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Human immune responses to oral micro-organisms

I. ASSOCIATION OF LOCALIZED JUVENILE PERIODONTITIS (LJP) WITH SERUM ANTIBODY RESPONSES TO ACTINOBACILLUS *ACTINOMYCETEMCOMITANS*

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SUMMARY

The association between periodontal disease in humans and serum and salivary antibody to *Actinobacillus actinomycetemcomitans* strain Y4 was determined. An ELISA was used to examine anti-Y4 antibody of the IgM, IgG, IgA and IgE isotypes in serum from 127 individuals and IgA in parotid saliva. Patients diagnosed as having localized juvenile periodontitis ($n=37$) had significantly higher levels and frequency of serum IgG antibodies to Y4 than all other groups. Serum and salivary IgA and serum IgE antibody levels were significantly increased in patients with both localized and generalized types of juvenile periodontitis ($n=48$) when compared to all other patient groups. Specificity studies suggested that the antigenic determinants that were differentiating the group responses were unique to the Y4 organism. These results indicate that serum antibodies to Y4 may reflect an infectious process with this micro-organism and that these responses may provide some diagnostic value in delineating different types of periodontal diseases.

INTRODUCTION

Juvenile periodontitis (JP) is an infrequent form of periodontal disease that occurs primarily in adolescents (Baer, 1971; Manson & Lehner, 1974). This disease progresses rapidly such that within a few years extensive loss of alveolar bone is noted (Baer, 1971; Manson & Lehner, 1974). Also, this disease has a predominant occurrence in specific sites (molar-incisor) of the oral cavity (Baer, 1971; Manson & Lehner, 1974). Anaerobic Gram-negative rods, which are associated with increased periodontal-type disease in monoinfected gnotobiotic rats (Irving, Socransky & Heeley, 1974), have been isolated from subgingival plaques of juvenile periodontitis patients (Newman & Socransky, 1977). One of these organisms, designated Y4, has subsequently been identified taxonomically as *Actinobacillus actinomycetemcomitans*. This organism has been frequently isolated from JP patients (Tanner *et al.*, 1979; Slots, 1979) and has been shown to produce a leucotoxin (Tsai *et al.*, 1979) that lyses human polymorphonuclear leucocytes.

In this study we examined the serum and salivary antibody response in humans to *A. actinomycetemcomitans* strain Y4. The immune response was related to different forms of periodontal disease by cross-sectional analysis. A modified enzyme-linked immunosorbent assay (ELISA) was used to analyse serum IgG, IgM, IgA and IgE, and salivary IgA antibodies to the micro-organisms. The results of these experiments indicate that a significant relationship exists

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between serum antibodies to *A. actinomycetemcomitans* strain Y4 and localized juvenile periodontitis.

MATERIALS AND METHODS

Patient samples. All serum and saliva samples were obtained from the patient populations of the Forsyth Dental Center and the School of Dentistry, State University of New York at Buffalo. Blood was drawn by venipuncture and serum collected by centrifugation (1,400 ***g***, 15 min) after clotting. Parotid saliva, stimulated with a lemon drop, was collected over a 10–15-min period using a standard Curby cup. Both saliva and serum samples were stored at −20°C until analysed.

Six groups of individuals were used in this study: a localized juvenile periodontitis (LJP) (Van Dyke *et al.*, 1980) group was composed of 38 young (mean 19·3, range 14–29 years) patients who had alveolar bone loss that was localized to the molars and incisors and not more than two additional teeth (< 14 teeth); a generalized juvenile periodontitis (GJP) group ($n = 11$; mean 22·0, range 13–29 years) had severe alveolar bone loss on ⩾ 14 teeth with no definite pattern of localized disease; an adult periodontitis (AP) group consisted of 39 patients (mean 32·4, range 27–63 years) who exhibited generalized severe alveolar bone loss with numerous vertical defects in the bone support; the acute necrotizing ulcerative gingivitis (ANUG) group ($n = 9$) had a mean age of 23·3 with a range of 17–29 years; the edentulous (ED) group had six patients all greater than 50 years of age; and a normal (N) group consisted of 24 healthy individuals (mean 24·6, range 18–37 years) with no evidence of bone loss or periodontal disease other than mild gingivitis.

