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The Inflammatory Cytokine Response to *Chlamydia trachomatis* Infection Is Endotoxin Mediated

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Chlamydia trachomatis* is a major etiologic agent of sexually transmitted diseases. Although *C. trachomatis* is a gram-negative pathogen, chlamydial infections are not generally thought of as endotoxin-mediated diseases. A molecular characterization of the acute immune response to chlamydia, especially with regard to the role of its lipopolysaccharide (LPS), remains to be undertaken. We extracted 15 mg of LPS from 5×10^{12} *C. trachomatis* elementary bodies (EB) for analysis of structure and biological activity. When methylated lipid A was subjected to high-pressure liquid chromatography followed by mass spectrometry, the majority of the lipid A was found to be pentaacyl. The endotoxin activities of whole *C. trachomatis* EB and purified LPS were characterized in comparison with whole *Salmonella minnesota* R595 and with *S. minnesota* R595 LPS and lipooligosaccharide from *Neisseria gonorrhoeae*. Both *C. trachomatis* LPS and whole EB induced the release of tumor necrosis factor alpha from whole blood ex vivo, and *C. trachomatis* LPS was capable of inducing the translocation of nuclear factor κB in a Chinese hamster ovary fibroblast cell line transfected with the LPS receptor CD14. In both assays, however, *C. trachomatis* was ~ 100-fold less potent than *S. minnesota* and *N. gonorrhoeae*. The observation that *C. trachomatis* is a weak inducer of the inflammatory cytokine response correlates with the clinical observation that, unlike *N. gonorrhoeae* infection, genital tract infection with *C. trachomatis* is often asymptomatic. The ability of specific LPS antagonists to completely inhibit the tumor necrosis factor alpha-inducing activity of whole *C. trachomatis* EB suggests that the inflammatory cytokine response to chlamydia infection may be mediated primarily through LPS. This implies that the role of other surface protein antigens, at least in terms of eliciting the proinflammatory cytokine response, is likely to be minor.

Chlamydiae are obligate intracellular gram-negative bacteria. One of the recognized species, *Chlamydia trachomatis*, is a major sexually transmitted pathogen in the United States, with a yearly incidence of approximately 3 million (45). In addition, trachoma is the leading cause of preventable blindness in the world (8). Infection with *C. trachomatis* is usually followed by an inflammatory response involving phagocytes and lymphocytes that, in turn, release various cytokines (19, 31). In contrast to *Neisseria gonorrhoeae*, genital tract infection with *C. trachomatis* is often chronic and is associated with few symptoms and a scant inflammatory exudate. Infected men will frequently notice dysuria or urethral discharge, prompting them to seek medical attention. Infected women, however, may be entirely asymptomatic. This can lead to a chronic, low-grade infection that produces widespread upper tract disease, including pelvic inflammatory disease, tubal scarring, infertility, and ectopic pregnancy.

As in all gram-negative bacteria, the outermost monolayer of *C. trachomatis* is composed of lipopolysaccharide (LPS), a complex glycolipid essential for bacterial survival (11). LPS, or endotoxin, is a potent activator of the acute inflammatory response, inducing mononuclear phagocytes to produce various inflammatory cytokines, such as tumor necrosis factor alpha (TNF-α) and interleukin-1β (26, 36, 39). Despite this, most efforts at elucidating the pathophysiology of chlamydial infections have focused on two protein antigens. The first is the major outer membrane protein (MOMP) of *C. trachomatis*, which is almost certainly involved in the earliest interactions of this organism with leukocytes during the course of natural infection. MOMP, an immunodominant molecule (7), constitutes almost 60% of the outer membrane protein of *C. trachomatis* (6) and appears to evoke a protective humoral response to infection (16, 52, 53). A second protein produced by pathogenic strains of *C. trachomatis* is the 57- to 65-kDa heat shock protein (Hsp-60), which induces purulent conjunctivitis in a guinea pig model of trachoma (27). Unfortunately, preparations of Hsp-60 and MOMP purified from bacteria are likely to include significant amounts of contaminating LPS, further complicating efforts to delineate the roles of these three unique antigens. The current study was predicated upon the belief that the relative contributions of the two proteins to the inflammatory responses to chlamydia infection cannot be defined without an understanding of the biological activity of *C. trachomatis* LPS (CT LPS).

