Granulocyte-Macrophage Colony-Stimulating Factor Amplification of Interleukin-1β and Tumor Necrosis Factor Alpha Production in THP-1 Human Monocytic Cells Stimulated with Lipopolysaccharide of Oral Microorganisms

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Cytokines, including granulocyte-macrophage colony-stimulating factor (GM-CSF), are used to assist in bone marrow recovery during cancer chemotherapy. Interleukin-1β (IL-1β) and tumor necrosis factor alpha (TNF-α) play important roles in inflammatory processes, including exacerbation of periodontal diseases, one of the most common complications in patients who undergo this therapy. A human monocytic cell line (THP-1) was utilized to investigate IL-1β and TNF-α production following GM-CSF supplementation with lipopolysaccharide (LPS) from two oral microorganisms, Porphyromonas gingivalis and Fusobacterium nucleatum. LPS of P. gingivalis or F. nucleatum was prepared by a phenol-water extraction method and characterized by sodium dodecyl sulfate-polyacrylamide gel electrophoresis and determination of total protein and endotoxin contents. Resting THP-1 cells were treated with LPS of P. gingivalis or F. nucleatum and/or GM-CSF (50 IU/ml) by using different concentrations for various time periods. Production of IL-1β and TNF-α in THP-1 cells was measured by solid-phase enzyme-linked immunosorbent assay. Reverse transcription (RT)-PCR was used to evaluate the gene expression of resting and treated THP-1 cells. IL-1β was not detected in untreated THP-1 cells. IL-1β production was, however, stimulated sharply at 4 h. GM-CSF amplified IL-1β production in THP-1 cells treated with LPS from both oral anaerobes. No IL-1β-specific mRNA transcript was detected in untreated THP-1 cells. However, IL-1β mRNA was detected by RT-PCR 2 h after stimulation of THP-1 cells with LPS from both organisms. GM-CSF did not shorten the IL-1β transcriptional activation time. GM-CSF plus F. nucleatum or P. gingivalis LPS activated THP-1 cells to produce a 1.6-fold increase in TNF-α production at 4 h over LPS stimulation alone. These investigations with the in vitro THP-1 model indicate that there may be an increase in the cellular immune response to oral endotoxin following GM-CSF therapy, as evidenced by production of the tissue-reactive cytokines IL-1β and TNF-α.

Inflammation of the supporting tissues of the teeth produces one of the most common groups of human diseases, periodontal diseases (26). The mechanisms associated with these common oral inflammatory diseases are poorly understood. Interaction of bacterial products and antigens of periodontal pathogens with host inflammatory cells results in the release of cytokines. Periodontitis may involve both the direct cytotoxic and proteolytic effects of oral microorganisms and the indirect pathologic consequences of the host immune response to these microorganisms (33, 43).

Periodontitis is a relatively common infectious disease, leading to tooth loss in adults worldwide. Porphyromonas gingivalis is considered to be one of the important pathogens in the etiology of rapidly progressive periodontitis and adult periodontitis (38, 39). Fusobacterium nucleatum is routinely isolated in high numbers from subgingival plaque in patients with periodontitis (17, 38–40). The role of the lipopolysaccharide (LPS) of these two oral microorganisms in cytokine-mediated inflammatory and destructive lesions of the gingiva and periodontium merits investigation.

Interleukin-1β (IL-1β) is an important mediator of various immunological and inflammatory reactions produced primarily by monocytes (3). As a prototype of the proinflammatory cytokines, IL-1β induces the expression of a variety of genes and the synthesis of several proteins, in turn inducing acute and chronic inflammatory changes (3). Higher levels of IL-1β have been demonstrated in periodontitis tissue (23). IL-1β may play a pivotal role in the pathogenesis and onset of chronic inflammatory periodontal disease (30). IL-1β is one of the factors known to stimulate bone resorption and secretion of proteinase and may be involved in the attachment loss and bone resorption which are characteristic features of periodontitis (25, 36, 46).

