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Effect of Subgingival Scaling on Systemic Antibody Responses to Oral Microorganisms

JEFFREY L. EBERSOLE,[1,2]* MARTIN A. TAUBMAN,[1,2] DANIEL J. SMITH,[1,2] AND ANNE D. HAFFAJEE[2,3]
Department of Immunology,[1] Department of Periodontology,[3] and Center for Clinical Research in Periodontal Disease,[2] Forsyth Dental Center, Boston, Massachusetts 02115

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The effects of scaling and root planing treatment on systemic antibody responses were studied in patients with periodontal disease and in normal subjects. Immunoglobulin G antibody in serum to a battery of oral microorganisms was assessed in an enzyme-linked immunosorbent assay before and after treatment in 31 individuals. The majority (96%) of the diseased patients exhibited elevated antibody to one or more of the microorganisms before the scaling regime. Significant increases in antibody levels in serum were noted in 16 of 19 patients after scaling, whereas only 2 of 12 nonscaled subjects showed similar changes during monitoring intervals of up to 3 years. The bacterial specificities of the increases were found to differ among the patients; however, a significant correlation to preexisting elevated antibody levels was observed. Peak levels of responses were noted at approximately 2 to 4 months posttreatment; antibody returned to pretreatment levels by 8 to 12 months. The predominant organisms for which changes were noted included the black-pigmented *Bacteroides* spp., *Eikenella corrodens*, *Campylobacter concisus*, and *Actinobacillus actinomycetemcomitans*. In 18 of 19 instances, the homologous microorganism was detected in the subgingival plaque when elevated antibody was present after treatment. These findings indicated that specific changes in host systemic responses accompany scaling and root planing treatment of periodontal disease patients. These alterations in the host response may provide an additional means by which successful therapy can be accomplished.

Systemic antibody responses are frequently seen as a manifestation of bacterial infections (25). Numerous studies have shown the presence of antibody in serum to microorganisms colonizing the oral cavity (7, 11, 21, 30, 32). Recent studies have also suggested that elevated responses to certain of these microorganisms are associated with periodontal diseases (7, 11, 21, 30, 32). In these studies, host responses to the organisms were examined by using cross-sectional analyses of antibody in serum from groups of diseased and normal subjects. However, the immune response to oral colonization is not necessarily static but may fluctuate during disease (12, 13) or treatment.

Mechanical removal of subgingival plaque (scaling) is a standard treatment for periodontal diseases (3, 17, 20). Frequently, bacteremias have been shown to accompany treatment of dental diseases (23). The purpose of the present study was to examine the longitudinal effects on systemic antibody levels that result from scaling the teeth of patients with periodontal disease.

MATERIALS AND METHODS

Patients and samples. Subjects examined in this study were patients from the Clinical Center for Periodontal Disease Research at Forsyth Dental Center. The individuals were clinically monitored as described by Haffajee et al. (14). Patients from the following four characteristic groups were included in the study: localized juvenile periodontitis (LJP); advanced destructive periodontitis (ADP); adult periodontitis (AP); and normal (N). The criteria for the disease classifications have been described previously (7, 8). Blood was collected at various intervals from the subjects by venipuncture, and serum was removed after centrifugation (1,400 × *g* for 10 min) of the clots. The serum was stored at −20°C until analyzed for antibody.

Microorganisms. The microorganisms used as antigens in the antibody analysis were cultured in broth, killed by formalinization (0.5% buffered formal saline), and harvested as described previously (5). The 16 organisms included in the assay were: *Actinomyces israelii* B11, *Actinomyces naeslundii* I, *Actinobacillus actinomycetemcomitans* Y4, *Bacteroides intermedius* 581, *Bacteroides gingivalis* 381, *Bacteroides gracilis* 1084, *Bacteroides oralis* ATCC 33321, *Bacteroides melaninogenicus* 287, *Campylobacter concisus* 484, *Capnocytophaga sputigena* 4, *Eikenella corrodens* 373 and 1073, *Fusobacterium nucleatum* 364, *Streptococcus mutans* Ingbritt, *Streptococcus sanguis* 254, and *Wolinella recta* 371.

Antibody analysis. Formalinized bacteria were used as antigens in microtiter wells of polystyrene plates (Linbro). Optimal conditions were determined for each microorganism as described previously (5). To estimate antibody activity in the samples, the human sera were incubated in the antigen-coated plates, followed by the addition of rabbit anti-human immunoglobulin G (IgG) (lot 010394; 2.25 mg/ml; Calbiochem-Behring). The system was developed by incubation of goat anti-rabbit IgG (G134; 14.8 mg/ml; Miles Laboratories) conjugated to alkaline phosphatase (9) and *p*-nitrophenylphosphate as substrate. The extent of reaction was determined at 405 nm with an enzyme-linked immunosorbent assay (ELISA) reader (ARTEK).

