A Novel Approach for Detecting an Immunodominant Antigen of *Porphyromonas gingivalis* in Diagnosis of Adult Periodontitis

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In the course of long-term infection with *Porphyromonas gingivalis* in adult periodontitis, a specific antibody response to this organism is generated. We describe a potential novel approach for identifying an immunodominant antigen in human periodontitis patients. First, various monoclonal antibodies (MAbs) were established from mice immunized with crude antigen preparations of *P. gingivalis* FDC 381. The antigen specificities of these MAbs were compared with those of serum antibodies of 10 periodontitis patients in a competitive enzyme-linked immunosorbent assay. The binding of one MAb (termed PF18) was readily inhibited by sera from all patients but not by sera from healthy volunteers. The antigen recognized by PF18 existed on the cell surface, presumably in the capsule layer, shown by immunoelectron microscopic analysis. Purification of the antigenic substance, termed PF18-Ag, was performed by immunoaffinity chromatography with the MAb. Characterization of PF18-Ag suggested that the epitope was composed of carbohydrates but not peptides and that the substance was different from lipopolysaccharide. Measurement of levels of serum antibody to PF18-Ag better discriminated periodontitis patients from healthy individuals than measurement of antibodies to crude antigen preparations of *P. gingivalis*. Immunoglobulin G2 was the predominant isotype among the antibodies to PF18-Ag in the patients’ sera. These results suggest that PF18-Ag, which is possibly a novel substance, is an important antigenic substance and is potentially useful for the clinical diagnosis of adult periodontitis. The approach that was used would also be relevant to detecting immunodominant antigens of other infectious microorganisms.

In the late stage of immune response, maturation of the antibody response is led in an antigen-driven manner and includes isotype switching and somatic mutation (10, 26, 30). In most cases of infectious diseases, specific antibodies are generated against immunologically dominant antigens of the pathogenic organisms. Detection of a specific antibody response in patients but not in healthy individuals is helpful for an efficient diagnosis of those diseases. Therefore, searching for an antigen that induces a specific serological reaction only in patients is of particular interest.

More than 400 different species of microorganisms grow in the oral cavity of every adult (33). Among those resident bacteria, *Porphyromonas gingivalis* has been implicated as an important etiologic agent in periodontal diseases, particularly adult periodontitis and rapidly progressive periodontitis (5, 24). A number of investigators have found elevated levels of immunoglobulin G (IgG) antibody to this organism in patients’ sera and suggested the feasibility of measuring antibody titers as a laboratory test that could delineate the states of periodontitis (6, 32). However, examination of the antibody response pattern has, so far, not been very useful for the categorization of individuals into clinical classifications. Some healthy individuals possess levels of anti-*P. gingivalis* antibody titers comparable to those in patients, while the levels in some patients stay within the range of those in healthy subjects (25). Presumably, cross-reactive antigens conserved over species interfere with the detection of a specific antibody response.

Measurement of levels of antibody to some purified antigens rather than to crude, complex preparations is expected to serve as a better means of determining the clinical states of the patients. In this regard, many putative pathogenic substances, such as lipopolysaccharide (LPS) (28), fimbriae (23, 39), trypsin-like protease (12), and hemagglutinin (22), were isolated and tested as antigens for the measurement of antibody levels in serum. However, the overall results were not particularly better than those obtained when the levels of antibody to the crude antigens were measured.

To identify a useful immunodominant substance, some investigators have paid greater attention to the host reaction than to the biological properties of microbial substances (16, 17, 36, 39). They have used immunoblot analyses to search for antigenic substances for clinical diagnosis. Several proteins were successfully purified and characterized, but the results obtained by this method are qualitative rather than quantitative in evaluations of the host response.

In the present study, we tested a novel approach to the search for a specific antigen to which only patients’ sera react, and in this report we discuss the potential of the newly identified antigen of *P. gingivalis* for the clinical diagnosis of human adult periodontitis.

