Research Article

Innate Immunity

Innate Immunity
17(6) 517–525

DOI: 10.1177/1753425910383999
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Neisseria meningitidis lipid A mutant LPSs function as LPS antagonists in humans by inhibiting TLR 4-dependent cytokine production

Tom Sprong[1,2], Peter van der Ley[3], Shahla Abdollahi-Roodsaz[4], Leo Joosten[2], Jos van der Meer[1,2], Mihai Netea[1,2] and Marcel van Deuren[1,2]

Abstract

Lipopolysaccharide is a major constituent of the outer membrane of Gram-negative bacteria and important in the induction of pro-inflammatory responses. Recently, novel LPS species derived from *Neisseria meningitidis* H44/76 by insertional inactivation of the *lpxL1* and *lpxL2* genes have been created with a lipid A portion consisting of five (penta-acylated *lpxL1*) or four (tetra-acylated *lpxL2*) fatty acids connected to the glucosamine backbone instead of six fatty acids in the wild-type LPS. We show that these mutant LPS-types are poor inducers of cytokines (tumor-necrosis factor-α, IL-1β, IL-10, IL-RA) in human mononuclear cells. Both penta- and tetra-acylated meningococcal LPSs were able to inhibit cytokine production by wild-type *Escherichia coli* or meningococcal LPS. Binding of FITC-labelled *E. coli* LPS TLR4 transfected Chinese hamster ovary (CHO) cells was inhibited by both mutant LPS-types. Experiments with CHO fibroblasts transfected with human CD14 and TLR4 showed that the antagonizing effect was dependent on the expression of human TLR4. In contrast to the situation in humans, *lpxL1* LPS has agonistic activity for cytokine production in peritoneal macrophages of DBA mice, and exacerbated arthritis in murine collagen induced arthritis model. *N. meningitidis* lipid A mutant LPSs *lpxL1* and *lpxL2* function as LPS antagonists in humans by inhibiting TLR4-dependent cytokine production but have agonistic activity in mice.

Keywords

Neisseria meningitidis, LPS, lpxL1, lpxL2, TLR4, cytokine

Date received: 25 October 2009; accepted: 17 August 2010

Introduction

Lipopolysaccharide is a major constituent of the outer membrane of Gram-negative bacteria and is important in the induction of inflammatory responses and a potent adjuvant in vaccine development.[1–3] In *Neisseria meningitidis*, the molecule consists of an oligosaccharide tail, a conserved saccharide inner core and a hexa-acylated lipid A portion. The lipid A portion is considered the toxic-moiety of LPS, responsible for the induction of pro-inflammatory signalling, although biological activity of neisserial LPS is also dependent on the saccharide tail and the inner 3-deoxy-D-*manno*-oct-2-ulosonic acid (Kdo) core.[4]

The cellular receptor complex for LPS is well established, consisting of CD14, TLR4 and MD-2.[5–9] After binding of LPS to this complex, a cascade of intracellular molecules is engaged. Activation of MyD88, TIRAP/MAL and IRAK4 ultimately leads

[1]Department of Internal Medicine, Radboud University Nijmegen Medical Centre, Nijmegen, The Netherlands
[2]Nijmegen Institute for Infection, Inflammation and Immunity (N4i), Radboud University Nijmegen Medical Centre, Nijmegen, The Netherlands
[3]National Institute for Vaccine Development, Bilthoven, The Netherlands
[4]Department of Rheumatology, Radboud University Nijmegen Medical Centre, Nijmegen, The Netherlands

Corresponding author:
Tom Sprong MD PhD, Department of Internal Medicine, Radboud University Nijmegen Medical Centre, Nijmegen, PO Box 9101, 6500 HB Nijmegen, The Netherlands.
Email: t.sprong@aig.umcn.nl

to the release of NF-κB and the induction of cytokine genes. In addition, for TLR4 also a MyD88 independent, TRIF-dependent pathway exists, that leads to activation of IRF-3 via TBK1 and IKK.[10] However, not all LPSs function as agonists for TLR4. Lipopolysaccharide with a penta- or tetra-acylated lipid A such as *Rhodobacter sphaeroides* LPS,[11] *Porphyromonas gingivalis* LPS,[12] LPS from the *Escherichia coli* msbB strain (this *E. coli* strain has a penta-acylated LPS as the msbB gene that is deficient in this strain encodes an enzyme that is crucial for the addition of a fatty acid side chain on the lipid A of *E. coli* LPS)[13] or synthetic LPSs such as lipid A-like compound E5531[14] are poor pro-inflammatory stimuli and can even act as antagonists of LPS-induced signals. Curiously, it has been shown previously that some of these LPS-antagonists in humans are LPS agonists in rodent cells, inducing a normal inflammatory response.[15] Lipopolysaccharide isolated from wild-type *N. meningitidis* is a specific and high-affinity ligand for TLR4/MD-2/CD14 whereas non-LPS components of meningococci can also induce cytokine via TLR4-independent pathways.[16–18] Interestingly, *N. meningitidis* LPS has also been reported to induce cytokines independently of TLR4 and CD14, especially at higher concentrations of meningococcal LPS (>100 ng/ml).[17,19] In addition to TLR4, TLR2 has also been reported as a receptor for some LPS-types, including meningococcal LPS.[20,21] However, it is uncertain whether this is caused by a contaminating substance in the LPS preparations tested.[22,23]

