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Porphyromonas gingivalis Lipopolysaccharide Is Both Agonist and Antagonist for p38 Mitogen-Activated Protein Kinase Activation

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Lipopolysaccharide (LPS) is a key inflammatory mediator. It has been proposed to function as an important molecule that alerts the host of potential bacterial infection. Although highly conserved, LPS contains important structural differences among different bacterial species that can significantly alter host responses. For example, LPS obtained from *Porphyromonas gingivalis*, an etiologic agent for periodontitis, evokes a highly unusual host cell response. Human monocytes respond to this LPS by the secretion of a variety of different inflammatory mediators, while endothelial cells do not. In addition, *P. gingivalis* LPS inhibits endothelial cell expression of E-selectin and interleukin 8 (IL-8) induced by other bacteria. In this report the ability of *P. gingivalis* LPS to activate p38 mitogen-activated protein (MAP) kinase was investigated. It was found that p38 MAP kinase activation occurred in response to *P. gingivalis* LPS in human monocytes. In contrast, no p38 MAP kinase activation was observed in response to *P. gingivalis* LPS in human endothelial cells or CHO cells transfected with human Toll-like receptor 4 (TLR-4). In addition, *P. gingivalis* LPS was an effective inhibitor of *Escherichia coli*-induced p38 MAP kinase phosphorylation in both endothelial cells and CHO cells transfected with human TLR-4. These data demonstrate that *P. gingivalis* LPS activates the LPS-associated p38 MAP kinase in monocytes and that it can be an antagonist for *E. coli* LPS activation of p38 MAP kinase in endothelial and CHO cells. These data also suggest that although LPS is generally considered a bacterial component that alerts the host to infection, LPS from *P. gingivalis* may selectively modify the host response as a means to facilitate colonization.

The innate host defense system protects mammalian hosts against microbial infection through an orchestrated response to the presence of nonself components (31, 32). Lipopolysaccharide (LPS), a component of the gram-negative bacterial cell wall, is a key structure recognized by a variety of different innate host defense proteins, allowing the host to "sense" a potential bacterial infection (8, 62). LPS is evolutionarily an ideal target, since it is a conserved structure found on a wide variety of pathogenic bacteria and is sufficiently different from host components to allow a safe selective response (38). However, there are important structural differences in LPS composition between different bacterial species, such as fatty acid acyl chain composition and charge, which can significantly affect the host response (33, 36, 42, 49, 64). Different binding affinities for LPS binding protein and CD14 may only partly explain the lowered inflammatory response to some LPS species that has been observed (14), consistent with the notion that the major role of LPS binding protein and CD14 is to concentrate LPS at host cell surfaces (65). Recently it has been demonstrated that cell surface Toll-like receptor (TLR) proteins participate in the ability of the host to discriminate different LPS structural features (6, 25, 30, 34, 47, 58, 66). These data suggest that one mechanism by which the innate host defense system recognizes different bacterial species relates to their unique LPS structural features.

Porphyromonas gingivalis is a gram-negative bacterium that is recognized as an important etiologic agent of human adult-type periodontitis (56). This bacterially induced chronic inflammatory disease affects a large proportion of the population and is characterized by resorption of alveolar bone surrounding the tooth root surface, resulting in the loss of permanent dentition (56). *P. gingivalis* LPS is unusual in that although it is able to activate human monocytes by a CD14-dependent mechanism (54) and binds sCD14 (14), it does not facilitate either E-selectin expression or interleukin 8 (IL-8) secretion from human umbilical cord vein vascular endothelial cells (16). In fact, this LPS is a natural antagonist for the human endothelial E-selectin and IL-8 response to *Escherichia coli* LPS and other oral bacteria (16). Studies with the mutant sCD14 proteins E47K and E47R that bind *E. coli* LPS but not *P. gingivalis* LPS (55) have demonstrated that sCD14 is not required for inhibition of E-selectin expression (13), ruling out competition for sCD14 binding as the mechanism for antagonism. Although the host inflammatory response to *P. gingivalis* LPS both in vivo (50) and in vitro (5) is significantly different from that observed with *E. coli* LPS, little is known concerning how this LPS may affect intracellular signaling pathways.

