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# Effect of Anti-CD14 Antibody on Experimental Periodontitis Induced by *Porphyromonas gingivalis* Lipopolysaccharide

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ABSTRACT—The lipopolysaccharide (LPS) released by *Porphyromonas gingivalis*, a Gram-negative bacterium found in the periodontal pockets of patients with periodontitis, induces bone resorbing activity in vivo. We previously showed that a receptor for LPS on human gingival fibroblasts and gingival epithelial cells is CD14. In this study, we established a mouse model of experimental periodontitis by applying a *P. gingivalis* LPS solution to the buccal region of mice. *P. gingivalis* LPS-induced bone resorption and interleukin-6 production in the gingival tissues were significantly inhibited by pretreatment with anti-CD14 antibody for 5 weeks prior to LPS treatment. This result suggests that anti-CD14 antibody may be usable as a prototype for the development of drugs for the treatment of periodontal disease.

*Keywords*: Lipopolysaccharide, CD14, Periodontal disease, Experimental periodontitis, Animal model

Periodontal disease comprises a group of infections that lead to inflammation of the gingiva and destruction of periodontal tissues; and in severe cases, they are accompanied by loss of alveolar bone with eventual exfoliation of teeth (1). Oral microbes are involved in the progression of periodontal disease that eventually causes tooth loss. Specifically, Gram negative bacteria have become widely recognized as the periodontopathic bacteria (2). Lipopolysaccharide (LPS), a component of the bacterial outer membrane, is the pathogenetic factor in a wide variety of periodontopathic bacteria (3, 4). For this reason, numerous studies have examined the immune response of periodontal tissue cells upon exposure to periodontopathic LPS. When periodontopathic bacteria proliferate in periodontal pockets, it is suggested that LPS is amply released, causing immunocytes such as macrophages and fibroblasts to steadily synthesize inflammatory cytokines including interleukin (IL)-1 and IL-6. These cytokines, in turn, aggravate inflammation, destroy periodontal tissues, and induce alveolar bone resorption (5 – 11).

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Since Mouton et al. reported that patients with adult periodontitis have a higher serum level of immunoglobulin G (IgG) antibodies against *P. gingivalis* than control individuals (12), many investigators have reported that patients with periodontitis have elevated levels of antibodies against sonicates of *P. gingivalis* in both the serum and gingival crevicular fluid. *P. gingivalis* is primarily found in deep periodontal pockets and, especially, in active sites of infection (13). *P. gingivalis* lipopolysaccharide (P-LPS) may also play an important role in the pathogenic mechanism leading to inflammation of the gingiva and alveolar bone loss in periodontal diseases. We have focused our attention on P-LPS (7 – 11) and *P. gingivalis* protease (14 – 16) and the immune response of gingival fibroblasts and gingival epithelial cells, which comprise most of the human periodontal tissue. We previously suggested that the receptor for P-LPS on gingival fibroblasts (7) and gingival epithelial cells is CD14 (11). To elucidate the mechanism of inflammatory bone resorption induced by P-LPS, using an in vitro culture system, we identified the sequence of events from P-LPS stimulation to bone resorption. Upon stimulation by P-LPS, fibroblasts produce IL-6, which in turn activates osteoclasts and eventually causes bone adsorption (9).

It is very important to develop a strategy for preventing periodontal disease. Having established an animal model of periodontitis by treating mice with P-LPS, we sought to examine if anti-CD14 antibody (Ab) inhibits bone resorption. In this study, we show that anti CD14 Ab inhibits alveolar bone resorption of the maxilla and IL-6 production in P-LPS induced periodontitis in mice.

## MATERIALS AND METHODS

### *P-LPS and anti-CD14 Ab*

P-LPS was prepared as previously described (17). Briefly, LPS was extracted from acetone-dried cells of *P. gingivalis* by the phenol-water method (17) and then purified by ultracentrifugation six times at 105,000 × *g* at 4°C for 16 h each and RNase treatment. Mouse anti-human CD14 monoclonal antibody (anti-CD14 Ab) (IgG1, clone MEM-18) was purchased from Monosan (Am Uden, Netherlands). P-LPS-induced IL-6 production in mice gingival fibroblasts was inhibited by anti-CD14 Ab, and the binding of LPS to the cells was abrogated by the antibody (data not shown). Thus, this antibody reacted to mice.