Although LPS preparations from enteric gram-negative aerobic bacilli uniformly stimulate neutrophils (24) and monocytes (13), preparations from nonenteric organisms have been reported to have little endotoxin activity. Moreover, some "endotoxin" preparations, like *Bacteroides fragilis* LPS and lipid A from *Rhodobacter sphaeroides* (RSLA), can act as inhibitors of enterically derived (e.g., *Escherichia coli* or *Pseudomonas*) LPS (13, 17, 24, 42). Several investigators have reported that whole chlamydial elementary bodies (EB) and LPS preparations are capable of stimulating mononuclear phagocytes (25, 41, 48, 49), but these studies have been limited in scope. No assess-

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ment of LPS purity was made, and qualitative comparisons with well-characterized endotoxin preparations, such as *Salmonella* LPS, have not been reported. As a result, little is known about the inflammatory potential of *C. trachomatis* LPS and the role it may play during infection.

Chlamydial LPS is chemically similar to rough forms of enterically derived LPS. It is composed of a pentasaccharide containing a lipid A core covalently attached to a unique trisaccharide of α-3-deoxy-D-*manno*-octulosonic acid (KDO)–(2-8)-αKDO–(2-4)-αKDO, which is antigenically dominant (2, 4, 14). Recent reports demonstrate that smooth forms of CT LPS can occur under some culture conditions, suggesting that variation in LPS structure may be related to pathogenesis (22). Although an unusual fatty acid, 3-hydroxydocosanoic acid (3-OH $C_{22:0}$), has been reported as a lipid A constituent (30), the precise molecular weight, the degree of lipid A heterogeneity, the location of the fatty acid substituents, and the linkages of CT LPS have not been defined.

In this article, we report on initial efforts to characterize the chemical structure of the lipid A moiety of CT LPS. We observed far less heterogeneity than was expected on the basis of previous reports (30). In addition, we demonstrate that CT LPS lacks potency, as assessed by its ability to activate mononuclear phagocytes and a Chinese hamster ovary (CHO)-K1 fibroblast cell line transfected with the LPS receptor CD14 (12). These data are the first demonstration of the effects of highly purified CT LPS on the LPS signal transduction pathway and the first to define the potency of CT LPS. The results of inhibition studies with specific LPS antagonists suggest that the proinflammatory cytokine response to *C. trachomatis* invasion may be mediated entirely through the interactions of its LPS with the CD14 signaling system.

MATERIALS AND METHODS

Solutions and plasticware. All solutions and plasticware were guaranteed sterile and pyrogen free by the manufacturer. Heparin sodium (5,000 U/ml) was obtained from Elkins-Sinn, Inc. (Cherry Hill, N.J.). The enzyme-linked immunosorbent assay (ELISA) 20× concentrate wash solution (phosphate-buffered saline [PBS]-Tween), ELISA 10× concentrate blocking solution (bovine serum albumin), and TMB peroxidase substrate and solution B (H_2O_2) were obtained from Kirkegaard and Perry Laboratories (Gaithersburg, Md.). Phosphoric acid was obtained from Fisher Scientific (Fair Lawn, N.J.).

PBS and Ham's F-12 medium were obtained from Bio-Whittaker (Walkersville, Md.). Macrophage-SFM medium was obtained from Gibco Laboratories (Grand Island, N.Y.). Bovine calf serum was obtained from HyClone Laboratories (Logan, Utah). Ciprofloxacin was a gift from Miles Pharmaceuticals (West Haven, Conn.). Recombinant $TNF\alpha^{hu}$ was obtained from Genzyme (Cambridge, Mass.).

Polypropylene 50- and 15-ml conical tubes and six-well tissue culture plates were obtained from Costar Corporation (Cambridge, Mass.). Falcon brand polystyrene round-bottom tubes (12 by 75 mm), 25- and 75-cm^2 tissue culture treated flasks, and 96-well tissue culture plates were obtained from Becton Dickinson (Lincoln Park, N.J.). Ninety-six-well ELISA plates were obtained from Dynatech (Chantilly, Va.). Borosilicate glass tubes (12 by 75 mm) and screw cap tubes (13 by 100 mm) were obtained from Gibco and baked at 200°C for 24 h to render them pyrogen free. Sterile, pyrogen-free pipette tips were obtained from Marsh Biomedical (Rochester, N.Y.).