A key component of many intracellular signaling pathways are the mitogen-activated protein (MAP) kinases (10, 45). This superfamily includes the extracellular signal response kinases (ERKs), c-jun N-terminal kinases, and the p38 family of kinases. Although LPS facilitates the activation of all three of these MAP kinases, numerous studies employing p38 MAP kinase-specific inhibitors have provided good evidence that many cellular responses to LPS require p38 activation (1, 2, 11, 41), consistent with a primary role for p38 activation in response to LPS (12, 26, 27, 29, 59). In this study the p38 MAP kinase response to *P. gingivalis* LPS was examined. It was found that p38 MAP kinase activation correlated with the ability of *P.*

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gingivalis to activate cells. In addition, *P. gingivalis* LPS was an effective inhibitor of *E. coli*-induced p38 MAP kinase phosphorylation.

MATERIALS AND METHODS

Bacterial strains and preparation of LPS. *P. gingivalis* ATCC 33277 was obtained from the American Type Culture Collection, Rockville, Md.; it was examined for purity, properly identified, and stored at −70°C. Cultures were made from frozen bacterial stocks to avoid repetitive subculture. Bacterial cells were grown for LPS isolation as follows: *P. gingivalis* was grown anaerobically at 37°C for 2 to 3 days in enriched Trypticase soy broth supplemented with heme, cysteine, and menadione. Strain 33277 was grown in a large batch culture by Lee Laboratories, Grayson, Ga., under similar conditions and shipped as frozen cell pellets.

P. gingivalis LPS was prepared by the cold $MgCl_2$-ethanol procedure (17) followed by lipid extraction (21) and conversion to sodium salts (46). Purified LPS from *E. coli* 0111:B4 (Sigma) was obtained by the phenol-water method and was further purified by a Folch extraction (21) to remove contaminating phospholipids. LPS preparations were suspended in distilled water, and the absence of contaminating nucleic acid and protein was verified by measuring the optical densities at 280 and 260 nm. Colloidal gold staining (Bio-Rad Laboratories, Hercules, Ca.) of the LPS preparations was performed as described by the manufacturer and revealed less than 1% protein contamination for both preparations. Gas chromatographic analysis for sugar and fatty acid composition revealed that the composition of these purified LPSs agreed with published data (42).

Cell culture. Human umbilical vein endothelial cells (HUVEC) were prepared by collagenase (Worthington Biochemical, Freehold, N.J.) treatment of umbilical cords. Cultures were maintained in RPMI 1640 (Whittaker, Walkerville, Md.) supplemented with 20% adult bovine serum (JRH Bioscience, Lenexa, Kans.), HEPES buffer solution (10 mM), sodium pyruvate (1 mM), L-glutamine (2 mM), nonessential amino acids solution (GIBCO BRL, Grand Island, N.Y.) (0.1 mM), endothelial cell growth supplement (25 μg/ml) (Collaborative Research, Lexington, Mass.), heparin (90 μg/ml) (Sigma), penicillin (100 U/ml), and streptomycin (100 μg/ml). Third-passage HUVEC plated in 100-mm-diameter tissue culture treated plates (Corning, Corning, N.Y.) and maintained at 37°C in a 5% CO_2 atmosphere were used in all experiments. Human peripheral blood mononuclear cells were prepared from the blood of healthy volunteers by Ficoll density centrifugation, and monocytes were subsequently purified from the mononuclear layer by adherence to plastic. Chinese Hamster Ovary (CHO) cells expressing human CD14 and human TLR4 were a gift of Douglas Golenbock, Boston University School of Medicine, Boston, Mass., and are described in reference 28. CHO cells were maintained in RPMI 1640 containing 10% fetal bovine serum and supplemented with hygromycin, puromycin, or G418 as required to maintain selection.

MAP kinase assays. Before stimulation, confluent HUVEC cultures were changed to an endothelial cell growth supplement-free medium with 10% adult bovine serum. The cells were stimulated with various concentrations of *E. coli* and/or *P. gingivalis* LPS. Similarly, CHO cells cultured overnight in RPMI 1640 containing 1% fetal bovine serum were stimulated for 30 min with LPS or other stimulants and treated as described for HUVEC. Monocytes were cultured with LPS or other stimulants for 30 min in RPMI 1640 containing 10% adult bovine serum.