Antibody levels in the samples are expressed as ELISA units (EU). A standard human serum for each microorganism was assigned a value of 100 EU. A linear reference curve was generated by comparing the optical density of the serum reaction with the log of the EU in the standard. EU levels in the experimental samples were determined by relating the optical density to the linear portion of the reference curve.

SeroELISA. The seroELISA was used to detect the presence of an organism in sites sampled from the patients (6). Briefly, species-specific antisera to the organisms were pre-

* Corresponding author.

TABLE 1. Summary of increases in antibody responses to specific microorganisms in scaled and nonscaled subjects

Patient[a]	Elevated antibody preexisting to[b]:	Increase in response to[c]:	Interval monitored (days)
Scaled			
LJP1	*Actinobacillus actinomycetemcomitans* Y4	*Actinobacillus actinomycetemcomitans* Y4 *Capnocytophaga sputigena* 4, *E. corrodens* 373	458
ADP1	*Actinobacillus actinomycetemcomitans* Y4, *Capnocytophaga sputigena* 4, *F. nucleatum* 364	*Actinobacillus actinomycetemcomitans* Y4, *Capnocytophaga sputigena* 4, *E. corrodens* 373	490
ADP2	*E. corrodens* 1073, *S. mutans* Ingbritt	*E. corrodens* 1073, *Campylobacter concisus* 484	477
ADP3	*E. corrodens* 1073	*Campylobacter concisus* 484, *E. corrodens* 1073	329
AP1	*B. gingivalis* 381, *E. corrodens* 1073	*B. gingivalis* 381, *Campylobacter concisus* 484	361
AP2	*E. corrodens* 1073	*E. corrodens* 1073	156
AP3	*B. gingivalis* 381, *B. intermedius* 581	*B. intermedius* 581	273
AP4	*Campylobacter concisus* 484, *F. nucleatum* 364	*B. intermedius* 581, *F. nucleatum* 364	390
AP5	*E. corrodens* 373, *F. nucleatum* 364	*F. nucleatum* 364	392
AP6	*B. gingivalis* 381	*B. oralis* ATCC 33321	283
AP7	*Campylobacter concisus* 484	*B. gingivalis* 381, *Campylobacter concisus* 484, *B. intermedius* 581, *W. recta* 371	444
AP8	*Campylobacter concisus* 484	*B. gingivalis* 381, *E. corrodens* 1073, *Actinomyces israelii* B11, *B. oralis* ATCC 33321	352
AP9	—[d]	—	115
AP10	*B. intermedius* 581	*B. intermedius* 581, *B. gracilis* 1084	364
AP11	*B. gingivalis* 381, *W. recta* 371	*B. gingivalis* 381, *B. intermedius* 581, *Capnocytophaga sputigena* 4	258
AP12	*B. gingivalis* 381, *F. nucleatum* 364	*B. gingivalis* 381, *B. gracilis* 1084	305
AP13	*S. sanguis* 254	—	371
AP14	*Actinobacillus actinomycetemcomitans* Y4, *E. corrodens* 373	*Actinobacillus actinomycetemcomitans* Y4, *Capnocytophaga sputigena* 4, *W. recta* 371	314
AP15	*B. intermedius* 581	—	315
Nonscaled			
N1	—	—	834
N2	—	—	1,275
N3	—	—	723
N4	—	—	575
N5	—	—	515
N6	—	—	605
AP16	*B. gingivalis* 381, *B. intermedius* 581	—	240
AP17	*Capnocytophaga sputigena* 4, *E. corrodens* 1073	—	250
AP18	*B. gingivalis* 381, *B. gracilis* 1084	—	315
AP19	*B. gingivalis* 381	—	195
AP20	*B. gingivalis* 381	*B. intermedius* 581	563
AP21	*E. corrodens* 1073	*E. corrodens* 1073	455

[a] Patient classifications: LJP, localized juvenile periodontitis; ADP, advanced destructive periodontitis; AP, adult periodontitis; N, normal.
[b] Antibody existing at a level greater than the mean plus 2 standard deviations of a normal population.
[c] Increase in antibody was defined as a level greater than the mean plus 2 standard deviations of the level in the patient before scaling.
[d] —, None detected.

pared in rabbits and conjugated to horseradish peroxidase. Organisms sampled from the subgingival area (22) were cultured anaerobically on blood agar plates. The colonies from the primary isolation plates were scraped and suspended in phosphate-buffered saline (0.02 M phosphate [pH 7.4]). The bacterial suspensions were attached to microtiter wells by centrifugation and addition of glutaraldehyde. The presence of the organism was detected by a positive reaction identified by a conversion of *N,N,N′,N′*-tetramethylbenzidine (19) to a blue precipitate.

