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Effect of Treatment on Titer, Function, and Antigen Recognition of Serum Antibodies to *Actinobacillus actinomycetemcomitans* in Patients with Rapidly Progressive Periodontitis

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Although periodontal treatment by scaling and root planing (SCRP) is known to induce bacteremia, the effect of this procedure on the host immune response is not known. We have determined pre- and post-SCRP immunoglobulin G antibody titers to antigens of *Actinobacillus actinomycetemcomitans* in the sera of 22 patients with rapidly progressive periodontitis. We also assessed the ability of these sera to enhance phagocytosis and killing of *A. actinomycetemcomitans* by human polymorphonuclear leukocytes by using a polymorphonuclear leukocyte chemiluminescence (CL) assay. Specific anti-*A. actinomycetemcomitans* antibody titers were significantly increased at 6 and 12 months after beginning treatment, and CL values were significantly increased at 12 months, whereas mean interproximal pocket depths were significantly decreased at 12 months after beginning treatment. When patients were classified as either seropositive (twice the median titer of control subjects; $n = 10$) or seronegative ($n = 12$), both median titers and CL values were significantly increased for the seronegative group at 6 and 12 months after treatment. In the seropositive group, only the median titer was significantly increased at 12 months. Western blot (immunoblot) patterns for six seronegative and six seropositive patients differed remarkably at the baseline. Before treatment, all of the seropositive patients recognized high-molecular-mass lipopolysaccharide (LPS) and a large number of protein components. Patterns were virtually unaffected by therapy. Before treatment, only one of the seronegative patients recognized the LPS smear and none reacted strongly with protein components. Following treatment, slight LPS staining was observed for five of six seronegative patients and detection of protein bands was enhanced in all cases. We conclude that treatment by SCRP induces a humoral immune response, especially in seronegative patients, and that response may play a role in the observed beneficial effects of periodontal treatment.

Rapidly progressive periodontitis (RPP) is an aggressive, early-onset form of periodontitis observed in young adults that affects most of the teeth (5, 16). *Actinobacillus actinomycetemcomitans*, a gram-negative, facultative anaerobic microorganism found in the subgingival flora of many patients, is considered to be etiologically involved in the disease (25, 26, 35). Many, although not all, RPP and localized juvenile periodontitis patients produce detectable levels of serum antibodies reactive with *A. actinomycetemcomitans* antigens during the course of their spontaneously occurring disease (8, 24, 32). The role these antibodies play in the progress and resolution of these periodontal diseases and in susceptibility to infection with *A. actinomycetemcomitans* is not understood. Several recent studies indicate that such antibodies may be protective; they may prevent subclinical infection from progressing to clinical disease, and they may limit progression of established disease (2, 13, 20, 24, 29, 30).

A recent study by Chen et al. (6) demonstrated that treatment modalities, including scaling and root planing (SCRP) and various forms of surgery, induce the production of serum antibodies specific for antigens of periodontopathic bacteria in RPP patients, specifically in patients who are seronegative prior to therapy, and that avidity of serum antibodies is significantly enhanced. However, in that study the type of therapy performed and the time posttherapy when serum samples were harvested were not standardized. Furthermore, direct measures of antibody function, such as opsonization of bacteria for polymorphonuclear leukocyte (PMN)-mediated killing, were not done.

The purpose of the present study was to assess the effects of therapy consisting only of SCRP on *A. actinomycetemcomitans*-specific antibodies in serum samples harvested prior to therapy and at 6 and 12 months after beginning therapy. Both antibody titers and the capacity of the antibodies to opsonize *A. actinomycetemcomitans* for phagocytosis and killing by human PMNs were assessed. In addition, we wanted to determine whether treatment-induced antibodies recognized new antigens or whether there was enhanced reactivity with antigens recognized by pretreatment sera.

