Activation of Toll-Like Receptor 2 (TLR2) and TLR4/MD2 by *Neisseria* Is Independent of Capsule and Lipooligosaccharide (LOS) Sialylation but Varies Widely among LOS from Different Strains

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Lipooligosaccharide (LOS) structure and capsular polysaccharide of *Neisseria meningitidis* each greatly influence the virulence of the organism and the quality of host innate immune responses. In this study, we found that production of the proinflammatory cytokine tumor necrosis factor (TNF) by a human monocyte-derived cell line (THP-1) exposed to strains of *N. meningitidis* lacking capsule and/or with truncated LOS was similar to that elicited by the isogenic wild-type strain. These mutants also exhibited no difference in induction of the interleukin-8 (IL-8) promoter in a transfected HeLa cell system of Toll-like receptor 2 (TLR2) and TLR4/MD2 signaling. However, purified LOS from diverse strains of *Neisseria* (both *N. meningitidis* and *N. gonorrhoeae*) caused widely variant levels of IL-8 promoter induction in cells expressing MD2 that correlated with the production of TNF from THP-1 cells. These data suggest that although modification of the oligosaccharide chain of LOS and/or absence of capsule do not affect cell signaling mediated by TLR4/MD2, fine-structural differences in the LOS do influence signaling through TLR4/MD2 and, through this pathway, influence some of the proinflammatory responses elicited by *Neisseria*.

Neisseria meningitidis is an important cause of sepsis syndrome and meningitis, the severity of which is correlated with the release of proinflammatory cytokines (50, 51). The lipooligosaccharide (LOS) of *N. meningitidis*, like other gram-negative bacteria, is a potent stimulator of the proinflammatory response, and plasma LOS levels correlate with the severity of disease (3). Neisserial LOS is composed of a hydrophobic lipid A portion that anchors it to the bacterial outer membrane, and this is linked to a hydrophilic oligosaccharide core, which is exposed on the surface. However, it does not possess the repeating O-polysaccharide region that is seen in the lipopolysaccharide (LPS) of many other bacteria. The outer core of the LOS is highly variable, and this forms the basis of strain immunotyping (41). The lipid A structure of both *N. meningitidis* (29) and *N. gonorrhoeae* (44) consists of a β-D-glucosaminyl-(1′→6)-D-glucosamine disaccharide backbone with variable patterns of phosphorylation and fatty acid acylation (23).

Addition of sialic acid to the terminal galactose moiety of the lacto-*N*-neotetraose of meningococcal LOS and the polysialic acid capsule of serogroup B organisms both determine virulence through the regulation of complement (10, 20, 22) and also by modification of a number of innate host immune responses, including expression of phagocyte adhesion molecules (26) and nonopsonic macrophage phagocytosis of the organism (38).

The Toll-like receptors (TLRs) have been shown to initiate innate proinflammatory signal transduction in response to a number of pathogen-associated molecular patterns (33, 34, 39). TLR4, with its cofactor MD2, has been shown to respond to LPS (30, 42), and TLR2 responds to lipoproteins (2) and peptidoglycan (45). TLR3, TLR5, and TLR9 respond to double-stranded RNA (1), flagellin (17), and bacterial unmethylated CpG DNA (18), respectively. The potential of different TLRs to form heteromeric complexes (35, 53) also dramatically increases the possible number of pathogen-associated molecular patterns that may be recognized in order for the cell to distinguish successfully between different pathogens.

We have previously shown that *N. meningitidis* signals through both TLR2 and TLR4/MD2 and that LOS is necessary for the response by TLR4/MD2, as an LOS-deficient mutant of *N. meningitidis* is unable to induce the interleukin-8 (IL-8) promoter via TLR4/MD2 (36). Because capsule and LOS structure each influence the host innate response, we have used mutants deficient in capsule and/or with altered LOS structure to test the hypothesis that this is due to modulation of the activation of signal transduction via TLR2 and/or TLR4/MD2.

