Serum levels of antibodies against *Actinobacillus actinomycetemcomitans* in various forms of human periodontitis

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Serum levels of IgG, IgA, and IgM antibodies against extracts from *Bacteroides gingivalis* PER8, *Actinobacillus actinomycetemcomitans* Y4, and *Bacteroides fragilis* NCTC 9343 were determined in three categories of periodontitis patients by means of enzyme-linked immunosorbent assay. The test groups comprised 10 patients with juvenile periodontitis (JP), 18 young patients with severe periodontitis (YP), and 31 patients with adult periodontitis (AP). Nine subjects with healthy periodontium (HP) served as a reference group. Increased frequencies of patients with significantly elevated IgG and IgA antibody values against *B. gingivalis* and *A. actinomycetemcomitans* were found in the three periodontitis groups as compared with the HP group. The AP group, however, showed lower IgM values than the other groups. The results support the contention that *A. actinomycetemcomitans* may play a contributory role in adult periodontitis and that *B. gingivalis* is a suspected periopathogenic bacterium in juvenile periodontitis. The clinical YP classification was not supported by the present serologic findings. □ Actinobacillus; Bacteroides; *immunoenzyme technics; immunoglobulins; juvenile periodontitis*

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Actinobacillus actinomycetemcomitans most likely plays a pathogenic role in juvenile periodontitis (1). Recent bacteriologic findings have shown that *A. actinomycetemcomitans* frequently occurs in progressing periodontal sites in patients with adult periodontitis as well (2), and it was proposed that *A. actinomycetemcomitans* might trigger periodontal breakdown regardless of age. *B. gingivalis* is, however, usually associated with adult periodontitis (3), but it has also been found in periodontal pockets in patients with juvenile periodontitis (4).

In the study of pathogenesis of periodontitis, the host reaction can give important clues complementary to bacteriologic investigations (5). In the present investigation we compared mean serum antibody levels of *A. actinomycetemcomitans* Y4 and *B. gingivalis* PER8 in different patient groups. Our aim was to find indirect evidence that *A. actinomycetemcomitans* also is involved in the process of adult periodontitis. A strain of *Bacteroides fragilis*, a commensal of the intestine, was used as a non-oral reference bacterium.

Materials and methods

Patients

Study groups were formed in accordance with the following criteria: 1) healthy periodontium (HP) ($n = 9$): patients older than 20 years of age with no clinical and radiographic evidence of periodontal breakdown, and with less than 10% bleeding gingival units after probing; 2) juvenile periodontitis (JP) ($n = 10$): patients between 13 and 20

years of age and with periodontal bone loss of at least one-third of the root length around one or more permanent first molar or incisor, as judged with a Schei ruler on intra-oral radiographs; 3) young adults with severe periodontitis (YP) ($n = 18$): patients between 21 and 35 years of age, with periodontal bone loss exceeding one-third of the root length around 12 or more teeth; and 4) adult periodontitis (AP) ($n = 31$): patients older than 35 years of age and with periodontal bone loss exceeding one-third of the root length around at least one tooth, as judged from intra-oral radiographs. A blood sample was taken from each patient, and the serum was collected and stored in small portions at −20° until used.

This study complies with the recommendations of the Declaration of Helsinki.

Antigens

Bacteroides gingivalis strain PER8 (6), *Actinobacillus actinomycetemcomitans* strain Y4 (7), and *Bacteroides fragilis* NCTC strain 9343 (8) were grown for 7 days on Columbia agar base (Oxoid, England) supplemented with 7% whole blood in an anaerobic glove box (ANEE, Heinicke-National Appliance Co.). Each growth was removed from the agar with a rubber scraper and washed twice in phosphate-buffered saline (PBS) at pH 7.3. Bacterial disruption was achieved by crushing the cell pellet at −40°C in an X-press (Biox AB, Nacka, Sweden). This preparation was cleared by centrifugation at 35,000 *g* for 15 min, and the supernatant was chromatographed on a Sephadex G-75 column (Pharmacia Fine Chemicals, Uppsala, Sweden) with twice distilled water containing 0.1% (w/v) NaN_3. The optical density (OD) of the eluted fractions was measured at 280 nm, and those corresponding to the void volume were pooled, lyophilized, and stored at −20°C until used.

