Leishmania-Induced Biphasic Ceramide Generation in Macrophages Is Crucial for Uptake and Survival of the Parasite

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The initial macrophage-Leishmania donovani interaction results in the formation of membrane platforms, termed lipid rafts, that help in the entry of the parasite. Therefore, it is imperative that the parasite designs a strategy to modulate its uptake and survival within the macrophages. Herein, we report Leishmania-triggered biphasic ceramide generation. In the first phase, L. donovani promastigotes induce activation of acid sphingomyelinase (ASMase), which catalyzes the formation of ceramide from sphingomyelin. Inhibition of ASMase resulted in reduced uptake and infection with the parasite. In the second phase, de novo synthesis generates ceramide that reduces the cellular cholesterol level and displaces the cholesterol from the membrane, leading to enhanced membrane fluidity, disruption of rafts, and impaired antigen-presentation to the T cells. The results reveal a novel role for ceramide in the perspective of L. donovani infection and help formulate an antileishmanial strategy that can possibly be applied to other intracellular infections as well.

Leishmania amastigotes are obligatorily intracellular protozoan parasites that reside and replicate within the macrophages of their mammalian host. The parasite survives by modulating host-cell signaling from various cell membrane receptors [1–3]. Therefore, it has 2 important phases in its survival: quick uptake by the host cell and survival once internalized. It has been shown recently that pretreatment of macrophages with β-methyl cyclodextrin (βMCD) or nystatin, which destroys small sphingolipid- and cholesterol-enriched membrane rafts, inhibits Leishmania internalization [4–6]. On the other hand, cholesterol is depleted from the membrane of the Leishmania-infected macrophages, causing disruption of the lipid raft [7]. Lipid rafts are stabilized by ceramide molecules [8–10], which separate spontaneously from other phospholipids and seem to exist as preformed entities in the cell membrane. Thus, these apparently contrasting observations provide a paradox with regard to the role of cholesterol and ceramide. However, the functions of ceramide-enriched membrane domains and the relationship of ceramide with membrane cholesterol in Leishmania infection of macrophages remain unknown.

Ceramide is a membrane lipid involved in many biological processes. Recent studies suggest that ceramide, a pleiotropic lipid molecule, plays a pivotal role in the establishment of pathogens inside host cells [11–13]. Ceramide-enriched membrane platform formation is initiated by activation of acid sphingomyelinase (ASMase), which has been shown to be stimulated by several receptors [14, 15] and by pathogenic bacteria and viruses [16–19]. These stimuli not only activate the enzyme but also induce the translocation of ASMase from an intracellular compartment to the extracellular leaflet of the cell membrane [20]. The hydrolysis of the membrane sphingomyelin by ASMase results in the generation of ceramide [21].
Ceramide is generated either by this catabolic pathway or by de novo synthesis [22]. Previous work from our laboratory demonstrated that infection with *Leishmania donovani* increases the de novo synthesis of ceramide in macrophages [11], which alters the normal antimicrobial machinery of the macrophages and helps in the establishment of parasite inside the cells.

Here, we demonstrate for the first time that *Leishmania* triggers a biphasic induction of ceramide and that *L. donovani* induces ASMase activation and the first phase of ceramide generation. The ceramide-enriched membrane platforms are then used for the parasite internalization. While the internalized parasites are transformed into amastigotes, the ceramide produced by de novo synthesis displaces the cholesterol from the membrane, enhancing the membrane fluidity but reducing antigen presentation to the T cells. The results indicate the bimodal generation of ceramide, associating each process to a particular function in macrophages. While the initial ASMase-dependent generation of ceramide is important for parasite uptake, the de novo synthesis of ceramide associates with the antigen-presenting function of macrophages. The model of *Leishmania* infection exemplifies how the dual regulation of ceramide can on, one hand, be targeted for therapeutics and, on the other hand, be targeted by the parasite to devise an immune evasion strategy.

**MATERIALS AND METHODS**

**Materials**

Anti-ceramide antibody (15B4; dilution, 1:50) was a kind gift from Prof. E. Gulbins (University of Essen, Germany). The peptide LEDARRLKAIYEKKK (12–26 residues of λ repressor protein [λR12–26]) and fetal bovine serum (FBS) were obtained from Invitrogen Life Technologies. Tissue culture reagents were from Gibco Laboratories. All other chemicals were from Sigma or Merck.