It has been shown that both gonococcal and meningococcal LOS exhibit strain-to-strain variation in clotting of *Limulus* amoebocyte lysate (LAL) (40). Because some of the mechanisms involved in endotoxicity are more fully understood now in the context of TLRs, in this study, we have also determined the relative activities of LOS derived from a variety of *Neisseria*

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TABLE 1. Neisserial LOS structures

Strain	LOS α-chain structure[a]	Monoclonal antibody 3F11 binding[b]	Heptose I/II PEA	Reference
GC PID2	Galβ1-4GlcNAcβ1-3Galβ1-4GlcNAcβ1-3Galβ1-4Glc	+	−	47
MGC 89I	(NeuNAcα2-3)Galβ1-4GlcNAcβ1-3Galβ1-4Glc	+[c]	+	27
GC F62	(GalNAcβ1-3)Galβ1-4GlcNAcβ1-3Galβ1-4Glc	+	+	54
GC 1291	Galβ1-4GlcNAcβ1-3Galβ1-4Glc	+	+	21
MGC 7880	Galβ1-4-Glcβ1-4Glc	−	+	48
MGC 7889	Galβ1-4-Glc	−	+	25
MGC 8032	ND[d]	+	ND	40
GC 56	ND	+	ND	31
GC DOV	ND	+	ND	31

[a] Residues in parentheses indicate that a portion of the LOS made by the strain contains that sugar.
[b] LOS that bind monoclonal antibody 3F11 are presumed to express lacto-N-neotetraose (54).
[c] After neuraminidase treatment.
[d] ND, not determined.

strains in stimulation of proinflammatory cytokine responses from monocytes and signaling through TLR4/MD2.

MATERIALS AND METHODS

Bacterial strains. The capsule and LOS outer core-deficient mutants of *N. meningitidis* (donated by Matthias Frosch, University of Würzburg, Würzburg, Germany) were derived from a clinical isolate of serogroup B, strain 1940 (immunotype L3, 7, 9) and have been described elsewhere (15, 16). In brief, the capsule-deficient strain (*siaD* mutant) was created by insertional inactivation of the polysialyltransferase gene *siaD*, the strain with a truncated LOS (*galE* mutant) was created by deletion of the *galE* gene, and the strain lacking both capsule and LOS outer core (*cps* mutant) was created by deletion of the entire *cps* region (including *galE*). The expression of the outer membrane components opa and pilin was identical to that of the parent in each case. Before each experiment, bacteria were removed from storage in liquid nitrogen and cultured overnight on blood agar (Columbia agar base with 5% horse blood; Oxoid). Two colonies were transferred to 10 ml of Mueller-Hinton broth (Oxoid) and incubated to mid-exponential phase (about 4 h) at 37°C in 5% CO_2, and viable counts were adjusted spectrophotometrically. Fixed bacteria were prepared by incubation in 2% paraformaldehyde for 10 min at 37°C and then washed with PBS by centrifugation at $10,000 \times g$ for 2 min and stored in PBS at 4°C before use within 24 h. Culture filtrates were prepared by resuspension of mid-log-phase bacteria at 2×10^8 CFU/ml in RPMI medium plus 10% heat-inactivated fetal calf serum (Invitrogen) and incubation at 37°C for 2 h before filtration of the culture through a 0.2-μm-pore-size filter.

LOS. LOS from clinical isolates of *N. meningitidis* (MGC 7889, MGC 89I, MGC 7880, and MGC 8032) and *N. gonorrhoeae* (GC 56, GC F62, GC 1291, GC DOV, and GC PID2) were isolated by hot phenol extraction by the method of Westphal and Jann (52). Structural information relating to the LOS expressed by these strains can be seen in Table 1; none of the strains for which the structures are known express β chain. *Salmonella enterica* serovar Minnesota Re595 LPS was obtained from List Biological Laboratories, Campbell, Calif., and *Escherichia coli* O55:B5 LPS was from Sigma Chemical Co., St. Louis, Mo.

Proinflammatory cytokine response of THP-1 cells. THP-1 cells are a human monocyte-like cell line derived from a patient with acute monocytic leukemia. For stimulation of THP-1 cells with bacteria, 10^5 cells per well were inoculated with bacteria to give a final concentration of 10^9 CFU per well and incubated at 37°C in 5% CO_2 for 2 h. The cells were then pelleted ($300 \times g$ for 15 min), and the supernatants was collected and stored at −70°C before tumor necrosis factor (TNF) levels were assayed by enzyme-linked immunosorbent assay (ELISA).

For stimulation of THP-1 cells with LOS, 10^5 cells per well were treated with 200 ng of LOS and incubated at 37°C in 5% CO_2 for 6 h, after which TNF concentrations in the supernatants were determined by ELISA.

To assay for TNF by ELISA, an anti-human TNF MAb (BD-Pharmingen) was used for capture, and a biotinylated anti-human TNF MAb (BD-Pharmingen) was used for detection in accordance with the manufacturer's recommended protocol. A standard curve was prepared by using recombinant human TNF (BD-Pharmingen).

