INFECTION AND IMMUNITY, Dec. 1993, p. 5219–5224 Vol. 61, No. 12
0019-9567/93/125219-06$02.00/0
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Expression of Monocyte Chemoattractant Protein 1 (MCP-1) in Adult Periodontal Disease: Increased Monocyte Chemotactic Activity in Crevicular Fluids and Induction of MCP-1 Expression in Gingival Tissues

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Received 8 February 1993/Returned for modification 31 March 1993/Accepted 24 September 1993

The present study shows that monocyte chemotactic activity in crevicular fluids increases with severity of the disease and that a monocyte chemoattractant, monocyte chemoattractant protein 1 (MCP-1), is expressed as the predominant cytokine of gingival tissues and their fibroblasts treated with *Porphyromonas (Bacteroides) gingivalis* lipopolysaccharide (P-LPS). High monocyte chemotactic activity in the crevicular fluids was neutralized significantly by antiserum specific for the JE/MCP-1 protein. Marked expression of the MCP-1 gene was observed in the gingival tissues of all adult periodontal patients tested, but not in those of healthy subjects. Monocyte chemotactic activity was observed in culture supernatants of human normal gingival tissues treated with P-LPS, and the chemotactic activity increased in a dose-related manner. Expression of MCP-1 in P-LPS-treated human gingival fibroblasts was further examined. P-LPS induced the MCP-1 gene expression in a dose- and treatment time-dependent manner. The MCP-1 gene product in the culture supernatant was detected as two forms with molecular masses of 11,000 and 15,000 Da by immunoprecipitation with the specific antiserum. The MCP-1 gene expression was induced in the fibroblasts treated with interleukin-1β and tumor necrosis factor alpha, but not with interleukin-6. These results suggest that gingival fibroblasts can participate in monocyte recruitment in gingival tissues of adult periodontal patients via the MCP-1 gene product and that MCP-1 plays an important role in the inflammatory reaction in the disease.

Adult periodontal disease has been characterized as one with alveolar bone loss and inflammation of periodontal tissues. Many recent studies (2, 5, 10, 11, 23, 25, 34) have demonstrated that interleukin-1 (IL-1) and tumor necrosis factor alpha (TNF-α) are potent inflammatory cytokines having bone-resorbing activity. Since these cytokines are produced by monocytes/macrophages, these cells localized in periodontal tissues may play a functional role in the pathogenic mechanism(s) of periodontal disease through production of these cytokines.

Early studies (4, 24, 29) have shown that monocytes and neutrophils infiltrate markedly into periodontal tissues in adult periodontal patients. As described above, since these cells produce some inflammatory cytokines that may be involved in the inflammatory reaction and alveolar bone loss, infiltration of these cells into periodontal tissues is an important event in this disease. However, the relationship between monocyte chemotactic activity and severity of disease in patients having this disease and the mechanisms specific for monocyte/macrophage recruitment into periodontal tissues are unknown.

The gene for monocyte chemoattractant protein 1 (MCP-1), a potent chemoattractant specific for monocytes, was cloned recently by several investigators (27, 37) who provided evidence that the MCP-1 gene is the human homolog of the mouse JE gene, one of the platelet-derived growth factor-inducible genes (7, 14, 26, 27). This specificity is an apparently unique property since other characterized chemoattractants such as leukotriene B_4, platelet-activating factor, and C_5 are not specific for monocytes.

In this study, we indicate that monocyte chemotactic activity in crevicular fluids of adult periodontal patients increases with severity of the disease and suggest that MCP-1 may contribute to the increased infiltration of monocytes into periodontal tissues observed for this disease.

