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Mini Review

Variations of *Porphyromonas gingivalis* fimbriae in relation to microbial pathogenesis

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Objectives: Periodontal disease is an infectious disorder caused by a small subset of periodontal pathogens including *Porphyromonas gingivalis*. Accumulated evidences show that the expression of *P. gingivalis* heterogenic virulence properties is dependent on its clonal diversity. *P. gingivalis* expresses two distinct fimbria molecules, major and minor fimbriae, on its cell surfaces, both of which seem to be involved in the development of periodontitis. In this short review, variations of fimbriae in relation to microbial pathogenesis are discussed.

Materials and Methods: Our recent findings are summarized to elucidate the relationship between clonal variation of fimbriae and bacterial pathogenicity of various strains.

Results: Major fimbriae were classified into six types (I to V and Ib) based on the diversity of *fimA* genes encoding FimA (a subunit of major fimbriae). A majority of periodontitis patients were found to carry type II *fimA* organisms, followed by type IV, and type II *fimA* organisms were significantly occurred with more severe forms of periodontitis. Studies of clones with type II *fimA* have revealed significantly greater adhesive and invasive capabilities to epithelial cells than other *fimA* type clones. Minor fimbriae induced interleukin-1α (IL-1α), IL-1β, IL-6, and tumor necrosis factor-α (TNF-α) cytokine expression in macrophages and were suggested to be a causative factor of alveolar bone resorption in animal models. The clonal diversity of minor fimbriae is unclear, however, distinct minor fimbria molecules were found in different strains.

Conclusion: The fimbria variations may have an influence on the development of periodontal disease.

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Chronic periodontitis is a destructive inflammatory disease resulting from the complex actions of a small subset of periodontal pathogens (1), though there are marked differences in the progression rate and severity of this infectious disorder, as well as response to therapy among patients. As a result, periodontitis is not considered to be a homogeneous disease, but rather intricately influenced by host susceptible differences and/or diversities in virulence among the organisms harbored by individuals (2, 3). Such aspects are not pathognomonical only for periodontitis, but they are commonly observed features of various infectious diseases caused by a great number of pathogens in humans. Accumulated evidence shows that *Porphyromonas gingivalis* is frequently detected in patients with periodontitis and definitely associated with various forms of periodontal

diseases (1). In addition, this microorganism has been shown to produce a number of virulence factors such as fimbriae, LPS, capsules, and proteases (4). Thus, of the various species harboring in subgingival microflora, *P. gingivalis* is considered to be a bona fide pathogen influencing disease initiation and progression.

P. gingivalis is reportedly detected at lower frequency in periodontally healthy individuals without marked gingival inflammation (5–8). Although host factors, such as genetic background and environmental stress, may have a determining influence in individuals infected by *P. gingivalis*, there are diversities in virulence among organisms harbored by individuals who are periodontally healthy and those with periodontitis. Further, it is possible that specific virulent clones of *P. gingivalis* exist in patients with strongly developed chronic and aggressive periodontitis. Recently, we reported a close association between periodontitis and *P. gingivalis* clones with specific genotypes (9–13). In the present short review, the relationships of clonal variations of *P. gingivalis* to bacterial virulence, along with the possible involvement of major and minor fimbriae as virulence factors in the microbial pathogenesis of periodontal disease, are discussed.

Distinct virulence of *P. gingivalis* strains

The heterogenic virulence properties of *P. gingivalis* were first examined using animal models with minimized host susceptibility factors by various investigators [reviewed in (14)], among which mouse and guinea pig abscess models have been extensively employed. Following subcutaneous infection of rodents with *P. gingivalis*, virulence is generally evaluated in relation to the size of the abscesses and/or eroded skin lesions, along with cachexia and death. In those studies, many strains of *P. gingivalis* were classified as either avirulent/non-invasive (such as strains ATCC 33277, 381, 2561, and HG1694) or virulent/invasive (ATCC 53977, A7A2-10, HG1690, and W83) (15–20). Although the factors regulating the expression of virulence in *P. gingivalis* have not been clearly elucidated, encapsulated strains appear to be more virulent and invasive. Further, six serogroups (K-antigen types; K1 to K6) of *P. gingivalis*, based on capsular antigens linked to pathogenicity in animal models, are currently recognized (21–23).