TLR signaling assay. To examine signaling through TLRs, HeLa cells (European Collection of Animal Cell Cultures 85060701) were seeded at 1.5×10^4 per well into 96-well tissue culture plates 24 h before transfection. HeLa cells express TLR4 but not TLR2 or MD2, which is necessary for the response by TLR4 (53). Signaling in response to bacteria or bacterial products was measured by using an

NFκB-responsive region (IL-8 promoter) linked to the firefly luciferase gene, and levels were normalized against the constitutive low-level expression of *Renilla* luciferase. Transient transfection of luciferase reporter plasmids and expression vectors for TLR2 and MD2 with an activated dendrimer (SuperFect; Qiagen) has been described previously (36). After 24 h following transfection, 10 μl of the agonists was added to the wells, and 30 h after transfection, luciferase reporter levels were measured with the Dual-Luciferase system (Promega).

RESULTS

Proinflammatory cytokine response of THP-1 cells elicited by *N. meningitidis* is not influenced by encapsulation or LOS truncation. After incubation with live group B *N. meningitidis* strain B1940 for 2 h, THP-1 cells produced TNF in amounts dependent on the inoculating dose of the organism (Fig. 1, wt bars). We have previously shown that a mutant of *N. meningitidis* completely deficient in LOS induces a proinflammatory response that is attenuated in comparison to that induced by wild-type organisms (36). The data presented here indicate that truncation of the LOS, and therefore removal of the terminal sialylation site, did not alter the proinflammatory response to the bacteria (Fig. 1, compare *galE* and wt bars) (*P*
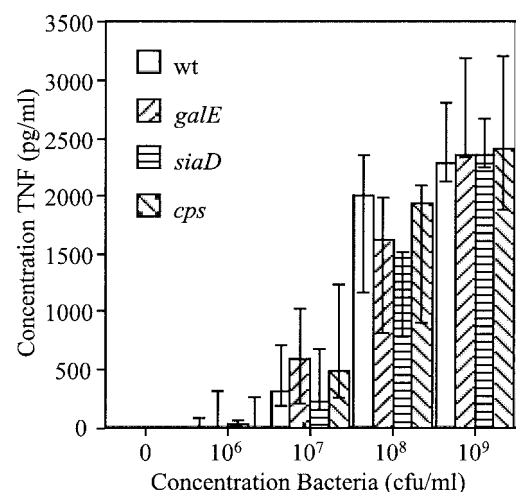

FIG. 1. Proinflammatory response of THP-1 cells to *N. meningitidis*. The magnitude of TNF release in response to live wild-type (wt) *N. meningitidis* and mutants with truncated LOS (*galE*), without capsule (*siaD*), or without capsule together with truncated LOS (*cps*) was measured by ELISA. Each bar represents the median and interquartile range of three separate experiments. *P* > 0.05 (Kruskal-Wallis test).

> 0.05 [Kruskal-Wallis test]). Absence of capsule and absence of capsule together with LOS truncation also did not modulate the proinflammatory response in these cells (Fig. 1, compare *siaD* and *cps* bars, respectively, with wt bars; $P > 0.05$ [Kruskal-Wallis test]).

Signaling through TLR2 and TLR4/MD2 is not influenced by encapsulation or LOS truncation. We have previously reported that although a mutant strain of *N. meningitidis* deficient in LOS does not signal through TLR4/MD2, the mutant is capable of inducing a signal through TLR2 (36). To examine whether signaling through these receptors is modified by truncation of LOS or absence of capsule, we inoculated transfected HeLa cells with mutant *N. meningitidis* strains that had been fixed with paraformaldehyde to give a final concentration of 3.3×10^7 CFU/ml and 10-fold serial dilutions down from this. Signaling through TLR2 was examined by introduction of a TLR2 expression vector and, after incubation with potential agonists for 6 h, subsequent induction of the IL-8 promoter was measured. Signaling through TLR4 was examined by introducing an MD2-expressing vector (this MD2 combines with the endogenous TLR4 expressed by the cells to produce a functional receptor), and induction of the IL-8 promoter was measured as for TLR2. The results were all compared to those obtained with empty-vector-transfected cells, which served as a control for activation of NFκB by other pathways. As shown in Fig. 2, truncation of the LOS (Fig. 2A), absence of capsule (Fig. 2B), or absence of capsule together with LOS truncation (Fig. 2C) did not affect signaling through TLR4/MD2 ($P > 0.05$ [Kruskal-Wallis test]). Similarly, signaling through TLR2 was also not affected by truncation of the LOS (Fig. 3A), absence of capsule (Fig. 3B), or absence of capsule together with LOS truncation (Fig. 3C) ($P > 0.05$ [Kruskal-Wallis test]). While these data support the lack of modulation of the cytokine response of THP-1 cells, it is not clear if TLR2 and TLR4/MD2 are the only receptors involved in this interaction.