### *Animals and experimental design*

The protocol employed here meets the guidelines of The Japanese Pharmacological Society. All efforts were made to minimize animal suffering and to reduce the number of animal used. Seven-week-old, male, specific pathogen-free C3H/HeN mice were purchased from Sankyo Laboratory Service Co. (Tokyo). All animals weighed between 20 and 22 g, and were divided into five groups (n = 5 in each group). The Group A mice were treated with P-LPS for 10 weeks. The Group B mice were treated with P-LPS + anti-CD14 Ab for 10 weeks. The Group C mice were pretreated with anti-CD14 Ab for 4 weeks and then treated with LPS + anti-CD14 Ab for 10 weeks. The Group D mice were pretreated with anti-CD14 Ab for five weeks, and then treated with LPS + anti-CD14 Ab for 10 weeks. The control group was not treated with P-LPS nor anti-CD14 Ab. The experimental schedules of the four experimental groups are shown in Fig. 1. To remove $NaN_3$, the antibody was dialyzed, and it was concentrated to a 1 mg /ml. The reagent [1 mg/ml of P-LPS dissolved in distilled water (D.W.) and/or 1 mg/ml of anti-CD14 Ab dissolved in D.W.] was swabbed onto the buccal region of each mouse in the quiescent state for 5 min with a swab, once every two days. The specified level of anti-body concentration was determined based on the concentrations used in our previous culture experiments of P-LPS stimulation of the IL-6-producing system (7). The approximate total amount of LPS or anti-CD14 Ab that was applied to each mouse during one application was 50 $\mu$l. During the experimental period, all mice were given demineralized food and water ad lib. All mice were weighed weekly. The mice in the four experimental groups were sacrificed on the day after the last day of treatment by general anesthesia using Nembutal (Abbott Laboratories, North Chicago, IL, USA). The control mice were sacrificed at the end of 15 weeks.

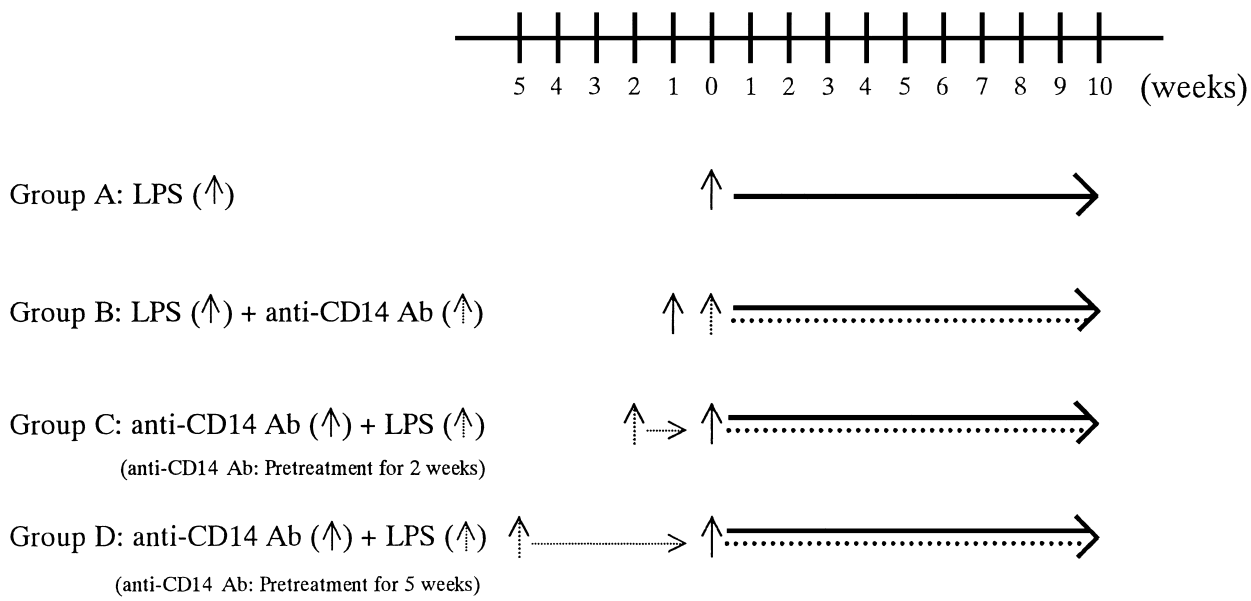


**Fig. 1.** Treatment schedules of LPS with or without anti-CD14 antibody in the mice of Groups A, B, C and D. The control mice were not treated with LPS or anti-CD14 antibody. The upward arrow represents the time point at which LPS or anti-CD14 antibody treatment was started.

*Measurement of the level of alveolar bone adsorption*

For preparation of the bone specimen, the left maxilla was excised from the mouse. After a 5-day fixation in a solution of 10% neutral formalin (Wako, Osaka) plus sodium hypochlorite (Wako), it was spread out. After neutralization and washing in 3% hydrogen peroxide in water, the soft tissue was mechanically removed from the left maxilla specimen under a stereoscopic microscope (Olympus, Tokyo). The tissue was sectioned. The sections were dyed in 5% basic fuchsin aqueous solution after they had dried, in order to clarify the cement at each animal boundary as a measurement of alveolar bone adsorption. The level of alveolar bone adsorption was determined using the procedures described by Sallay et al. (18) and Crawford et al. (19). The distance (mm) from the cement of one enamel boundary to the top of the alveolar bone was measured at seven locations in the first, second and third molars using a Paek Scale Lupe (Masuda Co., Osaka) on the maxilla primary mesial root buccal palate side, as shown in Fig. 2.

