Activation of Human Monocytic Cells by *Treponema pallidum* and *Borrelia burgdorferi* Lipoproteins and Synthetic Lipopeptides Proceeds via a Pathway Distinct from That of Lipopolysaccharide but Involves the Transcriptional Activator NF- κ B

MICHAEL V. NORGARD,¹ LESLIE L. ARNDT,¹ DARRIN R. AKINS,² LINDA L. CURETTY,²
DAVID A. HARRICH,² AND JUSTIN D. RADOLF^{1,2*}

Departments of Microbiology¹ and Internal Medicine,² The University of Texas Southwestern Medical Center, Dallas, Texas 75235

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There is increasing evidence that lipoproteins of *Treponema pallidum* and *Borrelia burgdorferi* are key inflammatory mediators during syphilis and Lyme disease. A principal objective of the present study was to identify more precisely similarities and divergences among lipopolysaccharide (LPS)- and lipoprotein-lipopeptide-induced immune cell signaling events. Like LPS, purified native *B. burgdorferi* OspA and synthetic analogs of OspA, OspB, and two *T. pallidum* lipoproteins (Tpp47 and Tpp17) all induced NF- κ B translocation in THP-1 human monocytoid cells. Acylation of OspA and the synthetic peptides was requisite for cell activation. Polymyxin B abrogated only the response to LPS. By using 70Z/3-derived pre-B-cell lines either lacking or expressing human CD14 (the LPS receptor), it was observed that expression of human CD14 imparted responsiveness to LPS but not to OspA or spirochetal lipopeptides (assessed by induction of NF- κ B and expression of surface immunoglobulin M). Finally, the biological relevance of the observation that *T. pallidum* lipoproteins-lipopeptides induce both NF- κ B and cytokine production in monocytes was supported by the ability of the synthetic analogs to promote human immunodeficiency virus replication in chronically infected U1 monocytoid cells; these observations also suggest a potential mechanism whereby a syphilitic chancre can serve as a cofactor for human immunodeficiency virus transmission. The combined data lend additional support to the proposal that spirochetal lipoproteins and LPS initiate monocyte activation via different cell surface events but that the signaling pathways ultimately converge to produce qualitatively similar cellular responses.

Syphilis and Lyme disease are chronic infections caused by the pathogenic spirochetes *Treponema pallidum* and *Borrelia burgdorferi*, respectively (35, 42). Although each is distinctive, syphilis and Lyme disease share a number of clinical features and are characterized by similar histopathological findings (35, 42). One potential explanation for the similar inflammatory responses induced by *T. pallidum* and *B. burgdorferi* is that both organisms synthesize abundant lipid-modified integral membrane proteins (10, 11, 56). By analogy with findings reported for the murein lipoprotein of *Escherichia coli* (22, 25), we and others have shown that spirochetal lipoproteins are potent activators of immune effector cells, including monocytes-macrophages, B cells, and endothelial cells (2, 14, 36, 37, 43, 50, 51, 53, 58, 63, 71). These observations, coupled with the fact that neither spirochete contains lipopolysaccharide (LPS) (20, 49, 64), support the contention that lipoproteins are important proinflammatory molecules of syphilis and Lyme disease.

A major problem in studying the proinflammatory properties of spirochetal lipoproteins arises from the difficulty in obtaining adequate quantities of these bacterial constituents under endotoxin-free conditions. As an extension of findings

by Bessler and colleagues (22, 25), we recently showed that this problem can be circumvented through the use of synthetic lipopeptides corresponding to the N-terminal domains of spirochetal lipoproteins (14, 51). Particularly intriguing were our findings that LPS and spirochetal lipopeptides elicited qualitatively similar cytokine patterns from human and murine macrophages even though they appeared to utilize distinct signaling pathways (50, 51). Prompted by the overall importance of elucidating the mechanisms by which spirochetal constituents induce inflammatory processes, we conducted additional studies to identify similarities and divergences among the LPS- and lipoprotein-induced cell signaling pathways. We found that both spirochetal lipoproteins and synthetic analogs induced the translocation of NF- κ B, a transcriptional activator heavily implicated in cytokine induction in monocytoid cells and in activation of human immunodeficiency virus (HIV) gene expression and replication (41, 54). In the absence of stimulation, NF- κ B is present in the cytoplasm in an inactive form complexed with the inhibitor protein I κ B (34). Upon appropriate stimulation, an alteration in I κ B allows dissociation of this complex. Active NF- κ B then translocates into the nucleus, where it binds to its DNA recognition sites to mediate gene transcription. Interestingly, lipoproteins-lipopeptides did not promote activation and translocation of NF- κ B through the CD14-dependent pathway utilized by LPS. The present studies, therefore, lend additional support to the contention that spirochetal lipoproteins and LPS initiate monocyte activation