Bacterial cultures. *Salmonella minnesota* R595 was obtained from American Type Culture Collection. A single colony from a fresh plate was inoculated into Luria-Bertani broth (1% tryptone, 0.5% yeast extract, 0.5% NaCl, 0.001 N NaOH) and grown to saturation. The number of bacteria was determined by serial dilutions and expressed as CFU per milliliter. Bacteria were washed three times in PBS and stored in 200-μl aliquots at −80°C. *C. trachomatis* serovar F (gift of H. Caldwell, Rocky Mountain Laboratories, Hamilton, Mont.) was grown in McCoy suspension cultures on microcarrier beads as described by Tam et al. (43). The organisms were purified by differential centrifugation followed by centrifugation through 32% Renografin in HSC buffer as previously described (44). Purified EB were stored at −80°C in PBS at a concentration of 10^9 inclusion-forming units per ml. Both *S. minnesota* and *C. trachomatis* suspensions were thawed on ice just prior to use.

Lipids. LPS was extracted from *S. minnesota* R595 (ReLPS), *C. trachomatis*, and *R. sphaeroides* (RSLA) by the Galanos method as modified by Qureshi et al. (34, 35). *N. gonorrhoeae* was grown as previously described (15), and lipooligosaccharide (LOS) was extracted from whole organisms by a modified hot phenol-water method (47). Synthetic lipid IV_A was purchased from ICN Biomedicals (Irvine, Ca.). Lipids were prepared as 1-mg/ml dispersed sonicates in pyrogen-free PBS and stored at −20°C. Prior to use, the suspensions were thawed and sonicated for 3 to 5 min in a water bath sonicator (Laboratory Supplies, Hicksville, N.Y.).

Characterization of CT LPS lipid A. CT LPS was suspended in 0.1 M HCl and acid hydrolyzed at 100°C for 15 min. The resulting crude monophosphoryl lipid A, (MPLA) was centrifuged at 8,000 × *g* for 15 min. The pellet was suspended in a two-phase chloroform-methanol-water (10:5:6, vol/vol) solvent and centrifuged again. The lower layer was recovered, filtered, and evaporated to dryness. The MPLA was converted into the free-acid form by using a Dowex column (H^+) and methylated by using diazomethane. The methylated lipid A mixture was fractionated by reverse-phase high-pressure liquid chromatography (HPLC) with two Waters 6000A solvent delivery systems (Waters Associates Inc., Milford, Mass.), a Waters 660 solvent programmer, U6K universal liquid chromatography injector, a variable wavelength detector (model LC-85B; Perkin-Elmer Corp., Norwalk, Conn.), and a radial compression module (model RCM-100, Waters Associates, Inc.). A Radial Pak A cartridge (8 mm by 10 cm) (C_{18} bonded, 10 μ, silica; Waters Associates, Inc.) was used at a flow rate of 2 ml/min. A linear gradient of 30 to 100% isopropanol in acetonitrile was used over a period of 60 min. The wavelength of the detector was set at 210 nm. Analytical thin-layer chromatography was performed on MPLA preparations by using Silica Gel H (250 μm) and the solvent system of chloroform–methanol–water–concentrated ammonium hydroxide (50:25:4:2, vol/vol). Hexaacyl and pentaacyl MPLA were prepared from the LPS of *E. coli* D31m4 in the same manner.

Stimulation of whole blood ex vivo. Whole blood was collected from a healthy volunteer aseptically into a heparinized syringe (75 U of heparin per ml of blood). Blood was plated at 180 μl per well in a 96-well tissue culture dish. LPS and bacterial dilutions were made as 10× concentrates in PBS, and 20 μl was added per well for overnight stimulation (16 h) at 37°C in 5% CO_2–95% air. When appropriate, inhibitors were premixed with blood prior to plating as follows: RSLA or lipid IV_A was added for a final concentration of 1 μg/ml; anti-CD14 monoclonal antibody 60 bca (gift of C. Moldow) was added for a final dilution of 1:30 (approximately 10 μg/ml). After centrifugation for 10 min at 400 × *g*, plasma (supernatant) was removed and TNF-α was measured by ELISA. Each sample was run in triplicate.