*The amount of IL-6 in the gingival tissues*

Gingival samples were biopsied from gingival tissues on the mouse. Biopsy specimens were dissected into 1 mm in diameter and then washed by phosphate-buffered saline (PBS) (pH 7.4). Gingival samples were plated onto 96 well culture plates (Coastar, Cambridge, MA, USA) with RPMI 1640 medium (Gibco, Grand Island, NY, USA) containing 10% heat inactivated fetal calf serum (FCS) (Gibco), 2 mM L-glutamine (Gibco), 100 U penicillin (Gibco) and 100 mg /ml streptomycin (Gibco), in a humidified atmosphere of 5% $CO_2$ at 37°C f or 48 h. The amount of IL-6 in 100 $\mu$l of the culture supernatant on gingival samples culture was measured using an enzyme-linked immunosorbent assay (ELISA), according to the manufacturer's instructions. The ELISA kit was purchased from Wako.

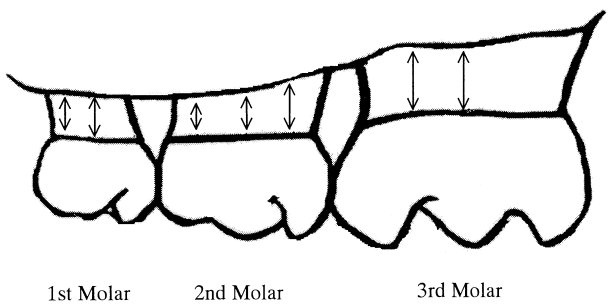


**Fig. 2.** Measurement of the alveolar bone level in the first, second and third molars of the left maxilla of a mouse. A diagram of the first, second and third molars of the left maxilla on the maxilla primary mesial root buccal palate side is shown. The distance between the cement of one enamel boundary and the top of the alveolar bone was measured at the seven locations shown in the figure. The level of alveolar bone in a mouse was taken to be the sum of the seven distances. The level of alveolar bone adsorption was determined using the procedures of Sallay et al. (18) and Crawford et al. (19).

*Statistical analyses*

All data are expressed as the mean ± S.E.M. Statistical analyses was performed by the Scheffe test; $P<0.05$ was considered significant.

## RESULTS

The weight of the mice in each experimental group did not significantly differ from that of the control group at each 1-week time point (Fig. 3). Therefore, application of anti-CD14 Ab and/or LPS does not affect the body weight of these laboratory animals.

Figure. 4 shows representative photographs of the left maxilla of a mouse in each experimental and control group under microscopic observation. A greater degree of bone resorption was observed in the mice in Groups A, B, and C in comparison with that in the control mice. The degree of bone resorption in the Group D mice was less than that in the mice of Groups A, B, and C, although it was greater than that in the control mice.

Figure. 5 shows the level of alveolar bone adsorption in each group. The level of alveolar bone adsorption in the A, B, C, D and control groups, was 0.47 ± 0.03 mm, 0.48 ± 0.05 mm, 0.47 ± 0.06 mm, 0.34 ± 0.08 mm and 0.12 ± 0.04 mm, respectively. The level of alveolar bone adsorption in the Group A mice which had been treated with LPS alone for 10 weeks, was significantly larger than that in the Group D mice ($P<0.05$). The level of alveolar bone adsorption among the mice in Group A, B or C did not significantly differ. The level of alveolar bone adsorption in the control mice was significantly smaller than that in the Group A, B, C or D mice ($P<0.05$). Treatment with anti-CD14 Ab alone over 10 weeks did not affect the level of alveolar bone adsorption (data not shown). The control mice also showed a small degree of alveolar bone adsorption; these data are within the normal range of bone adsorption. From these results (Figs. 4 and 5), treatment with P-LPS alone for 10 weeks enhanced alveolar bone adsorption.

The level of IL-6 was produced by P-LPS. The level of IL-6 in the control was significantly smaller than that in the group of pretretment for 4 and 5 weeks ($P<0.05$). Anti-CD14 Ab inhibited P-LPS IL-6 production in the gingival tissues. The level of IL-6 in the periodontal tissue started to decrease gradually in the third week of the pretreatment (Fig. 6).

Thus, pretreatment with anti-CD14 Ab for 5 weeks prior to P-LPS and anti-CD14 Ab treatment for 10 weeks inhibited P-LPS-induced bone adsorption and IL-6 production in the gingival tissues.

