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Initial Serum Antibody Titer to *Porphyromonas gingivalis* Influences Development of Antibody Avidity and Success of Therapy for Chronic Periodontitis

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This study assessed the effect of periodontal therapy on specific serum antibody concentration, expressed as titer, and antibody binding strength, expressed as relative avidity. The immune responses to *Porphyromonas gingivalis* and *Actinobacillus actinomycetemcomitans* were investigated. Antibody titer was assayed by enzyme-linked immunosorbent assay (ELISA) and relative avidity was measured by thiocyanate elution in 17 adult periodontitis patients before and after therapy. Immunoglobulin G (IgG) avidities (expressed as thiocyanate molarity) to *P. gingivalis* increased from 1.01 to 1.38 M ($P = 0.05$) and IgA titers (expressed as ELISA units [EU]) increased from 89 to 237 EU ($P = 0.012$). There were no significant changes in avidity to *A. actinomycetemcomitans*, but the titer of all three immunoglobulin classes increased significantly ($P < 0.03$). More specifically, when patients were divided into subgroups which had originally been either IgG seropositive (i.e., having an IgG titer to this organism >2 times the control median) or seronegative for *P. gingivalis*, only patients who were initially seropositive showed a significant increase in antibody avidity ($P = 0.026$; mean difference, 0.69 M). Patients who were originally seropositive in terms of IgG and IgA titer to *P. gingivalis* had demonstrably better treatment outcomes in terms of a reduced number of deep pockets and sites which bled on probing ($P < 0.05$). These findings suggest that periodontal therapy affects the magnitude and quality of the humoral immune response to suspected periodontopathogens, that this effect is dependent on initial serostatus, and that initial serostatus may have a bearing on treatment outcome.

Porphyromonas gingivalis and *Actinobacillus actinomycetemcomitans* have been shown to be important periodontopathogens in various types of periodontal disease (35, 36). In addition, a number of studies have demonstrated that antibody responses to these two organisms in particular are increased in patients with periodontal disease compared with subjects without disease (8, 9, 11, 24, 33, 38).

An early study by Taubman et al. (37) investigated the ability of serum antibody titers to distinguish subjects with periodontitis from healthy subjects and suggested that these responses could provide an appraisal of treatment effects in the management of periodontitis patients. Naito et al. (27) demonstrated that the serum titer to *P. gingivalis* was reduced in periodontitis patients posttherapy. This has been confirmed by Aukhil et al. (1). The treatment regimen employed in these studies included scaling, root planing, and oral hygiene instruction, followed by surgery or scaling and root planing under anesthesia.

However, Mouton et al. (25) reported that a reduction of 50% in antibody titers to *P. gingivalis* occurred within 1 year posttreatment in most patients, after an initial increase. A subgroup of patients, however, did not respond to the challenge of the mechanical treatment regimen by producing an increased antibody titer to *P. gingivalis.*

An investigation of antibody titers to *A. actinomycetemcomitans* in localized juvenile periodontitis (31) has shown that these decreased posttreatment. It has been shown that untreated adult periodontitis patients have significantly elevated antibody titers to *P. gingivalis* compared with treated adult periodontitis patients (26). Additionally, an investigation of serum titers to *P. gingivalis* lipopolysaccharide (LPS) in relation to treatment effects demonstrated levels reduced by 15 to 30% long term posttreatment (32). Ebersole et al. have reported that the homologous organism can be detected in subgingival plaque when antibody titer elevations are found postscaling (10) and also that multiple foci of infection must be successfully treated to have an effect on serum immunoglobulin G (IgG) levels (5). They have also demonstrated that the long-term reductions in antibody titers occur most frequently 8 to 10 months posttherapy, within a range of 2 months to 2 years (4).

A recent report by Ebersole et al. (7) quotes data showing that 60% of periodontitis patients exhibited increased titers to a battery of suspected periodontopathogens posttreatment and that these patients had fewer episodes of active disease during the monitoring interval (2 years) than those who did not demonstrate increased titers.

However, these studies have focused on antibody titer and have not considered antibody avidity in relation to treatment effects. Chen et al. (2) studied both the titer and avidity of IgG antibodies to *P. gingivalis* before and after treatment in rapidly progressive periodontitis patients. They found that although avidities are significantly lower in rapidly progressive periodontitis patients pretreatment than in controls, these avidities increased significantly posttreatment to levels higher than in controls. They concluded that many rapidly progressive periodontitis patients do not produce protective levels of biologically functional antibody during the course of natural infection, but that they may be stimulated to do so by treatment.

