

Serum Immunoglobulin G Antibody to *Porphyromonas gingivalis* in Rapidly Progressive Periodontitis: Titer, Avidity, and Subclass Distribution

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Porphyromonas gingivalis is a suspected pathogen in rapidly progressive periodontitis (RPP). We have determined the anti-*P. gingivalis* serum immunoglobulin G (IgG) isotype response and avidity and the subclass titer distributions for 30 RPP patients and 30 age-, sex-, and race-matched healthy subjects by using enzyme-linked immunosorbent assay technology. Patients and control subjects were classified as seropositive if their total IgG response to *P. gingivalis* was twofold or more than the median response in healthy subjects. The predominant antibody responses for both patients and healthy subjects were IgG2 and IgG3, with a subclass order of IgG2 > IgG3 > IgG1 > IgG4. The avidity of the IgG response was highest for the seropositive healthy subjects and was no different between seronegative and seropositive RPP patients. The subclass antibody responses did not depend on gender, and there were no correlations between titer, avidity, or subclass with disease severity in the RPP patients as measured by pocket depth or bone loss on dental X rays. The seronegative RPP patients exhibited antibody responses that were greater than the responses of seronegative healthy subjects for all four subclasses, while the seropositive RPP patients had higher IgG1 and IgG4 levels than seropositive healthy subjects. These findings are consistent with the hypothesis that both carbohydrate and protein antigens are important in the IgG response to *P. gingivalis*. The relative predominance of IgG2, a subclass which lacks strong complement fixation and opsonic properties, and the low avidity of patient anti-*P. gingivalis* IgG antibodies suggest that humoral responsiveness to infection with *P. gingivalis* may be ineffective in clearing this organism.

Periodontitis is a relatively common infectious disease leading to tooth loss in adults worldwide. An unusual form characterized by an early age of onset, involvement of most if not all of the teeth, and a rapid rate of tissue destruction is observed in young adults. This form has been designated rapidly progressive periodontitis (RPP) (4, 32). *Porphyromonas gingivalis* is considered important in the etiology of RPP (21, 39).

RPP patients and some periodontally healthy subjects manifest serum immunoglobulin G (IgG) antibodies to antigens of *P. gingivalis*. While some investigators have observed elevated antibody levels in almost all patients (42), others have reported a great diversity of responses (13, 17, 24), with many patients manifesting titers below mean values observed in periodontally healthy subjects (5, 40). In most of the studies reported so far, there has been no association between the presence and titer of anti-*P. gingivalis* antibodies and the arrest or prevention of disease progression, although one research group has claimed an inverse relationship between anti-*P. gingivalis* titer and disease severity in RPP patients (11).

Existing data do not resolve several critical issues, including why some patients mount a humoral immune response during the course of their infection while others do not and why the antibodies are not clearly protective in those patients with an elevated IgG anti-*P. gingivalis* antibody titer. Our study bears on both issues but focuses on the second.

IgG antibodies exist in serum as subclasses 1 to 4. These

differ greatly with regard to the chemical natures and biologic roles of the inducing antigens. We report here determinations of the total anti-*P. gingivalis* IgG antibody titers and avidities in samples of sera from 30 untreated RPP patients and age-, sex-, and race-matched control subjects and the IgG subclass distribution of these anti-*P. gingivalis* antibodies.

MATERIALS AND METHODS

Human subjects. Sera were obtained from 30 University of Washington patients with RPP according to general diagnostic criteria described previously (4, 32). The patients in this study were from 18 to 40 years of age, showed radiographic evidence of bone loss prior to age 35, had 20 or more teeth, and had at least 7 teeth with a minimum of 5 mm of attachment loss and 4-mm probing depths. Exclusionary criteria included recent use of nonsteroidal anti-inflammatory drugs, antibiotics, or antiplaque preparations; systemic diseases affecting the periodontium directly or indirectly by interfering with the ability to perform adequate oral hygiene; pregnancy, breastfeeding, or being fewer than 3 months postpartum; or a history of periodontal surgery. The patients consisted of 17 males and 13 females; 28 were Caucasian, 1 was black, and 1 was Asian.