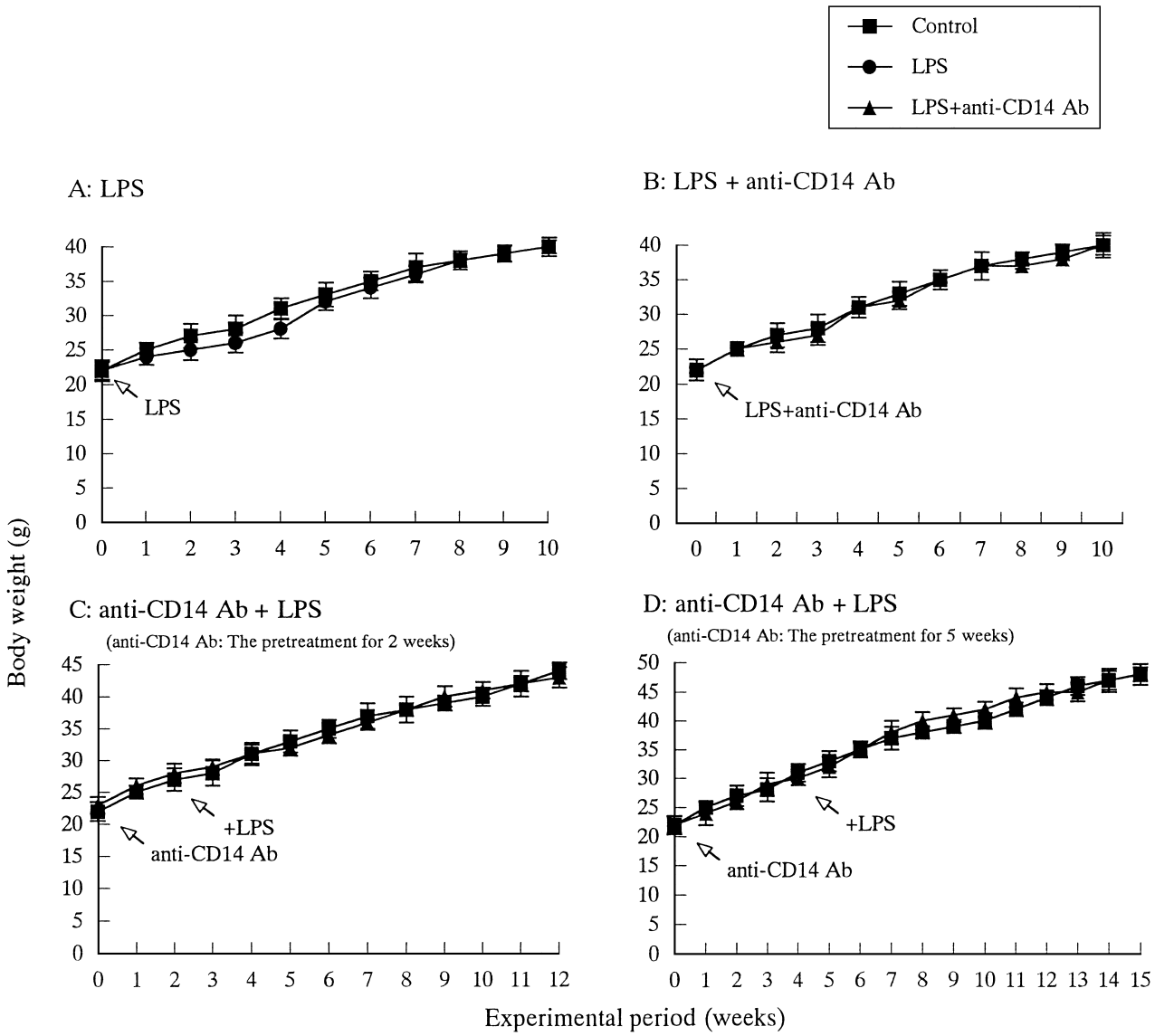


**Fig. 3.** Change in the body weight (g) of the mice in (A) Group A, (B), Group B, (C) Group C and (D) Group D over the study period. The body weight of the control mice is plotted in each panel for comparison. Each point represents the mean ± S.E.M. (n = 5).

## DISCUSSION

Many basic and clinical studies support the concept that human periodontitis is a disease caused by several types of Gram-negative bacteria that mainly exist in dental plaque (1). In the pathology of periodontal disease, it has been clarified that periodontal disease is the end result of the host immune response to LPS, rather than the result of one-sided invasion of a periodontopathic bacterium. The specificity of various pathogen fungi in causing periodontal disease was proven in an animal experiment system in which individual bacterial cells isolated from a human periodontitis lesion were infected in laboratory animals (20 – 24). The animal models of periodontal disease were recently reviewed by Weinberg and Bral (25). Many of these animals were infected by means of a bacterium-soaked silk ligature or the gavaging of bacterium. In this study, we established a mouse model of experimental periodontitis by applying a periodontopathic bacterium solution to the buccal region of mice. Namely, as P-LPS has been strongly implicated as one of the major causative agents of periodontal disease, we established a mouse model of experimental periodontitis with P-LPS. Mice that were stimulated with P-LPS for 10 weeks showed enhanced bone resorption.