Tumor necrosis factor (TNF), or cachectin, is a cytokine originally thought to play a role in host surveillance against neoplasms (7). Endotoxin-stimulated macrophages are the most important source of TNF. TNF alpha (TNF-α) was initially identified as a factor produced by leukocytes and was thought to be responsible for infection-induced cachexia. It has been recognized subsequently that TNF has a broader range of effects on host immune responsiveness, such as enhancing polymorphonuclear neutrophil-endothelial interactions and facilitating phagocytosis and bacterial killing. Recently, a role for TNF in the generation of free radicals and the pathophysiologic changes during sepsis and septic shock has been proposed (7).

The biological properties of TNF have remarkable similar-
ities to those of IL-1. Similar to IL-1, TNF induces fever by its ability to stimulate hypothalamic prostaglandin E2 synthesis directly (16). Levels of circulating TNF increase rapidly in humans injected with endotoxin (9). IL-1 acts synergistically with TNF to protect rats exposed to lethal hyperoxia or radiation. IL-1 cytokine effects on the insulin-producing beta cells of the islets of Langerhans are dramatically augmented by TNF. IL-1 can synergize with TNF to induce lethality in animal models, and in endotoxin-induced shock, the lethality is the result of the synergistic action of IL-1 and TNF rather than overproduction of TNF alone (15).

Human granulocyte-macrophage colony-stimulating factor (GM-CSF) is a glycoprotein functionally involved in the proliferation and differentiation of normal hematopoietic cells (35). This factor stimulates the growth and differentiation of granulocytes, monocytes, erythrocytes, and megakaryocytes (1) from progenitor cells, and it also activates mature granulocytes and macrophages (21, 35). In the last few years, recombinant human GM-CSF has been used in the treatment of chemotherapy-induced bone marrow suppression in patients undergoing transplantation for the treatment of cancer (8, 12). During chemotherapy and bone marrow recovery, with the administration of GM-CSF, these patients often suffer from periodontal infections involving complications. The relationship of GM-CSF-treated monocytes and their responses to LPS from two putative periodontal pathogens, P. gingivalis and F. nucleatum, were investigated by using a human monocytic leukemia cell line, THP-1 (49).

In the last few years, many studies have been done to investigate the role of LPS of aerobic bacteria of monocyte or THP-1 cell activation. There is little, if any, knowledge regarding IL-1β or TNF-α production by monocytes or THP-1 cells in response to LPS of putative periodontal pathogens. The complex interplay between the activation of monocyte-type cells and the release of these tissue-active cytokines in the complex oral environment was the focus of this study. The effect of GM-CSF on monocyte differentiation and activation in the presence of oral LPS has, in fact, never been investigated. It is hypothesized that GM-CSF-stimulated THP-1 cells are immunologically and functionally hyperactivated in the presence of oral LPS. Therefore, the purpose of this study was to elucidate the IL-1β and TNF-α expression of THP-1 cells after treatment with GM-CSF and in response to LPS of P. gingivalis and F. nucleatum to exploit this cell culture model, leading to more precise design of in vivo oral investigations.

MATERIALS AND METHODS
Preparation and characterization of LPS of P. gingivalis and F. nucleatum. LPS of P. gingivalis and F. nucleatum was prepared by the method of Westphal and Jahn (50). Briefly, P. gingivalis (ATCC 33277) and F. nucleatum (ATCC 25586) were grown in Trypticase soy broth containing 1.5% yeast extract, 5% containing 85% N2, 10% H2, and 5% CO2. The bacterial cells were then harvested in an anaerobic chamber (Coy Laboratory Products Inc., Ann Arbor, Mich.) and further purified by being dissolved three times in water to give a 3% (wt/vol) acetone (80°C heat-inactivated fetal bovine serum, 1-glutamine (2 mM), HEPES buffer (10 mM), and minimum essential medium containing sodium pyruvate (1 mM).