Bacteria. Actinobacillus actinomycetemcomitans strain Y4 is a small, Gram-negative anaerobic rod. It was originally isolated from a lower, first molar of a 13-year-old female patient diagnosed as having juvenile periodontitis. The organism was grown in Mycoplasma broth base (BBL Microbiology Systems) or in a chemically defined medium (Socransky, Smith & Manganello, 1973) and formalinized for use in the antibody analysis as described previously (Ebersole *et al.*, 1980). *Actinobacillus actinomycetemcomitans* ATCC 29523 was obtained from Dr B. F. Hammond (University of Pennsylvania). This has been identified as an Actinobacillus strain that produces little or no leucotoxin (Dr B. F. Hammond, personal communication). The organism was grown and harvested identically to the Y4 organism.

Actinomyces naeslundii strain I, *Streptococcus sanguis* strain 254, and *Bacteroides gingivalis* strain 381 were grown according to Walker *et al.* (1979) and formalin-treated similar to Y4.

Antibody analysis. Antibodies in serum were determined by a modified enzyme-linked immunosorbent assay (Engvall & Perlmann, 1972), the details of which have been described previously (Ebersole *et al.*, 1980). Formalinized Y4 or *A. actinomycetemcomitans* ATCC 29523 were used as antigens in the analyses.

Antibody activity in each serum is expressed as ELISA units (EU) which are defined by a standard curve. The standard serum yielded a known $OD_{400\ nm}$ for each immunoglobulin isotype when tested against the Y4 organism. This serum was assigned a value of 100 EU at an equal dilution for each isotype and a standard curve was prepared by a linear regression analysis $OD = m(\log_{10} EU) + b$. The antibody activity (EU) for each experimental serum was determined by comparing its OD in the linear range of the assay with the standard serum curve.

Salivary IgA levels were measured by single radial immunodiffusion (Mancini, Carbonera & Heremans, 1965). The salivary precipitin rings were compared to a serum IgA standard and were converted by a factor of 3·25 as described by Brandtzaeg, Fjellanger & Gjeruldsen (1970) to adjust for secretory (SIgA) levels.

Statistical analyses of the antibody levels were determined by the Kruskall–Wallis one-way analysis of variance and the Wilcoxon/Mann–Whitney rank sum test. Statistical analyses of the distribution of responding individuals in the disease categories were performed using a Kolmogorov–Smirnov one-sample test (Siegel, 1956).

Adsorption procedure. To determine specificity of the antibody response to the Y4 organism, adsorptions of sera with the formalinized micro-organisms were performed. A 1:25 dilution of each serum (1·0 ml) was incubated with varying concentrations of the appropriate organism in 1·0 ml of

PBS and mixed for 2 hr at 37°C. The organisms were centrifuged and the resulting 1:50 serum dilution was removed and used to test residual antibody activity. Four control serum samples were also subjected to three consecutive adsorptions with 1×10^9 micro-organisms. A mean baseline EU value was subsequently determined from the four sera. Positive samples were those with EU values greater than 2 standard deviations above the mean negative level.

Antisera and human proteins. Rabbit antisera to human IgG (RAHG), IgM (RAHM), IgA (RAHA), and IgE (RAHE) were purchased from Behring Diagnostics (Somerville, New Jersey). Goat anti-rabbit IgG (GARG) was obtained from Miles Laboratories (Elkhart, Indiana). RAHG (Lot 0312L, 1·80 mg antibody/ml ± 30%), RAHM (Lot 0919F, 4·50 mg antibody/ml ± 30%) and RAHA (Lot 0807G, 1·50 mg antibody/ml ± 30%) were tested for specificity against normal human serum by immunoelectrophoresis (IEP) and were each found to react with a band of material consistent with the electrophoretic mobility of IgG, IgM and IgA respectively, A single precipitin band of non-identity was noted among the three antisera when reacted with the human serum in gel-diffusion analysis. Rabbit anti-human IgE (Lot D4221) was also tested in an IEP procedure and showed a single band when reacted against a serum from an atopic individual. This band had the electrophoretic mobility characteristic of human IgE. Human IgG (Cohn fraction II, American Red Cross) was dissolved in PBS at 12·5 mg/ml. Human α_2-globulins (Lot W3515, Schwartz Mann, Orangeburg, New York) and albumin (Cohn V, Lot 1832, American Red Cross) were also dissolved in PBS for use in specificity tests.