Recently, by insertional inactivation of the *lpxL1* and *lpxL2* genes of *N. meningitidis* H44/76, mutant meningococci have been created expressing LPS with a lipid A portion consisting of five (penta-acylated *lpxL1*) or four (tetra-acylated *lpxL2*) fatty acids connected to the glucosamine backbone instead of six fatty acids in the wild-type H44/76 LPS. These mutant meningococcal strains have previously been shown to have reduced toxicity in comparison with the wild-type H44/76 strain, as shown by a decreased potency for the induction of TNF-α in a human macrophage cell-line.[24] In addition, a *N. meningitidis* LPS mutant (msbB) structurally equal to the *lpxL1* LPS has been shown to inhibit interleukin (IL)-8 production in TLR4 transfected HEK293 cells.[25] In the present study, we investigated the ability of the *lpxL1* and *lpxL2* LPS mutants to inhibit cytokine responses mediated by human TLR4. In addition, we tested these LPS mutants in mouse macrophages and a mouse model of arthritis.

Materials and methods

Bacterial strains and lipopolysaccharides

Construction and characterization of the meningococcal H44/76 *lpxL1* and *lpxL2* mutants have been described in detail elsewhere.[24] The parent strain *N. meningitidis* H44/76 is an international reference strain, isolated from a patient with invasive meningococcal disease,[26] serologically classified as B:15:P1.7,16, immunotype L3,7,9.[27] As analysed by MS, *lpxL1* makes LPS with penta- instead of hexa-acylated lipid A, in which the secondary lauroyl chain is missing from the non-reducing end of the GlcN disaccharide. Insertional inactivation of the other (*lpxL2*) gene was not possible in wild-type strain H44/76 expressing full-length immunotype L3 LPS but was achieved in a galE mutant expressing a truncated oligosaccharide chain. Structural analysis of *lpxL2* mutant lipid A showed a major tetra-acylated species lacking both secondary lauroyl chains and a minor penta-acylated species.[24] For isolation of LPS, the *N. meningitidis* strain H44/76 and its derivatives with inactivated *lpxL1* or *lpxL2* genes, were grown at 37°C in liquid medium supplemented with IsoVitaleX (Becton Dickinson) in a humid atmosphere containing 5% CO_2. Bacterial suspensions were heat inactivated for 30 min at 56°C. Lipopolysaccharide was isolated by the phenol/water extraction method as described by Westphal and Jann.[28] After extraction, LPS was treated with Proteinase K (Sigma-Aldrich Co), DNA-ase and RNA-ase (Roche diagnostics) for additional purification, recovered by ultracentrifugation, freeze-dried and dissolved in sterile PBS. Lipopolysaccharide purified and isolated in this way has previously been shown to be free of protein contamination (<1.0%, determined by Lowry-assay), whereas nucleic acid contamination is approximately 5%.[17] *Escherichia coli* O55:B5 LPS and FITC-labelled *E. coli* LPS was obtained from Sigma-Aldrich Chemical Co.

Cells and culture conditions

Human peripheral blood mononuclear cells (PBMCs). Blood for the isolation of PBMCs was drawn in 10 ml EDTA anti-coagulated tubes (Vacutainer System, Beckton Dickinson) from healthy human volunteers. Informed consent was obtained before each experiment, and the guidelines of the local ethics committee were followed in the conduct of these experiments. PBMCs were isolated by density gradient centrifugation over Ficoll-Hypaque (Pharmacia Biotech AB). The cells from the interphase were aspirated, washed 3 times in sterile phosphate buffered saline (PBS) and resuspended in culture medium RPMI 1640 (Dutch modification, Flow Labs) supplemented with L-glutamine (2 mmol), pyruvate (1 mmol) and gentamycin (50 μg/ml). Cells were incubated with the different stimuli for 24 h, unless otherwise indicated, whereafter the supernatants were collected and stored at –80°C until assay in one batch.

