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Short communication

Stimulation of epithelial cell matrix metalloproteinase (MMP-2, -9, -13) and interleukin-8 secretion by fusobacteria

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Background/aims: Bacterial pathogens involved in periodontal diseases exert their destructive effects primarily by stimulating the host cells to increase their secretion of proinflammatory cytokines and matrix metalloproteinases (MMPs). This study aimed to determine the epithelial cell matrix metalloproteinase and interleukin-8 (IL-8) secretion upon exposure to fusobacteria.
Methods: Eight different oral and non-oral *Fusobacterium* strains were incubated with HaCaT epithelial cells. Gelatin zymography and Western blot analysis were performed to detect collagenase 3 (MMP-13), gelatinase A (MMP-2), gelatinase B (MMP-9), and IL-8 secretion by epithelial cells.
Results: All *Fusobacterium* strains, especially *Fusobacterium necrophorum* ATCC 25286, *Fusobacterium nucleatum* ATCC 25586, and *Fusobacterium varium* ATCC 51644, increased MMP-9 and MMP-13 secretion. *Fusobacterium simiae* ATCC 33568, and to a lesser extent *F. nucleatum* and *F. necrophorum*, increased epithelial MMP-2 secretion. *F. nucleatum* and *F. necrophorum* also increased IL-8 secretion. *F. varium* ATCC 27725, a strain that only weakly stimulated MMP production, strongly increased the IL-8 production, suggesting that their expression is differently regulated.
Conclusion: We conclude that the pathogenic potential of fusobacteria may partly result from their ability to stimulate secretion of MMP-9, MMP-13, and IL-8 from epithelial cells.

Key words: *epithelial cell; Fusobacterium;* interleukin-8; matrix metalloproteinase

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During the onset of periodontal diseases pathogenic oral bacteria interact with cells in periodontal tissue and initiate inflammatory reactions that lead to periodontal destruction. The first targets of the bacteria accumulating in the gingival sulcus are the sulcular and junctional epithelial cells. Epithelial cells respond to the attack by altered cell signaling, leading to changes in cell behavior, such as cytokine and protease production, cell proliferation, and migration (1, 10, 12). Infected epithelial cells recruit phagocytic cells by producing chemotactic substances, most notably interleukin-8 (IL-8) (3, 5, 11). Activated epithelial cells produce also a number of proteolytic enzymes, including matrix metalloproteinases (MMPs) that modify inflammatory reactions and facilitate cell migration (12, 16). Fusobacteria are believed to play an important part in the pathogenesis of periodontal disease. *Fusobacterium nucleatum* forms a bridge between early and late colonizers of the subgingival biofilm. It also has several virulence determinants, including level of adhesion to human cells, coaggregation with a wide array of microorganisms, and induction of apoptosis in lymphocytes (2). *F. nucleatum* has been shown to be a potent inducer of collagenase 3 (MMP-13) production and of epithelial cell migration (17), as well as of IL-8 secretion (5, 8). Both the collagenase 3 and IL-8 production is at least partly regulated by p38 mitogen-activated protein (MAP) kinase

signaling in the epithelial cells infected by *F. nucleatum* (8, 17). Several other fusobacteria reside in the oral and pharyngeal tissues. Some of them are commensals and the others, notably *Fusobacterium necrophorum*, are pathogenic (2, 14). The aim of this study was to compare various *Fusobacterium* species and strains for their capability to stimulate secretion by epithelial cells of MMPs (gelatinases A and B and collagenase 3) and IL-8.

As a model cell for fusobacteria–epithelium interactions, we used the HaCaT cell line that consists of well-characterized, transformed but non-tumorigenic human keratinocytes (4). Epithelial cells were cultured on tissue culture plastic plates at 37°C in Dulbecco's modified Eagle's medium plus 10% fetal calf serum for 24 h to reach subconfluent density. The following bacteria were grown on *Brucella* blood agar enriched with hemin and vitamin K, and incubated at 37°C in anaerobic jars for 3 to 5 days: *F. nucleatum* AHN 9500 (an oral isolate), *F. nucleatum* ssp. *nucleatum* ATCC 25586^T, *Fusobacterium simiae* ATCC 33568^T, *Fusobacterium periodonticum* ATCC 33693^T, *F. necrophorum* ssp. *necrophorum* ATCC 25286, *Fusobacterium mortiferum* ATCC 9817, *Fusobacterium varium* ATCC 27725, and *F. varium* ATCC 51644 (formerly *Fusobacterium pseudonecrophorum*). *F. varium* and *Fusobacterium mortiferum* have not been detected in the oropharyngeal area (2). The bacteria were collected from the culture plates into phosphate-buffered saline (PBS) and added immediately to epithelial cell cultures to form an epithelial cell : bacteria ratio of about 1 : 100. The cultures were continued for 24 h in the absence of serum. All the experiments were repeated twice and the results from the different experiments were found to differ at most by 18% from each other.