Our previous work showed that culture filtrates prepared from the LOS-deficient mutant were not able to initiate signaling through TLR2 or TLR4/MD2 (36); therefore, culture filtrates of the B1940 wild-type strain and the isogenic *galE*, *siaD*, and *cps* mutant strains were also examined for the ability to signal through TLR2 and TLR4/MD2. Culture filtrates from these mutants were all capable of initiating signaling through TLR2 and TLR4/MD2, and the magnitude of the signal was not affected by LOS truncation, absence of capsule, or absence of capsule together with LOS truncation (data not shown; $P > 0.05$ [Kruskal-Wallis test]).

Strain-to-strain variation in proinflammatory cytokine responses elicited by neisserial LOS. We next examined whether there was strain-to-strain variation in the proinflammatory cytokine response of THP-1 cells (as measured by release of TNF) to purified LOS from different strains of *Neisseria*, including both *N. meningitidis* and *N. gonorrhoeae*. As shown in Fig. 4, the same concentration of LOS extracted from different strains induced the production of various levels of TNF from THP-1 cells. Within the LOS from *Neisseria*, the highest TNF release was seen in response to meningococcal LOS, but wide variation was observed when meningococcal and gonococcal LOS from different strains were used. The greatest production of TNF overall was elicited by LPS isolated from *E. coli*, and this was much more potent than the LPS from the deep rough
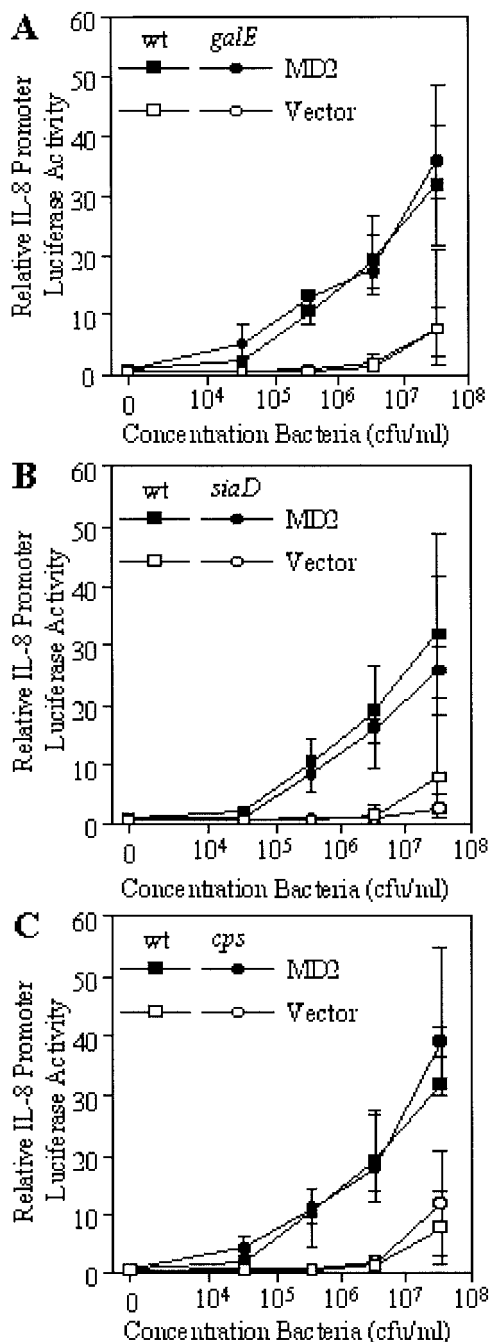

FIG. 2. Response of MD2-transfected HeLa cells to paraformaldehyde-fixed *N. meningitidis* as measured by induction of the IL-8 promoter. Cells were challenged with *N. meningitidis* mutants with truncated LOS (*galE*) (A), without capsule (*siaD*) (B), or without capsule together with truncated LOS (*cps*) (C), and the results were compared with the response of the wild-type (wt) strain. The median and interquartile range of IL-8 promoter induction of transfected HeLa cells are shown ($n = 6$). $P > 0.05$ (Kruskal-Wallis test).