MATERIALS AND METHODS

Patients. Informed consent was obtained from all study participants, in accordance with a protocol approved by the

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University of Washington Human Subjects Division of the Office of Research. Venous blood was collected at the baseline from 30 patients diagnosed as having RPP on the basis of published criteria (5, 16). Ages ranged from 25 to 40 (mean, 32.97 ± 4.77) years; 17 were male, and 13 were female; 1 was Asian, 1 was African-American, and the remainder were Caucasian. All patients had radiographic evidence of bone loss prior to age 35; they had 20 or more teeth, with at least 7 teeth other than first molars and central incisors having a minimum of 5 mm of attachment loss and 4-mm or greater probing depths. The mean interproximal pocket depth (IPPD) was 4.79 ± 0.97 (range, 3.6 to 8.3) mm. Mean bone loss was 26.48% ± 7.82% (range, 11.9 to 48.5%) (22). Titers and chemiluminescence (CL) values for baseline blood samples from this group and from matched controls have been reported previously (24). A subset of 22 individuals from that RPP patient group (12 male, 10 female) participated in the study we now report.

Treatment consisted of SCRP with a local anesthetic at intake and at 3, 6, 9, and 12 months. Maxillary and mandibular quadrants on one side were treated, and the quadrant on the other side was treated 1 week later. Venous blood was collected prior to beginning therapy and at 6 and 12 months after initial therapy and sera obtained after clotting and centrifugation were stored at −30°C until used. Longitudinal samples were assayed simultaneously, after heating for 30 min at 56°C to inactivate serum complement. Of the 22 subjects, 5 had serum samples from 6 and 12 months. An additional 7 had serum samples from 6 months only, and an additional 10 had samples from 12 months only. Mean IPPD scores were calculated from periodontal charting done at the time of initial examination and at 12 months by using standard periodontal procedures. Evaluation and treatment were carried out in the University of Washington Graduate Periodontics Clinic.

Bacteria. *A. actinomycetemcomitans* ATCC 43718 (serotype b, strain Y4) was grown and prepared as previously reported (24). Briefly, bacterial cultures were grown in brain heart infusion broth (Difco, Detroit, Mich.) in a reduced-O_2 atmosphere, harvested, and suspended in gelatin-Veronal-buffered saline (pH 7.5) supplemented with 5% glucose (GGVB++) (11) at a concentration of 3×10^7 to 1×10^8 CFU/ml. Broth cultures were diluted and plated on brain heart infusion plates for enumeration.

Complement source. A C5-depleted human serum preparation (Quidel Laboratories, San Diego, Calif.) was used as the complement source in these assays. Aliquots were stored and supplemented as described previously (24). The complement preparation was used at a final dilution of 1:500 in GGVB++ in all assays.

CL assay. A 5-amino-2,3-dihydro-1,4-phthalazinedione (luminol; Sigma Chemical Co., St. Louis, Mo.)-enhanced PMN CL assay was employed to measure opsonization as previously reported (24). Briefly, PMN from a periodontally normal donor (3×10^5 cells per ml), heat-inactivated serum (final dilution, 1:50), an *A. actinomycetemcomitans* suspension (final dilution, 3×10^6 CFU/ml), and complement were mixed in the presence of 1.2 mM luminol (total assay volume, 1 ml). All reagents were diluted in GGVB++. Measurements were taken on a Bioscan 1251 luminometer (Wallac, Inc., Gaithersburg, Md.) over a period of 60 to 90 min. The mean peak CL for each serum sample was calculated from three separate experiments.

Enzyme-linked immunosorbent assay (ELISA). Titers of immunoglobulin G (IgG) to a whole-cell homogenate of *A. actinomycetemcomitans* in serum were measured as previously described (33). One microgram (dry weight) of antigen was used per well, a known high-titer serum was used as a control, and IgG was quantitated by using alkaline phosphatase-conjugated goat anti-human IgG (A-5403; Sigma). ELISA units (EU) were calculated by the method of Butler (3).