Chinese hamster ovary fibroblasts (CHO) stably transfected with human CD14 (3E10) or CD14 and TLR4 (3E10-TLR4). Chinese hamster ovary fibroblasts (CHO) stably transfected with human CD14 (3E10), human CD14 and TLR2 (3E10-TLR2) or human CD14 and hTLR4 (3E10-TLR4), described previously in detail,[6] were a kind gift from Dr Robin Ingalls and Dr Douglas T Golenbock. It is important to note that 3E10 expresses also hamster TLR4 on the outer membrane, and is capable of LPS-mediated signalling. These cell-lines express inducible membrane CD25 under control of a region from the human E-selectin (ELAM-1) promoter containing NF-κB binding sites; this promoter element is absolutely dependent on NF-κB.[15] Cells were maintained at 37°C and 5% CO_2 in HAM's F12 medium (Gibco™, Invitrogen) supplemented with 10% FCS, 0.01% L-glutamine, 50 μg/ml gentamycin, 400 U/ml hygromycin and 0.5 mg/ml of G418 (for 3E10-TLR2) or 0.05 mg/ml of puromycine (for 3E10-TLR4). Expression of TLR 2 and TLR4 was confirmed by flow-cytometry (Coulter FACS-scan) using PE-labelled anti-TLR2 (clone TL2.1) or anti-TLR4 (clone HTA125; Immunosource). For stimulation experiments, 500 μl of cells in culture medium at a density of 1×10^5 cells/ml were plated in 24-well culture plates. After a 20-h incubation, cells were incubated with the various stimulants for 20 h, whereafter the cells were harvested using trypsin/EDTA (Cambrex) and prepared for flow-cytometry (Coulter FACS-scan). CD25 expression of the CHO-cells was measured using FITC labelled anti-CD25 (DAKO).

FITC-LPS binding experiments

lpxL1 and *lpxL2* LPSs (1 μg/ml) were pre-incubated with CHO 3E10-TLR4 for 15 min whereafter FITC labelled *E. coli* LPS was added at increasing concentrations. Cells were incubated for 1 h at 20°C in the dark, harvested using trypsin/EDTA and washed with sterile PBS. Read out was by flow cytometry (Coulter FACS-scan).

Murine model of arthritis

Collagen-induced arthritis (CIA) was induced in DBA-1 mice between 10–12 weeks of age.[29] Bovine type II collagen was dissolved in 0.05 M acetic acid to a concentration of 2 mg/ml and emulsified in an equal volume of Freund's complete adjuvant (2 mg/ml of *Mycobacterium tuberculosis* strain H37Ra; Difco laboratories, USA). Mice were immunized by intradermal injection of 100 μl of the emulsion at the base of the tail and were given an intraperitoneal booster injection of 100 μl of CII dissolved in phosphate-buffered saline without any adjuvant on day 21. Clinical onset and progression of arthritis was macroscopically evaluated by two blinded observers and scored on a scale between 0 and 2 for each paw as described previously.[29] Mice were treated using three i.p. injections of *lpxL1* (400 μg/kg body weight) once in 2 d started on day 22 of immunization before the clinical onset of the disease.

Stimulation of murine peritoneal macrophages

Murine macrophages were isolated from naïve DBA/1 mice by the lavage of the peritoneal cavity using 10 ml cold medium (DMEM + 10% FCS). Adherent cells were harvested and cultured for 4 d before use. Cells were incubated with *E. coli* LPS (10 ng/ml), *lpxL1* (100 ng/ml) or a combination of both.

Cytokine assays

Human TNF-α, IL-10 and IL-1RA were determined by ELISA (in-house 4-span ELISA for TNF-α,[30] Pelikine compact ELISA (Sanquin) for the other cytokines; lower limit of detection 20 pg/ml). Interleukin-1β was determined by radioimmunoassay (RIA) as described by Drenth *et al.*[31] The lower limit of detection was 40 pg/ml. Murine cytokines were measured using the Bioplex cytokine assays (Bio-Rad, USA) following the manufacturer's instructions.

Results

Cytokine induction by N. meningitidis *H44/76 LPS and* lpxL1 *or* lpxL2 *mutant LPSs*

Neisseria meningitidis H44/76 LPS was a potent inducer of the cytokines TNF-α and IL-10 in human PBMCs. In contrast, the *lpxL1* and *lpxL2* mutant LPS types were 100–1000 times less potent in the induction of these cytokines, inducing only minimal cytokine production at a concentration of 10 μg/ml (Figure 1). The mutant LPSs were also minimal inducers of IL-1β and IL-1RA (data not shown).

Antagonistic properties of lpxL1 and lpxL2 mutant LPSs

Similar to meningococcal LPS, *E. coli* LPS was a potent inducer of TNF-α and IL-10 in human PBMCs. Pre-incubation of the PBMCs with *lpxL1* or *lpxL2* LPSs (1 μg/ml) completely inhibited TNF-α and IL-10 production induced by *E. coli* LPS. This antagonistic effect was present at all concentrations of *E. coli* LPS tested, ranging from 0.1 ng/ml up to 100 ng/ml (Figure 2). *Neisseria meningitidis* H44/76 LPS was inhibited to a similar extent by the mutant LPSs (not shown). The antagonistic effect of *lpxL1* and *lpxL2* LPSs on cytokine induction by 10 ng/ml of *E. coli* LPS was present at concentrations of 10 ng/ml of *lpxL1* or *lpxL2* or higher. Complete inhibition was seen at concentrations of 100 ng/ml of *lpxL2* LPS and 1 μg/ml of *lpxL1* LPS (Figure 3). In addition, we examined whether pre-incubation was necessary for inhibition of LPS-induced cytokines by *lpxL1* and *lpxL2*. We found that LPS-induced cytokine

