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Short communication

Actinobacillus actinomycetemcomitans lipopolysaccharide stimulates collagen phagocytosis by human gingival fibroblasts

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Takahashi N, Kobayashi M, Takaki T, Takano K, Miyata M, Okamatsu Y, Hasegawa K, Nishihara T, Yamamoto M. Actinobacillus actinomycetemcomitans *lipopolysaccharide stimulates collagen phagocytosis by human gingival fibroblasts.*
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Introduction: Collagen phagocytosis by fibroblasts is involved in the intracellular pathway related to collagen breakdown in soft connective tissues. The possible role of lipopolysaccharide (LPS) in regulating this fibroblast function has not been elucidated so we investigated the effect of LPS from *Actinobacillus actinomycetemcomitans*, a periodontopathic bacterium, on collagen phagocytic activity in human gingival fibroblasts and associated regulatory mechanisms.
Methods: LPS pretreatment stimulated binding of collagen-coated beads to cells and, subsequently, their internalization.
Results: The LPS-activated collagen phagocytic process was enhanced in the presence of the soluble form of CD14 (sCD14) or LPS-binding protein (LBP), while the LPS/LBP treatment activated Akt and induced actin reorganization. Furthermore, these LPS/LBP-induced effects were partially suppressed by adding phosphatidyl-inositol-3 kinase (PI3K) inhibitors.
Conclusion: These results suggest that *A. actinomycetemcomitans* LPS disturbs the homeostasis of collagen metabolism within gingival tissue by facilitating collagen phagocytosis by gingival fibroblasts, and serum sCD14 and LBP positively regulate the action of LPS. In addition, the PI3K/Akt signaling is thought to partially mediate the LPS/LBP-stimulated collagen phagocytic pathway, which may be dependent on actin cytoskeletal rearrangement.

Key words: *Actinobacillus actinomycetemcomitans;* actin reorganization; collagen phagocytosis; human gingival fibroblasts; β_1-integrin; lipopolysaccharide; phosphatidyl-inositol-3 kinase/Akt

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Lipopolysaccharide (LPS) from *Actinobacillus actinomycetemcomitans* is well characterized as an important pathogenic component in the initiation and progression of periodontal disease, because it stimulates host cells to produce inflammatory cytokines and induces bone resorption (16, 22).

The breakdown of collagen is enhanced in inflamed gingival tissue, and both extracellular and intracellular pathways are involved in that breakdown. Of the two pathways, the intracellular pathway, which involves phagocytosis of collagen fibrils by fibroblasts and subsequent digestion by lysosomal cysteine proteinases, is suggested to be the major route of collagen degradation under steady-state conditions in soft connective tissues (6, 7). In particular, the intracellular collagen degradation is prevalent in connective tissues with a rapid turnover of collagen, including gingival tissue (21). Furthermore, inflammatory cytokines such as tumor necrosis

factor-α and interferon-γ decrease collagen phagocytosis by gingival fibroblasts (5, 27), suggesting that the inhibition by these inflammatory cytokines of fibroblast collagen phagocytosis contributes to collagen overgrowth in inflamed gingival tissue. However, little is known about the regulatory effect of LPS from periodontopathogens on collagen phagocytosis by fibroblasts and the associated regulatory mechanisms.

We first examined the modulation of phagocytic activity by *A. actinomycetemcomitans* LPS in human gingival fibroblasts (HGF). The HGF were isolated from attached gingival tissues, which had been obtained from three adult male volunteers, each with clinically healthy periodontium. The volunteers were fully informed of the nature and extent of the study, and their informed consent was obtained. The protocol for this human study was approved by the ethical committee of Showa University School of Dentistry, Tokyo, Japan. The collected tissues were cultured until confluent cell monolayers were formed. Typical HGF populations isolated from the tissues of each of the three volunteers were designated as HGF-1, -2, and -3. Collagen phagocytic activity in HGF was determined using an assay for internalization of collagen-coated beads, following the previously published method (27). Similar to the findings of previous studies (5, 27), when HGF were incubated with collagen-coated beads for 3 h in medium with 0.5% bovine serum albumin (BSA), the percentage of cells that contained ingested beads was increased (mean 22.3%) (data not shown). We next investigated the effect of LPS on the phagocytosis of collagen-coated beads by HGF. Our preliminary experiment showed that the stimulatory effect of LPS on collagen phagocytosis by HGF treated in medium with 10% fetal bovine serum was significantly stronger than that on phagocytosis by cells treated in medium with 0.5% BSA (data not shown). Then, to avoid the biological effects of growth factors and hormones, as well as a soluble form of CD14 (sCD14)/LPS-binding protein (LBP) contained in fetal bovine serum, we assessed the LPS action in medium with 0.5% BSA. When the cells were pretreated with *A. actinomycetemcomitans* Y4 LPS, which was provided by T. Nishihara (Department of Oral Microbiology, Kyushu Dental College, Fukuoka, Japan), for 72 h, the subsequent internalization of collagen-coated beads was weakly, but significantly, stimulated by that pretreatment at concentrations above 100 ng/ml (mean % of cells containing beads; 36.7% at 100 ng/ml and 39.3% at 1000 ng/ml vs. control, 26.2%) (Fig. 1A,B). Further, pretreatment with LPS (100 ng/ml) for 24–72 h stimulated the subsequent internalization of collagen-coated beads in a time-dependent manner (Fig. 1C). The percentage of cells with internalized collagen-coated beads was significantly increased by LPS pretreatment for each period (LPS vs. control; mean 31.1% vs. 24.1% after pretreatment for 24 h, 37.5% vs. 25.7% after pretreatment for 48 h, 39.5% vs. 26.2% after pretreatment for 72 h) (Fig. 1C). When the cells were pretreated with LPS from *Escherichia coli* 055 : B5 (100 ng/ml; Sigma, St Louis, MO) for 72 h, a similar percentage (mean 35.4%) of cells internalized collagen-coated beads (data not shown).