Table 1. Specificity of rabbit anti-human isotype sera

Protein preparation (concentration)*	Antisera isotype†	Treatment†	Antibody activity ELISA units	Per cent reduction in antibody activity
Human serum‡	IgG	−	103·1 ± 1·7§	
		+	7·7 ± 0·5	92·5
	IgM	−	26·5 ± 0·6	
		+	25·1 ± 0·8	5·3
	IgA	−	25·6 ± 3·6	
		+	26·0 ± 1·3	0
	IgE	−	5·0 ± 0·1	
		+	4·9 ± 0·2	3·9
Human IgG¶	IgG		12.2 ± 0·3	
	IgM		0·6 ± 0·1	
	IgA		0·7 ± 0·1	
	IgE		0·8 ± 0·1	
Human α_2-globulin (3 mg/ml)	IgG		0·1 ± 0·1	
Human serum albumin (45 mg/ml)	IgG		0·1 ± 0·1	

* Developing antisera were used at optimal concentrations for analysing each isotype of antibody.

† Normal goat serum pretreatment (−), pretreatment with goat anti-human IgG (+).

‡ The standard serum used had IgG activity 100 EU = $OD_{400\ nm}$ 1·258 at a 1:200 dilution; IgM: 25 EU = $OD_{400\ nm}$ 0·689 at a 1:50 dilution; IgA: 25 EU = $OD_{400\ nm}$ 1·336 at a 1:50 dilution; and IgE: 5 EU = $OD_{400\ nm}$ 0·844 at a 1:10 dilution.
5 EU = $OD_{400\ nm}$ 0·845 at a 1:10 dilution.

§ Values are means of triplicate determinations ± s.e.m.

¶ Human IgG was tested at a 1:25 dilution; α_2-globulin and human serum albumin were tested at a 1:10 dilution.

The specificity of the rabbit anti-human Ig serums (RAHG, RAHM, RAHA, and RAHE) was also tested in the ELISA. Intact formalinized Y4 were bound to the ELISA plates and the plates were then incubated with either an anti-Y4 containing human serum or with a pooled human IgG preparation (Cohn II) that had been shown by ELISA to have antibody activity against the Y4 organism. One series of samples was incubated with goat anti-human IgG (GAHG; Hyland, Costa Mesa, California) and a second series was incubated with normal goat serum (Colorado Serum Co., Denver, Colorado) for 2 hr prior to incubation with either RAHG, RAHM, RAHA or RAHE. A decrease in antibody activity due to prior blocking of the serum IgG antibodies with GAHG would indicate a degree of cross-reaction among the various rabbit antisera. The results in Table 1 show homologous blocking only between the two anti-IgG sera of nearly 93%. Also, only anti-IgG sera reacted with human IgG antibodies to Y4 (Table 1). Thus, within the limitations of the analyses used, each of the rabbit anti-human sera appeared to detect specifically only the appropriate antibody isotype designated.

RESULTS

Specificity of serum antibodies to Y4

To determine the specificity of the human serum antibodies reacting with Y4, high-titred sera were adsorbed with various other oral micro-organisms, as well as varying concentrations of the homologous bacteria (see Materials and Methods). The resulting adsorbed sera were then tested for residual IgG anti-Y4 activity in the ELISA (Table 2). Treatment of the serum with *A. naeslundii* str. I.

Table 2. Specificity of antibody reaction to intact *A. actinomycetemcomitans* strains

Treatment*	No. of bacteria	*vs* Y4		*vs* 29523	
		Mean EU†	% Reduction	Mean EU	% Reduction
None	—	102·3	—	98·7	—
A. naeslundii	10^9	101·8	0·5	110·3	0
B. gingivalis 381	10^9	102·1	0·2	93·0	5·8
Strep. sanguis 254	10^9	100·6	1·7	99·4	0
A. actinomycetemcomitans 29523	10^9	60·6	40·8	10·1	89·8
	10^8	81·5	20·3	32·6	67·0
	10^7	89·0	13·0	92·1	6·7
	10^6	95·7	6·5	97·8	1·0
	10^5	99·0	3·2	97·6	1·1
A. actinomycetemcomitans (Y4)	10^9	3·8	96·3	33·7	65·9
	10^8	9·9	90·3	85·9	13·0
	10^7	62·1	39·3	99·3	0
	10^6	71·3	30·3	98·7	0
	10^5	95·9	6·3	100·1	0

* Adsorptions of test sera ($n=5$) were performed as described under Materials and Methods with 1·0 ml of a 1:25 dilution of the sera treated with an equal volume of the micro-organisms or PBS (None).