Treatment. Thirty-one subjects were monitored clinically and immunologically at 2-month intervals for up to 27 months. Twelve individuals (nonscaled group) did not demonstrate significant periodontal attachment loss at any site during the monitoring period. These individuals did not receive any periodontal therapy during the monitoring period. Six of these individuals had evidence of prior destructive periodontal disease and were considered to be adult

TABLE 2. Relationship between preexisting antibody levels and response to scaling

Treatment group[a]	Ratio of elevated or normal antibody levels in serum to antibody increases after scaling: No. of elevated antibody responses with increase in antibody/total no. of elevated antibody responses	No. of normal antibody responses with increase in antibody/total no. of normal antibody responses
Scaled	16/38 (0.421)[b]	21/266 (0.076)[b]
Nonscaled	1/9 (0.111)	1/183 (0.005)

[a] Total numbers of elevated and normal antibody levels were determined by the number of patients in each group multiplied by the number of organisms tested. Therefore, for the scaled group, 19 × 16 = 304, and for the nonscaled group, 12 × 16 = 192.
[b] Statistically different: $P = 0.001$ (χ^2 test).

periodontitis subjects. The remaining six individuals in this group had no clinical evidence of destructive periodontal disease and were considered to be N subjects.

The scaled group consisted of 19 individuals (1 LJP, 3 ADP, 15 AP) who showed evidence of active destructive periodontal disease at one or more sites as determined by changes in attachment level measurements (14). These individuals were treated by scaling and root planing accompanied by repeated reinforcement of home care procedures. The scaling procedures required 1 to 4 h to complete, depending on the severity of the disease and the extent of the bacterial plaque and calculus accumulations. Some individuals received more than one course of scaling at intervals of approximately 3 to 4 months.

RESULTS

Distribution of systemic antibody. IgG antibody levels in serum to the battery of oral microorganisms were examined in 31 subjects. Pretreatment antibody response patterns were assessed in all patients. Nineteen subjects were scaled, and 12 received no scaling regime. Most individuals exhibited elevated antibody levels to organisms from the heterologous battery (Table 1). Subsequently, the sera from each subject was tested in triplicate, and an increase of >2 standard deviations above the mean antibody level from the same patient before scaling was selected as a positive change. A significant increase in the antibody level in serum was noted in 16 of 19 scaled patients, whereas only 2 of 12 untreated patients exhibited a change during the monitoring period (P = 0.01). The results suggest that the scaling treatment may provide an active immunization with bacteria colonizing the gingival crevice.

Microbial specificity of the systemic antibody. A significant increase in the number of patients with elevations in systemic antibody was noted after scaling. However, whether these were elevations to specific microorganisms in the flora or whether they represented a polyclonal stimulation of the host response was unclear. Table 1 shows the organisms to which alterations in antibody responses were detected, as well as the pre- and posttreatment intervals of monitoring for each of the patients. Increases in the response in individual patients were specific for only a few organisms. The monitoring intervals extended from 3 to 16 months in the treated patients. There was no generalized response to the organisms tested. Also, a significant predilection for increases in the antibody responses postscaling was associated with preexisting elevated antibody levels (Table 2). In more than 42% of the cases in which an initial elevated antibody level in serum was detected to a microbial species, an increase was demonstrated after treatment. In contrast, increases in antibody levels posttreatment were seen less than 8% of the time when levels of antibody were within the normal range pretreatment.