MATERIALS AND METHODS

**Bacterial strains.** *P. gingivalis* FDC 381 (supplied by S. S. Socransky) was grown in Todd-Hewitt broth containing hemin (5 mg/ml) and menadione (0.5 mg/ml) at 37°C for 48 h in an anaerobic atmosphere. The cells were then harvested by centrifugation (7,000 × *g*, 20 min) and washed three times with phosphate-buffered saline (PBS; pH 7.4) and twice with distilled water. Finally, the cells were lyophilized and stored. *Eikenella corrordens* FDC 1073, *Actinomyces*
viscous ATCC 29246, Actinomyces naeslundii ATCC 12104, Fusobacterium nucleatum ATCC 14364, and Actinobacillus actinomycetemcomitans FDC Y4 were previously grown in our laboratory and were stored in a lyophilized form (15). *P. gingivalis* ATCC 33277, W83, and TDC 16-1, *Porphyromonas endodontalis* ATCC 33540, *Bacteroides gracilis* ATCC 25260, *Prevotella intermedia* strain 25611, *Prevotella denticola* ATCC 33185, and *Bacteroides nodaceae* ATCC 3314 are all kind gifts from K. Okuda (Tokyo Dental College).

**Human subjects.** After informed consent was obtained, sera were obtained from 10 patients (mean age, 31 years; age range, 23 to 43 years) with advanced stages of periodontitis at Osaka University Dental Hospital and from 10 volunteers (mean age, 31 years; age range, 27 to 39 years) who were systemically and periodontally healthy and who had no history of periodontitis. All patients completed initial medical and dental histories, had thorough clinical and radiographic dental examinations, and were consequently diagnosed with adult periodontitis according to previously published criteria (27).

**Generation of MAbs.** BALB/c mice (female, 8 weeks; Japan SLC, Shizuoka, Japan) were immunized with either sonicated extracts, autokaryosolized extracts, or formalinized cells, which were prepared from lyophilized *P. gingivalis* FDC 381 cells suspended in 0.15 M NaCl at 2 mg (dry weight/ml). Each preparation was emulsified with an equal volume of complete Freund’s adjuvant (Difco, Detroit, Mich.), and 0.3 ml of each emulsion was subcutaneously injected into three mice. Two weeks later, the mice were immunized with the same preparations initially injected, but the preparation was emulsified in incomplete Freund’s adjuvant. Two weeks after the second injection, 0.1 ml of each preparation was injected intraperitoneally, twice a week, as a booster, for 6 days after the booster. Reaction, spleen cells were prepared and were fused with myeloma cell line NS/0 (9) by the method described by Köhler and Milstein (14). Hybridomas were screened for their levels of production of antibody to a sonicated suspension of *P. gingivalis* FDC 381 cells which was assumed to contain all kinds of native antigens by the enzyme-linked immunosorbent assay (ELISA) described below. Antibody-producing hybridomas were cloned more than twice by repeated limited dilution. Purification of monoclonal antibodies (MAbs) was accomplished by protein A affinity chromatography (Ampure PA kit; Amersham-Japan, Tokyo, Japan) from ascites. Fab fragments of MAbs were prepared and purified by using an Immunopure Fab purification kit (Pierce, Rockford, Ill.).

**Immunofluorescence analysis (IFA).** Cells of various bacterial strains were washed three times with PBS. Smeared cells were prepared and heat fixed. The cells were then incubated with purified MAb (5 μg/ml) for 1 h at 37°C in a humidified atmosphere. After three washes with PBS, the cells were incubated with fluorescein isothiocyanate (FITC)-conjugated goat anti-mouse IgG-Fc (used at a 1/100 dilution in PBS; Zymed, South San Francisco, Calif.) for 1 h at 37°C. After four washes with PBS, the preparations were mounted by using glycerol, and the staining profiles were observed under a fluorescence microscope. It was also confirmed that the FITC-conjugated antibody alone or in combination with a control MAb did not stain any strains of bacteria examined.

**ELISA.** Production of MAbs from hybridomas was determined by ELISA. Lyophilized *P. gingivalis* FDC 381 cells were suspended in 0.05 M carbonate buffer (pH 9.6) at 0.1 mg/ml and were subjected to sonication. The sonicated materials were transferred to nitrocellulose membranes following SDS-polyacrylamide gel electrophoresis (PAGE), and the membranes were then probed with the antibody to the antigens on the membranes. The treated membranes were submitted to immunological staining as described above. Electron microscopy. *P. gingivalis* FDC 381 cells were suspended in PBS, MAb PF18 was added to the suspension at 1 μg/ml, and the mixture was incubated at 37°C for 1 h. After washing with PBS, the cells were incubated with gold-labeled protein A (7 nm; E-Y Laboratories, San Mateo, Calif.) at 37°C for 1 h. The cells were washed with PBS, fixed in 1% glutaraldehyde in PBS for 1 h, and further fixed with 1% osmium tetroxide (Wako) overnight. They were dehydrated in ethanol and embedded in Epon (Poly/Bed 812; Polysciences, Warrington, Pa.). Ultrathin sections (80 nm) were stained with uranyl acetate and lead citrate and were observed with a transmission electron microscope.