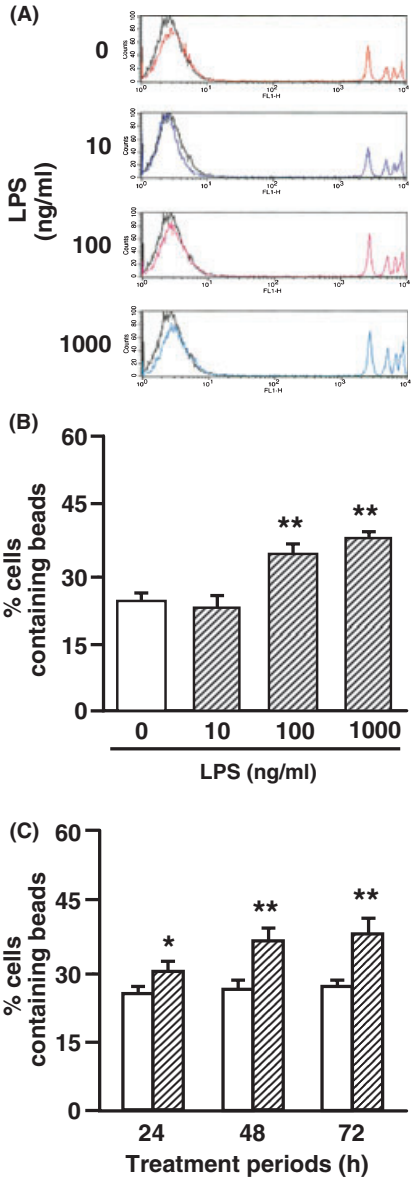

Fig. 1. LPS facilitates the phagocytosis of collagen-coated beads by HGF. Cells at confluence were pretreated with various concentrations of *Actinobacillus actinomycetemcomitans* LPS (10–1000 ng/ml) for 72 h (A, B) or pretreated with LPS (100 ng/ml) for 24–72 h (C) in medium with 0.5% BSA. Following each treatment, collagen-coated yellow-green (YG) fluorescent beads, which were prepared according to the method described by Lee et al. (18), were added to the cell cultures at a bead-to-cell ratio of 4 : 1 (2×10^6 beads) and incubated for 3 h in medium containing 0.5% BSA at 37°C. Following incubation, the cells were washed, detached with 0.01% trypsin at 37°C for 10 min, then centrifuged and resuspended in 1 ml phosphate-buffered saline. Internalization of the beads was then analyzed using a flow cytometer (FACScalibur™; Becton Dickinson, Mountain View, CA) with excitation set at 488 nm and a 530/30 nm band-pass filter in the emission path for the YG fluorescent beads. (A) Data are representative of the results of one of three separate experiments with HGF-1 cells. Similar results were obtained with HGF-2 and -3 cells (data not shown). (B, C) The graphs demonstrate the percentages of cells that contained beads. Values are shown as the mean ± SD of three separate experiments for each of the three populations of HGF. Differences from the values for untreated cells were considered to be significant at $^{**}P < 0.01$ and $^{*}P < 0.05$.

This stimulatory effect of LPS, therefore, may be a common characteristic of gram-negative bacteria.

Since the binding of collagen is an early and potentially rate-limiting step in collagen phagocytosis by fibroblasts, we further examined the effect of *A. actinomycetemcomitans* LPS on the binding of collagen-coated beads to HGF, according to the previously described method (27). Similar to our previous findings (27), the number of collagen-coated beads bound to the cell layer was increased 1 h after adding beads to the culture (data not shown). As expected, the increased bead binding was weakly, but significantly, enhanced by pretreatment with LPS at concentrations above 100 ng/ml for 72 h (vs. control; mean 1.3-fold increase at 100 ng/ml, 1.4-fold increase at 1000 ng/ml) (Fig. 2A). Furthermore, a significant increase in bead binding was observed with LPS pretreatment (100 ng/ml) for more than 24 h (Fig. 2B).