TNF-α ELISA. Antibodies for the ELISA were as follows. Mouse anti-$TNF\alpha^{hu}$ monoclonal antibody was obtained from Miles Pharmaceuticals. Polyclonal rabbit anti-$TNF\alpha^{hu}$ monoclonal antibody was obtained from Miles Pharmaceuticals. Polyclonal rabbit anti-$TNF\alpha^{hu}$ was obtained from Genzyme. Goat anti-rabbit immunoglobulin G-horseradish peroxidase was obtained from Southern Biotechnology Associates (Birmingham, Ala.). TNF-α ELISA was performed as described in detail by Lynn et al. (23). Optical density readings were converted to concentration of TNF-α by using the AssayZap software program (Biosoft, Cambridge, United Kingdom). The data are reported as mean concentrations of released TNF-α ± standard deviations.

Cell culture and stimulation conditions. The CHO-K1 cell line was obtained from the American Type Culture Collection and stably transfected with the genes for human CD14 and neomycin resistance (CHO/CD14) as described by Golenbock et al. (12). Cells were maintained in Ham's F-12 medium supplemented with 10% bovine calf serum and ciprofloxacin, 10 μg/ml (complete medium). They were grown as adherent monolayers in tissue culture dishes at 37°C in 5% CO_2–95% air and passaged twice a week to maintain logarithmic growth.

Prior to the day of experimentation, CHO/CD14 cells were trypsinized and plated in complete medium at a density of 10^6 per well in six-well tissue culture plates. After overnight incubation at 37°C in 5% CO_2–95% air, adherent monolayers were washed with medium alone and resuspended in 900 μl of Ham's F-12 supplemented with 2% heat-inactivated human serum and ciprofloxacin, 10 μg/ml. CT LPS and ReLPS dilutions were made as 10× concentrates in PBS. Cells were stimulated with 100 μl of the appropriate LPS suspension and returned to a 37°C, 5% CO_2–95% air environment for 60 min. At the end of the 60-min period, dishes were placed on ice to stop cellular activation during the preparation of nuclear extracts.

Preparation of nuclear extracts. The procedure used for the preparation of nuclear extracts has been described in detail by DeLude et al. (9). Briefly, adherent cells were washed in tissue culture plates with PBS containing 2% bovine calf serum, harvested with a rubber policeman, and pelleted in a microcentrifuge (Beckman Microfuge 11). Cells were resuspended in 0.4 ml of buffer I (10 mM Tris HCl [pH 7.8], 5 mM $MgCl_2$, 10 mM KCl, 0.5 mM dithiothreitol, 0.3 mM EGTA [ethylene glycol-bis(β-aminoethyl ether)-*N*,*N*,*N*′,*N*′-tetraacetic acid], 1 mM phenylmethylsulfonyl fluoride, 10 mM β-glycerolphosphate, 0.3 M sucrose, and 1.0 μg each of protease inhibitors aprotinin, antipain, leupeptin, chymostatin, and pepstatin per ml), incubated on ice for 15 min, and lysed by adding Nonidet P-40 to 0.5%. Nuclei were collected by centrifugation and resuspended in 50 μl of buffer II (20 mM Tris [pH 7.8], 5 mM $MgCl_2$, 320 mM KCl, 0.5 mM dithiothreitol, 0.2 mM EGTA, 0.5 mM phenylmethylsulfonyl fluoride, 10 mM β-glycerolphosphate, 25% glycerol, and 1.0 μg of each protease inhibitor per ml as above). After a 15-min incubation on ice, the nuclear extracts were cleared by centrifugation and transferred to a new tube. Protein concentration