**RESULTS**

**Establishment of MAbs to *P. gingivalis* and their antigen specificities.** Hybridomas were screened by ELISA for the production of antibody reactive to a crude sonicate of *P. gingivalis* FDC 381, and 19 clones were established. These MAbs were subjected to immunoblots to determine their antigen specificities. The majority of the MAbs produced complex profiles of multiple bands and/or smear patterns, although their mononodality was repeatedly confirmed by the limiting dilution method. Only four clones (clones 3, 4, 5, and 14) developed a predominant single band on the immunoblots. Eleven of the 19 MAbs could be categorized into 4 groups according to the similarities in their immunoblotting profiles, and the other 8 MAbs were not grouped because their staining patterns were unique or too weak in this assay. One representative clone was selected from each of the four groups according to its higher antibody productivity compared with those for the remainder of the clones in each group. These clones were PF18, PS2, PA20, and PF24 from groups A, B, C, and D, respectively. The results of immunofluorescence staining of the four MAbs against various bacteria (Table 1) suggested that PF18, PS2, and PA20 recognized an antigen of *P. gingivalis* common to the species, and PF24 recognized an antigen specific to strain FDC 381. PF18 was weakly reactive with *P. endodontalis*, an ascarachyonic oral species which shares several antigens with *P. gingivalis*.

**Competition between MAbs and serum antibodies from periodontitis patients.** We next examined by competitive inhibition ELISA whether the antigens recognized by the four MAbs were involved in the immune responses in human periodontitis (Fig. 2). The binding of MAb PF18 to the crude *P. gingivalis*...
extracts was remarkably inhibited in a dose-response manner by the sera from all patients tested. The binding of MAb PS2 was inhibited by the sera from half of the patients, while MAbs PA20 and PF24 were little inhibited by any of these sera. These findings imply that a humoral immune response to the epitope recognized by PF18 is commonly raised in periodontitis patients. On the other hand, no inhibition of PF18 binding was recorded for 10 serum samples obtained from healthy volunteers (Fig. 3). This critical difference suggests the possibility that the antibody to the epitope for PF18 was induced in periodontitis patients but was absent from healthy individuals.

Purification of PF18-Ag. The antigenic substance recognized by MAb PF18 was separated from sonic supernatants of P. gingivalis FDC 381 by affinity chromatography (Fig. 4A). The affinity-purified substance, termed PF18-Ag, was analyzed by SDS-PAGE under a reduced condition, and the profile was

<table>
<thead>
<tr>
<th>TABLE 1. Specificities of anti-P. gingivalis MAbs</th>
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<tbody>
<tr>
<td>Bacterial strain</td>
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<tr>
<td>------------------------</td>
</tr>
<tr>
<td>Porphyromonas gingivalis ATCC 33277</td>
</tr>
<tr>
<td>W83</td>
</tr>
<tr>
<td>FDC 381</td>
</tr>
<tr>
<td>TDC 16-1</td>
</tr>
<tr>
<td>Prevotella intermedia ATCC 25611</td>
</tr>
<tr>
<td>Prevotella loescheii ATCC 15920</td>
</tr>
<tr>
<td>Prevotella melaninogenica ATCC 25845</td>
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<tr>
<td>Prevotella denticola ATCC 33185</td>
</tr>
<tr>
<td>Bacteroides melaninogenicus ATCC 3314</td>
</tr>
<tr>
<td>Porphyromonas endodontalis ATCC 35406</td>
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<tr>
<td>Porphyromonas asaccharolytica ATCC 25260</td>
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<td>Eikenella corrodens FDC 1073</td>
</tr>
<tr>
<td>Actinomyces viscosus ATCC 19246</td>
</tr>
<tr>
<td>Actinomyces naeslundii ATCC 12104</td>
</tr>
<tr>
<td>Fusobacterium nucleatum FDC 1436</td>
</tr>
<tr>
<td>Actinobacillus actinomycetemcomitans FDC Y4</td>
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</table>

* Reactivity was microscopically determined by an indirect fluorescence immunosassay. Smears of the bacteria were incubated with purified MAb, followed by FITC-conjugated goat anti-mouse IgG. Staining profiles are expressed as follows: +, outline of the bacterial cells was clearly stained; ±, weak fluorescence was observed, but the outline of the cells was vague; −, no fluorescence was observed.