MATERIALS AND METHODS

Reagents. The α-modification of Eagle's minimum essential medium (α-MEM) was obtained from Flow Laboratories (McLean, Va.); methionine-free Eagle's MEM was obtained from Nissui Pharmaceutical Co., Tokyo, Japan; and fetal calf serum (FCS) was obtained from HyClone (Logan, Utah). 5′-[α-^{32}P]dCTP, a multiprime DNA labeling system, and [^{35}S]methionine (15 mCi/ml) were purchased from Amersham Japan (Tokyo, Japan). Formyl-methionyl-leucine-phenylalanine (FMLP) was from Sigma Chemical Co. (St. Louis, Mo.), and protein A-Sepharose beads were from Pharmacia-LKB Japan (Tokyo, Japan). *Porphyromonas gingivalis* lipopolysaccharide (P-LPS) was prepared as described previously (20). Human recombinant IL-1β was a gift from Otsuka Pharmaceutical Co. (Tokushima, Japan), human recombinant TNF-α was provided kindly by Suntory Co. (Osaka, Japan), and human recombinant IL-6 was purchased from Genzyme (Cambridge, Mass.).

Periodontal patients and removal of crevicular fluids. Adult periodontal patients at Meikai University Hospital were

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classified into three groups by their attachment loss: AL-1, attachment loss = 0 to 2.9 mm; AL-2, attachment loss = 3.0 to 5.9 mm; and AL-3, attachment loss ≥ 6.0 mm. None of the subjects had received dental treatment or antibiotic therapy for 6 months prior to this study. Crevicular fluid of the most advanced site of each subject was harvested with a microcapillary glass inserted into the base of the periodontal pocket. The harvested fluid was transferred to a microtube and centrifuged for 15 min at 4,000 × *g*. The supernatant fluids were diluted with Gey's balanced salt solution containing 1% bovine serum albumin (BSA). The diluted samples were used for the monocyte chemotactic assay.

Preparation of gingival tissues and their culture supernatants. Human gingival tissues were obtained from healthy and adult periodontal disease individuals. Their tissues were used for both the monocyte chemotactic assay and the Northern (RNA) blot analysis described below. Healthy tissues were prepared from gingiva of impacted third molars of healthy individuals. For the monocyte chemotactic assay, the gingival tissues from healthy individuals were transferred into each well of Falcon 24-well multiple plastic plates containing serum-free α-MEM in 5% CO_2 at 37°C and then cultured for 24 h with or without the selected doses of P-LPS. The culture supernatants were used for the monocyte chemotactic assay.

Preparation of human gingival fibroblasts. Human gingival tissues were cultured in Falcon 30-mm plastic plates in α-MEM containing 10% FCS under an atmosphere of 5% CO_2 at 37°C. The medium was changed every 6 days. After a confluent monolayer of cells that had migrated from the gingival tissues had formed, the cells were trypsinized and then again grown to confluence. After the fifth passage, typical gingival fibroblasts were harvested and used in this study. The subconfluent gingival fibroblasts in plastic plates with α-MEM containing 10% FCS were cultured for various times with or without the selected doses of P-LPS.

Monocyte chemotactic assay. Human peripheral blood mononuclear cells were isolated by the method utilizing Ficoll-Hypaque (Pharmacia Japan) and then suspended in 1% BSA containing Gey's balanced salt solution at a concentration of 5×10^6 cells per ml. Monocyte chemotactic activity was measured in a 48-well apparatus equipped with a 5-μm-pore-size polycarbonate filter (Nuclepore, Costar Corp., Cambridge, Mass.) as described previously (9). After the loaded microchamber had been incubated for 90 min at 37°C, the filter was removed and fixed in methanol and then stained with Diff-Quik (Kokusai Shiyaku, Kobe, Japan). Monocytes that had migrated through the bottom of the filter were counted in a 20-microgrid field under oil immersion (magnification, ×1,000), and then the total count was divided by 20. The chemotactic activity was expressed as the mean ± standard deviation (SD) of the total number of migrating monocytes per oil immersion field in triplicate assays. In each experiment, FMLP was used as a positive control.

In the neutralization test, culture supernatants from gingival tissues and fibroblasts were left untreated or treated with rabbit serum or anti-JE/MCP-1 antiserum (kindly supplied by B. J. Rollins, Dana-Farber Cancer Institute, Boston, Mass.) diluted with Gey's balanced salt solution containing 1% BSA. Thereafter, the test media were incubated for 90 min with the indicator cells, and then the filters were stained to assess the chemotactic activity. The characteristics and specificity of the anti-JE/MCP-1 antiserum were described previously (27, 28).