* Corresponding author. Mailing address: Department of Internal Medicine, The University of Texas Southwestern Medical Center, 5323 Harry Hines Blvd., Dallas, TX 75235-9113. Phone: (214) 648-6896. Fax: (214) 648-5476. Electronic mail address: jradol@mednet.swmed.edu.

via different cell surface events but that these signaling pathways ultimately converge to produce qualitatively similar cellular responses.

MATERIALS AND METHODS

Isolation of native OspA lipoprotein from *B. burgdorferi*. *B. burgdorferi* T11-EV, generously provided by Jorge Benach (State University of New York, Stony Brook), was used as the source for purification of native OspA; this strain expresses an OspAB chimera which includes most of OspA (amino acids 1 to 233) fused via an aspartic acid residue to a small portion of the C terminus of OspB (amino acids 257 to 296). *B. burgdorferi* was cultivated in BSK II medium at 34°C (6). Care was exercised to minimize contamination by environmental LPS during the purification of native OspA by utilizing baked glassware, disposable materials, and endotoxin-free water to prepare all buffers and reagents. OspA was purified by the method of Ma and Weis (37) as modified by Jones et al. (29), except that the fractions obtained by monoclonal antibody immunoaffinity chromatography were not dialyzed. Fractions were stored at -70°C. Protein was precipitated by addition of 10 volumes of cold acetone (and incubation overnight at -20°C), collected by centrifugation, and air dried. Protein content was analyzed by the bicinchoninic acid assay method (Pierce Chemical Company, Rockford, Ill.) and by comparison with known quantities of bovine serum albumin stained with either Coomassie brilliant blue or silver following sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis. The yield from approximately 4×10^{10} spirochetes was about 110 µg of protein. All lipoprotein samples were assayed for the presence of contaminating endotoxin by the *Limulus* amoebocyte lysate gelation assay (E-Toxate; Sigma Chemical Co., St. Louis, Mo.) under conditions recommended by the manufacturer; this assay routinely detected a minimum of 1.2 pg of endotoxin. Prior to addition to cell cultures, OspA was suspended by vigorous agitation in 0.1 ml of prewarmed (37°C) fetal bovine serum (FBS) (final endotoxin contamination due to FBS was less than 0.2 pg/ml) (51).

Isolation of nonlipidated recombinant OspA from *E. coli*. A nonacylated recombinant DNA-derived fusion protein of OspA with glutathione S-transferase was constructed in *E. coli* by using a strategy analogous to that described by Radolf et al. (48). Briefly, PCR amplification of *B. burgdorferi* T11-EV DNA was carried out with a 5' oligonucleotide primer for the *ospA* gene (5'-TTGGA TCCTGTAAAGCAAAATGTTAGCAGC-3'; contains a 5' *Bam*HI site) and a 3' reverse primer (5'-TTGAATTCCTAGCTGATGCCTTGTAGGG-3'; has no *Bam*HI site) corresponding to the 3' end of the *ospB* gene. The gel-purified amplicon was digested with *Bam*HI and then ligated into the *Bam*HI and *Sma*I sites of pGEX-2T (Pharmacia-LKB Biotechnology, Piscataway, N.J.). Following transformation in *E. coli* DH5α, one clone was selected (designated pDA49.2) which expressed an OspA-glutathione S-transferase fusion protein of the predicted size. The 5' and 3' junctions of the construct in pGEX-2T were sequenced (300 bp in each direction) to verify proper genetic construction. Expression and affinity column purification of the glutathione S-transferase fusion proteins were performed by standard methods (60). The nonacylated spirochetal proteins were cleaved and isolated from the glutathione S-transferase moieties by thrombin cleavage of the immobilized fusion proteins bound to glutathione resin (70). To remove residual *E. coli* LPS contaminants from the nonacylated recombinant proteins, protein solutions were subjected to Detoxi-Gel Endotoxin Removing Gel columns (Pierce Chemical Company) as recommended by the manufacturer. Proteins were tested by the *Limulus* amoebocyte lysate gelation assay to confirm endotoxin removal.