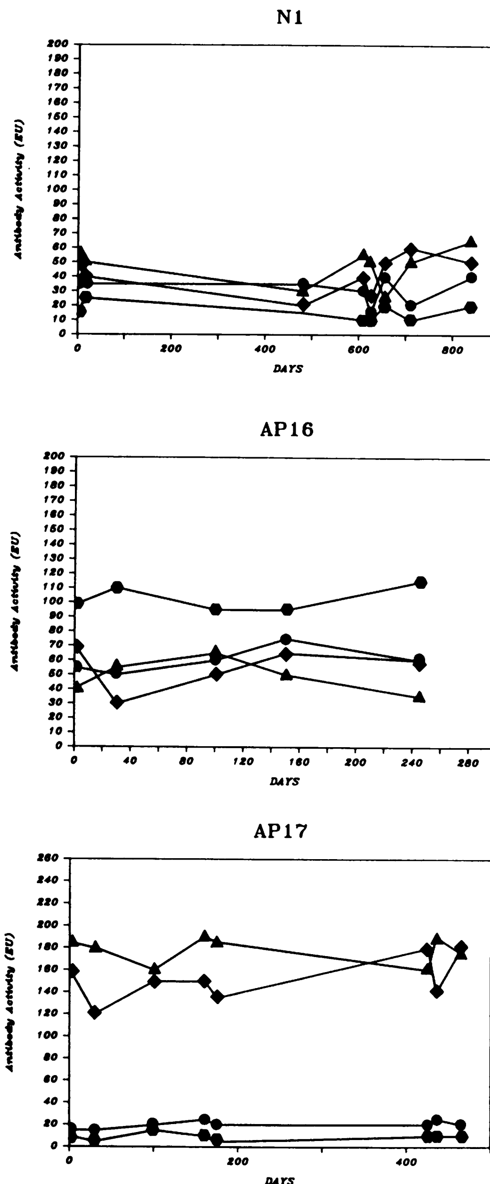

FIG. 1. Systemic antibody levels in untreated normal (N1) and adult periodontitis (AP16 and AP17) subjects. The points represent the means of triplicate determinations of antibody levels in serum to *Capnocytophaga sputigena* (▲), *Eikenella corrodens* 1073 (◆), *Actinobacillus actinomycetemcomitans* Y4 (●), and *B. gingivalis* (●). Standard errors of the means were less than 14%.

TABLE 3. Number of subjects in which significantly increased antibody responses were detected to specific microorganisms

Organism	No. of subjects showing increased response				
	Scaled			Nonscaled	
	AP	ADP	LJP	AP	N
B. gingivalis	5	—[a]	—	—	—
B. intermedius	5	1	—	1	—
E. corrodens	3	3	1	1	—
Campylobacter concisus	2	2	—	—	—
Capnocytophaga sputigena	2	1	1	—	—
B. oralis	2	—	—	—	—
B. gracilis	2	—	—	—	—
W. recta	2	—	—	—	—
Actinobacillus actinomycetemcomitans	1	1	1	—	—
Actinomyces israelii	1	—	—	—	—
F. nucleatum	1	—	—	—	—
S. mutans	1	—	—	—	—
S. sanguis	—	—	—	—	—
Actinomyces naeslundii	—	—	—	—	—
B. melaninogenicus	—	—	—	—	—

[a] —, No increase in antibody levels in any patient to these organisms.