Immunoprecipitation assay. MCP-1 protein synthesis was analyzed by metabolic labeling and immunoprecipitation. Human gingival fibroblasts were washed in phosphate-buffered saline, treated or not with P-LPS at 10 μg/ml, and incubated for 24 h in methionine-free Eagle's MEM containing [^{35}S]methionine. The culture supernatants (500 μl) were collected and supplemented with Triton X-100 (1%), EDTA (1 mM), and protease inhibitors (phenylmethylsulfonyl fluoride [0.8 mM] and leupeptin [10 μg/ml]). Each sample was centrifuged to remove any cellular debris, aliquoted, and incubated overnight at 4°C with normal rabbit serum or anti-JE/MCP-1 antiserum (1:100). Immune complexes were recovered by incubation with protein A-Sepharose beads (30 μl), washed five times with immunoprecipitation buffer (20 mM Tris-HCl [pH 7.4], 300 mM NaCl, 1 mM EDTA, 1% Triton X-100, 1% BSA, and 10 mM NaF) plus protease inhibitors, washed with distilled water, and then eluted with sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) sample buffer (2% SDS, 5% 2-mercaptoethanol, 0.06 M Tris-HCl [pH 6.8], 10% glycerol). The eluted proteins were boiled for 3 min and separated by SDS-PAGE on 17.5% polyacrylamide. The gels were treated with Amplify (Amersham Japan) for 20 min, dried, and exposed to Kodak XAR film (Eastman Kodak, Rochester, N.Y.) at −70°C for 2 days.

cDNA hybridization probe. A plasmid containing human MCP-1 sequences was a gift of B. J. Rollins. Also, a plasmid with β-actin sequences was purchased from Oncor Co. (Gaithersburg, Md.). The methods used for plasmid preparation were described earlier (22).

Preparation of total RNA and Northern blot analysis. Human gingival tissues (1 g [wet weight] from each subject) from healthy and adult periodontal subjects were homogenized individually in 5.5 M guanidine isothiocyanate for 3 min with a Teflon homogenizer. On the other hand, human gingival fibroblasts (10^6 cells) were cultured in α-MEM with 10% FCS in Falcon 90-mm plastic plates until nearly confluent. Then, the cells were washed, incubated for 24 h in 0.05% FCS-containing α-MEM, and then treated for the indicated periods in 0.5% FCS-containing α-MEM with or without test samples at various concentrations.

Total cellular RNA in human gingival tissues and fibroblasts was extracted by the guanidine isothiocyanate procedure (6). Northern blot analysis was carried out as follows. Briefly, total cellular RNA was separated by 1% agarose gel electrophoresis, blotted onto a nylon membrane, and hybridized to human MCP-1 and β-actin cDNA probes labeled with 5′-[α-^{32}P]dCTP by use of a multiprime DNA labeling system. After hybridization, the membranes were washed sequentially, dried, and then exposed to X-ray film (Eastman Kodak Co.) at −70°C. β-Actin was used as an internal standard for quantification of total mRNA on each lane of the gel.

Statistical analysis. All statistical analyses were made according to Student's *t* test.

RESULTS

Monocyte chemotactic activity of crevicular fluids of adult periodontal patients increases with severity of the disease. Initially, we examined whether or not monocyte chemotactic activity of crevicular fluids of periodontal patients is related to severity of the disease. The crevicular fluids of the periodontal patients were collected, centrifuged, and then assessed for monocyte chemotactic activity. As shown in Fig. 1, the chemotactic activity of the crevicular fluids increased with the progression of the disease. Although the activity in the AL-2 group tended to be greater than that in