Sera were also collected from 30 age-, sex-, and race-matched periodontally healthy subjects and used as controls. These individuals were predominantly University of Washington School of Dentistry patients who were undergoing care for limited restorative dentistry needs or University of Washington clinical or laboratory staff who had minimal or

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no gingival inflammation, no periodontal probing depths of >4 mm, and no evidence of alveolar bone loss on radiographs.

To assess the relationship between severity of disease and predominant subclass response, bone loss and mean interproximal pocket depths were determined for each RPP patient. Bone loss was calculated from full-mouth radiographs by using the method of Schei et al. (35), and mean interproximal pocket depth scores were calculated from complete periodontal charting done at the time of initial examination following standard periodontal procedures.

Bacterial antigen. Type strain *P. gingivalis* ATCC 33277 was used as antigen in the experiments described. Cultures were maintained on prereduced blood agar plates incubated at 37°C in Oxoid anaerobe jars containing an anaerobic atmosphere generated with Oxoid anaerobe gas packs (Oxoid, Ltd., London, England). Batch cultures were grown on enriched Trypticase soy agar plates (38). After 4 days of growth, cells were scraped from the plate surface with a sterile Dacron-tip swab, placed in 10 ml of phosphate-buffered saline, and washed twice in the same buffer and once in sterile distilled water. The washed cell pellets were suspended in sterile distilled water and heated in a boiling water bath for 30 min to deactivate heat-labile proteases. The cells were frozen, lyophilized, and stored at -20°C until needed. Prior to use, the lyophilized bacteria were suspended at a concentration of 10 mg (dry weight) per ml in distilled water, disrupted with a Cole-Parmer 4710 ultrasonic homogenizer (5 min, 50% pulse, 40% power) in an ice bath, and then diluted 10-fold with distilled water (final concentration, 1 mg (dry weight) per ml).

Antibody measurement. An enzyme-linked immunosorbent assay (ELISA) was used to measure the serum antibody titers against *P. gingivalis*. Whole-cell sonicate of *P. gingivalis* obtained as described above (1 mg/ml) was diluted 1:100 in carbonate coating buffer (0.1 M NaHCO₃, 0.02 M MgCl₂, pH 9.6), and 100 µl was added to each well of 96-well microtiter plates (EIA II, Flow Laboratories, McLean, Va.) except those wells designated blanks. We chose to use whole-cell homogenate rather than purified bacterial components because we wished to determine the overall activity profile of reactivity of the patients to this organism. The plates were incubated at room temperature for 1 h on a shaker at 140 rpm and washed 10 times with TES-Tween (14.5 mM NaCl, 20 mM TES, 0.1% Tween) to remove any unbound antigen. A serum with a known high IgG titer to *P. gingivalis* was used on each plate as a reference to allow for between-plate comparisons. Each patient serum in duplicate and the reference serum were diluted seven times down the plate at twofold change per dilution (1:50 to 1:3,200). The plates were incubated and washed as described above. One-tenth milliliter of a 1:1,000 TES-Tween dilution of biotinylated mouse anti-human IgG subclass antibody (clones 8c/6-39, anti-IgG1; HP-6014, anti-IgG2; HP-6050, anti-IgG3; HP-6025, anti-IgG4; Sigma Chemical Co., St. Louis, Mo.) was placed in each well. These anti-IgG subclass reagents had been tested in preliminary studies by ELISA against myeloma proteins IgG1, IgG2, IgG3, and IgG4 (Sigma product no. I-4014 [γ1], I-4264 [γ2], I-4764 [γ4], and Calbiochem product no. 400124 [γ3]) for specificity and were found to be non-cross-reactive. Furthermore, the antigen-binding capacities of the reagents at the 1:1,000 dilution were found to be approximately equal. For the total IgG response and the reference wells on every plate, a biotinylated mouse anti-human IgG (clone HP-6017; Sigma) diluted 1:1,000 was placed in the wells. After incubation and wash-

ing, 0.1 ml of avidin conjugated to alkaline phosphatase (Sigma) diluted 1:400 was added to all wells, and the plates were incubated for 1 h and then washed in carbonate buffer. Sigma substrate 104 mixed with carbonate buffer was then added to each well. The plates were read kinetically every 20 s for 8 min on a V_{max} plate reader (Molecular Devices, Menlo Park, Calif.), and the data were transferred to a Macintosh IICx computer with SoftMax software (Molecular Devices) for analysis. ELISA units (EU) were calculated by the method of Butler (3).