Enzyme-linked immunosorbent assay (ELISA)

Polystyrene microtiter plates (M 129 B, Dynatech, Plochingen, FRG) were incubated with 150 µl per well of a 10 µg/ml solution of bacterial extract in PBS. Each plate was sealed and stored at 4°C for at least 12 h. Before each assay the plates were washed with saline, containing 0.05% (v/v) Tween 20 (Koch-Light, England) and 0.02% (w/v) NaN_3, in an automatic washing machine (Microwash, Skatron, Lier, Norway). Then 200 µl of PBS containing 0.5% (w/v) bovine serum albumin (Behringwerke AG, Mahrburg, FRG (PBSB) was added to each well, and the plates were incubated at room temperature for 30 min. The wells were washed and incubated overnight at 4°C with 150 µl of patient serum appropriately diluted in PBSB. After being washed, 150 µl of a dilution of alkaline phosphatase-conjugated swine antibodies against human IgG, IgA, or IgM (heavy-chain-specific, Orion Diagnostica, Helsinki, Finland) in PBSB was added to each well. The plates were incubated at room temperature for 4 h and washed. Then 150 µl substrate solution (1 mg/ml disodium nitrophenol phosphate (Phosphatase substrate, Sigma Chemical Co., St. Louis, Mo., USA) in 1 M diethanolamine buffer at pH 9.8 and containing 0.5 mM $MgCl_2$ and 0.02% NaN_3) was added to each well. The OD was read at 405 nm in a photometer (Titertek Multiskan, Flow Laboratories, Scotland) at 10-min intervals. OD values obtained at 40, 50, and 60 min for IgG, IgA, and IgM, respectively, were used, except when values exceeded the limit of accuracy of the photometer. For these wells, OD values were calculated from earlier readings. For each bacterial extract and conjugate, standard curves were drawn by plotting the logarithm of the dilution of a standard serum versus the OD observed for that dilution. On the basis of these curves, experimental serum dilutions were chosen (1:3200 for IgG and 1:400 for IgA and IgM). A few sera containing particularly high activities were remeasured at higher dilutions, and sera that showed activities near the lower detection limits of the assay (the lower non-linear part of the standard curve) were remeasured at lower dilutions. Each serum was tested in duplicate.

Statistical evaluation

The data in Table 1 were first analyzed by

Table 1. Antibody values (OD, 405 nm) to *A. actinomycetemcomitans* Y4, *B. gingivalis* PER8, and *B. fragilis* NCTC 9343 in sera from healthy subjects and patients with juvenile, severe and adult periodontitis (mean and standard deviation)*

	Healthy periodontium ($n = 9$)	Juvenile periodontitis ($n = 10$)	Severe periodontitis in young adults ($n = 18$)	Adult periodontitis ($n = 31$)
A. actinomycetemcomitans Y4				
IgG	0.35	1.17†	0.73†	0.58†‡
	(0.26–0.47)	(0.49–2.80)	(0.31–1.72)	(0.33–1.02)
IgA	0.32	0.81†	0.68†	0.41†§
	(0.32–0.50)	(0.32–2.10)	(0.20–1.67)	(0.23–0.74)
IgM	0.47	0.71†	0.48‡	0.37‡§
	(0.31–0.71)	(0.49–1.04)	(0.28–0.84)	(0.26–0.53)
B. gingivalis PER8				
IgG	0.24	0.44†	0.36	0.44†
	(0.15–0.39)	(0.22–0.91)	(0.18–0.72)	(0.22–0.89)
IgA	0.29	0.52†	0.54†	0.61†
	(0.20–0.41)	(0.30–0.89)	(0.32–0.90)	(0.32–1.14)
IgM	0.57	0.76	0.61	0.47‡§
	(0.39–0.82)	(0.46–1.27)	(0.41–0.90)	(0.34–0.64)
B. fragilis NCTC 9343				
IgG	0.30	0.30	0.31	0.29
	(0.22–0.40)	(0.24–0.39)	(0.23–0.42)	(0.21–0.41)
IgA	0.25	0.27	0.30	0.26
	(0.19–0.34)	(0.20–0.35)	(0.22–0.42)	(0.22–0.31)
IgM	0.64	0.74	0.65	0.47†‡§
	(0.43–0.94)	(0.48–1.16)	(0.44–0.96)	(0.33–0.67)