Immunoblots. Subjects were classified as seropositive if their baseline IgG antibody titers were greater than twice the median of the control group (14.5 EU [24]) (6, 33) and as seronegative if titers were below this value. Twelve sera (six from each group) were selected to represent the range of IgG antibody titer responses among the seropositive and seronegative patients. Western immunoblots (immunoblots) were performed by the method of Towbin et al. (27). A whole-cell sonic extract of lyophilized *A. actinomycetemcomitans* was separated by polyacrylamide gel electrophoresis as previously described (17), with minor modifications. Fifty-microliter aliquots of samples containing 1 mg of protein per ml were separated in 7.5% polyacrylamide gels. Nitrocellulose blots were run at 150 mA and 30 V overnight. Blots were washed five times for 5 min each time in blocking buffer containing 1% bovine serum albumin in 10 mM *N*-tris(hydroxymethyl)methyl-2-aminoethanesulfonic acid (TES)–0.1% Tween 20 (pH 7.5), dried at room temperature, and cut into strips. The strips were incubated in individual patient sera diluted 1:500 or 1:1,000 in blocking buffer for 2 h at room temperature on a rocking platform. After being washed with blocking buffer, strips were incubated for 1 h in goat anti-human IgG-alkaline phosphate conjugate (Sigma) diluted 1:1,000, washed, and finally developed with 5-bromo-4-chloro-3-indolylphosphate–nitroblue tetrazolium phosphate (Kirkegaard & Perry Laboratories, Inc., Gaithersburg, Md.) until antibody-binding bands were visible. All samples were tested at the same time.

Statistical methods. Patients were divided into cohorts for analysis of data. Patients with serum samples at the baseline and at 6 months were termed the 6-month cohort, while patients with serum samples at the baseline and at 12 months were the 12-month cohort. The Wilcoxon matched-pair signed-rank test was used to compare the 6- or 12-month response to the respective cohort baseline response. Seroconversion was considered to occur if a serum classified as seronegative at the baseline reached a posttreatment IgG titer greater than twice the normal median value. Increases in values twofold or greater above the baseline for antibody titer and CL were considered to be significant for the purposes of this study.

RESULTS

Pre- and posttreatment antibody titers and CL values. When patients were analyzed by temporal cohort, we found that median levels of IgG antibody to *A. actinomycetemcomitans* by serum increased significantly after treatment over median values at intake in both the 6- and 12-month cohorts ($P < 0.01$) (Table 1). Although there was an increase in the median CL value at 6 months, it was not significantly different from the baseline ($P = 0.14$). The median CL value at 12 months was significantly greater than the baseline value, primarily because the baseline median value of this cohort was low ($P = 0.001$).

Antibody changes in seropositive and seronegative patients after treatment. The 22 patients enrolled in this study were part of a larger investigation which compared the titer and function of antibody to *A. actinomycetemcomitans* in RPP patients with the responses of a matched normal control

TABLE 1. IgG antibody titers and CL values for 6- and 12-month cohorts before and after initial treatment[a]

Cohort (no. of patients) and time of testing	IgG titer (EU)[b]	CL (mV)[c]	IPPD (mm)[d]
6 month (12)			
Intake	25.00, 37.42 (44.02)	34.83, 68.47 (112.82)	
Posttreatment	83.50, 104.83 (84.60)	62.63, 80.39 (49.88)	
12 month (15)			
Intake	23.00, 53.53 (66.19)	23.33, 57.71 (60.52)	4.85, 4.95 (0.98)
Posttreatment	87.00, 131.87 (132.35)	35.00, 103.00 (106.34)	4.20, 4.26 (0.60)

[a] Values are reported as follows: median, mean (standard deviation).
[b] The 6- and 12-month medians are significantly greater than the respective baseline medians at intake ($P < 0.001$).
[c] The 12-month median is significantly greater than the respective baseline median ($P < 0.01$). The 6-month median does not differ from the respective baseline median ($P = 0.14$).
[d] The 12-month median is significantly lower than the baseline median ($P = 0.0004$). $n = 20$.