Synthetic peptides and lipopeptides corresponding to the N termini of the spirochetal lipoproteins. Hexapeptides corresponding to the N termini of the 47-kDa (Cys-Gly-Ser-Ser-His-His) (70) and 17-kDa (Cys-Val-Ser-Cys-Thr-Thr) (2) lipoproteins of *T. pallidum* subsp. *pallidum* (designated Tpp47 and Tpp17, respectively) and the OspA (Cys-Lys-Gln-Asn-Val-Ser) and OspB (Cys-Ala-Gln-Lys-Gly-Ala) lipoproteins of *B. burgdorferi* (8) were synthesized on an Applied Biosystems (Foster City, Calif.) 430A peptide synthesizer using standard 9-fluorenylmethoxycarbonyl chemistry as recommended by the manufacturer. For use in cell stimulation assays, quantities of dry hexapeptides were solubilized by vortexing in sterile, endotoxin-free water. Inasmuch as palmitate is the principal long-chain fatty acid of the native spirochetal lipoproteins (7), lipohexapeptide analogs corresponding to the acylated N termini of the lipoproteins were synthesized as tripalmitoyl derivatives by using a solid-phase synthesis procedure (14). All synthetic peptides and lipopeptides were assayed for contaminating LPS by the *Limulus* amoebocyte lysate gelation assay after being dissolved or suspended in sterile, endotoxin-free water. As were the native lipoproteins, synthetic lipopeptides were dissolved in prewarmed FBS prior to addition to cell cultures.

Cell culture. The THP-1 human monocytic cell line (66), obtained from Robert S. Munford and Richard Kitchens (University of Texas Southwestern Medical Center, Dallas), was maintained and cultured as previously described (31). Some experiments were conducted with THP-1 cells to evaluate the influence of vitamin D₃ treatment (0.1 µM for 72 h, followed by retreatment for an additional 24 h) (31, 32) prior to stimulation of adherent cells for 4 h by LPS, peptides, lipoproteins, or lipopeptides. Rough bacterial LPS (from *Salmonella minnesota* R5 [Rc mutant]; molecular weight, ca. 4,000; Sigma Chemical Co. catalog no.

L-8893) was used as a positive control in all cell stimulation assays. Preincubation (15 min at room temperature) of proteins, lipoproteins, peptides, lipopeptides, and LPS with polymyxin B (PB; 10 µg/ml; Sigma Chemical Co.) was used to abrogate the effect of LPS or LPS contaminants.

Murine lymphoma 70Z/3 pre-B cells (45) transfected with either the human CD14 gene (hCD14) or the pRc/RSV expression vector alone (33) were generously provided by Richard Ulevitch (Scripps Research Institute, La Jolla, Calif.). 70Z/3-derived cell lines were cultured as previously described (30, 45).

U1 cells, a subclone of the promonocytic cell line U937 latently infected with HIV type 1 (HIV-1) (17), were used to assess induction of HIV-1 by *T. pallidum* lipopeptides. The cells were maintained in RPMI 1640 culture medium supplemented with 10% FBS, 1% glutamine, and a 1% penicillin-streptomycin solution. All reagents were determined to be free of endotoxin.

