A Potent Adjuvant Monophosphoryl Lipid A Triggers Various Immune Responses, but Not Secretion of IL-1β or Activation of Caspase-1

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Lipopolysaccharide is a major constituent of the outer membrane of Gram-negative bacteria. LPS consists of a polysaccharide termed the O-specific chain, a nonrepeating core oligosaccharide, and a hydrophobic lipid component termed lipid A (1). LPS is also known as an endotoxin, and lipid A is responsible for its toxicity (1). LPS from Gram-negative bacteria, such as Escherichia coli, trigger various pathophysiological responses via TLR-4 (2, 3). For example, LPS stimulates macrophages, resulting in the production of proinflammatory cytokines such as TNF-α, IL-1β, IL-6, IL-10, and IFN-γ (4). Excess production of these cytokines induced by LPS causes endotoxin shock, which is characterized by inflammation, abnormal coagulation, profound hypotension, and organ failure (5). In contrast, the ability of LPS to induce these immune responses has been suggested to make it useful as a potent adjuvant (6); however, the margin between its clinical benefit and unacceptable toxicity is exceedingly narrow.

In the 1980s, Ribi and coworkers (7) found a LPS-mimetic compound that exhibits potent adjuvant activity but is 100- to 10,000-fold less toxic than LPS (7, 8). This compound is monophosphoryl lipid A (MPL), a lipid A derivative that lacks the phosphate attached at the 1-position of the reducing-terminal glucosamine moiety. However, the molecular mechanism underlying the low toxicity of MPL remains to be elucidated.

IL-1β is a pleiotropic proinflammatory cytokine, and its secretion is partly responsible for endotoxin shock (9). IL-1β is produced as a cytoplasmic precursor (pro-IL-1β), and then the precursor is processed to an intermediate form (pre-IL-1β) and finally to the secretory mature form of IL-1β (10). The processing of IL-1β is catalyzed by caspase-1, which is constitutively expressed as a precursor in cells producing it. The LPS stimulus induces the processing of the caspase-1 precursor, which completely lacks cleavage activity (11), into an active form consisting of equimolar amounts of 10- and 20-kDa proteins (12). Although a recent study showed that muramyl dipeptide (MDP) contaminating an LPS preparation derived from E. coli is a potent stimulant that activates IL-1β secretion (13), it remains controversial as to whether LPS itself has the ability to induce IL-1β secretion.

To understand the molecular mechanism underlying the low toxicity of MPL, we examined the effects of lipid A and MPL on the production of IL-1β and the activation of caspase-1 in mouse peritoneal macrophages, and found that MPL is defective in the induction of IL-1β secretion and incapable of activating caspase-1. Because caspase-1 has been shown to be essential for the induction of endotoxin shock using caspase-1-deficient mice (14), our results suggest that the lack of caspase-1 activation in MPL-stimulated macrophages contributes to the low toxicity of this adjuvant. The Journal of Immunology, 2006, 176: 1203–1207.

Materials and Methods

Animals

C3H/HeN and C3H/HeJ mice (male, 6-wk old) were obtained from Clea Japan. The mice were housed in a specific pathogen-free environment with
a 12-h light-dark cycle in the animal house of the National Institute of Infectious Diseases.

Reagents

Lipid A from the E. coli F583 Rd mutant (E. coli lipid A), MPL, from the Rd mutant, and 7-amino-4-trifluoromethyl-coumarin (AFC) were purchased from Sigma-Aldrich. Chemically synthesized E. coli-type lipid A (synthetic lipid A), compound 506, was provided by Drs. S. Kusumoto and K. Fukase (Department of Chemistry, Graduate School of Science, Osaka University, Osaka, Japan) (15). Anti-mouse IL-1B Ab was purchased from BioSource International. Anti-human IL-1B Ab and anti-human caspase-1 were purchased from Santa Cruz Biotechnology. Anti-mouse caspase-1 p45 and anti-mouse caspase-11 mAbs were provided by Dr. M. Miura (Department of Genetics, Pharmaceutical Sciences, University of Tokyo, Tokyo, Japan) (16). Anti-mouse GAPDH Ab was purchased from Chemicon International. Acetyl-Tyr-Val-Ala-Asp-7-amino-4-trifluoromethyl-coumarin (AcYVAD-AFC), acetyl-Tyr-Val-Ala-Asp-7-amino-4-trifluoromethyl-aldehyde (AcYVAD-CHO), and PMA were purchased from Calbiochem-Novabiochem. ATP was purchased from Oriental Yeast.