group (24). For further analysis, subjects in this study were classified as seropositive if their baseline IgG antibody titers were greater than twice the median titer of the normal control group and seronegative if their titers were below the cutoff. Ten of the patients were seropositive and 12 were seronegative at the baseline. Four of the 10 patients who were seropositive showed a twofold rise in IgG titer over the baseline (Fig. 1), 3 had no change, and the remaining patients showed moderate-to-low titer increases. The median titer for the seropositive patients at 6 months (64 EU) was not significantly different from the baseline titer (45 EU; $P = 0.08$; $n = 5$), but at 12 months it was significantly higher than the baseline titer (106 EU; $P = 0.02$; $n = 7$). Nine of the 12 patients who were seronegative at the baseline showed a twofold or greater rise in titer (Fig. 2), while the remaining 3 showed no change or a slight decline. Seven seronegative patients became seropositive. The median titer at 6 months (103 EU) was significantly greater than the cohort baseline titer (14 EU; $P = 0.03$; $n = 7$). The median titer at 12 months (43 EU) was also significantly higher than the respective baseline median value (11 EU; $P = 0.02$; $n = 8$).

Only 1 of the 10 patients classified as seropositive at the baseline had a greater-than-twofold rise in the CL value over the baseline (Fig. 3). One patient showed a fourfold decrease in CL. There was no significant increase over baseline median CL values for either the 6- or the 12-month seropositive cohort. Eight of the 12 patients classified as seronegative at the baseline had twofold or greater increases in CL values (Fig. 4). The 6-month cohort median CL value (69.3 mV) was significantly greater than the baseline median value (32.0 mV; $P = 0.04$; $n = 7$), and the median CL value at 12

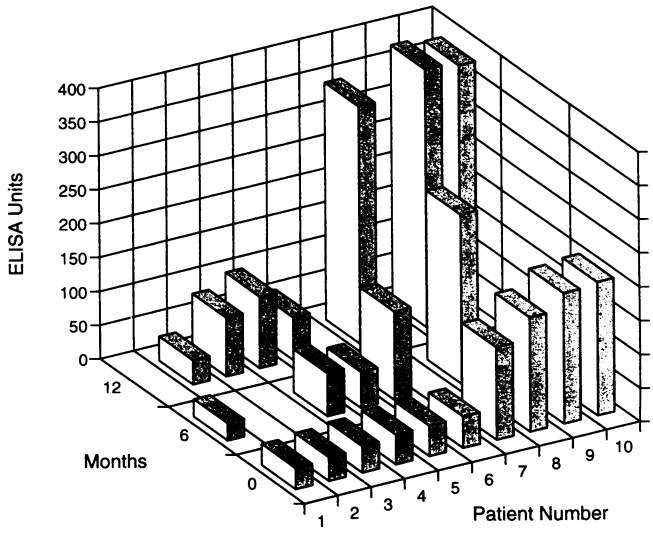

FIG. 1. IgG antibody levels in serum before and after treatment of seropositive RPP patients. The bars show IgG antibody levels in ELISA units. The blank spaces represent missing serum samples. Ten of the 22 RPP patients exhibited elevated amounts of antibodies against *A. actinomycetemcomitans* antigens at the baseline. Following SCRP therapy, 4 of the 10 patients (no. 3, 4, 6, and 7) showed a twofold or greater rise in specific antibody levels while 3 showed no change in titer (no. 1, 2, and 5). The median titer at 6 months (64 EU) was not significantly different from the baseline (45 EU; $P = 0.08$; $n = 5$). The median titer at 12 months (106 EU) was significantly higher than at the baseline (45 EU; $P = 0.02$; $n = 7$).

