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# Differential virulence and innate immune interactions of type I and II fimbrial genotypes of *Porphyromonas gingivalis*

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**Introduction:** The *fimA*-encoded fimbriae of the periodontal pathogen *Porphyromonas gingivalis* display genetic diversity. Type I fimbriated *P. gingivalis* (Pg-I) has been most widely studied at the molecular level, whereas Pg-II is the most frequent isolate from severe periodontitis.
**Methods:** To investigate virulence differences between Types I and II fimbriae, we examined strains 33277 (Pg-I) and OMZ314 (Pg-II), reciprocal swap mutants (i.e. expressing the heterologous fimbrial type), and their respective FimA-deficient derivatives. These organisms were tested in a mouse periodontitis model and in interactions with mouse macrophages, a cell type that plays important roles in chronic infections.
**Results:** Strain 33277 induced significantly more periodontal bone loss than OMZ314 and substitution of Type II fimbriae with Type I in OMZ314 resulted in a more virulent strain than the parent organism. However, the presence of Type II fimbriae was associated with increased proinflammatory and invasive activities in macrophages.
**Conclusion:** The inverse relationship between proinflammatory potential and ability to cause experimental periodontitis may suggest that an aggressive phenotype could provoke a host response that would compromise the persistence of the pathogen.

Key words: bone loss; fimbriae; inflammation; macrophages; *P. gingivalis*; periodontitis

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*Porphyromonas gingivalis* is a major pathogen in human periodontitis, an inflammatory disease leading to destruction of the tooth-supporting tissues (12, 18). This bacterium is moreover implicated in certain systemic conditions, such as atherosclerosis (9). Its pathogenicity is attributed to several virulence factors, including cysteine proteinases, hemagglutinins, and cell surface fimbriae (24). The fimbriae constitute adhesive filamentous appendages and comprise two types: *fimA*-encoded 'major' fimbriae and *mfa1*-encoded 'minor' fimbriae of considerably shorter length (37). The major fimbriae (henceforth referred to as 'fimbriae') are traditionally recognized as a critical colonization factor (24). It is now appreciated that the fimbriae contribute to *P. gingivalis* virulence also through immune subversion of innate host responses (17, 34).

On the basis of sequence diversity of the *fimA* gene, the fimbriae are currently classified into six genotypes (I–V and Ib) (3). *P. gingivalis* expressing Type II fimbriae (designated Pg-II) is the most common genotype, while the second most prevalent genotype has been variably found to be Type IV, Ib, or I depending on the ethnic population studied (reviewed in References 3 and 11). Type I fimbriae display 97% amino acid identity with Type Ib, 76% identity with Type II, but only 46% identity with Type IV (reviewed in Ref. 11). In terms of clinical significance, Pg-II is the most frequent isolate from deep periodontal pockets (2). Interestingly, despite lower prevalence, Pg-I is associated with diseased sites that are more refractory to periodontal treatment than Pg-II-infected sites (8).

Most studies examining the virulence properties of *P. gingivalis* have predomi-

nantly used Pg-I strains (e.g. 33277 or 381) or their purified fimbriae, which have been considered prototypical (4, 9, 13, 36, 37). However, because of diverse amino acid sequences of the various types of fimbriae and, therefore, the possibility that these may display distinct virulence features, there is a recent interest in comparative studies of *P. gingivalis* fimbrial genotypes. These studies have shown that Pg-II and, to a lesser extent, Pg-I are the most potent genotypes in terms of adhesive and cell-invasive activities (28, 29). However, Pg-II displays increased adhesion to and invasion of epithelial cells compared to Pg-I (28). Moreover, Pg-II was found to be more proinflammatory than Pg-I (6, 30).

*P. gingivalis* strains 33277 and OMZ314 have been widely studied as model Pg-I and Pg-II strains, respectively (14, 21, 29, 31). Although the genetic diversity of their *fimA* genes may contribute to virulence differences, additional factors may play a role. To control for potential differences in non-fimbrial virulence factors and, thereby, validly determine virulence variations attributed to specific fimbrial types, an elegant genetic system was developed (21). Specifically, the Type I and II *fimA* genes were exchanged between the 33277 and OMZ314 strains, resulting in two swap mutants, i.e. Type II *fimA*-expressing 33277 and Type I *fimA*-expressing OMZ314 (21). This study revealed that substitution of Type I FimA with Type II promotes *P. gingivalis* invasion of epithelial cells and enhances its proinflammatory properties (21). Conversely, the reverse substitution (Type II FimA replaced by Type I) rendered the modified strain less potent in the same assays (21).