Cells were incubated at 7°C in a humidified atmosphere consisting of 5% CO2. All of the media and ingredients used in the cell culture system were tested with the E-Toxate test (Sigma) and found to be negative for endotoxin activity. After 3 to 4 days of growth, THP-1 cells were harvested, THP-1 cells at 106/ml were distributed among the wells of a 24-well microtiter plate, and the cells were then considered to be ready for the various treatment experiments. Concentrations of GM-CSF (Collaborative Biomedical Products, Bedford, Mass.) of 500, 50, 5, and 0.5 IU/ml in RPMI 1640 CM were added to duplicate wells of 24-well microtiter plates containing THP-1 cells at 106/ml in RPMI 1640 CM. Different concentrations of P. gingivalis or F. nucleatum LPS or TNF-α were added. Comparable concentrations (10, 2, 10, and 100 pg/ml) of P. gingivalis or F. nucleatum LPS or TNF-α were added. Following centrifugation, the colorless upper aqueous phase containing RNA was transferred to a fresh tube. The RNA was precipitated from the aqueous phase by mixing with 0.5 ml of isopropyl alcohol. Samples were incubated at room temperature for 10 min and centrifuged at 4,500 x g for 10 min. The supernatant fluids of untreated THP-1 cells or those treated at different times and with different doses and substances were stored at 8°C until used for measurement of IL-1β and TNF-α with commercial enzyme-linked immunosorbent assay (ELISA) kits (R & D Systems, Minneapolis, Minn.). The basic principle of the ELISA was the quantitative sandwich enzyme immunoassay technique in which a monoclonal antibody specific for IL-1β or TNF-α was used to coat the microtiter plate provided in the kit. Duplicate readings for each standard, control, and sample were taken and averaged. The average absorbance for each duplicate set of standards, controls, and samples was calculated by using a standard curve. Results are expressed as picograms of IL-1β or TNF-α per milliliter of supernatant fluid.

Isolation of RNA. RNA was isolated with TriZol Reagent (GIBCO) by using a single-step isolation method originally developed by Chomczynski and Sacchi (10). RNase-free plastic and water were used throughout. THP-1 cells grown in 3 days in a 75-cm2 flask were harvested, and 3 x 106 THP-1 cells per ml were distributed among the wells of a 24-well microtiter plate. The cells were then treated with 100 μg of the LPS (5 ng/ml) final concentration (3) or with TNF-α (10 ng/ml) final concentration (or without GM-CSF) (50 U/ml) for 5, 15, or 30 min or 1 or 2 h. After each treatment period, the cells were harvested and lysed by resuspending the cell pellet with 1 ml of TriZol Reagent (GIBCO) and refrigeration on ice. A 100-μl volume of the lysis buffer was then added, and the samples were centrifuged at 4,500 x g for 30 min at 4°C.

Following centrifugation, the colorless upper aqueous phase containing RNA was transferred to a fresh tube. The RNA was precipitated from the aqueous phase by mixing with 0.5 ml of isopropyl alcohol. Samples were incubated at room temperature for 10 min and centrifuged at 4,500 x g for 20 min at 4°C. The RNA pellet was washed once with 75% ethanol. The concentration and purity of the RNA thus isolated were determined by measuring the optical density at 260 and 280 nm in a spectrophotometer and by agarose gel electrophoresis.

Determination of the optimal number of THP-1 cells and the purity of the RNA isolated. The number of THP-1 cells required to produce an optimal quantity of isolated total RNA was determined by using 1 x 106, 5 x 105, and 1 x 105 THP-1 cells in preliminary experiments. A concentration of 3 x 106 THP-1 cells was chosen for the subsequent RNA isolation procedures after evaluation of the amounts of RNA isolated from the different numbers of cells. In all reverse transcription (RT)-PCRs, the isolated total RNA was run in a 1.2% agarose gel with ethidium bromide to see if pure RNA was present prior to quantitation of the optical density at 260 and 280 nm in a spectrophotometer.