**Methods**

The λR12–26-specific 1-A4–restricted T-cell hybridoma 9H3.5 [23] was a kind gift from Prof. M.L. Gefter (MIT, Cambridge, MA). The interleukin 2 (IL-2)–dependent cell line HT-2 was from ATCC. All cells were maintained in Roswell Park Memorial Institute 1640 medium with 10% FBS and 2-ME (5 × 10⁻⁵ M) at 5% CO₂ in a humidified atmosphere.

BALB/c mice aged 4–6 weeks (National Center for Laboratory Animal Sciences, India) were used for each experiment, with 8–10 mice/group. Stationary-phase *L. donovani* (MHOM/IN/1983/AG83) promastigotes were used for infection. Amastigotes were prepared as described elsewhere [24]. Thioglycollate-elicited peritoneal macrophages were cultured as described by Fahey et al. [25].

The micellar ASMase assay involving exogenous radiolabeled SM was performed using the methods of Quintern and Sandhoff [26]. Ceramide synthase activity that involved exogenous radiolabeled [¹⁴C] palmitoyl-CoA was measured as previously described [27]. The ceramide assay was carried out with radiolabeled stearic acid, using the methods of Gamen et al. [28].

Following the manufacturer’s protocol, total RNA from macrophages was extracted in TRI Reagent (Sigma). Isolated RNA was reverse transcribed using Revert Aid M-MuLV Reverse Transcriptase (Fermentas). The resulting complementary DNA was used for real-time polymerase chain reaction (PCR) to detect 3-hydroxy-3-methylglutaryl coenzyme A reductase (HMGCR), using ABI 7500 real-time PCR with SYBR green dye (Applied Biosystems). Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was used as a reference. The forward and reverse primer sequences were as follows: HMGCR: forward 5’-ACAATAAGATGCTGGTGGAGATTATGGA-3’ and reverse 5’-GCTATGCTATGTTATGTCAGAAA-3’; GAPDH: forward 5’-CAAGGCTTGGGGCAAGGTC-3’ and reverse 5’-AGGTGGAAGATGGAGTTGCTG-3’. Relative changes in HMGCR expression were determined on the basis of a comparison with unstimulated control, were normalized to GAPDH, and were quantified by the 2⁻ΔΔCt method.

Total and membrane-specific cholesterol content in uninfected and infected macrophages were assayed with use of the Amplex Red cholesterol assay kit (Invitrogen). The assay was performed according to the manufacturer’s instructions.

Control and infected macrophages were harvested, washed, and resuspended in cold wash buffer (phosphate-buffered saline/0.1% NaN₃/1% FBS), centrifuged at 350g for 5 minutes, and resuspended in 50 µL of wash buffer [29]. Cells were stained with fluorochrome-conjugated antibodies according to the manufacturer’s protocol. The cells were then fixed with 1% paraformaldehyde, mounted with 90% glycerol on a glass slide, and observed under a laser-scanning microscope (LSM 510; Zeiss).

Macrophages were stained with anti-ceramide mouse monoclonal antibody (dilution, 1:50) followed by FITC-conjugated anti-mouse secondary antibody [17]. The cells were analyzed on a FACS Calibur.

The membrane fluorescence and lipid fluidity of cells were measured in accordance with the method described by Shnitzky and Inbar [30, 31]. The fluorescence anisotropy (FA) value was calculated using the equation FA = (Iₚ−1) / (Iₚ+2I⊥), where Iᵦ and I⊥ are the fluorescent intensities oriented parallel and perpendicular, respectively, to the direction of polarization of the excited light.

The antigen-presenting ability of macrophages was studied by their ability to present λR12–26 to T-cell hybridoma (9H 3.5) and produce IL-2, as described originally by Roy et al. [24].

**Statistical Analysis**

The data, represented as mean ± SD, are from 1 experiment, which was performed at least 3 times. The Student’s t test was used to assess the significance of the differences between the
mean values of control and experimental groups. At least 6 mice were included per group for the experiments in vivo.

RESULTS

*L. donovani* Induces Sphingolipid-Enriched Platforms on the Macrophage Membrane

To determine whether sphingolipid-rich membrane platforms are involved in *L. donovani* infection of macrophages, we infected *Leishmania*-susceptible BALB/c-derived peritoneal macrophages with *L. donovani* and determined the distribution of sphingolipid-rich membrane domains by staining the cells with FITC-labeled cholera toxin (CTX-B-FITC), a well-known lipid raft marker that binds to the typical raft-associated ganglioside GM1 [32]. Within 1 hour of infection of macrophages with *L. donovani* promastigotes, a rapid fusion of the CTX-B-FITC+ domains was triggered to generate larger platforms on the macrophage membrane. These CTX-B-FITC+ domains started dispersing around 12 hours after infection and were unidentifiable 24 hours after the infection (Figure 1A). Similar observations were noted with another raft marker, anti-CD48-PE [33] (Figure 1B). These data show that the initial parasite-macrophage interaction triggers the reorganization of sphingolipid-enriched membrane domains into larger platforms.