In this paper, using 33277 and OMZ314 in parallel with their *fimA* swap mutants, we determined for the first time the relative contribution of Type I and II fimbriae to *P. gingivalis*-induced bone loss in the mouse periodontitis model. At the host cellular level, we examined the phagocytic uptake of Type I and II clones and, moreover, investigated their intracellular fate in correlation with the macrophage antimicrobial response. Our findings suggest that an overt aggressive/proinflammatory phenotype could compromise the ability of *P. gingivalis* to persist and cause disease in the mouse periodontitis model.

## Materials and methods

### Bacteria

*P. gingivalis* was grown anaerobically at 37°C in gifu anaerobic medium (GAM) medium, containing 5 μg/ml hemin and 1 μg/ml menadione (Nissui Pharmaceutical, Tokyo, Japan). The Type I and II *fimA* genes were exchanged between strains 33277 and OMZ314, as previously described (21), resulting in two swap mutants (SM), i.e. Type II *fimA*-expressing 33277 (strain KDC2; designated SM I → II) and Type I *fimA*-expressing OMZ314 (strain KDC1; SM II → I). The swapped *fimA* genes were expressed from their native promoters and the expected fimbrial phenotype was confirmed genetically, biochemically, and morphologically (21). The strains used also included FimA-deficient ('non-fimbriated') isogenic mutants of 33277 and OMZ314 (Table 1). Type I fimbriae were extracted by a washing method and purified as previously described (38). Type II fimbriae were extracted from bacterial cell lysates using a French Press and ultracentrifugation. The extracts were then subjected to 50% saturated ammonium sulfate precipitation, dialysed against 20 mM Tris–HCl, pH 8.0, and purified by gel filtration (Sephacryl S-400; GE Healthcare, Piscataway, NJ) and ion-exchange chromatography (diethylaminoethyl–Sepharose; GE Healthcare). The fimbrial preparations were free of contaminating substances on silver-stained sodium dodecyl sulfate–polyacrylamide gel electrophoresis, and tested negative for endotoxin (< 6 EU/mg protein) according to the BioWhittaker limulus amebocyte lysate assay (BioWhittaker, Walkersville, MD).

*Table 1. Porphyromonas gingivalis* strains used in this study

| Strain | Remarks | FimA phenotype | Designation used in this study | Source/ reference |
|---|---|---|---|---|
| 33277 | Wild-type | Type I | WT I | ATCC |
| KDC2 | Type I *fimA* substituted by Type II Isogenic with 33277 | Type II | SM I → II[1] | (21) |
| JI-1 | Non-fimbriated Isogenic with 33277 | Not applicable | FimA(-)I | (34) |
| OMZ314 | Wild-type | Type II | WT II | (29) |
| KDC1 | Type II *fimA* substituted by Type I Isogenic with OMZ314 | Type I | SM II → I[1] | (21) |
| KDC3 | Non-fimbriated Isogenic with OMZ314 | Not applicable | FimA(-)II | (21) |

[1]Swap mutants (SM); the arrow indicates the current FimA type after substitution.

### Mammalian cell culture

Thioglycollate-elicited macrophages were isolated from the peritoneal cavity of BALB/cByJ mice (The Jackson Laboratory, Bar Harbor, ME) (15). Mouse macrophages and monocytic THP-1-Blue™ cells (InvivoGen, San Diego, CA) were cultured in RPMI-1640 (Invitrogen, Carlsbad, CA) containing 10% heat-inactivated fetal bovine serum, 2 mM L-glutamine, 10 mM HEPES, 100 U/ml penicillin G, 100 μg/ml streptomycin, and 0.05 mM 2-mercaptoethanol. Cell viability was monitored by the CellTiter-Blue™ assay (Promega, Madison, WI). None of the treatments affected cell viability compared to medium-only control.

