Serum antibody levels to oral microorganisms in children and young adults with relation to the severity of gingival disease

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Abstract

The purpose of this study was to describe and compare the IgG and IgM serum antibody levels to 10 microorganisms involved in periodontal diseases in children and young adults with no systemic disease, but with either minimal/none or severe gingival inflammation. Blood samples from 15 children and 14 young adults were collected. Seven children and nine adults had minimal or no gingival inflammation; the rest had severe gingivitis. Kruskal-Wallis H analysis indicated significant differences among the age groups in the IgM values for all microorganisms and for most organisms in the IgG values. The contrast coefficient matrix values between children and young adults were significant for all the microorganisms for the IgM values and for most of the organisms for the IgG values when the severity of the disease was not taken into consideration. However, when the severity of the disease was also considered, the contrast coefficient matrix values for most of the microorganisms were not significant for both the IgG and IgM values. Age had a more significant influence on the serum antibody levels than the severity of the disease, mostly in the IgM values. (Pediatr Dent 13:267–72, 1991)

Introduction

Clinical and histologic studies indicate that the prevalence and severity of gingival disease increase from infancy to adulthood due to an age-dependent reactivity to plaque (with similar amounts of plaque children have less severe gingivitis than older individuals). In adults, chronically inflamed gingival tissue presents the histologic characteristics of an established periodontal lesion, while the same tissue in children presents the characteristics of an early lesion (Longhurst et al. 1977; Matsson 1978; Longhurst et al. 1980; Klinge et al. 1983; Matsson and Goldberg 1985).

Periodontal diseases are the expression of an inflammatory reaction of the periodontal tissues to microbial plaque products and their manifestation depends on the interaction of several local and systemic factors including the immunological reaction of the individual (Genco and Slots 1984; Page 1986; Bimstein and Ebersole 1989). Previous studies indicate that serum levels of antibodies to microorganisms involved in periodontal diseases appear to increase gradually from infancy to adulthood (Mouton et al. 1981; Tolo and Schenck 1985) and with the severity of disease (Ebersole et al. 1987). These findings suggest that a more intense serum antibody reaction may take place associated with an increase in:

1. Severity of disease, within individuals of the same age
2. Age, within individuals with the same disease severity.

No attempt was made in previous studies to compare the antibody levels in adults and children with similar severity of gingivitis or periodontitis, so findings do not indicate the differences (if any) in the degree and/or type of immunological response in these inflammatory diseases at different ages. The purpose of the present study was to describe and compare the serum antibody levels to 10 microorganisms involved in periodontal diseases in children and young adults with relation to two most distinct degrees of severity of gingival inflammation.

Materials and Methods

After Institutional Review Board approval (University of Texas Health Science Center in San Antonio, Texas), blood was obtained by venipuncture for serum antibody analysis from children and young adults. The children were selected from a population of patients who had routine appointments at the Continuing Care Pediatric Clinic in the Brady Green Clinics in San Antonio. The adults were selected from students, staff, or patients at the Dental School, UT Health Science Center in San Antonio. Pregnant women were not included in the study. At the time of the examination, no subject had systemic disease or had received medication for at least three months.

Informed consent was obtained for all subjects. Immediately before the blood sample, based on the patients age, gingival index (GI, Löe 1967) and extent of the gingival disease, the patients were classified in one of the following groups:

Group 1. Children with a mixed dentition and a GI score of 0 or 1 in all quadrants.

Group 2. Children with a mixed dentition and a GI score of 2 and/or 3 adjacent to several teeth per quadrant in at least two quadrants.

Group 3. Adults ages 20–30 years with a GI score of 0 or 1 in all quadrants.

Group 4. Adults ages 20–30 years with a GI score of 2 and/or 3 adjacent to several teeth per quadrant in at least two quadrants.

Individuals who clearly did not meet the criteria for age or severity of disease, or who had clinical evidence of periodontitis as shown by abnormal periodontal probing depths were not included in the study.

