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Full Length Research Paper

Heterogenic abilities in adhesion and invasion among clinical isolates of *Porphyromonas gingivalis* with Type II fimbriae

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Porphyromonas gingivalis (P. gingivalis) **fimbriae are classified into six types (Types I to V and Ib) based on the *fimA* genes encoding FimA (a subunit of fimbriae), and they play a critical role in bacterial adhesion to and invasion of gingival epithelium. Accumulated evidences suggest that *P. gingivalis* strains with Type II fimbriae (designated Pg-II) are more virulent than other types. This study aimed to compare the abilities in adhesion to and invasion of oral epithelial cells of Pg-II strains. *P. gingivalis* strains were isolated from Chinese patients with chronic periodontitis using conventional anaerobic separation techniques. Adherent and invasive properties of *P. gingivalis* were measured by standard methods, and confirmed by both scanning and transmission electron microscopy. Analysis of eight Pg-II strains showed significant differences in their adherent and invasive capabilities, but there was no significant correlation between the *fimA* genotype and the adhesion and invasion abilities of the bacteria. The results suggest that adherent and invasive heterogeneities exist among Pg-II strains and that there must be other factors that influence the virulence of *P. gingivalis* with distinct *fimA* genotypes.**

Key words: *P. gingivalis*, fimbriae, *fimA* genotype, adhesion, invasion.

INTRODUCTION

Porphyromonas gingivalis (P. gingivalis), a Gram-negative anaerobe present in subgingival plaque, is one of the major pathogens responsible for causing chronic periodontitis (Holt and Ebersole, 2005). Previous research has revealed a clonal heterogeneity in virulence among various *P. gingivalis* strains, which can be used to classify *P. gingivalis* stains into disease-associated and non-disease-associated strains (Genco et al., 1991; Laine et al., 1998; Amano et al., 2004).

One of the primary virulence factors of *P. gingivalis* is fimbriae, which play a critical role in the interaction of *P. gingivalis* with host cells. The *fimA* gene, which encodes the fimbrillin (FimA) protein of the major fimbriae, has been classified into six variants (Types I to V and Ib) based on the nucleotide sequences of their open reading frame (Fujiware et al., 1994; Nakagawa et al., 2000, 2002). Previous studies suggest that *P. gingivalis* strains with different *fimA* genotypes display distinct degrees of virulence. Several epidemiological surveys have reported the Type II fimbriae-expressing *P. gingivalis* (designated Pg-II) to be the predominant *fimA* genotype in periodontitis patients (Amano et al., 2000; Beikler et al., 2003; Missailidis et al., 2004; Zhao et al., 2007). An additional study performed by Amano et al. (1999) has shown that Pg-II is the most frequently isolated species from deep periodontal pockets. Other research has revealed that Pg-II plays an important role in enhancing periodontal tissue inflammation and destruction (Nakano et al., 2004; Sugano et al., 2004; Wang et al., 2009).

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Collectively, these studies indicate that the *fimA* genotype of *P. gingivalis* is associated with bacterial virulence and that the Pg-II genotype represents a highly virulent strain closely related with periodontitis. However, Inaba et al. (2008) have found that pathogenic heterogeneity exists among Pg-II strains.

Gingival epithelial cells (GECs) form the first barrier against bacterial invasion into underlying tissues. The initial, and thus crucial steps in most infectious diseases involves the adhesion of pathogens to host tissues and subsequent invasion beyond the physiological barriers, strongly implicating the adherent and invasive abilities of pathogens in bacterial virulence (Andrian et al., 2006). *P. gingivalis* is an invasive, intracellular pathogen that is able to remain resident in the perinuclear region for prolonged periods without causing cell death (Lamont and Jenkinson, 1998). However, in doing so, *P. gingivalis* disrupts the physiological equilibrium of the host cell by interfering with cellular signal transduction pathways (Moffatt and Lamont, 2011). Previous *in vitro* findings have shown that Pg-II strains can adhere to and invade epithelial cells much more effectively than strains with other *fimA* genotypes (Nakagawa et al., 2006; Kato et al., 2007). However, whether this correlation between the Pg-II genotype and increased adherent and invasive properties applies to all Pg-II strains remains unknown. This study aimed to answer this question in addition to whether any of the distinct *fimA* genotypes in *P. gingivalis* strains can be correlated with adhesion and invasion abilities.

MATERIALS AND METHODS

Isolation and culture of clinical *P. gingivalis* isolates

Clinical *P. gingivalis* strains were individually separated from the periodontal pockets of patients suffering from chronic periodontitis. Subjects who used antibiotics or accepted periodontal therapy within the last three months were excluded. All subjects involved in this study willingly signed an informed consent form. This study was approved by the Sichuan University Health Guidelines for Studies Involving Human Subjects.

Subgingival plaque samples were collected from the periodontal pockets (depth ≥ 5 mm**)** using sterile paper points and immersed immediately in a sterile plastic tube with mercaptoethanol salt. Sample diluents were inoculated into brain heart infusion (BHI) blood agar (OXIOD, UK) supplemented with 5% sheep blood, 1% hemin and vitamin K. Inoculated plates were cultured in an anaerobic chamber (80% N_2, 10% H_2, 10% CO_2) at 37°C for 5-7 days. Black pigment-producing colonies were selected, gram-stained and further genotyped in additional assays. The reference strains *P. gingivalis* ATCC 33277 (*fimA* genotype I) and W83 (*fimA* genotype IV) were provided by the State Key Laboratory of Oral Diseases in China and were used as positive controls for the detection of *P. gingivalis* and *fimA* genotyping.