LPS mutant *S. enterica* serovar Minnesota Re595, which lacks the O antigen, the outer core, and part of the inner core. This is consistent with other studies that have shown that the proinflammatory cytokine response of THP-1 cells to *E. coli* LPS is at least 20-fold higher than the response to Re595 LPS (46).
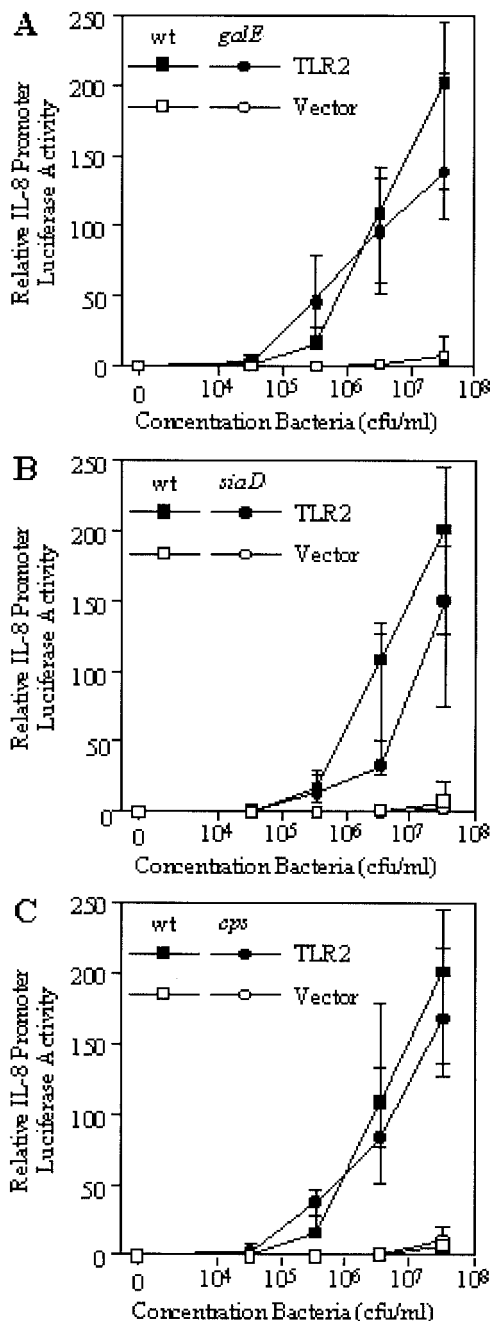
FIG. 3. Response of TLR2-transfected HeLa cells to fixed *N. meningitidis* as measured by induction of the IL-8 promoter. Cells were challenged with *N. meningitidis* mutants with truncated LOS (*galE*) (A), without capsule (*siaD*) (B), or without capsule together with truncated LOS (*cps*) (C), and the results were compared with the response of the wild-type (wt) strain. The median and interquartile range of IL-8 promoter induction of transfected HeLa cells are shown (n = 6). P > 0.05 (Kruskal-Wallis test).
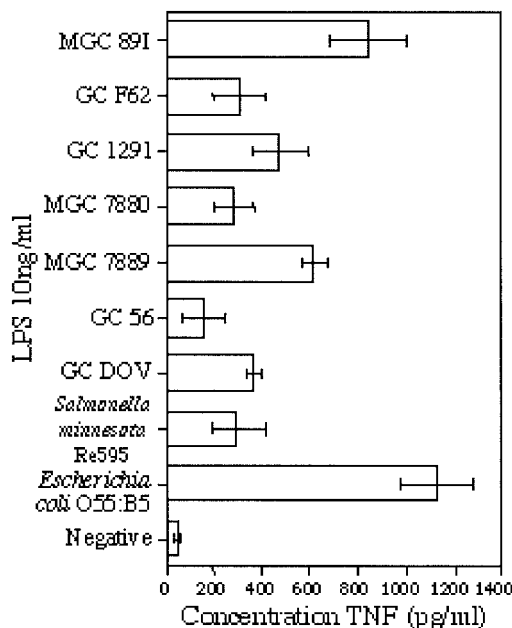

FIG. 4. Proinflammatory cytokine (TNF) production by THP-1 cells in response to LOS from *Neisseria*. Cells were challenged with 200 ng of LOS isolated from *N. meningitidis*, *N. gonorrhoeae*, *E. coli*, or *S. enterica* serovar Minnesota Re595 for 6 h, after which TNF concentrations in the supernatants were determined by ELISA. The neisserial LOS are ranked in order of decreasing oligosaccharide chain length (where known). Each bar represents the mean of quadruplicate data points ± the standard deviation.