Preparation of lipid A and MPL suspensions

Each lipid in chloroform-methanol (2:1, v/v) was dried in a glass tube under a stream of nitrogen, resuspended in PBS, and then subjected to sonication for 5 min in a bath-type sonicator (Branson Type 2) on ice water.

Cell culture and stimulation

Peritoneal exudate macrophages from C3H/HeN and C3H/HeJ mice were cultured in RPMI 1640 medium (Invitrogen Life Technologies) containing 10% heat-inactivated FBS (Equitech-Bio) in 24-well tissue culture plates at 5 × 104 cells/well. RAW264.7 cells were maintained in the medium (17) and cultured in 24-well tissue culture plates at 5 × 105 cells/well. THP-1 cells were maintained in the medium. For differentiation into macrophages, the cells were incubated with 100 nM PMA for 2 days. The adherent cells were washed twice with culture medium and incubated for 3 days. The cells were then cultured in 24-well tissue culture plates at 5 × 105 cells/well.

Sonicated suspensions of lipids at appropriate concentrations were added to cultures of the murine peritoneal macrophages, RAW264.7 cells, and the differentiated THP-1.

Assaying of IL-1B and TNF-α released from macrophages by MPL and lipid A

After the addition of E. coli lipid A, synthetic lipid A, or MPL, cells were further incubated for 12 h for determination of IL-1B release, and for 6 h for that of TNF-α release. The amounts of IL-1B and TNF-α released from the stimulated cells into the culture medium were determined by ELISA (BioSource International) as specified by the manufacturer. When the secretion of IL-1B in THP-1 cells was examined, ATP (at a final concentration of 5 mM) was added to the THP-1 cell cultures preincubated with lipid A or MPL (18). The ATP-supplied THP-1 cell cultures were incubated further for 90 min, and then the secreted IL-1B was quantified.

Measurement of the IL-1B and TNF-α mRNA levels by RT-PCR

Peritoneal exudate macrophages from C3H/HeN or C3H/HeJ mice (5 × 105 cells/well) were allowed to adhere to wells, and then suspensions of lipids were added at appropriate concentrations. After 3 h of culture, total cellular RNA was extracted from the macrophages using an RNaseasy Mini kit (Qiagen), according to the manufacturer’s instructions, and then quantified spectrophotometrically. PCR was performed with 0.3 μg of cDNA, and then 22 or 25 cycles for amplification were performed. The following PCR primers were used in this study: IL-1B primer: sense strand, 5′-TACGAGCTCCCGAGATGAACAACAA-3′, antisense strand, 5′-TGGGGGAAGCATTAGAAACACTCC-3′; TNF-α primer: sense strand, 5′-ATGAGCAGAAGAACGAGATC-3′, antisense strand, 5′-TACAGGCTTGTACCTGGAAT-3′; β-actin primer: sense strand, 5′-TACGTAGGTTGTTAGATGCATCTCGT-3′, and antisense strand, 5′-CTCTGTAACGACTTGGTGGTCAGTG-3′.

Detection of IL-1B, caspase-11, and caspase-1 proteins on Western blot analysis

After peritoneal exudate macrophages from C3H/HeN mice and PMA-differentiated THP-1 cells had been treated with E. coli lipid A, synthetic lipid A, or MPL, the cells were washed with PBS at 37°C, and then scraped off with a rubber policeman. The cells suspended in 500 μl of ice-cold PBS were centrifuged at 1000 × g for 10 min at 4°C. The supernatant was removed and the cells were suspended in 50 μl of lysis buffer consisting of 20 mM Tris-acetate buffer (pH 7.5), 1% Triton X-100, 0.1 mM EDTA, 1 mM EGTA, 1 mM Na2VO4, 10 mM sodium glycerophosphate, 50 mM NaF, 5 mM sodium pyrophosphate, 1 mM benzamidine, 270 mM sucrose, and a protease inhibitor mixture, Complete EDTA Free (Roche Diagnostics). Cell suspensions were placed on ice for 20 min, followed by centrifugation at 10,000 × g for 15 min at 4°C. The supernatants were stored at −80°C. The protein concentrations of the samples were determined with a biinchoninic acid protein assay reagent kit ( Pierce Biotechnology) according to the manufacturer’s instructions. Then the blot was incubated with anti-IL-1B, -caspase-11, -caspase-1, or -GAPDH Abs, and subsequently with anti-rabbit, goat, rat, or mouse IgG Abs linked to HRP. Cross-reactive proteins were detected with ECL Plus Western blotting detection reagents (Amersham Biosciences) and a luminescence analyzer, LAS1000plus or LAS3000 (Fuji Film).