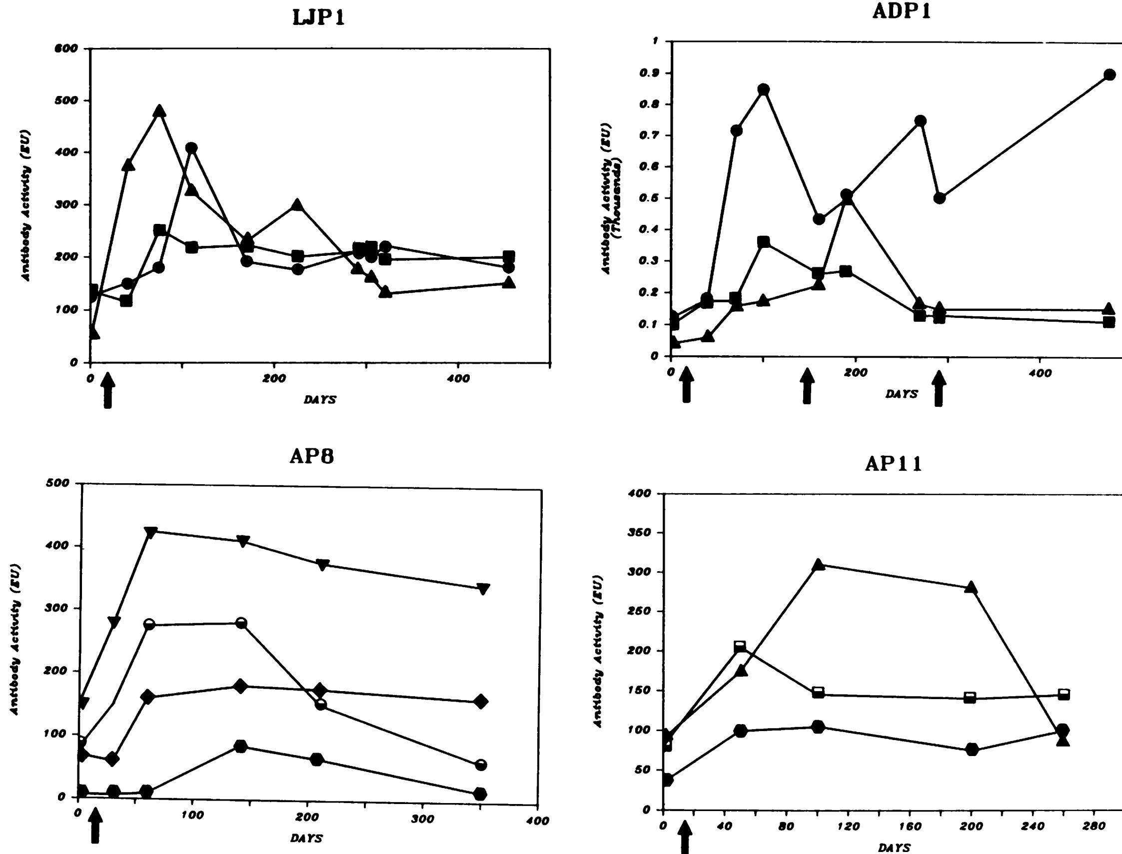

FIG. 2. Systemic antibody levels in localized juvenile periodontitis (LJP1), advanced destructive periodontitis (ADP1), and adult periodontitis (AP8 and AP11) patients that were scaled (↑) at least once during the monitoring interval. The points represent the means of triplicate determinations of antibody levels in serum to *Capnocytophaga sputigena* (▲), *E. corrodens* 373 (■) and 1073 (◆), *Actinobacillus actinomycetemcomitans* (●), *B. oralis* (▼), *Actinomyces israelii* (⊖), *B. gingivalis* (⬣), and *B. intermedius* (⊟). Standard errors of the means were less than 17%.

Since recent microbiological and immunological evidence has indicated that some microorganisms may be associated with certain periodontal disease processes, the frequency of increases in antibody levels to specific microorganisms was examined (Table 3). The results suggested that changes were most frequently associated with the black-pigmented *Bacteroides* spp. group (*B. gingivalis* and *B. intermedius*), *E. corrodens*, and *Actinobacillus actinomycetemcomitans*. These findings are consistent with the high prevalence of these organisms in periodontal lesions.

Kinetics of the systemic antibody response. Longitudinal monitoring of antibody in sera of both treated and untreated patients indicated a consistent pattern of antibody changes. Figure 1 shows the consistency of antibody levels in representative untreated subjects monitored over a 2-year interval. This pattern was noted in the untreated individuals, whether the initial levels were elevated or within the normal range of antibody activity.

In contrast to the finding in untreated subjects, significant increases of antibody in serum to subgingival microorganisms are shown for four representative patients after a scaling regimen (Fig. 2). Generally, the response levels peaked approximately 2 to 4 months after scaling. In patients in which increases to multiple organisms were noted, the kinetics of the responses were similar. Additionally, in patients that received multiple episodes of scaling, an increase in antibody levels was detected to some oral microorganisms after each treatment (Fig. 2). Continued monitoring of the responses suggested that the levels returned to prescaling levels by 8 to 12 months posttreatment.

Relationship of systemic antibody increase to the presence of the organism. Before the scaling regime, 9 of the 19 treated patients were monitored for the presence of the organisms the scaling regime. The seroELISA was used to detect a species of organism cultured from suspected lesions in the patients. In the nine patients, changes in IgG antibody levels in serum to 19 oral microorganisms were demonstrated after scaling (Table 4). Eighteen of the organisms to which a significant increase in level was noted were detected in plaque samples from the respective patients. Therefore, significant increases in antibody levels in serum after scaling were probably indicative of a specific response to a microorganism that is present in the periodontal flora of the host before scaling. In addition, at least one microorganism to which the host did not show a change in response was always identified in the bacterial samples from the patients.