Correlation of TLR4/MD2 responsiveness and proinflammatory cytokine release following stimulation of cells by LOS derived from different strains of *Neisseria*. To examine whether differences in proinflammatory cytokine release by THP-1 cells in response to these LOS was mirrored by differences in TLR4/MD2 activation, HeLa cells transfected with an MD2-express-ing vector (or an empty vector as a control) were inoculated with a fixed concentration of LOS from each strain (10 ng/ml). The TLR2-stimulating activity of purified LOS was not examined, as our previous work indicated that neisserial LOS signals through TLR4/MD2 (36). The data presented here (Fig. 5) show that LOS from different strains of *Neisseria* all induced signaling through TLR4/MD2, but there were significant differences in the level of the signal generated by the different strains (P < 0.01 [Kruskal-Wallis test]). As was shown with the production of TNF from THP-1 cells, the activities of the meningococcal and gonococcal LOS overlap. When the level of production of TNF by THP-1 cells was compared with the level of signaling through TLR4/MD2 for the LOS from each strain (Fig. 6), a significant positive correlation between these two values was seen (Spearman's rank correlation coefficient ρ = 0.867; P < 0.01).

Two gonococcal LOS with distinct activities were examined over a wide range of doses for the ability to stimulate signaling through TLR4/MD2. We observed that the LOS with the highest activity at a single dose of 10 ng/ml (GC 1291) stimulated higher levels of signaling across the whole dose range than did the LOS with the lowest activity (GC 56) (Fig. 7). The GC 1291 LOS also reached a plateau of signaling activity at a concentration of 100 ng/ml, whereas the GC 56 LOS still only induced minimal signaling at 1 μg/ml.

DISCUSSION

Our previous work (36), and that of others (19), has shown that TLR4/MD2 signaling by meningococci is dependent on
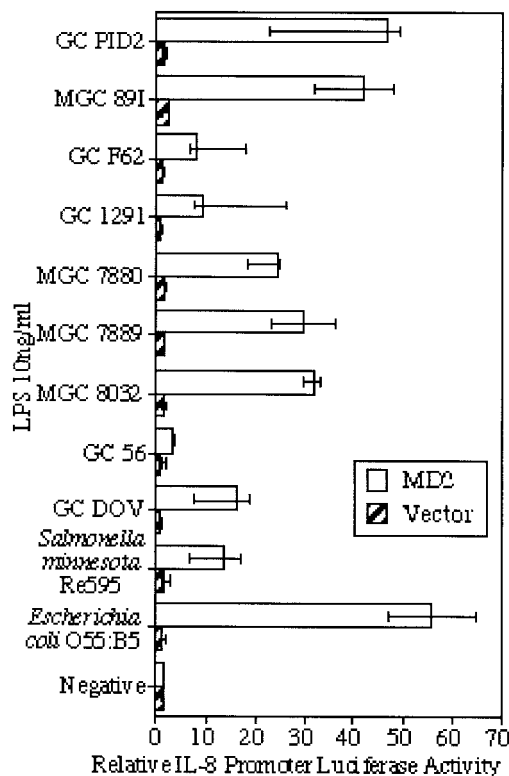
FIG. 5. Response of MD2-transfected HeLa cells to LOS from *Neisseria* as measured by induction of the IL-8 promoter. Cells were challenged at 10 ng/ml with LOS isolated from *N. meningitidis*, *N. gonorrhoeae*, *E. coli*, or *S. enterica* serovar Minnesota Re595, and the response of MD2-transfected cells was compared with the response of empty-vector-transfected cells. The neisserial LOS are ranked in order of decreasing oligosaccharide chain length (where known). The data represent the median and interquartile range of transfected HeLa cells ($n = 3$). Significant variation between strains was found ($P < 0.01$ [Kruskal-Wallis test]).
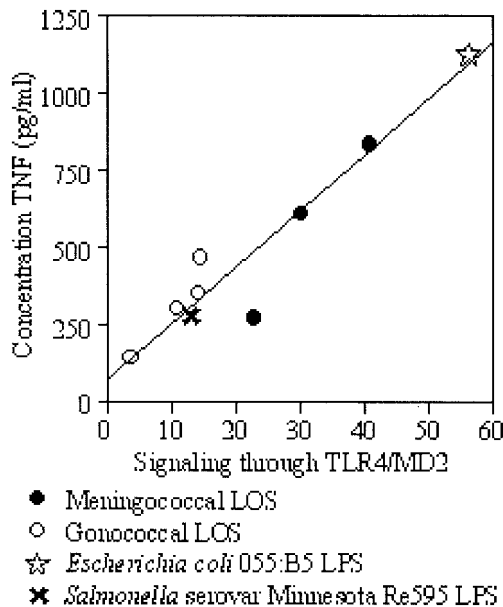