Figure 1. *Leishmania donovani* infection induces sphingolipid-enriched platform formation on macrophage membrane. A and B, Macrophages (MΦ) cultured and challenged with *L. donovani* promastigotes (LD) and cultured for the indicated periods. Cells were stained with either cholera toxin B-FITC (CTX-B-FITC; A) or anti-CD48-PE (B). Cells were observed under a confocal laser-scanning microscope (LSM 510; Zeiss). The lower panel shows light transmission microscopy images. Control staining with irrelevant isotype-matched antibodies was negative. Results are representative of 3 independent experiments in which at least 100 cells per sample were analyzed.

Sphingolipid-Rich Membrane Platforms Are Essential for *L. donovani* Internalization

To assess the significance of the formation of sphingolipid-rich rafts in the host cell membrane during the initial macrophage-*Leishmania* interaction, we disrupted the rafts by preincubating BALB/c-derived peritoneal macrophages with nystatin or β-methyl cyclodextrin. α-cyclodextrin, the inactive isomer of βMCD, was used as a negative control. Disruption of sphingolipid-enriched rafts prevented the entry of *L. donovani* inside the host macrophages. We found that *L. donovani* infection of nystatin- or βMCD-pretreated macrophages but not of α-cyclodextrin–treated macrophages significantly decreased parasite internalization (*P* < .001) (Figure 2A).

Because ceramide is a major sphingolipid that helps in the raft organization, we examined the role of ceramide in the raft-mediated entry of *Leishmania* by enhancing raft formation with exogenous C16 ceramide (10 μM) or by inhibiting endogenous ceramide generation by use of the ASMase-specific inhibitor imipramine [34]. C16 ceramide significantly increased the parasite internalization (*P* < .001) (Figure 2A). In contrast, in...
Figure 3. *Leishmania donovani* infection triggers biphasic induction of ceramide in the host macrophage. A, *L. donovani* infection induces the activation of acid sphingomyelinase (ASMase). Macrophages were cultured and infected with the promastigotes for indicated periods, as described in the legend of Figure 1. ASMase activity was determined as described in Materials and Methods. Results are expressed as mean ± SD of 4 replicate experiments. Asterisks indicate statistically significant induction (*P* < .003) of ASMase activity as compared to 0-hour infected cells. B, Surface ceramide reside inside the membrane rafts. Cells were stained with anti ASMase-FITC and anti-ceramide-PE and were observed under a confocal laser-scanning microscope. Yellow fluorescence depicts colocalization. Results are representative of 3 independent experiments in which at least 100 cells per sample were analyzed. C, *L. donovani* infection triggers ceramide production in the host cells membrane. Cells were infected with promastigotes, and the membrane ceramide production was analyzed by flow cytometry. D, The cytosolic and membrane fractions were separated by ultracentrifugation, and the ceramide level was quantitated (see Materials and Methods). Results are expressed as mean ± SD of 3 replicate experiments. Asterisks indicate statistically significant induction of ceramide level as compared to 3-hour infected cells (*P* < .001, **P** < .005). E, *L. donovani* infection induces...
imipramine-treated cells, parasite internalization was significantly reduced ($P < .001$) (Figure 2A), compared with the untreated cells. Likewise, parasite burden was significantly reduced in ASMase siRNA-transfected macrophages but not in the control siRNA-transfected macrophages ($P < .005$) (Figure 2B). These observations indicate that ASMase-generated ceramide is required for the raft-reliant internalization of the parasite.