FIG. 2. Inhibition of MAb reaction by antibodies from sera from patients with periodontitis. Sera from 10 patients diluted 1/10, 1/100 and 1/1,000 in PBS-T were added to microtiter plates which were precoated with sonicated P. gingivalis, and the plates were incubated for 30 min at 25°C. After removal of unbound antibodies, the plates were incubated with four different MAbs: PF18 (A), PS2 (B), PA20 (C), and PF24 (D). The MAbs were diluted to concentrations that gave half-maximum binding without inhibitors, which were determined in preliminary experiments. The binding of MAbs was determined by using a mouse IgG-Fc-specific antibody conjugated with AP (no cross-reactivity to human immunoglobulins). Binding of the MAbs was expressed as follows: (mean A₄₀₅ for triplicate test wells incubated with human serum prior to the incubation with MAbs/mean A₄₀₅ for triplicate control wells without human sera) × 100 (to give a percent).

FIG. 3. Marginal inhibition of MAb PF18 reaction by antibodies from sera from healthy individuals. A competitive inhibition assay (ELISA) was performed for MAb PF18 and sera from 10 healthy individuals as described in the legend to Fig. 2.
compared with those of fimbriae and LPS, which are well-characterized antigens of *P. gingivalis* (Fig. 4B). The SDS-PAGE profile of PF18-Ag had some similarity to that of fimbriae; the major band of PF18-Ag showed a molecular mass close to that of fimbrillin. Therefore, the antigenicities of the two preparations were compared. In the ELISA, PF18 did not bind to fimbriae coating the plates and polyclonal antifimbrial antibodies showed no reaction to PF18-Ag (data not shown).

**Chemical properties of the epitope for PF18.** PF18-Ag was treated with periodate or trypsin to characterize the chemical properties of the antigenic determinant for PF18 (Fig. 5A). MAb PF18 did not react to the periodate-treated PF18-Ag, but it was able to react to the antigen treated with trypsin. Purified fimbriae and a polyclonal rabbit antibody raised against its monomeric subunit (40) were used as controls for this experiment. Fimbriae contain no carbohydrates, so the epitope for the antifimbrillin antibody is considered to be proteinaceous (41). As was anticipated, the epitope for the rabbit antibody was not affected by periodate treatment but was destroyed by proteolytic trypsin treatment (Fig. 5B). The antigenicity of PF18-Ag was heat stable, even after PF18-Ag was autoclaved at 120°C for 20 min (data not shown). Collectively, the epitope recognized by PF18 is likely to be composed of carbohydrates.

**Difference between PF18-Ag and LPS.** Since PF18-Ag was suggested to be composed of carbohydrates, we reexamined the reactivities of all 19 anti-*P. gingivalis* MAbs to the LPS of *P. gingivalis*, the best-characterized carbohydrate substance of the organism. MAbs exhibiting an A450 more than twice the standard deviation over the mean for negative control wells incubated with 5 μg of OX35, a mouse MAb specific to rat CD4 (IgG2a, kappa), per ml were judged to be positive. The MAb numbers and the group names correspond to those in the lanes of Fig. 1 (e.g., 1 = PF18).

**FIG. 4.** Immunoaffinity purification of PF18-Ag and its profile by SDS-PAGE. (A) Sonic supernatants of *P. gingivalis* FDC 381 were subjected to a column of PF18-coupled Sepharose. After extensive washing with PBS and PBS containing 0.5 M NaCl, the substance that specifically bound to the MAb was obtained by acid elution. The concentration of protein in each fraction was monitored by measuring the A280 (dotted line), and the activity as the ligand for MAb PF18 was determined by a sandwich ELISA as described in Materials and Methods (solid line). (B) SDS-PAGE profiles of various preparations of *P. gingivalis* FDC 381. Sonic extract (lane 1), affinity-purified PF18-Ag (lane 2), fimbriae (lane 3), and LPS (lane 4) were subjected to SDS-PAGE under reducing conditions, and the gel was developed by silver staining. Relative molecular masses obtained by using a molecular mass marker (Pharmacia) are indicated on the left.