After stimulation with *E. coli* and/or *P. gingivalis* LPS for various time periods, the cell monolayer was washed twice with cold phosphate-buffered saline. Total cellular protein was then extracted by lysing the cells in 500 μl of lysis buffer (20 mM Tris, 137 mM NaCl, 2 mM EDTA, 10% glycerol, 1% Triton X-100, 1 μM sodium orthovanadate, 100 μM dithiothreitol, 200 μM phenylmethanesulfonyl fluoride, 10 μg of leupeptin/ml, 0.15 U of aprotinin/ml, 50 mM sodium fluoride, 10 mM sodium pyrophosphate, 2.5 μg of pepstatin A/ml, 1 mM benzamidine) at 4°C. The protein concentration was determined by the (bicinchoninic acid) protein assay (Pierce, Rockford, Ill.). Twenty micrograms of protein was subjected to sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) in 10% acrylamide gels and transferred to a Hybond-ECL nitrocellulose membrane (Amersham Pharmacia Biotech, Piscataway, N.J.). The membrane was blocked for 1 h at room temperature with 5% nonfat milk and then incubated overnight with a dual phospho-specific ERK-1/ERK-2 MAP kinase (Thr202/Tyr204) monoclonal antibody (New England Biolabs, Beverly, Mass.) or dual phospho-specific p38 MAP kinase (Thr180/Tyr182) polyclonal antibody (New England Biolabs) at 4°C. The dual phospho-specific antibodies detect MAP kinase only when activated by dual phosphorylation. These antibodies, which are raised against human p38, are known to cross-react with mouse and rat p38 and were found to cross-react with hamster p38 as well. All membranes were then incubated with a horseradish peroxidase-conjugated secondary antibody for 1 h at room temperature. The blots were developed using the SuperSignal chemiluminescent substrate (Pierce) and exposed to Kodak XAR-5 film (Eastman Kodak Company, Rochester, N.Y.). Densitometry by the NIH image program was used to quantitate optical density of the blots.

Statistics. Each experiment generated one immunoblot that was subjected to densitometry; the number of times that each experiment was performed varied and is indicated in the figure legends. The data in each figure are presented as the mean and standard of error of the mean. Significant differences in either ERK or p38 MAP kinase phosphorylation levels due to the different experiential conditions were determined by the two-tailed *t* test employing the combined raw data. Differences were considered statistically significant at *P* values of <0.05.

RESULTS

***P. gingivalis* LPS does not activate ERK or p38 MAP kinase in human endothelial cells.** *E. coli* LPS induces phosphorylation of both ERK and p38 MAP kinase in human endothelial cells (4, 52), which is necessary for *E. coli* LPS activation of IL-8 (27, 29). Since *P. gingivalis* LPS does not facilitate the activation of this inflammatory mediator (15) or E-selectin (16), initial experiments were performed to determine the effect of *P. gingivalis* LPS on MAP kinase phosphorylation. Various concentrations of either *E. coli* LPS or *P. gingivalis* LPS were added to human endothelial cells, and the ratios of phosphorylated ERK and p38 MAP kinase to the levels for a unstimulated control were determined after a 30-min incubation (Fig. 1). *E. coli* LPS activated both ERK and p38 MAP kinase in a dose-dependent manner. The ratio of *E. coli* LPS-induced MAP kinase activation to the unstimulated control level was higher for p38 than for ERK MAP kinase. In contrast, *P. gingivalis* LPS either failed to induce phosphorylation levels of either MAP kinase over control values or induced minor increases at selected LPS concentrations.

Time-dependent MAP kinase activation experiments were performed to determine if *P. gingivalis* LPS activated ERK or p38 MAP kinase with kinetics significantly different from that observed for *E. coli* LPS. *E. coli* LPS (0.1 μg/ml) or *P. gingivalis* LPS (1.0 μg/ml) was added to endothelial cells, and increases in the amounts of phosphorylated ERK and p38 MAP kinase compared to those for an unstimulated control were determined at select times for 2 h (Fig. 2). Maximum MAP kinase activation was observed for both ERK and p38 at 30 min following the addition of *E. coli* LPS. ERK phosphorylation decreased to unstimulated control values by the end of the 2-h time course, whereas p38 levels remained significantly elevated throughout the experiment. *P. gingivalis* LPS induced minor changes in ERK phosphorylation at early time points and had little or no effect on p38 phosphorylation throughout the 2-h experiment.

