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Lipopolysaccharides of *Bacteroides intermedius* (*Prevotella intermedia*) and *Bacteroides* (*Porphyromonas*) *gingivalis* Induce Interleukin-8 Gene Expression in Human Gingival Fibroblast Cultures

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Lipopolysaccharides (LPS) prepared from *Bacteroides intermedius* (*Prevotella intermedia*) and *Bacteroides* (*Porphyromonas*) *gingivalis* by hot phenol-water extraction induced interleukin-8 (IL-8) mRNA in normal human gingival fibroblast cultures, as demonstrated by Northern (RNA) blot analysis. IL-8 mRNA levels began to increase after a 2-h exposure, reached a maximum after 12 h, and then dropped to the unstimulated level at 48 h. IL-8 mRNA levels were also enhanced in a dose-dependent manner. By contrast, LPS specimens from various *Salmonella* species with S and R chemotypes and from bacterial and synthetic lipid A preparations did not increase IL-8 mRNA levels in fibroblasts. Although recombinant human IL-1α induced IL-8 mRNA expression in fibroblast cultures, an antiserum to recombinant human IL-1α did not decrease the IL-8 mRNA accumulation induced by *B. intermedius* LPS. Fibroblasts primed with natural human gamma interferon (IFN-γ) expressed higher IL-8 mRNA levels upon stimulation with *B. intermedius* LPS, but not with *Salmonella* LPS, compared with nontreated cells. Natural human IFN-β exhibited a similar priming effect on the fibroblasts, and antiserum to IFN-β added to the cultures together with *B. intermedius* LPS decreased the IL-8 mRNA levels. Therefore, endogenous IFN-β enhanced IL-8 mRNA production in response to *B. intermedius* LPS in fibroblasts.

Some black-pigmented *Bacteroides* species, such as *Bacteroides* (*Porphyromonas*) *gingivalis* and *Bacteroides intermedius* (*Prevotella intermedia*), are implicated in periodontal diseases (7, 26, 41). The lipopolysaccharides (LPS) of these organisms possess unique chemical structures and show characteristic biological properties different from those of the usual LPS found in members of the family *Enterobacteriaceae* (12, 13). Fibroblasts are the predominant cell type found in periodontal tissues. The cells are regulated by various cytokines, and they themselves are capable of producing various inflammatory cytokines, such as interleukin (IL)-1, IL-6, and IL-8, which may in turn initiate and augment periodontal diseases (36). In fact, ample evidence indicates the presence of higher levels of IL-1 and IL-6 in diseased than in normal periodontal sites (4, 15–17, 24). In a previous study, we demonstrated that oral *Bacteroides* LPS induced cell-associated IL-1α and cell-free IL-1β and IL-6 in human gingival fibroblast cultures (37), whereas LPS from the other bacterial species had no or only weak activity in this respect. Induction of IL-1β and IL-6 by human gingival fibroblasts stimulated with *B. gingivalis* LPS was also reported by Sisney-Durrant and Hopps (31) and Bartold and Haynes (1), respectively.

IL-8, another important inflammatory cytokine, is chemotactic and stimulatory for neutrophils; it induces neutrophil degradation and increases expression of the complement receptor CR-1 and the cell adhesion molecule Mac-1 (CD11b/CD18) in neutrophils (reviewed by Oppenheim et al. [27] and van Damme [40]). Various cells, including fibroblasts, are capable of producing IL-8 upon stimulation with IL-1 and tumor necrosis factor (TNF) (reviewed by van Damme [40]). LPS from *Enterobacteriaceae* also stimulates IL-8 production in human monocytes (29, 42, 44), endothelial cells (28, 33), and neutrophils (2, 32) but not in fibroblasts (30, 34). As described above, *Bacteroides* LPS differed from *Enterobacteriaceae* LPS with respect to effects on fibroblasts. Therefore, we examined the IL-8-inducing activity of LPS from *B. intermedius* and *B. gingivalis* by measuring the steady-state levels of IL-8 mRNA in normal human gingival fibroblast cultures compared with that of endotoxins from *Enterobacteriaceae*. The IL-8 gene was expressed by gingival fibroblasts stimulated with *Bacteroides* LPS, providing additional evidence of the pathological involvement of LPS in periodontal diseases.