Detection of interleukin-1β (IL-1β) transcripts. Total RNA was extracted from stimulated cells by using the Ultraspec RNA isolation system (Biotecx Laboratories, Inc., Houston, Tex.) as recommended by the manufacturer. RNA was dissolved in sterile, deionized water (treated with 0.1% diethylpyrocarbonate) and quantitated spectrophotometrically (55). To 20 µg of RNA in 11 µl of water were added 5 µl of 10× MOPS buffer [0.4 M 3-(N-morpholino)propane-sulfonic acid, 0.1 M sodium acetate, 0.01 M EDTA], 9 µl of 12.3 M formaldehyde, and 25 µl of deionized formamide. The mixture was incubated for 15 min at 65°C and chilled on ice, and then 10 µl of formaldehyde loading buffer (1 mM EDTA, 0.25% [wt/vol] bromophenol blue, 50% glycerol) was added. RNA in this mixture was resolved by using standard formaldehyde buffer (1× MOPS buffer, 0.666% [vol/vol] formaldehyde) and 1% agarose gel electrophoresis conditions (55). A lane containing RNA ladder molecular size markers (0.24 to 9.5 kb) (Gibco-BRL, Gaithersburg, Md.) was trimmed away from the gel, stained with ethidium bromide, and photographed for estimation of molecular sizes of hybridizing RNA transcripts. RNA was transferred to a Hybond-N⁺ charged nylon membrane (Amersham, Arlington Heights, Ill.) in 20× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate) buffer via the capillary method and UV cross-linked to the membrane. Hybridization and washing of the membranes were carried out essentially as previously described (55). Briefly, hybridization was performed at 42°C overnight in 5× SSC buffer-5× Denhardt's solution-1% SDS-50% formamide-1 mg of salmon sperm DNA per ml. Probes for IL-1β (American Type Culture Collection catalog no. 67024) (5) and glyceraldehyde-3-phosphate dehydrogenase (American Type Culture Collection catalog no. 57090) (72) were prepared from the relevant plasmid DNAs; they were radiolabeled with [α -³²P]dCTP by using the Boehringer Mannheim (Indianapolis, Ind.) random DNA primer labeling kit in accordance with the manufacturer's instructions. Washing of filters was performed at room temperature by two serial washes with 2× SSC buffer-0.1% SDS followed by one wash at 42°C with 0.2× SSC-0.1% SDS. Membranes were then dried and subjected to autoradiography at -70°C for various times.

NF-κB assays. The active form of NF-κB, translocated into the nuclei of stimulated cells, was detected by incubating nuclear extracts from stimulated cells with a radiolabeled target NF-κB oligonucleotide followed by a gel retardation assay; cell stimulations, nuclear extracts from cell lysates, and mobility shift assays were performed as previously described (31). THP-1 cells typically were stimulated for 1 h, and 70Z/3 cells were stimulated for 30 min.

Surface immunoglobulin M (IgM) expression by activated 70Z/3 cells. Surface IgM expression by stimulated 70Z/3 cells was assessed by fluorescent cell staining and flow cytometry essentially as previously described (59). Briefly, 70Z/3 cells were plated at a final density of 2×10^6 per well in 24-well plates. After stimulation for 24 h with various agents, cells were harvested by centrifugation, suspended in phosphate-buffered saline (PBS)-1% FBS, and enumerated, and 10^6 cells were distributed into sterile polystyrene tubes. After being washed twice by centrifugation at room temperature in PBS-1% FBS, the cells were stained for 30 min (4°C) by addition of fluorescein isothiocyanate-conjugated goat anti-mouse IgM, washed thrice by centrifugation in cold PBS-1% FBS, and resuspended in 2 ml of cold PBS-1% FBS. They then were transferred to fresh polystyrene tubes (kept on ice and protected from light) and analyzed with a FACScan flow cytometer (Becton Dickinson Immunocytometry Systems, Mansfield, Mass.).

Assay for HIV-1 p24 antigen. To assay for HIV-1 induction in U1 cells, triplicate cultures containing 2×10^5 cells per ml were grown in complete culture medium or the same medium supplemented with either LPS (200 ng/ml) or spirochetal peptides or lipopeptides (30 µM). Cell-free culture supernatants were collected 48 h poststimulation and assayed for the HIV-1 p24 antigen with the Alliance HIV-p24 ELISA Kit (Du Pont, Boston, Mass.). Statistical analysis of the assay results was performed by using Fisher's least significant difference.