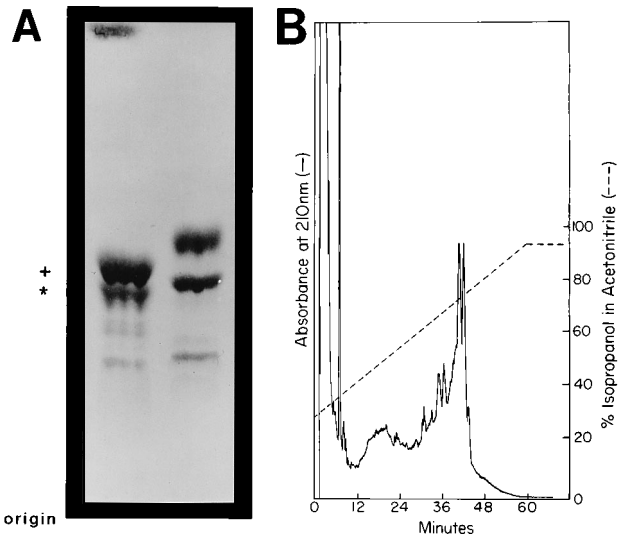

FIG. 1. (A) Thin-layer chromatography. Twenty-five micrograms each of CT LPS and *E. coli* LPS were run on Silica Gel H plates by using a chloroform-methanol-water-ammonia (50:25:42:2) solvent system. Left lane, MPLA from CT LPS. The main bands are pentaacyl (+) and tetraacyl (*) MPLA. Right lane, LPS from *E. coli* D31m4. (B) HPLC. Methylated CT MPLA (1.0 mg) was analyzed by reverse-phase HPLC. A C_{18}-bonded silica cartridge was developed with a linear gradient of isopropyl alcohol in acetonitrile. The major lipid A constituent was the pentaacyl MPLA peak (40 min).

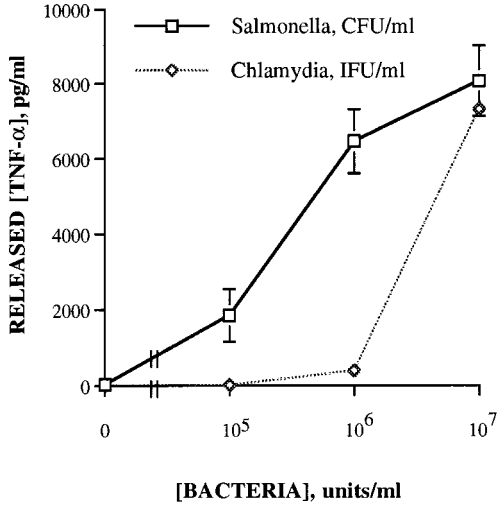

FIG. 2. Effect of whole *C. trachomatis* EB on TNF-α release from whole blood in vitro. *C. trachomatis* EB induced the release of TNF-α from whole blood ex vivo. *C. trachomatis* EB were less potent than whole *S. minnesota* R595. IFU, inclusion-forming units.

was determined by using the Bio-Rad (Hercules, Calif.) protein assay dye reagent concentrate.

Electrophoretic mobility shift assay. Two synthetic oligonucleotides containing the consensus sequence for nuclear factor κB (NF-κB) binding from the murine immunoglobulin κ light-chain gene enhancer (Oligos Etc., Guilford, Conn.) were annealed and labeled as described by DeLude et al. (9). Four micrograms of crude nuclear extracts and 0.2 ng of a DNA probe (20,000 cpm) were used in a DNA binding reaction mixture containing 50 μg of poly(dI-dC) per ml, 5% glycerol, and 1× band shift buffer (10 mM Tris HCl [pH 7.8], 1 mM EDTA, 40 mM KCl, 1 mM dithiothreitol). The reaction mixture was incubated at room temperature for 30 min and size fractionated by gel electrophoresis on a 4% native-polyacrylamide gel (Protogel brand acrylamide-bisacrylamide [30: 0.8, vol/vol]; National Diagnostics, Atlanta, Ga.) run at 10 V/cm for 45 min at room temperature. Gels were transferred to 3-mm filter paper (Whatman, Ltd.), immediately dried under vacuum at 80°C, and exposed to XAR2 X-ray film (Eastman Kodak Co., Rochester, N.Y.) overnight at −80°C with an intensifying screen.