Genotypic characterization is also performed to isolate specific periodontitis-related clones from periodontitis patients, based on restriction fragment length polymorphism, multilocus enzyme electrophoresis, arbitrarily primed polymerase chain reaction, and amplified fragment length polymorphism methods (24–33). Results from those studies have revealed an extensive heterogeneity and as many as 100 different clonal types of *P. gingivalis* isolates have been found to infrequently display genomic clonality, in contrast to many other types of bacterial pathogens. For example, 73 *P. gingivalis* strains could be divided into 23 or 45 genotypes by arbitrarily primed polymerase chain reaction, depending on the primers used (31), whereas another analysis of 100 *P. gingivalis* strains from humans and animals revealed 78 different multilocus enzyme electrophoresis types (28). The consensus of those studies is that there is considerable heterogeneity among *P. gingivalis* isolates, whereas intra-individual heterogeneity is very low.

A recent study showed that phylogenetic trees derived from the sequencing of several housekeeping genes in many strains were completely different and it was concluded that *P. gingivalis* has a non-clonal population structure, with its genotypic diversity derived from an accumulation of genetic changes or mutations that are subject to ecological selective pressures in periodontal lesions (34, 35). These genetic changes may be caused by a variety of genetic mechanisms, including the movement of insertion sequence elements and/or recombination between non-mobile repeated DNA sequences (36–40). Notably, it was also suggested that the virulence of *P. gingivalis* was not confined to a distinct evolutionary lineage and that particular genotypes, possibly with increased pathogenic potential, are able to spread successfully in humans (34). The search for virulence diversities will therefore necessarily focus on individual genes or operons.

Major fimbriae of *P. gingivalis*

P. gingivalis expresses a number of potential virulence factors that may contribute to the pathogenesis of periodontitis. Among them, major fimbriae of *P. gingivalis* are recognized as a critical virulence factor influencing disease initiation and progression (41). Major fimbriae are filamentous components on the cell surface and their subunit protein, fimbrillin (FimA), reportedly acts on bacterial interactions with host tissues by mediating bacterial adhesion and colonization in targeted sites (42). Major fimbriae are capable of binding specifically to and activating various host cells such as human epithelial cells, endothelial cells, spleen cells, and peripheral blood monocytes, resulting in the release of cytokines including interleukin-1 (IL-1), IL-6, IL-8, and tumor necrosis factor-α (TNF-α) (41, 43, 44), as well as cell adhesion molecules including intercellular adhesion molecule 1 (ICAM-1), vascular cell adhesion molecule 1 (VCAM-1), and P- and E-selectins (45, 46). In addition, *P. gingivalis* major fimbriae have been shown necessary for bacterial invasion to host cells [reviewed in (47)].

Clonal variations of major fimbriae in relation to bacterial virulence

The clonal variations of major fimbriae (FimA) and the *fimA* gene encoding FimA protein among *P. gingivalis* strains have been studied in relation to bacterial virulence diversity. Lee *et al.* (48) first reported the variation of FimA proteins and divided a number of *P. gingivalis* strains into four types based on their N-terminal amino acid sequences. *P. gingivalis fimA* genes have been further classified into six variants (types I to V, and Ib) on the basis of nucleotide sequences (10, 13, 49). In our study, we unexpectedly noticed that the clonal variation of *fimA* genes might have a relationship with virulent traits,

which were previously evaluated using subcutaneous injection rodent models (15–22). The strains evaluated as virulent/invasive consisted of a large number of type II *fimA* strains, such as ATCC 53977, A7A2-10, HG1690, HG184, and HW24D1, and type IV *fimA* strains including W50, W83, and 9-14K-1. In contrast, avirulent traits were expressed by type I *fimA* strains such as ATCC 33277, 381, 2561, 1432, and 1112. Our recent study using a mouse abscess model also showed the same tendency, as type II *fimA* organisms caused the most significant serum sialic acid concentration as a quantitative inflammatory parameter, as well as other infectious symptoms, followed by types Ib, IV, and V. In contrast, type I and III strains caused weaker host responses, whereas *fimA* mutants of type II strains clearly lost their infectious abilities (50).

