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Immunoglobulin G subclass antibody profiles in *Porphyromonas gingivalis*-associated aggressive and chronic periodontitis patients

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Background/aims: The immunoglobulin G (IgG) antibody response is considered to be protective and beneficial for the control of periodontal lesions. This study analysed IgG subclass antibody levels of *Porphyromonas gingivalis* in patients with both aggressive periodontitis (AgP) and chronic periodontitis (CP).

Methods: Subgingival plaque and peripheral blood samples were collected from patients with localized AgP ($n = 13$), generalized AgP ($n = 28$) and generalized CP ($n = 27$) and from 14 periodontally healthy controls. *P. gingivalis* was identified in subgingival pockets using a polymerase chain reaction. Simultaneously, serum IgG subclass antibody against *P. gingivalis* whole cells/*P. gingivalis* fimbriae were measured using enzyme-linked immunosorbent assay.

Results: *P. gingivalis* was frequently detected in periodontitis patients. Anti-*P. gingivalis* whole cell IgG1 was elevated in all *P. gingivalis*-positive patients in the three periodontitis groups. Although increased anti-*P. gingivalis* IgG1 was also observed in the bacterium-positive healthy controls, the level was lower than that found in the three periodontitis groups. Levels of IgG1, IgG2, IgG3 and IgG4 to *P. gingivalis* did not differ among bacterium-positive patients in the three periodontitis groups; a significant increase of IgG2 level was not observed in localized AgP. Anti-fimbriae IgG subclass levels of IgG1, IgG2 and IgG4 did not differ among bacterium-positive subjects in all groups, while the anti-fimbriae IgG3 level in generalized CP was significantly higher than that in localized and generalized AgP.

Conclusions: *P. gingivalis* infection elicited an IgG subclass antibody response in both periodontitis patients and healthy subjects, while higher anti-*P. gingivalis* IgG1 levels were found in the three periodontitis groups compared with the healthy control group.

Key words: *Porphyromonas gingivalis*; immunoglobulin G; periodontitis

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Periodontitis is an inflammatory disease leading to the destruction of periodontal tissues, which support the teeth. The interaction between plaque bacteria and the host immune system is responsible for most periodontal inflammation and different interactions present various patterns and severities of periodontal tissue destruction.

Several bacterial species have been implicated in the etiology of periodontitis (54). We previously reported that *Porphyromonas gingivalis*, which is one of the periodontopathic bacteria, was frequently detected in aggressive periodontitis (AgP) and chronic periodontitis (CP) patients in the Japanese population (47). While immunoglobulin G (IgG) is a major immunoglobulin of human serum, the IgG antibody response to *P. gingivalis* antigens has been considered beneficial for the control of *P. gingivalis*-mediated periodontitis (5, 12, 13, 24, 41). However, no clear correlation has been shown between IgG antibody levels and severity of disease (3). Some studies have reported increased levels of IgG antibody to *P. gingivalis* antigens in periodontitis patients (21, 25, 30, 34, 38, 40, 43, 51). However, others have suggested similar or lower levels of anti-*P. gingivalis* antibody in periodontitis patients compared to healthy controls (8, 9, 19). Few studies have simultaneously examined the presence of *P. gingivalis* and specific IgG titers in patients, which may result in inconsistent results.

The purpose of this study was to examine IgG antibody responses against *P. gingivalis* in AgP and CP patients in a Japanese population. The presence or absence of *P. gingivalis* and the serum levels of IgG subclass antibodies to antigens from *P. gingivalis* were examined.

Materials and methods

Subjects

Periodontitis patients attending the Tokyo Medical & Dental University hospital were selected for this study: 13 demonstrated localized AgP, 28 demonstrated generalized AgP and 27 demonstrated generalized CP. Fourteen periodontally healthy subjects served as controls. Periodontitis patients were diagnosed using previously detailed criteria (47), which were based on the American Academy of Periodontology (AAP) Workshop for Classification held in 1999 (10, 50). Teeth with attachment loss or a probing pocket depth >3 mm were not seen in healthy subjects. None of the subjects had received any periodontal treatment during the previous 6 months or antimicrobial medication during the last 3 months. Informed consent was obtained from each subject; all were in good general health and were non-smokers.