### Phagocytosis

Flow cytometry was performed to assess the uptake of *P. gingivalis* (34). Briefly, mouse macrophages were incubated at 37°C with fluorescein isothiocyanate (FITC) -labeled *P. gingivalis* for 30 min [multiplicity of infection (MOI) 10 : 1; $5 \times 10^5$ macrophages used per assay]. Phagocytosis was stopped by cooling the incubation tubes on ice. After washing to remove non-adherent bacteria, in some groups, extracellular fluorescence (representing attached but not internalized bacteria) was quenched with 0.2% trypan blue. The cells were washed again, fixed, and analysed by flow cytometry [% positive cells for FITC-labeled *P. gingivalis* and mean fluorescence intensity (MFI)] using the FACSCalibur and the CELLQUEST software (Becton-Dickinson, Franklin Lakes, NJ). Association (i.e. representing both adherence and phagocytosis) or phagocytic indices were calculated using the formula (% positive cells × MFI)/100.

### Intracellular survival assay

The capacity of phagocytosed *P. gingivalis* for intracellular persistence in macrophages was determined by an antibiotic protection-based survival assay, as previously described (34). Briefly, viable counts (colony-forming units; CFU) of internalized *P. gingivalis* were determined by plating serial dilutions of macrophage lysates on

blood agar plates subjected to anaerobic culture. Before macrophage lysis, extracellular non-adherent bacteria were removed by washing, while extracellular adherent bacteria were killed by addition of gentamicin and metronidazole.

#### Cell activation assays

Induction of cytokine release in culture supernatants of activated mouse macrophages was measured by enzyme-linked immunosorbent assay (eBioscience, San Diego, CA). Induction of nitric oxide (NO) was assessed by measuring the amount of $NO_2^-$ (a stable metabolite of NO) in stimulated culture supernatants using a Griess reaction-based assay (R&D Systems, Minneapolis, MN). Activation of nuclear factor-$\kappa$B (NF-$\kappa$B) was assessed using THP-1-Blue™ cells stably transfected with NF-$\kappa$B-inducible reporter system, following the manufacturer's protocol (InvivoGen). In all three assays, a MOI of 10 : 1 was used ($2 \times 10^6$ bacteria and $2 \times 10^5$ host cells).

#### Confocal microscopy

Confocal laser scanning microscopy was used to determine colocalization of *P. gingivalis* with lysosomes, as previously described (33). Briefly, macrophages were exposed for 30 min to FITC-labeled *P. gingivalis* (MOI = 10 : 1; $5 \times 10^5$ macrophages), washed, and incubation was allowed to proceed for an additional 90 min at 37°C. Subsequently, the cells were stained with LysoTraker Red DND-99, which targets acidified late endosomes and lysosomes. The cells were then fixed and imaged on an Olympus FV500 confocal microscope. Figure 5 shows representative single optical sections and two-color overlay (merge) confocal images, as well as additional overlay with differential interference contrast (DIC) images. The degree of colocalization of *P. gingivalis* with lysosomes was quantified using the IMAGE J software with the Intensity Correlation Analysis plugin (National Institutes of Health; http://rsb.info.nih.gov/ij).

#### Mouse periodontitis model

BALB/cByJ mice (10–12 weeks old; The Jackson Laboratory) were orally infected with *P. gingivalis* strains (Table 1) for induction of periodontal bone loss, essentially as originally described by Baker et al. (5) with slight modifications (34). Briefly, upon suppression of the normal oral flora with antibiotics, mice were infected by oral gavage three times at 2-day intervals with $10^9$ CFU *P. gingivalis* suspended in 2% carboxymethylcellulose/phosphate-buffered saline. Sham-infected controls received 2% carboxymethylcellulose alone. Mice were sacrificed 6 weeks later and assessment of periodontal bone loss in defleshed maxillae was performed under a dissecting microscope (× 40) fitted with a video image marker measurement system (VIA-170K; Nikon Instruments, Melville, NY). Specifically, the distance from the cementoenamel junction (CEJ) to the alveolar bone crest (ABC) was measured on 14 predetermined points on the buccal surfaces of the maxillary molars. To calculate bone loss, the 14-site total CEJ–ABC distance for each mouse was subtracted from the mean CEJ–ABC distance of sham-infected mice. The results were expressed in millimeters and negative values indicate bone loss relative to sham-infected controls. This morphometric method is well established and validated (5, 10) and correlates very well with histomorphometric and micro-computed tomographic analyses (all three methods were found to accurately quantify periodontal bone loss)(25). All animal procedures were approved by the Institutional Animal Care and Use Committee, in compliance with established Federal and State policies.

#### Statistical analysis

Data were evaluated by analysis of variance and the Tukey–Kramer multiple comparisons test using the GRAPHPAD INSTAT software. Where appropriate (comparison of two groups only), two-tailed *t*-tests were also performed. $P < 0.05$ was taken as the level of significance.