Both sCD14 and LBP are serum proteins and play key roles in LPS recognition. The sCD14 binds LPS monomers from LPS–LBP complexes, and participates in cell activation by transferring monomeric LPS to membrane CD14 (mCD14) (14) or directly to MD-2/Toll-like receptor 4 (TLR4) complex on mCD14-negative cells (9, 25). LBP also

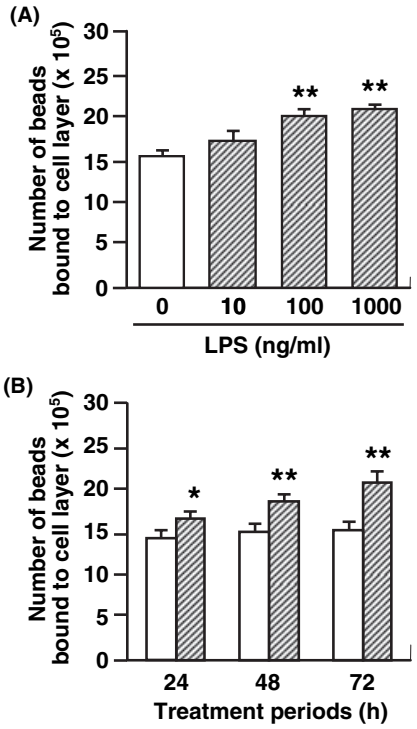

Fig. 2. LPS stimulates collagen-coated bead binding to HGF. Cells at confluence were pretreated with various concentrations of LPS (10–1000 ng/ml) for 72 h (A) or pretreated with LPS (100 ng/ml) for 24–72 h (B) in medium with 0.5% BSA. Following each treatment, collagen-coated beads were added to the cell cultures at a bead-to-cell ratio of 4 : 1 (2×10^6 beads) and incubated for 1 h in medium containing 0.5% BSA at 37°C. The incubation medium was then gently collected, and the number of beads in each medium sample (unbound beads) was counted with a hemocytometer. The number of beads bound to the cell layer was then determined by subtracting the number of unbound beads from the number of added beads. The graphs demonstrate the number of beads bound to the cell layers. Values are shown as the mean ± SD of three separate experiments for each of the three populations of HGF. Differences from the values for untreated cells were considered to be significant at $^{**}P < 0.01$ and $^{*}P < 0.05$.

transfers LPS monomers to the binding sites of either mCD14 or sCD14 (33), and the resulting interaction of LPS with sCD14 or mCD14 activates signaling through MD-2/TLR4. The activities of sCD14 and LBP are generally concentration-dependent, because low concentrations of sCD14 and LBP augment responses to LPS, whereas high concentrations of these are inhibitory (9, 14, 17, 25). In gingival tissue, the LPS-increased collagen phagocytosis by fibroblasts may be modulated by the sCD14 and LBP contained in serum. Flow cytometric analysis demonstrated that the three populations of HGF expressed weak but similar levels of mCD14 (% positive cells; mean 13.8%) and TLR4 (% positive cells; mean 25.1%) on the cell surfaces (Fig. 3A), suggesting that the weak responsiveness to LPS in the present HGF may have been the result of the relatively low frequency of expression of mCD14 and TLR4. Then, we investigated the regulatory effects of sCD14 and/or LBP on *A. actinomycetemcomitans* LPS-stimulated binding and internalization of collagen-coated beads. Before the addition of collagen-coated beads, the cells were pretreated for 48 h with LPS (100 ng/ml) or nothing in the presence or absence of sCD14 and/or LBP (1–100 ng/ml) in medium with 0.5% BSA. Neither sCD14 (100 ng/ml) nor LBP (100 ng/ml) had a significant effect on the subsequent bead binding for 1 h or on internalization for 3 h, while each of the proteins at concentrations of 100 ng/ml significantly enhanced LPS-stimulated binding and internalization of the beads (binding – sCD14: mean 1.3-fold increase, LBP: mean 1.5-fold increase; internalization – sCD14: mean 1.2-fold increase, LBP: mean 1.3-fold increase, vs. pretreatment with LPS alone) (Fig. 3B,C). However, the synergistic enhancement by sCD14 and LBP could not be observed (Fig. 3B,C).

Actin, a major cytoskeletal protein, may be required for LPS-activated cellular signaling and its functions. Actually, LPS induces reorganization of actin filaments in various cell types, while the actin arrangement in response to LPS leads to changes in cellular functions, such as adhesion, spreading, phagocytosis, cytokine production, and barrier dysfunction (1, 3, 4, 12, 32). However, since an involvement of actin in LPS-modulated collagen phagocytosis by fibroblasts has not been elucidated, we examined cytoskeletal changes induced by co-treatment with *A. actinomycetemcomitans* LPS and LBP in HGF. At the start of treatment (Time 0), cultured HGF-1 cells showed abundant stress fibers stained with rhodamine–phalloidin (Fig. 4). Following co-treatment with LPS (100 ng/ml) and LBP (100 ng/ml) for 12 h, the cells exhibited reorganization of actin filaments. Namely, most stress fibers were eliminated, whereas only staining of actin filaments subjacent to the plasma membrane, in part, as well as perinuclear actin staining could be detected (Fig. 4). Such cytoskeletal changes were also observed after treatment for 24, 48, and 72 h (data not shown). Similar results were obtained with HGF-2 and HGF-3 cells (data not shown).