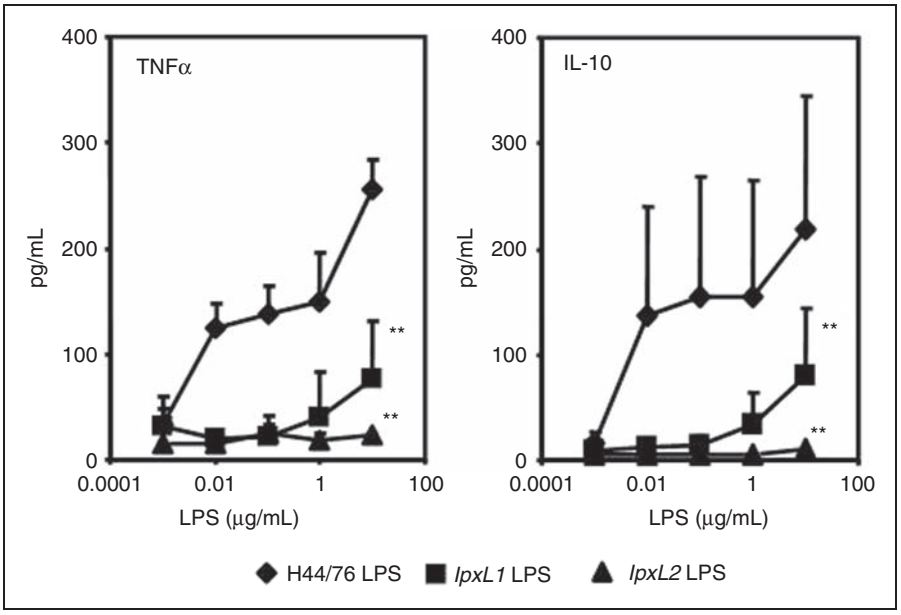

Figure 1. Tumor necrosis factor-α and IL-10 production after stimulation of human PBMCs for 24 h with *N. meningitidis* H44/76 LPS, and the mutant *lpxL1* and *lpxL2* LPSs. Mean values and SD are shown, $n = 3$. $^{**}P < 0.01$ for the comparison of *lpxL1* and lpx LPSs with *N. meningitidis* H44/76 LPS by two-way ANOVA.

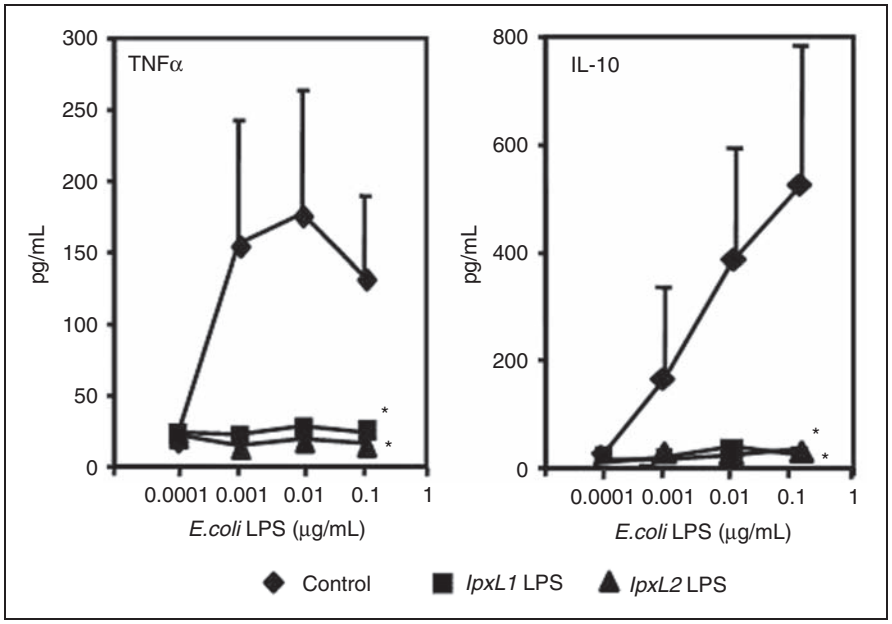

Figure 2. *Escherichia coli* induced TNF-α and IL-10 production by human PBMCs in the absence or presence of the mutant *lpxL1* and *lpxL2* LPSs (1 μg/ml). *lpxL1* and *lpxL2* LPSs were pre-incubated with the PBMCs for 30 min prior to stimulation with *E. coli* LPS. Mean values and SD are shown, $n = 3$. $^{*}P < 0.05$ for the comparison of *lpxL1* and *lpxL2* LPSs with the control (RPMI) by two-way ANOVA.

production was inhibited by the *lpxL* mutants equally well when they were pre-incubated with the PBMCs or simultaneously added with the *E. coli* LPS (Figure 4).