Bacterial detection

Subgingival plaque samples were collected from the deepest pockets in each quadrant with a sterile paper point. Sampling sites of healthy subjects were the mesiobuccal surface of teeth #3, #9, #19 and #25. The plaque suspension was washed as previously described (48) and used to detect *P. gingivalis* by polymerase chain reaction (PCR) based on the amplification of the signature sequence of bacterial 16S rRNA genes. The design of primers and the conditions used in PCR amplification and electrophoresis followed the procedures of Ashimoto et al. (2). Subjects were designated carriers of *P. gingivalis* if they had at least one *P. gingivalis*-positive sample.

Measurement of IgG subclass antibodies

Peripheral blood was collected from each subject. The serum was separated and stored at −20°C until measurement. IgG subclass antibody titers in sera to sonicated *P. gingivalis* ATCC 33277 whole cells or to the bacterial fimbriae were measured using enzyme-linked immunosorbent assay as previously described (33). Fimbriae as antigen were prepared by the method of Yoshimura et al. (53). The top two rows of each 96-well assay plate (Coaster, Cambridge, MA) were coated with 0.1 ml of serial two-fold dilution (10 μg/ml to 0.019 μg/ml) of the purified monoclonal antibody (human IgG1, IgG2, IgG3 or IgG4 kappa and lambda; Sigma, St Louis, MO), which were used as the standard. The remaining rows were coated with 0.1 ml of 10 μg/ml of each antigen in carbonate buffer, and incubated overnight at 4°C. The plates were washed three times with phosphate-buffered saline (PBS), and non-specific binding sites were blocked with 200 μl 1% bovine serum albumin. The plates were then washed three times with PBS. Serial dilutions of serum were prepared, 100 μl was added to antigen-coated wells in duplicate, and the plate was incubated for 2 h at 37°C. Following incubation, the wells were washed three times with PBS. The secondary antibody consisted of 100 μl of each concentration of monoclonal anti-human biotin conjugate for IgG1 (HP6069, Sigma), IgG2 (HP6002), IgG3 (HP6047) and IgG4 (HP6023). Plates were incubated for 2 h at 37°C, and then washed three times with PBS. Then, 100 μl of horseradish peroxidase-conjugated streptavidin (Genzyme TECHNE, Minneapolis, MN) at 1:400 dilution was added. After a 1-h incubation and washing, plates were developed with the tetramethylbenzidine liquid substrate system (Sigma). After 30 min, 100 μl of the stop solution (1 mol/l H_2SO_4 ; Sigma) was added to each well. Data were analysed by comparison with standard curves, and titers are expressed as μg immunoglobulin/ml.

Statistical analysis

Differences in IgG subclass antibody titers between bacterium-positive and bacterium-negative subjects were tested by Mann–Whitney *U*-test. The Kruskal–Wallis test with Bonferroni-type multiple comparisons was used to compare IgG subclass antibody titers among the four groups (three periodontitis and one healthy control). The test was two-tailed, and $P < 0.05$ was considered statistically significant.

Results

Detection frequencies of *P. gingivalis* were very high in the three periodontitis groups but low in the healthy controls. All localized AgP patients were found to be positive for *P. gingivalis* (Table 1).

Titers of IgG subclass antibodies to *P. gingivalis* whole cells are summarized in Table 2. In the generalized AgP, generalized CP and healthy control groups, mean levels of IgG subclass antibodies were higher in *P. gingivalis*-positive subjects than in the bacterium-negative subjects. In the three periodontitis groups, the predominant IgG subclass antibody to *P. gingivalis* was IgG1 in bacterium-positive patients. Increased anti-*P. gingivalis* IgG1 was also observed in *P. gingivalis*-positive healthy controls but at a level significantly lower than that in generalized AgP and generalized CP patients. IgG1 titer in *P. gingivalis*-positive localized AgP was also elevated, to the same level as in the generalized AgP and generalized CP groups, although there was no significant difference in IgG1 titers between the localized AgP group and the controls. There was no significant difference in levels of anti-*P. gingivalis* IgG2, IgG3 and IgG4 among the bacterium-positive subjects of all four groups.