* The levels can be compared within immunoglobulin isotype, but not among them.
† Statistically significant differences as compared with the healthy group (t test, $P < 0.05$).
‡ Statistically significant differences as compared with the juvenile periodontitis group (t test, $P < 0.05$).
§ Statistically significant differences as compared with the severe periodontitis in young adults group (t test, $P < 0.05$).

means of regression analyses, analyses of variance (ANOVA) and covariance, and then by t tests and discriminant analysis. All tests were based on the logarithm of the separate OD measurements. A statistical program package was used (9). The frequencies of values exceeding the mean plus 1.7 times the standard deviation in the HP group were calculated within groups, and differences among the groups were analyzed by means of the G test of independence (10). The level of significance chosen was 0.05.

Results

As compared with the HP group, the periodontitis groups showed significantly ($P < 0.05$) raised mean IgG and IgA values to *A. actinomycetemcomitans* Y4 and *B. gingivalis* PER8. In contrast, the AP group showed the lowest average IgM values. Very consistent mean values were found for *B. fragilis* NCTC 9343, except for the lowered IgM mean value in the AP group. The mean OD values of the study groups, the corresponding standard deviations, and the results of final t tests on group pairs are shown in Table 1.

A comparison of the results for *B. gingivalis* PER8 and *A. actinomycetemcomitans* Y4 showed that the HP group had significantly lower mean values of specific IgG and IgA antibodies than the JP, YP, and AP groups. We used two-way ANOVAs on the variates patient group and bacterial extract

Table 2. Percentages of subjects in each group displaying antibody levels exceeding the mean plus 1.7 times the standard deviations as calculated from the group with healthy periodontium

	Healthy periodontium ($n = 9$)	Juvenile periodontitis ($n = 10$)	Severe periodontitis in young adults ($n = 18$)	Adult periodontitis ($n = 31$)
A. actinomycetemcomitans Y4				
IgG	11	70*	55*	58*
IgA	0	60*	33*	23*†
IgM	0	30*	17†	0†
At least 1 raised IgG, IgA or IgM value	11	70*	67*	61*
B. gingivalis PER8				
IgG	0	40*	22*	42*
IgA	0	30*	39*	52*
IgM	0	30*	11	0†
At least 1 raised IgG, IgA or IgM value	0	50*	50*	61*
B. fragilis NCTC 9343				
IgG	0	0	6	3
IgA	0	10	11	0
IgM	0	20	11	3‡
At least 1 raised IgG, IgA or IgM value	0	30*	22	6

* Statistically significant differences as compared with the healthy group (G test of independence, $P < 0.05$).

† Statistically significant differences as compared with the group with juvenile periodontitis (G test of independence, $P < 0.05$).

‡ Statistically significant differences as compared with the severe periodontitis in young adults group (G test of independence, $P < 0.05$).

(yielding for IgG, $F = 13.07$, $F = 6.24$, and $F = 7.40$, and for IgA, $F = 9.10$, $F = 11.57$, and $F = 8.01$, respectively; $P < 0.05$). The mean IgM activities in the AP group were significantly lower than those in the HP, JP, and YP groups ($F = 4.27$, $F = 21.02$, and $F = 7.79$, respectively; $P < 0.05$).