***P. gingivalis* LPS activates p38 MAP kinase in human monocytes.** In contrast to studies with human endothelial cells, human monocytes respond to *P. gingivalis* LPS by the CD14-dependent secretion of inflammatory mediators (16, 54). It was therefore of interest to determine if human monocytes respond to *P. gingivalis* LPS by the activation of p38 MAP kinase. Human monocytes were stimulated with various concentrations of either *E. coli* LPS or *P. gingivalis* LPS (Fig. 3). In three separate experiments, these cells responded to *P. gingivalis* LPS by the stimulation of p38 MAP kinase with a response

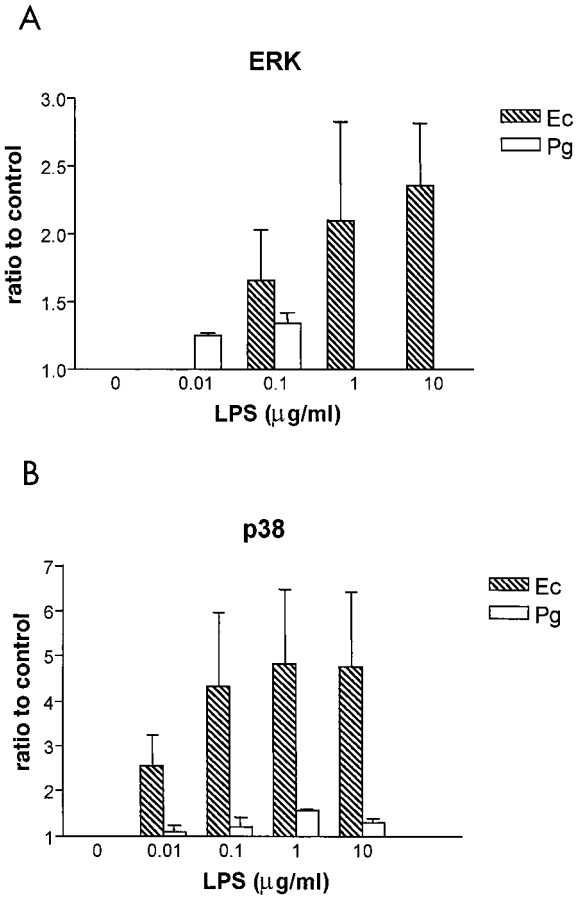

FIG. 1. MAP kinase activation in response to various doses of either *E. coli* or *P. gingivalis* LPS. HUVECs were stimulated with various concentrations of LPS (Ec, *E. coli*; Pg, *P. gingivalis*) as indicated on the horizontal axis. Total cellular protein was harvested after 30 min and subjected to SDS-PAGE followed by immunoblotting with dual phosphospecific ERK (A) and p38 (B) antibodies. Gels were scanned for densitometry analysis, and the ratio of each LPS dose to an unstimulated control is shown. The data are presented as an averages from three separate experiments (each error bar indicates the standard error of the mean).

similar to that observed for *E. coli* LPS. These data demonstrate that *P. gingivalis* LPS can elicit the activation of p38 MAP kinase in human monocytes, although no response was observed when human endothelial cells were exposed to this LPS under identical conditions.