† The serum sample was tested for IgG anti-Y4 at a dilution of 1:800 in the assay. The standard serum curve was described by the linear equation $OD_{400\ nm} = 1{\cdot}366\ (\log_{10} EU) - 0{\cdot}891$; ($r^2 = 0{\cdot}998$), and 100 EU had an $OD_{400\ nm}$ of 1·289. Mean EU was determined from triplicate analyses of each treatment sample and the standard errors for each test were no greater than 9%.

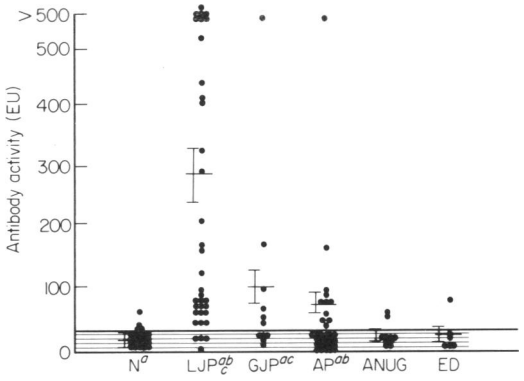

Fig. 1. Serum IgG antibody to *A. actinomycetemcomitans* strain Y4. Shaded area represents the mean negative serum value plus 2 standard deviations (38·1 EU). The horizontal lines represent the group mean and the bars 2 s.e.m. The antibody activity was determined from a standard serum curve OD = 1·246 ($\log_{10}$ EU) − 0·922; ($r^2 = 0{\cdot}973$). The standard serum yielded an $OD_{400\ nm} = 1{\cdot}266$ at a 1:200 dilution which was assigned a value of 100 EU. Statistical analysis for corresponding letters: a & $b = P < 0{\cdot}001$, $c = P < 0{\cdot}02$.

B. gingivalis str. 381 or *Strep. sanguis* str. 254 had no effect on the anti-Y4 activity. Adsorption of the sera with 10^9 *A. actinomycetemcomitans* ATCC 29523 (non-leucotoxin-producing strain) removed only 35% of the anti-Y4 activity while adsorption of the serum with only 10^7 homologous Y4 organisms removed nearly 40% of the IgG antibody activity and 10^9 organisms inhibited the ELISA reactivity by 96%. Furthermore, antibody reactivity in four human sera to Y4 grown in either PPLO (Mycoplasma) broth or in chemically defined medium was found to be similar. The results suggest that the antigenic determinants detected on the micro-organism were not media contaminants.

Serum antibody to Y4 in periodontal disease patients

IgG. IgG antibodies in the human sera showed a significantly increased level in the LJP patients compared to all other diseased or non-diseased groups (Fig. 1). Nearly 90% of the LJP patients had detectable IgG antibodies to Y4 which was significantly different than each of the other groups ($P < 0{\cdot}05$–$0{\cdot}001$) (Table 3).

IgM. The levels of IgM antibody in the periodontal disease groups were significantly greater than the normal patients. A significant difference was noted in the level of IgM between the juvenile periodontitis (LJP = 36·5 ± 6·5; GJP = 39·2 ± 9·2 EU) and adult periodontitis (25·2 ± 1·9 EU) groups, although no significant difference was found between the LJP and GJP groups. Serum IgM antibodies were detected in 70% (61 of 87) of patients diagnosed as having clinical symptoms of periodontal disease (LJP, GJP and AP) (Table 3). In contrast, N, ANUG and ED groups showed one-third or less of the individuals with a detectable level of IgM anti-Y4 activity. Also, there was no significant difference in the percentage of JP (LJP and GJP) and AP patients who had detectable IgM antibody.

IgA. The levels of IgA activity in the LJP group (201·2 ± 58·6 EU) were significantly greater than the AP, ED, ANUG and N groups (Table 3). Similar to IgG antibody levels, a significantly increased percentage of LJP patients (67·6%) had IgA antibodies to Y4 compared to all other groups tested with the exception of the GJP patients who also exhibited IgA antibody to Y4.

