogen that is known to survive within host macrophages by residing in phagosomes and preventing its fusion with lysosomes by employing a variety of mechanisms (Russell, 2007). Sphingolipids have been identified to play a crucial role in growth, replication and survival of mycobacteria in host macrophages (Lovewell et al., 2016; Speer et al., 2015; Toledo and Benach, 2015; Vromman and Subtil, 2014; Wipperman et al., 2014). Importantly, sphingolipids such as ceramide, sphingosine and sphingomyelin have been shown to inhibit intracellular growth of *M. tuberculosis* in mouse macrophages (Anes et al., 2003) *via* different mechanisms (Gutierrez et al., 2009). In addition, reduction in sphingosine-1-phosphate (S1P) production by inhibiting the activity and localization of sphingosine kinase has been identified as a crucial determinant of survival of *M. tuberculosis* in host macrophages (Heung et al., 2006; Kusner, 2005; Sharma and Prakash, 2017).

Although several studies have demonstrated that host sphingolipids are crucial players in the pathogenicity of mycobacteria, the role of sphingolipids in the entry of mycobacteria into host macrophages has not been explored. Our present results clearly demonstrate that host macrophage sphingolipids are essential for the entry of mycobacteria. Importantly, we show that this process is specific to mycobacterial entry since such dependence was not observed in case of entry of *E. coli*. As mentioned above, previous results from our laboratory (Viswanathan et al., 2015) and others (Gatfield and Pieters, 2000; Han, 2009; Lobato et al., 2014; Martens et al., 2008; Miner et al., 2009; Parihar et al., 2014) showed the requirement of host macrophage cholesterol for mycobacterial entry. In addition, we demonstrated the requirement of optimum host plasma membrane cholesterol for the

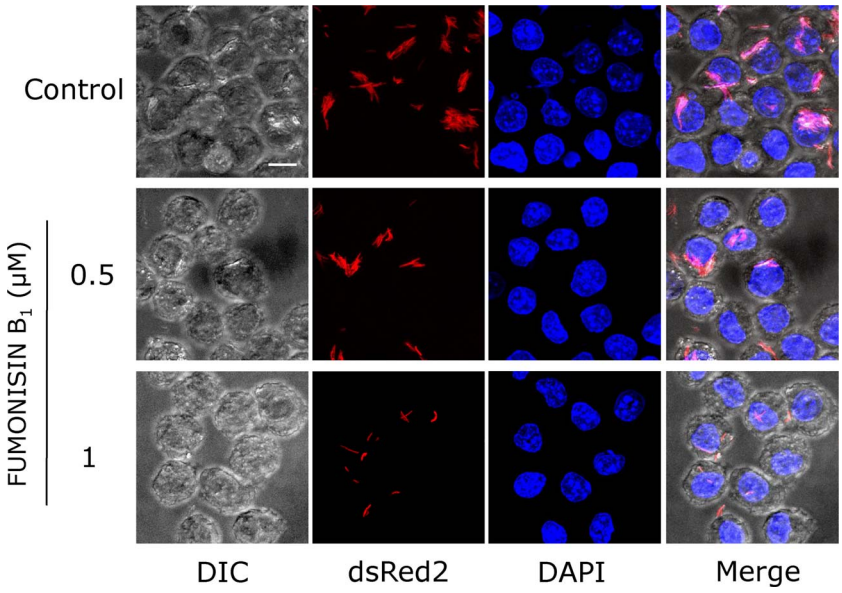

Fig. 4. Confocal microscopic imaging confirms the requirement of macrophage sphingolipids for the entry of *M. smegmatis*. Representative confocal microscopic images showing entry of *M. smegmatis* into untreated (control), and FB_1-treated macrophages. The panels show macrophages (DIC images) infected with *M. smegmatis* expressing dsRed2 (red). The nucleus of macrophages was stained with DAPI (blue). The merged images are shown in the last panel. The scale bar represents 10 μm. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

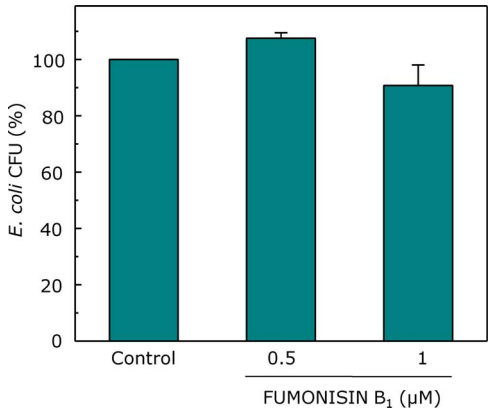

Fig. 5. Entry of *E. coli* into macrophages remains invariant upon metabolic depletion of sphingolipid biosynthesis. Macrophages (control and sphingolipid-depleted) were exposed to *E. coli* DH5α at a multiplicity of infection of 100:1 (bacteria to macrophage) for 2 h. After lysing macrophages, intracellular bacilli were cultured and the colony forming units (CFUs) were counted. Values are normalized to CFU counts obtained from untreated (control) cells. See Section 2 for more details.

entry of mycobacteria into macrophages (Viswanathan et al., 2015). Taken together, our present results on the requirement of sphingolipids in the entry of mycobacteria, along with our previous observations on cholesterol-dependent mycobacterial entry (Viswanathan et al., 2015), suggest a synergism between these essential functional membrane lipids in mycobacterial entry.

We recently proposed a possible mechanism by which membrane lipids could modulate the entry of intracellular pathogens (Kumar et al., 2016a; Viswanathan et al., 2015). As mentioned above, a number of candidate receptors on host macrophage cell surface have been implicated in recognition and entry of mycobacteria into host cells (Ernst, 1998; Killick et al., 2013). Importantly, earlier work by us and others has shown that membrane cholesterol and sphingolipids modulate the organization and function of several membrane receptors, particularly GPCRs (Gimpl, 2016; Jafurulla and Chattopadhyay, 2013, 2015; Oates and Watts, 2011; Paila and Chattopadhyay, 2010; Pucadyil and Chattopadhyay, 2006). We therefore envisage that the conformation of membrane receptors implicated in mycobacterial entry into host cells could possibly be modulated by membrane cholesterol and sphingolipids. It is interesting to note that a recent study has shown that mycobacteria suppress the host cell signaling pathways by manipulating GPCRs (Alaridah et al., 2017).

Several proteins that interact with sphingolipids have been reported to contain a consensus amino acid sequence, termed the 'sphingolipid binding domain' (SBD). SBD was initially identified and characterized in HIV-1 surface envelope glycoprotein gp120 and amyloid proteins (Mahfoud et al., 2002). It was later identified in a wide range of proteins including receptors, toxins and viral proteins (Chattopadhyay et al., 2012; Fantini, 2003; Fantini and Barrantes, 2009). The SBD motif typically consists of a characteristic combination of aromatic, basic and turn-inducing residues. It would be interesting to check whether membrane receptors responsible for mycobacterial entry contain SBD motifs in their sequence.

In summary, our present results highlight, for the first time, the specific requirement of host membrane sphingolipids in mycobacterial entry. These results, along with previous reports, clearly demonstrate the requirement of host cell sphingolipids for the entry and survival of mycobacteria. Modulation of sphingolipids of host cells could therefore potentially lead to novel therapeutic strategies against mycobacterial infection. This approach could have a major advantage to counter the emerging challenge of drug resistance in the treatment of mycobacterial infection (Dye, 2009; Gandhi et al., 2006; Keshavjee and Farmer, 2012), since the focus of treatment would be on the host rather than the parasite.

Conflict of interest

The authors declare no conflict of interest.

Acknowledgments

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