Correlation coefficients were calculated within each group between specific antibody levels, age, and radiographic variables. Statistically significant negative correlation coefficients were found between age and specific anti-bacterial IgM values ($r < -0.50$, $P < 0.05$). Hence, analysis of covariance was performed on the IgM data with age as a covariate. According to these tests, none of the mean IgM values in the AP group were significantly lower than the IgM values in the other groups; that is, the age factor explained the low IgM values observed.

Table 2 shows the percentage of patients in each group with antibody values exceeding the means plus 1.7 times the standard deviation as calculated for the HP group.

A discriminant classification function using *B. gingivalis* PER8 and *A. actinomycetemcomitans* Y4 measurements placed 89% of the HP subjects, 60% of the JP patients, and 61% of the AP patients correctly into their clinical groups (jack-knifed classification; that is, a procedure involving a new classification function for each subject, based on all subjects except the one being tested). None of the YP patients were classified as such.

Discussion

Other work has shown that increased levels of serum antibodies to *B. gingivalis* frequently occur in adult periodontitis, whereas

raised antibody values to *A. actinomycetemcomitans* are prevalent in juvenile periodontitis (6, 11–18). The present study agrees with those findings. Juvenile periodontitis patients emerged as a group with significantly raised mean concentrations of anti-*A. actinomycetemcomitans* Y4 antibodies, whereas AP had significantly increased mean values of anti-*B. gingivalis* PER8 antibodies (Table 1).

In addition, we found that AP displayed elevated average anti-*A. actinomycetemcomitans* Y4 IgG values, indicating past exposure of patients to this bacterial species. To some extent, the results accord with those bacteriologic findings that showed *A. actinomycetemcomitans* to be present in many sites with progressing periodontal breakdown in adult patients (2). Similarly, JP showed moderately raised anti-*B. gingivalis* PER8 IgG and IgA mean values. The results indicate that *B. gingivalis* at times might be involved in the disease process of juvenile periodontitis and at times is a useful supplementary serologic marker of the disease. Low antibody values against *B. fragilis* NCTC 9343, the reference bacterium, was found in all groups. The latter results support the contention that there is an association between elevated antibody activity in serum and past or present infection with oral bacterial colonizers. Antibody response among adult periodontitis patients is not limited to a single bacterial species (15–17, 19). In the present study, most of the patients in the periodontitis groups showed at least one significantly raised IgG, IgA, or IgM antibody value to the test bacteria (Table 2). We agree with those authors who propose that such findings rather suggest that combinations of opportunistic bacteria trigger periodontal breakdown, or that the more virulent pathogens attack sequentially (20, 21). Moreover, the extent to which all relevant subgingival bacteria release sufficient antigens to produce raised antibody levels, as detected by ELISA, is unknown.

Inverse correlations were found between age and specific IgM levels. When analysis of covariance was used, the differences in specific IgM levels between AP and the other groups appeared insignificant. Since the relationship with age involved *B. fragilis* as well, reduced IgM values with advancing age might reflect a general phenomenon. Other studies have shown that cell proliferation, interleukin-2 production, and responsiveness to lymphokines triggering cell growth and differentiation decrease with advancing age (22–24). Such lessened reactivity might also reduce IgG and IgA production in adults. Moreover, terminal differentiation in IgM-secreting B cells is impaired in older persons (25). Increased production of anti-idiotype antibodies might also contribute to reduced amounts of specific antibodies in elderly people (24).