RESULTS

Characterization of *Chlamydia* lipid A. Approximately 5×10^{12} EB were collected, yielding 15 mg of LPS. A protein assay of the LPS preparation failed to identify any significant amounts of protein (data not shown). Twenty-five micrograms of CT MPLA was run against MPLA from *E. coli* D31m4 LPS on Silica Gel H plates by using a chloroform-methanol-water-ammonia (50:25:4:2) solvent system (Fig. 1A). Methylated *C. trachomatis* MPLA (1.0 mg) was also analyzed by reverse-phase HPLC. A C_{18}-bonded silica cartridge was developed with a linear gradient of isopropyl alcohol in acetonitrile. The MPLA fractions (HPLC peaks at 40 min; Fig. 1B) were collected and subjected to fast atom bombardment mass spectrometry. Mass spectrometry revealed molecular ions at *m/z* 1,900 to 1,980, and together with the results of fatty acid analysis described by others (30) suggested that the chlamydial lipid A is predominantly pentaacyl. Minor amounts of lipid A corresponding in molecular weight to tetraacyl compounds were also observed (33).

***Chlamydia* EB induced production of TNF-α in a dose-dependent manner.** Like whole gram-negative organisms, *C. trachomatis* EB induced the in vitro release of TNF-α from whole blood. Overnight incubation of *Chlamydia* EB with whole blood resulted in the dose-dependent production of TNF-α in the plasma, as measured by ELISA (Fig. 2). Minimal endotoxin activity was seen at concentrations below 10^7 inclusion forming units per ml, with a maximum response of 23,000 pg of released TNF-α per ml at 10^8 inclusion-forming units per ml. In comparison, equivalent numbers of whole *S. minnesota* R595 were more potent inducers of TNF-α release by approximately 10-fold and markedly more efficacious than *C. trachomatis* in inducing a cytokine response at concentrations below 10^6 CFU/ml.

***Chlamydia* LPS induced the production of TNF-α in a dose-dependent manner.** Overnight incubation of whole blood with 0.1 to 1,000 ng of CT LPS per ml resulted in the dose-dependent production of TNF-α in the plasma, as measured by ELISA. The dose-response curve for CT LPS parallels that of the gram-negative LPS dose-response curves for *S. minnesota* and *N. gonorrhoeae* but with approximately 100 times less potency (Fig. 3). Minimal activity was seen below 100 ng of CT LPS per ml, while the maximum response required ≥1,000 ng of CT LPS per ml. In contrast, activity was seen with ReLPS at concentrations as low as 0.1 ng of LPS per ml, and a maximum response was achieved with 10 ng of LPS per ml.

***Chlamydia* LPS activated NF-κB in CHO/CD14 transfectants.** We sought to determine if CT LPS activated cells through the same signaling pathways as LPS preparations from enteric gram-negative bacilli. One possible explanation for the lack of potency observed with CT LPS might be that it fails to activate CD14-mediated signaling but is capable of activating alternative (and as yet unidentified) receptors (50).

CHO cells are normally unresponsive to LPS. However, when transfected with CD14 (CHO/CD14), they acquire macrophage-like sensitivity to endotoxin (12). We tested the ability of CT LPS to signal through the CD14 pathway by incubating CHO/CD14 cells with LPS and then assaying nuclear supernatants for the induction of NF-κB translocation. Our results (Fig. 4) demonstrated that CT LPS, like ReLPS, was capable of signaling through CD14-mediated pathways but, again, was ~100-fold less potent. Additional evidence that CT LPS can stimulate cells via the CD14 pathway is that monoclonal anti-

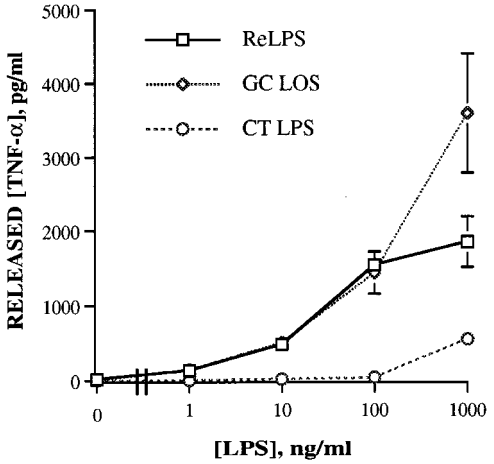

FIG. 3. Comparison of ReLPS-, CT LPS-, and *N. gonorrhoeae* (GC) LOS-induced TNF-α release from whole blood in vitro. CT LPS induced TNF-α release from whole blood ex vivo and was at least 100-fold less potent than either *S. minnesota* ReLPS or GC LOS.