RT-PCR detection of cytokine mRNA. RT-PCR was performed to determine whether IL-1β, which was not detectable by the ELISA, could be detected by RT-PCR. For TNF-α, RT-PCR was performed to detect the presence of mRNA in untreated and treated THP-1 cells. RT-PCR was also utilized to determine the start time of IL-1β transcription after stimulation of THP-1 cells with LPS of P. gingivalis or F. nucleatum. IL-1β sense (upstream): 5′-ATGAAAGGCTTCTCCTTCACT-GTG-3′ and antisense (downstream): 5′-TCAGTTTTC-3′ and TNF-α sense (upstream: 5′-GGAATCACCAGTGGGCAAG-3′) and antisense (downstream: 5′-TGGGAGATAGGTGAGTAGCACG-3′) primers were selected from published sequences (11). The primers were prepared by Oligo (Woburn, Mass.). An RT reaction was prepared by using a thermostable rTth DNA reverse transcriptase PCR Kit.
RESULTS

Characterization of the LPS of P. gingivalis and F. nucleatum. The endotoxin content of F. nucleatum LPS was determined to be 3 x 10^{-6} EU/mg, and that of P. gingivalis LPS was 6 x 10^{-6} EU/mg. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis of the isolated LPS displayed low- and intermediate-molecular-weight (10,000 to 60,000) bands. P. gingivalis LPS showed a prominent band of lipid smudges which was not seen in the F. nucleatum LPS preparation (data not shown).

Results of IL-1β estimation. The effects of P. gingivalis and F. nucleatum LPS (100, 10, 1, and 0.1 μg/ml), PMA (10^{-7}, 10^{-8}, 10^{-9}, and 10^{-10} mol/ml), and GM-CSF (500, 50, 5, and 0.5 IU/ml) on IL-1β production by THP-1 cells were evaluated. Dose-response experiments with THP-1 cells after 24 h of incubation with GM-CSF (500, 50, 5, and 0.5 IU/ml) demonstrated that treatment with GM-CSF at 500 μg/ml produced IL-1β at 0.5 pg/ml, while 8 pg/ml was produced by 500 μg/ml and 5.2 pg/ml was produced by 5 μg/ml (Fig. 1). A GM-CSF concentration of 500 μg/ml was selected for further experimentation (Fig. 1). Similarly, a PMA dose of 10^{-8} mol/ml was chosen. F. nucleatum LPS at 10 μg/ml produced IL-1β at 525 pg/ml, 1 μg/ml produced 421 pg/ml, and 0.1 μg/ml produced 223 pg/ml (Fig. 1). One microgram of F. nucleatum LPS per milliliter was selected as a suitable concentration for IL-1β stimulation. P. gingivalis LPS at 10 μg/ml produced IL-1β at 665 pg/ml, 1 μg/ml produced 595 pg/ml, and 0.1 μg/ml produced 386 pg/ml (Fig. 1). A P. gingivalis LPS dose of 1 μg/ml was also selected for further experimentation.

Untreated THP-1 cells did not produce IL-1β (Fig. 2). There was a sharp rise in IL-1β production to 600 pg/ml after 4 h of treatment with the LPS (1 μg/ml) of F. nucleatum (Fig. 2). Significantly greater (P < 0.05) IL-1β production resulted when GM-CSF was used along with P. nucleatum LPS than when F. nucleatum LPS alone was used. GM-CSF had a synergistic effect when combined with treatment with the LPS of F. nucleatum from 1 to 7 days (Fig. 2). LPS (1 μg/ml) of P. gingivalis produced a similar trend in IL-1β production (Fig. 2). There was also a significant (P < 0.05) increase in IL-1β production due to supplementation with GM-CSF (50 IU/ml) (Fig. 2).

PMA (10^{-8} mol/ml) treatment caused a gradual increase in IL-1β production to 600 pg/ml after 24 h (Fig. 2). When GM-CSF (50 IU/ml) was added with PMA, IL-1β production peaked at 950 pg/ml at 24 h and then declined to 700 pg/ml at 7 days. There was significantly (P < 0.05) greater IL-1β production when PMA treatment was supplemented with GM-CSF than when PMA alone was used at the 12-h, 24-h, and 2-day time points (Fig. 2).