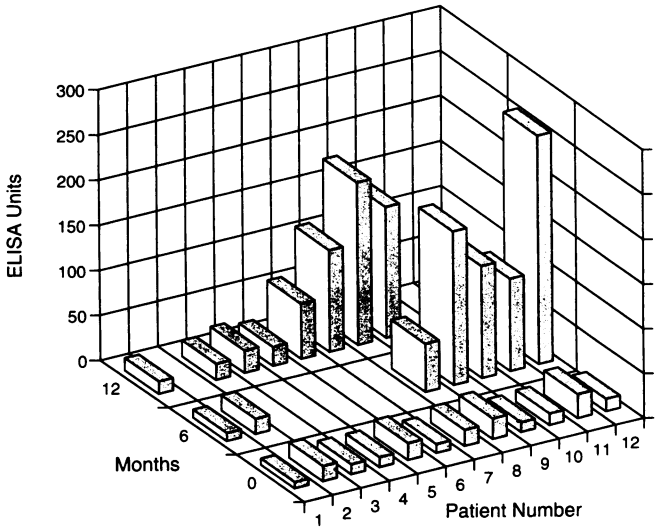

FIG. 2. IgG antibody levels in serum before and after treatment of seronegative RPP patients. Twelve of the 22 patients showed negligible baseline levels of antibody against *A. actinomycetemcomitans*. After therapy, 9 of the 12 showed a twofold or greater rise in titer (no. 1, 4, 6, 7, 8, 9, 10, 11, and 12). The median titer at 6 months (103 EU) was significantly greater than the cohort baseline titer (14 EU; $P = 0.03$; $n = 7$). The median titer at 12 months (43 EU) was also significantly higher than the respective baseline median value (11 EU; $P = 0.02$; $n = 8$).

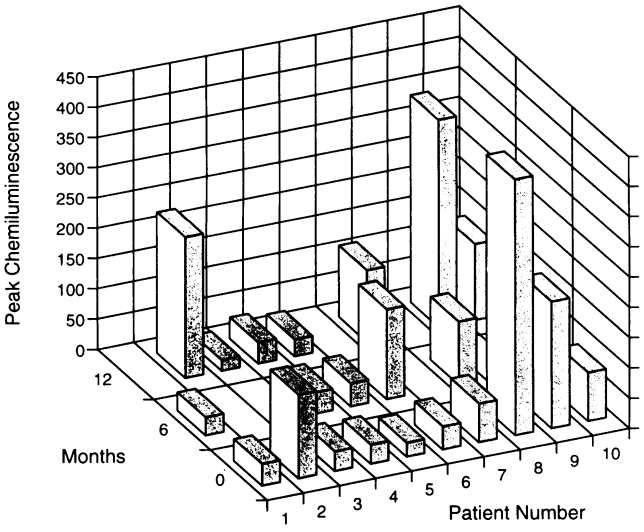

FIG. 3. Serum opsonic activity before and after treatment of seropositive RPP patients. The bars show opsonic activity as peak CL values in millivolts. The blank spaces represent missing serum samples. Only 1 of 10 seropositive patients had a greater-than-twofold rise in CL over the baseline (no. 6), while 1 patient showed a fourfold decrease (no. 8). All others showed either no change or modest increases. There was no significant increase over baseline median CL values at either 6 or 12 months.