**FIG. 5.** Effects of treatment with periodate or trypsin on antigenicities of PF18-Ag and fimbrillin. Reaction of MAb PF18 to modified PF18-Ag (A), reaction of a rabbit antifimbrillin antibody to modified fimbriae (B), and reaction of PF18 to unmodified fimbriae (C) were examined in immunoblot analyses. Lane 1, unmodified antigens; lane 2, antigens treated with periodate; lane 3, antigens treated with trypsin. The strips of the membrane were incubated with PF18 (5 μg/ml) followed by an incubation with AP-conjugated anti-mouse IgG-Fc (1/2,000; A and C) or with the rabbit antibody (diluted 1/500), followed by incubation with AP-conjugated anti-rabbit IgG (1/3,000; B).

**TABLE 2.** Reaction of MAbs against *P. gingivalis* PF18-Ag and LPS<sup>a</sup>

<table>
<thead>
<tr>
<th>Antigen</th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>D</th>
<th>Miscellaneous</th>
<th>Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>PF18-Ag</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>LPS</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Crude <em>P. gingivalis</em></td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

<sup>a</sup> Triplicate ascites of 20 MAbs (1/100 dilution) were tested for their binding to purified PF18-Ag, LPS, and a sonicated cell suspension in an ELISA. MAbs exhibiting an A450 more than twice the standard deviation over the mean for negative control wells incubated with 5 μg of OX35, a mouse MAb specific to rat CD4 (IgG2a, kappa), per ml were judged to be positive. The MAb numbers and the group names correspond to those in the lanes of Fig. 1 (e.g., 1 = PF18).
this bacterium, in order to clarify any possible relationship between the two independent preparations (Table 2). The MAbs categorized into group A, including PF18, definitely reacted to PF18-Ag but showed no reaction to LPS. On the other hand, the MAbs in group C definitely reacted to LPS but not to PF18-Ag. These results support the conclusion that PF18-Ag is a substance different from LPS. In addition, no similarity in their silver staining profiles was noted by SDS-PAGE analysis (Fig. 4).

Localization of PF18-Ag. Localization of PF18-Ag was determined by transmission electron microscopic analysis (Fig. 6). Colloidal gold deposits were observed at the outermost layer of the cell membrane; the antigen therefore existed in the surface layer of P. gingivalis.

Antibodies in human sera reactive to PF18-Ag. Twenty human serum samples, 10 from patients with advanced periodontitis (P) and 10 healthy volunteers (H) were assessed by ELISA for their levels of antibody to PF18-Ag (Fig. 7). Simultaneously, levels of antibody to whole P. gingivalis cells in these serum samples were determined. Mann-Whitney U-test analysis suggested that the levels of anti-PF18-Ag and anti-whole P. gingivalis cells were both significantly elevated in the patients in comparison with those in the healthy subjects (P < 0.01). However, the antibody response to PF18-Ag showed about a 1,000-fold difference between patients and healthy subjects, with the exception that one patient had a marginal level of anti-PF18-Ag. In contrast, only about a 10-fold difference was observed between patients and healthy subjects when antibody levels were measured by using the whole-cell preparation as an antigen in the ELISA; the small difference was essentially due to the relatively strong reactions to the crude antigen recorded for sera from healthy volunteers.

The IgG subclass distribution of anti-PF18-Ag antibodies was investigated for individual patients. The distributions varied highly among the patients, and 8 of the 10 patients lacked at least one IgG subclass antibody to PF18-Ag and for the crude antigen preparation, whose antibody titers for the two antigens were regarded as 100 ELISA units (EU). Each circle represents a result for one subject. Background values (broken horizontal lines), median values (thick horizontal lines), 25 and 75% fractiles (solid vertical lines with thin horizontal lines), and 10 and 90% fractiles (broken vertical lines with thin horizontal lines) are indicated.
DISCUSSION

An immunodominant saccharide cell surface antigen of P. gingivalis that predominantly induces an IgG2 antibody response in periodontitis patients was identified by a new MAb-affinity chromatography approach. It was surprising that, at first, the majority of the MAbs which were generated against crude antigen preparations of P. gingivalis displayed staining profiles of multiple bands and/or smear patterns on Western blots, although their monoclonality was repeatedly confirmed. To rule out the possibility of fragmentation of proteins by intrinsic protease, a cocktail of protease inhibitors was added to the preparation throughout the antigen extraction. Several other inhibitors were also added, but the results were the same (data not shown). Afterward, evidence that the epitope for MAb PF18 was composed of carbohydrates was obtained; hence, a plausible explanation for the complex immunoblot profiles was that other MAbs may also react with carbohydrate epitopes that may be found on multiple molecular isoforms on the cell surface.