Antibody avidity. An estimate of avidity was made by measuring the degree of ammonium thiocyanate-induced dissociation of antibody-antigen binding by the method of Lopatin et al. (23). Plates were coated with antigen as in the ELISA procedure. A single dilution of serum from each subject was chosen from the midpoint (linear portion) of the log-logit curves plotted from values obtained from the ELISA optical densities (y axis) versus serum dilutions (x axis). The serum-containing plates were incubated for 30 min at room temperature on a rotator platform and washed three times with TES-Tween buffer, and then graded concentrations (0.25, 0.5, 0.75, 1.0, 1.5, 2.0, and 2.5 M) of ammonium thiocyanate diluted in TES-Tween were added to different wells of the plate. Control wells received TES-Tween without thiocyanate. After incubation for 1 h and 10 washes with TES-Tween, alkaline phosphatase-conjugated goat anti-human IgG was added and the procedure was continued as described for the ELISA. Percentage of antibody binding was calculated for each concentration of thiocyanate by using the following formula: (optical density of thiocyanate-treated well/optical density of control well) × 100%. The concentration of thiocyanate required to dissociate (or inhibit) 50% of the bound antibody (ID₅₀) was calculated by linear regression analysis of curves generated by plotting the percentages of antibody binding versus the log₁₀ of the thiocyanate concentration.

Statistics. Patients and control subjects were classified as seropositive or seronegative by using a cutoff of total IgG antibody titer to *P. gingivalis* twofold greater than that of the median total IgG antibody response in the healthy subjects.

The predominant subclass for a given subject was defined as the subclass yielding the highest antibody response as determined by EU calculations. The percent distributions of specific anti-*P. gingivalis* IgG subclass responses (see Table 3) were based on median subclass responses. The presence or absence of detectable amounts of *P. gingivalis* in plaque samples was determined by cDNA probe and/or culture (26).

The Mann-Whitney U statistic was used for between-group comparisons. Correlations were evaluated by Spearman's rank correlation. Relationships of the subclass responses within an individual were evaluated by using Friedman's two-way analysis of variance with multiple-comparisons adjustments to statistically assess the ordering of subclass responses. All tests were performed at the α = 0.05 level. Box plots of the ELISA results were produced by using STATVIEW 5.12⁺ statistical software for Macintosh computers. This program determines the lengths of the whiskers (vertical lines extending above and below the box) by the 10th and 90th percentiles.

RESULTS

Total IgG antibody response to *P. gingivalis*. The total anti-*P. gingivalis* IgG antibody responses quantified in EU for the 30 RPP patient sera and their matched control sera are presented in Fig. 1 and 2, respectively. For both groups,

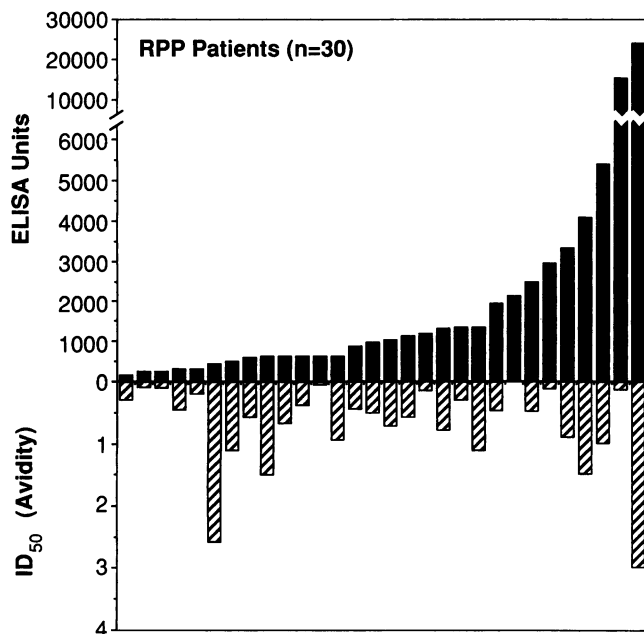


FIG. 1. IgG antibody response and avidity. The titer (ELISA units) and avidity (ID_{50}) of total IgG reactive with *P. gingivalis* are shown for each RPP patient.