Gelatin zymography, which was performed using 20-µl aliquots of culture medium, showed prominent gelatinolytic bands at 92 kDa, 72 kDa, and 52 kDa corresponding to proforms of gelatinase B (MMP-9), gelatinase A (MMP-2), and collagenase 3 (MMP-13), respectively (15, 17). Western blot using an anticollagenase 3 antibody (15, 17) showed that collagenase 3 comigrated with the 52-kDa band in zymography of medium samples treated with *F. nucleatum* (Fig. 1A). Control cultures in the absence of fusobacteria did not react with the collagenase 3 antibody. There were variable increases in MMP secretion with the different fusobacteria. *F. necrophorum*, which is a major pathogen causing severe necrotic infections

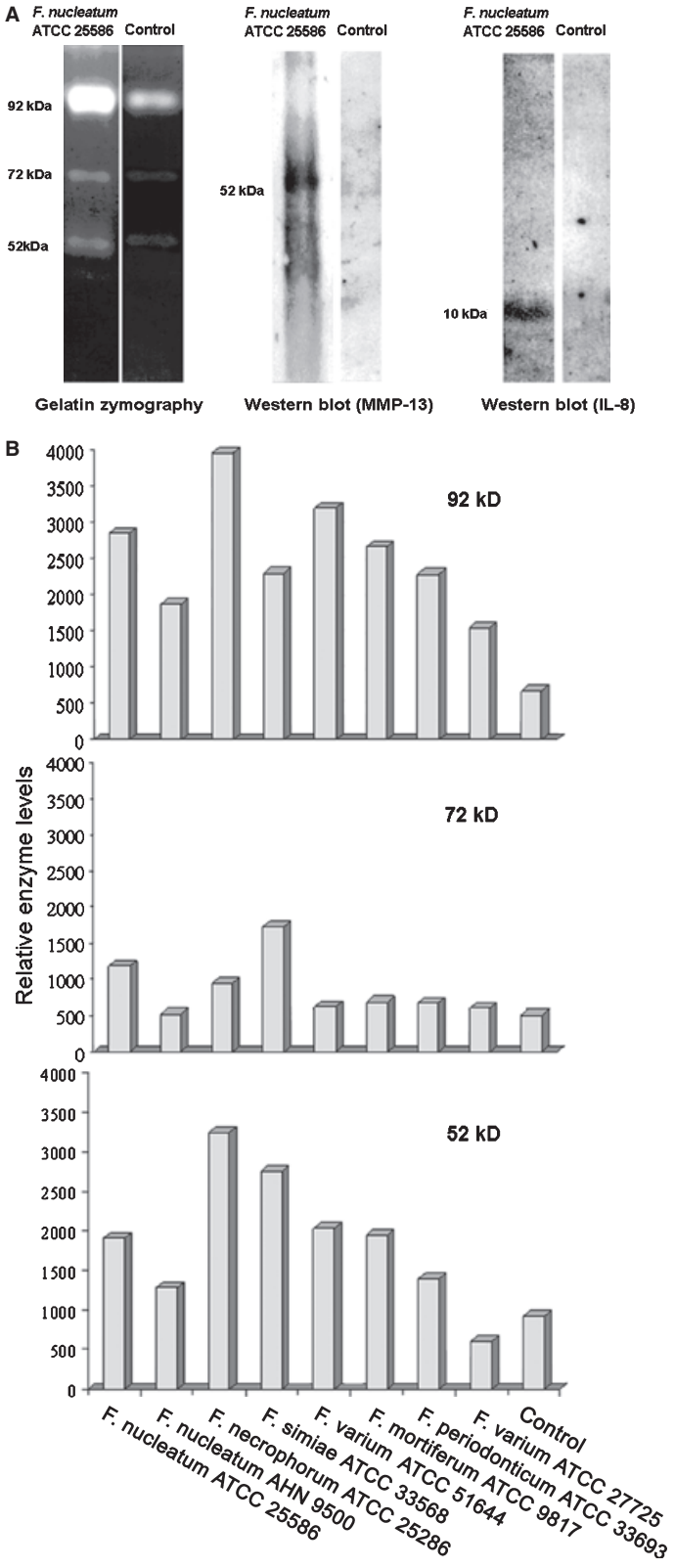

Fig. 1. (A) After 24 h of incubation of HaCaT epithelial cells in the absence of serum and in the presence of *Fusobacterium nucleatum*, secretion of matrix metalloproteinases (MMPs) was analyzed in medium samples using gelatin zymography and by Western blot analysis with anticollagenase-3 (MMP-13) antibody (Calbiochem). Secretion of interleukin-8 (IL-8) was measured by Western blot (antibody from Chemicon, Temecula, CA). (B) MMP-9 (92 kDa), MMP-2 (72 kDa), and MMP-13 (52 kDa) secretion by epithelial cells in the presence of various *Fusobacterium* species was quantified by densitometric scanning of the gelatin zymography gels.

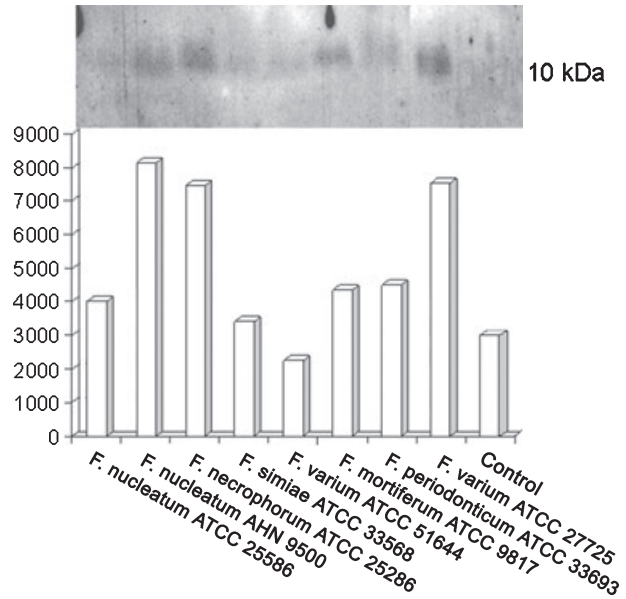

Fig. 2. Interleukin-8 (IL-8) expression by HaCaT epithelial cells incubated in the presence of various fusobacteria for 24 h. The upper part of the picture shows the Western blot analysis of medium samples and the lower part represents the densitometric scanning of the IL-8 bands.

in human and animals (2), was the strongest inducer of MMP-9 and MMP-13. *F. nucleatum* ATCC 25586, *F. varium* ATCC 27725, *F. mortiferum*, and *F. simiae* were also clearly increasing the amount of MMP secreted. These MMPs have been implicated in cell migration as well as in the behavior of malignant cells (6). The promoter regions of the genes for MMP-9 and MMP-13 are very similar and they are often induced