Polymerase chain reaction (PCR)

After growth, a 3 µL bacteriological loop of cells was suspended in sterile water and lysed by immersing the tubes in boiling water for 10 min. After centrifugation, the supernatants containing DNA were acquired (Umeda et al., 2006).

The detection of *P. gingivalis* was performed by PCR using primers specific for the 16S rRNA gene, as described by (Tran and Rudney, 1996). The *fimA* type-specific primers, designed by Amano (Amano et al., 2000), were used for *fimA* typing and detection. The total 25 µL PCR reaction system consisted of 2 µL of template DNA, 100 pmol of each primer (TaKaRa Biontech Bo, Ltd, Japan), 2.5 µL of 10x buffer, 3 mM $MgCl_2$, 200 µM dNTPs, 0.2 µL of Taq DNA polymerase (Fermentas Life Science, Vilnius, Lithuania) and sterile Tris-buffered water. The PCR amplification protocol used in this study has been described elsewhere (Ashimoto et al., 1996; Tran and Rudney, 1996). *P. gingivalis* ATCC 33277 (*fimA* I) and W83 (*fimA* IV) were used as positive controls. When both Type I and Type II *fimA* were simultaneously detected, another PCR amplification was performed using the Type Ib primer pair. The amplified fragments were then digested with RsaI (Fermentas Life Science) to distinguish the Type Ib *fimA* genotype.

Cell cultures

The human oral keratinocyte (HOK, 2610) cell line (ScienCell Research Laboratories) was grown in Dulbecco's Modified Eagle Medium (DMEM) (Gibco, USA) supplemented with 10% fetal calf serum (Hyclone, USA) and 0.4 µg/ml hydrocortisone. HOK cells were grown at 37°C in the presence of 5% CO_2.

Adhesion and invasion assay

The adhesion to and invasion of HOK cells by *P. gingivalis* was quantified using a colony formation assay and an antibiotic protection assay as described by Nakagawa with slight modifications (Nakagawa et al., 2006). All strains of *P. gingivalis* with different *fimA* genotypes were cultivated in BHI liquid medium supplemented with 1% hemin and vitamin K for 24 h. The bacterial suspensions were centrifuged and resuspended in DMEM. Bacteria were counted using a Petroff-Hausser counting chamber and were diluted to 10^8 colony forming units (CFU)/mL. Bacterial suspensions were added to monolayers of HOK cells (5×10^5 /well) in a 24-well culture plate (MOI=200:1) and incubated at 37°C in the presence of 5% CO_2 for 90min. External non-adherent bacteria were removed by washing the cells three times with phosphate-buffered saline (PBS). The cells were then disrupted by adding distilled water and were incubated at 37°C for 20 min. Serial dilutions of the disrupted mixture were plated on blood agar plates and incubated for 7 days allowing the number of adherent bacteria to be determined. To determine the number of invasive bacteria, *P. gingivalis*-infected cells were incubated with medium containing gentamicin (0.3 mg/ml) and metronidazole (0.2 mg/ml) for 1 h to kill all extracellular bacteria. Control wells containing no cells were also included to verify the antibiotic efficacy. Infected cells were washed three times with PBS, and the number of internalized bacteria was determined as described above. Adhesion and invasion efficiencies were calculated using the formula: adherent/invasive rate = (number of colonies on the plate × dilution multiple)/initial inoculums ×100%. All of the assays were performed in triplicate.

Scanning electron microscopy (SEM)

Scanning electron microscopy was performed to confirm the adhesion of Pg-II strains to HOK cells. Bacterial suspensions (1×10^8 CFU/mL) were added to single layers of HOK cells on a round coverslip in a 24-well culture plate (5×10^5 /well). The mixtures were incubated for 90 min, washed with PBS three times, and then fixed in 2.5% glutaraldehyde overnight at 4°C. After gradient