a large amount of variability was observed in the total responses. The RPP patients (median = 1021.75 EU) exhibited twofold-greater total IgG responses to *P. gingivalis* than did healthy subjects (median = 452.3 EU; $P < 0.01$). Since antibody produced by high responders may differ from that produced by low responders in avidity and subclass distribution, subjects were classified as seropositive when the total IgG antibody exceeded twice the median titer observed

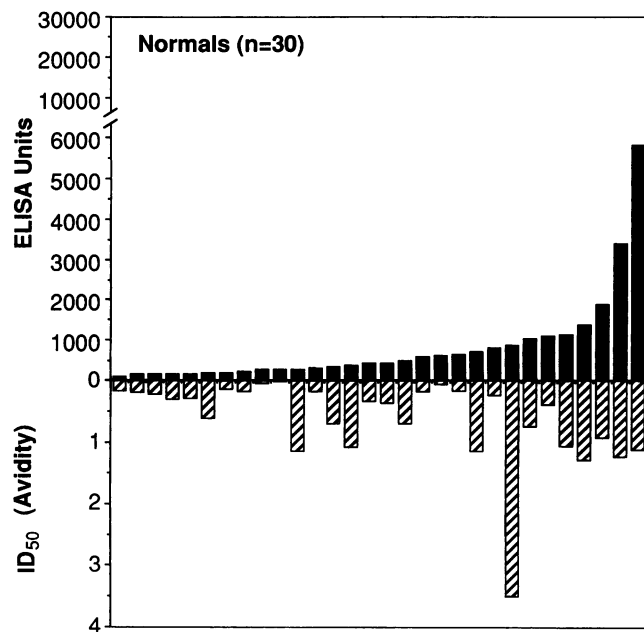


FIG. 2. IgG antibody response and avidity. The titer (ELISA units) and avidity (ID_{50}) of total IgG reactive with *P. gingivalis* are shown for each healthy (normal) subject.

TABLE 1. Median avidities by serostatus for healthy controls and RPP patients

| Serostatus | RPP patients | | Healthy controls | |
|--------------|--------------|----------------|------------------|-------------------|
| | <i>n</i> | Median avidity | <i>n</i> | Median avidity |
| Seropositive | 16 | 0.53 | 7 | 1.06 ^a |
| Seronegative | 14 | 0.44 | 23 | 0.24 |

^a $P < 0.01$ when seropositive controls are compared with seronegative controls. Seropositive healthy subjects show a trend ($P < 0.10$) toward greater avidity than RPP seropositive and seronegative groups. The small number of seropositive healthy subjects in our study was sufficient for detecting only very large differences in avidity levels (at the $\alpha = 0.05$ level).

in healthy subjects (i.e., titer > 904.6 EU) or as seronegative when the titer was lower. According to this definition, 7 (23%) of the healthy subjects and 16 (53%) of the RPP patients were seropositive.

Avidity is a term used to describe the overall binding power of a heterogeneous population of antibodies directed against multivalent antigens. Weaker antibody-antigen bonds are dissociated more readily and rapidly than strong bonds by mild denaturing conditions (16). Stronger concentrations of denaturing agents are needed to dissociate stronger antibody-antigen bonds (33). We used various concentrations of thiocyanate to get an estimate of the strength of binding of the total serum anti-*P. gingivalis* antibodies with *P. gingivalis* antigens. Avidity in terms of ID_{50} (the concentration of thiocyanate needed to cause 50% dissociation) is shown directly below the corresponding total anti-*P. gingivalis* titers in Fig. 1 and 2. The ID_{50} s were similar in the patients (median = 0.49) and the healthy subjects (median = 0.35). There was no statistically significant correlation ($r = 0.16$) between the ID_{50} and the total IgG titer in patients, but there was in healthy subjects ($r = 0.56$, $P < 0.01$). In seropositive healthy subjects, avidity was significantly elevated over avidity in the seronegative group, with a trend toward elevation over avidity in seropositive and seronegative patients (Table 1).