TNF-α estimation results. The dose-response effect of TNF-α was initially evaluated by using the LPS of P. gingivalis or F. nucleatum (100, 10, 1, and 0.1 μg/ml), PMA (10^{-7}, 10^{-8}, 10^{-9}, and 10^{-10} mol/ml), and GM-CSF (500, 50, 5, and 0.5 IU/ml) to select a minimum concentration at which measurable TNF-α was produced. The maximum concentration (100 μg/ml) of P. gingivalis LPS produced 2,887 pg of TNF-α per ml, while 1 μg/ml produced 2,700 pg/ml. Hence, P. gingivalis LPS at 1 μg/ml was selected for later TNF-α experimentation. Similarly, 1 to 100 μg of F. nucleatum per ml produced >3,000 pg of TNF-α per ml (Fig. 3) after 8 h of stimulation. Therefore, 1 μg of F. nucleatum LPS per ml was also selected for later TNF-α experiments. A GM-CSF concentration of 50 IU/ml produced a maximum response of 112 pg of TNF-α per ml and was selected as suitable for experimentation (Fig. 3). A PMA concentration of 10^{-7} mol/ml produced 1,965 pg of TNF-α per ml, 10^{-8} mol/ml produced 1,155 pg/ml, and 10^{-9} mol/ml produced 358 pg/ml at 8 h of stimulation (Fig. 3). A PMA concentration of 10^{-8} mol/ml was selected for future experimentation.

A very low level of TNF-α was detected in 2-h culture supernatant fluids of untreated THP-1 cells (Fig. 4). This indi-
cated that THP-1 cells produced TNF-α constitutively, a finding which was supported later by our RT-PCR results. The TNF-α level rose sharply after 4 h of treatment with F. nucleatum LPS with or without GM-CSF treatment and then gradually declined to the baseline level at 7 days (Fig. 4). The TNF-α level after treatment with P. gingivalis LPS (1 μg/ml) and GM-CSF significantly (P < 0.05) greater IL-1β production was observed at 8 h of treatment and afterwards when P. gingivalis LPS (1 μg/ml) was supplemented with GM-CSF (50 IU/ml) than when P. gingivalis LPS or GM-CSF alone was used. The data points represent average values of two replicative samples. (C) IL-1β production after treatment with PMA (10^{-8} mol/ml) and GM-CSF. Significantly (P < 0.05) greater IL-1β production was observed at 8 h after treatment when PMA was supplemented with GM-CSF (50 IU/ml) than when PMA or GM-CSF alone was used. The data points represent average values of two replicative samples.

FIG. 3. Different dose-response experiments for TNF-α assay. THP-1 cells (10^6/ml) were treated with different doses of GM-CSF for 24 h, PMA for 12 h, and F. nucleatum or P. gingivalis LPS for 8 h, and supernatant fluids were tested for TNF-α. A 50-IU/ml GM-CSF concentration (concn) was chosen for future experimentation. A 10^{-8} mol/ml PMA concentration was chosen as optimal. One microgram of F. nucleatum or P. gingivalis LPS was used for TNF-α stimulation.

RT-PCR detection of IL-1β mRNA. The RT-PCR products of IL-1β from THP-1 cells (Fig. 5) revealed the 300-bp IL-1β cDNA in THP-1 cells after 2 h of treatment with either P. gingivalis or F. nucleatum LPS. Untreated, GM-CSF (50 IU/ml)-treated, P. gingivalis or F. nucleatum LPS (1 μg/ml)-treated, and PMA (10^{-8} mol/ml)-treated THP-1 cells were studied over different periods of time for IL-1β-specific cDNA. P. gingivalis or F. nucleatum LPS stimulated IL-1β mRNA transcription after 2 h of stimulation (Table 1). Untreated THP-1 cells produced no mRNA within 24 h, indicating that IL-1β was not produced constitutively by THP-1 cells.

RT-PCR detection of TNF-α mRNA. An agarose gel (1.2%) containing the TNF-α RT-PCR product (Fig. 6) showed a 250-bp TNF-α cDNA in untreated and treated THP-1 cells, indicating constitutive production of TNF-α mRNA in THP-1 cells. The negative control (no band) was in the other half of the gel (not shown). All THP-1 cells, including untreated, GM-CSF (50 U/ml)-treated, P. gingivalis and F. nucleatum LPS (1 μg/ml)-treated, and PMA (10^{-8} mol/ml)-treated cells, produced a 250-bp TNF-α-specific cDNA.