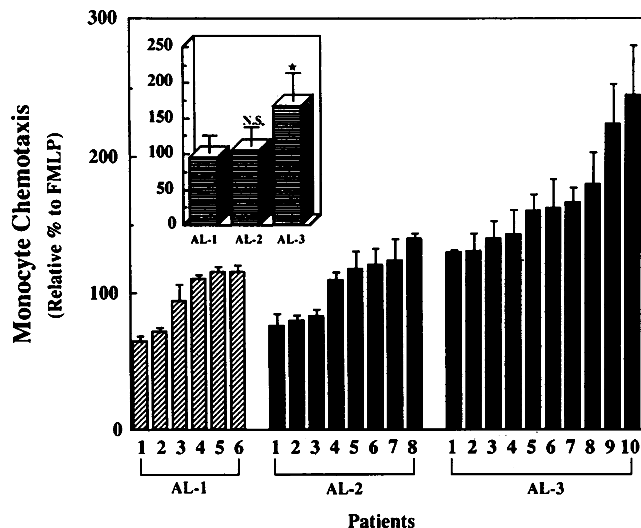

FIG. 1. Monocyte chemotactic activity of crevicular fluids of adult periodontal patients. Crevicular fluids of adult periodontal patients were harvested, centrifuged, and then diluted 100-fold. The diluted samples were tested in the monocyte chemotactic assay. The data are expressed as the mean ± SD of the total number of migrating monocytes per oil immersion field in triplicate assays. Monocyte chemotaxis of FMLP was 140.6 ± 6.7. Inset shows mean ± SD of monocyte chemotaxis for each AL group. *, $P < 0.01$, AL-1 group versus AL-3 group; N.S., not significant for AL-1 group versus AL-2 group.

the AL-1 group, the difference was not significant. However, the chemotactic activity of the AL-3 group was increased significantly compared with that of the AL-1 group. These results suggest that monocyte chemotactic activity of crevicular fluids may increase with the severity of the disease.

Neutralizing effect of anti-JE/MCP-1 antiserum on monocyte chemotactic activity of crevicular fluids of adult periodontal patients. It is very important for an understanding of the mechanism underlying inflammation in periodontal disease to explain whether MCP-1, a chemoattractant specific for monocytes, contributes to the chemotactic activity of crevicular fluids. We examined this point by using anti-JE/MCP-1 antiserum. Crevicular fluids of nine patients in the AL-3 group were left untreated or treated with normal rabbit serum or anti-JE/MCP-1 antiserum and then assessed for monocyte chemotactic activity. Table 1 shows that the increased monocyte chemotactic activity seen in the crevicular fluids was neutralized significantly by anti-JE/MCP-1 antiserum, though the chemotactic activity was not significantly affected by normal rabbit serum. These results suggest that MCP-1 may be involved in the chemotactic activity of crevicular fluids of adult periodontal patients.

Expression of MCP-1 gene in gingival tissues of adult periodontal patients. We wanted to explore the possibility that MCP-1 in the crevicular fluids may be derived from gingival tissues. Therefore, expression of the MCP-1 gene in gingival tissues of adult periodontal patients was examined. Gingival tissues of healthy subjects and periodontal patients in the AL-3 group were collected and homogenized, and then total RNA in these tissues was prepared. The MCP-1 gene expression was analyzed by the Northern blot assay. As shown in Fig. 2, marked expression of the MCP-1 gene was observed in the gingival tissues from all of the periodontal patients tested. However, such gene expression was not detected in those of healthy subjects.

TABLE 1. Neutralization by anti-JE/MCP-1 antiserum of monocyte chemotactic activity of crevicular fluids from adult periodontal patients[a]

Test patient	Monocyte chemotaxis (cells/oil immersion field) with treatment:		
	None	Normal serum	Anti-JE/MCP-1 antiserum
1	63 ± 11	52 ± 5	22 ± 4*
2	64 ± 8	55 ± 10	39 ± 6*
3	57 ± 6	47 ± 6	36 ± 3*
4	60 ± 4	48 ± 8	36 ± 3*
5	52 ± 4	58 ± 4	38 ± 4*
6	67 ± 9	50 ± 4	39 ± 3*
7	72 ± 5	67 ± 5	42 ± 5*
8	61 ± 5	55 ± 7	40 ± 3*
9	55 ± 8	48 ± 5	30 ± 6*

[a] Crevicular fluids of adult periodontal patients were harvested, centrifuged, and then diluted 1,000-fold. The diluted samples were treated for 2 h with anti-JE/MCP-1 rabbit antiserum (1:1,000) or normal rabbit serum and then tested for monocyte chemotactic activity. The data are expressed as the mean ± SD of the total number of migrating monocytes per oil immersion field in triplicate assays. Monocyte chemotaxis of the control was 19 ± 2. Monocyte chemotaxis of FMLP was 78 ± 3.5. *, $P < 0.01$, no treatment versus anti-JE/MCP-1 antiserum treatment.