Finally, we tested whether the observed inhibitory effect on cytokine production was dependent on inhibition of LPS-binding to TLR4. Figure 5 shows that binding of FITC-labelled *E. coli* LPS to CHO cells transfected with hCD14 and hTLR4 was completely inhibited by the *lpxL1* and *lpxL2* mutant LPSs. This indicates that the antagonistic effect is dependent on TLR4 binding by the two *lpxL* mutant LPSs.

Expression of human TLR4 is necessary for LPS antagonism

To test whether the observed antagonistic effect of *lpxL1* and *lpxL2* LPS was dependent on differences in TLR4-mediated signalling, we made use of the

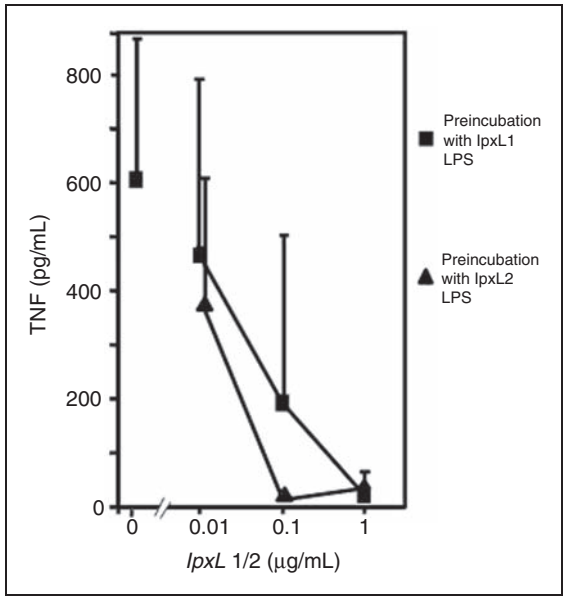

Figure 3. *Escherichia coli* (10 ng/ml) induced TNF-α production by human PBMCs in the presence of different concentrations of *lpxL1* and *lpxL2* LPSs. *lpxL1* and *lpxL2* LPS were pre-incubated with the PBMCs for 30 min prior to stimulation with *E. coli* LPS. Mean values and SD are shown, $n = 3$. *lpxL1* and *lpxL2* did not significantly differ in their ability to inhibit *E. coli* induced TNF-α (two-way ANOVA).

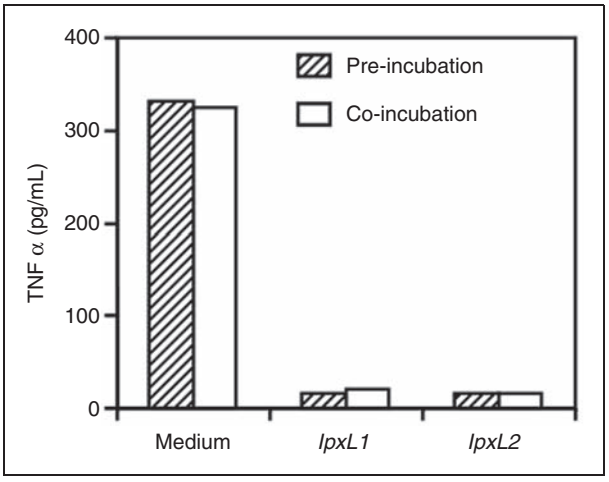

Figure 4. *Escherichia coli*-induced TNF-α production by human PBMCs in the absence (RPMI) or presence of *lpxL1* and *lpxL2* mutant LPSs, pre-incubated with the PBMCs for 30 min or added directly together with the *E. coli* LPS. Mean values are shown, $n = 2$.

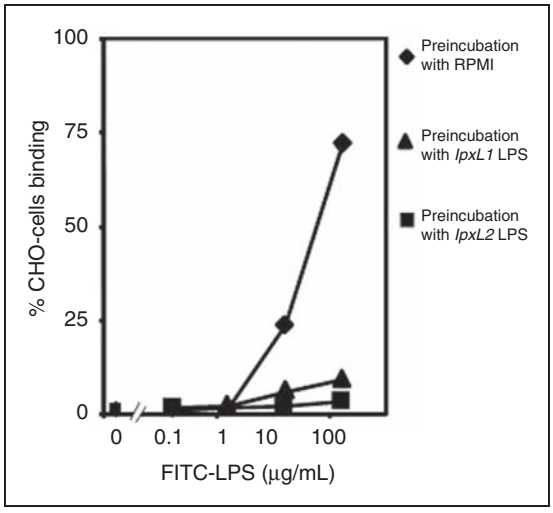

Figure 5. Inhibition of binding of FITC LPS to CHO 3E10-TLR4 by *lpxL1* and *lpxL2* LPSs. Representative experiment of three performed is shown.

differences in TLR4-dependent signalling between human and hamster TLR4, employing a CHO cell line stably expressing human CD14 and either hamster TLR4 (3E10) or overexpressing human TLR4 (3E10-TLR4).