IgE. IgE antibody activity was noted in the sera from the juvenile periodontitis groups (LJP = 44·4 ± 12·6; GJP = 13·0 ± 2·7 EU) that was significantly greater than all other groups tested (Table 3). In general, the IgE antibody levels in other diseased groups (AP, ANUG) were similar to normal, control patients. Serum IgE anti-Y4 activity was also detected in a larger percentage of the LJP (75·7%) and GJP (81·2%) patients than all other groups ($P < 0{\cdot}01$).

Serum antibody to A. actinomycetemcomitans *ATCC 29523 in periodontal disease patients*

Serum samples from the diseased and normal groups were analysed for serum IgG antibody activity

Table 3. Serum antibody to *A. actinomycetemcomitans* strain Y4 in humans

	IgG	IgM		IgA		IgE	
Patient group	No. pos. patients/total patients*	Antibody activity (EU)†	No. pos. patients/total patients‡	Antibody activity (EU)¶	No. pos. patients/total patients**	Antibody activity (EU)††	No. pos. patients/total patients‡‡
Localized juvenile periodontitis	34/38[a, b, c]	36·5±6·5§[a, e]	28/37[e]	201·2±58·6[a]	25/37[e]	44·4±12·6[a, b]	28/37[e]
Generalized juvenile periodontitis	6/11[b, d]	39·2±9·2[a, d]	9/11[e]	119·9±87·0[f, d]	7/11[e]	13·0±2·7[a, b]	9/11[e]
Adult periodontitis	10/38[c]	25·2±1·9[a, d, e]	24/38[e]	9·4±1·1[a, d]	13/38[e]	3·2±0·4[b, d]	13/38[e]
ANUG	2/9	9·8±2·1	3/9	10·5±2·2	4/9	1·7±0·4	1/9
Edentulous	1/6	4·6±1·0	0/6	5·3±2·2	1/6	1·4±0·5	2/6
Normal	3/24[a, d]	9·0±1·0[a]	8/24[e]	6·0±1·0[a, f]	3/22[e]	1·2±0·4[a, d]	4/24[e]

* Positive samples for IgG gave antibody values >2 s.d. above the mean (18·3 EU) of four sera extensively adsorbed with homologous organisms (see Materials and Methods).

† Antibody activity for IgM was determined from a standard serum curve with the linear equation $OD_{400\ nm} = 0{\cdot}569\ (\log_{10} EU) - 0{\cdot}499$. The standard serum was assigned a value of 25 EU which yielded an $OD_{400\ nm}$ of 0·701 at a 1:50 dilution.

‡ Positive samples for IgM gave antibody values > 2 s.d. above the mean (11·1 EU) of four sera extensively adsorbed with homologous organisms (see Materials and Methods).

§ Values are means ± s.e.m.

¶ Antibody activity for IgA was determined from a standard serum curve with the linear equation $OD_{400\ nm} = 1{\cdot}189\ (\log_{10} EU) - 0{\cdot}991$; ($r^2 = 0{\cdot}922$). The standard serum was assigned a value of 25 EU which yielded an $OD_{400\ nm}$ of 1·510 at a 1:50 dilution.

** Positive samples for IgA gave antibody values > 2 s.d. above the mean (10·3 EU) of four sera extensively adsorbed with homologous organisms (see Materials and Methods).

†† Antibody activity for IgE was determined from a standard curve with the linear equation $OD_{400\ nm} = 0{\cdot}978\ (\log_{10} EU) - 0{\cdot}284$; ($r^2 = 0{\cdot}978$). The standard serum was assigned a value of 5 EU with an $OD_{400\ nm}$ of 0·404 at a 1:10 dilution.

‡‡ Positive samples for IgE gave antibody values >2 s.d. above the mean (3·8 EU) of four sera extensively adsorbed with homologous organisms (see Materials and Methods).

Corresponding lettered pairs are statistically different at: a, b, c = $P < 0{\cdot}001$, d = $P < 0{\cdot}05$, e = $P < 0{\cdot}01$ and f = $P < 0{\cdot}003$.