Four MAbs were selected from among the 19 MAbs. We made an assumption that substances recognized by more than one MAb were more likely to be immunodominant over other substances that responded to a single MAb. One hybridoma was chosen from each of the four groups (Fig. 1A to D). All four MAbs were specific to P. gingivalis, as far as we tested, using a battery of strains and species of oral bacteria (Table 1). The positive results by IFA suggested that antigens to which these MAbs reacted were on the cell surface. The localization of PF18-Ag was further confirmed by immunoelectron microscopic analysis. The outermost layer probed by the MAb seemed to be the capsule, as described by Cutler et al. (5). We finally selected PF18 for further investigation, because its reaction was readily inhabitable in competition with sera from periodontitis patients; the result implied that an antibody response to the antigen recognized by this MAb was raised in the patients.

The substance to which MAb PF18 reacts was affinity purified by using the MAb, and its immunochemical characteristics were examined. The periodate-sensitive nature of the epitope suggests involvement of a sugar moiety in PF18-Ag but no relationship to fimbriae, in spite of some similarity in their SDS-PAGE profiles. PF18 was strongly reactive to an autoclaved extract of P. gingivalis in an ELISA (data not shown). This is consistent with the notion that PF18-Ag consists of carbohydrates, since carbohydrate antigens are generally heat resistant. Among several monosaccharides tested, N-acetylgalcosamine partially inhibited the binding of PF18 (data not shown), suggesting that the monosaccharide, which is known to construct the core region of LPS or that of cell wall peptidoglycan, is also involved in the epitope for PF18. Carbohydrate antigens are known to preferentially induce IgG2 isotype antibody in adults. In our experiment, the dominant IgG subclass among patients’ antibodies reactive to PF18-Ag was IgG2. This also supports the notion that PF18-Ag is a carbohydrate antigen.

We proved that MAb PF18 did not react to a conventional LPS preparation in an ELISA (Table 2), while three MAbs in group C and MAb PS7 clearly reacted to LPS. Although some similarity between PF18 and anti-LPS antibodies was shown in the immunoblot profiles (Fig. 1, group C and PS7 [no. 12] in this study; anti-LPS antibodies reported by Naito et al. [21] also showed a similar pattern), the SDS-PAGE profiles of purified PF18-Ag and LPS were completely different. In addition, PF18-Ag was soluble in chloroform-methanol (1:2) solution, but LPS was not (data not shown). Consequently, we conclude that PF18-Ag is not the major component of the conventional LPS preparation. The localization of PF18-Ag on the cell surface suggests that it is not from the peptidoglycan layer of the gram-negative bacterium. Furthermore, PF18-Ag is unlikely to be the polysaccharide antigens reported by Schifferle et al. (29) because they were readily detected in the conventional phenol-water-extracted LPS preparation, nor was it the K antigen reported by van Winkelhoff et al. (35), since those investigators could not find the substance in strain FDC 381, which we used in this study. Thus, PF18-Ag is presumably a novel substance, although further characterization is needed.

The average titer of IgG to PF18-Ag in serum was about 1,000-fold higher in patients than in healthy subjects (Fig. 7). On the other hand, the average antibody titer measured for total P. gingivalis antigens indicated about a 10-fold difference between the two groups, and some overlap was observed. All the sera from healthy subjects showed a substantial reaction to the crude antigen preparation. A high background is commonly observed in healthy subjects and becomes a reason for not classifying the disease on the basis of antibody titers (16). This high background presumably reflects cross-reactive responses to common antigens between P. gingivalis and other resident bacteria in the oral cavity or other mucosal origins. The greater specificity observed in the serologic assay with purified PF18-Ag suggests the potential of this substance in the development of more useful diagnostic methods. However, five healthy subjects showed low but significantly positive reactions to PF18-Ag. It could be speculated that these healthy people might have already been colonized with the organism at low levels but had no clinical signs of the disease. They might have differential susceptibilities to periodontal disease compared with those of other subjects who showed negative responses. Further study is required to elucidate a possible relationship between titers of antibody to PF18-Ag in serum and severity of disease.