IgG subclass antibody titers to *P. gingivalis* fimbriae, which is one of antigens in this bacterium, were also recorded (Table 3). Higher anti-fimbriae IgG1 titers were revealed in the bacterium-positive subjects of the generalized AgP and generalized CP groups when compared to those of the localized AgP and control groups, although there was no statistically significant difference. There was no significant difference in anti-fimbriae IgG2 and IgG4 levels among the bacterium-positive

Table 1. Microbiological data by PCR

	Aggressive periodontitis		Chronic periodontitis	
	Localized	Generalized	Generalized	Healthy
No. of subjects	13	28	27	14
Age (years; mean ± SD)	23.6 ± 5.3	28.0 ± 5.6	55.3 ± 6.5	25.6 ± 1.8
Detection rate, *P. gingivalis* (%)	100	78.6	88.9	28.6

Table 2. Serum IgG subclass titers against *P. gingivalis* whole cells

	P. gingivalis	Localized AgP	Generalized AgP	Generalized CP	Healthy
Number	(+)	13	22	24	4
	(−)	0	6	3	10
IgG1	(+)	147.7 ± 43.4	166.7 ± 71.1	155.0 ± 41.2	78.6 ± 7.8
	(−)	–	54.3 ± 12.4	135.4 ± 25.0	41.0 ± 8.0
IgG2	(+)	66.4 ± 16.8	62.6 ± 14.6	59.7 ± 14.6	82.4 ± 26.6
	(−)	–	32.4 ± 10.5	36.2 ± 19.3	53.7 ± 28.7
IgG3	(+)	8.0 ± 5.2	8.2 ± 5.7	8.2 ± 5.2	6.3 ± 2.4
	(−)	–	4.9 ± 4.8	6.6 ± 6.2	3.8 ± 3.1
IgG4	(+)	62.6 ± 35.6	64.8 ± 41.2	87.9 ± 50.0	41.7 ± 49.3
	(−)	–	7.0 ± 4.6	35.6 ± 60.6	4.9 ± 1.8

Mean ± SD (μg/ml).
*$P < 0.05$.

Table 3. Serum IgG subclass titers against *P. gingivalis* fimbriae

	P. gingivalis	Localized AgP	Generalized AgP	Generalized CP	Healthy
Number	(+)	13	22	24	4
	(−)	0	6	3	10
IgG1	(+)	11.0 ± 10.7	48.7 ± 82.3	44.6 ± 79.9	9.5 ± 5.0
	(−)	–	1.6 ± 2.2	18.3 ± 16.1	1.1 ± 2.0
IgG2	(+)	18.2 ± 22.4	20.4 ± 19.4	22.4 ± 16.0	10.8 ± 15.7
	(−)	–	15.2 ± 12.6	8.0 ± 6.0	6.4 ± 7.9
IgG3	(+)	4.3 ± 6.7	5.1 ± 6.9	17.9 ± 16.7	4.5 ± 1.5
	(−)	–	1.3 ± 1.2	9.8 ± 8.7	3.3 ± 2.2
IgG4	(+)	4.3 ± 4.3	6.5 ± 10.0	5.1 ± 7.9	12.9 ± 22.4
	(−)	–	1.8 ± 0.4	2.3 ± 0.4	0.8 ± 0.5

Mean ± SD (μg/ml).

subjects of all four groups. Anti-fimbriae IgG3 levels in bacterium-positive localized and generalized AgP patients were significantly lower than in the generalized CP patients. Furthermore, the anti-fimbriae IgG3 level in bacterium-positive control subjects was also lower than that in generalized CP patients, although the difference was not significant. The distribution of anti-fimbriae IgG subclass antibody was IgG1 > IgG2 > IgG3 ≈ IgG4 in *P. gingivalis*-positive generalized AgP and generalized CP patients, while the distribution was IgG2 > IgG1 > IgG3 ≈ IgG4 in localized AgP patients. In controls, anti-fimbriae IgG1 and IgG4 levels in *P. gingivalis*-positive subjects were higher than those in bacterium-negative subjects.