In periodontitis lesions, the human gingival fibroblasts

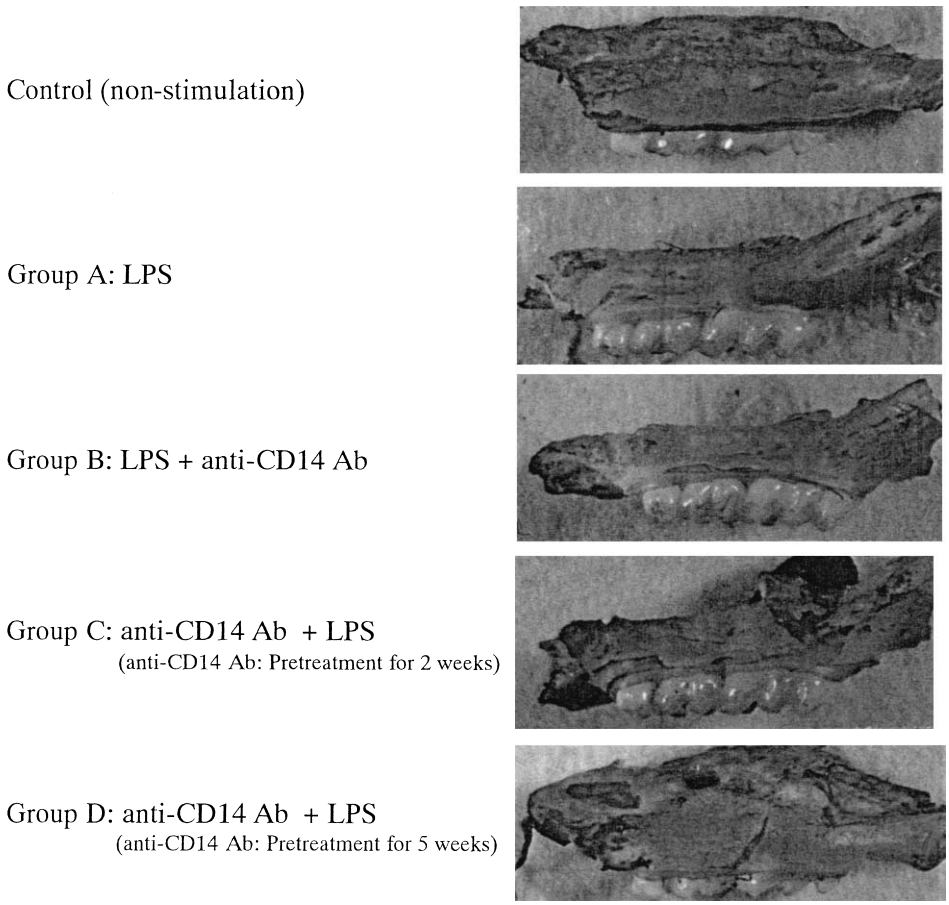


**Fig. 4.** Morphological changes of bone formation after completion of LPS treatment for 10 weeks (A), LPS and anti-CD14 antibody treatment for 10 weeks (B), or pre-treatment with anti-CD14 antibody for 4 weeks (C) or 5 weeks (D), and then treatment with LPS and anti-CD14 antibody for 10 weeks. Photographs of the left maxilla of a representative mouse in each group are shown.

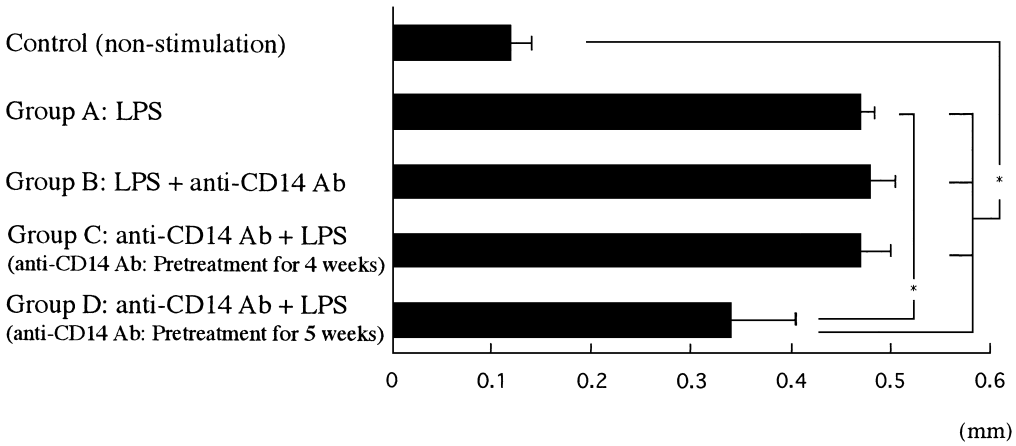


**Fig. 5.** Level of alveolar bone adsorption (mm) in the four experimental groups and the control group. Each bar represents the mean ± S.E.M. (n = 5). **P*<0.05.

and gingival epithelial cells in the oral cavity, which are the major constituents of gingival connective tissue, may interact directly with bacteria and bacterial products including LPS. It is, therefore, suggested that these cells play