DISCUSSION

F. nucleatum and P. gingivalis LPSs were selected for THP-1 cell stimulation in an attempt to develop a model system which would allow the study of monocyte-macrophage activation in oral diseases. There are few publications related to macro-
phage interactions with LPS from these periodontal pathogens, as most investigators have used *Escherichia coli* LPS in their studies (5, 32, 41). The composition of *P. gingivalis* LPS is unique in that it contains phosphorylated 2-keto-3-deoxyoctonate, which is not in the LPS of *E. coli* (20). *F. nucleatum* LPS differs from the classical *E. coli* LPS in that it contains a significant amount of heptose and small quantities of 2-keto-3-deoxyoctonate (27).

The cytokine IL-1 has a central role in many biologic processes, including inflammation (44). We observed a sharp rise in IL-1β production after 4 h of treatment with *F. nucleatum* LPS or *P. gingivalis* LPS. The production reached a peak at 24 h and lasted for 4 days after treatment. An amplified effect was produced when GM-CSF was used with *F. nucleatum* or *P. gingivalis* LPS compared to treatment with LPS alone. These data agree with those of Hart et al. (28), who reported that the combination of GM-CSF and LPS (*E. coli*) induced synergistic IL-1 release by THP-1 cells and human monocytes. In combination with *E. coli* LPS, GM-CSF was reported to be a weak inducer of monocyte IL-1β activity (28).

No evidence of IL-1β gene expression in circulating peripheral blood mononuclear cells of healthy subjects has been obtained by Northern hybridization, in situ hybridization, or PCR (47). Many reports of “spontaneous” IL-1 production in various disease states, such as AIDS, or in the laboratory by infection of mononuclear cells with the human immunodeficiency virus are likely artifactual because of endotoxin contamination (37).

In our investigations, the 300-bp IL-1β cDNA was found in THP-1 cells after 2 h of treatment with *P. gingivalis* or *F. nucleatum* LPS but was absent in untreated cells and cells treated for 2 h with GM-CSF. Hence, IL-1β mRNA was not produced constitutively by the THP-1 cells. GM-CSF alone did not induce IL-1β mRNA production by 2 h, but *F. nucleatum* or *P. gingivalis* LPS induced IL-1β mRNA production at 2 h with or without GM-CSF supplementation. Other investigators (24) could not detect IL-1β mRNA in both unstimulated control monocytes and macrophages, whereas a marked accumu-

**FIG. 4.** (A) TNF-α production after treatment with *F. nucleatum* LPS and/or GM-CSF. There was a sharp rise in TNF-α production at 4 h of *F. nucleatum* LPS (1 µg/ml) stimulation, and the level gradually returned to the baseline by 48 h. Significantly (P < 0.05) greater TNF-α production was observed from 4 to 48 h when *F. nucleatum* LPS (1 µg/ml) was supplemented with GM-CSF (50 IU/ml) than when *F. nucleatum* LPS or GM-CSF alone was used. The data points represent average values of two replicative samples. (B) TNF-α production after treatment with *P. gingivalis* LPS and/or GM-CSF. TNF-α production rose sharply at 4 h after stimulation with *P. gingivalis* LPS (1 µg/ml) and then gradually returned to the baseline level after 48 h. Significantly (P < 0.05) greater TNF-α production was observed from 4 to 48 h when *P. gingivalis* LPS (1 µg/ml) was supplemented with GM-CSF (50 IU/ml) than when *P. gingivalis* LPS alone was used. The data points represent average values of two replicative samples. (C) TNF-α production after treatment with PMA (10⁻⁸ mol/ml) and/or GM-CSF. TNF-α production gradually increased up to 24 h of stimulation with PMA (10⁻⁸ mol/ml) and then gradually declined. The data points represent average values of two replicative samples. Concn, concentration.