Anti-*P. gingivalis* subclass response. The distribution of the individual subclass-specific antibody responses to *P. gingivalis* are reported as box plots in Fig. 3, in which the box represents the middle 50% of the distribution and the line within the box represents the median response level. For both patients and healthy subjects, the IgG2 and IgG3 subclass responses were higher, but they were also more variable than the IgG1 and IgG4 responses. Healthy subjects exhibited relatively low IgG1 and IgG4 levels and generally low variability. The RPP patients exhibited greater antibody responses to *P. gingivalis* ($P < 0.05$) in all subclasses (Table 2), but these responses were likely due in part to the higher total IgG responses in RPP patients. However, relative to their total subclass response, healthy subjects and RPP patients showed significant differences ($P < 0.05$) only in their IgG1 and IgG4 responses. Notably, however, several patients had minimal levels of antibody of all subclasses to *P. gingivalis* antigens, and several healthy subjects demonstrated relatively high anti-*P. gingivalis* IgG2 and IgG3 responses.

According to the observed median responses, IgG2 $>$ IgG3 $>$ IgG1 $>$ IgG4 was the subclass order for both patients and healthy subjects. In both groups, IgG2 was the predominant subclass response (80% of healthy subjects, 63% of RPP patients). The second most common predominant response was IgG3 (20% of healthy subjects, 30% of RPP