MCP-1 expression in human gingival tissues treated with P-LPS. Next, we postulated as a possibility that the marked expression of the MCP-1 gene in gingival tissues of adult periodontal patients may be induced by cell components of *P. gingivalis*, an important pathogenic bacterium for the disease. Since it is well known that P-LPS is a potent inducer for production of inflammatory cytokines such as IL-1, TNF-α, and IL-6, LPS may be an influential factor contributing to the induction of MCP-1 expression in gingival tissues. Therefore, we used P-LPS as an inducer for chemoattractant expression. The gingival tissues of human healthy donors were treated for 24 h with P-LPS, and the culture supernatants were prepared and then assessed for monocyte chemotactic activity. Figure 3A (a and b) shows that culture supernatants of P-LPS-treated gingival tissues exhibited the chemotactic activity in a dose- and dilution-related manner. Therefore, using anti-JE/MCP-1 antiserum, we examined the involvement of MCP-1 in the chemotactic activity of P-LPS-treated gingival tissues. As shown in Fig. 3B, anti-JE/MCP-1 antiserum markedly neutralized the chemotactic activity in culture supernatant of P-LPS-treated gingival tissues. These results suggest that MCP-1 contributes predominantly to the P-LPS-induced chemotactic activity of gingival tissues.

MCP-1 expression in human gingival fibroblasts treated with P-LPS. Several studies (7, 21, 30, 33) have demon-

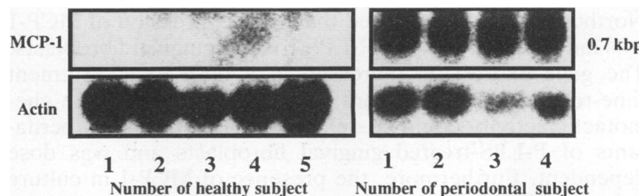

FIG. 2. Expression of MCP-1 gene in human gingival tissues of adult periodontal patients. Human gingival tissues were prepared from healthy subjects and adult periodontal patients in group AL-3 and homogenized in 5.5 M guanidine isothiocyanate. Then, their total RNA was prepared. Northern blot analysis was performed with MCP-1 and β-actin cDNAs used as probes.