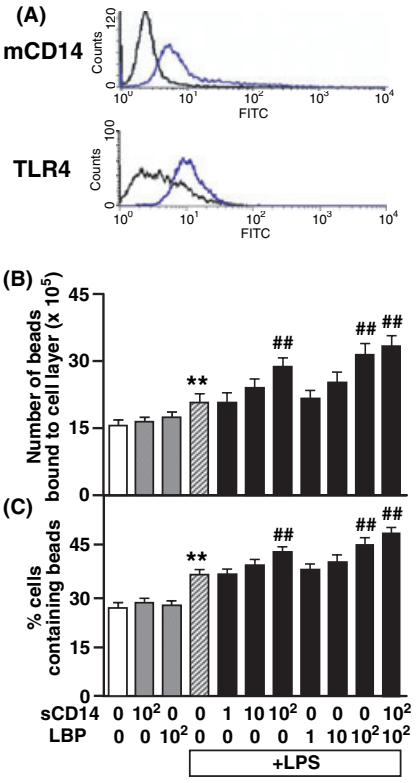

Fig. 3. LPS-stimulated binding and phagocytosis of collagen-coated beads are enhanced in the presence of sCD14 or LBP. (A) For analysis of mCD14 expression, cells at confluence were stained with fluorescein isothiocyanate (FITC)-conjugated anti-CD14 monoclonal antibody MY4 (blue line; Coulter Co., Miami, FL) or FITC-conjugated mouse immunoglobulin G2b (IgG2b; black line; Coulter) as a control at 4°C for 30 min. For analysis of TLR4 expression, the cells were stained with the anti-TLR4 monoclonal antibody HTA125 (blue line; provided by Dr K. Miyake; The Institute of Medical Science, The University of Tokyo, Tokyo, Japan) or mouse IgG2a (black line; Coulter) as a control at 4°C for 30 min, followed by FITC-conjugated goat anti-mouse IgG (Biosource International Inc., Camerillo, CA) at 4°C for an additional 30 min. Flow cytometric analysis was performed using the FACScalibur™. Data are representative of the results of one of three separate experiments with HGF-1 cells. Similar results were obtained with HGF-2 and -3 cells (data not shown). (B, C) Cells at confluence were pretreated for 48 h with LPS (100 ng/ml) in the presence or absence of sCD14 (1–100 ng/ml) and/or LBP (1–100 ng/ml) in medium with 0.5% BSA, before incubation with collagen-coated YG fluorescent beads for 1 h (B) or 3 h (C). The graphs demonstrate the number of beads bound to the cell layer (B) and the percentages of cells containing beads (C). Values are shown as the mean ± SD of three separate experiments for each of the three populations of HGF. Differences from the values for the control cells and LPS-treated cells were considered to be significant at $^{**}P < 0.01$ and $^{\#\#}P < 0.01$, respectively.