The latter study also indicated that convincing increases in IgG titer to *P. gingivalis* occurred only in seronegative subjects (i.e., those whose baseline titer was <2 times the control subject median). Significantly, this accords with the finding of Mouton et al. (25) that patients who initially had high serum antibody levels to *P. gingivalis* (seropositive) responded better

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to therapy than those who initially had low levels (seronegative).

The present study aimed to build on these observations by conducting an analysis of specific antibody titer and avidity to *P. gingivalis* and *A. actinomycetemcomitans* in adult periodontitis patients before and after periodontal therapy. More specifically, it aimed to investigate any differences between seropositive and seronegative patients in terms of treatment response and changes in antibody response.

MATERIALS AND METHODS

Patients. Seventeen subjects (nine males and eight females; age range, 33 to 53 years) suffering from advanced periodontal disease (probing depths [PDs] of >5.5 mm in at least one site per sextant and at least two nonadjacent sites per quadrant with a PD of >4 mm) were recruited for this investigation. These subjects had no history of systemic conditions which could influence the course of periodontal disease and had not received antibiotics during the previous 3 months.

Two nonadjacent sites per quadrant with a PD of >4 mm were selected in each subject and monitored during the course of this study. Nonadjacent sites were used so as to reduce the possibility of contamination and ensure independence of the gingival crevicular fluid and plaque samples. At the baseline appointment, PDs and bleeding on probing (BOP) at these sites were assessed with the Florida probe (13), and blood samples were taken. The blood was allowed to clot and centrifuged at 750 × *g* for 15 min, and the sera were separated, aliquoted, and stored at −300°C. Patients were treated by an experienced periodontist (E.A.) and given oral hygiene instruction and quadrant scaling and root planing under local anesthesia (hygiene phase therapy [HPT]).

Subjects were reassessed with conventional probing charts between 6 and 7 weeks after HPT, a blood sample was taken, and PD and BOP measurements were recorded at the eight preselected sites with the Florida probe. The duration of HPT ranged between 6 weeks and 7 months, with a mean of 5.5 months. All of the patients were reassessed after 3 months (±2 weeks), which was the time of the first maintenance visit. A blood sample was also taken during this appointment. Only the eight study sites were used to assess the response to treatment in terms of percent of sites that have a PD of >3.5 mm and BOP. It should be noted, however, that the initial assessment of periodontal disease status was made on the basis of a larger number of sites.

Control subjects. The controls were 23 periodontally healthy individuals (age range, 20 to 40 years; 12 males and 11 females). The immunological data from these individuals were used to define the serostatus of the patients as described by Chen et al. (2). Seropositive patients are those with titers >2 times the control median.

Bacteria. *P. gingivalis* NCTC 11834 was grown under anaerobic conditions (85% N_2, 10% H_2, 5% CO_2), and *A. actinomycetemcomitans* ATCC 29523 (serotype a) was grown in CO_2 at 37°C on Columbia blood agar. This medium was routinely supplemented in our laboratory with vitamin K and hemin for the growth of black-pigmenting gram-negative anaerobes. Otherwise, fastidious anaerobe agar should be used. *P. gingivalis* was harvested after 5 days and *A. actinomycetemcomitans* after 24 h into phosphate-buffered saline with 1 mM disodium EDTA, pH 7.4 (PBSE), washed by centrifugation, and fixed for 1 h in 10% formal saline. The cells were then washed twice in PBSE and once in 0.1 M sodium carbonate-bicarbonate buffer containing 0.02% NaN_3 at pH 9.6 (coating buffer). Fixed cells were stored in coating buffer at 4°C until use.

Collection of subgingival plaque samples. Subgingival plaque samples were taken with a single stroke, using a separate sterile curette for each sample to prevent cross-contamination, and collected in tubes containing 0.5 ml of fastidious anaerobe broth (FAB; Lab M, Bury, England). Samples were immediately transported to the laboratory, where they were Vortex mixed for 30 s. Broths were then stored at −70°C until PCR analysis was performed.