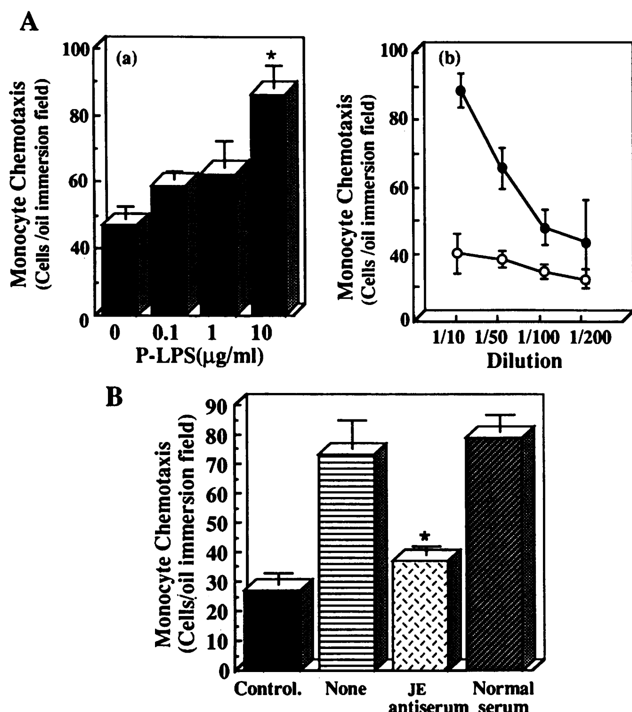

FIG. 3. Monocyte chemotactic activity in culture supernatants of P-LPS-treated human gingival tissues. (A) (a) Human gingival tissues were prepared from healthy subjects and incubated for 24 h with or without various doses of P-LPS. The culture supernatant at a 1/10 dilution was tested for monocyte chemotactic activity. *, $P < 0.01$, control versus P-LPS treatment. (b) Human gingival tissues were incubated for 24 h with (●) or without (○) P-LPS at 10 μg/ml. The culture supernatant was diluted to various degrees and then tested for monocyte chemotactic activity. Monocyte chemotaxis of FMLP was 129.5 ± 5.9. (B) The culture supernatants (1/10 dilution) of human gingival tissues were prepared as described for Fig. 2; they were left untreated (none) or treated for 2 h with anti-JE/MCP-1 antiserum (1:1,000) or normal rabbit serum and then tested in the monocyte chemotactic assay. *, $P < 0.01$, no treatment of supernatant versus anti-JE/MCP-1 antiserum treatment. Monocyte chemotaxis of FMLP was 162.5 ± 8.9. The data in these experiments are expressed as the mean ± SD of the total number of migrating monocytes per oil immersion field in triplicate assays.

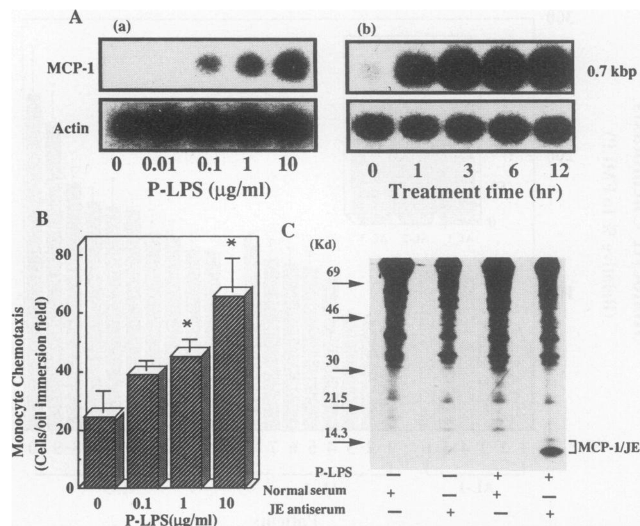

FIG. 4. MCP-1 expression of human gingival fibroblasts treated with P-LPS. (A) (a) The cells were incubated with or without various doses of P-LPS, and total RNA was prepared at 3 h after the initiation of treatment. (b) The cells were incubated for the selected times with or without P-LPS at 10 μg/ml, and then their total RNA was prepared. Northern blot analysis was performed with MCP-1 and β-actin cDNAs used as probes. Exposure time to the X-ray film was 6 h for subpanel a and 18 h for subpanel b. (B) Human gingival fibroblasts were incubated with or without various doses of P-LPS, and the culture supernatants were collected at 24 h after the initiation of treatment. Then, the supernatants were diluted to 1/20 and were tested in the monocyte chemotactic assay. *, $P < 0.01$, control versus P-LPS treatment. Monocyte chemotaxis of FMLP was 78.4 ± 4.5. The data are expressed as the mean ± SD of the total number of migrating monocytes per oil immersion field in triplicate assays. (C) The cells were treated or not with P-LPS (10 μg/ml) and labeled with [^{35}S]methionine, and the culture supernatants were harvested after 24 h. Then, the supernatants were applied to the immunoprecipitation assay with anti-JE/MCP-1 antiserum (1:100) as described in Materials and Methods.