The ELISA method chosen was based on time-dependent OD readings and therefore permitted semiquantitative comparisons of OD values within each immunoglobulin class. The OD values found were also transformed to serum dilutions by aid of ELISA standard curves (data not shown). Mean IgG and IgA reactivities to *A. actinomycetemcomitans* Y4 in the JP group (1.17 and 0.81 in Table 1) were thus raised 10 and 15 times, respectively, as compared with the mean level in HP. In contrast, the mean antibody values to *A. actinomycetemcomitans* Y4 and *B. gingivalis* PER8 in the groups YP and AP were increased two to five times. Those results emphasize the strongly increased reactivity to *A. actinomycetemcomitans* Y4 in juvenile periodontitis (14) versus the more moderate increases found to oral bacterial species in other forms of periodontitis. Perhaps the local microbial flora in juvenile periodontitis produces immunomodulators different from those produced by the complex microbiota in adult periodontitis (26).

The group YP was formed in accordance with clinical criteria given by others (13, 16, 27–31). Smith et al. (27) reported a nonspecific hyperresponsiveness to polyclonal B-cell activators in such patients. The serologic characteristics found in the present YP group were, however, largely comparable with those of AP (Table 1). YP showed higher mean serologic reactivity to *A. actinomycetemcomitans* Y4 than to *B. gingivalis* PER8, albeit less than that observed in JP. Classification by means of a discriminant function failed to classify YP patients as a

particular group. Thus the data from this study comply with the view of Tew et al. (16) that YP hardly comprises a separate disease entity.

In sum, raised serum antibody levels to *A. actinomycetemcomitans* Y4 were found in patients with juvenile periodontitis as well as in adults with periodontitis. The present findings suggest that *A. actinomycetemcomitans*, to which an etiologic role is ascribed in the pathogenesis of juvenile periodontitis (31), also might be related to other adult forms of periodontitis. Similarly, raised levels of anti-*B. gingivalis* PER8 antibodies were found in periodontitis patients regardless of age, indicating that *B. gingivalis* is a useful general marker of periodontal breakdown.