PCR. For PCR analysis, 90 μl of Vortex-mixed subgingival plaque was added to 10 μl of 10× lysis buffer (100 mM Tris-HCl [pH 8.0], 10 mM EDTA, 10% Triton X-100) and boiled for 5 min. Then 10 μl of this lysate was used in each PCR. The primers used for PCR identification of *A. actinomycetemcomitans* targeted the leukotoxin A gene as previously described (15): LKT2 (5′-GGAAT TCCTAGGTATTGCGAAACAATTTGATC-3′) and LKT3 (5′-GGAATTC CTGAAATTAAGCTGGTAATC-3′), which give an expected amplification product of 262 bp. For identification of *P. gingivalis*, the primers used targeted the fimbrillin gene as previously described (39): FIM1 (5′-ATAATGGAGAA CAGCAGGAA-3′) and FIM2 (5′-TCTTGCCAACCAGTTCCATTGC-3′), which gave an expected amplification product of 131 bp. PCR amplification with primers LKT2 and LKT3 was carried out in a reaction volume of 100 μl, consisting of 10 μl of sample lysate and 90 μl of reaction mixture, containing 1× PCR buffer (10 mM Tris-HCl [pH 8.8], 2.5 mM $MgCl_2$, 50 mM KCl, 0.1% Triton X-100), 2.5 U of DynaZyme DNA polymerase (Flowgen), 0.2 mM each dATP, dCTP, dGTP, and dTTP, and 50 pmol of each primer. When the primer pair FIM1 and FIM2 was used, PCR conditions were as above except that the reaction mixture contained 1.5 mM $MgCl_2$. Primers were separated from the

TABLE 1. Pre- and posttreatment antibody analysis for entire patient group ($n = 17$)[a]

Antibody	*P. gingivalis*						*A. actinomycetemcomitans*					
	Avidity (ID_{50})			Titer (EU)			Avidity (ID_{50})			Titer (EU)		
	Baseline	*P*	Post-HPT	Baseline	*P*	Post-HPT	Baseline	*P*	Post-HPT	Baseline	*P*	Post-HPT
IgG	1.01 (0.68–1.20)	0.05	1.38 (0.70–2.00)	700 (306–4,471)	0.62	1,375 (518–3,176)	0.77 (0.63–0.90)	0.43	0.67 (0.43–0.66)	128 (16–407)	0.02	724 (88–54,423)
IgM	0.75 (0.66–0.86)	0.17	0.98 (0.64–1.12)	346 (214–512)	0.92	461 (264–716)	0.49 (0.34–0.62)	0.42	0.53 (0.47–0.64)	198 (103–460)	0.03	461 (140–3,803)
IgA	1.27 (1.01–1.44)	0.81	1.22 (0.80–1.58)	89 (30–951)	0.01	237 (149–2,750)	0.83 (0.57–1.14)	0.72	0.80 (0.65–0.92)	9 (4–14)	0.02	19 (11–54)

[a] Mean titers are given, with interquartile ranges in parentheses. *P* values for the comparisons are given for the paired *t* test (avidity) or Wilcoxon signed rank test (titer).

TABLE 2. Analysis of treatment effect on IgG, IgM, and IgA antibodies to *P. gingivalis* by subgroups[a]

Antibody	Subgroup (*n*)	Avidity (ID_{50})				Titer (EU)			
		Baseline	Post-HPT	*P*	*D*	Baseline	Post-HPT	*P*	*D*
IgG	Seropositive (9)	1.14 (0.82–1.44)	1.82 (0.88–2.81)	0.026	+0.69	2,559 (1,477–48,590)	2,725 (1,066–14,502)	0.53	−568
	Seronegative (8)	0.85 (0.63–1.09)	0.85 (0.54–1.14)	1.00	0.00	306 (195–547)	518 (293–2,000)	0.08	+474
IgM	Seropositive (3)	0.76 (0.69–0.87)	0.68 (0.58–0.75)	0.25	−0.08	941 (853–1,054)	721 (501–724)	0.18	−288
	Seronegative (14)	0.75 (0.66–0.87)	1.05 (0.65–1.24)	0.14	+0.30	290 (186–489)	362 (232–658)	0.45	+31
IgA	Seropositive (11)	1.38 (1.00–1.65)	1.21 (0.67–1.56)	0.57	−0.18	602 (89–1,226)	625 (196–3,488)	0.056	+652
	Seronegative (6)	1.06 (0.99–1.14)	1.24 (0.87–1.62)	0.38	+0.18	26 (21–33)	128 (62–2,871)	0.036	+12