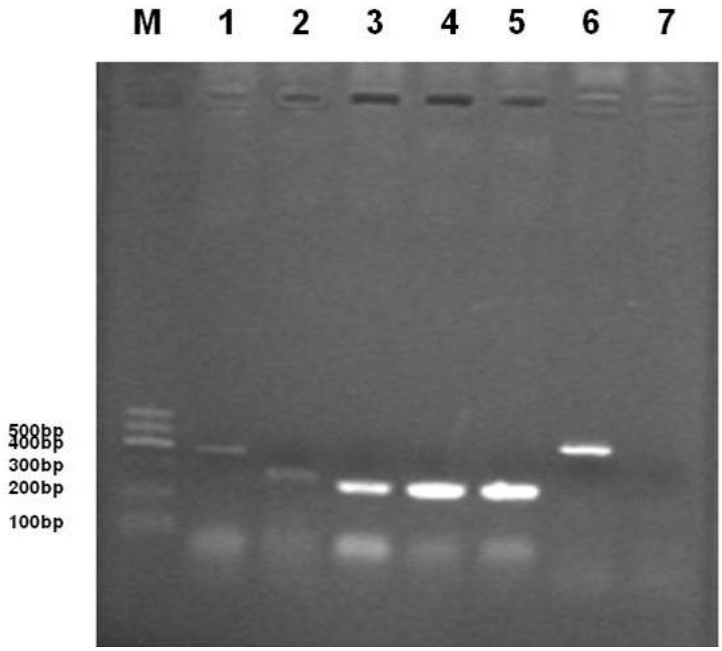

Figure 1. Electrophoresis of amplification products of PCR on a 3% agarose gel. Lanes 1–7, polymerase chain reaction products using primers specific for gene *fimA* Type I (392 bp), Type Ib (271 bp), Type II (257 bp), Type III (247 bp), Type IV (251 bp), Type V (462 bp) and the negative control, respectively. M: molecular weight marker (100 bp DNA ladder).

ethanol dehydration, samples were placed in isoamyl acetate for rehydration. Finally, the samples were sectioned and viewed by SEM (FEI INSPECT F, Netherlands).

Transmission electron microscopy (TEM)

Transmission electron microscopy was performed to confirm Pg-II strains invasion of HOK cells. Bacterial suspensions and HOK monolayers were co-cultured (MOI=200:1) for 2.5 h. The cultures were centrifuged for 15 min at 2000 rpm. The pellet was colleted and cultures were fixed with 0.5% glutaraldehyde for 30 min at 4°C, and then centrifuged for 15 min at 130000 rpm. Cells were fixed in 3% glutaraldehyde and then incubated in with OsO_4. The cells were dehydrated by ethanol gradient and embedded in Epon. Thin sections were placed on copper grids stained with uranyl acetate-lead citrate, and then examined by TEM (H-600IV HITICHI, Japan).

Statistical analysis

All data was presented as the mean ± standard deviations. Analysis of variance (ANOVAS) was used to test whether there were significant differences among the log of CFU of *P.gingivalis* after adhesion and invasion assays.

RESULTS

Isolation and culture of *P. gingivalis* strains

Fifteen clinical strains were isolated and classified by *fimA* type (Figure 1). There were eight strains of Type II, three strains of Type IV, and one strain of Types I, Ib, III, and V, respectively.

Adherent and invasive abilities of *P. gingivalis* with different *fimA* genotypes

The data for adhesion to and invasion of HOK cells by *P. gingivalis* strains were shown in Table 1. All seventeen strains in this study were capable of adhering to and invading HOK cells. Among these strains, two strains exhibited an adherent rate between 0 and 1%, nine strains exhibited an adherent rate between 1 and 10%, and six strains demonstrated an adherent rate of more than 10%. According to the standard in a study performed by Dorn et al. (2000), seven strains exhibited low invasive ability (0.001-0.1%), six strains exhibited moderate invasive ability (0.1-1%) and four strains exhibited high invasive ability (>1%). Comparison of adherent and invasive abilities among various *fimA* genotypes showed that the difference was not statistically significant ($P>0.05$). Among the eight Pg-II strains, adherent and invasive rates varied considerably, the adherent rate ranged from 0.665% (6506) to 37.154% (7205), the invasive rate ranged from 0.040% (6104) to 3.750% (7205). These data for the Pg-II strains demonstrated statistically significant differences among individual Pg-II isolates ($P<0.01$).

Table 1. Adherent and invasive rates (%) of *P. gingivalis* with different *fimA* genotypes ($\bar{x}$±s).

fimA type	Strains	Adherent Rate ($\bar{x}$±s)*	Invasive Rate ($\bar{x}$±s)*
I	ATCC33277	8.279±3.117	0.213±0.009
	WH1103	11.376±0.766	0.089±0.003
Ib	WH4702	3.459±0.937	0.254±0.013
	WH4404	7.943±0.471	0.137±0.002
	WH5601	24.322±3.659	2.371±0.083
II	WH6104	3.133±0.069	0.040±0.014
	WH6506	0.665±0.132	0.057±0.007
	WH7002	0.904±0.148	0.072±0.021
	WH7205	37.154±5.638	3.750±1.325
	WH7904	29.376±2.593	2.805±0.167
	WH115	9.682±0.762	0.596±0.087
III	WH1.5	4.031±0.717	0.069±0.003
IV	W83	6.327±0.784	0.039±0.019
	WH1904	11.117±1.137	0.330±0.083
	WH2103	3.119±0.893	0.475±0.076
	WH559	25.290±2.234	1.173±0.093
V	WH3501	7.432±0.617	0.037±0.003

*Values represent means plus or minus standard deviations of tripliacate. Experiments were performed in triplicate. a Comparison among eight Pg-II stains showed that the difference was significant ($P<0.01$). b Comparison among various kind of *fimA* genotype showed that the difference was not significant ($P > 0.05$).

Scanning electron microscopy and transmission electron microscopy

Results of SEM and TEM showed that all strains were capable of adhering to and invading HOK cells. SEM analysis revealed distinct adherent abilities among Pg-II strains (Figure 2). Differences in bacterial invasion of the Pg-II strains were observed by TEM investigations (Figure 3).