**FIG. 5.** Agarose (1.2%) gel stained with ethidium bromide containing the 300-bp IL-1β RT-PCR product from treated and untreated THP-1 cells. Lanes: 1, 100-bp DNA ladder; 2, THP-1 cells treated for 2 h with *F. nucleatum* LPS (1 µg/ml); 3, THP-1 cells treated for 2 h with *P. gingivalis* LPS (1 µg/ml); 4, negative control; 5, untreated THP-1 cells; 6, THP-1 cells treated with GM-CSF for 2 h.

**TABLE 1.** RT-PCR results for IL-1β gene expression in THP-1 cells after treatment with different substances for different durations of timea

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a +, IL-1β mRNA transcription in THP-1 cells detected by RT-PCR. −, IL-1β mRNA transcription in THP-1 cells not detected by RT-PCR. NT, not tested.
b GM-CSF at 50 IU/ml.
c *F. nucleatum* LPS at 1 µg/ml.
d *P. gingivalis* LPS at 1 µg/ml.
e PMA at 10⁻⁸ mol/ml.
The network of cytokines is complex. Since TNF-α can also induce the synthesis of IL-1β by monocytes-macrophages, it is possible that the observed TNF-α effect was dependent on the synthesis of IL-1β (4). The kinetics of cytokine production by LPS-stimulated monocytes differed for IL-1β and TNF-α (34). In our study, TNF-α reached a plateau by 8 h after stimulation and then gradually declined. Also, all of our untreated, GM-CSF-treated, and P. gingivalis or F. nucleatum LPS-treated THP-1 cells showed a 250-bp TNF-α cDNA. This finding suggested that TNF-α is produced constitutively in THP-1 cells, as determined by RT-PCR. This is in conformity with the results of others (13, 19, 32), who also found low levels of TNF-α mRNA in untreated THP-1 cells.

The findings of Asakura et al. (2) and Essner et al. (18) are consistent with our finding that TNF-α, but not IL-1β, may be produced constitutively in human monocytes. In the THP-1 cell system, this appears to be the case. The findings of Terao et al. (48) are also consistent with our in vitro model. In sarcoidosis patients treated with GM-CSF, an enhanced inflammatory response, as evidenced by increased production of TNF-α and IL-1β, might be relevant to the pathogenesis of the disease. Periodontal disease and the cellular response to the LPS of oral microorganisms might likewise be affected by GM-CSF treatment.

Hays and Zoon (29) referred to a “priming effect” of GM-CSF on human monocytes. In our in vitro model with LPS of oral microorganisms, the goal was to identify any synergism between LPS and GM-CSF. The clinical importance in our model is that preexisting periodontal disease may predispose cancer or other patients to periodontal complications following GM-CSF therapy due to previous stimulation with the LPS of oral organisms. In fact, Perkins et al. found that patients receiving continuous infusion of GM-CSF demonstrated enhanced production of TNF-α and IL-1β in monocytes (45). Those researchers concluded that this effect may enhance the patient’s resistance to new infection, but based on our model, in an inflammatory disease like periodontal disease, exacerbation of disease activity might be observed.

The sequence of events in periodontal diseases is still in need of in-depth study. The studies described herein have evaluated the effect of the growth factor GM-CSF and the LPSs of two putative periodontal pathogens on macrophage lineage cells. The results of this study imply that macrophages have an active role in acute and chronic periodontal exacerbations in the presence of the GM-CSF growth factor and LPS. Periodontal diseases are clearly multifactorial, perhaps beginning with the activation of the immune system at the cellular level by the LPS of a potential pathogen such as F. nucleatum or P. gingivalis. Simultaneously, genes are up-regulated to express tissue-active inflammatory cytokines such as IL-1β, IL-6, and TNF-α. The events become cyclic, leading to periodontal attachment and tissue damage. The effects of these agents clearly are directly related to the oral disease activity observed clinically in immunologically healthy and immunocompromised patients. Activation and differentiation of THP-1 cells by oral LPS in the presence of GM-CSF may suggest a role for human macrophages in acute and chronic periodontal diseases.

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REFERENCES