Isolated *E. coli* LPS and wild-type *N. meningitidis* H44/76 LPS (not shown) induced NF-κB activation in both cell lines. In contrast, *lpxL1* and *lpxL2* LPSs induced a similar response to *E. coli* LPS in the CD14-transfected CHO cells expressing hamster TLR4, but when these CHO cells overexpress human TLR4, the NF-κB response was blunted (Figure 6A). These results indicate that NF-κB activation by the *lpxL1* and *lpxL2* mutant LPSs differs between species, and its absence in humans is dependent on TLR4.

Antagonistic activity of *lpxL1* and *lpxL2* LPSs was dependent on expression of human TLR4, as the *lpxL* mutants were able to inhibit *E. coli* LPS-induced NF-κB activation in hCD14-transfected CHO cells overexpressing human TLR4 but not in hCD14-transfected CHO cells expressing hamster TLR4 (Figure 6B). To investigate TLR2-dependent signalling by the H44/76 *N. meningitidis* LPS and the *lpxL1* and *lpxL2* mutant LPS-types, we tested whether they could induce signalling in TLR2 transfected CHO-cells. Figure 7 shows that TLR2-transfected CHO-cells do not show an increased response to all three LPS types compared to CHO cells transfected with CD14 alone, whereas Pam3Cys (a known TLR2 agonist) induces a very strong signal in TLR2 transfected cells.

Taken together, these results indicate that that *lpxL1* and *lpxL2* LPSs bind hamster and human TLR4, inducing a strong NF-κB response by hamster TLR4, but no NF-κB dependent signalling by human TLR4. In this way, they can inhibit binding of agonist-LPS to human TLR4, antagonizing the effects of agonist-LPS on NF-κB dependent cytokine production in humans.

lpxL1 *is not an antagonist in mice, and exacerbates arthritis in a murine model of arthritis*

Because of the antagonistic nature of the *lpxL1* LPS in human cells, we investigated whether *lpxL1* LPS was

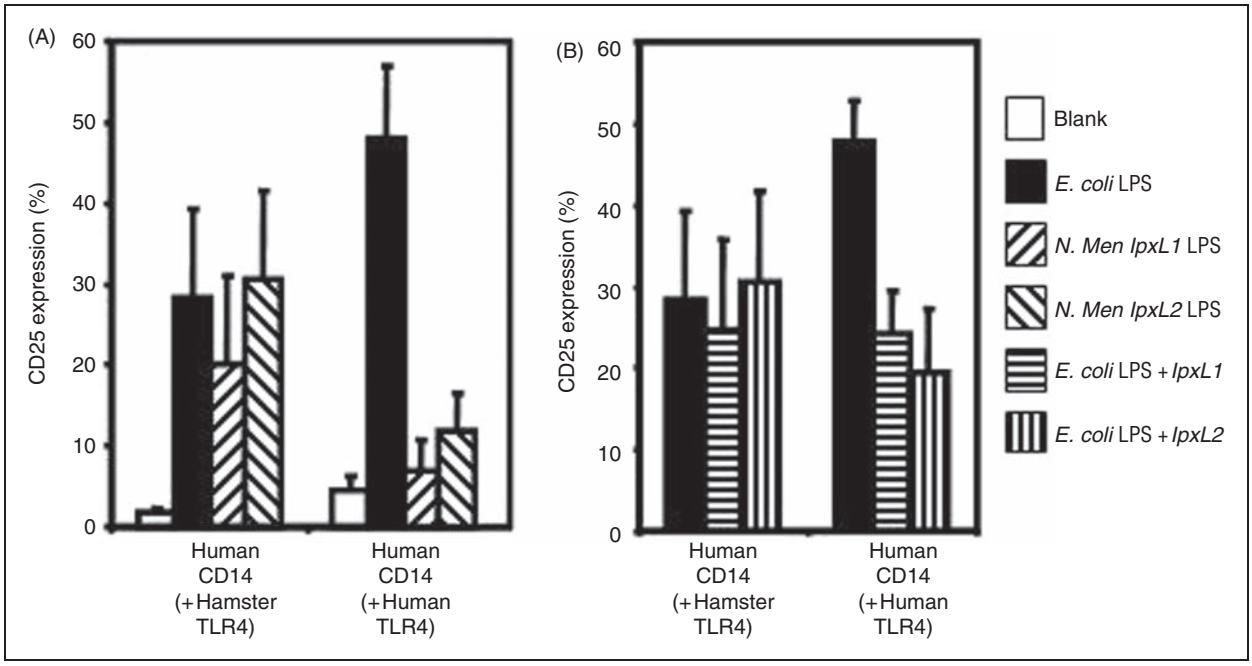

Figure 6. Nuclear factor-κB dependent CD25 expression on CHO cells stably transfected with human CD14 alone or human CD14 and human TLR4. Human CD14 transfected CHO cells also express endogenous hamster TLR4, whereas the human CD14 and TLR4 transfected cells overexpress human TLR4. Cells were incubated with RPMI (empty bars), *E. coli* LPS, *lpxL1* LPS or *lpxL2* LPS at 1 μg/ml for 20 h (A) or pre-incubated with the *lpxL1* and *lpxL2* LPSs for 30 min and stimulated with *E. coli* LPS (1 μg/ml) for 20 h (B). Average and SD are presented of three experiments performed.