**L. donovani** Infection Triggers Biphasic Induction of Ceramide in the Host Macrophage

Because ASMase can release ceramide from sphingomyelin in the membrane raft, we first examined whether *L. donovani* infection affected ASMase activity. We observed that ASMase activity started 30 minutes after the infection (Figure 3A). ASMase activity was almost undetectable in uninfected cells. The surface ceramide clustered in a pattern similar to that of ASMase, as confirmed by its colocalization with ASMase (Figure 3B). Flow cytometry analyses revealed a significantly higher amount of ceramide in the membrane of the infected cells as compared to the uninfected cells (Figure 3C). Although there was significantly enhanced ceramide production in the membrane of 3 hour-infected macrophages (Figure 3D), the ceramide levels gradually decreased to the basal level. The membrane ceramide level increased again in 24-hour-infected macrophages ($P < .001$) (Figure 3D), suggesting a biphasic induction of ceramide in *Leishmania*-infected macrophages. This second wave of ceramide generation is due to the *de novo* synthesis. We observed that the activity of ceramide synthase, the main enzyme of *de novo* synthesis, started 12 hours after the infection (Figure 3E). Pretreatment of cells with FB1, a specific blocker of the *de novo* synthesis of ceramide, followed by infection for 24 hours, resulted in significant reduction in membrane ceramide level ($P < .005$). Interestingly, FB1 treatment did not affect the initial ceramide burst (Figure 3D). To conclusively prove that the retained ceramide at 24 hours is *de novo* synthesized, we radiolabeled sphingomyelin, the precursor of ASMase-dependent ceramide, and palmitoyl CoA, the precursor of *de novo* synthesized ceramide, and studied their retention in the cell membrane (Figure 3F). The results showed that the retained ceramide at 24 hours was indeed from *de novo* synthesis, because of the incorporation of radio-labeled C$^{14}$ from palmitoyl CoA, the precursor in the *de novo* synthesis pathway. Moreover, the generation of ceramide via ASMase stopped after 6 hours, so the retained ceramide at 24 hours was clearly from the *de novo* synthesis pathway and not from the sphingomyelin breakdown (Figure 3F).

To examine whether ceramide was localized in the raft structure, we assessed colocalization of fluorescence-labeled anti-ceramide antibody and anti-CD48-PE antibody. The confocal microscopy revealed that ceramide moved inside the rafts ≤1 hour after infection (Figure 3G). Pretreatment of macrophages with imipramine resulted in the disappearance of ceramide microdomains from the membrane (Figure 3H). In contrast, 24 hours after the infection, the raft structure completely disappeared in spite of elevated ceramide levels in the host cell membrane. Pretreatment of macrophages with FB1, a selective inhibitor of the *de novo* pathway, reduced the ceramide level but recovered normal raft architecture (Figure 3H). Taken together, these data indicate that ceramide can be generated in at least 2 different modes, by ASMase and by FB1-sensitive *de novo* synthesis, which are involved, respectively, in the initial uptake of the parasite via the ceramide-enriched membrane domains and in the late function of the macrophages, where raft disruption may play an important role.

**De novo**-Synthesized Ceramide Reduces HMGCR Expression and Membrane Cholesterol in *L. donovani*-Infected Macrophages

The above-mentioned data present the paradox that although ceramide is necessary to form the raft structure, rafts are disrupted in the macrophages 24 hours after infection, despite the enhanced ceramide level in the membrane. We addressed this paradoxical role of ceramide in the modulation of raft in *L. donovani*-infected macrophages. Because cholesterol is a key component in the lipid raft, we first measured the total cellular and membrane cholesterol levels in the infected...
macrophages and the ceramide-treated uninfected macrophages. While *L. donovani* infection, exogenous ceramide, and imipramine significantly reduced both membrane and cellular cholesterol levels, FB1 did not exert similar effects (Figure 4A). It is possible that an enhanced ceramide concentration may reduce the cholesterol level in macrophages during *L. donovani* infection.

Because cell-permeable ceramide reduced the activity of HMGCR, the rate-limiting enzyme of cholesterol biosynthesis [35], we studied the effect of ceramide on HMGCR expression.

*Enhanced Ceramide Increases the Membrane Fluidity but Decreases the Antigen Presentation by the Infected Macrophages*

Because the FB1-sensitive *de novo*-synthesized ceramide extruded cholesterol from the membrane of the *L. donovani*–infected macrophages, and because cholesterol is a major factor that regulates membrane fluidity, we examined whether the enhanced ceramide affected the membrane fluidity of the infected cells (Figure 5A). We measured FA as an indicator of membrane fluidity and observed that the FA was significantly diminished 24 hours after the infection (Figure 5B), suggesting enhanced fluidity of the membrane of *L. donovani*–infected macrophages. FB1 pretreatment of the infected macrophages restored the FA of the membrane in *L. donovani*–infected cells to the level observed in the uninfected macrophages (Figure 5B). These observations indicated that the elevated ceramide levels regulated membrane fluidity in the infected macrophages.