RESULTS

For our comparative studies of cell activation by LPS and spirochetal lipoproteins and lipopeptides, it was considered advantageous to employ the well-characterized THP-1 human monocytoid cell line (3, 66). At the outset, it was necessary to determine whether this cell line was responsive to spirochetal lipopeptides and lipoproteins. As shown in Fig. 1, lipopeptides

FIG. 1. Responsiveness of THP-1 cells to graded doses of spirochetal lipopeptides. THP-1 cells were either treated (+) or not treated (–) with vitamin (Vit.) D₃; 1-ml subcultures were then stimulated for 4 h with various doses (shown in micrograms per milliliter at the top) of LPS (positive control) or lipopeptides corresponding to Tpp17 (17L) and OspA (OspA-L). RNA was isolated and hybridized in Northern (RNA) blots with a radiolabeled probe for either IL-1 β or glyceraldehyde-3-phosphate dehydrogenase, the gene for which is constitutively expressed. For lipopeptides, 1.0 μ g/ml corresponds to ca. 0.71 μ M.

based upon the N termini of OspA and Tpp17 induced IL-1 β mRNA in a dose-dependent fashion. Vitamin D₃ maturation, which has been shown to enhance the LPS responsiveness of THP-1 cells (31, 32), also enhanced the sensitivity of the cells to lipopeptides. Consistent with earlier findings (14, 51), the corresponding nonlipidated peptides were without effect (data not shown). Essentially identical results were obtained with lipopeptides based upon OspB and Tpp47 (data not shown), as well as with purified OspA (32 nM; ca. 1 μ g/ml) (data not shown), which, by the *Limulus* amoebocyte lysate gelation assay, contained a level of endotoxin contamination (<12 pg/ μ g of protein) well below that capable of activating THP-1 cells (Fig. 1). (OspA was selected as a representative spirochetal lipoprotein because of the relative ease with which it can be purified without endotoxin contamination.) Upon the basis of the above-described findings, subsequent experiments utilized only vitamin D₃-treated cells.

We next sought to determine whether signaling events associated with LPS-mediated cell activation also occur during cell activation by spirochetal lipoproteins-lipopeptides. We chose to study the activation and translocation of the transcription factor NF- κ B, a sentinel event in LPS-mediated signaling (34, 41), which has been examined extensively in THP-1 cells (31). In our earlier studies, it was established that lipopeptides at 10 μ M and LPS at 10 nM were approximately equivalent with respect to induction of cytokine production by murine and human macrophages (51). THP-1 cells were treated for 1 h with either LPS or four representative spirochetal lipopeptides (as well as nonlipidated peptide controls), and nuclear extracts were prepared for gel retardation assays. As shown in Fig. 2, LPS and all four acylated peptides promoted NF- κ B translo-

FIG. 2. Synthetic lipopeptides corresponding to *T. pallidum* (A) and *B. burgdorferi* (B) lipoproteins induce NF- κ B. THP-1 monocytoid cells were stimulated for 1 h with 10 μ M (ca. 14 μ g/ml) of lipopeptides corresponding to Tpp47 (47L), Tpp17 (17L), OspA (OspA-L), and OspB (OspB-L) or 10 μ M (ca. 6 μ g/ml) nonacylated control hexapeptides (lanes 47, 17, OspA, and OspB) in either the presence (+) or the absence (–) of PB. LPS (10 nM; 40 ng/ml) was used as a positive control. Activated NF- κ B in nuclear extracts was assessed by gel retardation assay; the arrow indicates the retarded radiolabeled target oligonucleotide. Neg., unstimulated control culture.

cation, whereas the nonlipidated peptides did not. Consistent with prior findings (50, 51), PB abrogated only the effect of the LPS. Additional kinetic studies revealed that NF- κ B translocation in response to both LPS and lipopeptides was detectable within 15 min poststimulation (data not shown).

To obtain further evidence supporting the use of lipopeptides as surrogates for lipoproteins in studies of immune cell activation (14, 51), we also examined the translocation of NF- κ B in response to OspA purified from *B. burgdorferi* (Fig. 3A). Similar to the OspA lipopeptide, translocation of NF- κ B after incubation with OspA was not affected by the presence of PB (Fig. 3B). It is of particular importance that, as with the synthetic analogs, OspA-mediated NF- κ B translocation was observed only with the lipid-modified polypeptide (Fig. 3B).