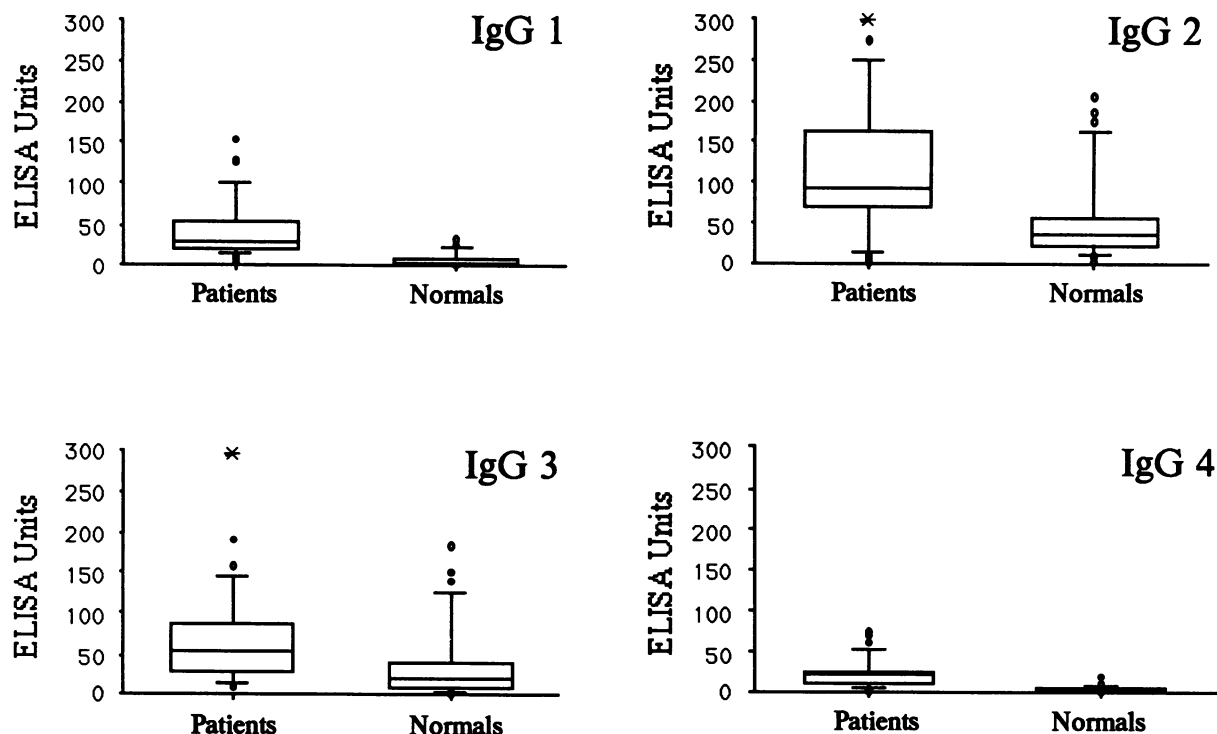


FIG. 3. IgG subclass distribution in patients and healthy (normal) subjects in ELISA units. Box plots of antibody response indicate the 10th, 25th, 50th, 75th, and 90th percentiles (solid bars). Each dot indicates an outlier, and * indicates extreme outliers which were not plotted (two for IgG2 [1,050 and 2,184 EU]; one for IgG3 [552.6 EU]).

patients). Two RPP patients (7%) had IgG1 as their predominant response. However, statistical analysis of these findings with Friedman's two-way analysis of variance revealed that IgG2 did not significantly predominate over IgG3 ($P = 0.10$) in either patients or healthy subjects. The same was true for IgG1 and IgG4. In healthy subjects, IgG2 and IgG3 significantly predominated over IgG1, but this was not the case in RPP patients.

Proportionate percent distribution of IgG subclass response. There is general agreement (for a review, see Hamilton [14]) on the percentage of each IgG subclass in healthy adult human serum. The predominant subclass, IgG1, is followed by IgG2, which averages one-half to one-third the IgG1

level, while IgG3 and IgG4 together represent less than 10% of the total serum IgG (Table 3). The specific anti-*P. gingivalis* subclass distributions were clearly different from this normal distribution. The total subclass response was dominated by IgG2, which was followed by the IgG3 response, which was approximately one-third to two-thirds that of the IgG2 response (except in the small group of seropositive normal subjects). IgG1 and IgG4 represented 12 to 26% of the total subclass responses. There were no striking differences between seropositive and seronegative subjects.

Clinical parameters and subclass responses. We considered the possibility that predominant subclass responses might be influenced by severity of periodontal disease. However, we found no significant correlation between severity of bone loss (as determined from periapical radiographs), pocket

TABLE 2. Median IgG subclass antibody responses to *P. gingivalis* by serostatus groups and combined groups

| Subject and IgG subclass | Median response ^a | | |
|--------------------------|------------------------------|--------------|----------|
| | Seropositive | Seronegative | Combined |
| RPP patients | | | |
| IgG1 | 32.89 | 18.71 | 28.69 |
| IgG2 | 155.95 | 74.90 | 91.96 |
| IgG3 | 52.93 | 47.25 | 51.92 |
| IgG4 | 22.23 | 20.71 | 21.58 |
| Healthy controls | | | |
| IgG1 | 4.14 | 5.19 | 4.94 |
| IgG2 | 97.48 | 28.11 | 38.18 |
| IgG3 | 87.96 | 16.26 | 20.95 |
| IgG4 | 2.66 | 2.32 | 2.90 |

^a There were 16 seropositive and 14 seronegative RPP patients and 7 seropositive and 23 seronegative healthy controls.

TABLE 3. Percent distribution of specific anti-*P. gingivalis* IgG subclass responses by serostatus and disease status

| Serostatus | Subject (n) | % Response | | | | Subclass distribution |
|---------------------------------|--------------|------------|------|------|------|-----------------------|
| | | IgG1 | IgG2 | IgG3 | IgG4 | |
| Seropositive | RPP (16) | 13 | 59 | 20 | 8 | 2 > 3 > 1 > 4 |
| | Healthy (7) | 2 | 51 | 46 | 1 | 2 > 3 > 1 > 4 |
| Seronegative | RPP (14) | 12 | 46 | 29 | 13 | 2 > 3 > 4 > 1 |
| | Healthy (23) | 10 | 54 | 31 | 5 | 2 > 3 > 1 > 4 |
| Combined | RPP (30) | 15 | 47 | 27 | 11 | 2 > 3 > 1 > 4 |
| | Healthy (30) | 8 | 57 | 31 | 4 | 2 > 3 > 1 > 4 |
| Normal human serum ^a | | 66 | 25 | 7 | 2 | |