## Results

#### Type I vs. Type II fimbriae and *P. gingivalis*-induced bone loss

Type I fimbriae play an important virulence role in *P. gingivalis*-induced periodontal bone loss in mice (9, 34). To determine and compare the relative contributions of Type I and Type II fimbriae to *P. gingivalis*-induced bone loss, mice were orally infected with Pg-I (33277) or Pg-II (OMZ314) strains, their reciprocal swap mutants (SM I → II and SM II → I), and their respective non-fimbriated mutants [FimA(-)I and FimA(-)II] (Table 1). Periodontal bone loss was induced to varying degrees by all the tested strains (Fig. 1). As expected, strain 33277 caused significantly more bone loss than its isogenic FimA(-)I mutant ($P < 0.001$) but, interestingly, was also more potent in this activity than the OMZ314 strain ($P < 0.05$). The latter finding indirectly suggested that Type I fimbriae may confer additional virulence potential over Type II in this model. This inference was substantiated by the observation that substitution of Type II fimbriae with Type I resulted in a more virulent strain (SM II → I) than the parent organism, OMZ314 ($P < 0.001$; Fig. 1). However, the observed reduction in virulence by the reverse substitution (i.e. 33277 vs. SM I → II) did not reach statistical significance ($P > 0.05$; Fig. 1), suggesting that factors other than FimA also play a role in pathogenesis. Quite unexpectedly, OMZ314 was not significantly more virulent than its isogenic FimA(-)II mutant ($P > 0.05$); rather, this comparison indicated that the presence of Type II fimbriae tended to decrease induction of bone loss (Fig. 1). In summary, at least in the mouse periodontitis model, the Type I fimbriae appear to contribute to *P. gingivalis* virulence more than the Type II fimbriae.

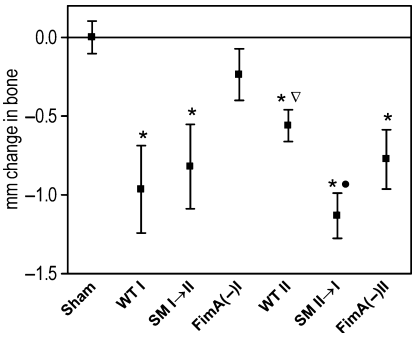


*Fig. 1*. Induction of periodontal bone loss by Type I or Type II *Porphyromonas gingivalis* strains or mutants. BALB/cByJ mice were orally infected with wild-type *P. gingivalis* 33277 (WT I) or OMZ314 (WT II), their reciprocal fimbrial swap mutants (SM I → II and SM II → I), and their isogenic FimA-deficient mutants (FimA(-)I and FimA(-)II), or treated with vehicle only (sham). At the end of the experiment, the distance from the cementoenamel junction to the alveolar bone crest was measured at 14 predetermined buccal sites on the maxillary molars and the values were transformed to directly indicate bone loss. Data are means ± SD ($n = 5$). Asterisks indicate statistically significant differences ($P < 0.001$) between infected and sham-infected mice. The inverted triangle denotes significant difference ($P < 0.05$) between WT II and WT I, and the black circle shows significant difference ($P < 0.001$) between SM II → I and WT II.

#### Fimbrial genotype-dependent differences in proinflammatory activities

The reduced *P. gingivalis* virulence associated with Type II fimbriae in the mouse

periodontitis model (Fig. 1) might be the result of stronger protective host responses induced by Type II compared to Type I fimbria-expressing strains. We previously showed that *P. gingivalis* is susceptible to NO-mediated macrophage killing, although it is resistant to reactive oxygen species (16). We therefore investigated potentially differential induction of NO by mouse macrophages in response to the strains used in the *in vivo* study. All strains tested were capable of inducing NO production to varying degrees, although the FimA(-)I and FimA(-)II were the least potent (Fig. 2A). However, WT-II (i.e. OMZ314) induced significantly higher NO levels than either WT-I (33277) or SM II → I ($P < 0.01$) but comparable NO levels to SM I → II (Fig. 2A). Therefore, the expression of Type II fimbriae results in stronger induction of NO. This conclusion is further supported by the significant increase of NO production upon substitution of Type I fimbriae with Type II ($P < 0.05$ for SM I → II vs. 33277; Fig. 2A). The induction of NO is NF-κB-dependent so we examined whether the NO-inducing capacities of the various strains tested correlated with differential NF-κB activation. Indeed, induction of NF-κB activation followed a similar pattern, i.e. expression of Type II fimbriae was associated with stronger NF-κB activation than expression of Type I fimbriae (Fig. 2B). This notion was confirmed by direct comparison for the first time of purified Type I and II fimbriae. Specifically, Type II fimbriae from OMZ314 were significantly more potent in activating NF-κB than Type I fimbriae from 33277 (Fig. 2C).