Measurement of caspase-1 activity in mouse peritoneal macrophages

Caspase-1 activity was measured as described previously (12). In brief, cells were suspended in 50 μl of lysis buffer (100 mM HEPES, containing 10 mM DTT, 0.5 mM EDTA, 10% sucrose, 0.1% CHAPS- NaOH buffer (pH 7.4), 5 μg/ml aprotinin, 10 μg/ml pepstatin A, 10 μg/ml leupeptin, and 0.5 mM 4-amidino-PMFS) and then placed on ice for 15 min, followed by centrifugation at 10,000 × g for 10 min at 4°C. The supernatant (50 μg of protein) was suspended in 100 μl of assay buffer (50 mM HEPES- NaOH buffer (pH 7.5), 100 mM NaCl, 10 mM DTT, 10% (v/v) glycerol, 0.1% CHAPS, and 50 μM caspase-1 substrate AcYVAD-AFC) supplemented with or without caspase-1 inhibitor AcYVAD-CHO (500 μM). After incubation at 37°C for 90 min, the fluorescence of the released fluorescent peptide was measured with excitation and emission wavelengths of 400 and 505 nm, respectively. Specific activity was determined as units per microgram of protein. One unit was defined as the activity that released 1 pmol AFC/min.

Results

MPL lacks the ability to induce IL-1B release from mouse macrophages

The ability of MPL, in addition to that of E. coli-type lipid A (both E. coli and/or synthetic lipid A), to induce the release of IL-1B and TNF-α from mouse peritoneal macrophages or RAW264.7 cells was examined. TNF-α (150 pg/ml) was detected in the culture medium of mouse macrophages cultivated in the presence of 0.5 μg/ml MPL, and the release reached the maximum level (~600 pg/ml) in the presence of 3 μg/ml MPL (Fig. 1B). On the other hand, IL-1B was hardly detected in the culture medium of macrophages cultivated in the presence of 5 μg/ml MPL (Fig. 1A). In contrast, 0.5 μg/ml E. coli lipid A induced the release of both IL-1B (200 pg/ml) and TNF-α (500 pg/ml), and 5 μg/ml synthetic lipid A also induced the release of both IL-1B (200 pg/ml) and TNF-α (600 pg/ml) (Fig. 1, A and B). With respect to the induction of TNF-α and IL-1B release, similar results were obtained with RAW 264.7 cells (Fig. 1, C and D).

To elucidate the mechanism underlying the lack of ability of MPL to induce the release of IL-1B into the culture medium, the level of IL-1B mRNA in macrophages in cultivation of the presence of MPL was examined by RT-PCR. E. coli lipid A, synthetic lipid A, and MPL, respectively, greatly elevated the levels of both IL-1B mRNA and TNF-α mRNA in the macrophages from C3H/HeN mice (Fig. 2A), this being consistent with a previous study showing that MPL induces IL-1B gene expression in mouse peritoneal macrophages (19). No elevation of the IL-1B mRNA level by these stimulants was observed in C3H/HeJ mice, which possess null TLR-4 (Fig. 2B). These results indicate that MPL can induce
IL-1β gene expression in a TLR-4-dependent manner, but that it cannot induce the release of IL-1β.

IL-1β processing efficiency in MPL-stimulated cells is much lower than that in lipid A-stimulated macrophages

