Rhizobium Sin-1 Lipopolysaccharide (LPS) Prevents Enteric LPS-induced Cytokine Production*

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Endotoxin (lipopolysaccharide (LPS)), a component of Gram-negative bacteria, is among the most potent proinflammatory substances known. The lipid-A region of this molecule initiates the production of multiple host-derived inflammatory mediators, including cytokines (e.g. tumor necrosis factor-α (TNFα)). It has been a continuous effort to identify methods of interfering with the interaction between enteric LPS and inflammatory cells using natural and synthetic LPS analogs. Some of these LPS analogs (e.g. Rhodobacter spheroides LPS/lipid-A derivatives) are antagonists in human cells but act as potent agonists with cells of other species. Data reported here indicate that structurally novel LPS from symbiotic, nitrogen-fixing bacteria found in association with the root nodules of legumes do not stimulate human monocytes to produce TNFα. Furthermore, LPS from one of these symbiotic bacterial species, Rhizobium sp. Sin-1, significantly inhibits the synthesis of TNFα by human cells incubated with Escherichia coli LPS. Rhizobium Sin-1 LPS exerts these effects by competing with E. coli LPS for binding to LPS-binding protein and by directly competing with E. coli LPS for binding to human monocytes. Rhizobial lipid-A differs significantly from previously characterized lipid-A analogs in phosphate content, fatty acid acylation patterns, and carbohydrate backbone. These structural differences define the rhizobial lipid-A compounds as a potentially novel class of LPS antagonists that might well serve as therapeutic agents for the treatment of Gram-negative sepsis.

Based on recent estimates, circulatory shock resulting from microbial sepsis accounts for at least 100,000 human deaths annually in the United States (1, 2). The development of circulatory shock is often linked to a systemic inflammatory response to endotoxin (lipopolysaccharide; LPS)1 in the blood of affected patients, strongly implicating endotoxemia as a critical factor in pathogenesis. LPS, a component of Gram-negative bacteria, is among the most potent proinflammatory substances known, with its lipid-A region initiating the production of multiple host-derived inflammatory mediators (3, 4). LPS causes these effects after binding to CD14 on mononuclear phagocytes or to soluble CD14 and then to cells lacking CD14 (5–8). Recent evidence (9–11) indicates that cell signaling events are initiated after an interaction among LPS, CD14, Toll-like receptor 4, and the Toll-like receptor 4-associated protein, MD-2. A plasma protein termed LPS-binding protein (LBP) facilitates the CD14-dependent interactions by transferring LPS monomers from LPS aggregates to CD14 expressed on the surface of mononuclear cells (5, 12, 13). CD14-independent pathways for cellular activation by high concentrations of LPS may involve direct interaction with the Toll-like receptors, other less well characterized receptors, or intracellular effectors such as Nod1 and -2 (14–16). Considerable efforts have been expended to identify methods to interfere with the interaction between LPS and inflammatory cells using a limited number of natural and/or synthetic LPS analogs (17–21). These analogs have agonistic and/or antagonistic properties in LPS-responsive cells, depending upon the species. In addition, some of these analogs are purported to have limited shelf lives (e.g. R. spheroides LPS/lipid-A derivatives) (17, 22). Having identified a structurally novel LPS from nitrogen-fixing bacteria, we were interested in determining the effects of these novel rhizobial LPS molecules on human mononcytic cells, and whether individual rhizobial LPS might antagonize enteric LPS-induced synthesis of TNFα by these cells (23, 24). In particular, the lipid-A of Rhizobium Sin-1 differs significantly from other well characterized lipid-A analogs in its unique carbohydrate backbone, fatty acid acylation patterns, and the lack of phosphate (39). These structural differences define Rhizobium Sin-1 lipid-A as a potentially novel LPS antagonist that might serve as a therapeutic agent for the prevention of circulatory shock due to Gram-negative sepsis.

EXPERIMENTAL PROCEDURES

Materials—Escherichia coli O55:B5 LPS and 1H- and unlabeled E. coli LDE25 LPS were purchased from List Biologicals (Campbell, CA). Alexa Fluor 488-conjugated E. coli O55:B5 LPS was purchased from Molecular Probes (Eugene, OR). Anti-CD14 monoclonal antibody, MY4, was obtained from Beckman Coulter (Miami, FL), and another monoclonal anti-CD14 antibody, 60bca, was generously provided by Dr. Philip Boschler, University of Tennessee. Tissue culture media, antibiotics, and endotoxin-free fetal calf serum were purchased from BioWhittaker (Walkerville, MD), and OptEIA human TNFα ELISA kits were from Pharmingen. Recombinant CD14 and LPS-binding protein (LBP) were obtained from R & D Systems (Minneapolis, MN). Hbt Endoblock LBP assay kits were obtained through Cell Sciences (Norwood, MA). All other reagents and chemicals were obtained from Sigma and were of highest analytical grade available.