After blood clotting, the serum was collected by centrifugation to identify IgM and IgG serum levels with an enzyme-linked immunosorbent assay (ELISA) technique (Ebersole et al. 1980) to the whole cells of the following bacteria: *Actinobacillus actinomy-cetemcomitans* (ATCC 29523), (Y4) and (ATCC 33384), *Bacteroides gingivalis* (ATCC 33277), *Bacteroides intermedius* (ATCC 25611), *Capnocytophaga gingivalis* (ATCC 33624), *Capnocytophaga ochracea* (ATCC 33596), *Eikenella corrodens* (ATCC 23834), *Fusobacterium nucleatum* (ATCC 25586) and *Wolinella recta* (ATCC 33238).

Antibody analysis

An ELISA was used to determine the antibody activity in the human serum to the various microorganisms. Briefly, the bacterial sonicates (formalinized microorganisms) were attached to polystyrene microtiter plates at 37°C for 3 to 4 hr, after which the plates were stored at 4°C until used. The antigen-coated plates were rinsed and incubated for 2 hr at room temperature with appropriate dilutions of human sera. After the plates were washed, rabbit antihuman serum isotype-specific antisera for IgG and IgM (RAHG and RAHM, Calbiochem Behring Diagnostics, Somerville, NJ) were added and incubated for 2 hr at room temperature. The plates were washed again and the reaction was developed by incubation for 16 to 18 hr with goat antirabbit IgG (GARG, Miles Laboratories, Elkhart, IN) conjugated to alkaline phosphatase and addition of substrate (p-nitrophenylphosphate). The reactions were terminated at 30 min by addition of NaOH and the extent of the reaction, expressed as color intensity, was examined by spectrophotometric determination at 405 nm with an automated ELISA plate reader (Dynatech USA, Alexandria, VA). The serum antibody activity then was expressed in ELISA units (EU), which are defined by a reference curve prepared by a linear regression analysis obtained from standard dilutions for each microorganism. Thus, the color intensity to a certain microorganism could be extrapolated so that the value of 1 EU for each microorganism was related to the same extent of reaction (Ebersole et al. 1980; Ebersole et al. 1986).

Statistical analysis

A Student's *t*-test from a standard statistical analysis computer package (512+ StatView™, BrainPower Inc., Calabasas, CA) was used to examine the difference in age between the children or adult groups. The Kruskal-Wallis H nonparametric test (BMPD Statistical Software, Inc., Los Angeles, CA) was used to disclose the significance of differences between groups in the antibody levels to each microorganism. The serum antibody level differences between children and young adults, with and without taking into consideration the severity of the disease, were examined using a contrast coefficient matrix (SPSS-X Release 3.1 for IBM VM/CMS 4.0). A pairwise *t*-test using Bonferroni alpha/6 significance levels (BMPD Statistical Software, Inc. Los Angeles, CA) was used to examine the differences between antibody levels for each microorganism, among the different groups.

Results

Blood samples from 15 children (mean age 9.8, SD 1.5, range 7–12 years) and 14 young adults (mean age 23.7, SD 2.1, range 20–27) were collected. Seven children and nine adults had minimal or no gingival inflammation; the rest showed clear evidence of severe gingivitis. No significant differences in age (Student's *t*-test, $P > 0.05$) were found between children or adults with minimal/no and with severe gingival disease.

The IgM and IgG serum antibody levels expressed in Elisa Units (EU) for the 10 microorganisms by the group of patients are presented in Tables 1 and 2 (see next page). The Kruskal-Wallis H analysis indicated significant differences among the groups for the IgM EU values of all microorganisms, and for most of the microorganisms in the IgG values (Tables 1 and 2).

The contrast coefficient matrix between children and young adults for the IgM values was significant for all the microorganisms when the severity of the disease was not considered, and only for *C. gingivalis* and *C. ochracea* when the severity of the disease was considered (Table 3, see page 270). The contrast coefficient matrix between children and young adults for the IgG values was significant for eight microorganisms when the severity of the disease was not taken into consideration and only for *A. actinomycetemcomitans* (Y4) when the severity of the disease was considered (Table 4, see page 270).