able to attenuate arthritis in a murine model of collagen-induced arthritis, similar to previously published results for *Bartonella quintana* LPS.[32] However, in contrast to the findings with *Bartonella* LPS, we found that the *lpxL1* LPS exacerbated arthritis in DBA mice, as evidenced by higher arthritis scores in the mice injected with *lpxL1* LPS in comparison with control mice injected with saline (Figure 8A). As previous studies have shown that agonist *E. coli* LPS also causes exacerbation of arthritis, we investigated whether the *lpxL1* LPS is an agonist for cytokine production in DBA mice. Experiments with murine peritoneal macrophages demonstrated that the *lpxL1* LPS induced potent TNF-α, IL-1β and IL-6 responses, although *lpxL1* LPS was approximately 10 times less potent then *E. coli* LPS and was not able to inhibit cytokine production by *E. coli* LPS (Figure 8B, results for TNF-α are shown). This indicates that, in DBA mice, *lpxL1* LPS functions as an agonist for TLR4, in contrast to the situation in humans where *lpxL1* LPS is antagonistic.

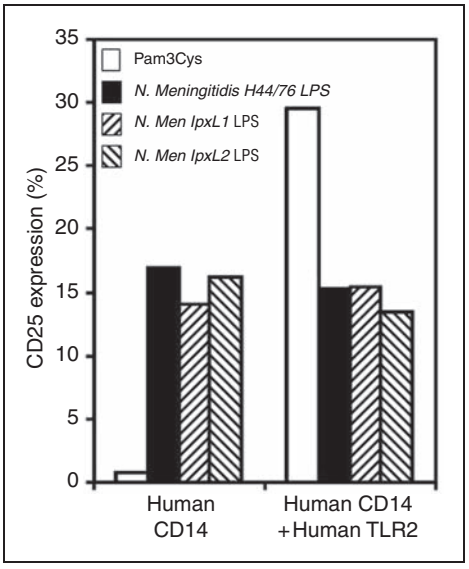

Figure 7. Nuclear factor-κB dependent CD25 expression on CHO cells stably transfected with human CD14 alone or human CD14 and human TLR2. Cells were incubated with Pam3Cys (empty bars), *N. meningitidis* H44/76 LPS, *lpxL1* LPS or *lpxL2* LPS at 1 μg/ml for 20 h. Representative experiment of three performed is shown.

Discussion

In the present study, we show that lipid A mutant LPS, derived from genetically engineered meningococci expressing penta- or tetra-acylated LPS, have a strongly reduced capacity to induce NF-κB activation and cytokine production by human TLR4. In contrast, they function as LPS-antagonists in humans, by binding TLR4 and competitively inhibiting cytokine production by agonist LPS. This effect on TLR4 transmitted signals is species-specific. Penta-acylated mutant *lpxL1* LPS exacerbates disease in a murine arthritis model and is an agonist for TLR4-mediated cytokine

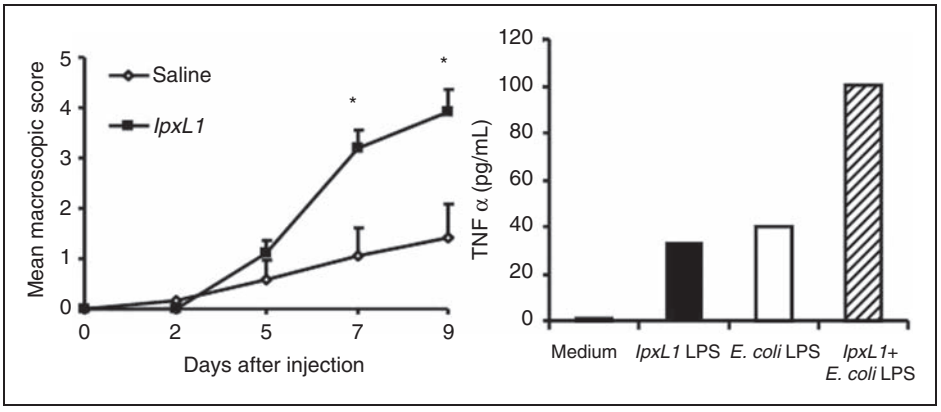

Figure 8. Exacerbation of arthritis in a collagen-induced arthritis model in DBA mice by *lpxL1* LPS (panel A). Mean and SEM of arthritis scores in 11 mice are shown; filled squares, mice challenged with collagen and three intraperitoneal injections of *lpxL1* LPS at day 0, 2 and 5, open circles, mice injected with 0.9% saline solution. Panel B shows TNF-α production by murine peritoneal macrophages 24 h after stimulation with DMEM, *lpxL1* LPS (100 ng/ml), *E. coli* LPS (10 ng/ml) or a combination of *lpxL1* LPS (100 ng/ml) and *E. coli* LPS (10 ng/ml). Representative experiment of two performed is shown. *$P < 0.05$ by Mann–Whitney test.

production in murine peritoneal macrophages and hamster cells.