Purification of Rhizobial Lipopolysaccharides—To purify LPS from...
the rhizobial bacteria (Rhizobium galegae, Rhizobium etli CE3, and Rhizobium Sin-1), the bacteria were pelleted by centrifugation and extracted with hot phenol, and the aqueous phase was dialyzed extensively against water. The LPS preparations were purified further by Sepharose 6B-CL column chromatography followed by affinity chromatography over polymyxin B-agarose (24). Fractions were assayed for 3-deoxy-o-manno-2-octulosonic acid and heziose by thiobarbituric acid and anthrone assays, respectively (25). Fractions containing purified rhizobial LPS were pooled, dialyzed against water, and stored lyophilized. The purity of the LPS was determined by deoxycholate-PAGE analysis and silver staining, as well as by glycosyl and fatty acid residue analysis as described previously (39).

Cell Culture Techniques—Mono Mac 6 cells, a human monocyte cell line, were kindly provided by Dr. H. W. L. Ziegler-Heitbrock (University of Munich, Germany) (26, 27). The cells were cultured in RPMI 1640 medium supplemented with 2 g/liter NaHCO3, 2 mM L-glutamine, 200 units/ml penicillin, 200 μg/ml streptomycin, non-essential amino acids (product number 043-01140 H; Invitrogen), 1% OPI supplement (containing oxalacetic acid, sodium pyruvate, and insulin), and 10% fetal calf serum and were maintained in a humid 5% CO2 atmosphere at 37 °C. New batches of frozen cell stock were grown every 2 months, and growth morphology was checked. Two days prior to each experiment the cells were treated with 10 ng/ml calciotin to up-regulate CD14 expression.

TNFα secretion by Mono Mac 6 cells was assayed by culturing duplicate aliquots of cells (1 × 106/ml) for 6 h in a humid 5% CO2 atmosphere at 37 °C in the presence or absence of LPS from either E. coli, the rhizobial species, or both. Thereafter, cell supernatants containing secreted TNFα were harvested by centrifugation and stored at −70 °C until analysis. Cells treated with LPS diluent alone served as negative controls.

Parental Chinese hamster ovary (CHO) cells (CD14−) or transfected CHO cells expressing human CD14 on their surface (CD14+) were kindly provided by Dr. P. Tobias (Scripps Research Institute, La Jolla, CA). The cells were routinely cultured at 37 °C in Ham’s F-12 nutrient media supplemented with 10% fetal calf serum, 100 units/ml penicillin, 100 units/ml streptomycin, and 2 mM L-glutamine.

A commercial TNFα ELISA kit was used to measure TNFα antigen in the supernatants from the Mono Mac 6 cells. In selected experiments, a WEHI TNFα biosassay, utilizing WEHI 164 clone G cells, performed as described previously (28, 29), was used to confirm TNFα bioactivity in the supernatants.

Competitive Binding Assays—Binding assays were performed according to the method described by Kitchens and Munford (30). Briefly, Mono Mac 6 cells were preincubated in 20 mM HEPES (pH 7.4), 150 mM NaCl, 1 mM EDTA, 300 μg/ml bovine serum albumin, 2 mM NaF, 5 mM deoxyglucose, 10 mM NaN3, SEDBEE buffer (prevent lipid internalization). Trinitiated E. coli LPS (30 μg/ml final concentration) was mixed with increasing concentrations of either unlabeled E. coli or Rhizobium Sin-1 LPS in the presence of 10% fetal calf serum as a source of LBP prior to addition to the cells. These mixtures were then incubated with the cells at 37 °C for 30 min. The cells were harvested by centrifugation, washed with ice-cold SEDBEE buffer, solubilized in scintillation mixture, and cell-bound [3H]LPS was quantified by liquid scintillation counting. In preliminary experiments, pretreatment of Mono Mac 6 cells with the anti-CD14 neutralizing antibody, 60bca (1.4 μl aliquots in 380 μl of PBS, pH 7.4), for 30 min reduced binding of the trinitiated E. coli LPS by >85% (data not shown).