MATERIALS AND METHODS

LPS and lipid A preparations. *Bacteroides* LPS were prepared from *B. intermedius* ATCC 25611 and *B. gingivalis* 381 cultured in GAM broth (Nissui Seiyaku Co., Tokyo, Japan) at 37°C for 18 h in an N_2-H_2-CO_2 (85:10:5) atmosphere in an anaerobic culture system (MIP-1025; Sanyo, Tokyo, Japan) by hot phenol-water extraction (43). The chemical analytical data of LPS were generally similar to those reported previously by Hamada et al. (11). No visible bands of protein were observed in Coomassie brilliant blue-stained sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoregrams of these LPS preparations. The silver-

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stained migration patterns of *B. intermedius* and *B. gingivalis* LPS on deoxycholate-polyacrylamide gel electrophoresis (18) showed an intense, low-molecular-mass band typical of lipooligosaccharide and the typical ladder-form pattern of smooth (S)-form LPS, respectively, as described previously (35). The LPS from *Salmonella abortus-equi* (S form), *Salmonella minnesota* S519 (S form), and *S. minnesota* R595 (Re form) and the lipid A prepared from *S. minnesota* R595 were provided by C. Galanos, Max-Planck-Institut für Immunbiologie (Freiberg, Germany) (9). The synthetic *Escherichia coli*-type lipid A, LA-15-PP (14), was a gift from S. Kusumoto (Osaka University, Osaka, Japan).

Cytokines, antibodies, and chemicals. Recombinant human IL-1α (rHuIL-1α) and rabbit antiserum to rHuIL-1α were supplied by Dainippon Pharmaceutical Co. (Osaka, Japan). The rabbit antiserum to rHuIL-1β was a gift from Otsuka Pharmaceutical Co. (Tokushima, Japan). Natural human interferon-β (nHuIFN-β; 32,000 U/ml) and rabbit antiserum to nHuIFN-β were generously provided by Toray Co. (Tokyo, Japan). nHuIFN-γ (specific activity, 3.1×10^8 IU/mg of protein) was supplied by Hayashibara Bioscience Institute (Okayama, Japan). Cycloheximide was purchased from Sigma Chemical Co. (St. Louis, Mo.).

cDNA probes. A human IL-8 cDNA clone containing a 0.5-kb insert (5′ end) (25) was supplied by H. A. Young (Laboratory of Experimental Immunology, NCI-Frederick Cancer Research and Development Center, Frederick, Md.). The human IL-1α and IL-1β cDNA plasmids, huIL-1αH2H3 containing a 0.66-kb *Hin*dIII-*Hin*cII restriction fragment and huIL-1β:pGEMI containing a 0.57-kb *Sst*I-*Sma*I restriction fragment (23), were generously provided by S. Gillis (Immunex Research and Development Co., Seattle, Wash.). A human glyceraldehyde-3-phosphate dehydrogenase (GADPH) cDNA clone (39) was provided by S. Sakiyama (Chiba Cancer Center Research Institute and Hospital, Chiba, Japan). For Northern (RNA) blot analysis, inserts were excised with appropriate restriction enzymes and then purified from vector sequences by agarose gel electrophoresis.