[a] *P* values for paired comparison were derived from the paired *t* test (avidity) or Wilcoxon signed rank test (titer). Interquartile ranges are given in parentheses. *D*, mean difference.

other components of the reaction mixture by a layer of wax (hot-start PCR), preventing the reaction from starting until the wax melted upon commencement of PCR cycling and thus improving the specificity and yield of reaction products. PCR cycling was carried out in a Hybaid OmniGene thermal cycler. After an initial denaturation step of 95°C for 5 min, 33 amplification cycles of denaturation at 95°C for 1 min, annealing of primers at 55°C for 1 min, and primer extension at 72°C for 1 min were carried out, followed by a final primer extension step at 72°C for 10 min. Reaction products were stored at −20°C or analyzed immediately by gel electrophoresis. Each reaction product (20 μl) was fractionated on a 2% agarose gel containing ethidium bromide (0.5 μg/ml) and visualized on a UV transilluminator.

In order to avoid contamination, strict procedures were employed when carrying out PCR. Separate rooms were used for sample preparation, setting up of PCRs, and analysis of PCR reaction products. Filter tips were used at all stages of the process except when adding the sample to the reaction mixture, when a positive-displacement pipette was used. Negative and positive PCR controls were included with each batch of samples being analyzed by PCR. The negative control was a standard PCR mixture with the sample replaced by 10 μl of sterile water; the positive control used was a standard PCR mixture containing 100 ng of genomic DNA from *A. actinomycetemcomitans* or *P. gingivalis* instead of the sample.

ELISA. Specific antibody titers were measured by enzyme-linked immunosorbent assay (ELISA) by the method of Ebersole et al. (6), using formalinized whole cells at an absorbance which had previously been determined as optimal to coat microtiter plates. Immulon 1 plates (Dynatech) were used because of their low protein-binding characteristics. After coating, the plates were treated with PBS containing 0.1% bovine serum albumin, 0.05% Tween 20, and 5% skimmed milk to remove background binding. Serum serially diluted in this buffer was then added, and the plates were subsequently incubated with biotin-conjugated anti-human IgG, IgA, or IgM (Sigma) and thereafter with Extravidin-peroxidase (Sigma). The reaction was visualized with *o*-phenylenediamine dihydrochloride substrate and stopped with 1 M H_2SO_4. Samples were assayed in duplicate, and results were calculated with a regression line and derived equation from serial dilutions of a reference serum (23). Results were expressed as ELISA units (EU) (14).

Dissociation assay. The dissociation assay to determine antibody avidity was performed as follows. After incubation with serum as described above, the wells were treated with increasing concentrations of ammonium thiocyanate (0.2 to 8.0 M). The concentration of thiocyanate required to dissociate 50% of bound antibody was determined by linear regression analysis. This concentration was termed the 50% inhibitory dose (ID_{50}) and provides a measure of relative avidity, as previously reported (21, 28).

Statistical analysis. Mean values are given for antibody avidities throughout, whereas median values are given for titers because these were not normally distributed.

Student's paired *t* tests were used to assess whether differences in antibody avidity were significant between baseline, post-HPT, and the first maintenance visit. Because antibody titers were not normally distributed, the nonparametric Wilcoxon signed rank test was used to perform the parallel assessments. When seropositive and seronegative subgroups were directly compared in terms of treatment outcome, two-sample *t* tests were used.

RESULTS

An analysis of the entire patient group before and after HPT is given in Table 1. This analysis demonstrates a tendency towards an increase in avidity of IgG to *P. gingivalis* and significant increases in titer to *A. actinomycetemcomitans* after therapy. For comparison only, since this study did not attempt to relate diseased and healthy individuals, the corresponding healthy control data for IgG, IgA, and IgM avidity (mean, interquartile range) and titer (median, interquartile range), respectively, to these organisms were as follows. For *P. gingivalis*, these values were: IgG, 1.00 (0.69 to 1.12) and 320 (118 to 587); IgA, 1.18 (0.60 to 1.69) and 13 (7 to 30); and IgM, 0.70 (0.56 to 0.86) and 412 (188 to 1139). For *A. actinomycetemcomitans*, they were: IgG, 0.81 (0.60 to 1.00) and 749 (171 to 3,217); IgA, 1.38 (0.75 to 2.02) and 8 (4 to 153); and IgM, 0.64 (0.38 to 1.04) and 152 (95 to 298).