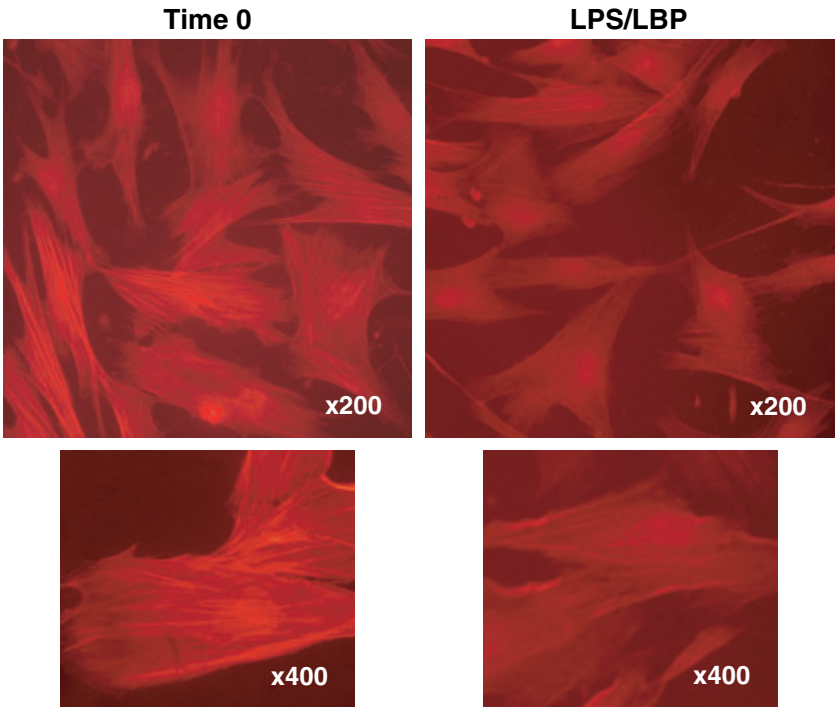

Fig. 4. Co-treatment with LPS and LBP induces actin reorganization. Cells seeded on eight-well chamber slides were co-treated with LPS (100 ng/ml) and LBP (100 ng/ml) in medium with 0.5% BSA for 12 h. Following each treatment, the cells were fixed with 4% formaldehyde, permeabilized with 0.1% Triton X-100 in phosphate-buffered saline and stained with rhodamine–phalloidin (diluted 1 : 500: Molecular Probes, Eugene, OR). Photographs were taken with an epifluorescence microscope. Each photograph is representative of the results of one of three separate experiments with HGF-1 cells. Similar results were obtained with HGF-2 and -3 cells (data not shown).

Cytochalasin D inhibits actin polymerization by binding to the barbed end of actin filaments, thereby preventing actin filament formation. Furthermore, cytochalasin D has been shown to inhibit collagen-coated bead binding in gingival fibroblasts (26). We, therefore, assessed the effects of cytochalasin D on increased binding and internalization of collagen-coated beads by pretreatment with both LPS and LBP in HGF. As described in the previous study (26), pretreatment for 30 min with cytochalasin D (1 μM) significantly reduced the subsequent binding and internalization of collagen-coated beads as compared with the control cells pretreated with dimethylsulfoxide alone (data not shown). Furthermore, LPS/LBP-stimulated binding and internalization of collagen-coated beads were also significantly suppressed to nearly the levels of the control by cytochalasin D (data not shown).

Phosphatidyl-inositol-3 kinase (PI3K) is activated in response to LPS stimulation, and it has been shown to both positively and negatively regulate LPS-induced production of cytokines and matrix metalloproteinase-9 (MMP-9) in monocytes/macrophages (13, 19, 23). PI3K is also widely implicated in the regulation of the actin cytoskeleton (30). In addition, this kinase plays an important role in various cellular responses, such as lens cell differentiation (31) and keratinocyte migration (24), through reorganization of the actin cytoskeleton. Then, to investigate the possible role of PI3K in *A. actinomycetemcomitans* LPS/LBP-facilitated binding and internalization of collagen-coated beads in HGF, the cells were incubated with LY294002 (25 μM) or wortmannin (50 μM) (PI3K inhibitors) for 30 min before pretreatment with both LPS and LBP. LY294002 and wortmannin each significantly suppressed the subsequent binding and internalization of collagen-coated beads as compared with the control cells (Fig. 5A,B). Furthermore, each inhibitor partially, but significantly, reduced LPS/LBP-stimulated bead binding and internalization (Fig. 5A,B). Since Akt is one of the well-characterized downstream effectors of PI3K, we also determined the LPS activation of Akt in HGF by Western blotting. HGF-1 cells treated with LPS (100 ng/ml) showed a weak increase in the phosphorylated form of Akt expression, which peaked after 60 min stimulation with LPS (Fig. 5C). Akt phosphorylation facilitated by LPS treatment for 15 min was enhanced in the presence of sCD14 (100 ng/ml) or LBP (100 ng/ml), while the synergistic enhancement of LPS-activated Akt by simultaneous treatment with sCD14 and LBP was not observed (Fig. 5D). Similar results were obtained with HGF-2 and -3 cells (data not shown). Finally, we assessed whether PI3K mediates LPS/LBP-induced actin reorganization in HGF. Pretreatment with wortmannin (50 μM) for 30 min abolished actin reorganization, such as the disappearance of stress fibers, induced by co-treatment with LPS and LBP for 12 h in HGF-1 cells (Fig. 5E). Similar results were obtained with HGF-2 and -3 cells (data not shown).

The present study is the first to demonstrate that *A. actinomycetemcomitans* LPS activates the collagen phagocytic pathway in gingival fibroblasts. The stimulation by LPS of collagen phagocytosis by fibroblasts is supported by a previous *in vivo* study, which found that an application of LPS to a gingival sulcus stimulated phagocytic activity of collagen fibrils in fibroblasts that existed within the periodontal ligament area (15). On the other hand, Bodet et al. (2) recently demonstrated that *A. actinomycetemcomitans* LPS stimulated the production of a urokinase-type plasminogen activator, MMP-2, and to lesser extent MMP-3, by gingival fibroblasts. They also showed that stimulating the cells with the LPS resulted in an increased production of tissue inhibitor of metalloproteinases-1 (TIMP-1); however, this up-regulation of TIMP-1 was likely not sufficient to compensate the increased MMP expression (2). Therefore, *A. actinomycetemcomitans* LPS may enhance collagen degradation in gingival tissue by stimulating both extracellular and intracellular pathways in gingival fibroblasts.