DISCUSSION

Adhesion to and invasion of oral epithelial cells by *P. gingivalis* is a key step in the colonization of periodontal pockets. To investigate the adherent and invasive abilities of *P. gingivalis*, previous studies used KB cells and HeLa cells to simulate the oral epithelium. However, limitations to the use of the KB cell line as an oral epithelial cell model include the facts that the KB cell line was originally derived from an epidermal carcinoma of the mouth and that it was found to be contaminated with HeLa cells at the time that it was established (Jiang et al., 2009). An obvious limitation to the use of the HeLa cell line as a model for oral epithelium is that it was derived from a uterine cervical carcinoma. HOK cells were isolated from human oral mucosa, which simulates the keratinized stratified squamous cells of the gingival epithelium. Thus, we rationalized the use of HOK cells in this study in order to enhance the credibility and the reproducibility of our study. The results showed that all strains could adhere to and invade HOK cells, adherent rate ranged from 0.665 to 37.154% and invasive rate ranged from 0.037 to 3.750%. Among the seventeen strains, adherent and invasive abilities were significantly different. These findings agree with the results from previous studies (Belton et al., 1999; Nakagawa et al., 2006; Umeda et al., 2006).

Compared with other *fimA* genotypes, Pg-II was thought to be not only the predominant *fimA* genotype in chronic periodontitis but also the most frequent genotype in chronic apical periodontitis and bacteremia (Pérez-Chaparro et al., 2009; Wang et al., 2010). Furthermore, a study on the distribution of *fimA* genotypes in cardio-vascular specimens from Japanese patients indicated the possible involvement of Pg-II in the initiation and progression of cardiovascular diseases (Nakano et al., 2008). As suggested by the researchers aforementioned, in comparison to other *fimA* genotypes, Pg-II showed a stronger correlation with periodontitis and other diseases and was shown to be more virulent. However, Inaba et al found that pathogenic heterogeneity existed among the Pg-II strains; the strong correlation with pathogenicity was not uniformly conserved as a typical characteristic of every Pg-II strain (Inaba et al., 2008). The results of this investigation showed that there were significant differences in adherent and invasive capabilities among the Pg-II strains. Among the eight Pg-II strains, adherent ability varied significantly and ranged from 0.665 to 37.154%, whereas invasive abilities ranged from 0.040 to

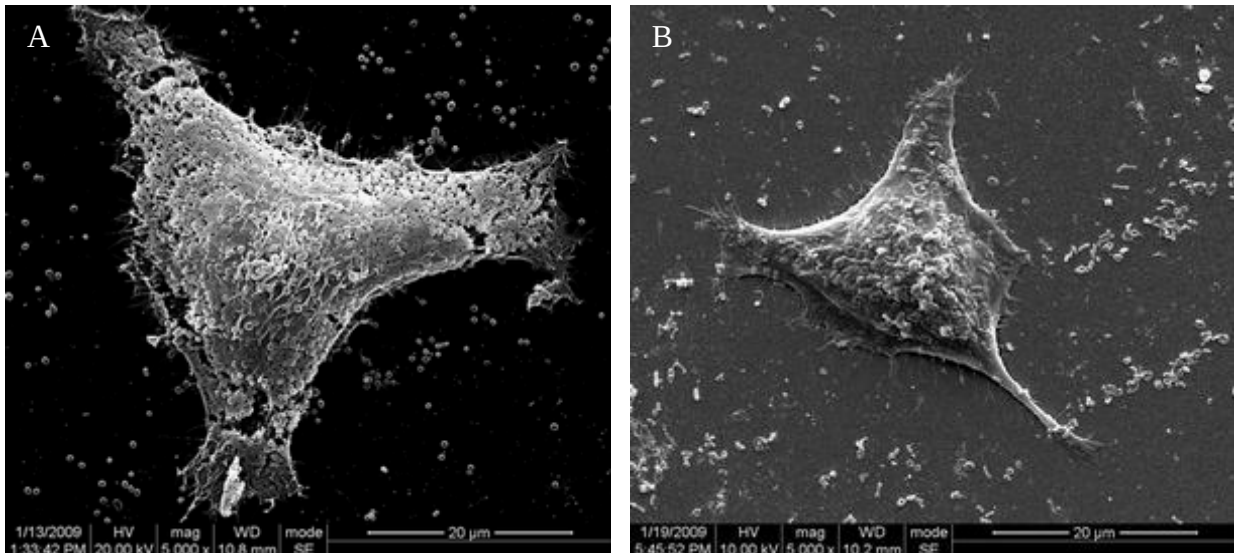

Figure 2A-B. Scanning electron micrographs showing Pg-II adhesion to HOK cells. The spots on the cell surface are adherent bacteria. The adherence rates were 37.154% (A) and 0.665% (B). (Magnification ×5000).