Previous evidence suggested that LPS and spirochetal lipoproteins-lipopeptides stimulate cytokine production via dif-

FIG. 3. Native OspA from *B. burgdorferi* induces NF- κ B. (A) Samples (0.75 μ g) of either native OspA purified from *B. burgdorferi* or nonacylated, recombinant OspA isolated from *E. coli* were resolved by SDS-polyacrylamide gel electrophoresis and stained with silver. Molecular masses are indicated on the left in kilodaltons. (B) Translocation of NF- κ B in THP-1 cells incubated for 1 h with 32 nM (ca. 1 μ g/ml) native or nonacylated recombinant OspA (nOspA and rOspA, respectively) or LPS (10 nM; 40 ng/ml) in the presence (+) or absence (-) of PB. Neg., unstimulated control culture.

ferent cell surface events (51). To garner additional support for this notion, we examined the role of the CD14-dependent pathway utilized by LPS in cell activation by spirochetal lipoproteins-lipopeptides. Binding of LPS (complexed to LPS-binding protein) to the CD14 receptor is the initial event in macrophage activation (67); CD14 also has been implicated as a pattern recognition receptor for other bacterial cell wall constituents with immunostimulatory activities (47). Experiments were performed with the CD14-negative murine pre-B-cell line 70Z/3 and the same cells transfected with hCD14 (33). As previously described (33), LPS at 40 ng/ml failed to induce NF- κ B translocation during a 30-min incubation with CD14-negative 70Z/3 cells (data not shown). Lipopeptides corresponding to OspA and Tpp17 also were without effect at concentrations of up to 100 μ M (data not shown). In contrast, transfection of 70Z/3 cells with hCD14 imparted responsiveness to the same low dose of LPS but not to the two spirochetal lipopeptides (Fig. 4A). The same results were obtained by

using cells incubated with LPS and lipopeptides for 1 h (data not shown). The observation that CD14 transfection imparted responsiveness to LPS but not to lipopeptides was supported by examining the induction of surface IgM expression as an alternative activation marker for these same two cell lines (33). LPS concentrations on the order of 100 ng/ml were required to induce surface expression of IgM in CD14-negative cells (via the CD14-independent pathway), whereas a low dose of LPS (10 ng/ml) readily induced surface IgM expression by hCD14-transfected cells (Fig. 4B). Consistent with the NF- κ B results (Fig. 4A), surface IgM was not induced in either cell line following a 24-h incubation with the OspA or Tpp17 lipopeptide (Fig. 4B). Analogous results for NF- κ B activation (not shown) and surface IgM expression (Fig. 5) were obtained when CD14-negative and hCD14-transfected cells were incubated with 32 nM (ca. 1 μ g/ml) native OspA.

Cells of the monocyte-macrophage lineage, which are abundant in syphilitic lesions (28, 35), are important targets of HIV infection (24, 54). Furthermore, binding of NF- κ B to the HIV promoter is associated with enhanced HIV gene expression and replication of virus within infected monocytes (4, 46, 54). The observation that *T. pallidum* lipoprotein analogs induce the translocation of NF- κ B, coupled with the association between syphilitic genital ulcers and transmission of HIV (26, 38, 69), prompted us to examine whether treponemal lipopeptides could stimulate HIV replication. U1 monocyotoid cells chronically infected with HIV-1 were incubated for 48 h with either LPS, lipopeptide Tpp47 or Tpp17, or the corresponding nonacylated peptide control. LPS, which has previously been shown to induce HIV gene expression via NF- κ B activation (46), provided the positive control for these experiments (Fig. 6). As with LPS, the treponemal lipopeptides, but not their nonacylated counterparts, induced HIV-1 replication as assessed by detection of the p24 antigen (Fig. 6). This effect likely was not due to secondary autocrine effects from elicited cytokines, inasmuch as U937 cells, the parental cell line of U1, first must be induced to differentiate with phorbol myristate acetate before they can produce IL-1 β , IL-6, and tumor necrosis factor alpha (at least in response to LPS stimulation) (21).

DISCUSSION

The absence of classical endotoxin in both *T. pallidum* and *B. burgdorferi* has provided impetus for identifying the constituents of these two pathogens which elicit inflammatory responses. Such studies, however, have been impeded by the lack of systems for genetically manipulating these two spirochetal pathogens. Building upon the experimental strategies pioneered by Bessler and colleagues in their studies of murein lipoprotein of *E. coli* (22, 25), investigators of spirochetal disease immunopathogenesis have utilized a number of tissue culture systems to demonstrate that various *T. pallidum* and *B. burgdorferi* lipoproteins and synthetic analogs have comparable proinflammatory properties (2, 14, 36, 37, 43, 50, 51, 53, 58, 63, 71). In addition, following intradermal injection into mice and rabbits, lipopeptides derived from both borrelial and treponemal lipoproteins elicited histopathological responses which resembled those in syphilis and Lyme disease (43). Taken together, these observations constitute a strong body of evidence that spirochetal lipoproteins are key inflammatory mediators in syphilis and Lyme disease and, as such, should be considered among the components of bacterial cell envelopes, in addition to LPS, which possess immunostimulatory activities.