Moreover, expression of Type II fimbriae was associated with stronger induction of cytokine responses as revealed by comparison of the OMZ314 and 33277 strains ($P < 0.01$; Fig. 3). In addition, depending on the type of FimA substitution, cytokine induction tended to be either positively (SM I → II) or negatively (SM II → I) influenced, although some of these differences did not always reach statistical significance (Fig. 3). Taken together, the data from Figures 1–3 suggest an inverse relationship between induction of bone loss and activation of NF-κB-dependent activities, such as production of NO and proinflammatory cytokines.

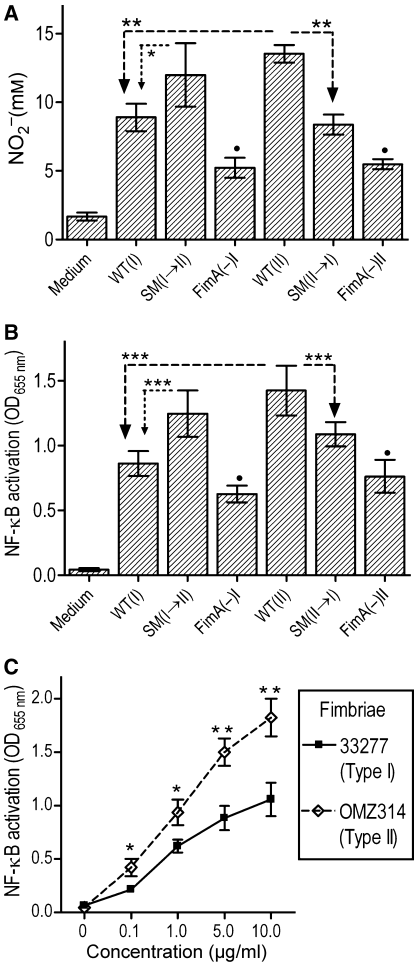


*Fig. 2.* Induction of nitric oxide (NO) production and activation of nuclear factor-κB (NF-κB) by Type I or Type II *Porphyromonas gingivalis* strains or mutants. Primary mouse macrophages (A) or the reporter THP-1-Blue™ cell line (B) were incubated with medium only or with the indicated strains of *P. gingivalis* (multiplicity of infection 10 : 1). After 24 h, production of $NO_2^-$ (stable metabolite of NO) was assayed by the Griess reaction (A) and NF-κB activation was determined colorimetrically by measuring the activity of NF-κB-inducible alkaline phosphatase secreted in culture supernatants (B). In (C), a similar NF-κB assay was performed using purified Type I or Type II fimbriae, at the indicated concentrations. Data are means ± SD ($n = 3$) from one of three sets of independent experiments that yielded consistent results. Asterisks show statistically significant differences (*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$) among wild-type (WT) and swap mutant (SM) strains (A, B), or between purified Type I and II fimbriae (C). Black circles indicate significantly reduced activities ($P < 0.05$) in FimA-deficient mutants compared with their corresponding parent strains (A, B).