***P. gingivalis* LPS inhibits endothelial cell p38 MAP kinase phosphorylation in response to *E. coli* LPS but not in response to tumor necrosis factor alpha (TNF-α).** As mentioned above, activation of p38 MAP kinase is necessary for *E. coli* LPS activation of inflammatory mediators, including IL-8 (27, 29). Also, *P. gingivalis* LPS inhibits endothelial cell E-selectin and IL-8 responses to *E. coli* LPS by a mechanism which does not involve competition for binding to sCD14 (13). Therefore, one possible mechanism for inhibition of *E. coli* LPS endothelial cell responses by *P. gingivalis* LPS would be the blocking of p38 MAP kinase activation by interfering in the activation pathway after CD14 presentation of LPS to the cell. The ability of *P. gingivalis* LPS to inhibit endothelial cell p38 MAP kinase activation in response to *E. coli* LPS was examined next (Fig. 4). Various combinations of *E. coli* and *P. gingivalis* LPS representing different ratios of these two LPS species were added to endothelial cells, and increases in the amount of phosphorylated p38 MAP kinase compared to an that of the unstimulated control were determined after a 30-min incubation (Fig. 4A). *E. coli* LPS (0.1 μg/ml), when added alone, stimulated phosphorylation of p38 MAP kinase; however, when combined with *P. gingivalis* LPS (1 μg/ml), p38 MAP kinase phosphorylation was significantly attenuated ($P < 0.001$ with nonpaired Student's *t* test). At a ratio of 100-fold excess *P. gingivalis* LPS to *E. coli* LPS, *E. coli* LPS-induced p38 MAP kinase phosphorylation was completely blocked by *P. gingivalis* LPS (Fig. 4A).

Although *P. gingivalis* LPS is an effective inhibitor of endothelial cell E-selectin (16) responses to *E. coli* LPS, it does not block either E-selectin expression or transcription in response to TNF-α (16), indicating that the mechanism of inhibition is unique to the LPS activation pathway. Therefore, the ability of *P. gingivalis* LPS

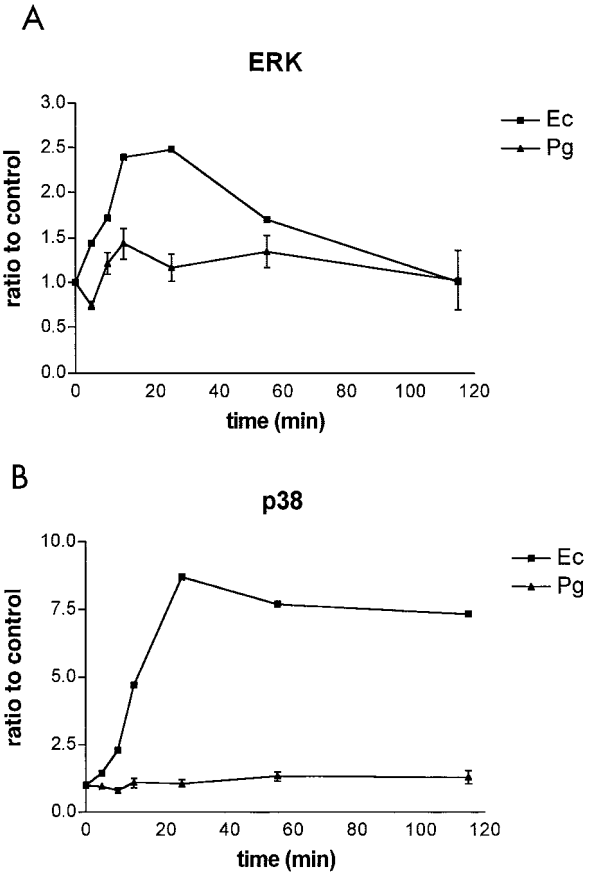

FIG. 2. Time course of MAP kinase activation in response to either *E. coli* or *P. gingivalis* LPS. HUVECs were stimulated with *E. coli* LPS at a concentration of 0.1 μg/ml (triangles) or with *P. gingivalis* LPS at a concentration of 1 μg/ml (squares). Total cellular protein was harvested at the times indicated on the horizontal axis and subjected to SDS-PAGE followed by immunoblotting with dual phosphospecific ERK (A) and p38 (B) antibodies. Gels were scanned for densitometry analysis, and the ratio of each LPS dose to an unstimulated control is shown. The data are presented as an average from two (*E. coli*) or four (*P. gingivalis*) separate experiments (each error bar indicates the standard error of the mean).