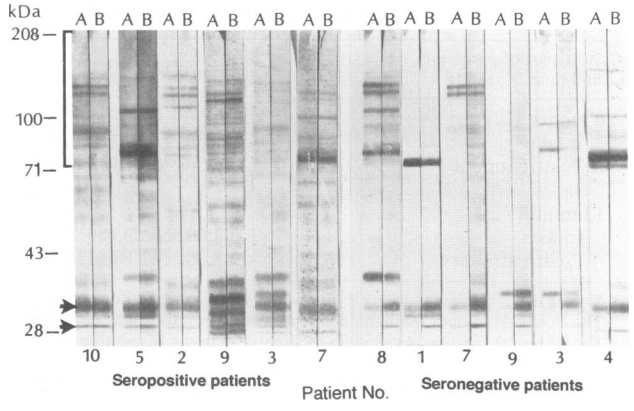

FIG. 5. Western blots of RPP patient sera obtained before and 12 months after SCRP treatment. Fifty micrograms of protein of an *A. actinomycetemcomitans* whole-cell homogenate per lane was separated by sodium dodecyl sulfate–7.5% polyacrylamide gel electrophoresis and blotted overnight onto nitrocellulose. Blots were developed by using a 1:1,000 dilution of test serum. The first six lane pairs are serum samples from patients classified as seropositive pretreatment. There is little difference between the antigenic profiles seen at the baseline (column A) and the profiles seen 12 months after treatment (column B). The second six lane pairs are serum samples from patients classified as seronegative pretreatment. Sera obtained 12 months after treatment (B) were generally more reactive than baseline sera (A). There was a slight response to what may represent the high-molecular-mass LPS antigen (bracket) in five of the six posttreatment-seronegative sera. In addition, there was an increased response at 12 months posttherapy to 30- and 36-kDa antigens (arrows) in all six sera.

months (34 mV) was significantly greater than the respective baseline value (21.5 mV; $P = 0.02$; $n = 8$).

Change in median IPPD after treatment. The median value for whole-mouth IPPD measurements taken on 20 of the 22 patients (data were incomplete for 2 patients) was significantly lower at 12 months (4.20 mm) than the baseline median value at intake (4.85 mm; $P = 0.0004$) (Table 1). Both baseline-seropositive and baseline-seronegative patients showed significant decreases in median IPPD values at 12 months (seropositive, 4.85 mm at baseline and 4.25 mm at 12 months [$P = 0.02$]; seronegative, 4.75 mm at baseline and 4.25 mm at 12 months [$P = 0.008$]). However, there was no significant difference between the two groups in the amount of IPPD decrease ($P = 0.06$).

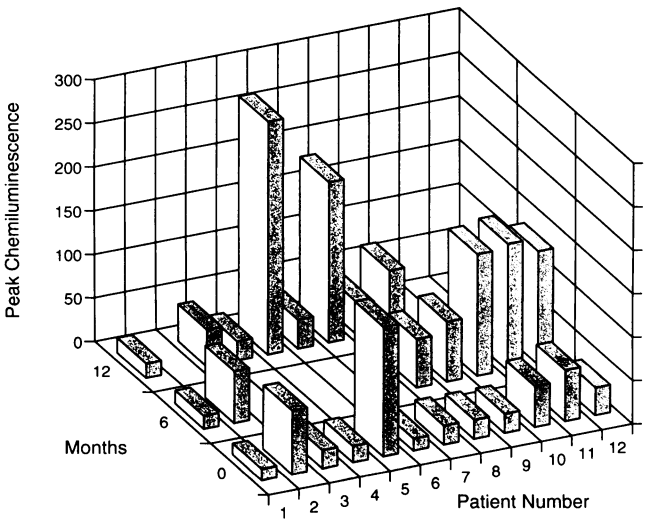

FIG. 4. Serum opsonic activity before and after treatment of seronegative RPP patients. Eight of the 12 patients had twofold or greater increases in CL values (no. 3, 6, 7, 8, 9, 10, 11, and 12). The 6-month median CL value (69 mV) was significantly greater than the cohort baseline median value (32 mV; $P = 0.04$; $n = 7$). The median CL value at 12 months (34 mV) was significantly greater than the respective baseline value (21.5 mV; $P = 0.02$; $n = 8$).

Immunoblots of patient sera against *A. actinomycetemcomitans* before and after treatment. Sera drawn at the time of initial examination and at 12 months after treatment from six baseline-seropositive patients and six baseline-seronegative patients were used as primary antisera in a Western blot analysis (Fig. 5). All samples that were seropositive before beginning treatment (seropositive A lanes) showed antibody binding to a smear in the high-molecular-mass zone of the gels (Fig. 5, brackets) (a component previously shown by us and others to be high-molecular-mass lipopolysaccharide (LPS) [4, 17, 23, 34]) and to a variety of protein bands ranging in size from approximately 30 to approximately 140 kDa. No differences in band pattern between 1:500 and 1:1,000 dilutions of primary antisera were observed. Although there were minor variations among patients, treatment appeared to have little or no effect on the Western blot antibody-binding pattern for patients who were seropositive before therapy (seropositive B lanes). In contrast, antibody binding from the pretreatment-seronegative samples (seronegative A lanes) to the high-molecular-mass smear material was observed in only one (seronegative 8A) of six samples, and antibody bound to many fewer protein bands than observed in the pretreatment-seropositive samples. Binding to protein bands by pretreatment-seronegative samples ranged from a single band (seronegative 9A) to approxi-