A principal goal of the present study was to obtain further support for the proposal that LPS and spirochetal lipoproteins and lipopeptides initiate immune cell activation via different

FIG. 4. Spirochetal synthetic lipopeptides fail to activate hCD14-transfected 70Z/3 cells. (A) NF- κ B translocation in hCD14-transfected 70Z/3 cells incubated for 30 min with a 10 μ M concentration of a lipopeptide corresponding to either OspA (OspA-L) or Tpp17 (17L) in the presence (+) or absence (-) of PB. NF- κ B induced by LPS (10 nM; 40 ng/ml) is indicated by the arrowhead. Nonacylated hexapeptides corresponding to OspA (lane OspA) and Tpp17 (lane 17) were included as negative controls. Unstim., unstimulated. (B) Flow cytometric analysis of surface IgM expression in untransfected (CD14⁻) or hCD14-transfected (CD14⁺) 70Z/3 cells following incubation for 24 h with either the same two lipopeptides, the corresponding nonacylated peptide controls, or a high (100 ng/ml) or low (10 ng/ml) dose of LPS. All of the samples tested are listed in the upper right-hand corner of each graph; for simplicity, only the curves corresponding to the LPS samples are labeled, inasmuch as all of the other samples yielded negative results for both CD14⁻ and CD14⁺ cells. Surface IgM expression is indicated by a rightward curve shift (left graph, LPS at 100 ng/ml; right graph, LPS at 10 and 100 ng/ml). The slight rightward shift in one of the other histograms (OspA-L; unlabeled) was not statistically significant from unstimulated cultures. UM, micromolar.

FIG. 5. Flow cytometric analysis of surface IgM expression in untransfected (CD14⁻) or hCD14-transfected (CD14⁺) 70Z/3 cells following incubation for 24 h with native OspA (32 nM; ca. 1 μg/ml), an equivalent concentration of recombinant (nonacylated) OspA, and high (100 ng/ml) and low (10 ng/ml) doses of LPS. OspA lipopeptide and nonacylated peptide (each at 10 μM) also were included for comparison with native OspA. As in Fig. 4B, surface expression of IgM in response to LPS is indicated by a rightward shift.

cell surface events. The most important prior evidence for this was the observation that synthetic lipoproteins and lipoprotein analogs activated peritoneal macrophages from LPS-nonresponsive (C3H/HeJ), as well as LPS-responsive (C3H/HeN), mice (50, 51). Because there is strong evidence that C3H/HeJ macrophages express normal levels of CD14 (13), our results

FIG. 6. Synthetic *T. pallidum* lipopeptides induce HIV-1 in U1 cells. U1 cells were stimulated for 48 h with either LPS (200 ng/ml), lipopeptides representative of Tpp47 (47L) and Tpp17 (17L) (30 μM), or corresponding nonacylated peptide (bars 47 and 17) controls (30 μM). Culture supernatants were assayed for HIV-1 by quantitative enzyme-linked immunosorbent assay for p24 antigen (Ag). Data from three trials are expressed as the average ± the standard deviations. Levels of p24 antigen only from cultures treated with LPS ($P = 0.003$), 47L ($P = 0.005$), and 17L ($P = 0.002$) were significantly different (asterisks) from those of unstimulated (Neg.) cultures (Fisher's least significant difference).

implied that the CD14-dependent pathway utilized by LPS is not involved in cell activation by spirochetal lipoproteins and lipopeptides. Here we showed that hCD14 expression, which conferred LPS sensitivity on 70Z/3 cells, did not impart responsiveness to OspA or to two different spirochetal lipopeptides. These results were buttressed by our use of two different indicators of cell activation, NF-κB translocation and surface IgM expression, representative of early signaling events and a phenotypic endpoint following prolonged stimulation with these compounds, respectively. While our results are most easily explained by the lack of CD14 involvement in lipoprotein-lipopeptide-mediated cell activation, our experiments have not eliminated the possibility that these spirochetal constituents utilize an alternative, but undescribed, CD14-dependent pathway. Experiments with macrophages from CD14-deficient mice should be instrumental in resolving this issue (23).