Because *L. donovani* infection impairs the antigen-presentation capability of macrophages, owing to enhanced membrane fluidity of the antigen-presenting cells [36], we tested the role of *de novo*-generated ceramide on the antigen-presentation capability of the infected macrophages. We assessed the presentation of the *ρ*R12–26 peptide by the uninfected and *L. donovani*–infected macrophages to the peptide-specific T cell hybridoma. It was observed that FB1 treatment of the infected macrophages significantly enhanced their antigen-presenting functions (Figure 5C).

Because major histocompatibility complex (MHC) class II molecules are constitutively present on the lipid rafts during antigen presentation [37], we studied the colocalization of MHC class II (anti-I-A<sup>d</sup>) molecules and CTX-B FITC. Although MHC class II (anti-I-A<sup>d</sup>) molecules were not colocalized with CTX-B in the infected macrophages, FB1 treatment brought these 2 groups of molecules together (Figure 5A), indicating that the enhanced intracellular ceramide disrupted lipid rafts and impaired antigen presentation by the infected macrophages.

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**Figure 4.** Endogenously elevated ceramide during *Leishmania donovani* infection reduces 3-hydroxy-3-methylglutaryl coenzyme A reductase (HMGCR) expression and subsequently decreases the membrane cholesterol level at a later period of infection. A, Total and membrane cholesterol content of uninfected and infected macrophages treated with imipramine or FB1, relative to the content in uninfected untreated cells, set as 100%. Results are expressed as mean ± SD of 3 replicate experiments (**P**< .005; *P*< .001). (B) Real-time polymerase chain reaction (PCR) analysis of HMGCR messenger RNA in uninfected and infected macrophages pretreated with imipramine or FB1 or C2 ceramide. The densitometric analysis of the PCR run on agarose gel is shown in the bottom panel. Data are from 1 representative experiment, which was performed at least 3 times.
Ceramide Reduction Imparts Significant Resistance to
*L. donovani* in BALB/c Mice

The results from the above-stated experiments clearly revealed the importance of ceramide-enriched membrane platforms and the intracellular ceramide level in the cellular uptake and intracellular survival, respectively, of the parasite. This prompted us to study whether disrupting the above 2 events could abrogate the intracellular parasitic growth in a host susceptible to *L. donovani* infection. Because both ASMase- and FB1-sensitive pathways of ceramide generation are equally important for this protozoan parasite, we studied the effect of ASMase siRNA, which reduces ASMase expression, and FB1, which reduces de novo ceramide synthesis, on the intracellular ceramide generation and subsequently on intracellular parasitic growth. *L. donovani*-infected BALB/c mice were either left untreated or were pretreated with ASMase siRNA or FB1, individually or in combination. We observed higher levels of ceramide generation in the splenic macrophages 7 and 21 days after *L. donovani* infection, compared with the uninfected control (*P* < .01) (Figure 6A). However, reduced ceramide levels in ASMase siRNA–treated, FB1-treated, or ASMase siRNA– and FB1-treated mice were observed in the splenic macrophages 7 and 21 days after the infection, compared with the only infected sets (*P* < .01) (Figure 6A); the reduction was even stronger in the mice treated with ASMase siRNA plus FB1 (*P* < .005) (Figure 6A).

Because ASMase siRNA and FB1 modulated ceramide levels in *vivo*, we examined whether these treatments conferred significant protection against *L. donovani* infection. ASMase siRNA treatment reduced the splenic parasite burden by 66% and 89% 7 days and 21 days, respectively, after the infection (*P* < .005) (Figure 6B). In contrast, FB1-pretreated mice showed no significant decrease in parasite burden 7 days after the infection, but a decrease of 80% was found 21 days after the infection (*P* < .005) (Figure 6B). Mice treated with ASMase siRNA plus FB1 had highly significant protection (75% and 93% reduced spleen parasitic burden 7 days and 21 days, respectively, after the infection; *P* < .005) (Figure 6B). Taken together, these data indicate for the first time that the rationale for the ceramide-targeted therapy of *L. donovani* infection is a spatiotemporal function of ceramide generation in the host cell.

**DISCUSSION**

Our observations reveal that *Leishmania* induces biphasic ceramide activation in the host cell. *L. donovani* attachment with macrophage membrane activates ASMase, which hydrolyzes

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sphingomyelin to ceramide, thereby helping in the formation of the membrane microdomains responsible for *Leishmania* internalization [4]. However, the second phase of ceramide generation, by the FB1–sensitive de novo pathway, efficiently reduces cholesterol biosynthesis and displaces cholesterol from the lipid raft, resulting in impaired antigen-presentation by the *L. donovani*–infected macrophages. These data provide the first evidence that *Leishmania*-induced biphasic ceramide activation causes sequential association and disruption of the rafts at early and later phases, respectively, of the infection.