Human fibroblast cultures. A specimen of normal human gingival tissue (wet weight, ca. 500 mg) was obtained from a male patient (10 years old) during a fenestration operation. The minced explants were cultured in alpha minimal essential medium (α-MEM; Flow Laboratories, McLean, Va.) supplemented with 10% fetal bovine serum (FBS; GIBCO Life Technologies Inc., Grand Island, N.Y.) in plastic culture dishes as described previously (37). The fibroblasts that were homologous, slim, and spindle shaped, growing in characteristic swirls, were designated GF-5 and used at subculture levels 5 through 10. The GF-5 cells were plated into 55-cm^2 dishes (Iwaki Glass, Tokyo, Japan) and grown to confluence in α-MEM containing 10% FBS and 200 mg of kanamycin per liter at 37°C in a 5% CO_2 atmosphere. Confluent cells were rinsed in serum-free α-MEM and preincubated in α-MEM containing 1% FBS for 18 h. For IFN priming, 1,000 U of nHuIFN-β or nHuIFN-γ per ml was added to the preincubation medium. The medium was replaced with that containing test materials and incubated for the indicated periods.

RNA isolation and Northern blot analysis. Total cellular RNA was prepared from cells according to the guanidium isothiocyanate-cesium chloride procedure (5). Isolated RNA was suspended in diethyl pyrocarbonate-treated water, and 15 μg of each was fractionated on 1.2% agarose gels containing 0.66 M formaldehyde and transferred to a nylon membrane (Zeta-Probe; Bio-Rad Laboratories, Richmond, Calif.) by electroblotting (38). The RNA bound to the membrane was prehybridized overnight at 42°C with 50% formamide–4× SSPE (1× SSPE is 180 mM NaCl, 10 mM $Na_2HPO_4 \cdot 7H_2O$, and 1 mM EDTA)–1% SDS–0.5% nonfat powdered milk–0.5 mg of salmon sperm DNA (Sigma) per ml. Hybridization was performed at 42°C for 18 to 24 h in 50% formamide–4× SSPE–1% SDS–0.5% nonfat powdered milk–0.5 mg of salmon sperm DNA per ml with 10^6 cpm of ^{32}P-labeled cDNA probes per ml. Probes were labeled by using random primers, Klenow fragment (Boehringer, Mannheim, Germany), and [α-^{32}P]dCTP (NEN, Wilmington, Del.) as described by Feinberg and Vogelstein (8). The specific activity of the labeled cDNA was 1×10^8 to 5×10^8 cpm/μg of DNA. Membranes were washed three times with 2, 0.5, and 0.1× SSC (1× SSC is 150 mM NaCl plus 15 mM trisodium citrate, pH 7.0) containing 0.1% SDS at room temperature for 20 min each time. The filters were exposed to X-ray film (medical X-ray film; Konika, Tokyo, Japan) with intensifying screens at −70°C for several days. In some experiments, relative intensities of exposures of two lanes on a film were determined by densitometry scanning.

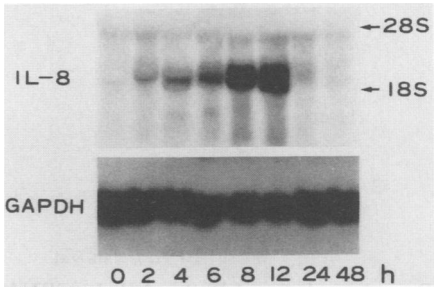

FIG. 1. Time course of IL-8 mRNA expression in human gingival fibroblast cultures stimulated with *B. intermedius* LPS in GF-5 human gingival fibroblasts. Confluent, quiescent GF-5 cells were stimulated with *B. intermedius* LPS (100 μg/ml) for the indicated periods. Total cellular RNA (15 μg per lane) was electrophoresed in agarose-formaldehyde gels, blotted onto nylon membranes, and then hybridized with a ^{32}P-labeled IL-8 probe as described in Materials and Methods. Arrows indicate the locations of 18S and 28S rRNAs. As a control, the same blot was reprobed for GAPDH mRNA.

