Mycobacterium tuberculosis lipoarabinomannan enhances LPS-induced TNF-α production and inhibits NO secretion by engaging scavenger receptors

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ABSTRACT

Lipoarabinomannan capped with terminal oligomannosides (ManLAM) is a component of mycobacteria cell wall enabling Mycobacterium tuberculosis to infect macrophages. We found that short treatment (3.5 h) of macrophage-like J774 cells and thioglycollate-elicited peritoneal murine macrophages with ManLAM and its deacylated form enhanced LPS-stimulated release of tumor necrosis factor-α (TNF-α). In contrast, prolong incubation of J774 cells with ManLAM (16 h) led to inhibition of LPS-stimulated TNF-α production. LPS-triggered secretion of nitric oxide (NO) was suppressed by ManLAM and its deacylated form. Effects of ManLAM and its deacylated derivative were mimicked by dextran sulfate, a general ligand of scavenger receptors. The enhancement of LPS-induced TNF-α production by dextran sulfate was partially reversed by an antibody neutralizing scavenger receptor SR-PSOX/CXCL16 while the stimulatory activity of deacylated ManLAM was reversed by an antibody neutralizing class B scavenger receptor CD36. Our data suggest that CD36 mediates the activity of ManLAM and its deacylated form leading to TNF-α release in LPS-stimulated J774 cells and peritoneal murine macrophages, while NO production is modulated by unknown scavenger receptors.

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1. Introduction

Mycobacterium tuberculosis (Mtb) is a highly successful intra-cellular pathogen infecting an estimated one-third of the world's population and responsible for more deaths yearly than any other bacterial pathogen. Although most of the infected individuals do not develop active disease, their immune system fails to eradicate the bacteria which remain in a dormant state in granulomas for life [1].

The interactions between Mtb and the host have been found to be very complex and are still far from being fully understood. Mtb infects primarily macrophages and survives and replicates within these cells whose function is to kill internalized bacteria. Several macrophage receptors have been reported to mediate interactions with Mtb. Activation of macrophages by isolated components of mycobacteria is mediated mainly by Toll-like receptors (TLR), TLR2 and TLR4 [2,3].

Other macrophage receptors implicated in the interactions with Mtb include the mannose receptor [4], DC-SIGN and its murine homo-logues SIGNR1 and SIGNR3 [5–11], complement receptor 3 (CR3) and CR4 [4,9,12] and different scavenger receptors (SRs) [12–14]. This list is likely to expand because the response of murine macrophages to viable, virulent Mtb has been demonstrated to be largely independent of TLR, the mannose receptor, CR3, CR4 and SR-A [15].

Lipoarabinomannan (LAM), a component of mycobacterial cell wall, has been identified as a major virulence factor of Mtb [7], enabling Mtb to infect macrophages [9,16] and survive within their hostile intracellular environment by inhibiting the phag-osome—lysosome fusion [17], apoptosis [18] and cellular-type immune responses to mycobacteria [19,20]. LAM is composed of a mannosyl-phosphatidyl-myo-inositol plasma membrane anchor (MPI) to which is attached a mannan core followed by an arabinan domain. LAMs isolated from different species of mycobacteria are usually classified based on the structure of motifs located at the nonreducing ends of the arabinofuranosyl chains. Mannosylated LAM (ManLAM), which is synthesized by slow-growing, patho-genic mycobacteria, such as Mtb, ends with α(1 → 2)-linked oligo-mannosides [21]. In contrast, LAM from fast-growing, saprophitic mycobacterial species, such as Mycobacterium smegmatis, is capped with phospho-myo-inositol residues (PILAM) [22].

Lipomannans are biosynthetic precursors of LAM, lacking the arabinan domain. Recognition of lipomannans isolated from different mycobacteria species as well as of PILAM by macrophages was shown to be mediated by TLR2 [2,3,23,24]. In contrast, although structurally similar, ManLAM seems unable to activate TLR2. It has been suggested that the presence of the arabinan domain in ManLAM prevents its interaction with TLR2, presumably through steric hindrance [24]. ManLAM, rather than inducing pro-inflammatory activation typical of TLR ligands, has been reported to antagonize the activation of macrophages and dendritic cells by other factors, such as enterobacterial lipopolysaccharide (LPS), a ligand of TLR4 [3,5,19,25]. These anti-inflammatory and imm-unosuppressive effects of ManLAM have been suggested to be mediated by mannose-binding C-type lectins, the mannose receptor and DC-SIGN, recognizing the oligomannoside capping of ManLAM [5–8,16]. The engagement of the mannose receptor by ManLAM during the phagocytic process has been demonstrated to be also required for the blockade of the phagosome–lysosome fusion induced by pathogenic mycobacteria [26].

Despite the ability of Mtb to subvert the host immune responses in its own favor [27], the host immune system attempts to combat Mtb by producing pro-inflammatory mediators like tumor necrosis factor-α (TNF-α) and nitric oxide (NO). Mice with disrupted genes for TNF-α or for the 55 kDa TNF-α receptor, or treated with neutralizing anti-TNF-α antibody were unable to control infection with Mtb: they exhibited severely decreased survival and impaired granuloma formation associated with increased bacteremia and necrosis [28,29]. An important mecha-nism of the TNF-α protective effect seems to be promotion of macrophage apoptosis [30] which deprives Mtb its preferred growth niche, decreases the viability of mycobacteria [31], makes them susceptible to killing by freshly recruited macrophages [32] as well as enables processing and presentation of Mtb antigens [33]. A high level of TNF-α production in macrophages was reported to be induced by both lipomannans and PILAM, acting through TLR2 [2,3,23,24]. Another mediator, NO, was shown to participate in Mtb killing by activated macrophages and probably contributed to the infection-induced apoptosis of macrophages in murine models [34]. In humans the role of NO in the killing or restricting growth of Mtb remains controversial [35]. In contrast to TNF-α generation, the induction of NO production by Mtb in macrophages does not seem to depend on lipomannans, TLR2-specific ligands [2,15]. Recently, macrophage-inducible C-type lectin (Mincle) has been reported to mediate Mtb-stimulated NO production upon binding a mycobacterial cell wall glycolipid called cord factor (trehalose-6,6′-dimycolate) [36]. Also TLR4 may contribute to the NO induction by Mtb in macrophages upon activation by a yet unidentified heat-sensitive cell-associated mycobacterial factor [2].