The pairwise *t*-test using Bonferroni alpha/6 significance levels indicated that the differences between children with and without gingivitis (Groups 1 and 2) were not significant for most of the microorganisms in IgM

Table 1. IgM antibody activity levels (Elisa Units) by type of microorganism, in children with minimal or no (Group 1) and with severe gingival disease (Group 2), and in adults with minimal or no (Group 3) and with severe gingival disease (Group 4)

Microorganism	*Group 1*		*Group 2*		*Group 3*		*Group 4*	
	mean	*SD*	*mean*	*SD*	*mean*	*SD*	*mean*	*SD*
Aa, 29523	2.6	1.9	1.7	1.0	8.6	2.0	10.0	1.9*
Aa, Y4	1.7	0.7	2.1	1.5	10.3	2.9	8.8	1.4*
Aa, 33384	1.5	0.5	0.8	0.5	6.1	1.7	6.4	1.2*
B. gingivalis	1.8	0.7	1.4	0.6	9.5	3.2	8.4	1.8*
B. intermedius	5.3	1.1	5.7	4.6	24.8	8.2	23.7	5.4*
C. gingivalis	4.9	4.3	15.1	5.8	18.9	7.7	16.2	1.4*
C. ochracea	8.5	4.3	25.9	13.0	50.8	10.6	48.4	14.5*
E. corrodens	4.0	1.0	2.2	1.2	11.8	4.1	12.2	2.6*
F. nucleatum	4.2	2.2	2.2	2.6	15.7	6.8	13.9	3.9*
W. recta	2.2	2.2	1.6	0.8	8.9	2.8	10.3	3.5*

* Significant differences among groups, Kruskall-Wallis H test, $P \leq 0.004$.

Table 2. IgG serum antibody activity levels (Elisa Units) by type of microorganism, in children with minimal or no (Group 1) and with severe gingival disease (Group 2), and in adults with minimal or no (Group 3) and with severe gingival disease (Group 4)

Microorganism	*Group 1*		*Group 2*		*Group 3*		*Group 4*	
	mean	*SD*	*mean*	*SD*	*mean*	*SD*	*mean*	*SD*
Aa, 29523	4.8	5.3	3.4	2.8	22.8	9.9	34.3	24.1*
Aa, Y4	2.3	2.2	2.7	1.6	26.2	8.9	18.8	1.7*
Aa, 33384	1.3	2.3	1.3	1.2	24.4	7.7	18.6	7.4*
B. gingivalis	2.3	2.1	2.8	2.2	6.9	4.3	6.8	6.2*
B. intermedius	22.2	13.9	20.2	8.8	58.4	16.1	48.2	19.0*
C. gingivalis	43.9	24.7	48.4	45.5	38.4	20.4	42.1	16.9
C. ochracea	13.9	12.2	15.5	9.5	14.4	12.1	19.0	12.6
E. corrodens	3.9	5.3	4.5	3.5	11.1	3.8	16.9	5.3*
F. nucleatum	5.4	3.8	4.6	2.2	23.6	7.9	14.3	12.0*
W. recta	3.6	3.8	5.1	2.2	22.1	7.0	28.5	7.6*

* Significant differences among groups, Kruskall-Wallis H test, $P \leq 0.01$.

and none in the IgG values; adults with and without gingivitis (Groups 3 and 4) were not significant for both IgG and IgM in all microorganisms; children with no disease (Group 1) and adults with no disease (Group 3) were significant for all IgM values and most of the IgG values; children with disease (Group 2) and adults with disease (Group 4) were significant for most IgM values but not significant for half of the IgG values (Tables 5 and 6, see page 271).

Discussion

The idea that a child with incipient periodontal disease may become an adult with advanced periodontal diseases originates with the fact that periodontal diseases are chronic, and a connection between periodontal diseases in the primary and the permanent dentitions may exist. Sjödin et al. (1989) in a recent retrospective radiographic study showed that most of the 13- to 22-year-old patients with alveolar bone loss in the permanent dentition had alveolar bone loss in the primary dentition. Prevention and/or early diagnosis and treatment of periodontal diseases in children and adolescents becomes a major goal for improving oral health. Delineation of the means by which periodontal diseases are established and progress may provide efficient prevention and treatment.