RESULTS

IL-8 mRNA expression by fibroblasts stimulated with *Bacteroides* LPS. We initially examined the effect of LPS from *B. intermedius* on the expression of IL-8 mRNA by the human gingival fibroblast GF-5 cell cultures. Confluent cells were rendered quiescent by an 18-h incubation in α-MEM containing 1% FBS and then stimulated with *B. intermedius* LPS for 0 to 48 h. The time-dependent accumulation and clearance of IL-8 mRNA are shown in Fig. 1. IL-8 mRNA levels began to increase after 2 h of exposure and reached a maximum after 12 h but declined to the unstimulated levels by 48 h, whereas basal IL-8 mRNA was undetectable in unstimulated cells. The IL-8 mRNA was 2.0 kb in size. The control gene assessed for GAPDH was not affected by stimulation with *B. intermedius* LPS. As shown in Fig. 2, the IL-8 mRNA level in the culture was enhanced in a dose-dependent manner within a range between 1 and 100 μg of *B. intermedius* LPS per ml in culture at 12 h. The expression of a 2.2-kb IL-1α mRNA was also induced by *B. intermedius* LPS in a dose-dependent manner similar to that of IL-8 mRNA. By contrast, the expression of a 1.7-kb IL-1β

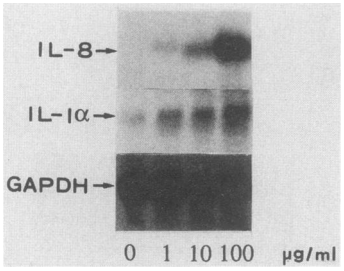

FIG. 2. Dose dependence of IL-8 and IL-1α mRNA expression in human gingival fibroblast cultures stimulated with *B. intermedius* LPS. Confluent, quiescent GF-5 cells were stimulated with the indicated doses of *B. intermedius* LPS for 12 h. Total cellular RNA (15 μg per lane) was electrophoresed in agarose-formaldehyde gels, blotted onto nylon membranes, and then hybridized with ^{32}P-labeled IL-8, IL-1α, and GAPDH probes as described in Materials and Methods.

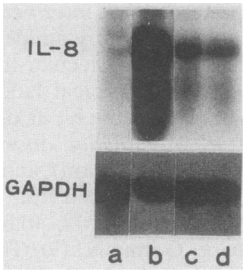

FIG. 4. Effects of the antiserum to rHuIL-1α and cycloheximide on *B. intermedius* LPS-induced IL-8 mRNA expression by fibroblasts. GF-5 cells were unstimulated (lane a) or stimulated for 12 h with *B. intermedius* LPS (10 μg/ml) together with cycloheximide (10 μg/ml) (lane b), *B. intermedius* LPS (10 μg/ml) alone (lane c), and *B. intermedius* LPS (10 μg/ml) together with the antiserum to rHuIL-1α (1:100) (lane d). Other details are given in the legend to Fig. 1.

mRNA was only slightly induced by *B. intermedius* LPS (data not shown).

GF-5 cells were stimulated with various endotoxins for 12 h. Total RNA was extracted and assayed by Northern blotting for IL-8 mRNA accumulation (Fig. 3). Cultures stimulated with rHuIL-1α as a positive control had strongly induced IL-8 mRNA expression. *B. gingivalis* LPS induced definite mRNA expression, while other materials (the S-form LPS of *S. abortus-equi* and *S. minnesota* S519, Re-form LPS of *S. minnesota* R595, lipid A from *S. minnesota* R595, and synthetic *E. coli*-type lipid A) did not increase IL-8 mRNA levels. IL-8 mRNA was not detected even at the longest exposure of the cells to these endotoxins of *Enterobacteriaceae* (data not shown).

Low-level involvement of IL-1 in IL-8 production by fibroblasts stimulated with *Bacteroides* LPS. To elucidate whether the induction of IL-8 mRNA expression was exerted via IL-1 produced by fibroblasts stimulated with *Bacteroides* LPS, GF-5 cells were stimulated with *B. intermedius* LPS (10 μg/ml) in the presence or absence of the authentic antisera to rHuIL-1α and rHuIL-1β, alone or in combination, for 12 h. Addition of anti-rHuIL-1α did not inhibit IL-8 mRNA accumulation in fibroblasts stimulated with *B. intermedius* LPS (Fig. 4). Anti-rHuIL-1β (1:100), either alone or in combination with anti-IL-1β and anti-IL-1α (1:100 each), inhibited IL-8 mRNA expression very little (data not shown). To determine whether the effects of *B. intermedius* LPS on IL-8 mRNA induction required protein synthesis, we treated fibroblasts with *B. intermedius* LPS in the presence of cycloheximide (10 μg/ml). Induction of IL-8 mRNA by *B. intermedius* LPS was also resistant to cycloheximide, and superinduction of IL-8 mRNA was observed (Fig. 4).