Flow Cytometry—Binding assays were performed with CD14-transfected CHO cells expressing high levels of human CD14 (31). Cells were released from the flasks with trypsin, washed twice, and incubated for 30 min at 37 °C. SEDBEE buffer. Cell viability and number were assayed by trypan blue exclusion. Ligands (Alexa Fluor E. coli LPS and unlabeled E. coli or Rhizobium Sin-1 LPS) were incubated for 15 min at 37 °C in fetal calf serum to form LPS-LBP complexes prior to addition to the cells. Complexes were incubated for 1 h at 37 °C with 2 × 106 cells suspended in SEDBEE buffer. Thereafter, the cells were washed three times with SEDBEE buffer, fixed on ice, and analyzed on a Beckman Coulter Epics XL flow cytometer. Expression of CD14 by cells was monitored with fluorescein isothiocyanate-conjugated anti-CD14 antibody, MY-4.

LPS-binding Protein Assay—To assess binding of LPS to LBP, we used a commercially available LBP competitive binding ELISA assay. This ELISA is that described by Scott et al. (32) to demonstrate competition between polymyxin B or cationic peptides and LPS for binding to LBP. Briefly, biotinylated E. coli LPS was mixed with increasing concentrations of either E. coli or Rhizobium Sin-1 LPS. These complexes were then incubated with antibody-immobilized LBP in wells of a microtiter plate. Wells were washed, and binding of the biotinylated E. coli LPS to the LBP was detected colorimetrically at A450 with streptavidin-peroxidase conjugate and tetramethylbenzidine as substrate.

Native PAGE Mobility Shift Assays—To demonstrate competitive binding of unlabeled Rhizobium Sin-1 or E. coli LPS to purified recombinant CD14, we used native PAGE mobility shift assays as described by Hailman et al. (33). Briefly, 1 μg/ml [3H]E.coli LPS was incubated in the presence or absence of either 250 ng/ml unlabeled E. coli LPS or Rhizobium Sin-1 LPS in Mg2+/Ca2+-free phosphate-buffered saline containing 1 mM EDTA. Thereafter, 1 μg of recombinant CD14 was added to the tubes and incubated for 2 h at 37 °C. Complexes were resolved by 4–20% native PAGE at 150 V for ~2 h. The gels were fixed in 30% methanol, 10% acetic acid, soaked in Amplify (Amersham Biosciences) for 30 min, and exposed to Kodak XAR film for 2 days.

Autoradiographs were scanned densitometrically, and changes in the binding of the radiolabel to the CD14 was reported as arbitrary values. Inclusion of LBP during the incubations did not significantly alter the binding of the radiolabel to CD14 or the competition of either unlabeled LPS for binding to CD14 during these assays (data not shown). As a specificity control, 100 μg/ml bovine serum albumin was incubated with the [3H]E.coli LPS; no mobility shift of the radiolabel was evident.

Statistical Analysis—All values are listed as means ± S.E. (S.E.). Analyses were performed with GraphPad Prism™ software. The data were analyzed with analysis of variance with Bonferroni’s post hoc test. Significance was set at p < 0.05.

RESULTS

Comparative Effects of Rhizobial and E. coli LPS on TNFα Secretion—Incubation of Mono Mac 6 cells with LPS at concentrations of 10 and 100 ng/ml resulted in significant secretion of TNFα only in response to E. coli LPS. LPS from Rhizobium Sin-1 and R. galegae did not induce TNFα secretion, whereas LPS from R. etli CE3 was a weak agonist (Fig. 1). Concentrations of Rhizobium Sin-1 LPS up to 1 μg/ml failed to induce TNFα secretion by the Mono Mac 6 cells (data not shown).

Rhizobium Sin-1 LPS Reduces E. coli LPS-induced TNFα Secretion by Mono Mac 6 Cells—Based on the lack of response to Rhizobium Sin-1 LPS, its potential to act as an antagonist of E. coli LPS-induced TNFα secretion was explored. To this end, E. coli LPS concentration-response curves were determined in the absence and presence of three increasing concentrations of Rhizobium Sin-1 LPS (100, 200, or 300 ng/ml; Fig. 2A). In the absence of Rhizobium Sin-1 LPS, E. coli LPS produced a concentration-dependent increase in TNFα synthesis, which plateaued at concentrations exceeding 32 ng/ml. Preincubation with Rhizobium Sin-1 LPS produced parallel shifts of the E. coli LPS-response curve to “the right.” The latter findings indicate that Rhizobium Sin-1 LPS antagonized the E. coli LPS-induced stimulation of the cells. Further analysis of these data indicated that the EC50 (concentration of E. coli LPS required to induce 50% maximal TNFα synthesis) increased 3.9-, 9.7-, and 10.1-fold at the three concentrations of Rhizo-
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**bium Sin-1 LPS (E. coli alone, 3.1 ng/ml; E. coli /H11001 Rhizobium Sin-1 100 and 12.1 ng/ml; E. coli /H11001 Rhizobium Sin-1 200 and 30.1 ng/ml; E. coli /H11001 Rhizobium Sin-1 300 and 31.5 ng/ml).**