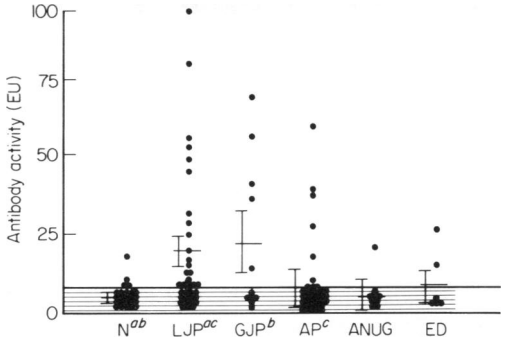

Fig. 2. Serum IgG antibody to *A. actinomycetemcomitans* ATC 29523. Shaded area represents the mean negative serum value plus 2 standard deviations (10·1 EU). Any serum values > 10·1 were considered positive. The horizontal lines represent the group mean and the bars 2 s.e.m. The antibody activity was determined from a standard serum curve OD = 1·031 ($\log_{10}$ EU) − 0·693. The standard serum yielded an $OD_{400\ nm} = 1{\cdot}591$ at a 1 : 200 dilution which was assigned a value of 100 EU. Statistical analysis for corresponding letters: $a = P < 0{\cdot}001$, $b = P < 0{\cdot}01$, $c = P < 0{\cdot}04$.

against a second strain of *A. actinomycetemcomitans* (ATCC 29523). A significantly greater percentage of LJP patients (57·9%) showed antibody to this organism compared to the AP (25·0%), ANUG (11·1%), ED (33·3%) or normal group (12·5%) of patients. The antibody levels in the JP patients were somewhat greater than the control group levels; however, the difference between LJP and GJP patients was not significant and the percentages of individuals demonstrating antibody in the GJP compared to the N group were similar (Fig. 2). The sera from each of these patients were also compared to determine the relationship between serum IgG antibody responses to the two Actinobacillus strains. The results suggested a significant positive correlation between the responses to the two organisms in the LJP and GJP patients. In both cases, nearly 65% of the patients responded to both strains. Also, very few JP individuals (LJP none of 34, GJP one of 11) responded to the 29523 strain without a concomitant response to the Y4 organism.

Table 4. Level of IgA and IgA anti-Y4 activity in parotid saliva of periodontal disease patients

Patient group	No. tested	IgA (mg/100 ml)	IgA anti-Y4* (EU)	No. pos./total
JP†	11	4·24 ± 0·81‡	$26{\cdot}4 \pm 10{\cdot}3^a$	$10/11^b$
AP	17	4·00 ± 0·65	4·8 ± 1·3	7/17
N	13	4·58 ± 0·93	$2{\cdot}0 \pm 0{\cdot}6^a$	$3/13^b$
IgA-deficient	2	0·03	0·7 ± 0·7	0/2

* IgA antibody activity against intact Y4 was determined from a standard saliva curve with the linear equation OD = 0·681 ($\log_{10}$ EU) − 0·608. The standard saliva yielded an $OD_{400\ nm} = 0{\cdot}607$ at a 1 : 5 dilution and was assigned a value of 100 EU. The negative saliva reactions were determined from triplicate measurements of two IgA-deficient individuals. Any antibody levels greater than 2 standard deviations above this mean (2·1 EU) were considered positive.

† The JP group consisted of nine LJP and two GJP patients.

‡ Values are mean ± s.e.m.

Statistical analyses of the corresponding lettered pairs: ^{a} & $^b = P < 0{\cdot}01$.

Salivary IgA antibody to Y4 in periodontal disease patients

Studies of patients with or without diagnosed periodontal disease (Table 4) from the Forsyth patient population showed normal levels of IgA in parotid saliva. However, a significantly greater frequency of combined JP patients (LJP+GJP) had salivary IgA antibodies to Y4 and a significantly higher level of salivary IgA antibody was detected in this JP group (Table 4). No differences were noted between the adult periodontitis group levels and the control samples.

DISCUSSION

Actinobacillus actinomycetemcomitans has been reported to be a constituent of the human oral microflora (Kilian & Schiott, 1975). *A. actinomyecetemcomitans* have been frequently isolated as a portion of the predominant cultivable flora from patients with juvenile periodontitis (Newman & Socransky, 1977; Tanner *et al.*, 1979; Slots, 1976; Slots, 1979). In contrast, this micro-organism was seldom detected in subgingival plaque from adult periodontitis or normal patients (Tanner *et al.*, 1979).