Priming effects of IFN-β and IFN-γ on IL-8 mRNA expression by fibroblasts. IL-8 mRNA accumulation was investigated when GF-5 cells were primed with nHuIFN-β or nHuIFN-γ for 18 h and then stimulated with *B. intermedius* LPS for 8 h. Fibroblasts that had been primed with nHuIFN-γ induced higher (102% on the basis of densitometory analysis) levels of IL-8 mRNA expression than did nontreated fibroblasts upon stimulation with *B. intermedius* LPS (Fig. 5, lanes e and d), while GF-5 cells treated with nHuIFN-γ alone did not induce IL-8 mRNA accumulation (lane c). Furthermore, GF-5 cells that had been primed with nHuIFN-γ for 18 h did not express IL-8 mRNA in response to stimulation with LPS from *S. abortus-equi*, *S. minnesota* S519, and *S. minnesota* R595 as well as bacterial and synthetic lipid A (lanes f to j). nHuIFN-β also exhibited a similar but weak (17.1%) priming effect on the fibroblasts

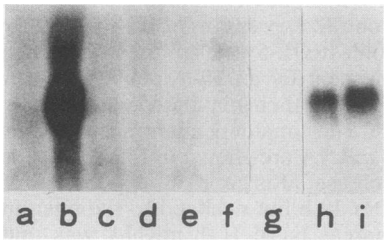

FIG. 3. Expression of IL-8 mRNA in human gingival fibroblast cultures stimulated with rHuIL-1α and various endotoxin specimens. Confluent, quiescent GF-5 cells were untreated (lane a) or were incubated for 12 h with rHuIL-1α (10 ng/ml) (lane b), synthetic *E. coli*-type lipid A (LA-15-PP) (1 μg/ml) (lane c), *S. minnesota* R595 (Re-form) LPS (10 μg/ml) (lane d), *S. minnesota* R595 lipid A (1 μg/ml) (lane e), *S. minnesota* S519 (S-form) LPS (10 μg/ml) (lane f), *S. abortus-equi* LPS (10 μg/ml) (lane g), *B. gingivalis* LPS (10 μg/ml) (lane h), and *B. intermedius* LPS (10 μg/ml) (lane i). Other details are given in the legend to Fig. 1.

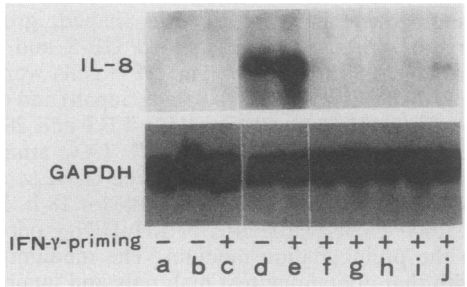

FIG. 5. Priming effects of nHuINF-γ on *B. intermedius* LPS-induced IL-8 mRNA expression by fibroblasts. GF-5 cells were preincubated with medium alone (lanes a, b, and d) or with nHuIFN-γ (1,000 U/ml) (lanes c and e to j) for 18 h and then not stimulated (lanes a and c) or stimulated for 8 h with nHuIFN-γ (1,000 U/ml) (lane b), *B. intermedius* LPS (10 μg/ml) (lanes d and e), *S. abortus-equi* LPS (10 μg/ml) (lane f), *S. minnesota* R595 LPS (10 μg/ml) (lane g), *S. minnesota* S519 LPS (10 μg/ml) (lane h), synthetic lipid A (LA-15-PP) (1 μg/ml) (lane i), and *S. minnesota* R595 lipid A (1 μg/ml) (lane j). Other details are given in the legend to Fig. 1.