A possible connection between the intensity of the immunologic response and the age-dependent reactivity of the periodontal tissues to plaque is suggested by the fact that antibody levels to periopathogens may be related to the longevity of the disease (Mouton et al. 1981; Tolo and Schenck 1985). However, one must consider that elevations in cell-mediated or humoral responses to plaque microorganisms also may be associated with the bacterial load (Ebersole et al. 1987; Vincent et al. 1987) and/or increased severity of the disease regardless to age (Ebersole et al. 1987). In the studies of Mouton et al. (1981) and Tolo and Schenck (1985), a comparison between the serum antibody levels of children and adults with a similar periodontal condition was not performed, so a connection between an increase in the serum antibody level and the age-dependent reactivity of the periodontal tissues to plaque was not established.

The effect of the longevity of disease would be examined better in a longitudinal study, but we are limited to cross-sectional studies, like the present one, in which the age groups and the severity of the gingival disease criteria allow for the comparison of the immunological

Table 3. Significance of contrast coefficient matrix* in IgM serum antibody activity to 14 microorganisms by age and by age and severity of the gingival disease

	By Age	*By Age and Severity of Disease*
Aa, 29523	≤ 0.001	NS
Aa, Y4	≤ 0.001	NS
Aa, 33384	≤ 0.001	NS
B. gingivalis	≤ 0.001	NS
B. intermedius	≤ 0.001	NS
C. gingivalis	≤ 0.001	0.002
C. ochracea	≤ 0.001	0.046
E. corrodens	≤ 0.001	NS
F. nucleatum	≤ 0.001	NS
W. recta	≤ 0.001	NS

* *Contrast coefficient matrix, T-two-tailed probability, $P \leq 0.05$.*

Table 4. Significance of contrast coefficient matrix* in IgG serum antibody activity to 14 microorganisms by age and by age and severity of the gingival disease

	By Age	*By Age and Severity of Disease*
Aa, 29523	0.008	NS
Aa, Y4	≤ 0.001	0.025
Aa, 33384	≤ 0.001	NS
B. gingivalis	≤ 0.001	NS
B. intermedius	≤ 0.001	NS
C. gingivalis	NS	NS
C. ochracea	NS	NS
E. corrodens	≤ 0.001	NS
F. nucleatum	≤ 0.001	NS
W. recta	≤ 0.001	NS

* Contrast coefficient matrix, T-two-tailed probability, $P \leq 0.05$.

response in children and adults with two distinct categories of gingival inflammation. It was expected that in the present study, with an increase in the severity of gingivitis and longevity of disease process, the serum antibody levels would be higher. However, none of the EU values for the 10 microorganisms agreed with this theory (Tables 1 and 2).

The more significant contrast between children and adults is found when the severity of the disease is not considered than when it is (Tables 3 and 4). The fact that more significant differences were found between the two age groups than between the different subgroups of the same age (Tables 5 and 6), indicates that longevity of the disease had a more significant influence than the severity of gingivitis in the serum antibody levels. While these levels were increased in the young adults relative to the children, they were within the range of a larger systemically healthy populations, aged 18 to 37 years (Ebersole et al. 1983; Ebersole et al. 1986)

The finding that the differences between the groups for IgM were more significant than those of the IgG serum levels, may be related to the fact that IgM is more prominent in primary type and T cell-independent immune responses, whereas IgG is a T cell-regulated response and the major antibody of secondary immune responses (Goodman et al. 1987; Roitt et al. 1985). This connection is further suggested by previous findings which indicate that:

1. Adult gingival tissue contains equal numbers of plasma cells and lymphocytes whereas children's tissue is dominated by small B lymphocytes — T-cells rarely are encountered (Longhurst et al. 1977; Longhurst et al. 1980; Gillett et al. 1986).
2. The majority of inflammatory cells in gingivitis associated with the primary dentition children have a B-cell phenotype (Seymour et al. 1982).

An increase in the IgG response at older ages may be regulated by an increase in the relative amount of T-cells in the inflammatory cell infiltrates (Longhurst et al. 1977; Longhurst et al. 1980; Gillett et al. 1986). Also, these cells may exert great influence on the clinical expression of the inflammation, as T-cells have the capability to produce lymphokines which may exacerbate tissue destruction (Genco et al. 1974; Horton et al. 1974). Furthermore, recent studies have indicated that the presence of increased levels of macrophage-derived cytokines may, in part, be related to altered PMN function in cases of localized juvenile periodontitis (Agarwal et al. 1990; Agarwal and Suzuki 1991).