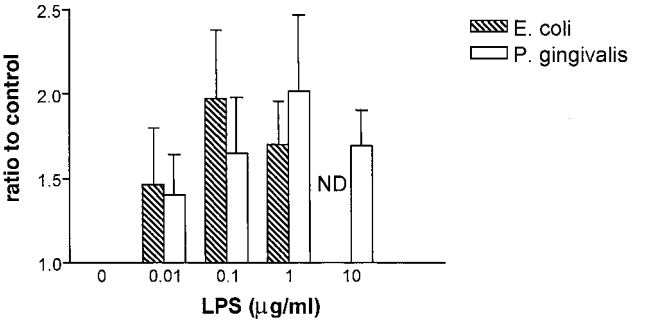

FIG. 3. Human monocyte p38 MAP kinase activation in response to various doses of either *E. coli* or *P. gingivalis* LPS. Human monocytes were stimulated with various concentrations of LPS (from *E. coli* or *P. gingivalis*) as indicated on the horizontal axis. Total cellular protein was harvested after 30 min and subjected to SDS-PAGE followed by immunoblotting with dual phosphospecific p38 antibody. Gels were scanned for densitometry analysis, and the ratio of each LPS dose to an unstimulated control is shown. The data are presented as averages from three separate experiments (each error bar indicates the standard error of the mean).

to modulate p38 MAP kinase phosphorylation in response to TNF-α was examined (Fig. 4B). Initial experiments determined that 0.01 μg/ml represented the lowest dose of TNF-α that yielded maximal phosphorylation of p38 MAP kinase (data not shown). Next, combinations of *P. gingivalis* LPS and TNF-α representing different ratios of these two components were added to endothelial cells, and the status of p38 MAP kinase phosphorylation was determined after a 30-min incubation (Fig. 4B). In contrast to the data obtained with *E. coli* LPS, combinations containing excess *P. gingivalis* LPS did not result in any significant decrease in the amount of p38 MAP kinase phosphorylation in response to TNF-α. This observation is consistent with the notion that *P. gingivalis* LPS interferes with endothelial cell activation at a site unique in the LPS activation pathway.

***P. gingivalis* LPS is a TLR-4 antagonist in CHO cells cotransfected with TLR-4 and mCD14.** TLR-4 represents a possible site where *P. gingivalis* LPS may act as an antagonist in the *E. coli* LPS activation pathway. First, TLR-4 is a key component of the CD14/*E. coli* LPS activation pathway (8, 32, 38, 62). Second, independent studies have demonstrated that different *P. gingivalis* LPS preparations may employ either TLR-4 (58) or TLR-2 (6, 30) to activate cells depending upon the cell type and or in vitro system employed. These studies indicate that although *P. gingivalis* LPS has the ability to interact with TLR-4, it may not always facilitate cellular activation. Therefore, the potential of *P. gingivalis* LPS to antagonize TLR-4-mediated *E. coli* LPS activation was examined in the CHO cells cotransfected with human TLR-4 and mCD14.

E. coli LPS (100 ng/ml) both with and without the addition of *P. gingivalis* LPS (10 μg/ml) was added to the CHO cell lines cotransfected with human TLR-4 and mCD14. The amount of p38 MAP kinase phosphorylation was determined after 30 min of coincubation by immunoblotting CHO cell extracts (Fig. 5). It was found that addition of *E. coli* LPS resulted in an increase in the amount of p38 MAP kinase phosphorylation (Fig. 5, lane 2). However, when the same concentration of *E. coli* LPS was premixed with *P. gingivalis* LPS prior to addition to the CHO cells, p38 MAP kinase phosphorylation was nearly abolished (Fig. 5, lane 4). This experiment demonstrates that p38 MAP kinase is activated in response to TLR-4 engagement of *E. coli* LPS in CHO transfected cells and that this activation is inhibited by *P. gingivalis* LPS.