Here we examined the effect of LAM on TNF-α and NO production in J774 cells, which do not express the mannose receptor, and in thioglycollate-elicited mouse peritoneal macrophages, which express the mannose receptor, but not SIGNR1 [37,38] and attempt-ted to identify the receptors involved. Surprisingly, we found that ManLAM modulates the release of TNF-α and NO in LPS-stimulated macrophages apparently acting through SRs.

2. Results

2.1. J774 cells exposed to ManLAM produce low amounts of TNF-α and NO without participation of TLR2

J774 cells challenged with ManLAM released low amounts of TNF-α and NO. At 20 μg/ml ManLAM, about 2 ng/ml TNF-α and 0.12 μg/ml NO were produced (Fig. 1A, B). It had been indicated earlier that

mycobacteria interact with macrophages by engaging TLR2, TLR4 and mannose-binding lectin receptors [2–4,10,11]. However, responses of J774 to Pam3CSK4 — a TLR2 agonist, to Escherichia coli LPS — a TLR4 agonist, and to mannan — a mannose receptor agonist, were different from those to ManLAM. At 50 ng/ml Pam3CSK4 stimulated a relatively high level of TNF-α, about 6.8 ng/ml (Fig. 1A), and no NO production (Fig. 1B), whereas 100 ng/ml LPS-stimulated high production of both TNF-α (13.5 ng/ml) and NO (1.5 μg/ml) (Fig. 1A, B). Mannan (100 μg/ml) stimulated neither TNF-α nor NO generation (Fig. 1A, B). As expected, blocking of TLR2 functions with anti-TLR2 mAb abolished TNF-α production stimulated by Pam3CSK4 (Fig. 1C). In contrast, the antibody had no effect on the TNF-α generation stimulated by Man-LAM (Fig. 1D), indicating that TLR2 is not involved in the ManLAM effect.

2.2. TLR2 mediates effects of PILAM in J774 cells

The pro-inflammatory activity of ManLAM found in J774 cells was unexpected, therefore we examined the response of these cells to PILAM. Instead of the mannan cap present in ManLAM, PILAM has a phospho-myo-inositol cap and is an agonist of TLR2 [3]. The response of J774 cells to PILAM differed markedly from that to ManLAM. PILAM stimulated higher level of TNF-α production than ManLAM; at 20 μg/ml of PILAM, 7 ng/ml TNF-α was released. In contrast, PILAM did not induce NO production (Fig. 2A, B). The effects of PILAM on TNF-α and NO production resembled the action of Pam3CSK4 (compare Fig. 2A, B with Fig. 1A, B). When the cells were treated with PILAM together with LPS, their effects on TNF-α production were additive (Fig. 2A). In contrast, NO production stimulated by LPS was not significantly affected by PILAM (Fig. 2B). Both the TNF-α production stimulated by PILAM alone and the enhancement of LPS-stimulated TNF-α production by PILAM were fully reversed by anti-TLR2 mAb (Fig. 2C, D). The data indicate that TLR2 is engaged in the activation of J774 cells by PILAM.

2.3. ManLAM enhances LPS-stimulated TNF-α and inhibits NO production in J774 cells independently of TLR2 and mannose-binding receptors

ManLAM dose-dependently enhanced the LPS-stimulated TNF-α production in J774 cells when the cells were challenged with both compounds simultaneously for 3.5 h. The effect was especially pronounced at 20 μg/ml of ManLAM which increased the LPS-induced TNF-α release by about 80% (Fig. 3A). In contrast, ManLAM inhibited the LPS-stimulated NO production in these cells by 15% at 20 ng/ml (Fig. 3B). Neither the TLR2 agonist Pam3CSK4, nor mannan, a general ligand of mannose-binding receptors mimicked the effects of ManLAM exactly. Pam3CSK4 increased the LPS-stimulated production of TNF-α by about 90% and did not affect significantly NO production (Fig. 3D, E). On the other hand, mannan did not affect the LPS-stimulated release of these mediators (Fig. 3D, E). Anti-TLR2 mAb did not affect the ManLAM-mediated enhancement of the LPS-stimulated TNF-α release (Fig. 3F). In the presence of anti-TLR2 antibody, ManLAM still inhibited the production of NO induced by LPS (Fig. 3G). The data confirmed a lack of TLR2 involvement in the responses of J774 cells to ManLAM.

The biological activities of mycobacterial lipoglycans have been reported to be highly dependent on the integrity of their lipid moieties. Acyl residues of the MPI plasma membrane anchor of ManLAM were found essential for their immunosuppressive activity as well as for their binding to the mannose receptor or DC-SIGN [2,6]. In contrast, in our study deacylated ManLAM enhanced the LPS-stimulated TNF-α release (Fig. 3A) and inhibited NO production (Fig. 3B) in J774 cells even more potently than the intact molecule. This higher biological activity of deacylated ManLAM as compared to