Prevalence of specific fimbria genotypes and periodontal health status

A sensitive polymerase chain reaction assay using *fimA* type-specific primer

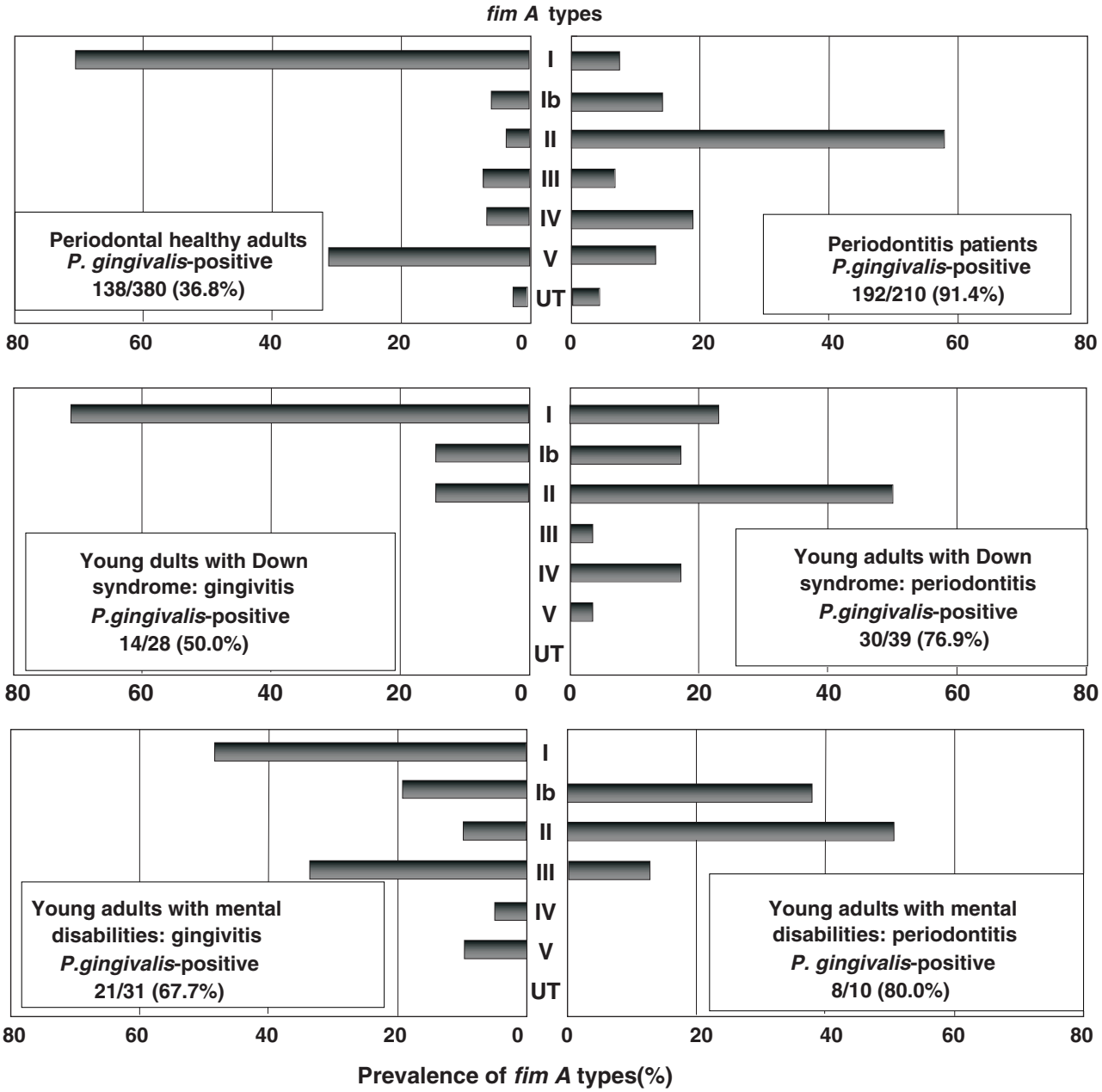

Fig. 1. Prevalence of *Porphyromonas gingivalis fimA* types in periodontitis and non-periodontitis populations. Clinical specimens were obtained from 380 periodontally healthy adults and 210 periodontitis patients, and analyzed using a polymerase chain reaction method to identify *P. gingivalis fimA* genotypes (11, 13). The same analyses were performed in young adults with Down syndrome and those with mental disabilities who suffered from gingivitis or periodontitis (12). Since some of the samples contained organisms with multiple *fimA* genotypes, the total percentage per subject number exceeds 100% in each group.

sets was developed to differentiate the six types of *fimA* genes found in organisms in saliva and dental plaque samples (9–13). Using that method, we surveyed the distribution of *P. gingivalis* in terms of genomic diversity of the *fimA* gene in periodontitis patients and periodontally healthy adults (Fig. 1). A majority of the patients were found to carry type II *fimA* organisms, followed by type IV, and the occurrence of type II *fimA* organisms was significantly increased with the more severe forms of periodontitis (9). In contrast, the most prevalent *fimA* type of *P. gingivalis* in the healthy adults was type I (11). Similar findings were observed in both Down syndrome patients, who are congenitally susceptible to periodontal diseases, and young adults with mental disability, which is a major factor in determining oral hygiene (12). Other reports have also shown that type II *fimA* organisms are predominantly prevalent in periodontitis patients (51, 52). These findings indicate that there are disease-associated and non-disease-associated *P. gingivalis* organisms, and that clonal *fimA* variations are related to the bacterial infectious traits that influence disease development.