Schild regression analysis (log (dose ratio /H11002 1) versus log (Rhizobium Sin-1 LPS)) of these data (Fig. 2B) yielded a slope of 1, indicating that Rhizobium Sin-1 LPS is a competitive inhibitor of E. coli LPS. Furthermore, the x-intercept reveals an apparent dissociation constant of ~40 ng/ml for Rhizobium Sin-1 LPS.

**Rhizobium Sin-1 LPS Prevents Binding of [3H]E. coli LPS to Mono Mac 6 and CD14 CHO Cells**—The results of competitive binding assays performed with either unlabeled E. coli LPS or Rhizobium Sin-1 LPS in Mono Mac 6 cells (Fig. 3A) demonstrate that both Rhizobium Sin-1 LPS and E. coli LPS compete with the [3H]E. coli LPS-binding site on Mono Mac 6 cells. Although Rhizobium Sin-1 LPS was somewhat less potent than E. coli LPS, increasing concentrations provided complete displacement of [3H]E. coli LPS-specific binding, indicating that binding of the two LPS species is mutually exclusive.

The results of flow cytometric assays (Fig. 3B) with CHO cells expressing CD14 indicate that unlabeled Rhizobium Sin-1 LPS, unlabeled E. coli LPS, and MY4 prevented binding of fluorescently labeled E. coli LPS; fluorescently labeled E. coli LPS did not bind to CHO cells not expressing CD14 (data not shown). The fact that MY4 is a CD14-specific monoclonal antibody supports the conclusion that E. coli LPS is binding to these CD14 CHO cells via CD14 and that inhibition of this binding by Rhizobium Sin-1 LPS occurs by preventing the binding of E. coli LPS to CD14.

**Rhizobium Sin-1 LPS Competes for Binding to Purified CD14**—To determine whether Rhizobium Sin-1 LPS competes with E. coli LPS at the level of CD14, we used native PAGE mobility shift assays with Rhizobium Sin-1 LPS, tritiated E. coli LPS, and purified CD14. Incubation of tritiated E. coli LPS with CD14 caused an increased mobility of the radiolabel on native polyacrylamide gels through the formation of an LPS:CD14 complex (Fig. 4). Inclusion of a 250-fold excess of either
unlabeled *E. coli* LCD25 or *Rhizobium* Sin-1 LPS during the incubation decreased binding of [3H]LPS to purified CD14. Native PAGE mobility shift assays were used to demonstrate that both unlabeled *E. coli* and *Rhizobium* Sin-1 LPS compete for binding of [3H]LPS to recombinant CD14. Briefly, radiolabeled *E. coli* LPS was incubated for 2 h with recombinant CD14 in the presence or absence of a 250-fold excess unlabeled *E. coli* or *Rhizobium* Sin-1 LPS. In lanes 5–7 purified LBP was included during this incubation, and bovine serum albumin (BSA, lane 8) was used to demonstrate the specificity of binding. Complexes formed were resolved by 4–20% native PAGE before the dried gel was exposed to x-ray film. Autoradiographs were scanned, and relative densitometry values (RDV) were recorded (n = 2).

**Fig. 4.** Unlabeled *Rhizobium* Sin-1 and *E. coli* LPS compete in vitro for binding of tritiated *E. coli* LPS to purified CD14. Native PAGE mobility shift assays were used to demonstrate that both unlabeled *E. coli* and *Rhizobium* Sin-1 LPS compete for binding of [3H]LPS to recombinant CD14. Briefly, radiolabeled *E. coli* LPS was incubated for 2 h with recombinant CD14 in the presence or absence of a 250-fold excess unlabeled *E. coli* or *Rhizobium* Sin-1 LPS. In lanes 5–7 purified LBP was included during this incubation, and bovine serum albumin (BSA, lane 8) was used to demonstrate the specificity of binding. Complexes formed were resolved by 4–20% native PAGE before the dried gel was exposed to x-ray film. Autoradiographs were scanned, and relative densitometry values (RDV) were recorded (n = 2).