IL-1β is produced as a cytoplasmic precursor (31 kDa), referred to as pro-IL-1β, and then the precursor is processed to an intermediate form (28 kDa), referred to as pre-IL-1β, and finally to the mature form IL-1β (17 kDa) by caspase-1 (10). To elucidate the molecular mechanism underlying the inability of MPL to induce IL-1β release despite its ability to induce IL-1β gene expression in mouse peritoneal macrophages, we examined the processing of pro-IL-1β by Western blotting analysis using anti-IL-1β Abs. Consistent with the results for IL-1β gene expression, production of 31-kDa pro-IL-1β was detected in macrophages stimulated with 5 μg/ml MPL for 6 h, similar to that observed with 0.5 μg/ml E. coli lipid A and 5 μg/ml synthetic lipid A (Fig. 3A). When the macrophages were stimulated with 0.5 μg/ml E. coli lipid A for 20 h, the production of 28-kDa pre-IL-1β was clearly detected, and the intensity of the 28-kDa band was higher than that of 31-kDa pro-IL-1β (Fig. 3B). In contrast, the intensity of the 28-kDa pre-IL-1β band for the cells stimulated with 5 μg/ml MPL for 20 h was lower than that of 31-kDa pro-IL-1β (Fig. 3B), and was much lower than that for the cells stimulated with 0.5 μg/ml E. coli lipid A for 20 h. These results indicate that the efficiency of processing of pro-IL-1β into pre-IL-1β is much lower in MPL-stimulated mouse peritoneal macrophages than in E. coli lipid A-stimulated macrophages.

MPL does not induce caspase-1 activation in mouse peritoneal macrophages

The poor IL-1β precursor processing in MPL-stimulated cells indicated the possibility that the MPL stimulus could not activate caspase-1. To examine this possibility, we analyzed the caspase-1 activity in a lysate of macrophages stimulated with MPL using a synthetic substrate. Compared with the basal level detected in non-stimulated control cells, both E. coli lipid A (0.5 μg/ml) and synthetic lipid A (5 μg/ml) enhanced the activity of caspase-1 by 3- to 5-fold (Fig. 4A). In contrast, MPL (5 μg/ml) did not enhance the activity at all (Fig. 4A).

Caspase-1, a constitutively produced 45-kDa inactive precursor in macrophages, is processed into active caspase-1 consisting of equimolar amounts of 10- and 20-kDa proteins upon LPS stimulation (20). To examine the ability of MPL to induce procaspase-1 processing, we analyzed the processing by determining the amounts of procaspase-1 in macrophages. The level of procaspase-1 in MPL-stimulated cells remained similar to that in unstimulated cells, while the level of procaspase-1 was markedly decreased in the E. coli-type lipid A-stimulated cells, suggesting the production of mature caspase-1 (Fig. 4B). Collectively, these results indicate that MPL, unlike E. coli type lipid A, cannot induce the processing of procaspase-1 into the active form.

MPL increases the level of caspase-11 in mouse peritoneal macrophages

Caspase-11 is highly induced by LPS stimulation via an NF-κB-dependent pathway (21, 22). The induction of caspase-11 is required for events leading to the processing of caspase-1 in response to LPS (16). We examined whether MPL caused up-regulation of the level of caspase-11 by Western blotting analysis. Stimulation of macrophages with 5 μg/ml MPL for 6 h clearly elevated the caspase-11 level, as seen for macrophages stimulated with either E. coli lipid A (0.5 μg/ml) or synthetic lipid A (5 μg/ml) (Fig. 5). These findings indicate that MPL as well as E. coli-type lipid A have the ability to induce caspase-11 in mouse peritoneal macrophages.
FIGURE 3. Processing of pro-IL-1β in lipid A- or MPL-stimulated mouse peritoneal macrophages. Cell lysates were obtained for mouse peritoneal macrophages (A and B) stimulated with E. coli lipid A (0.5 μg/ml), synthetic lipid A (5 μg/ml), or MPL (5 μg/ml) for 6 h (A), or the indicated times (B). Lysates (5 μg protein/lane) were subjected to Western blot analysis using anti-IL-1β Abs. Indicated are the sizes of pro-IL-1β (upper lane) and GAPDH protein (lower lane), A and pro-IL-1β (31 kDa) and pre-IL-1β (28 kDa), B.

Effect of MPL on the production of IL-1β protein in human-derived cultured cells

Some TLR-4 ligands, such as Taxol and lipid IVα, have been shown to exhibit species-specific discrimination (23, 24). To determine whether MPL lacks the ability to induce IL-1β secretion from human cells, we stimulated human macrophage-like strain THP-1 cells with MPL or E. coli-type lipid A. LPS is an efficient stimulant for the release of mature IL-1β from a murine macrophage-like cell line (RAW264.7) (25). However, human circular monocytes cultured in vitro and a human macrophage-like cell line (THP-1) show very little release of IL-1β with the LPS stimulus alone (26), and require additional extracellular effectors such as ATP for the efficient release of IL-1β (18, 27). Consistently, although stimulation with E. coli lipid A (0.5 μg/ml) alone induced only a tiny release of IL-1β (~25 ng/ml) from PMA-differentiated THP-1 cells (data not shown), a high level of IL-1β (600 ng/ml) release was observed for lipid A-primed THP-1 cells in the presence of 5 mM ATP (Fig. 6A). In contrast, IL-1β was hardly secreted from MPL-primed THP-1 cells even in the presence of ATP (Fig. 6A). On the other hand, MPL, as well as E. coli-type lipid A, induced the release of TNF-α (Fig. 6B).