Our data reveal that ceramide-enriched membrane platforms are a crucial component during the early stages of host-parasite interaction. Ceramide spontaneously self-aggregates into microdomains and triggers fusion of microdomains into larger and more stable platforms, facilitating the entry of *L. donovani* inside the host cells [4, 5]. Restriction of parasite entry by silencing the ASMase gene or by using the pharmacological ASMase-inhibitor imipramine [34] indicates that, in addition to cholesterol, ceramide is crucial lipid that regulates the parasite internalization.

To establish the mechanism of ceramide-mediated raft disruption in the membrane, we studied the effect of ceramide on the cholesterol level in the membrane of the *L. donovani*–infected macrophages. During infection, ceramide reduced not only membrane cholesterol but also cellular cholesterol (Figure 4A and 4B). While the reduction in cellular cholesterol might have been caused by the significantly reduced HMGCR expression in the infected macrophages [35], the reduction in membrane cholesterol could result from the competition with ceramide to complex with other membrane lipids. For example, because both cholesterol and ceramide are hydrophobic, with the polar head groups much shorter than the other membrane phospholipids, they are generally nucleated inside the hydrophobic microenvironment in the membrane lipids. Very high ceramide biosynthesis in the *L. donovani*–infected macrophages may alter the ceramide:cholesterol stoichiometric relationship with the other membrane phospholipids beyond the permissible range. Coupled with reduced cholesterol biosynthesis in *L. donovani* infection, ceramide drives the stoichiometry in its favor and thereby replaces cholesterol from the membrane [36]. However, the differences in the physicochemical properties between ceramide and cholesterol change the overall biophysical properties, such as anisotropy of the *L. donovani*–infected macrophage membrane, affecting T cell responses.

Indeed, increased membrane fluidity and disruption of lipid rafts inhibit the antigen-presentation capability of macrophages [37]. It appears that during the later phase of the infection, enhanced ceramide generation increased membrane fluidity and disrupted the lipid rafts. Thus, the anisotropy reduced with ceramide accumulation in the membrane (Figure 5B). Because MHC class II molecules are constitutively present inside the membrane lipid rafts and execute antigen presentation [29], we studied the colocalization of MHC class II molecules and CTX-B-FITC. The failure of these 2 molecules to colocalize in infected cells suggests the disruption of lipid rafts due to an increased ceramide level in the macrophage membrane during the later phase of the infection. Indeed, recovery of the raft architecture by FB1 clearly

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Figure 6. Acid sphingomyelase (ASMase) siRNA and FB1 pretreatment impart significant resistance to *Leishmania donovani* infection in BALB/c mice. Mice were pretreated with either ASMase siRNA (100 µg/kg of body weight) or FB1 (10 mg/kg of body weight), followed by infection with *L. donovani*. Mice were sacrificed on days 7 and 21 after infection. A, Levels of ceramide production in the splenic macrophages were analyzed by flow cytometry. Asterisks indicate statistically significant reduction as compared to control cells (**P < .005). B, Levels of parasite burden in spleen; samples are expressed in Leishman-Donovan units (LDU). Results are from 3 independent experiments and represent the mean values ± standard errors of the means for 5 animals per group per time point. Asterisks indicate statistically significant reduction as compared to infected sets (**P < .005).
indicated that ceramide disrupted the lipid rafts, dissociated the MHC class II organization in rafts, and eventually impaired antigen presentation. This inability to present antigen may contribute to the defective cell-mediated immune response in L. donovani–infected BALB/c mice and favored survival of the parasite. Indeed, our in vivo experiments demonstrate that ASMase siRNA and FB1 treatment could significantly lessen the intracellular ceramide generation and could restrict the splenic parasite growth 7 days after the infection or for 21 days following infection. Other pathogens, such as Mycobacterium [38], Pseudomonas [17], Plasmodium [39], Neisseria gonorrhoeae [16], and human immunodeficiency virus [40], also use rafts or raft-like structures for their internalization into their mammalian host cells, but the involvement of ceramide in these infections remains uncovered. Thus, unravelling the biphasic, spatiotemporal regulation of ceramide in L. donovani infection—both in vitro and in vivo—turns out to be the first prototype for the rationale for ceramide-targeted therapy for infectious diseases.

Notes

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