by the same cytokines (18). In contrast, gelatinase A (MMP-2) is regarded as less inducible (16). It was interesting to find, therefore, that *F. simiae*, and to a lesser extent *F. nucleatum* and *F. necrophorum*, increased epithelial MMP-2 secretion. MMP-2 (gelatinase A) has several functions in the control of inflammation (16). It is also related to the migration of keratinocytes (9).

A crucial part of the epithelial defense system is the efficient recruitment of professional defense cells, especially of neutrophils, to the infection site. IL-8 is a key cytokine in this process. We studied the amount of IL-8 in the medium of fusobacteria-treated epithelial cells using Western blot analysis of the medium after 24-h culture. All *Fusobacterium* species were able to induce IL-8 *in vitro*. The best inducers were *F. necrophorum* ATCC 25286, *F. nucleatum* AHN 9500, and *F. varium* ATCC 27725 (Fig. 2). Expression of MMP-13 and IL-8 is regulated by p38 MAP kinase (8, 17); it was unexpected to find a different induction pattern among different fusobacteria. In fact, it has been shown that various cell-signaling pathways are involved in IL-8 induction (7). It is uncertain whether continuous effective recruitment of neutrophils is beneficial or detrimental in terms of progression of periodontitis. It has been suggested that an excessive inflammatory reaction that involves persistent neutrophil activity plays an important part of the tissue destruction during periodontitis (1, 13).

In conclusion, we have shown that various fusobacteria specifically induce MMPs and IL-8. In this respect the species and strain differences were considerable. This study did not address the question of whether the observed differences are related to the ability of the bacteria to form aggregates in solutions, to stay alive longer in aerobic conditions or some other properties. Nevertheless, it seems that *F. necrophorum* and *F. nucleatum* are among the strongest inducers of destructive MMPs. This property may be important in the pathogenic potential of these bacteria.

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