^a Midpoint of range (14).

depth, and titer levels in any of the subclasses. Although not all of the patients in this study had detectable levels of *P. gingivalis* in their periodontal microflora at the time of initial examination (11 of the 30 were *P. gingivalis* negative), those patients known to be infected with *P. gingivalis* ($n = 19$) did not exhibit greater subclass responses than those who were *P. gingivalis* negative. The subclass responses were not different for males and females. These clinical results held for both serostatus groups.

Disease severity and antibody level. Among all RPP patients, there was no significant correlation between total IgG response and bone loss ($r = 0.17$) or pocket depth ($r = 0.03$). In seronegative RPP patients, these correlations were negative but not significant (bone loss, $r = -0.22$; pocket depth, $r = -0.39$). However, in seropositive RPP patients, there was a significant positive correlation between total IgG response and bone loss ($r = 0.51$, $P = 0.05$) but not between total IgG response and pocket depth ($r = 0.34$).

DISCUSSION

Our data show that the humoral immune response against a whole-cell homogenate of *P. gingivalis* in RPP patients, as well as in matched control subjects that mount a response, is dominated by IgG2 followed by IgG3. This observation extends but is in general agreement with previous observations regarding anti-*P. gingivalis* lipopolysaccharide antibody levels in adult periodontitis patients (30, 36) and RPP patients (30). Furthermore, this finding is consistent with results obtained in numerous other studies of IgG subclass responses in nonoral infections caused by a variety of bacteria (for a review, see Hammarström and Smith [15]).

The nature of the subclass response may have important implications concerning the role of serum antibodies in the disease process and the nature of the inducing antigen(s). The IgG subclasses differ greatly with regard to their functional activities. IgG2 is considered less effective than IgG1 (and IgG3) as a bacterial opsonin because of its lower binding efficiency to the Fc γ receptors of phagocytic cells (34). IgG2 is also less effective than IgG1 and IgG3 in complement fixation (1). However, IgG2 is not devoid of opsonic activity (2), and IgG2 anticarbohydrate antibodies may be more important than IgG1 antibodies in resistance to infections with encapsulated bacteria (15). IgG2 production is induced predominantly in response to carbohydrate antigens, while IgG1 and IgG3 are generally responses to protein antigens (15), although anticarbohydrate antibodies of the IgG1 subclass have been reported in some studies (15). Thus, most, although not all, RPP patients and control subjects appear to be responding most strongly to carbohydrate components of *P. gingivalis*. However, the seropositive (high-responder) patients show relative increases in IgG1 and IgG4 compared with seropositive controls, perhaps indicating a greater participation of protein antigens in these patients.

It is notable that 16 of 30 patients and 7 of 30 control subjects manifested positive (twice the median of controls) total IgG anti-*P. gingivalis* antibody responses. The failure of half or more of patients to mount an elevated response has been observed previously by us (5) and others (27, 28, 40, 41). The reasons are unclear. Since numerous microbial species other than *P. gingivalis* can be found in the subgingival flora of RPP patients, it is possible that seronegative patients were not infected with *P. gingivalis*. However, the absence of infection seems not to account for our observations. Of our 30 patients, 19 tested positive for *P. gingivalis*

in the subgingival plaque at the time serum was harvested. There was, however, no significant association between the presence of *P. gingivalis* and total IgG *P. gingivalis*-specific antibody or subclass response. Our observations are in agreement with those of previous investigators, who also failed to find such associations (5, 43), but at variance with the report by Ebersole et al. (8), who found a significant association between serum IgG antibody-specific titers and the microbial species found in the subgingival flora. Our data are consistent with the concept that while some RPP patients do respond to *P. gingivalis* infection by producing high titers of IgG antibodies reactive with the antigens of *P. gingivalis*, others appear not to have that capacity. Although the median total IgG titer for seronegative patients was by definition significantly lower than that of control subjects, the levels of all four subclasses were significantly elevated relative to the subclasses in seronegative controls, indicating that a response of some sort had occurred in the patients.