FIG. 6. Correlation of signaling through TLR4/MD2 and production of TNF from THP-1 cells in response to LOS from different strains of *Neisseria*. The data used for this analysis derive from the TNF concentrations in Fig. 4 and the relative luciferase activities in Fig. 5. Spearman's rank correlation coefficient $\rho = 0.867$ ($P < 0.01$).

the presence of LOS but that organisms lacking LOS are still able to initiate activation of NFκB via TLR2, presumably because of the presence of other agonists, such as PorB and lipoproteins (32). The results of the present study show that meningococci stimulate a proinflammatory TNF cytokine response and engage both TLR4/MD2 and TLR2 independently of the presence of a full-length LOS or the presence of the sialic acid capsule. A recent report shows that the TLR2 response to meningococci is mediated, at least in part, by the meningococcal porin PorB (32). This may explain the lack of difference in TLR2 signaling induced by the mutants used in this study, as no detectable differences in surface components between these mutant strains have been described (16). Our findings are supported by a report that suggests that cytokine induction in dendritic cells, although dependent on the presence of LOS, is not influenced by encapsulation (28).

Our observation that major alterations to the meningococcal surface do not alter TLR2 or TLR4/MD2 signaling is somewhat surprising. The mutants that we have used have been shown to be more potent activators of endothelial cells and neutrophils than the parent organism, as assessed through changes in adhesion molecule expression (6, 26). In the case of

the mutant with a truncated LOS structure, this upregulation is reversible by recombinant bactericidal–permeability-increasing protein, which has antiendotoxin properties, suggesting that adhesion molecule upregulation is mediated at least partly by LOS (6). Adhesion molecule expression is transcriptionally regulated by NFκB and other transcription factors, including ATF-2 (37). Although these signals can also be transduced via cytokine receptors (5), it may be considered that TLR4 activation by LOS would be a potent stimulatory signal leading to adhesion molecule changes. However, our data suggest that
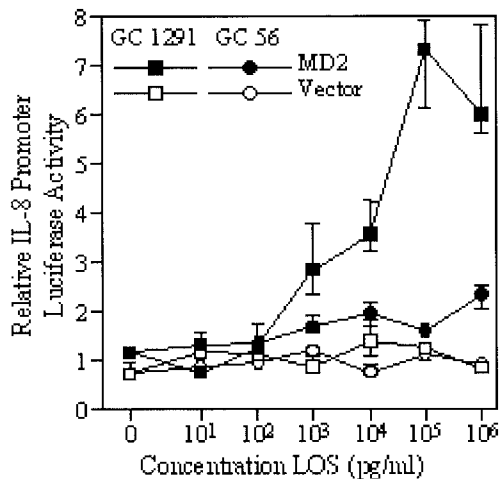

FIG. 7. Dose-related response of MD2-transfected HeLa cells to LOS from two strains of *N. gonorrhoeae*. IL-8 promoter induction in transfected HeLa cells stimulated by a range of concentrations of the two LOS is shown. The data points represent the median and interquartile range of three pools of transfected HeLa cells.

such changes are unlikely to result from modulation of TLR4/MD2- or TLR2-mediated activation of NFκB.

In the absence of an effect of LOS truncation on TLR4/MD2 signaling in the context of the whole organism, we investigated the inflammatory responses to purified LOS from a panel of neisserial strains. In contrast to the lack of an effect of LOS truncation, we found that LOS extracted from different meningococcal and gonococcal strains exhibited differential stimulatory activity in terms of both TNF production and signaling through TLR4/MD2. TNF production was significantly correlated with signaling through TLR4/MD2, indicating that the induction of a proinflammatory response by LOS is mediated primarily through TLR4/MD2 and that the magnitude of the proinflammatory response induced by a LOS is dependent on the magnitude of the signal generated through TLR4/MD2. The observed differences in proinflammatory response and TLR4/MD2 signaling did not appear to be related to oligosaccharide structure. A wide variation in these responses was induced by the LOS from strains GC 1291, GC F62, and MGC 89I, all of which express lacto-*N*-neotetraose α chains and add phosphoethanolamine (PEA) to the diheptose core of the oligosaccharide (Table 1). In general, the presence of terminal lacto-*N*-neotetraose or addition of PEA to the heptose of the inner core did not correlate with an increased or decreased response, as LOS in possession of these moieties covered the spectrum of observed activities. This contrasts with other data showing an increase in proinflammatory cytokines associated with the presence of PEA (4). There was also no pattern in TLR4/MD2 signaling or TNF production by THP-1 cells in response to increasing length of the LOS (Fig. 4 and 5, LOS are ordered in decreasing oligosaccharide chain length). Taken with the data in response to LOS truncation in intact organisms, we suggest that variation in the oligosaccharide portion of neisserial LOS is not responsible for modulation of endotoxic activity.