mately 7 bands (seronegative 8A). The major effect of treatment on the seronegative samples was to enhance antibody binding to the high-molecular-mass LPS and to two protein components. Faint binding to LPS was observed after treatment in five of six cases (seronegative lanes 8B, 7B, 9B, 3B, and 4B). A band at approximately 30 kDa (Fig. 5, lower arrow) that stained in all pre- and posttreatment-seropositive samples, but in none of the pretreatment-seronegative samples, appeared in all six posttreatment-seronegative samples. In addition, a band at approximately 36 kDa (Fig. 5, upper arrow) seen in all seropositive samples both pre- and posttreatment was detected for the first time or increased in staining intensity in all six posttreatment-seronegative samples. While SCRP clearly stimulates a humoral immune response, especially in seronegative patients, the procedure was insufficient to make the immune response of the patients that were seronegative pretreatment comparable to that of the seropositive patients.

DISCUSSION

The most commonly used therapy for chronic periodontitis is SCRP of the teeth. This procedure generally results in significant resolution of gingival inflammation, reduction in probing pocket depth, and in some cases, increased levels of clinical periodontal attachment (1, 12). In the present study, as expected, SCRP resulted in significant reduction in IPPD. The effectiveness of this therapy is considered to result from removal of microbial deposits from root surfaces and cementum, although the molecular means by which efficacy is accomplished is unknown. Our data demonstrate that SCRP in some patients with RPP induces a systemic humoral immune response to antigens of *A. actinomycetemcomitans*. Both median antibody titers and antibody function, as assessed by CL, were significantly enhanced following therapy. These findings confirm and extend those of Chen et al. (6), who reported that treatment of RPP patients by a variety of modalities enhanced antibody titers in serum and avidity for antigens of *Porphyromonas gingivalis*, especially in patients who were seronegative for these antigens prior to treatment.

In most studies of antibody levels in serum from untreated early-onset periodontitis patients, investigators have found that while some patients manifest high titers of antibodies to antigens of the infecting bacteria, others have low titers and in some cases the titers are below those of periodontally normal control subjects (6–8, 10, 14, 15, 23, 24, 30, 32, 33). The reason why some individuals are immunologically responsive during the course of their disease while others are not remains unknown, and the clinical implications are not understood. It has been suggested that a poor humoral immune response or failure to respond may be genetically determined and could be a host susceptibility factor of considerable importance (6). On the other hand, seronegative patients may not be infected with the pathogen under study. This seems not to be the case, since some, although not all, seronegative patients seroconvert following therapy (Fig. 2) (6).

Others have found elevated serum IgG, IgM, and IgA antibody responses to antigens of plaque bacteria following treatment in some early-onset periodontitis patients (9, 21, 31, 32), although antibody levels generally returned to pretreatment levels after 12 months. Our own finding of significantly elevated titers of IgG antibodies to *A. actinomycetemcomitans* at 12 months in the sera of both seropositive and seronegative groups probably resulted from repeated SCRP treatments over an extended time period.

When PMNs phagocytose opsonized bacteria, they manifest a metabolic burst which is accompanied by an emission of energy which can be amplified and measured as CL (28). We have previously shown (24) that CL is directly proportional to destruction of *A. actinomycetemcomitans*, demonstrating that CL is a surrogate measurement for phagocytosis and killing of *A. actinomycetemcomitans* by PMNs. In the present study, patient antibody titers were compared to values observed for matched periodontally normal control subjects (24). Patients were designated seropositive or seronegative by using a cutoff of two times the median titer for normal subjects. The 22 patients available for this study accurately represented the composition of the original RPP patient group in terms of gender, race, clinical status, and median IgG antibody titer at the baseline.