A proportion of the periodontally healthy subjects in our study had a significantly elevated serum anti-*P. gingivalis* IgG response. This is also consistent with previous reports (9, 11, 40) and may indicate that periodontal (or other site) infection sufficient to provoke a typical immune response may have occurred but that clinically manifest disease did not develop for reasons discussed below.

Antibody affinity, the intrinsic association constant that characterizes the bond strength between an antibody and its antigen, affects the functionality of the antibody. This is perhaps because immune complexes of high-affinity antibody and antigen are more stable and hence more likely to form the larger lattices which are needed for complement fixation (18) and which may also promote phagocytosis. Avidity as measured in our studies yields a reasonable approximation, although not an accurate measurement, of the strength of bonding between *P. gingivalis* antigens and anti-*P. gingivalis* antibodies. Our data show that while avidity of IgG anti-*P. gingivalis* antibodies in seropositive patients did not significantly differ from values for seronegative patients or seronegative control subjects, median avidity for seropositive control subjects was elevated relative to values for the other groups. This observation may be important in explaining why some individuals when exposed to oral *P. gingivalis* colonization and outgrowth do not develop clinical disease while others do.

Our findings of overall low-avidity antibodies in RPP patients are in agreement with previous reports. Serum antibodies in periodontitis patients have been reported to be of much lower avidity than antibodies to the antigens of the same microbial species raised in rabbits (23). Further, as shown by others, anti-*P. gingivalis* antibodies in patient serum had much lower avidities for their antigens than did antibodies reactive with tetanus toxoid and streptokinase in the same sera (22). The reason for low avidity values in periodontitis patients is not known. Lopatin et al. (23) have speculated that chronic antigen overload (20, 37) or antigen exposure in utero (10) may be responsible. It is also conceivable that certain individuals have a genetically determined diminished capacity to respond to antigens of certain types, as described above, since several RPP patients infected with *P. gingivalis* had low-titer, low-avidity IgG antibody against Pg.

We failed to find an association between total IgG anti-*P. gingivalis* titer or subclass levels and severity of bone loss or pocket depth among all the RPP patients. We did note a significantly positive correlation between total IgG antibody to *P. gingivalis* and severity of bone loss but not pocket

depth in the seropositive RPP subset. These observations contrast with those of Murayama et al. (28) and Gunsolley et al. (11, 12), who reported negative correlations between anti-*P. gingivalis* IgG titer and disease severity. However, Murayama stated that significant negative correlations were found only in RPP patients with active disease (28), and Gunsolley's report used "probing attachment loss" as the clinical measurement for comparison rather than pocket depth or bone loss as we used. Our observations do agree with those of Mansheim et al. (24) and Haffajee et al. (13), who found no association between IgG antibody titer and disease severity in RPP patients. Lack of agreement among various studies may be the result of differences in disease severity measurements, stage of disease when serum sample was taken, high variability among patients, and the relatively small size of the patient groups in all of these studies, including ours.

These findings support the hypothesis that humoral immunity to *P. gingivalis* in RPP patients is of less than optimal value in protecting these patients against periodontal infection by this organism. If *P. gingivalis* is definitely identified as a primary pathogenic agent in RPP, the need to boost immunity against *P. gingivalis* in these patients, and perhaps in other individuals at high risk for RPP, should be considered. Indeed, experimental immunization has been tested in rodent (6, 19, 31) and nonhuman primate (7, 25, 29) systems and appears to render some degree of protection against infection. Among the challenges we face in this endeavor is to determine which *P. gingivalis* antigens are most useful in provoking an antibody response of appropriate subclass and avidity to render a high degree of protection against infection and disease caused by this organism and provide a safe and effective means of delivering such a vaccine.

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