Discussion

Methods of bacterial sampling and identification are important for discriminating the immune responses against bacterial antigens in subjects with or without *P. gingivalis*. Collecting a large number of plaque samples from subjects is advantageous for identifying target bacteria accurately, although it is costly. So we collected subgingival plaque samples from the deepest pockets in each quadrant, as described in Savitt et al. (42), who found that sampling of the deepest sites would result in successful recovery of *P. gingivalis* in subjects who harbored this bacterium. In addition, we used PCR to reduce the risk of underestimation because this method has the best detection limit among the various bacterial detection methods (4). In this study there was a high prevalence of *P. gingivalis* in the Japanese population of localized and generalized AgP and generalized CP patients, and levels of anti-*P. gingivalis* whole cell IgG subclass antibodies were elevated by the bacterial infection. Anti-*P. gingivalis* IgG1 was predominantly elicited among the four IgG subclasses in the bacterium-positive patients, and increased anti-*P. gingivalis* IgG1 was also observed in *P. gingivalis*-positive healthy subjects. Predomination of anti-*P. gingivalis* IgG1 antibody was reported in periodontitis patients (25), though the presence or absence of the bacterium was not examined. IgG1 antibody is generally responsive to protein antigens (15), and it is possible that protein antigens of *P. gingivalis* mainly stimulate the immune response of IgG in subjects colonized with *P. gingivalis*.

As a result of the high titer of serum IgG2 to *Actinobacillus actinomycetemcomitans* in localized AgP but not in generalized AgP, some researchers have proposed that IgG2 antibody suppresses widespread periodontal lesions in localized AgP (27, 52). Many studies, mainly in North America and Europe, have reported *A. actinomycetemcomitans* to be the major periodontal pathogen of localized AgP (45); but a low detection frequency of *A. actinomycetemcomitans* in localized AgP has been shown in some clinical studies (16, 26, 32). In addition, we have reported a much lower prevalence of this bacterium than of *P. gingivalis* (20% vs. 90%) in localized AgP in Japanese patients (47). Thus, in the present study, we focused on IgG antibody responses to *P. gingivalis*. The anti-*P. gingivalis* IgG2 level was similar among the *P. gingivalis*-positive patients of the three periodontitis groups, and a specific increase in IgG2 titers in localized AgP patients was not observed. IgG2 production is induced predominantly in response to carbohydrate antigens (15), and higher production of lipopolysaccharide (LPS)-reactive IgG2 antibody has been observed in patients with *A. actinomycetemcomitans*-associated localized AgP (27, 52). While, Chen et al. (6) found that smaller amounts of serum IgG antibody reacted with *P. gingivalis* LPS in periodontitis patients, although a strong binding activity to *P. gingivalis* whole-cell protein was observed. The LPS of *P. gingivalis* may be a poor IgG2 inducer compared with the LPS of *A. actinomycetemcomitans*, and this could be the reason for the low IgG2 level in

patients with *P. gingivalis*-associated localized AgP. Interferon-γ is the key cytokine for IgG2 production (49), and the amount of interferon-γ produced is dependent on the amount of interleukin-12 production by monocytes/macrophages (22). Structures of *P. gingivalis* LPS and its lipid A are different from those of *Escherichia coli* or *A. actinomycetemcomitans* (23, 28, 35), and induction of interleukin-12 in macrophages stimulated with *P. gingivalis* LPS was markedly weak compared to that stimulated with *E. coli* LPS (17).