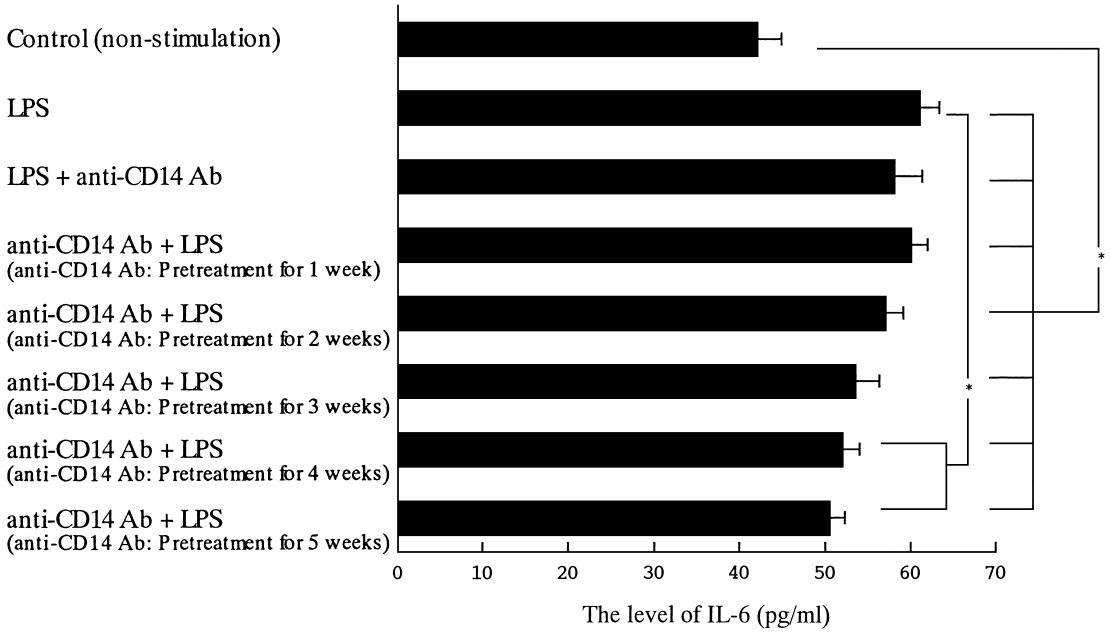


**Fig. 6.** Level of IL-6 in the gingival tissues. The concentration of IL-6 in the culture supernatant was measured by ELISA. Each bar represents the mean ± S.E.M. (n = 5). **P*<0.05.

important roles in the host responses of patients with periodontitis to LPS. Upon stimulation by oral black-pigmented bacteria such as *P. gingivalis* and their components including LPS, gingival fibroblasts and gingival epithelial cells produce various inflammatory cytokines including IL-1, IL-6 and IL-8 (7, 11, 26, 27). These cytokines may, in turn, initiate and exacerbate periodontal inflammatory diseases. The present study showed P-LPS induced gingival tissues to produce IL-6 in a mouse model of experimental periodontitis.

CD14 is one of several receptors for LPS and is expressed predominantly on the surface of monocytes, macrophages, and neutrophils (28 – 30). Shapira et al. (31) showed that P-LPS stimulates human monocytes in a CD14-dependent manner. In addition, we (7, 11) and other groups (30, 31) have demonstrated that the signal transduction pathway stimulated by P-LPS in human gingival fibroblasts and gingival epithelial cells, acts through CD14. We recently found that LPS stimulation of human gingival fibroblasts induces protein tyrosine phosphorylation of several intracellular proteins including extracellular signal-regulated kinase 2 (ERK2) (7). These events were suppressed by anti-CD14 Ab (7). Furthermore, we demonstrated in vitro that P-LPS stimulates the production of IL-6, which in turn activates osteoclasts (9). Thus, cytokines play a crucial role as mediators of inflammation in periodontal disease. More recently, we demonstrated in vitro that IL-10 inhibits the inflammatory response through the IL-10 receptor on gingival fibroblasts in P-LPS-initiated periodontal disease (10). Furthermore, several studies have shown that various types of antibiotics are effective for the treatment of chronic inflammatory disease. Golub et al. (32) suggested that a non-antimicrobial action of tetracycline inhibits both the production of gingival matrix metalloproteinase and bone loss in *P. gingivalis*-induced periodontitis in rats. These studies suggest that an antibiotic(s), cytokine(s) or anti-CD14 Ab may be able to prevent the development of periodontal disease by inhibiting P LPS-stimulated expression of inflammatory cytokines such as IL-1 and IL-6 that are involved in the pathogenesis of periodontal disease. Our present study demonstrate that P-LPS stimulates bone resorption and that anti-CD14 Ab inhibits P-LPS-induced bone resorption and the level of IL-6 in the gingival tissues when administered for 5 weeks prior to P-LPS treatment in an animal model of periodontitis.