Although it is recognized that LPS from different species of bacteria differ in endotoxic activity (8), it is somewhat surprising that LOS from isolates of a single species should elicit such a broad spectrum of activity. However, a similarly wide range of abilities of neisserial LOS to clot LAL has been observed (40). These differences were shown to be associated with the lipid A portion of the LOS rather than the oligosaccharide components, as purified lipid A showed a potency similar to that of the parent LOS (40). It is therefore likely that the variation we see in the TNF response of THP-1 cells and in TLR4/MD2 signaling induced by LOS from different *Neisseria* strains is due to differences in the lipid A portion of the molecule. There have been many reported alterations of lipid A structure leading to modulation of its endotoxic activity as measured by various assays (4, 9, 43, 49). It has been shown that the acylation pattern of lipid A is an important determinant of endotoxin activity. In neisseriae, this has been demonstrated by inactivation of genes involved in lipid A acylation (*lpxL1* and *lpxL2*), resulting in lipid A that is penta-acylated (*lpxL1*) or tetra-acylated (*lpxL2*) rather than hexa-acylated; in both cases, the LOS induced less TNF from a human macrophage cell line (49). Also, neisserial LOS deacylated by the leukocyte enzyme acyloxyacyl hydrolase was less potent in the LAL assay, in the ability to stimulate neutrophil adherence to endothelial cells, and in mitogenic ability for splenocytes (9).

Another modification of lipid A that may play a role in altering endotoxic activity is that of fatty acid chain length. Replacement of the neisserial *lpxA* gene with that from other gram-negative bacteria results in lipid A with shorter (*lpxA* from *Pseudomonas aeruginosa*) or longer (*lpxA* from *E. coli*) O-linked 3-OH fatty acids, both of which show reduced endotoxic activity (43).

Modification of lipid A has been shown to occur during the growth of bacteria. For example, the growth temperature of *Yersinia pestis* alters the lipid A acylation pattern, with growth at a higher temperature (37°C compared to 27°C) reducing the amount of acylation and reducing the potency of the LPS (24). *S. enterica* serovar Typhimurium has also been shown to regulate lipid A acylation in response to Mg^{2+}-deficient conditions (indicative of mammalian infection) (12), and this increases resistance to innate defenses (11, 13). *P. aeruginosa* isolated from the airways of cystic fibrosis (CF) patients was shown to be hexa-acylated with additional palmitate and aminoaribinose, compared to penta-acylated lipid A from other clinical isolates and laboratory strains (7). The LPS from CF airway isolates stimulated a more potent proinflammatory response from human endothelial cells (7) and also human TLR4 signals to a greater extent in response to the hexa-acylated CF isolate LPS (14). It is possible that modification of lipid A during infection by bacteria, as a result of environmental conditions, may result in modification of the stimulation of TLR4 and therefore the production of proinflammatory cytokines that could influence the progression or severity of disease. Indeed, a recent publication suggests that some immunotypes of *N. meningitidis* that have LOS modifications that induce a greater proinflammatory response are also associated with more severe disease (4).

In conclusion, by using an isogenic mutant system, we have found that truncation of the oligosaccharide does not influence the ability of meningococcal LOS to signal via TLR4/MD2 and induce TNF production. Furthermore, removal of the bacterial capsule also does not influence such activation. We suggest that the differences in proinflammatory signals initiated by these mutants, as observed by others (6, 26), must occur via other signaling pathways. By using purified LOS, we have observed major differences in the proinflammatory signal elicited by different *Neisseria* strains, and these did not appear to be related to the structure of the oligosaccharide. By inference from published differences in the activity of neisserial LOS in LAL assays due to differing lipid A, we conclude that natural strain variation in lipid A structure is likely to be responsible for the differences in TLR4/MD2 signaling and cytokine output that we observed. We are currently examining the structure of the LOS from the neisserial strains used in this study to determine the role of structural differences in the observed range of signaling through TLR4 and correlate induction of proinflammatory cytokines. Our observations highlight the complexity of the human inflammatory response to *Neisseria*.

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