**Fig. 5.** *Rhizobium* Sin-1 LPS competes more avidly with biotinylated *E. coli* LPS for binding to immobilized LPS-binding protein than does unlabeled *E. coli* LPS. Biotinylated *E. coli* LPS was mixed with increasing concentrations of either unlabeled *Rhizobium* Sin-1 (A) or *E. coli* (B) LPS and then incubated with antibody-immobilized LBP. Unbound LPS was removed by extensive washing and binding of the bound biotinylated LPS detected colorimetrically with streptavidin horseradish peroxidase (n = 4).

**Fig. 6.** The various lipid-A structures of the rhizobial LPSs. Shown are the four major lipid-A structures that are likely present in *R. etli* CE3 (37, 38) (A) and in *Rhizobium* sp. Sin-1 LPS (39) (B). For both *R. etli* CE3 and *Rhizobium* Sin-1, the structures shown are those that are likely to be present in the intact LPS. Additional structures are present in lipid-A isolated by mild acid hydrolysis of the LPS that may be a result of the hydrolysis procedure (37–39). There are also variations on these structures due to the fact that the N-acyl substituents can be 3-OHC14:0, 3-OHC16:0, or 3-OHC18:0 (A) and that the proximal 2-aminoglucuronate residue may also be present as a 2-amino-1,5-gluconolactone (B). In the *R. etli* CE3 lipid-A the N-acyl residues are primarily β-OHC14:0, and in the *Rhizobium* Sin-1 lipid-A these residues are primarily β-OHC16:0.
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LPS (34.7 and 121.1 ng/ml, respectively, for Rhizobium Sin-1 LPS and E. coli LPS).

DISCUSSION

In this study, two hypotheses were tested, namely that structurally novel LPS from nitrogen-fixing rhizobial bacteria would not induce, or would only minimally induce, TNFα synthesis by human monocytes and that the rhizobial LPS inducing the least synthesis of TNFα would antagonize the pro-inflammatory effects of E. coli LPS. These hypotheses were based on the unusual lipid-A structures of the rhizobial LPSs and on the fact that alterations in the structural features of E. coli lipid-A (based on studies using LPS from other bacterial species or natural and synthetic lipid-A analogs (19, 35)) have been associated with reductions in the endotoxicity and with the acquisition of endotoxin antagonist activity.

The rhizobial LPSs were obtained from R. etli CE3, R. galegae, and Rhizobium Sin-1. The lipid-A portions of these three different LPSs have different, but closely related, structures. The structures of R. etli CE3 lipid-A have been reported (23, 37, 38) and are shown in Fig. 6A, whereas the structures of Rhizobium Sin-1 lipid-A are shown in Fig. 6B (39). The structures of R. galegae lipid-A are still under investigation. The LPSs from these rhizobial species were of interest due to the fact that their lipid-A structures proved to be very different from those of enteric bacteria, as well as from some other rhizobial species, in that they do not have a bis-phosphorylated glucosamine disaccharide backbone and have very different fatty acylation patterns. In addition, the R. etli CE3 lipid-A differs from that of Rhizobium sp. Sin-1 in several ways. First, the R. etli CE3 lipid-A carbohydrate backbone has a galacturonic acid residue at the 4'-position of the distal glucosamine. Second, a significant portion of the R. etli CE 3 lipid-A structures contain glucosamine as a proximal residue, i.e. it is not oxidized to 2-amino glucuronic acid. Third, the principle N-fatty acyl residue of R. etli CE3 lipid-A is β-OH14:0 rather than β-OH16:0. Fourth, a significant percentage of the Rhizobium Sin-1 lipid-A molecules lack a fatty acyl residue at the 3'-position. Fifth, the 27-OHC28:0 residue of the Rhizobium Sin-1 lipid-A is not acylated with β-hydroxybutyrate. Although the R. galegae lipid-A structure (not shown) has not yet been completed, it appears to differ from that of Rhizobium Sin-1 only in the possible presence of a glucosyl residue at the 4'-position.