As shown in the present study, P-LPS-stimulated periodontitis in mice was not completely inhibited by anti-CD14 Ab. This suggests that upon P-LPS stimulation, another pathway(s) in addition to the CD14 pathway, is involved in the development of experimental periodontitis in mice, because LPS also binds to receptors other than CD14 on immunocytes such as monocytes, macrophages, and neutrophils and fibroblasts in the gingiva. On the other hand, we have already reported that anti-CD14 Ab inhibits IL-6, which is an inflammatory cytokine produced by P-LPS stimulants in human gingival fibroblasts (7). Therefore, in the present study, we expected to find that P-LPS and anti-CD14 Ab jointly inhibit bone resorption and

cytokines in animal experiments. Contrary to our expectation, the results showed no inhibition. This led us to try pretreatment with anti-CD14 Ab. When pretreated with anti-CD14 Ab, inhibition of bone resorption and cytokine was observed. However, we are uncertain why the administration of the anti-CD14 Ab inhibited bone resorption significantly more when it had been pretreated with antibody for 5 weeks than when it had been pretreated for 4 weeks. On the other hand, the level of IL-6 in the periodontal tissue started to decrease gradually in the third week of the pretreatment. Our previous study reported that inflammatory cytokine accelerates bone rebosorption in vitro (9). From this study, this suggests that the inflammatory cytokine such as IL-6 can be inhibited by the antibody. Although we have not yet reached a definite explanation of the result, one possibility would be that a long term storage of anti-CD14 Ab in periodontal tissues contributes in some way to the inhibition of bone resorption and cytokine. Another possiblity would be that the anti-CD14 Ab, causing LPS-like effects, had B cell produce some anti-LPS antibody. Thus, the mechanism of the residual bone resorption after anti-CD14 Ab treatment, remains to be elucidated.

This study is the first to demonstrate that anti-CD14 Ab inhibits the development of P-LPS induced periodontitis in mice. Our results raise the possibility that anti-CD14 Ab can be used as a prototype for developing remedial drugs for the treatment of periodontal disease.

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REFERENCES

1 Consensus report. Periodontal diseases: Pathogenesis and microbial factors. Ann Periodontol **1**, 926 – 932 (1996)
2 Offenbacher S: Periodontal diseases: Pathogenesis. Ann Periodontol **1**, 821 – 878 (1996)
3 Hamada S, Fujiwara T and Mihara J: Bacterial endotoxic substances and their effects on host cells. *In* Molecular Pathogenesis of Periodontal Disease, Edited by Genco R, Hamada S, Sehner T, McGhee J and Mergenhagen S, pp 105 – 117, ASM Press, Washington DC (1994)
4 Tobias PS, Gegner J, Tapping R, Orr S, Mathison J, Lee JD, Kravchenko V, Han J and Ulevitch RJ: Lipopolysaccharide dependent cellular activation. J Periodont Res **32**, 99 – 103 (1997)
5 Takada H, Mihara J, Morisaki I and Hamada S: Production of cytokines by human gingival fibroblasts. *In* Periodontal Disease, Edited by Hamada S, Holt SC and McGhee JR, pp 265 – 276, Quintessence Publishing Co Press, Tokyo (1991)
6 Takada H, Mihara J, Morisaki I and Hamada S: Induction of interleukin-1 and -6 in human gingival fibroblast cultures stimulated with *Bacteroides* lipopolysaccharides. Infect Immun **59**, 295 – 301 (1991)
7 Wang PL, Sato K, Oido M, Fujii T, Kowashi Y, Shinohara M, Ohura K, Tani H and Kuboki Y: Involvement of CD14 on human gingival fibroblasts in *Porphyromonas gingivalis* LPS-mediated IL 6 secretion. Arch Oral Biol **43**, 687 – 694 (1998)
8 Oido M, Wang PL, Ohura K, Fujii T, Kuboki Y and Kowashi Y: Effects of LPS and protease from *Porphyromonas gingivalis* on cultured human gingival fibroblasts. J Hard Tissue Biol **8**, 9 – 14 (1999)
9 Wang PL, Shirasu S, Shinohara M, Daito M, Kikuchi M, Suetsugu Y, Tanaka J and Ohura K: The signal transduction pathway from *Porphyromonas gingivalis* LPS-stimulated gingivalis fibroblasts to osteoclast activation. Med Sci Res **27**, 311 – 313 (1999)
10 Wang PL, Shirasu S, Shinohara S, Azuma Y, Daito M, Yasuda H and Ohura K: IL-10 inhibits *Porphyromonas gingivalis* LPS-stimulated human gingival fibroblasts production of IL-6. Biochem Biophys Res Commun **263**, 372 – 377 (1999)
11 Wang PL, Sato K, Oido M, Fujii T, Ohura K, Kowashi Y, Tani H and Kuboki Y: Destruction of LPS receptor by *Porphyromonas gingivalis* protease is prevented by histatin 5, a specific protease inhibitor. J Hard Tissue Biol **8**, 27 – 32 (1999)
12 Mouton C, Hammond PG, Slots J and Genco RJ: Serum antibodies to oral *Bacteroides asaccharolyticus (Bacteroides gingivalis)*: relationship to age and periodontal disease. Infect Immun **31**, 182 – 192 (1981)
13 Van Winkelhoff AJ, van Steenbergen TJ and de Graaff J: The role of black-pigmented *Bacteroides* in human oral infections. J Clin Periodontol **15**, 145 – 155 (1988)
14 Yoshioka M, Wang PL and Ohura K: Effect of proteases from *Porphyromonas gingivalis* on adhesion of human periodontal ligament fibroblasts. Folia Pharmacol Jpn (Nippon Yakurigaku Zasshi) **110**, 347 – 355 (1997) (text in Japanese with English abstract)
15 Wang PL, Shirasu S, Shinohara M, Daito M, Oido M, Kowashi Y and Ohura K: Induction of apoptosis in human gingival fibroblasts by a *Porphyromonas gingivalis* protease preparation. Arch Oral Biol **44**, 337 – 342 (1999)
16 Wang PL, Sato M, Oido M, Kowashi Y, Ohura K, Tani H and Kuboki Y: *Porphyromonas gingivalis* protease degrades cell adhesion molecules of human gingival fibroblasts. J Hard Tissue Biol **8**, 1 – 5 (1999)
17 Kumada H, Haishima Y, Umemoto T and Tanamoto K: Structural study on the free lipid A isolated from lipopolysaccharide of *Porphyromonas gingivalis*. J Bacteriol **177**, 2089 – 2106 (1999)
18 Sallay K, Sanavi F and Ring I: Alveolar bone destruction in the immunosuppressed rat. J Periodont Res **17**, 263 – 274 (1982)
19 Crawford J, Martin M and Taubman A: The effects of local immunization with periodontopathic microorganisms on periodontal bone loss in gnotobiotic rats. J Periodont Res **13**, 445 – 459 (1978)
20 Chen PB, Davern LB, Schifferle R and Zambon JJ: Protective immunization against experimental *Bacteroides (Porphyromonas)* gingivalis infection. Infect Immun **58**, 3394 – 3400 (1990)
21 Kesavalu L, Ebersole JL, Machen RL and Holt SC: *Porphyromonas gingivalis* virulence in mice: induction of immunity to bacterial components. Infect Immun **60**, 1455 – 1464 (1992)
22 Genco CA, Kapczynski DR, Cutler CW, Arko RJ and Arnold