strated that monocyte chemotactic factor(s) was produced by fibroblasts treated with a variety of stimulators. Since fibroblasts are the major cell component in gingival tissues, we suspected that fibroblasts would be a potent producer of MCP-1 if treated with P-LPS. To test this proposition, we examined MCP-1 expression in human gingival fibroblasts treated with P-LPS. As shown in Fig. 4A (a and b), the Northern blot assay showed that gene expression of MCP-1 was induced markedly in P-LPS-treated gingival fibroblasts. The gene expression increased in a dose- and treatment time-related manner. Figure 4B shows that monocyte chemotactic activity could be demonstrated in culture supernatants of P-LPS-treated gingival fibroblasts and was dose dependent. Furthermore, the presence of MCP-1 in culture supernatants of the fibroblasts was examined by an immunoprecipitation assay using anti-JE/MCP-1 antiserum. And, as shown in Fig. 4C, the MCP-1 was found to exist in two forms with molecular masses of 11,000 and 15,000 Da. These two forms were also previously demonstrated by Rollins et al. (27).

Inducing effect of inflammatory cytokines on MCP-1 gene expression in human gingival fibroblasts. Since it is well known that LPS is a potent inducer of inflammatory cytokines in fibroblasts, we explored the possibility that the MCP-1 expression in gingival tissues of adult periodontal patients is mediated by the action of inflammatory cytokines produced by monocytes and fibroblasts in gingival tissues stimulated with cell components of *P. gingivalis* such as LPS and fimbriae. Therefore, finally, the inducing ability of inflammatory cytokines for MCP-1 gene expression in gingival fibroblasts was examined. Under the experimental conditions tested, IL-1β and TNF-α markedly induced MCP-1 gene expression in the tissues, but IL-6 did not do so (Fig. 5).

DISCUSSION

This study has demonstrated that monocyte chemotactic activity of crevicular fluids of adult periodontal patients increases with an increase in severity of this disease and also suggests that MCP-1, a chemoattractant specific for monocytes, produced by the gingival tissues, may be involved in the mechanism of monocyte recruitment from the circulating pool into periodontal tissues.

Infiltration of macrophages and neutrophils into periodontal tissues of adult periodontal patients has been demonstrated by several investigations (4, 10, 24, 29). Migration of

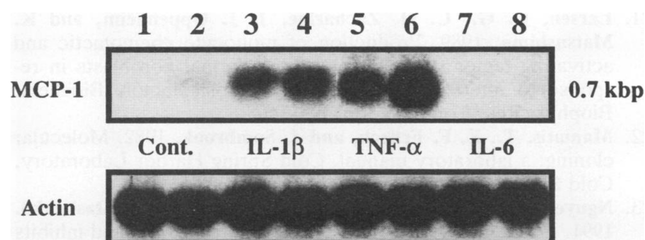

FIG. 5. Effect of inflammatory cytokines on MCP-1 gene expression of human gingival fibroblasts. The cells were incubated singly with or without selected cytokines, and total RNA was prepared at 1 (lanes 1, 3, 5, and 7) or 3 (lanes 2, 4, 6, and 8) h after the initiation of treatment. Northern blot analysis was performed with MCP-1 and β-actin cDNAs used as probes. Cont., control.

these cells into inflammatory regions has also been noted in rheumatoid arthritis and osteoarthritis. Monocytes/macrophages are a source of multiple regulatory factors such as inflammatory cytokines and growth factors, and they release arachidonic acid metabolites, oxygen radicals, and proteases. In view of these multiple functions, monocytes/macrophages may be involved in initiation and development of the inflammatory reactions and alveolar bone loss observed in adult periodontal disease. Therefore, analysis of the mechanisms of monocyte recruitment into periodontal tissues is an important step toward understanding the pathogenesis of this disease.

The high monocyte chemotactic activity in crevicular fluids obtained from the periodontal patients of group AL-3 was significantly neutralized by treatment with anti-JE/MCP-1 antiserum. These observations suggest that MCP-1 predominantly may be involved in the chemotactic activity in the crevicular fluids. However, since the chemotactic activity in the crevicular fluids was not neutralized completely by the specific antiserum, other chemoattractants such as leukotriene B_4 and C_{5a} may also contribute to the chemotactic activity. Although we are not now able to demonstrate whether MCP-1 in the crevicular fluids increases with severity of adult periodontal disease, the answer to this question is very important for furthering our understanding of the functional role of MCP-1 in periodontal disease.