Influence of *fimA* variations on bacterial interactions with host cells

Although scant biological explanation was given for the differences in pathogenic potential of various *P. gingivalis* strains with different *fimA* genotypes, we generated recombinant FimA (rFimA) proteins corresponding to their clonal variants, and characterized their capabilities of adhesion/invasion to human gingival fibroblasts (HGF) and a human epithelial cell line (HEp-2 cells) (53). There were no significant differences in the adhesion ability of microspheres (MS) coated with these rFimAs to HGF. However, that of type II rFimA-MS to HEp-2 cells was significantly greater than those of other rFimA types. It was also observed that type II rFimA-MS markedly invaded the epithelial cells and accumulated around the nuclei. A similar level of accumulation was also observed upon invasion of viable *P. gingivalis* whole cells into the epithelial cells (53). The adhesion/invasion activities of type II rFimA-MS were abrogated by the addition of antibodies against type II rFimA or α5β1-integrin. These results suggest that type II FimA is most able to efficiently promote bacterial invasion to the cells through specific host receptor(s), including α5β1-integrin.

P. gingivalis can internalize in normally non-phagocytic gingival epithelial cells, then uniformly accumulates in the perinuclear region (54). Invasion is associated with the phosphorylation of c-jun N-terminal kinase (JNK) and down-regulation of extracellular signal-regulated kinase (ERK1/2), as well as transient elevation of intracellular Ca^{2+} ion levels, however, nuclear factor kappa B (NF-kappaB) is not activated and secretion of IL-8 is inhibited [reviewed in (47)]. The major fimbriae of *P. gingivalis* are involved in both adhesion to epithelial cells and the subsequent signalling events associated with invasion (47, 55). The organism is also known to cause proteolysis of focal contact components such as paxillin and focal adhesion kinase (FAK), which are signaling molecules that regulate adhesion, survival, proliferation, differentiation, and migration (56). These bacterial effects are suggested to be mainly due to gingipains and, in part, fimbriae. Our recent study showed that type II *fimA* organisms

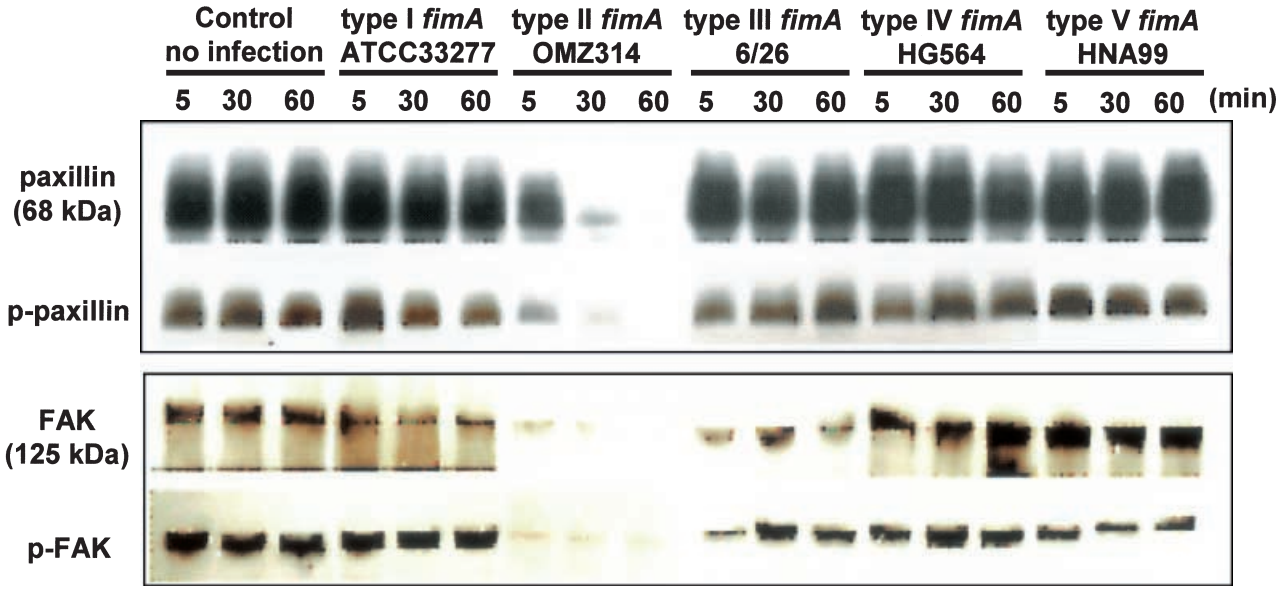

Fig. 2. Effect of *Porphyromonas gingivalis* infection on cellular focal adhesion complex proteins, paxillin, and focal adhesion kinase (FAK). HEp-2 cells(1×10^5 per well) were infected with *P. gingivalis* strains possessing distinct *fimA* types (1×10^7 cells) for the time periods indicated. HEp-2 cells were lysed following washing, and then immunoblotted. Total and phosphorylated (p-) proteins were probed with anti-paxillin or anti-p-paxillin (upper panel) and anti-FAK or anti-p-FAK(lower panel) (Nagakawa *et al.*, pers. comm.).