References

1. Zambon JJ. *Actinobacillus actinomycetemcomitans* in human periodontal disease. J Clin Periodontol 1985;12:1–20.
2. Slots J. Bacterial specificity in adult periodontitis. A summary of current work. J Clin Periodontol 1986;13:912–7.
3. Moore WEC. Microbiology of periodontal disease. J Clin Periodontol 1987;22:335–41.
4. Moore WEC, Holdeman LV, Cato EP, Smibert RM, Burmeister JA, Palcanis KG, Ranney RR. Comparative bacteriology of juvenile periodontitis. Infect Immun 1985;48:335–41.
5. Socransky SS. Criteria for the infectious agents in dental caries and periodontal disease. J Clin Periodontol 1979;6:16–21.
6. Schenck K. IgG, IgA and IgM serum antibodies against lipopolysaccharide from *Bacteroides gingivalis* in periodontal health and disease. J Periodont Res 1985;20:368–77.
7. Baehni P, Tsai CC, McArthur WP, Hammond BF, Taichman NS. Interaction of inflammatory cells and oral microorganisms. VIII. Detection of leukotoxic activity of a plaque-derived gram-negative microorganism. Infect Immun 1979;24:233–43.
8. Loesche W, Socransky SS, Gibbons RJ. *Bacteroides oralis,* proposed new species isolated from the oral cavity of man. J Bacteriol 1964;88:1329–37.
9. Dixon WJ, ed. BMDP statistical software. Berkeley: University of California Press, 1981.
10. Sokal RR, Rohlf FJ. Biometry. New York: W. H. Freeman and Co., 1981.
11. Mouton C, Hammond PG, Slots J, Genco RJ. Serum antibodies to oral *Bacteroides asaccharolyticus (Bacteroides gingivalis)*: relationship to age and periodontal disease. Infect Immun 1981;31: 182–92.
12. Listgarten MA, Lai C-H, Evian CI. Comparative antibody titres of *Actinobacillus actinomycetemcomitans* in juvenile periodontitis, chronic periodontitis and periodontally healthy subjects. J Clin Periodontol 1981;8:155–64.
13. Ranney RR, Yanni NR, Burmeister JA, Tew JG. Relationship between attachment loss and precipitating serum antibody to *Actinobacillus actinomycetemcomitans* in adolescents and young adults having severe periodontal destruction. J Periodontol 1982;53:1–7.
14. Ebersole JL, Taubman MA, Smith DJ, Genco RJ, Frey DE. Human immune responses to oral microorganisms. I. Association of localized juvenile periodontitis with serum antibody responses to *Actinobacillus actinomycetemcomitans*. Clin Exp Immunol 1982;47:43–52.
15. Tolo K, Schenck K. Activity of serum immunoglobulins G, A, and M to six anaerobic, oral bacteria in diagnosis of periodontitis. J Periodont Res 1985;20:113–21.
16. Tew JG, Marshall DR, Moore WEC, Best AM, Palcanis KG, Ranney RR. Serum antibody reactive with predominant organisms in the subgingival flora of young adults with generalized severe periodontitis. Infect Immun 1985;48:303–11.
17. Ebersole JL, Taubman MA, Smith DJ, Frey DE. Human immune response to oral microoganisms: patterns of systemic antibody levels to *Bacteroides* species. Infect Immun 1986;51:507–13.
18. Tollefsen TT, Schenck K, Tolo K. Cross-sectional study of the effects of immunosuppressive agents on humoral immune responses to six oral microorganisms in humans. J Periodont Res 1986;21:553–62.
19. Ebersole JL, Taubman MA, Smith DJ, Frey DE, Haffajee AD, Socransky SS. Human serum antibody responses to oral microorganisms. IV. Correlations with homologous infection. Oral Microbiol Immunol 1987;2:53–9.
20. Theilade E. The non-specific theory in microbial etiology of inflammatory periodontal diseases. J Clin Periodontol 1986;13:905–11.
21. Ranney RR, Best AM, Breen TJ, Moore WEC, Moore LVH. Bacterial flora of progressing periodontitis lesions. J Periodont Res 1987;22:205–6.
22. Weksler ME. The senescence of the immune system. Hosp Pract 1981;16:53–64.
23. Doggett DL, Chang M-P, Makinodan T, Strehler BL. Cellular and molecular aspects of immune system aging. Mol Cell Biochem 1981;37:137–56.
24. Weksler ME, Siskind GW. The cellular base of immune senescence. Monogr Dev Biol 1984;17: 110–21.
25. Ennist DL, Jones KH, St Pierre RL, Whisler RL. Functional analysis of the immunosenescence of the human B cell system: dissociation of normal activation and proliferation from impaired terminal differentiation into IgM immunoglobulin-secreting cells. J Immunol 1986;136:99–105.
26. Shenker BJ. Immunologic dysfunction in the pathogenesis of periodontal diseases. J Clin Periodontol 1987;14:489–98.
27. Smith S, Bick PH, Miller GA, Ranney RR, Rice

PL, Lalor JH, Tew JG. Polyclonal B-cell activation: severe periodontal disease in young adults. Clin Immunol Immunopathol 1980;16:354–66.

28. Ranney RR, Ruddy S, Tew JG, Welshimer HJ, Palcanis KG, Segreti A. Immunological studies of young adults with severe periodontitis. J Periodont Res 1981;16:390–402.
29. Burmeister JA, Best AM, Palcanis KG, Ciane FA, Ranney RR. Localized juvenile and generalized severe periodontitis: clinical findings. J Clin Periodontol 1984;11:181–92.
30. Tew JG, Smibert RM, Scott EA, Burmeister JA, Ranney RR. Serum antibodies in young adult humans reactive with periodontitis associated treponemes. J Periodont Res 1985;20:580–90.
31. Genco RJ, Zambon JJ, Murray PA. Serum and gingival fluid antibodies as adjuncts in the diagnosis of *Actinobacillus actinomycetemcomitans*-associated periodontal disease. J Periodontol 1985;56: 41–50.

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