Analyses were performed for the effect of treatment on avidity and titer of antibody to the two organisms after division of the treatment group into subgroups based on serostatus at baseline. The data for the response to *P. gingivalis* (Table 2) and *A. actinomycetemcomitans* (Table 3) are given. A large and statistically significant increase in avidity of IgG to *P. gingivalis* (0.69 M, $P = 0.026$) was detected only in the seropositive subgroup. By contrast, IgG and IgA titers to this organism tended to increase in the seronegative subgroup. However, Table 3 shows that there were no significant increases in anti-

TABLE 3. Analysis of treatment effect on IgG, IgM, and IgA antibodies to *A. actinomycetemcomitans* by subgroup[a]

Antibody	Subgroup (*n*)	Avidity (ID_{50})				Titer (EU)			
		Baseline	Post-HPT	*P*	*D*	Baseline	Post-HPT	*P*	*D*
IgG	Seropositive (5)	0.73 (0.51–0.99)	0.60 (0.42–0.78)	0.24	−0.14	631 (407–106)	105 (2,139–105)	0.79	+3,754
	Seronegative (12)	0.81 (0.63–0.92)	0.68 (0.42–0.64)	0.45	−0.13	27 (11–138)	391 (27–7,291)	0.006	+518
IgM	Seropositive (8)	0.53 (0.32–0.76)	0.50 (0.29–0.65)	0.73	−0.03	459 (320–745)	601 (159–7,094)	0.29	+914
	Seronegative (9)	0.45 (0.34–0.60)	0.56 (0.48–0.65)	0.08	+0.11	118 (69–168)	461 (124–3,014)	0.03	+511
IgA	Seropositive (11)	0.82 (0.59–1.16)	0.69 (0.64–0.77)	0.59	−0.13	32 (27–37)	20 (16–32)	0.42	−11
	Seronegative (6)	0.84 (0.52–1.16)	0.82 (0.62–0.96)	0.89	−0.02	8 (4–10)	18 (10–147)	0.003	+22

[a] See Table 2, footnote *a*.

TABLE 4. Analysis of clinical status according to serostatus for *P. gingivalis* and *A. actinomycetemcomitans*[a]

Antibody	*P. gingivalis*				*A. actinomycetemcomitans*			
	Seropositive subjects		Seronegative subjects		Seropositive subjects		Seronegative subjects	
	Mean PD (SD)	% BOP (SD)	Mean PD (SD)	% BOP (SD)	Mean PD (SD)	% BOP (SD)	Mean PD (SD)	% BOP (SD)
IgG	5.5 (0.8)	87 (19)	5.4 (0.6)	77 (21)	6.2* (0.5)	87 (21)	5.1* (0.4)	81 (22)
IgM	5.8 (0.9)	80 (21)	5.3 (0.7)	96 (7)	5.7 (0.6)	87 (14)	5.2 (0.7)	79 (14)
IgA	5.5 (0.2)	86 (18)	5.3 (0.8)	77 (23)	5.7 (0.8)	85 (21)	5.4 (0.7)	75 (12)

[a] Mean BOP and mean PD (millimeters) are given for seropositive and seronegative subjects for the three main immunoglobulin classes. Comparisons were made by the two-sample *t* test, and significant differences are denoted by an asterisk.

body avidity to *A. actinomycetemcomitans*, but titers in all three classes increased significantly only in the seronegative subgroup.

An analysis of the differences between seropositive and seronegative patients at baseline in terms of clinical parameters was also performed (Table 4). This showed a tendency towards higher mean pocket depth and higher percentage of BOP sites in seropositive subjects. Since the inclusion criteria demanded that all sites should be >4 mm at baseline, 100% of sites had a PD of >3.5 mm at this stage when related to later time points in Tables 5 and 6. This table is presented to show that seropositive patients did not have better clinical presentation at baseline, which may have contributed to their better response to treatment.