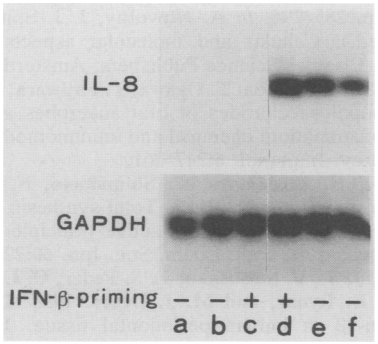

FIG. 6. Priming effects of nHuINF-β and effects of antiserum to nHuINF-β on *B. intermedius* LPS-induced IL-8 mRNA expression by fibroblasts. GF-5 cells were preincubated with medium alone (lanes a, b, e, and f) or with nHuIFN-β (1,000 U/ml) for 18 h (lanes c and d) and then not stimulated (lanes a and c) or stimulated for 8 h with nHuINF-β (1,000 U/ml) (lane b), *B. intermedius* LPS (10 μg/ml) (lanes d and e), and *B. intermedius* LPS (10 μg/ml) together with antiserum to nHuINF-β (1:200) (lane f). Other details are given in the legend to Fig. 1.

(Fig. 6, lanes f and e), while the cells treated with nHuIFN-β alone did not express IL-8 mRNA (lane c).

Possible involvement of IFN-β in IL-8 production by fibroblasts. To identify the involvement of IFN-β in induction of IL-8 mRNA by fibroblasts stimulated with *Bacteroides* LPS, GF-5 cells were incubated with *B. intermedius* LPS in the presence of the antiserum to nHuIFN-β. Anti-nHuIFN-β serum decreased (22.1%) IL-8 mRNA accumulation by fibroblasts stimulated with *B. intermedius* LPS (Fig. 6, lane f compared with lane e).

DISCUSSION

IL-8 is produced by a variety of cells, including fibroblasts, monocytes, endothelial cells, synoviocytes, chondrocytes, and keratinocytes (40). IL-1 and TNF are capable of stimulating these cells to express IL-8 mRNA or to release IL-8 (40). In contrast, LPS has been reported to induce IL-8 only in monocytes (29, 42, 44), endothelial cells (28, 33), and neutrophils (2, 32). Strieter et al. (34) revealed that rHuTNF, rHuIL-1α, or rHuIL-1β stimulated human foreskin fibroblasts to express IL-8 mRNA and to secrete chemotactic activity for human neutrophils, while LPS from *E. coli* failed to induce fibroblast-derived IL-8. Schröder et al. (30) also reported that LPS from *S. minnesota* was inactive with respect to stimulating the release of IL-8-related neutrophil chemotactic proteins, one of which might be comparable to an extended form of IL-8, in human dermal fibroblast cultures, in which rHuIL-1α and rHuIL-1β exhibited definite activity in this respect. In this study, we demonstrated that LPS from oral *Bacteroides* species induced IL-8 mRNA expression in human gingival fibroblast cultures. This is the first report describing a bacterial IL-8 inducer in fibroblasts. Endotoxins from *Enterobacteriaceae*, including S- and R-form LPS and bacterial and synthetic lipid A specimens, were also unable to induce IL-8 mRNA expression in our assay. Thus, the ability to induce IL-8 in fibroblasts appeared to be peculiar to *Bacteroides* LPS, though which structural moieties of *Bacteroides* LPS are responsible for the activity is not known at present. A similar tendency, however, was observed with IL-1 and IL-6 production by human gingival fibroblasts (36). Namely, *Bacteroides* LPS induced the cell-associated form of IL-1α and the cell-free form of IL-1β in human gingival fibroblast cultures, whereas LPS from other bacterial species, including *Enterobacteriaceae*, were scarcely active in inducing both forms of IL-1. Furthermore, *Bacteroides* LPS induced definite IL-6 activity in the system, while other LPS induced only weak IL-6 activity.