The present study identified human serum antibodies to *A. actinomycetemcomitans* strain Y4 and correlated these antibodies with the various types of periodontal disease exhibited by the patients in the experiment. Serum IgM antibodies were increased in patients with periodontal disease when compared to the normal group, ANUG patients or edentulous patients. IgA and IgE antibodies in serum were significantly greater in JP (LJP and GJP) patients when compared to adult periodontitis or control patients. Furthermore, serum IgG antibodies differentiated juvenile periodontitis patients with localized disease from all other patient groups tested. The results indicated that serum antibodies to Y4 were associated with juvenile periodontitis. In addition, the isotype of the antibody appears to be important in distinguishing LJP patients from patients with other forms of periodontal disease.

IgG, and especially IgA and IgE antibodies seem to correspond to more specialized aspects of humoral immunity (Benedict, 1979). In this regard, IgG, IgA and IgE antibodies to Y4 were found predominantly in patients with juvenile periodontitis. These findings may suggest that infection with this micro-organism is more chronic and that a greater diversity of antibody isotypes are elicited.

Salivary IgA antibodies to Y4 were also reflective of juvenile-type periodontal disease. Little information is available on the relationship of salivary secretory IgA (SIgA) antibodies to periodontal disease. The concept of SIgA as a 'blocking antibody' in protection of mucosal surfaces has been well established (Fubura & Freter, 1973; Williams & Gibbons, 1972); however, it is not known whether this ability is interfering with the production of pathogenic plaques in periodontal disease.

In contrast to the findings with *A. actinomycetemcomitans* strain Y4, the IgG antibody levels to a second strain of *A. actinomycetemcomitans* ATCC 29523 did not differentiate between the types of periodontal disease. While the levels of IgG antibody to *A. actinomycetemcomitans* 29523 in the LJP and GJP groups were significantly greater than the normal group, the percentages of patients that exhibited levels greater than the control sera were not distributed differently among the periodontal-diseased and non-diseased groups. These results may indicate that important antigenic determinants on Y4 that enable identification of the LJP from the other groups are missing from *A. actinomycetemcomitans* 29523. This suggestion is supported by the relative inability of *A. actinomycetemcomitans* 29523 to remove serum antibody activity to Y4. Recent investigations have suggested that *A. actinomycetemcomitans* strains comprise at least three serogroups (Tsai *et al.*, 1981). The Y4 and ATCC 29523 represent strains from two different groups.

The detection of IgE antibodies to the Y4 organism in the patients with periodontal disease is of special interest because of the *in vivo* manifestations of reaginic (IgE) antibody (Ishizaka & Dayton, 1973). Previous reports have attempted to relate the pathology of immediate hypersensitivity to the symptoms of periodontal disease (Nisengard, 1974; Nisengard & Beutner, 1970; Genco, 1970). Specific antibodies to Y4 of the IgE isotype are present and seem to be associated predominantly with juvenile periodontitis. Since the pathogenesis of periodontal disease appears to be a highly

localized phenomena in the gingiva, the importance of immediate hypersensitivity reactions in periodontal disease awaits the demonstration of increased IgE antibody levels at the local site of disease activity.

Use of the ELISA procedure enabled quantitative analysis of large numbers of serum samples for antibodies of different isotypes. A significant positive correlation was noted in individual LJP patients among the serum antibody isotypes. In addition, some patients from groups other than the LJP responded to Y4 with IgG antibody as well as antibody responses of other isotypes. Although few individuals from the other periodontal-disease groups exhibited this pattern, we feel that these individuals may also have a disease aetiology similar to the responsive LJP patients. Thus, while clinical diagnostic parameters recognized these individuals as having different diseases, the laboratory findings may support the concept that these patients are manifesting different stages of the same disease. Also, there were a few patients diagnosed clinically as having LJP with low to negligible antibody responses to the Y4 organism. In this case the clinical parameters may have assigned these individuals into a disease category that is inappropriate relative to the laboratory findings. The finding that humoral antibodies to this micro-organism do not strictly follow clinical diagnosis of LJP may suggest that evaluation of this disease entity should also incorporate immunological laboratory indices to aid in the description and eventual treatment of the periodontal diseases.

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