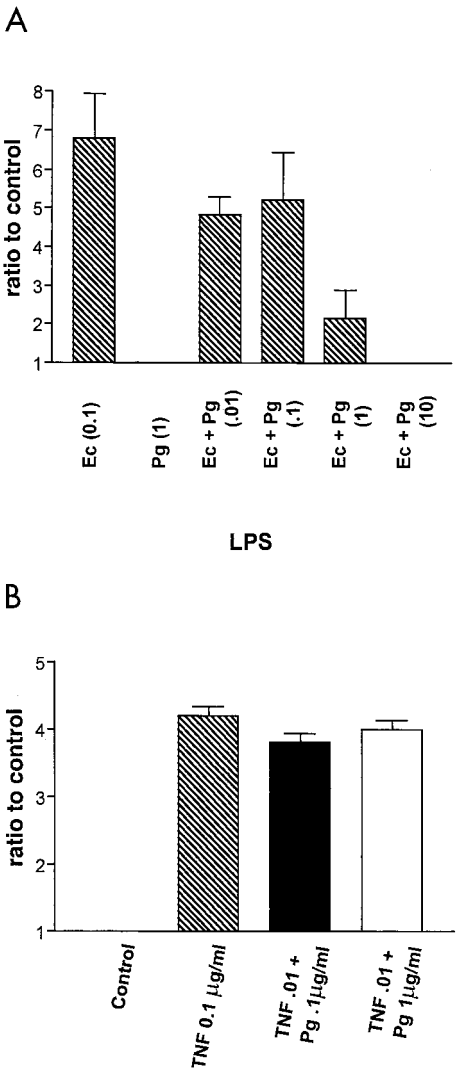

FIG. 4. Inhibition of p38 MAP kinase phosphorylation by *P. gingivalis* LPS in response to *E. coli* LPS or TNF-α. (A) *E. coli* LPS (0.1 μg/ml) was mixed with various concentrations of *P. gingivalis* LPS (indicated on the horizontal axis) prior to the addition to HUVECs. *E. coli* (0.1 μg/ml) and *P. gingivalis* (1 μg/ml) LPSs were also added alone as indicated on the horizontal axis. (B) *P. gingivalis* LPS did not inhibit p38 MAP kinase phosphorylation in response to TNF-α. TNF-α (0.01 μg/ml) alone and premixed with *P. gingivalis* LPS (0.1 and 1 μg/ml) was added to HUVECs. Total cellular protein was harvested after 30 min and subjected to SDS-PAGE followed by immunoblotting with dual phosphospecific p38 antibodies. Gels were scanned for densitometry analysis, and the ratio of TNF-α alone and in combination with *P. gingivalis* LPS to an unstimulated control is shown. The data are presented as an average from four (A) or three (B) separate experiments (each error bar indicates the standard error of the mean).

DISCUSSION

Although it is now clear that the TLR family of membrane proteins facilitates activation of host cells by LPS (34, 47, 58,

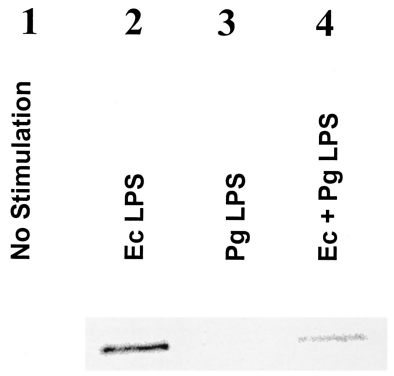

FIG. 5. P. gingivalis LPS is a TLR4 antagonist in CHO cells co-transfected with TLR4 and mCD14. CHO cells transfected with TLR4 and mCD14 were incubated with *E. coli* LPS (100 ng/ml) with or without 10 μg of *P. gingivalis* LPS/ml for 30 min in the presence of 1% normal human serum and lysed for determination of p38 by immunoblotting with phospho-specific p38 antibodies as described in the text. A representative blot was chosen from four identical experiments.