It has been reported previously (16) that some patients lack an antibody response to a purified substance of P. gingivalis, while they exhibit substantial reactions to a sonic extract of the antigen. Similarly, in our present study, one patient had an undetectable level of antibody to PF18-Ag but had a significant reaction to the crude P. gingivalis preparation (Fig. 7). This is not very surprising, because the directions of immune responses are genetically controlled by major histocompatibility complex restriction; thus, the capacity to produce antibodies to a certain molecule varies among individual patients. However, it is controversial why the serum from that patient inhibited the reaction of MAb PF18 but showed no reaction to PF18-Ag. A possible explanation is that an epitope in a molecule different from PF18-Ag might be located close to PF18-Ag on the cell surface and serum antibodies to the irrelevant epitope might block the reaction of the MAb by steric hindrance.

Since Mouton et al. (20) reported that the level of serum IgG reactivity against P. gingivalis was elevated in patients with adult periodontitis or generalized juvenile periodontitis, a number of reports have supported this observation. The paradox is why the humoral response against P. gingivalis is ineffective in halting periodontal disease. The predominance of the IgG2 isotype against P. gingivalis in patients’ sera (5, 25, 37) appears to suggest some clues to the answer to this question. IgG2 has a low affinity for Fc receptors on polymorphonuclear leukocytes and poorly fixes complement (34). The functions of polymorphonuclear leukocytes are essential for maintaining periodontal health (38). P. gingivalis appears to resist phagocytosis by these cells by constructing a unique polysaccharide capsule and secreting proteases which can destroy IgG and C3 (4). The characteristics of the polysaccharide capsule of P. gingivalis are known to be a major component of the conventional LPS preparation. The localization of PF18-Ag on the cell surface suggests that it is not from the peptidoglycan layer of the gram-negative bacterium. Furthermore, PF18-Ag is unlikely to be the polysaccharide antigens reported by Schifferle et al. (29) because they were readily detected in the conventional phenol-water-extracted LPS preparation, nor was it the K antigen reported by van Winkelhoff et al. (35), since those investigators could not find the substance in strain FDC 381, which we used in this study. Thus, PF18-Ag is presumably a novel substance, although further characterization is needed.

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Since Mouton et al. (20) reported that the level of serum IgG reactivity against P. gingivalis was elevated in patients with adult periodontitis or generalized juvenile periodontitis, a number of reports have supported this observation. The paradox is why the humoral response against P. gingivalis is ineffective in halting periodontal disease. The predominance of the IgG2 isotype against P. gingivalis in patients’ sera (5, 25, 37) appears to suggest some clues to the answer to this question. IgG2 has a low affinity for Fc receptors on polymorphonuclear leukocytes and poorly fixes complement (34). The functions of polymorphonuclear leukocytes are essential for maintaining periodontal health (38). P. gingivalis appears to resist phagocytosis by these cells by constructing a unique polysaccharide capsule and secreting proteases which can destroy IgG and C3 (4). The characteristics of the polysaccharide capsule of P.
gengivalis are largely unknown at present. PF18-Ag seemed to be one of the capsule antigens; therefore, it may also be a useful tool for studying the unique capsule of P. gengivalis.

Progress in molecular biological techniques has resulted in our increased interest in proteins which are directly encoded by DNA. Recently, a number of novel proteins specific to P. gengivalis have been identified and thoroughly investigated, i.e., proteases (2, 7, 11, 31), a fibroblast-activating factor (19), heat shock protein (18), etc. However, the roles of these proteins in vivo remain speculative, and their usefulness as diagnostic tools has not been established. Purification and characterization of unknown carbohydrate substances are usually more difficult than purification and characterization of proteins. Our present approach has the potential to allow for a search for immunologically dominant antigens, regardless of their chemical properties. The only requirement is that they be immunogenic. The MABs generated by the protocol will facilitate the isolation of substances of interest by immunoaffinity purification. This approach, based on the immunological specificity of host responses, may be a relevant and useful method for identifying clinically important microbial antigens in some other infectious diseases.

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