The highly similar cytokine profiles elicited in macrophages by spirochetal lipoproteins-lipopeptides and LPS (36, 51, 63, 65) suggested that despite the differences in initial signaling events, the two pathways eventually overlap or possibly converge. To investigate this, we chose to study translocation of the transcriptional activator NF-κB, a critical distal event in the pathway(s) by which LPS induces cytokine production (41, 54). Like LPS, spirochetal lipoproteins and lipopeptides induced NF-κB; moreover, the relatively rapid time course (data not shown) was compatible with a direct effect (as in the case of LPS), as opposed to indirect, autocrine stimulation by elicited cytokines. There is considerable evidence for the involvement of tyrosine phosphorylation events (particularly mitogen-activated protein kinases) in LPS signaling (15, 18, 19); some evidence also exists for the involvement of similar phosphorylation events in the responses to both LPS and bacterial lipoproteins or lipopeptides (15, 44, 52). Thus, the stage is set

for a detailed comparative analysis of upstream events in the LPS and lipoprotein-lipopeptide signaling pathways to identify points of convergence and/or overlap.

Epidemiological studies have implicated syphilis and other genital ulcer diseases as cofactors in the transmission of HIV (26, 27, 61, 69). At least two features of chancres are likely to contribute to this phenomenon. First, the breach in the protective epithelial barrier would either expose HIV-infected body fluids or create a portal of entry in a non-HIV-infected individual. Second, the influx of large numbers of macrophages and T cells into syphilitic ulcers (35) could facilitate HIV acquisition by providing an environment rich in immune cells expressing receptors for HIV. By activating immune cells at such local sites of infection, it also is plausible that *T. pallidum* provides an antigenic stimulus which "drives" HIV replication within these lesions, thereby promoting transmission. The proinflammatory activities of the treponemal lipoproteins could amplify this process both by directly inducing NF- κ B in macrophages and by stimulating macrophages to produce cytokines, such as tumor necrosis factor alpha, IL-1 β , and IL-6, which enhance HIV replication (16). Moreover, because syphilis, unlike other genital ulcer diseases, is almost always a disseminated infection, the possibility exists that syphilitic infection, particularly in the secondary stage, may be capable of stimulating systemic HIV replication, thereby accelerating the course of HIV disease.

Work by us (2, 14, 50, 51, 53) and by others (22, 25, 36, 37, 58, 63, 71) has shown that a variety of bacterial lipoproteins and lipopeptides can activate immune effector cells. While this common biological property presumably reflects a shared biochemical structure or configuration, it is noteworthy that tripalmitoyl-S-glycerylcysteine, the only moiety common to both lipoproteins and synthetic analogs, lacks proinflammatory activity (9, 51). Acyl modification via this configuration, on the other hand, appears to be necessary, although not sufficient, for biological activity of this class of compounds (9, 51). Because the covalently bound lipids provide membrane anchors for the native spirochetal proteins (29, 39), we have proposed that the lipids also promote insertion of the proteins and/or peptides into eukaryotic cell membranes, where they interact with one or more unidentified components of the signaling apparatus. While it remains conjectural (and somewhat paradoxical) that these diverse compounds may initiate cell activation via specific receptor-ligand-type interactions, the polymorphic nature of this phenomenon has important implications for Lyme disease pathogenesis. There is a substantial body of evidence that *B. burgdorferi* does not express OspA and OspB within mammalian hosts (40, 57, 62) and that other, antigenically unrelated proteins are expressed following tick transmission (1, 12, 40, 57, 62, 68). The observation that proinflammatory activity is a "generic" property of spirochetal lipoproteins raises the intriguing possibility that lipoproteins unrelated to OspA or OspB which are expressed exclusively during the course of infection can promote the inflammatory processes that engender clinical manifestations in chronic Lyme disease. This contention can be evaluated by examining the proinflammatory properties of borrelial lipoproteins expressed exclusively during the mammalian phase of the spirochete life cycle.

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