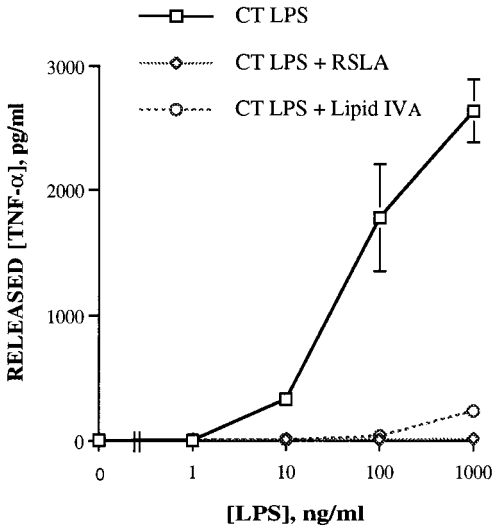

FIG. 5. Effects of RSLA and lipid IV_A on CT LPS-induced TNF-α release from whole blood ex vivo. Both RSLA and lipid IV_A inhibited CT LPS-induced TNF-α release from whole blood in vitro.

body to CD14 (60 bca) inhibited LPS-induced TNF-α release from whole blood in vitro (data not shown).

RSLA and lipid IV_A inhibited the TNF-α-inducing activity of *Chlamydia* LPS and whole EB. RSLA and lipid IV_A have been shown to specifically antagonize the effects of LPS (13, 17, 20, 42). The antagonism appears to be competitive in nature, although lipid IV_A fails to prevent the binding of LPS to CD14 at concentrations at which LPS-induced events are inhibited (18). We tested RSLA and lipid IV_A in an inhibition assay against CT LPS. Lipid IV_A or RSLA was coincubated with CT LPS in whole blood in vitro overnight, and TNF-α release was determined by ELISA. Both compounds proved to be potent inhibitors of CT LPS cytokine induction (Fig. 5). The possibility that antigens other than LPS might be contributing to the acute response to chlamydia remained. We have previously demonstrated that the stimulatory activities of protein antigens such as a purified protein derivative or whole gram-positive bacteria, which lack endotoxin, are not inhibited by RSLA or lipid IV_A (13). In order to determine if non-LPS moieties in *C. trachomatis* contribute to the cytokine response, we tested the ability of RSLA to block the activity of whole *C. trachomatis* EB in the TNF-α ELISA. We found that TNF-α release was completely blocked by coincubation with this LPS antagonist (Fig. 6). Thus, the LPS moiety present on the outer membrane of *C. trachomatis* EB appears to be the major determinant of the acute response.

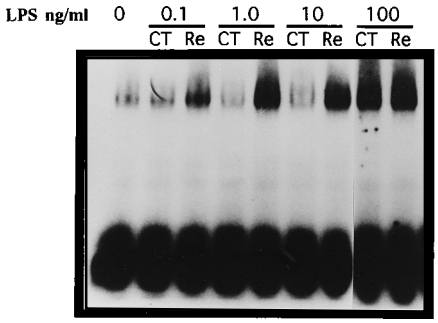

FIG. 4. CT LPS-induced NF-κB translocation in CHO/CD14. CHO/CD14 cells were incubated with increasing doses of either ReLPS or CT LPS. Nuclear extracts were prepared and analyzed for localization of NF-κB by the electrophoretic mobility shift assay as described in the text.

DISCUSSION

The outer surface of chlamydiae contains two major antigens, LPS and MOMP. During the course of natural and experimental infection, both antigens interact with the immune system. It is known, for example, that antibodies directed against MOMP (16, 51, 52) and LPS (3, 4) can be found in the sera of infected patients. A second protein associated with pathogenic *C. trachomatis* is the heat shock protein, Hsp-60, which produces inflammatory changes in experimental models of trachoma (27, 28). The role of these protein antigens, relative to LPS, in the acute inflammatory response to infection has never been well characterized. Indeed, chlamydial infections have never been considered endotoxin-mediated diseases, and no systematic characterization of CT LPS has ever been undertaken.