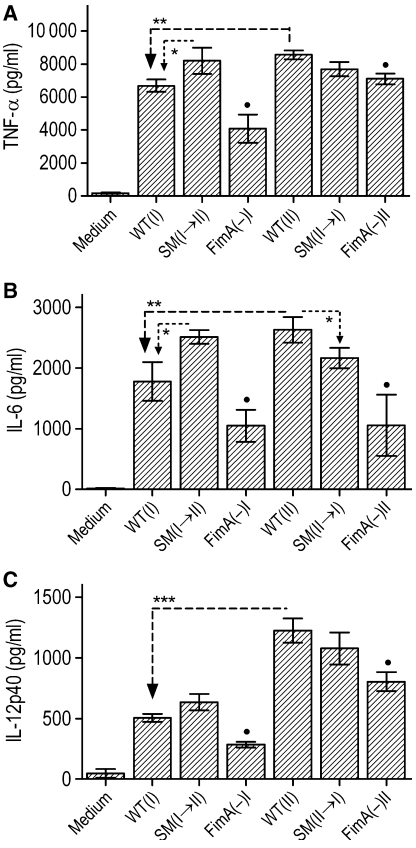


*Fig. 3.* Induction of proinflammatory cytokine production by Type I or Type II *Porphyromonas gingivalis* strains or mutants. Primary mouse macrophages were incubated with medium only or with the indicated strains of *P. gingivalis* (multiplicity of infection 10 : 1). After 24 h, induction of release of tumor necrosis factor-α (TNF-α) (A), interleukin-6 (IL-6) (B), or IL-12p40 in culture supernatants was determined by enzyme-linked immunosorbent assay. Data are means ± SD ($n = 3$) from one of two experiments with consistent results. Asterisks show statistically significant differences (*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$) among wild-type (WT) and swap mutant (SM) strains. Black circles indicate significantly reduced activities ($P < 0.05$) in FimA-deficient mutants compared with their corresponding parent strains.

### Effects of FimA type on *P. gingivalis* intracellular entry into macrophages

Although macrophages are professional phagocytes that actively internalize bacteria, the status of Type I fimbriae (presence or absence, or genetically altered) dictates the degree of *P. gingivalis* uptake (34). To further characterize potential functional differences of Type I and II fimbriae, we examined macrophage uptake of 33277 and OMZ314, their reciprocal swap mutants (SM I → II and SM II → I), and their respective FimA-deficient mutants (Table 1). As expected, the FimA-deficient mutants were significantly less active than their corresponding parent strains in both macrophage association and uptake assays ($P < 0.001$; Fig. 4A). However, no significant differences were noted between the wild-type strains in the way they associate or are taken up by macrophages, although substitution of Type I FimA with Type II (clone SM I → II) resulted in enhanced *P. gingivalis* association with and phago-

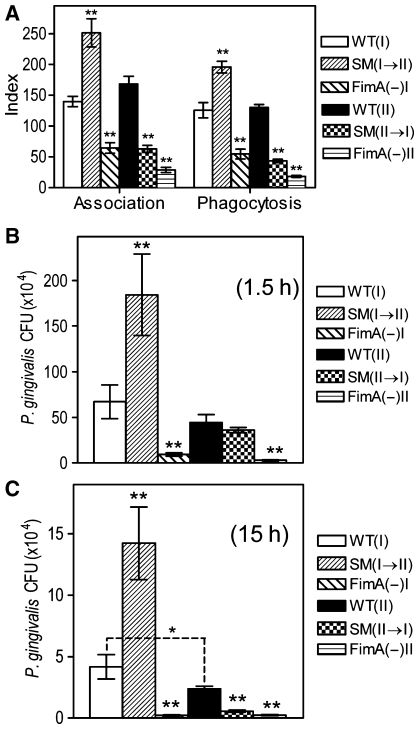


*Fig. 4*. Role of FimA type on the uptake and intracellular fate of *Porphyromonas gingivalis* in mouse macrophages. (A) Primary mouse macrophages were incubated for 30 min with the indicated fluorescein isohtiocyanate-labeled *P. gingivalis* strains (multiplicity of infection 10 : 1). Association (i.e. representing both adherence and phagocytosis) or phagocytic indices were determined by flow cytometry, using the formula % positive cells mean fluorescence intensity/100. (B, C) Primary mouse macrophages were incubated at a multiplicity of infection of 10 : 1 with Type I or II *P. gingivalis* or mutants, for 1.5 h (B) or 15 h (C). The persistence of viable internalized bacteria was determined using an antibiotic protection-based survival assay. Data are means ± SD ($n = 3$), from one of three independent sets of experiments that yielded consistent results. Double asterisks indicate statistically significant differences ($P < 0.01$) between FimA-deficient or swap mutants compared with their corresponding wild-type strains. In (C), a single asterisk denotes a significant ($P < 0.05$) difference between the indicated wild-type groups.