In PMA-differentiated THP-1 cells, both E. coli lipid A and MPL increased the intracellular pro-IL-1β level without a supply of the secretion enhancer ATP (Fig. 6C). These results are consistent with our observation for mouse macrophages (Fig. 3A). After E. coli-type lipid A-primed THP-1 cells had been treated with ATP, pro-IL-1β was hardly detected in the cells, confirming efficient secretion of IL-1β after stimulation by E. coli-type lipid A and ATP in the human macrophage-like cells (Fig. 6C). By contrast, in the PMA-primed THP-1 cells treated with ATP, the level of intracellular IL-1β in the cells remained higher than that in the lipid A-primed ones (Fig. 6D). These results indicated that both E. coli-type lipid A and MPL induce the production of pro-IL-1β in PMA-differentiated THP-1 cells. MPL, unlike E. coli-type lipid A, is also incapable of inducing IL-1β secretion from human macrophage-like cells in the presence of the effector ATP.

Discussion

LPS causes a serious problem in Gram-negative bacterial infections, while it exhibits potent adjuvant activity. In contrast, MPL is much less toxic than LPS, and is not only an effective adjuvant but also a prophylactic drug for endotoxin shock (28-30). However, no reports have appeared concerning the mechanism underlying the low toxicity of MPL. In the present study, we focused on MPL-induced macrophage activation, including the production of inflammatory cytokines, because inflammatory cytokine production is relevant to the toxicity of LPS. We found that MPL has the ability to induce the secretion of TNF-α but not IL-1β, whereas MPL is able to enhance the mRNA levels of both TNF-α and IL-1β in a TLR-4-dependent manner (Figs. 1 and 2). Furthermore, MPL, unlike E. coli-type lipid A, was found not to activate caspase-1, which is required for the processing of pro-IL-1β into its secretory mature form (Figs. 3 and 4). Based on these results, we suggest that MPL does not activate or very poorly activates caspase-1, thereby being incapable of inducing the secretion of IL-1β, despite activating expression of the IL-1β gene.

Macrophages treated with ATP alone have been reported to exhibit no detectable increase in IL-1β release of protein production, but an increase in activation of NF-κB and MAPK (31, 32).
THP-1 cells were preincubated with sizes of pro-IL-1. Insufficient for the processing or exocytosis of IL-1 monocytes. Our results suggested that the MPL-priming signal is min. Cell lysates (5 μg/ml) before and after being incubated with 5 mM ATP for 90 min. Supernatants of the cell treatment with lysate preparations. ATP triggers P2X7-purinergic receptor-dependent signaling cascades that induce protein secretion, including IL-1 release from MPL-primed THP-1 cells (Fig. 6A), but led to faint and smear pro-IL-1β bands on SDS-PAGE analysis (Fig. 6D). Andrieu and coworkers (34) have reported that ATP engagement of P2X7 triggers both nonspecific degradation and processing of pro-IL-1β as well as exocytosis of mature IL-1β in LPS-primed human monocytes. Our results suggested that the MPL-priming signal is insufficient for the processing or exocytosis of IL-1β with the ATP stimulus but sufficient for the nonspecific degradation of pro-IL-1β.

Upon stimulation of macrophages by LPS, TLR-4 initiates two signaling cascades, i.e., MyD88-dependent and MyD88-independent ones (2, 3). Transcriptional activation of IL-1β and TNF-α in response to LPS is a MyD88-dependent event (35). In addition, B cells prepared from MyD88 knockout mice do not proliferate in response to LPS (35). These results demonstrated the importance of the TLR-4/MyD88-dependence signaling pathway for LPS responses. The requirement of functional TLR-4 for the MPL response was similar with that of LPS (Fig. 2B). Another study showed that both LPS and MPL stimulate the proliferation of B cells derived from BALB/c mice, but not TLR-4-defective C3H/HeJ mice (29). These studies, taken together, suggest that MPL activates a TLR-4/MyD88-dependent signaling pathway. Nevertheless, MPL failed to induce caspase-1 activation. A possible explanation for this inability is that MPL, unlike LPS and lipid A, does not activate a caspase-1-related branch of TLR-4-dependent signaling cascades.