The effect of treatment on the immune response of seronegative patients was markedly different from that on the immune response of seropositive patients. The median IgG antibody titer for the seropositive group was significantly greater than the baseline titer at 12 but not at 6 months, while the median IgG antibody titer for the seronegative group was significantly greater than the baseline titer at both 6 and 12 months. Similarly, the median CL value for the seropositive group was not significantly enhanced at either 6 or 12 months, whereas the median CL values for the seronegative group were significantly increased at both time points. More than twice as many seronegative patients as seropositive patients showed greater-than-twofold increases in antibody titer and CL. Thus, on the basis of antibody titer and CL, treatment effects on immune responsiveness were seen mostly in seronegative patients.

Seropositive and seronegative patients also differed greatly with regard to the antigens their sera detected on Western blots. As might be expected, blot patterns of sera from seropositive patients were very similar to previously published patterns in that there was antibody binding to a smear of antigen in the region of the gels known to contain high-molecular-mass LPS (4, 17, 23, 34), as well as to a large number of bands known to be protein because of their sensitivity to destruction by proteinase K (17, 23). Treatment appeared to have virtually no reproducible effect on the Western blot patterns of seropositive patients but did affect the patterns of seronegative patients. Only one of the six pretreatment-seronegative patient serum samples manifested binding to high-molecular-mass LPS, and all of the samples studied detected far fewer protein components than did the seropositive samples. Following therapy, five of six samples manifested faint although clearly detectable binding to high-molecular-mass LPS and increased reactivity to two low-molecular-mass protein bands. In no case studied did posttreatment sera from previously seronegative patients yield blot patterns manifesting the staining intensity or the wide range of detected components observed in the seropositive samples. Notably, treatment did not induce an immune response that permitted detection of new antigenic components not detected by the pretreatment-seropositive sera.

Our data show that treatment by SCRP induces enhancement of specific anti-*A. actinomycetemcomitans* antibody titers and CL and, by direct implication, enhancement of phagocytosis and killing of *A. actinomycetemcomitans* by PMNs. The clinical implications of these findings remain unknown. However, when they are combined with other findings it seems likely that the enhanced immune responsiveness may make an important contribution to the effec-

tiveness of SCRP in arresting the progression of periodontal destruction. In both RPP and juvenile periodontitis patients, anti-*A. actinomycetemcomitans* antibodies in serum are effective in enhancing phagocytosis and killing by PMNs in the presence of complement (24, 30). SCRP in RPP patients also enhanced the titers and avidities of antibodies reactive with *P. gingivalis*, and avidity correlated negatively and significantly with pocket depth and bone loss, especially in patients who were seronegative prior to treatment (6). Ranney et al. (20) demonstrated in adolescents with severe periodontal destruction that those with precipitating antibodies for *A. actinomycetemcomitans* had fewer involved teeth than did patients without such antibodies. Similarly, Gunsolley et al. (13) reported an inverse relationship between levels of antibodies specific for *A. actinomycetemcomitans* and *P. gingivalis* in serum and disease severity in young adults. We have also demonstrated a negative correlation between spontaneously occurring anti-*P. gingivalis* antibody and amounts of *P. gingivalis* in the subgingival microflora in the nonhuman primate *Macaca fascicularis* (18). By using *M. fascicularis* as a model system, we have demonstrated that vaccination with killed *P. gingivalis* as the antigen significantly suppresses alveolar bone loss in ligature-induced periodontitis (19). When taken together, these data support the idea that serum antibodies reactive with antigens of periodontal pathogens may be protective and that use of vaccines to strongly enhance antibody titers and function may provide a valuable new approach to the prevention and control of periodontal diseases.

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