Various LPS-induced cellular functions in fibroblasts and dental pulp cells, as well as in monocytes, have been shown to be enhanced by adding low concentrations (< 1 μg/ml) of sCD14 or LBP (8, 10, 20). Also in the present study, sCD14 and LBP at 100 ng/ml each significantly augmented the *A. actinomycetemcomitans* LPS-increased binding and internalization of collagen-coated beads in HGF. Since sCD14 and LBP are present in serum at concentrations > 2 μg/ml (28, 34) and 10> μg/ml (11, 29), respectively, the present data suggest that physiological concentrations of serum sCD14 and LBP positively regulate the LPS-activated

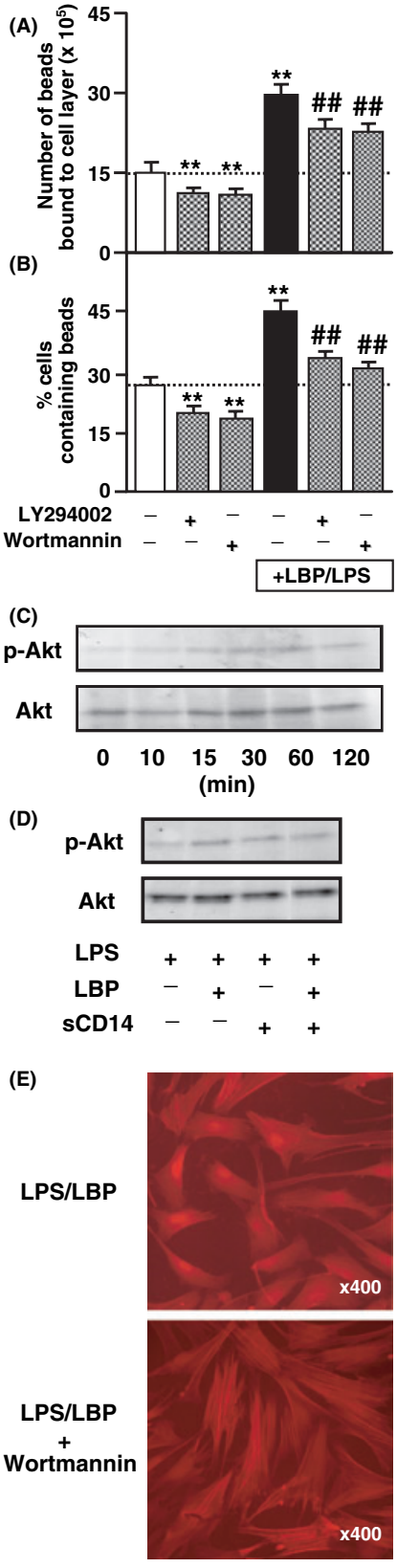

Fig. 5. PI3K/Akt mediates collagen-coated bead binding and phagocytosis stimulated by LPS/LBP. (A, B) Cells at confluence were pretreated with LY294002 (25 μM; Calbiochem, San Diego, CA) or wortmannin (50 μM; Calbiochem) (PI3K inhibitors) for 30 min and then pretreated with both LPS (100 ng/ml) and LBP (100 ng/ml) for 72 h, before incubation with collagen-coated YG fluorescent beads for 1 h (A) or 3 h (B). The graphs demonstrate the number of beads bound to the cell layer (A) and the percentages of cells containing beads (B). Values are shown as the mean ± SD of three separate experiments for each of the three populations of HGF. Differences from the values for the control cells and LPS-treated cells were considered to be significant at $^{**}P < 0.01$ and $^{\#\#}P < 0.01$, respectively. (C, D) Cells were treated for 10–120 min with LPS (C) or treated for 15 min with LPS alone or in combination with sCD14 (100 ng/ml) and/or LBP (D). Following each treatment, the phosphorylation of Akt was determined by a conventional method for Western blotting, which used each polyclonal antibody against Akt and phosphorylated Akt (diluted 1 : 1000; Cell Signaling Technology Inc., Beverly, MA) and horseradish peroxidase (HRP)-linked anti-rabbit immunoglobulin G (IgG; diluted 1 : 3000; Cell Signaling). (E) Cells were pretreated with wortmannin or nothing for 30 min before simultaneous treatment with LPS and LBP for 12 h. Following each treatment, the cells were fixed, permeabilized, and stained with rhodamine–phalloidin. Photographs were taken under an epifluorescence microscope. (C–E) Each photograph is representative of the results of one of three separate experiments with HGF-1 cells. Similar results were obtained with HPL-2 and -3 cells (data not shown).

collagen phagocytic process in gingival fibroblasts.