66), the subsequent pathways that lead to inflammatory mediator production are still being elucidated. Evidence demonstrates that both a Rac-1 (3) and a p38 MAP kinase dependent pathway (51) are activated after LPS or LTA interactions with TLRs. Endothelial cell secretion of IL-8 in response to LPS is dependent upon both pathways (29), which is consistent with other studies that have shown a key role for p38 MAP kinase in endothelial cells responses to *E. coli* LPS (27, 41, 52, 59). However, LPSs obtained from different bacterial species have significant variations in their abilities to activate host cells (5, 9, 16, 22, 40, 57), and it is not known if this correlates to their ability to activate p38 MAP kinase. For example, *P. gingivalis* LPS obtained from a variety of different strains (33277, 5083, 381, A7-A128, and A7436) in contrast to *E. coli* LPS fails to elicit E-selectin expression or IL-8 secretion 16; also unpublished observations) from human endothelial cells. However, both LPS species activate human monocytes, although the response to LPS obtained from *P. gingivalis* is both less potent (7, 16, 22, 43, 44, 60) and elicits a different pattern of inflammatory mediators (43, 60) relative to *E. coli* LPS. The present study demonstrated that p38 MAP kinase phosphorylation correlated with the ability of monocytes and endothelial cells to elicit secretion of inflammatory mediators in response to *P. gingivalis* LPS. Since p38 MAP kinase phosphorylation is one of the earliest events after LPS presentation to host cells, this correlation suggests that these two different cell types detect *P. gingivalis* LPS differently.

Two of the major differences between how monocytes and endothelial cells recognize LPS are the utilization of either the membrane (mCD14) or soluble (sCD14) form of CD14 (48) and TLR engagement (20). It has been demonstrated that mCD14 and sCD14 display different structural requirements for LPS binding (63). Therefore, it is possible that although *P. gingivalis* LPS binds both forms of CD14 (14, 54), it may bind them differently, leading to activation with mCD14 in monocytes (54) and failure to functionally engage *P. gingivalis* LPS/sCD14 complexes in the endothelial cell activation pathway (14). In addition, endothelial cells express a higher TLR-4/TLR-2 ratio than do human monocytes, and the ratio of these two TLRs may explain in part why endothelial cells respond to microbial components that engage TLR-4 but not TLR-2 (20). Therefore, the failure of *P. gingivalis* LPS to activate p38 MAP kinase in human endothelial cells is consistent with the notion that sCD14/*P. gingivalis* LPS complexes cannot functionally engage TLR-4 in these cells.

Existing data support the notion that TLR-4 may facilitate *P. gingivalis* LPS activation in some cell types but not others. For example, *P. gingivalis* LPS can employ TLR-4 (58) to facilitate gingival fibroblast activation, presumably by employing mCD14 that is expressed on these cells. However, the lack of p38 MAP kinase activation by *P. gingivalis* LPS in the TLR-4 mCD14 CHO transfected line reported here (Fig. 5), and previous observations with these cells by us and others where neither PGE_2 secretion (6) nor CD25 expression (30) was detected in response to this LPS, indicate that TLR-4 may not always be able to facilitate cell activation with this LPS. Furthermore, in this report it is demonstrated that *P. gingivalis* LPS is an effective antagonist for *E. coli* LPS activation of p38 MAP kinase in the TLR-4/mCD14-transfected CHO and endothelial cells. Based upon these observations, we propose that in some cell types *P. gingivalis* LPS can be a functional antagonist for TLR-4 and forms an incomplete binding complex (19) that remains "locked" and unable to activate or dissociate effectively, blocking *E. coli* and other bacterial components from functionally interacting with this receptor, whereas in others cell types or cells expressing a different repertoire of innate host defense response molecules, *P. gingivalis* LPS can elicit inflammatory responses.

One possible in vivo manifestation of *P. gingivalis* LPS antagonism with endothelial cells may be modulation of the dynamic balance that exists between commensal dental plaque bacteria and the host response. The innate host response is critical to maintaining a clinically healthy periodontium (18, 61), and studies have shown that in healthy individuals a highly orchestrated protective response is found (18, 61). *P. gingivalis,* which resides in close proximity to the gingival epithelial cell surface, releases copious amounts of outer membrane vesicles containing LPS (23, 24) which can penetrate periodontal tissue (37, 39, 53) and have direct inhibitory effects on the highly vascularized tissue adjacent to the tooth root surface. The interruption of endothelial cell responses to LPS is similar to earlier observations (15, 35) demonstrating inhibition of IL-8 secretion after invasion of epithelial cells by viable *P. gingivalis*. Both of these observations are consistent with a bacterial colonization strategy that creates localized areas of paralysis of innate host defense by direct interaction with host cells. We propose that disruption in the periodontium of inflammatory mediator secretion that normally serves to protect the host from infection may lead to disease.

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