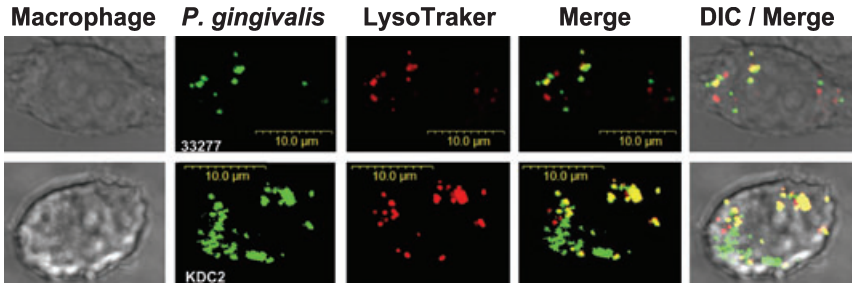


*Fig. 5*. Colocalization of *Porphyromonas gingivalis* strains 3227 and KDC2 with lysosomes. Primary mouse macrophages were exposed to fluorescein isothiocyanate-labeled *P. gingivalis* 33277 (Type I FimA strain) or its isogenic KDC2 mutant (Type I *fimA* substituted by Type II) at a multiplicity of infection of 10 : 1 for 30 min. After washing, incubation was allowed to proceed for an additional 90 min at 37°C. Subsequently, lysosomes were stained with LysoTraker Red for 15 min followed by cell fixation and imaging by confocal microscopy. Representative single optical sections are shown; green fluorescent bacteria colocalizing with red fluorescent lysosomes are manifested as yellow spots in merged images.

cytosis by macrophages ($P < 0.001$; Fig. 4A). Conversely, the reverse substitution (clone SM II → I) resulted in decreased association or uptake activities ($P < 0.001$; Fig. 4A). Therefore, all other factors being equal, Type II fimbriae endow *P. gingivalis* with increased adhesive/invasive activities, although, alternatively, Type I fimbriae may impede phagocytosis.

We then followed the intracellular fate of these *P. gingivalis* strains, to determine whether the type of fimbriae impacts on their survival capacity within macrophages. Viable CFU counts for the two FimA-deficient mutants were recovered at significantly lower levels than their corresponding parent strains at the 1.5-h time-point (Fig. 4B) and were hardly recoverable following overnight incubation for 15 h (Fig. 4C). There were no significant differences between 33277 and OMZ314 in terms of recovered CFU counts after 1.5-h incubation (Fig. 4B), although modest but statistically significant differences were noted at the 15-h time-point ($P < 0.05$; Fig. 4C).

Whether Type I fimbriae confer a survival advantage on *P. gingivalis* can be more validly determined by comparing the wild-type strain with its isogenic swap mutant. However, 33277 did not display increased intracellular survival compared with SM I → II (Fig. 4B,C). In fact, SM I → II CFU counts were about threefold higher than the counts corresponding to 33277 (Fig. 4B,C), which may reflect the higher uptake efficiency of SM I → II compared with 33277 (Fig. 4). Indeed, no significant differences in intracellular survival were noted between SM I → II and 33277 when the MOI values were adjusted to allow equilibration of the initial intracellular bacterial load (not shown). Moreover, although confocal microscopy confirmed the higher uptake efficiency of SM I → II (i.e. strain KDC2), it did not support possible enhanced survival of this strain (Fig. 5). Specifically, the proportions of 33277 and SM I → II bacteria trafficking to lysosomes (where they are presumably degraded) were comparable (Fig. 5). In support of this visual observation, when the confocal colocalization of LysoTraker-labeled lysosomes with FITC-labeled 33277 or SM I → II was quantified by IMAGEJ analysis, no significant differences were found (% lysosome colocalization of 33277 and SM I → II were 56.1 ± 11.3 and 64.7 ± 10.7, respectively, upon examining 15 macrophages from each group). Consequently, although the Type of FimA has a major impact on the internalization of *P. gingivalis* by macrophages, it does not significantly influence the subsequent intracellular fate of the pathogen.

## Discussion

It is becoming increasingly evident from this and other work that *P. gingivalis* represents a heterogeneous group of strains with distinct properties, in terms of proinflammatory potential, immune evasion capacity, cell invasive activity, and ability to cause disease (7, 20, 21, 30, 37). The genetic diversity of the *fimA* gene may, at least in part, mediate some of the virulence differences among distinct strains.