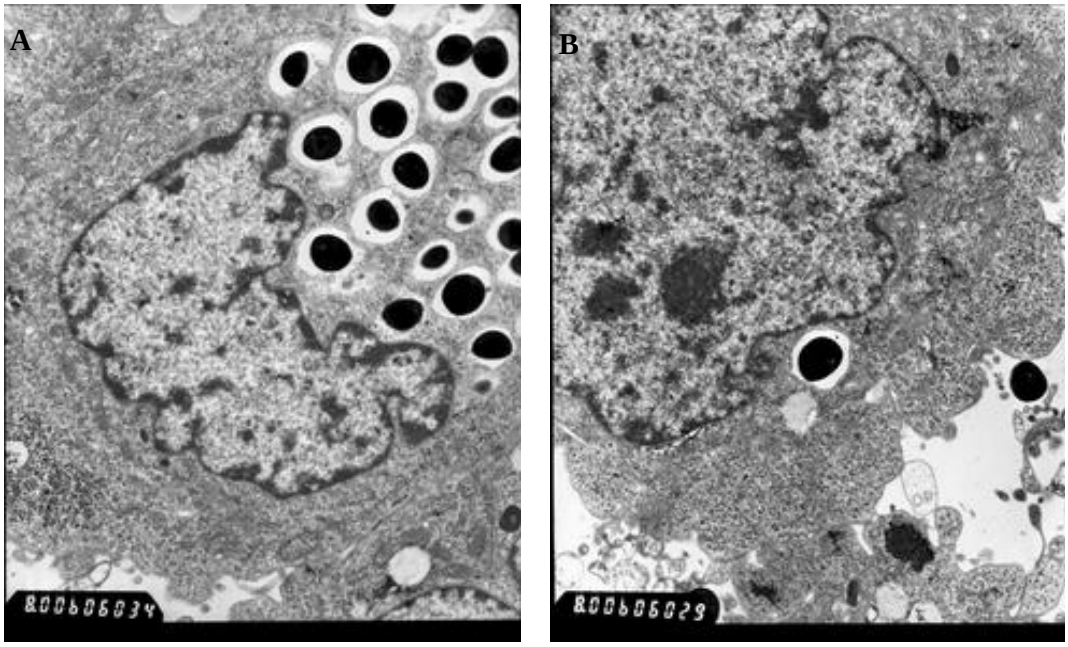

Figure 3A-B. Transmission electron micrograph showing the invasion to HOK cells by Pg-II. The perinuclear black spots are invasive bacteria. The invasive rates were 3.750% (A) and 0.040% (B). (Magnification ×80000).

3.750%. SEM and TEM results further demonstrated that adherent and invasive heterogeneities existed among clinical isolates of the Pg-II strains. Interestingly, Dorn et al. (2000) found that *P. gingivalis* strain W50 invaded KB cells at a rate 170 times greater than strain AJW4. *P. gingivalis* strain W50 expresses the same Type IV fimbriae as AJW4, yet strain AJW4 demonstrated an adherence rate 8.9 times greater than that of strain W50. This difference suggests that even among strains with the same type of *fimA*, adherence and invasive abilities can vary dramatically. The possible reasons could be various. Although fimbriae play an important role in the process of

adhesion and invasion of *P. gingivalis*, fimbriae-deficient mutants of *P. gingivalis* can still adhere to and invade epithelial cells (Njoroge et al., 1997). Yilmaz et al. (2002) found that invasion of epithelial cells of a fimbriae-deficient mutant was not completely blocked by the anti-β1 integrin antibodies, which suggested that there may be other fimbriae-independent pathways mediating the bacterial invasion of host cells. At present, *P. gingivalis* invasion can be divided into fimbriae-dependent and fimbriae-independent pathways (Andrian et al., 2006), and fimbriae are not the indispensable condition of bacterial invasion. In addition to fimbriae, other components may also affect the adherent and invasive abilities of *P. gingivalis*. Gingipains, a kind of secreted proteins found on the bacterial cell surface, contribute to the abilities of adhesion and invasion of *P. gingivalis* in a multifactorial way, and have been recognized as the major adhesion in the process of fimbriae-independent way (Katz et al., 2002; Tada et al., 2003; O'Brien-Simpson et al., 2009). It has also been shown that invasive efficiency of Pg-II strains was related to the activities of gingipains which were extracellularly secreted (Inaba et al., 2008). Moreover, capsule (Watanabe et al., 1992; Singh et al., 2011), vesicles (Duchesne et al., 1995; Furuta et al., 2009), abnormal endopeptidase Pep0 (Ansai et al., 2003), glycosyltransferase (Narimatsu et al., 2004), hemagglutinin A(Bélanger et al., 2011), hemin-binding protein 35 (Hiratsuka et al., 2010), hemagglutinating adhesion-antigen2 (Chandad et al., 1995; Du et al., 1997) and other bacterial cell-surface structures have all been implicated in the process of adhesion and invasion. Noiri (Noiri et al., 2004) have suggested that *P. gingivalis*-carrying fimbriae were strongly related to adhesion to the root surface at the bottoms of periodontal pockets rather than the pocket epithelium. This attachment site indicated that the function of *P. gingivalis* fimbriae regarding adhesion and invasion is only one aspect of its virulence. In addition, even among the same *fimA* genotype of *P. gingivalis,* the transcription levels of the *fimA* gene could be different. There is a correlation between the FimA expression level and binding activity to oral surface (Zheng et al., 2011), which is likely to be responsible for the differential adherent ability of the Pg-II strains.