As mentioned above, *Bacteroides* LPS induced a cell-associated IL-1 activity, which was identified as IL-1α, in human gingival fibroblast cultures (37); we showed that *B. intermedius* LPS induced IL-1α mRNA expression in human gingival fibroblast cultures (Fig. 2). IL-8 mRNA expression was clearly induced by rHuIL-1α in our assay system (Fig. 3). It seemed that the IL-1α induced by *Bacteroides* LPS, in turn, stimulated fibroblasts to express IL-8 mRNA. However, the cascade was not true, because anti-HuIL-1α serum did not inhibit IL-8 mRNA expression by fibroblasts stimulated with *B. intermedius* LPS (Fig. 4). These results accorded with the fact that de novo protein synthesis was not required for fibroblasts to synthesize IL-8 mRNA upon stimulation with *Bacteroides* LPS, because cycloheximide did not inhibit IL-8 mRNA expression by the fibroblasts stimulated with *B. intermedius* LPS (Fig. 4); instead, cycloheximide increased IL-8 mRNA expression in the assay. Similar findings were reported for IL-8 mRNA expression by human foreskin fibroblasts (FS-4) in response to rHuTNF (21). Together, these findings suggested that *Bacteroides* LPS directly stimulated fibroblasts to produce IL-8, though the involvement of other factors in the response was not necessarily ruled out (see below). In this context, we found that protein kinase C was not involved in the response, because the protein kinase C inhibitor H-7 did not inhibit IL-8 mRNA induction in fibroblasts stimulated with *B. intermedius* LPS (data not shown).

Although neither nHuIFN-β nor nHuIFN-γ induced IL-8 mRNA expression in fibroblasts in our assay (Fig. 5 and 6), those pretreated with nHuIFN-β and nHuIFN-γ expressed higher levels of IL-8 mRNA upon stimulation with *B. intermedius* LPS than did nontreated cells (Fig. 5 and 6). Furthermore, IFN-β may be required for fibroblasts to produce IL-8 efficiently in response to *Bacteroides* LPS, because IL-8 mRNA accumulation declined when fibroblasts were stimulated with *B. intermedius* LPS in the presence of anti-HuIFN-β serum (Fig. 6). Similar findings have been obtained for IL-1α production by human gingival fibroblasts stimulated with *B. intermedius* LPS (37). These findings suggest that a common mechanism regulates the production of IL-1α and IL-8 by fibroblasts, although further studies are necessary to elucidate this regulatory mechanism.

Finally, this study revealed an additional activity of oral *Bacteroides* LPS, which may be related to the etiology of human periodontal diseases. The IL-8 produced by gingival fibroblasts may induce neutrophil chemotaxis (29, 44), with resulting accumulation of neutrophils in periodontal tissues. This IL-8 is then capable of inducing neutrophil degradation accompanying the release of inflammatory agents in the granules (29), eliciting a respiratory burst of neutrophils (29), and increasing expression of CD11b/CD18 glycoprotein on the neutrophil cell surface, with a resulting increased adhesion of neutrophils to endothelial cells (3, 10). In addition, IL-8 is chemotactic for T lymphocytes and basophils (20, 22) and stimulates IL-3-primed basophils to release histamine and leukotrienes (6). As a whole, IL-8 in periodontal tissues could be involved in the initiation and augmentation of periodontal diseases, although the presence of IL-8 in diseased periodontal tissues has not yet been reported. Kris-

tensen et al. (19) compared IL-8 mRNA levels in cultured endothelial cells, fibroblasts, monocytes, and keratinocytes stimulated with rHuIL-1α and noted that endothelial cells produced the highest level of IL-8 mRNA, followed by fibroblasts, monocytes, and keratinocytes, in that order. This study, however, indicated that the IFN-primed fibroblasts express very high levels of IL-8 mRNA upon stimulation with *Bacteroides* LPS. Therefore, fibroblasts might be the major IL-8 producers in periodontal tissues in which *Bacteroides* species have colonized heavily, and various cytokines, including IFNs, might be released by inflammatory and immunologically competent cells.

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