The LPSs from the three rhizobial species all proved to have a greatly reduced capacity to induce TNFα. Both the R. galegae and Rhizobium Sin-1 LPSs were unable to induce TNFα synthesis even at mg/ml concentrations, whereas a 10 ng/ml concentration of E. coli LPS was a potent agonist of TNFα synthesis. However, the LPS from R. etli CE3 did induce TNFα synthesis at concentrations of 100 ng/ml and was, therefore, a weak agonist. This difference between the R. etli CE3 and Rhizobium Sin-1 (or R. galegae) activities is most likely due to one or more of the above-mentioned structural differences between the two different lipid-A. For example, it may be that the 4'-galacturonosyl of R. etli CE3 lipid-A provides a negative charge at that position (e.g. in place of phosphate), which results in the slight agonist activity. Because the purpose of this work was to pursue those lipid-A structures which minimize agonist activity and maximize antagonist activity, the structural basis for the difference between the R. etli CE3 and Rhizobium Sin-1 LPS activities was not investigated further. Thus, the LPS from Rhizobium Sin-1 was selected to perform the remaining experiments because it lacked agonist activity at all concentrations tested. The Rhizobium Sin-1 LPS preparation used contains a variety of lipid-A moieties with minor structural differences. The mixture of possible lipid-A structures in Rhizobium Sin-1 LPS are described in the companion paper (39) and shown in Fig. 6B.

In this series of studies, LPS from Rhizobium Sin-1 functioned as a potent antagonist of E. coli LPS in Mono Mac 6 cells. The data indicated that Rhizobium Sin-1 LPS acts as a competitive antagonist of E. coli LPS, with an apparent dissociation constant of ~40 ng/ml. When compared with the reported dissociation constant of ~30 ng/ml for E. coli LPS (36), the results of our study suggest that the affinities of Rhizobium Sin-1 and E. coli LPS for monocytic cells are similar. This competitive binding affinity is particularly interesting because the Rhizobium Sin-1 LPS preparation is a mixture of potentially eight or more molecules (see Fig. 6), and therefore, it is possible that only one or two of these structures is responsible for the antagonistic activity. If so, that structure(s) would have an even greater affinity than the observed 40 ng/ml value.

Further experiments were done to determine the mechanism by which Rhizobium Sin-1 LPS acts as an antagonist. As described in the Introduction, two initial proteins involved in the signal transduction pathway leading to the synthesis of TNFα are CD14 and LBP. It was shown that Rhizobium Sin-1 LPS prevents the binding of E. coli LPS to both CD14 and LBP. Interference with the binding of E. coli LPS to CD14 was shown by the fact that Rhizobium Sin-1 LPS competed with tritiated E. coli LPS for binding to Mono Mac 6 cells and prevented binding of fluorescently labeled E. coli LPS to transfected CHO cells expressing CD14. In addition, native PAGE assays showed that Rhizobium Sin-1 LPS significantly reduced the binding of tritiated E. coli LPS to purified CD14. It was next shown, using immobilized LBP, that Rhizobium Sin-1 LPS binds more tightly to LBP than E. coli LPS. These findings suggest that Rhizobium Sin-1 LPS may reduce the availability and delivery of E. coli LPS monomers to CD14 on mononuclear cells, as well as prevent the binding of E. coli LPS to CD14, thereby accounting for the reduction in E. coli LPS-induced synthesis of TNFα.

In conclusion, we have demonstrated that structurally unique LPS from rhizobial bacteria caused either no measurable, or only slight, induction of TNFα in human monocytes and that LPS from one of these rhizobial species, Rhizobium Sin-1, antagonizes the effects of E. coli LPS through at least two probable mechanisms. Rhizobium Sin-1 LPS avidly competes with E. coli LPS for binding to LBP, which is intimately involved in delivering LPS monomers to CD14 on the cell surface. Furthermore, Rhizobium Sin-1 LPS competes with E. coli LPS for binding sites on human mononuclear cells and CHO cells expressing CD14. Pharmacologic analysis of the concentrations of E. coli LPS required to induce TNFα synthesis in the presence of Rhizobium Sin-1 LPS indicate that Rhizobium Sin-1 is an effective competitive inhibitor of E. coli LPS.

In the companion paper (39), it is shown that the Rhizobium Sin-1 LPS used in this work contains eight or more different lipid-A structures (Fig. 6). These structures vary from one another in their fatty acylation pattern and as to whether or not the proximal glycosyl residue exists as 2-amino glucuronate or as 2-amino glucurono-1,5-lactone. Given the multiple dissimilarities between the rhizobial and enteric bacterial lipid-A structures, it is not possible to determine which specific structural feature(s) of the rhizobial LPS is/are most responsible for the results obtained in the present study. Therefore, studies with pure synthetic lipid-A analogs (synthesized by Dr. Geert-Jan Boons of the Complex Carbohydrate Research Center) are in progress to clearly define the structure/function relationship with regard to endotoxin antagonistic activity.