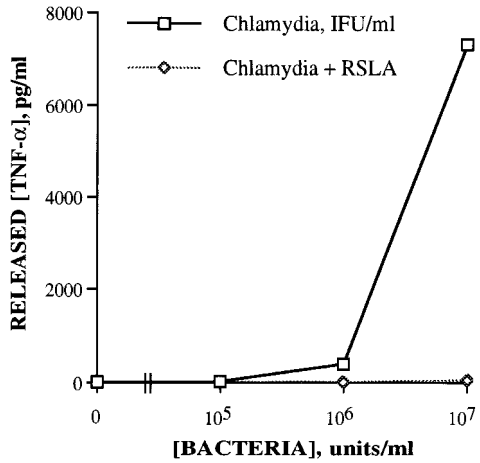

FIG. 6. Effect of RSLA on CT EB-induced TNF-α release from whole blood ex vivo. RSLA completely blocked the CT EB-induced TNF-α release from whole blood in vitro. IFU, inclusion-forming units.

Our data demonstrate that CT LPS, like the LPS from enteric gram-negative bacilli, is capable of stimulating phagocytic cells. It is, however, significantly less potent than *Salmonella* ReLPS and gonococcal LOS. Despite this quantitative difference, CT LPS has much in common with enteric LPS. CHO/CD14 transfectants respond to CT LPS with the translocation of NF-κB, indicating that signaling occurs through interaction with CD14. Furthermore, the ability of RSLA and lipid IV_A to inhibit CT LPS stimulation in mononuclear cells confirms that the same pathways utilized by ReLPS are involved in cell signaling by CT LPS. The observation that the cytokine-inducing activity of whole *C. trachomatis* EB can be completely blocked by a specific LPS antagonist would imply that the immunostimulatory activity observed with intact *C. trachomatis* EB may be accounted for primarily by the activity of its LPS. The role of MOMP or any other surface protein present on intact *C. trachomatis* EB, at least in the activation of the acute inflammatory response, is probably minor. There remains the possibility that proteins which are secreted from chlamydiae during the course of natural infection may also be capable of eliciting a proinflammatory cytokine response.

It is well established that the lipid A moiety of enteric gram-negative LPS is responsible for its biological activity (10, 37, 38). The data presented here suggest that most *C. trachomatis* lipid A is pentaacyl, similar to RSLA. In part, the degree of acylation per lipid A moiety might account for the reduced biological activity of CT LPS. Rietschel et al. recently reported that loss or gain of one acyl group to yield pentaacyl or heptaacyl *E. coli* lipid A reduced the bioactivity of the molecule by a factor of 100 (40). Mass spectrometry and fatty acid analyses of the major lipid A populations have been hampered by the slow growth of *C. trachomatis* in tissue culture and the difficulty of producing adequate quantities of CT LPS. However, preliminary analyses of the fatty acids associated with CT lipid A confirm that they are of an unusually long chain length, as proposed by others (5, 30). The biological significance of such long fatty acyl groups remains to be determined.

There are several clinical correlates to our observations. The finding that whole *C. trachomatis* EB and CT LPS are weak inducers of the acute immune response may explain why lower genital tract infection with *C. trachomatis* is often asymptomatic. This inability to induce a brisk inflammatory cytokine response may perpetuate the infection, allowing the organism to gain access to previously sterile sites. Women are at an especially high risk for morbidity from undetected chlamydial cervicitis, as progression to upper genital tract infection can quickly lead to irreversible scarring of the fallopian tubes. In addition, there is a growing appreciation for the interrelationships between various sexually transmitted diseases, including that caused by the human immunodeficiency virus (46). Two prospective cohort studies of African prostitutes found a significant association between chlamydial cervicitis and human immunodeficiency virus seroconversion (21, 32). In addition, there is also a potential role for chlamydiae in enhancing human immunodeficiency virus replication through the activation of NF-κB (1, 29).

Modulating the inflammatory cytokine response to LPS in endotoxin-mediated diseases, such as gram-negative bacterial sepsis, has been proposed as one approach to reducing the high mortality associated with this often fatal syndrome. Like sepsis, antibiotic therapy alone will not prevent many of the most tragic complications of chlamydial infections, such as tubal scarring and infertility in women. An antiendotoxin strategy might be useful for treatment of pelvic inflammatory disease, since many of the long-term sequelae are likely mediated through the release of induced cytokines by LPS-activated tissue macrophages. By delineating the role of endotoxin in those immune events which are initiated as a result of bacterial invasion, adjuvant therapies can be rationally designed to reduce the inflammation, scarring, and infertility associated with chlamydial infection.

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