It has been suggested that the bacterial composition of dental plaque is related to the serum antibody levels (Ebersole et al. 1987). Therefore, the possibility that the present findings may be connected with age-related changes in the dental plaque should be considered. In addition, whether the genera/species of bacteria that elicit this antibody response are important in the development of gingivitis in children, or emerge in response to increased exudates during the inflammatory process remains to be determined.

The authors thank Mr. Israel Einot from the Statistics Department, Hebrew University in Jerusalem for his most valuable help in the statistical analysis of the data.

Table 5. Significance of differences* between pairs of groups † in IgM serum antibody activity

	Pairs of groups‡				
	1 vs 2	*1 vs 3*	*1 vs 4*	*2 vs 3*	*2 vs 4*
Aa, A 29523	NS	≤0.001	≤0.001	≤0.001	≤0.001
Aa, Y4	NS	≤0.001	≤0.001	≤0.001	≤0.001
Aa, A 33834	NS	≤0.001	≤0.001	≤0.001	≤0.001
B. gingivalis	NS	≤0.001	≤0.001	≤0.001	≤0.001
B. intermedius	NS	≤0.001	≤0.002	≤0.001	≤0.001
C. gingivalis	0.002	≤0.001	≤0.001	NS	NS
C. ochracea	0.006	≤0.001	0.003	≤0.001	NS
E. corrodens	0.008	≤0.001	0.002	≤0.001	≤0.001
F. nucleatum	NS	≤0.001	0.003	≤0.001	≤0.001
W. recta	NS	≤0.001	0.003	≤0.001	0.005

* Pairwise T test IgG. (parametric), Bonferroni significance levels (alpha/6 significance level).

† Group 1 = children without gingival disease, Group 2 = children with gingival disease, Group 3 = adults without gingival disease, Group 4 = adults with gingival disease.

‡ No differences were found in the EU values between Groups 3 and 4.

Table 6. Significance of differences* between pairs of groups † in IgG serum antibody activity

	Pairs of groups‡				
	1 vs 2	*1 vs 3*	*1 vs 4*	*2 vs 3*	*2 vs 4*
Aa, A 29523	NS	≤0.001	NS	≤0.001	NS
Aa, Y4	NS	≤0.001	≤0.001	≤0.001	≤0.001
Aa, A 33834	NS	≤0.001	0.006	≤0.001	0.006
B. gingivalis	NS	0.016	0.007	NS	NS
B. intermedius	NS	≤0.001	NS	≤0.001	NS
C. gingivalis	NS	NS	NS	NS	NS
C. ochracea	NS	NS	NS	NS	NS
E. corrodens	NS	NS	0.003	0.002	0.003
F. nucleatum	NS	≤0.001	0.003	≤0.001	0.004
W. recta	NS	≤0.001	≤0.001	≤0.001	0.002

* Pairwise T test IgG. (parametric), Bonferroni significance levels (alpha/6 significance level).

† Group 1 = children without gingival disease, Group 2 = children with gingival disease, Group 3 = adults without gingival disease, Group 4 = adults with gingival disease.

‡ No differences were found in the EU values between Groups 3 and 4.

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New bike handlebars may lead to injury

New bicycle handlebars built for speed may be dangerous for the inexperienced rider, according to a letter published in the July 24, 1991 issue of the *Journal of the American Medical Association.*

The handlebars have an inverted V shape that "has allowed riders to increase their speeds by adopting a more aerodynamic position," wrote Michael P. Resnick, MD, and Ralph A. Yates, DO, both of the Providence Medical Center, Portland, OR.

They said the handlebars' hazards include changing weight distribution to one less stable than traditional posture, and allowing riders to attain higher speeds, which can inhibit maneuverability, visibility, and braking ability.

According to the letter, Yates, an experienced cyclist, suffered multiple facial injuries from a fall while traveling downhill at 50 kilometers per hour on a bicycle equipped with these handlebars.

The authors suggested that bicycles equipped with inverted V handlebars should not be used in areas of heavy traffic, and that the aerodynamic position should be abandoned when riding on rough terrain and when negotiating turns. They also recommended that cyclists should view roads before racing, to identify obstacles and hazards.