These results showed that there was no correlation between the differences of adherent and invasive abilities and *fimA* genotypes. Current opinion differs as to whether there is any difference in adherent and invasive abilities among *P. gingivalis* strains with distinct *fimA* genotypes. Nakagawa et al. (2006) and Kato et al. (2007) believe that there is a certain relationship between the *fimA* genotype and the adhesion and invasion abilities of *P. gingivalis*. In contrast, Dorn et al. (2000) and Umeda et al. (2006) did not observe any correlation between adhesion and invasion and specific *fimA* types in *P. gingivalis*. In Nakagawa's research (Nakagawa et al., 2006), a Type II *fimA* strain was found to adhere to and invade significantly more in Hep-2 cells than strains with other *fimA* genotypes. However, for each *fimA* type, there was only one strain investigated in the study, which cannot accurately and comprehensively represent the characteristics of that genotype. The results, which showed that there was no difference in adherent and invasive abilities among seventeen *P. gingivalis* strains with different *fimA* types, supported the opinions of Dorn et al. (2000) and Umeda et al. (2006).

It must be noted that the adherent/ invasive ability is not the only determinant correlated with bacterial virulence. Therefore, the stronger adherent and invasive abilities did not equate with stronger virulence. For example, the adherent and invasive abilities of *P. gingivalis* ATCC 33277 and 381 were much stronger than W83 and W50, whereas the latter had greater virulence (Sandros et al., 1994). No major difference in the adherent abilities was found between the non-virulent strains and virulent strains, indicating the inconsistency between adherent and invasive abilities and virulence (Pathirana et al., 2007). *P. gingivalis* is a periodontal pathogen that expresses a wide range of potential virulence factors, such as fimbriae, outer membrane protein, capsule, protease, endotoxin lipopolysaccharide, cytotoxic metabolites and other unknown virulence factors (Nakayama 2003; Grenier and La, 2011). Therefore, virulence of *P. gingivalis* is manifested in many other aspects in addition to the adherent and invasive abilities.

In conclusion, the results of this study indicate that adherent and invasive heterogeneities exist among the Pg-II stains. There is no significant correlation between *P. gingivalis fimA* genotypes and abilities in adhesion and invasion, suggesting that other factors may influence the virulence of strains of *P. gingivalis* with distinct *fimA* genotypes.

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REFERENCES

Amano A, Kuboniwa M, Nakagawa I, Akiyama S, Morisaki I, Hamada S (2000). Prevalence of specific genotypes of *Porphyromonas gingivalis fimA* and periodontal health status. J. Dent. Res., 79(9): 1664–1668.

Amano A, Nakagawa I, Kataoka K, Morisaki I, Hamada S (1999). Distribution of *Porphyromonas gingivalis* strains with fimA genotypes in periodontitis patients. J. Clin. Microbiol., 37(5):1426–1430.

Amano A, Nakagawa I, Okahashi N, Hamada N (2004). Variations of *Porphyromonas gingivalis* fimbriae in relation to microbial pathogenesis. J. Periodont. Res., 39(2):136–142.

Andrian E, Grenier D, Rouabhia M (2006). *Porphyromonas gingivalis*-Epithelial Cell Interactions in Periodontitis. J. Dent. Res., 85(5): 392-403.