The response to treatment was assessed after division of the test group into subgroups according to serostatus at baseline. It should be noted that one patient dropped out of the study between post-HPT and the first maintenance visit. Table 5 presents the data according to serostatus for *P. gingivalis*, and Table 6 presents the data for *A. actinomycetemcomitans*. Seropositive subjects for IgG against *P. gingivalis* demonstrated a significantly lower percentage of sites with a PD of >3.5 mm at the end of the hygiene phase of therapy. Similarly, seropositive subjects for IgA against *P. gingivalis* demonstrated a tendency towards fewer sites with a PD of >3.5 mm at post-HPT and significantly fewer deep and bleeding sites at the first maintenance visit. No significant differences in the response to treatment were observed between seropositive and seronegative subjects in terms of antibodies to *A. actinomycetemcomitans.*

The PCR analysis showed that 5 of 17 (29%) patients were *P. gingivalis* positive and 8 of 17 (47%) were *A. actinomycetemcomitans* positive in at least one site.

DISCUSSION

The results presented here suggest that the further development of the humoral immune response to suspected periodontopathogens is predicated on serostatus before treatment commences. For example, Table 2 demonstrates a large and statistically significant increase in IgG avidity in *P. gingivalis* for initially seropositive patients, whereas seronegative patients showed no change in avidity but a tendency toward increased titers (+474 EU, $P = 0.08$) which failed to reach statistical significance. Initial serostatus is dependent on a number of factors, including timing and extent of previous exposure to the subgingival microflora, the nature of the latter, and host susceptibility.

The effect of treatment on antibody responsiveness may result from an inoculation effect during scaling and root planing or from the reduction in antigen load resulting from these procedures and improved oral hygiene, or from a combination of these two mechanisms. Reduction in antigen load is known to result in selection of B-cell clones producing higher-avidity antibodies (19). Treatment may also permit the development of a normal maturation of the immune response, perhaps mediated by an inoculation effect, leading to antibodies of increasingly higher avidity.

Patient subgrouping was performed by Chen et al. (2) in their investigation of treatment effects on rapidly progressive periodontitis patients. They found that avidities of IgG antibody to *P. gingivalis* increased in their seropositive subgroup after therapy, whereas titers decreased. Therefore, the present study tends to confirm this effect in adult periodontitis patients. It should also be noted that antibody avidities to periodontopathogens are generally low in humans compared with those to other commonly occurring antigens and also compared with avidities observed in animals immunized with periodontopathogens (2, 20, 40).

The present study, being concerned with the systemic immune response, did not set out to relate site microbiology to site or systemic immune response. However, plaque samples were analyzed by PCR in order to provide some data on this point. Mombelli et al. (22) showed that when whole-mouth analysis for *P. gingivalis* was carried out in periodontal disease patients, 30% of patients were organism negative at all sites. Moreover, a further 30% of patients had this organism in only 1 to 10% of sites. This suggests that at any particular time, a maximum of 40% of patients with periodontal disease would be classified as *P. gingivalis* positive by a representative sample of eight sites. Our PCR analysis showed 29% of patients were *P. gingivalis* positive and 47% were *A. actinomycetemcomitans* positive. This compares well with another recent study in our laboratory showing that 12 of 43 (28%) patients were *P. gin-*

TABLE 5. Response to treatment by initial serostatus in terms of IgG, IgM, and IgA antibodies to *P. gingivalis*[a]

Antibody	Subgroup	% of sites (SD)					
		Post-HPT			Maintenance visit		
		n	PD >3.5 mm	BOP	*n*	PD >3.5 mm	BOP
IgG	Seropositive	9	52 (18)	40 (20)	9	28 (23)	24 (16)
	Seronegative	8	73 (22)	39 (27)	7	43 (28)	45 (24)
	P		0.047	0.958		0.288	0.082
IgA	Seropositive	11	55 (22)	42 (15)	10	28 (23)	23 (16)
	Seronegative	6	75 (16)	35 (34)	6	54 (29)	50 (21)
	P		0.053	0.689		0.048	0.025
IgM	Seropositive	3	58 (44)	46 (7)	3	34 (31)	37 (37)
	Seronegative	14	63 (17)	38 (25)	13	30 (23)	32 (19)
	P		0.872	0.337		0.317	0.834

[a] The average percentage of sites with a PD of >3.5 mm and BOP at the end of HPT and at the first maintenance visit are given. Comparisons between seropositive and seronegative subgroups were made by two-sample *t* tests.