Recent studies have demonstrated that MCP-1 contributes to an increased presence of alveolar monocytes/macrophages and inflammation in human idiopathic pulmonary fibrosis and also to monocyte/macrophage recruitment in inflamed synovial tissue (1, 3, 35). These studies have suggested that MCP-1 may play an important role in producing the pathogenic state of inflammatory disease. Since a marked infiltration of monocytes/macrophages into periodontal tissues of adult periodontal patients has been shown, it was of much interest for us to demonstrate whether MCP-1 contributes to infiltration of these cells into periodontal tissues. Therefore, we examined MCP-1 gene expression in gingival tissues of adult periodontal patients by the Northern blot assay. The marked MCP-1 gene expression was detected in gingival tissues from all adult periodontal patients tested, but not at all in those from healthy subjects. These observations strongly suggest that MCP-1 may play a functional role in marked infiltration of monocytes into periodontal tissues of periodontal patients.

It is postulated as a possibility that MCP-1 gene expression in gingival tissues of adult periodontal patients may be induced by the action of cell components of *P. gingivalis*. Since it is well known that LPS, growth factors, and inflammatory cytokines are potent inducers of MCP-1 expression (13, 18, 19, 28), we examined P-LPS to test our assumption that periodontopathic bacteria may be involved in induction of MCP-1 expression in gingival tissues, because *P. gingivalis* is an important bacterium implicated in adult periodontal disease. In this regard, P-LPS increased in a dose-dependent manner monocyte chemotactic activity in culture supernatants of gingival tissues and fibroblasts from healthy subjects. The chemotactic activity in these culture supernatants was neutralized markedly by anti-JE/MCP-1 antiserum. The immunoprecipitation assay also evidenced that P-LPS-treated gingival fibroblasts secrete MCP-1 into the extracellular medium. These observations demonstrate that P-LPS is a potent inducer of MCP-1 production by gingival tissues and fibroblasts. We further examined the inducing effect of P-LPS on expression of the MCP-1 gene of fibroblasts derived from gingival tissues of a healthy subject. The MCP-1 gene expression of the gingival fibroblasts was markedly induced by P-LPS, and the expression was dose and treatment time dependent. These results imply that MCP-1 produced by *P. gingivalis*-stimulated gingival fibroblasts may be involved predominantly in recruitment of monocytes from the circulating pool into periodontal tissues of adult periodontal patients. On the other hand, several studies (8, 28, 30) have demonstrated that MCP-1 is expressed in endothelial cells and monocytes as well. Therefore, we propose the possibility that MCP-1 detected in crevicular fluids may be derived from fibroblasts, monocytes, and endothelial cells in the gingival tissues.

P-LPS is a potent inducer of inflammatory cytokines of fibroblasts and macrophages (15, 16, 31). Some inflammatory cytokines are also stimulators of MCP-1 expression in these cells. Therefore, we considered the possibility that P-LPS directly induces MCP-1 expression in gingival fibroblasts and indirectly does so via action of inflammatory cytokines induced by itself. Our experimental results showed that IL-1β and TNF-α markedly induced MCP-1 gene expression of human gingival fibroblasts. Our previous study showed that transforming growth factor beta induced JE/MCP-1 expression of mouse osteoblasts (18), and most recently, Williams et al. (36) and we (17, 32) observed that the cells also expressed JE/MCP-1 by treatment with IL-1β and TNF-α. Therefore, MCP-1 expression in periodontal tissues of adult periodontal patients may be induced via actions of cell components of *P. gingivalis* and of inflammatory cytokines produced by cells composing periodontal tissues stimulated by the organism.

In summary, we suggest that MCP-1 plays an important role in monocyte recruitment into periodontal tissues of adult periodontal patients.

ACKNOWLEDGMENT

We express our gratitude to B. J. Rollins of the Dana-Farber Cancer Institute for providing the human MCP-1 cDNA probe and anti-mouse JE/MCP-1 antiserum.

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