DISCUSSION

The presence of systemic humoral (7, 11, 21, 30, 32) and cellular (15, 16, 26) reactivities to a variety of oral microorganisms have been found in subjects with different forms of periodontal disease. In general, in these studies, the immune responses were examined as if they represented a static phenomenon during the course of active disease or treatment or both (12, 13). However, since recent evidence

TABLE 4. Relationship between presence of microorganism and increase in antibody levels after scaling[a]

Patient	Increase in antibody, organism detected	No increase in antibody, organism detected	Increase in antibody, no organism detected
LJP1	*Actinobacillus actinomycetemcomitans* Y4, *Capnocytophaga sputigena* 4, *E. corrodens* 373	*F. nucleatum* 364, *B. intermedius* 581	—[b]
ADP1	*Actinobacillus actinomycetemcomitans* Y4, *Capnocytophaga sputigena* 4, *E. corrodens* 373	*F. nucleatum* 364	—
ADP2	*E. corrodens* 1073, *Campylobacter concisus* 484	*B. intermedius* 581, *F. nucleatum* 364	—
ADP3	*E. corrodens* 1073, *Campylobacter concisus* 484	*F. nucleatum* 364, *W. recta* 371, *B. gracilis* 1084	—
AP2	*E. corrodens* 1073	*F. nucleatum* 364, *Campylobacter concisus* 484	—
AP3	*B. intermedius* 581	*E. corrodens* 1073, *B. gracilis* 1084, *F. nucleatum* 364, *W. recta* 371	—
AP6	—	*B. gingivalis* 381, *B. melaninogenicus*, *F. nucleatum* 364, *W. recta* 371	*B. oralis* ATCC 33321
AP8	*B. gingivalis* 381, *E. corrodens* 1073, *B. oralis* ATCC 33321	*F. nucleatum* 364, *B. gracilis* 1084	—
AP14	*Actinobacillus actinomycetemcomitans* Y4, *Capnocytophaga sputigena* 4, *W. recta* 371	*F. nucleatum* 364, *B. intermedius* 581	—

[a] Subgingival plaque samples were collected, dispersed, and diluted, and three dilutions of each sample were cultured on blood agar plates. All colonies were scraped from the surface of each dilution plate and duplicate wells tested with each of the conjugated species-specific rabbit antisera. Negative samples were determined by the absence of reactivity in any of the seroELISA wells. A positive reaction was defined as duplicate positive reactions noted for any of the dilutions plated.

[b] —, None detected.

suggests a cyclical nature for periodontal disease lesions (12), the possibility existed that treatment might simultaneously affect both the clinical parameters and the immunological responses to specific microorganisms. The results of the present investigation indicate that mechanical debridement of subgingival plaque (scaling) can elicit a significant increase in circulating antibody levels to various periodontal disease-associated microorganisms. Similar changes in cell-mediated immune responses have also been found postscaling (24). Greater than 84% of the scaled patients exhibited changes in IgG antibody in serum to at least one microorganism, and the responses were generally limited to specific organisms within each patient. Peak levels in responses were consistently observed 2 to 4 months after scaling regardless of the microorganism eliciting the response. In previous studies from our laboratory, antibody response patterns have been identified in the serum of patients with periodontal disease (8). In this regard, the patients in this study exhibited distinct antibody patterns to oral microorganisms (Table 1). A significant relationship existed between increases in antibody levels in serum and preexisting elevated antibody levels in the patients (Table 2). These findings further support the association between specific microorganisms and periodontal disease. However, the primary emphasis concerning microbial involvement in the disease appears to be directed toward the individual patient and not necessarily associated with arbitrary disease classifications. Further studies are required to better define the individual host-parasite interactions in the development of periodontal disease.

Numerous investigations have shown the efficacy of scaling and plaque control in the treatment of gingivitis (2, 18, 31) and periodontitis (3, 17, 20). Indeed, scaling and plaque control have been shown to be as effective as surgical modalities in the treatment of certain forms of periodontal disease (3, 17, 20). The success of scaling and plaque control has most often been attributed to qualitative and quantitative changes in the microflora (10, 28, 29). However, it is well documented that manipulation of oral tissues frequently results in the passage of plaque microorganisms into the circulation (23). Parenteral immunization with bacterial vaccines has long been shown to elicit protective immunity (1, 4, 27). The present study indicates that scaling may provide a means of active immunization with periodontal microorganisms.

In almost every instance, the microbial species eliciting the elevated humoral immune response could be detected in samples of subgingival plaque from that subject. However, in many instances, microbial species could be detected in subgingival plaques which did not elicit an increased antibody response after treatment. These data reflect the specificity of the elevated responses to particular microorganisms. The factors which control this specificity in response have not yet been elucidated.

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