TABLE 6. Response to treatment by initial serostatus in terms of IgG, IgM, and IgA antibodies to *A. actinomycetemcomitans*[a]

Antibody	Subgroup	% of sites (SD)					
		Post-HPT			Maintenance visit		
		n	PD >3.5 mm	BOP	*n*	PD >3.5 mm	BOP
IgG	Seropositive	5	66 (19)	39 (19)	5	44 (28)	26 (31)
	Seronegative	12	60 (24)	40 (25)	11	31 (25)	36 (17)
	P		0.600	0.952		0.403	0.527
IgA	Seropositive	3	52 (13)	40 (24)	3	30 (18)	18 (16)
	Seronegative	14	64 (23)	39 (23)	13	36 (27)	37 (22)
	P		0.278	0.971		0.662	0.154
IgM	Seropositive	8	70 (29)	45 (22)	7	43 (29)	33 (29)
	Seronegative	9	56 (13)	35 (24)	9	28 (21)	34 (16)
	P		0.229	0.384		0.282	0.931

[a] See Table 5, footnote *a*.

givalis positive and 40% were *A. actinomycetemcomitans* positive based on representative site sampling (30).

There are few data on the relevance of differences in immune response to periodontopathogens to clinical improvement. However, Mouton et al. (25) demonstrated that initially seropositive patients responded better to therapy than those who were initially seronegative. Moreover, a recent study by our group suggested that the tendency to develop further attachment loss in maintenance patients is related to antibody avidity to *P. gingivalis* (23). In an earlier report, we demonstrated that gingival crevicular fluid IgG titers to *P. gingivalis* were lower in deeper pockets and more inflamed sites in maintenance patients (18). This suggested that failure of production of antibody at the systemic and/or local level combined with failure of deployment at the local level, perhaps caused by low binding strength (avidity) and/or degradation or inactivation of immunoglobulin by microorganisms (12, 15), may lead to further periodontal breakdown on a site-specific basis.

A recent experimental gingivitis study by Danielsen et al. (3) showed that reductions in serum IgG titers to *P. gingivalis* during the experiment were predominantly attributable to subjects who had high initial titers and went on to develop marked gingival inflammation during plaque accumulation. They suggested that the development of more extensive gingival inflammation in these subjects, perhaps equivalent to seropositive or previously sensitized individuals, could be a prerequisite for successful reduction or elimination of the organism through protective immune and inflammatory mechanisms.

Moreover, Ranney et al. (29) and Gunsolley et al. (16) demonstrated less disease in patients with higher antibody titers than in those with lower titers. However, a contradictory finding by Chen et al. (2) was the observation that there was a statistically significant, albeit weak, negative correlation between IgG antibody avidity to *P. gingivalis* whole-cell sonicate and both severity of bone loss and pocket depth. This latter study, however, investigated rapidly progressive periodontitis rather than adult periodontitis subjects, and the negative correlation did not apply to *P. gingivalis* LPS or protein. The significance of this study is therefore difficult to assess.

The present study indicates that the characteristics of the humoral immune responses to *P. gingivalis* and *A. actinomycetemcomitans* serotype a may be quite different. Avidities to *P. gingivalis* tended to increase after therapy in seropositive patients, whereas titers to *A. actinomycetemcomitans* tended to increase in seronegative patients.

Table 1 demonstrates that the increase in IgG avidity to *P. gingivalis* during the course of therapy in the entire patient group is, when considered with the data in Table 2, attributable to the influence of the seropositive subgroup. Our data suggest that seropositive subjects, in terms of IgG and IgA against *P. gingivalis*, demonstrate a tendency toward a higher chance of clinical improvement after conventional periodontal therapy (Tables 5 and 6). This is despite the finding that seropositive subjects tended to have deeper mean PDs at baseline (Table 4). Although the significance of our findings is weak, our data agree with the observation of Mouton et al. (25), who demonstrated that initially seropositive patients responded better to therapy than those who were initially seronegative. This observation would also concur with those of Ebersole et al. (4) and Danielsen et al. (3). The suggestion is that the prior development of a protective (although in an attenuated fashion) humoral immune response has a positive contribution to disease resolution during and after therapy. A recent study by Sjostrom et al. has also indicated that scaling and root planing induce a humoral immune response which has a role in the beneficial effects of periodontal treatment (34).

There are indications that the humoral immune response to periodontopathogens may be unique in character. There have also been indications from previous studies and the present study that previous exposure and host susceptibility have a bearing on both the development of periodontal disease and response to treatment. However, further work on the effects of periodontal treatment on the humoral immune response is required to elucidate this phenomenon.

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