Both an increase in actin synthesis and actin reorganization have been demonstrated upon LPS treatment in endothelial cells (12) and fibroblasts (4). We also showed here that HGF co-treated with *A. actinomycetemcomitans* LPS and LBP exhibited similar reorganization of actin filaments. Furthermore, cytochalasin D, which prevents actin filament formation, significantly suppressed LPS/LBP-stimulated internalization as well as binding of collagen-coated beads to almost the control levels. These findings, therefore, suggest an involvement of actin reorganization in LPS/LBP-enhanced collagen binding and phagocytosis in gingival fibroblasts. On the other hand, *A. actinomycetemcomitans* LPS weakly stimulated the phosphorylation of Akt in HGF, and the LPS activation of Akt was enhanced by separate co-treatments with sCD14 and LBP. This may result in the enhancement by sCD14 or LBP of PI3K/Akt-mediated cellular responses to LPS treatment. In addition, PI3K inhibitors partially, but significantly, attenuated the increased collagen-coated bead binding and internalization by pretreatment with both LPS and LBP. The present results, therefore, suggest the involvement of the PI3K/Akt signaling pathway in the LPS/LBP-facilitated collagen phagocytic process in HGF. PI3K is thought to mediate the LPS/LBP-induced actin reorganization in HGF because actin reorganization induced by both LPS and LBP was abolished by a PI3K inhibitor. Together, *A. actinomycetemcomitans* LPS/LBP may induce actin reorganization, leading to enhancement of the phagocytic route of collagen in gingival fibroblasts through the PI3K/Akt signaling pathway, at least in part.

In conclusion, *A. actinomycetemcomitans* LPS may disturb the homeostasis of collagen metabolism within gingival tissue by facilitating collagen phagocytosis by fibroblasts, and sCD14 and LBP in serum may positively regulate the action of LPS. In addition, the PI3K/Akt signaling pathway is thought to partially mediate the LPS/LBP-stimulated collagen phagocytic pathway, which may be dependent on actin cytoskeletal rearrangement.

Acknowledgments

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References

1. Abd-el Basset E, Fedoroff S. Effect of bacterial wall lipopolysaccharide (LPS) on morphology, motility, and cytoskeletal organization of microglia in cultures. J Neurosci Res 1995: **41**: 222–237.
2. Bodet C, Andrian E, Tanabe S, Grenier D. Actinobacillus actinomycetemcomitans lipopolysaccharide regulates matrix metalloproteinase, and tissue inhibitors of matrix metalloproteinase, and plasminogen activator production by human gingival fibroblasts: a potential role in connective tissue destruction. J Cell Physiol 2007: **212**: 189–194.
3. Burns S, Hardy SJ, Buddle J, Yong KL, Jones GE, Thrasher AJ. Maturation of DC is associated with changes in motile characteristic and adherence. Cell Motil Cytoskeleton 2004: **57**: 118–132.
4. Chakravortty D, Kumar KSN. Bacterial lipopolysaccharide induces cytoskeletal rearrangement in small intestinal lamina propria fibroblasts: actin assembly is essential for lipopolysaccharide signaling. Biochim Biophys Acta 2000: **1500**: 125–136.
5. Chou DH, Lee W, McCulloch CA. TNF-alpha inactivation of collagen receptors:

implications for fibroblast function and fibrosis. J Immunol 1996: **156**: 4354–4362.
6. Everts V, Beertsen W. The cellular basis of tooth eruption: the role of collagen phagocytosis. In: Davidovitch Z, ed. The biological mechanisms of tooth eruption and root resorption. Birmingham: EBSCO Media, 1988: 237–242.
7. Everts V, Hembry RM, Reynolds JJ, Beertsen W. Metalloproteinases are not involved in the phagocytosis of collagen fibrils by fibroblasts. Matrix 1989: **9**: 266–276.
8. Flo TH, Ryan L, Kilaas L et al. Involvement of CD14 and β_2-integrins in activating cells with soluble and particulate lipopolysaccharides and mannuronic acid polymers. Infect Immun 2000: **68**: 6770–6776.
9. Frey EA, Miller DS, Jahr TG et al. Soluble CD14 participates in the response of cells to lipopolysaccharide. J Exp Med 1992: **176**: 1665–1671.
10. Fukuoka K, Kumagai N, Yamamoto K, Fujitsu Y, Chikamoto N, Nishida T. Potentiation of lipopolysaccharide-induced chemokine and adhesion molecule expression in corneal fibroblasts by soluble CD14 or LPS-binding protein. IOVS 2005: **46**: 3095–3101.
11. Gallay P, Barras C, Tobias PS et al. Lipopolysaccharide (LPS)-binding protein in human serum determines the tumor necrosis factor response of monocytes to LPS. J Infect Dis 1994: **170**: 1319–1322.
12. Goldblum SE, Ding X, Brann TW, Campbell-Washington J. Bacterial lipopolysaccharide induces actin reorganization, intercellular gap formation, and endothelial barrier dysfunction in pulmonary vascular endothelial cells: concurrent F-actin depolymerization and new actin synthesis. J Cell Physiol 1993: **157**: 13–23.
13. Guha M, Mackman N. The phosphatidylinositol 3-kinase-Akt pathway limits lipopolysaccharide activation of signaling pathways and expression of inflammatory mediators in human monocytic cells. J Biol Chem 2002: **277**: 32124–32132.
14. Hailman E, Vasselon T, Kelley M et al. Stimulation of macrophages and neutrophils by complexes of lipopolysaccharide and soluble CD14. J Immunol 1996: **156**: 4384–4390.
15. Ijuhin N, Miyauchi M, Ito H, Takata T, Ogawa I, Nikai H. Enhanced collagen phagocytosis by rat molar periodontal fibroblasts after topical application of lipopolysaccharide – ultrastructual observations and morphometric analysis. J Periodontal Res 1992: **27**: 167–175.
16. Ishihara Y, Nishihara T, Maki E, Noguchi T, Koga T. Role of interleukin-1 and prostaglandin in *in vitro* bone resorption induced by *Actinobacillus actinomycetemcomitans* lipopolysaccharide. J Periodontal Res 1991: **26**: 155–160.
17. Lamping N, Dettmer R, Schröder NW et al. LPS-binding protein protects mice from septic shock caused by LPS or Gram-negative bacteria. J Clin Invest 1998: **101**: 2065–2071.
18. Lee W, Sodek J, McCulloch CA. Role of integrins in regulation of collagen phagocytosis by human fibroblasts. J Cell Physiol 1996: **168**: 695–704.
19. Lu Y, Wahl LM. Production of matrix metalloproteinase-9 by activated human monocytes involves a phosphatidylinositol 3-kinase/Akt/IKKalpha/NF-kappaB pathway. J Leukoc Biol 2005: **78**: 259–265.
20. Matsushita K, Motani R, Sakuta T et al. Lipopolysaccharide enhances the production of vascular endothelial growth factor by human pulp cells in culture. Infect Immun 1999: **67**: 1633–1639.
21. Melcher AH, Chan J. Phagocytosis and digestion of collagen by gingival fibroblasts *in vivo*: a study of serial sections. J Ultrastruct Res 1981: **77**: 1–36.
22. Mochizuki S, Kobayashi M, Suzuki T et al. IFN-γ enhances expression of CD14/MyD88 and subsequent responsiveness to lipopolysaccharide from *Actinobacillus actinomycetemcomitans* in human gingival fibroblasts. J Periodontal Res 2004: **39**: 333–343.
23. Ojaniemi M, Glumoff V, Haraju K, Liljeroos M, Vuori K, Hallman M. Phosphatidylinositol 3-kinase is involved in toll-like receptor 4-mediated cytokine expression in mouse macrophages. Eur J Immunol 2003: **33**: 597–605.
24. Pankow S, Bamberger C, Klippel A, Werner S. Regulation of epidermal homeostasis and repair by phosphoinositide 3-kinase. J Cell Sci 2006: **119**: 4033–4046.
25. Pugin J, Schürer-Maly C-C, Leturcq D, Moriarty A, Ulevitch RJ, Tobias PS. Lipopolysaccharide activation of human endothelial and epithelial cells is mediated by lipopolysaccharide-binding protein and soluble CD14. Proc Natl Acad Sci U S A 1993: **90**: 2744–2748.
26. Segal G, Lee W, Arora PD, McKee M, Downey G, McCulloch CAG. Involvement of actin filaments and integrins in the binding step in collagen phagocytosis by human fibroblasts. J Cell Sci 2001: **114**: 119–129.
27. Takaki T, Kobayashi M, Okubo KS et al. Interferon-γ inhibits collagen phagocytosis in human fibroblasts by inducing subcortical actin assembly and reducing activity of β_1 integrin to bind to collagen. Inflamm Res 2006: **55**: 534–542.
28. Takeshita S, Nakatani K, Tsujimoto H et al. Increased levels of circulating soluble CD14 in Kawasaki disease. Clin Exp Immunol 2000: **119**: 376–381.
29. Tobias PS, Mathison JC, Ulevitch RJ. A family of lipopolysaccharide binding proteins involved in response to Gram-negative sepsis. J Biol Chem 1988: **263**: 13479–13481.
30. Vanhaesebroeck B, Leevers SJ, Panayotou G, Waterfield MD. Phosphoinositide 3-kinases: a conserved family of signal transducers. Trends Biochem Sci 1997: **22**: 267–272.
31. Weber GF, Menko AS. Phosphatidylinositol 3-kinase is necessary for lens fiber cell differentiation and survival. Invest Ophthalmol Vis Sci 2006: **47**: 4490–4499.
32. Williams LM, Ridley AJ. Lipopolysaccharide induces actin reorganization and tyrosine phosphorylation of Pyk2 and paxillin in monocytes and macrophages. J Immunol 2000: **164**: 2028–2036.
33. Wright SD, Ramos RA, Tobias PS, Ulevitch RJ, Mathison JC. CD14, a receptor for complexes of lipopolysaccharide (LPS) and LPS binding protein. Science 1990: **249**: 1431–1433.
34. Wuthrich B, Kagi MK, Joller-Jemelka H. Soluble CD14 but not interleukin-6 is a new marker for clinical activity in atopic dermatitis. Arch Dermatol Res 1992: **284**: 339–342.