Ansai T, Yu W, Urnowey S, Barik S, Takehara T (2003). Construction of

a pepO gene-deficient mutant of *Porphyromonas gingivalis*: potential role of endopeptidase O in the invasion of host cells. Oral. Microbiol. Immunol., 18(6): 398-400.
Ashimoto A, Chen C, Bakker I, Slots J (1996). Polymerase chain reaction detection of 8 putative periodontal pathogens in subgingival plaque in gingivitis and advanced periodontitis lesions. Oral. Microbiol. Immunol., 11(4): 266–273.
Beikler T, Peters U, Prajaneh S, Prior K, Ehmke B, Flemmig TF (2003). Prevalence of *Porphyromonas gingivalis fimA* genotypes in Caucasians. Eur. J. Oral. Sci., 111(5): 390–394.
Bélanger M, Kozarov E, Song H, Whitlock J, Progulske-Fox A (2011). Both the unique and repeat regions of the *Porphyromonas gingivalis* hemagglutinin A are involved in adhesion and invasion of host cells. Anaerobe., [Epub ahead of print]
Belton CM, Izutsu KT, Goodwin PC, Park Y, Lamont RJ (1999). Fluorescence image analysis of the association between *Porphyromonas gingivalis* and gingival epithelial cells. Cell. Microbiol., 1(3): 215-223.
Chandad F, Mouton C (1995). Antigenic, structural, and functional relationships between fimbriae and the hemagglutinating adhesin HAAg2 of *Porphyromonas gingivalis*. Infect. Immun., 63(12): 4755-4763.
Dorn BR, Burks JN, Seifert KN, Progulske-Fox A (2000). Invasion of endothelial and epithelial cells by strains of *Porphyromonas gingivalis*. FEMS. Microbiol. Lett., 187(2): 139-144.
Duchesne P, Grenier D, Mayrand D (1995). Demonstration of adherence properties of *Porphyromonas gingivalis* outer membrane vesicles using a new microassay. Oral. Microbiol. Immunol., 10(2): 76-80.
Du L, Pellen-Mussi P, Chandad F, Mouton C, Bonnaure-Mallet M (1997). Fimbriae and the hemagglutinating adhesin HA-Ag2 mediate adhesion of *Porphyromonas gingivalis* to epithelial cells. Infect. Immun., 65(9): 3875-3881.
Fujiwara T, Nakagawa I, Morishima S, Takahashi I, Hamada S (1994). Inconsistency between the fimbrilin gene and the antigenicity of lipopolysaccharides in selected strains of *Porphyromonas gingivalis*. FEMS. Microbiol. Lett., 124(3): 333-341.
Furuta N, Takeuchi H, Amano A (2009). Entry of *Porphyromonas gingivalis* outer membrane vesicles into epithelial cells causes cellular functional impairment. Infect Immun., 77(11):4761-4770.
Genco CA, Cutler CW, Kapecaynski D (1991). A novel mouse model to study the virulence of and host response to *Porphyromonas (Bacteroides) gingivalis*. Infect. Immun., 59(4): 1255–1263.
Grenier D, La VD (2011). Proteases of *Porphyromonas gingivalis* as important virulence factors in periodontal disease and potential targets for plant-derived compounds: a review article. Curr Drug Targets., 12(3):322-331.
Hiratsuka K, Kiyama-Kishikawa M, Abiko Y (2010). Hemin-binding protein 35 (HBP35) plays an important role in bacteria-mammalian cells interactions in *Porphyromonas gingivalis*. Microb. Pathog., 48(3-4):116-123.
Holt SC, Ebersole JL (2005). *Porphyromonas gingivalis*, *Treponema denticola*, and *Tannerella forsythia*: the “red complex,” a prototype polybacterial pathogenic consortium in periodontitis. Periodontol. 2000., 38: 72–122.
Inaba H, Nakano K, Kato T, Nomura R, Kawai S, Kuboniwa M, Ishihara K, Ooshima T, Amano A (2008). Heterogenic virulence and related factors among clinical isolates of *Porphyromonas gingivalis* with type II fimbriae. Oral. Microbiol. Immunol., 23(1): 29-35.
Kato T, Kawai S, Nakano K, Inaba H, Kuboniwa M, Nakagawa I, Tsuda K, Omori H, Ooshima T, Yoshimori T, Amano A (2007). Virulence of *Porphyromonas gingivalis* is altered by substitution of fimbria gene with different genotype. Cell. Microbiol., 9(3): 753-765.
Katz J, Yang QB, Zhang P, Potempa J, Travis J, Michalek SM, Balkovetz DF (2002). Hydrolysis of epithelial junctional proteins by *Porphyromonas gingivalis* gingipains. Infect. Immun., 70(5): 2512-2518.
Jiang L, Zeng X, Wang Z, Chen Q (2009). Cell line cross-contamination: KB is not an oral squamous cell carcinoma cell line. Eur. J. Oral. Sci., 117(1):90-91.
Laine ML, van Winkelhoff AJ (1998). Virulence of six capsular serotypes of *Porphyromonas gingivalis* in a mouse model. Oral. Microbiol. Immunol., 13(5): 322–325.
Lamont RJ, Jenkinson HF (1998). Life below the gum line: pathogenic mechanisms of *Porphyromonas gingivalis*. Microbiol. Mol. Biol. Rev., 62(4):1244-1263.
Missailidis CG, Umeda JE, Ota-Tsuzuki C, Anzai D, Mayer MPA (2004). Distribution of *fimA* genotypes of *Porphyromonas gingivalis* in subjects with various periodontal conditions. Oral. Microbiol. Immunol., 19(4): 224–229.
Moffatt CE, Lamont RJ (2011). *Porphyromonas gingivalis* induction of microRNA-203 expression controls suppressor of cytokine signaling 3 in gingival epithelial cells. Infect. Immun., 79(7):2632-2637.
Nakagawa I, Amano A, Kimura RK, Nakamura T, Kawabata S, Hamada S (2000). Distribution and molecular characterization of *Porphyromonas gingivalis* carrying a new type of *fimA* gene. J. Clin. Microbiol., 38(5): 1909–1914.
Nakagawa I, Amano A, Ohara-Nemoto Y, Endoh N, Morisaki I, Kimura S, Kawabata S, Hamada S (2002). Identification of a new variant of *fimA* gene of *Porphyromonas gingivalis* and its distribution in adults and disabled populations with periodontitis. J. Periodont. Res., 37(6): 425–432.
Nakagawa I, Inaba H, Yamamura T, Kato T, Kawai S, Ooshima T, Amano A (2006). Invasion of epithelial cells and proteolysis of cellular focal adhesion components by distinct types of *Porphyromonas gingivalis* fimbriae. Infect. Immun., 74(7):3773-3782.
Nakano K, Inaba H, Nomura R, Nemoto H, Takeuchi H, Yoshioka H, Toda K, Taniguchi K, Amano A, Ooshima T (2008). Distribution of *Porphyromonas gingivalis* fimA genotypes in cardiovascular specimens from Japanese patients. Oral. Microbiol. Immunol., 23(2):170-172.
Nakano K, Kuboniwa M, Nakagawa I, Yamamura T, Nomura R, Okahashi N, Ooshima T, Amano A (2004).Comparison of inflammatory changes caused by *Porphyromonas gingivalis* with distinct *fimA* genotypes in a mouse abscess model. Oral. Microbiol. Immunol., 19(3): 205-209.
Nakayama K (2003). Molecular genetics of *Porphyromonas gingivalis*: gingipains and other virulence factors. Curr. Protein. Pept. Sci., 4(6): 389-395.
Narimatsu M, Noiri Y, Itoh S, Noguchi N, Kawahara T, Ebisu S (2004). Essential role for the gtfA gene encoding a putative glycosyltransferase in the adherence of *Porphyromonas gingivalis*. Infect. Immun., 72(5): 2698-2702.
Njoroge T, Genco RJ, Sojar HT, Hamada N, Genco CA (1997). A role for fimbriae in *Porphyromonas gingivalis* invasion of oral epithelial cells. Infect. Immun. 65(5): 1980-1984.
Noiri Y, Li L, Yoshimura F, Ebisu S (2004). Localization of *Porphyromonas gingivalis*-carrying fimbriae in situ in human periodontal pockets. J. Dent. Res., 83(12): 941-945.
O'Brien-Simpson NM, Pathirana RD, Walker GD, Reynolds EC (2009). *Porphyromonas gingivalis* RgpA-Kgp proteinase-adhesin complexes penetrate gingival tissue and induce proinflammatory cytokines or apoptosis in a concentration-dependent manner. Infect Immun., 77(3):1246-1261.
Pathirana RD, O'Brien-Simpson NM, Visvanathan K, Hamilton JA, Reynolds EC (2007). Flow cytometric analysis of adherence of *Porphyromonas gingivalis* to oral epithelial cells. Infect. Immun., 75(5): 2484-2492.
Pérez-Chaparro PJ, Lafaurie GI, Gracieux P, Meuric V, Tamanai-Shacoori Z, Castellanos JE, Bonnaure-Mallet M (2009). Distribution of *Porphyromonas gingivalis* fimA genotypes in isolates from subgingival plaque and blood sample during bacteremia. Biomedica., 29(2):298-306.
Sandros J, Papapanou PN, Nannmark U, Dahlén G (1994). *Porphyromonas gingivalis* invades human pocket epithelium *in vitro*. J. Periodontal. Res., 29(1): 62–69.
Singh A, Wyant T, Anaya-Bergman C, Aduse-Opoku J, Brunner J, Laine ML, Curtis MA, Lewis JP (2011). The capsule of *Porphyromonas gingivalis* leads to a reduction in the host inflammatory response, evasion of phagocytosis, and increase in virulence. Infect. Immun., 79(11):4533-4542.
Sugano N, Ikeda K, Oshikawa M, Sawamoto Y, Tanaka H, Ito K (2004). Differential cytokine induction by two types of *Porphyromonas gingivalis*. Oral. Microbiol. Immunol., 19(2):121-123.