Fimbriae appearing on the bacterial cell surface contribute to bacterial adhesion to host tissue cells. Although the pathogenicity of *P. gingivalis* fimbriae has been reported (14, 18), there is limited information regarding the IgG response to this protein antigen in periodontitis patients (11, 36, 46). As mentioned earlier, protein antigens have been demonstrated to induce mainly IgG1 responses, and the anti-fimbriae IgG1 titer was indeed higher than any other IgG subclass antibody in the *P. gingivalis*-positive generalized AgP and generalized CP patients in this study. Furthermore, the increase in anti-fimbriae IgG1 titer was moderate, and IgG2 titer was the highest IgG subclass titer of *P. gingivalis*-positive localized AgP patients. The anti-fimbriae IgG2 level of *P. gingivalis*-positive localized AgP patients was similar to that of the generalized AgP and generalized CP patients. In the rat model, the IgG2b level was rapidly elevated compared to IgG1 against local gingival challenge with *P. gingivalis* (20). Anti-fimbriae IgG2 may be produced before IgG1, and consequently IgG1 production would be accelerated when the bacterial stimulus is not completely removed after IgG2 production.

Ogawa et al. (36) reported increased serum IgG3 to *P. gingivalis* fimbriae in CP patients. In the present study, significantly higher levels of anti-fimbriae IgG3 were observed in the *P. gingivalis*-positive generalized CP patients compared to the localized and generalized AgP patients. However, no difference in the levels of the four IgG subclasses to *P. gingivalis* whole cells was observed among the three periodontitis groups. In addition, a lack of antibodies reacting with fimbriae in patients has not been defined as increasing disease risk (39). Although the reason for low levels of anti-fimbriae IgG3 in localized and generalized AgP patients is at present unknown, it is of note that a poor IgG3 response to fimbriae was observed compared with CP patients.

An increase in anti-fimbriae IgG1 and IgG4 was observed in our *P. gingivalis*-positive healthy controls. Prolonged exposure of protein antigens reportedly induces an IgG4 response (1, 29, 44). Although controls did not present with clinical symptoms of periodontitis, some were chronically exposed to *P. gingivalis*, and immunoglobulin class switch of fimbria-specific B lymphocytes to IgG1/IgG4 occurred in the *P. gingivalis*-positive controls. However, we remain cautious about this finding because the number of *P. gingivalis*-positive controls was limited, and the standard deviation for anti-fimbriae IgG4 titer was high.

Host immune responses against periodontopathic bacteria seem to affect the destructive forms of periodontitis. However, the difference of three periodontitis forms could not be determined in terms of IgG subclass antibody levels to *P. gingivalis*. Thus the question arises as to what factor may strongly affect the disease forms of periodontitis. Some studies have suggested that the quality of antibody responses to bacteria would affect the periodontal status. Chen et al. (5) mentioned that the avidity of IgG antibody produced from many generalized AgP (formerly rapidly progressive periodontitis) patients was low, and that this was a disadvantage for the host response. In addition, Mooney et al. (31) reported the difference of antibody avidity to *P. gingivalis* in generalized AgP and CP patients. We evaluated only the IgG levels against *P. gingivalis* in the present study, whereas more than 500 species of bacteria can reside in an oral cavity (37), and any of these species may be pathogenic. We cannot neglect the contribution of other bacteria. As the complex interactions between subgingival bacterial flora and the host response have been suggested (7), further studies which evaluate both quality and quantity of antibodies to multiple bacterial species are required.

In summary, infection with *P. gingivalis* elicited an IgG subclass antibody response in both periodontitis patients and healthy subjects. Anti-*P. gingivalis* IgG1 was the most strongly induced in IgG subclass antibodies of our Japanese AgP and CP patients, and the level was higher than that in healthy controls. Increased IgG2 titers to *P. gingivalis* were not observed in localized AgP patients. Different patterns of the four IgG subclass antibodies to *P. gingivalis* were not observed among the localized AgP, generalized AgP and generalized CP groups.

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