Recently, Teghanemt *et al.*[25] reported that the meningococcal msbB LPS mutant, structurally equivalent to the *lpxL1* LPS mutant used in the present study, bound MD2 and subsequently TLR4 in a similar manner as wild-type meningococcal LPS but was a partial antagonist of TLR4 dependent IL-8 production in HEK293 cells. We expand these findings with our observations that the antagonistic effect is also present in isolated human cells for a range of pro- and anti-inflammatory cytokines, that the *lpxL2* LPS has a similar antagonistic effect as the *lpxL1* LPS and that this effect is species-specific, not present in DBA mice or hamster cells. Teghanemt *et al.*[25] proposed that the mechanism behind the antagonistic effect of the meningococcal msbB LPS was an alteration of the interaction of the MD2–LPS complex with the TLR4 receptor, although it has also been shown that penta-acylated meningococcal LPS has a reduced binding affinity for MD2.[33] In contrast, recent crystallographic data showed that the LPS antagonist eritoran, derived from the lipid A portion of *R. sphaeroides* LPS, binds MD2 and TLR4 similar to LPS, but prevents heterodimerisation of the MD2–TLR4 complex. For *E. coli* msbB LPS, Coats *et al.*[13] found that MD-2 and TLR4 were the only components of the receptor complex necessary to mediate msbB LPS antagonism of TLR4 dependent signalling by wild-type *E. coli* LPS. Interestingly, the CHO cells we used in the present study only overexpress human CD14 and TLR4, whereas MD-2 is not overexpressed, although it may be present endogenously.[34] Thus, the antagonistic property of the *lpxL1* and *lpxL2* LPS mutants in the CHO cells overexpressing human TLR4 is independent of human MD-2. This suggests that the TLR4 molecule itself is central to the antagonistic effect of the *lpxL1* and *lpxL2* LPSs.

An important feature of LPS molecules that exhibit antagonistic properties is that this effect is species-specific. For instance, *R. sphaeroides* LPS and lipid IVa are agonists for cytokine production in hamster cells,[35] whereas in mouse, *R. sphaeroides* LPS is an antagonist and lipid IVa is an LPS mimetic.[11] We have previously shown that cytokine production by meningococcal LPS depends on TLR4 in murine macrophages.[17] In the present study, we found that the *lpxL1* and *lpxL2* LPSs are antagonists in humans, are LPS-mimetics in CHO cells expressing hamster TLR4 but are antagonists again in CHO cells overexpressing human TLR4. Interestingly, in DBA mice, *lpxL1* LPS was an agonist for cytokine responses, and exacerbated disease in a murine model of arthritis. This is a well-known effect of agonistic LPS[29,36] and shows that the *in vitro* differences in antagonistic properties between species for these LPS types also confer to *in vivo* functional effects. Our results are in agreement with the differential signalling of *lpxL1* LPS through murine and human TLR4 as reported by Steeghs *et al.*,[37] who showed that the reduction in activity of *lpxL1* as compared to wild-type meningococcal LPS is much more pronounced in the human than the mouse system. There are important differences in leucine-rich repeats (LRRs) found in TLR4 between species; these LRRs are important for the interaction of TLR4 with ligands and co-receptors. This difference may be structural to the differences in antagonistic behaviour of penta- and tetra-acylated LPS in humans and rodents.[38,39]

The antagonistic nature of penta- or tetra-acylated LPS has received significant attention as potential therapy for human diseases such as septic shock,[40,41] rheumatoid arthritis,[32,42] asthma[43] and inflammatory bowel

disease.[44] The beneficial effects may be caused by inhibition of the interaction of Gram-negative bacteria with cells of our immune system, but there is increasing evidence that LPS is not the only ligand for the TLR4 complex, and it is possible that antagonists for LPS will also be antagonists for these other ligands.[45]

Conclusions

Neisseria meningitidis lipid A mutant LPSs *lpxL1* and *lpxL2* function as LPS antagonists in humans by inhibiting TLR4-dependent cytokine production. As such, *lpxL1* and *lpxL2* mutant LPSs might be of therapeutic value in TLR4-dependent inflammatory conditions. Differences between human and murine TLR4, however, preclude the use of mouse models in studies addressed to this issue.

Acknowledgement

Financial support was provided by the Nederlandse Organisatie voor Wetenschappelijk Onderzoek (NWO) grant number 920-03-176. The authors thank Trees Janssen, Liesbeth Jacobs and Johanna van der Ven-Jongekrijg for excellent technical assistance.

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