Tada H, Sugawara S, Nemoto E, Imamura T, Potempa J, Travis J, Shimauchi H, Takada H (2003). Proteolysis of ICAM-1 on human oral epithelial cells by gingipains. J. Dent. Res., 82(10): 796-801.

Tran SD, Rudney JD (1996). Multiplex PCR using conserved and species-specific 16S rRNA gene primers for simultaneous detection of *Actinobacillus actinomycetemcomitans* and *Porphyromonas gingivalis*. J. Clin. Microbiol., 34(10):2674–2678.

Umeda JE, Missailidis C, Longo PL, Anzai D, Wikström M, Mayer MP (2006). Adhesion and invasion to epithelial cells by *fimA* genotypes of *Porphyromonas gingivalis*. Oral. Microbiol. Immunol., 21(6): 415-419.

Wang M, Liang S, Hosur KB, Domon H, Yoshimura F, Amano A (2009). Differential virulence and innate immune interactions of Type I and II fimbrial genotypes of *Porphyromonas gingivalis*. Oral. Microbiol. Immunol., 24(6):478-484.

Wang Q, Zhou XD, Zheng QH, Wang Y, Tang L, Huang DM (2010). Distribution of *Porphyromonas gingivalis* fimA genotypes in chronic apical periodontitis associated with symptoms. J. Endod., 36(11):1790-1795.

Watanabe K, Yamaji Y, Umemoto T (1992). Correlation between cell-adherent activity and surface structure in *Porphyromonas gingivalis*. Oral. Microbiol. Immunol., 7(6): 357–363.

Yilmaz O, Watanabe K, Lamont RJ (2002). Involvement of integrins in fimbriae-mediated binding and invasion by *Porphyromonas gingivalis.* Cell. Microbiol., 4(5): 305-314.

Zhao L, Wu YF, Meng S, Yang H, OuYang YL, Zhou XD (2007). Prevalence of *fimA* genotypes of *Porphyromonas gingivalis* and periodontal health status in Chinese adults. J. Periodontal. Res., 42(6): 511-517.

Zheng C, Wu J, Xie H (2011). Differential expression and adherence of *Porphyromonas gingivalis* FimA genotypes. Mol. Oral. Microbiol., 26(6):388-95.