degrade both paxillin and FAK more quickly than other *fimA* type strains, resulting in an inhibition of phosphorylation by these molecules (Nagakawa *et al*., pers. comm.) (Fig. 2). These findings may provide an explanation for the relationship of *fimA* variations to bacterial virulence.

Minor fimbriae of *P. gingivalis*

Major fimbriae were first determined in 1984 by Yoshimura *et al*. (57), and thereafter a secondary fimbrial structure termed minor fimbriae was found in 1996 (58) and shown to be short fimbria-like appendages in an *fimA* (major fimbria-deficient) mutant of strain ATCC 33277. A subunit protein of a minor fimbriae (Mfa1) encoding the *mfa1* gene was shown to be different in size (67 kDa in contrast to 41 kDa of major fimbria subunit) and antigenicity from that of major fimbriae (58–60). Although a *fimA* mutant revealed a significant reduction of adhesive potential to saliva-coated hydroxyapatite, gingival epithelial cells, and fibroblasts, as well as bone adsorption capability, in an orally infected rat model [reviewed in (41)], minor fimbriae purified from *P. gingivalis* ATCC 33277 markedly induced IL-1α, IL-β, IL-6, and TNF-α cytokine expression in mouse peritoneal macrophages (59). Thus, minor fimbriae were further investigated for their possible involvement in microbial virulence.

To identify the influence of major and minor fimbriae on bacterial virulence, isogenic mutants of *P. gingivalis* were constructed and inoculated into the oral cavities of rats (60). The mutants were *fimA* (major fimbriae) knockout (MPG1), *mfa1* (minor fimbriae) KO (MPG67), and double KO (MPG4167) mutants. *P. gingivalis* ATCC 33277 (wild-type strain) significantly induced periodontal bone loss, which was clearly suppressed by double fimbria deletion and, to the same degree, by minor fimbria deletion. MPG1 caused greater bone loss than MPG67. In addition, the wild-type strain adhered to KB cells in clumps by auto-aggregation and MPG67 formed larger clumps than the wild-type strain (60). In contrast, MPG1 did not show any auto-aggregation and lower levels of adherence to single cells, whereas MPG4167 completely lost adhesive capability. These findings indicate that production of both major and minor fimbriae is required for the expression of pathogenic traits by *P. gingivalis*.

Clonal variations of minor fimbriae

Although clonal variations of the *mfa1* gene have been investigated using a variety of laboratory strains, we found out a slightly varied sequence alignment (unpublished data). Recent analyses showed that the Mfa1 molecule is the same as that of the 75 kDa outer membrane protein (61), the 67 kDa major outer membrane protein (62), and Pg-II (a 72 kDa cell surface protein) (63) in strain ATCC 33277. However, a 53 kDa protein isolated from strain 381 was shown to be another minor fimbriae (64), which was demonstrated to be the same molecule as a 53 kDa major outer membrane protein in other reports (62, 65), and a major immunodominant protein likely to contribute to host–bacterial interaction (61, 66). Those two types of minor fimbrial proteins showed no immunological cross-reactivity. Thus, strain 33277 has 67 kDa minor fimbriae, and strain 381 has 53 kDa minor fimbriae. These distinct molecules are currently being investigated regarding their clonal heterogenicity in relation to bacterial virulence.

Conclusion

Genomic variations of the fimbria structures of *P. gingivalis* are likely involved in the initiation and progression of human periodontitis. It would be of value for periodontal therapy and assessment of prognosis if the disease-contributing strains could be differentiated based on clonal variations of the *fimA* gene. However, a number of reports (24–40) have demonstrated a wide variety of chromosomal genotypes of *P. gingivalis*, suggesting that possible variations of other pathogenic genes are involved in its pathogenicity. In addition, environmental conditions also seem to alter its virulence (67) and the expression of virulence factors, including several proteolytic enzymes such as gingipains, is influenced by several factors (68). Further studies regarding these aspects are necessary to better understand the virulence variations of *P. gingivalis* clones.

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