RR: Influence of immunization on *Porphyromonas gingivalis* colonization and invasion in the mouse chamber model. Infect Immun **60**, 1447 – 1454 (1992)

23 Baker PJ, Toddevans R and Roopenian DC: Oral infection with *Porphyromonas gingivalis* and induced alveolar bone loss in immunocompetent and severe combined immunodeficient mice. Arch Oral Biol **39**, 1035 – 1040 (1994)

24 Bird PS, Gemmell E, Polak B, Paton G, Sosroseno W and Seymour GJ: Protective immunity to *Porphyromonas gingivalis* infection in a murine model. J Periodontol **66**, 351 – 362 (1995)

25 Weinberg MA and Bral M: Laboratory animal models in periodontology. J Clin Periodontol **26**, 335 – 340 (1999)

26 Watanabe A, Takeshita A, Kitano S and Hanazawa S: CD14-mediated signal pathway of *Porphyromonas gingivalis* lipopolysaccharide in human gingival fibroblasts. Infect Immun **64**, 4488 – 4494 (1996)

27 Sugawara S, Sugiyama A, Nemoto E, Rikiishi H and Takada H: Heterogeneous expression and release of CD14 by human gingival fibroblasts: characterization and CD14-mediated interleukin-8 secretion in response to lipopolysaccharide. Infect Immun **66**, 3043 – 3049 (1998)

28 Wright SD, Ramos RA, Tobias PS, Ulevitch RJ and Mathison JC: CD14, a receptor for complexes of lipopolysaccharide (LPS) and LPS binding protein. Science **249**, 1431 – 1433 (1992)

29 Dentener AM, Bazil V, Asmuth UV, Ceska M and Buurman WA: Involvement of CD14 in lipopolysaccharide-induced tumor necrosis factor-$\alpha$, IL-6 and IL-8 release by human monocytes and alveolar macrophages. J Immunol **150**, 2885 – 2891 (1993)

30 Ziegler-Heitbrock HW and Ulevitch RJ: CD14: cell surface receptor and differentiation marker. Immunol Today **14**, 121 – 125 (1993)

31 Shapira L, Takashiba S, Champagne C, Amar S and Dyke ET: Involvement of protein kinase C and protein tyrosine kinase in lipopolysaccharide-induced TNF-$\alpha$ and IL-1 production by human monocytes. J Immunol **153**, 1818 – 1824 (1994)

32 Golub LM, Evans RT, McNamara TF, Lee HM and Ramamurthy NS: A non-antimicrobial tetracycline inhibits gingival matrix metalloproteinases and bone loss in *Porphyromonas gingivalis* induced periodontitis in rats. Ann NY Acad Sci **732**, 96 – 111 (1994)