The findings that substitution of Type I FimA with Type II (clone SM I → II) results in enhanced *P. gingivalis* phagocytosis by macrophages, whereas the reverse substitution (clone SM II → I) decreases these activities, suggest that Type II fimbriae interact more efficiently with macrophage phagocytic receptors than Type I fimbriae. However, there were no substantial differences between the original strains (33277 and OMZ314) in terms of phagocytosis, despite expressing different fimbrial types. This may be related to the fact that OMZ314, but not 33277, is encapsulated (19). Indeed, the presence of a hydrophilic capsule is negatively correlated with phagocytosis, whereas surface hydrophobicity, which is strongly associated with the presence of fimbriae, promotes *P. gingivalis* interactions with phagocytes (32, 35). In the OMZ314 strain, therefore, the increased interactive potential of its Type II fimbriae with

phagocytes may be offset by the presence of its capsule, which is also a potential virulence factor. In this regard, although the potential contribution of the capsule of *P. gingivalis* to the induction of periodontal bone loss has not been addressed, it is known that this factor contributes to disseminating infections in the mouse subcutaneous abscess model (23, 32).

In assays of macrophage activation, the immunostimulatory potential of *P. gingivalis* 33277 was significantly lower than that of OMZ314, which is in agreement with the differential *in vivo* cytokine responses upon systemic *P. gingivalis* infection with these strains (21). The findings that both strains displayed altered proinflammatory activities upon mutual exchange of fimbrial type indicate that their fimbriae are largely responsible for their cell activation capacity. The fimbriae could influence these activities either indirectly, by facilitating *P. gingivalis*–macrophage interactions, or directly by activating fimbria-specific signaling. The latter possibility is supported by our findings that purified Type II fimbriae were more potent than Type I in cytokine induction, reflecting the activities of their respective strains. These differences in proinflammatory cytokine release were modest despite being statistically significant and reproducible. However, it is likely that initial small changes in cytokine production (upon initial encounter of the pathogen with phagocytes) are amplified *in vivo* through mobilization and activation of additional cells or cell types.

Activated macrophages may help control bacterial infection, but can also contribute to periodontal tissue destruction, depending on the quality, magnitude, and duration of activation (22). At least in certain *in vivo* models, induction of macrophage NO responses correlates with increased clearance of *P. gingivalis* (16, 17). Studies with additional pathogens also support the notion that suppressed activation of innate host responses leads to enhanced bacterial survival (27). It could be speculated, therefore, that reduced activation of the immune system by 33277 relative to OMZ314 [as seen here *in vitro* or *in vivo* in a previous report (30)] may allow 33277 to persist at higher levels than OMZ314 in the mouse host and eventually cause greater bone loss. Consistent with this notion, when the immunostimulatory potential of *P. gingivalis* OMZ314 was suppressed upon substitution of its Type II FimA with Type I (clone SM II → I), this resulted in enhanced induction of periodontal bone loss. At least in part, the decreased Type II FimA-associated virulence could be the result of reduced colonization by the OMZ314 strain, although Type II FimA is more adhesive than Type I FimA (1). It is conceivable, however, that possible reduced colonization by OMZ314 could be the result of an enhanced innate host response, as suggested above.

Despite their aggressive phenotype, which could provoke a clearance response in mice, Pg-II may have more opportunities to establish chronic infection in humans, perhaps through supportive interactions with other members of the subgingival biofilm that may be absent from the murine oral cavity. Indeed, according to epidemiological studies, Pg-II strains represent the most frequently isolated strains from periodontal lesions in diverse populations (2, 26), although more studies are warranted to fully validate the generality of these observations. Periodontitis can be modeled in certain animal species, although no model can faithfully reproduce all aspects of periodontal pathogenesis. However, each of the currently validated models can be very informative in terms of their capacity to test specific hypotheses, and different models can measure distinct aspects of bacterial virulence (10). For example, the subcutaneous abscess model can measure the potential of a periodontal pathogen for systemic dissemination, whereas the oral gavage model can assess the ability of a pathogen to persist in the oral cavity and cause bone loss (10).

This study has provided novel insights into the apparent heterogeneity in *P. gingivalis* virulence properties. Our findings in macrophages indicate that Type II fimbriae endow *P. gingivalis* with increased invasive and proinflammatory capacity compared with Type I fimbriae. However, there is an inverse relationship between proinflammatory activity and ability to induce periodontal bone loss. It is possible that different *P. gingivalis* strains may have developed distinct virulence strategies to cope with the mammalian host (12). Certain strains may be overtly aggressive, aiming to overwhelm host defense mechanisms, whereas other may have evolved immunosuppressive strategies to evade immune elimination. For example, strain 33277 uses its Type I fimbriae to suppress Toll-like receptor 2-dependent killing by instigating an inhibitory cross-talk with CXCR4 (17). More studies are warranted to further elucidate intricate virulence variations among distinct *P. gingivalis* fimbrial genotypes.

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