A previous study indicated that activation of caspase-1 occurs upon the assembly of an intracellular complex, designated as the inflammasome (36). The inflammasome includes caspase-1, caspase-11 (the mouse ortholog of human caspase-5), NALP (NACHT, LRR, and PYRIN protein), and ASC (apoptosis associated speck-like protein). The MPL stimulus up-regulates the level of caspase-11, even with a defect in caspase-1 activation (Figs. 4 and 5). Although it remains unknown how LPS is involved in the assembly of the inflammasome, MPL may somehow be defective in the triggering of inflammasome assembly. For confirmation of this hypothesis, more studies are needed.

Recently, Martinon et al. (13) showed that MDPcontaminating crude LPS preparations derived from E. coli is the primary stimulus for the induction of IL-1β secretion in response to crude LPS preparations, this being consistent with our observation that an E. coli lipid A preparation was more potent than synthetic lipid A for

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**FIGURE 5.** Expression of caspase-11 protein in lipid A- or MPL-stimulated mouse peritoneal macrophages. Cell lysates were obtained for macrophages stimulated with E. coli lipid A (0.5 μg/ml), synthetic lipid A (5 μg/ml), or MPL (5 μg/ml) for 6 h. The lysates were analyzed by Western blot analysis using anti-caspase-11 Abs.

**FIGURE 6.** Secretion of IL-1β and production of pro-IL-1β in lipid A- or MPL-stimulated human THP-1 cells. A and B, Cultures of PMA-differentiated THP cells were set up in 24-well tissue culture plates, 5 × 10⁵ cells/well. After the addition of the indicated amounts of E. coli lipid A (C), synthetic lipid A ( ), or MPL ( ), the cells were incubated for 12 h for IL-1β release (A), or for 6 h for TNF-α release (B). To induce the release of IL-1β, ATP was added to these primed cells, followed by incubation for 90 min. Supernatants of the cell cultures wells after stimulation were assayed for IL-1β and TNF-α, as described under Materials and Methods. The results are shown as the averages of triplicate determination with the SD. C and D, PMA-differentiated THP-1 cells were preincubated with E. coli lipid A (0.5 μg/ml), synthetic lipid A (5 μg/ml), or MPL (5 μg/ml) for 12 h: the cells were treated with lysate preparations before and after being incubated with 5 mM ATP for 90 min. Cell lysates (5 μg protein/lane) were analyzed by Western blotting. Each blot was incubated with anti-IL-1β Abs for 12 h (C) or 48 h (D). Indicated are the sizes of pro-IL-1β (upper lane) and GAPDH protein (lower lane).
stimulating the release of the proinflammatory cytokines (Fig. 1). However, whereas Martinon et al. (13) further reported that pure LPS derived from E. coli failed to induce IL-1β processing and secretion, we here observed that synthetic lipid A clearly induced the activation of caspase-1 and the release of IL-1β in macrophages (Figs. 1 and 4). A possible explanation for this discrepancy is that E. coli lipid A itself can activate caspase-1, but that the potency of lipid A is much weaker than that of MDP as to caspase-1 activation. The possibility should also be pointed out that if LPS is dephosphorylated during an extensive purification process, it would cause a loss or severe reduction of its ability to activate caspase-1.

The activation of caspase-1 has been shown to be essential for the induction of endotoxin shock using caspase-1-deficient mice (14). As shown in the present study, MPL is capable of activating the TLR-4/MyD88-dependent pathway, but incapable of activating caspase-1. Thus, we suggest that the difference in the ability to activate caspase-1 between E. coli lipid A and MPL is related to the difference in their toxicity. Most Ags fail to induce productive immune responses unless certain microbial products are administered along with appropriate adjuvants (28). However, extreme toxicity associated with microbial products has been a major problem for their use for vaccines (5). Our understanding of the mechanism underlying the low toxicity of MPL will facilitate research for the development of effective adjuvants without toxicity.

Disclosures

The authors have no financial conflict of interest.

References