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Elevated serum IgG titer and avidity to *Actinobacillus actinomycetemcomitans* serotype c in Japanese periodontitis patients

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Aim: The purpose of this study was to characterize serum antibody responses to different serotypes of *Actinobacillus actinomycetemcomitans* strains in various forms of periodontitis and to determine whether any specific type of *A. actinomycetemcomitans* was associated with any specific form of periodontitis in a Japanese population.
Methods: Sonicated whole cell and autoclaved serotype antigens of *A. actinomycetemcomitans* were used. Serum IgG titer and avidity to *A. actinomycetemcomitans* were measured by enzyme-linked immunoabsorbant assay (ELISA) and ammonium thiocyanate-dissociation ELISA, respectively, in 46 aggressive periodontitis patients (8 localized, 38 generalized), 28 chronic periodontitis patients, and 18 periodontally healthy subjects. The presence of *A. actinomycetemcomitans* in plaque and saliva samples was determined using polymerase chain reaction.
Results: Generalized aggressive and chronic periodontitis patients exhibited significantly higher IgG titers than healthy subjects to both sonicated and autoclaved antigens of serotype c strains, while IgG titer to serotype b (Y4) was significantly higher in localized aggressive periodontitis patients compared to healthy subjects. No *A. actinomycetemcomitans* was detected in localized aggressive periodontitis patients. *A. actinomycetemcomitans*-positive patients exhibited significantly higher IgG titer and avidity to serotype c than *A. actinomycetemcomitans*-negative patients. In *A. actinomycetemcomitans*-positive patients, a significantly positive correlation was observed between antibody titer and avidity to serotype c. *A. actinomycetemcomitans*-positive patients with generalized aggressive periodontitis showed lower IgG avidities to serotype c than those with chronic periodontitis, though no statistically significant difference was found.
Conclusion: *A. actinomycetemcomitans* serotype c may play a significant role in chronic and generalized aggressive periodontitis, while *A. actinomycetemcomitans* serotype b may be associated with localized aggressive periodontitis in a Japanese population.

Key words: *Actinobacillus actinomycetemcomitans*; avidity; IgG; periodontitis; serotype

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Periodontitis is an infectious disease that results from the interplay between specific subgingival bacteria and host immune responses. *Actinobacillus actinomycetemcomitans*, which possesses a series of strong virulence factors (11), is considered to be the main etiologic agent of localized juvenile periodontitis (4, 38, 46), and can also contribute to adult periodontitis (30, 33).

The American Academy of Periodontology revised the classification of periodontal diseases in 1999, and the terms 'early onset or juvenile periodontitis' and 'adult periodontitis' were replaced by 'aggressive periodontitis' and 'chronic periodontitis' (2).

A. actinomycetemcomitans is classified into five serotypes (a–e) (14, 34, 47). They have been detected at different frequencies in infected subjects and serotypes a, b and c are more predominant than serotypes d and e (14, 34, 47). Serotype b strains were frequently isolated from localized juvenile periodontitis patients in the United States (46) and in Finnish (4) subjects with periodontal disease. A predominance of serotypes other than b, however, has been reported from other parts of the world (8, 34, 35). Chung et al. (8) reported approximately equal distributions of serotypes a, b and c in Korean localized juvenile periodontitis patients. Serotype c was reported to be predominant in Chinese periodontally healthy or diseased subjects (26). A previous study from our laboratory showed that serotypes a and c were predominant in Japanese patients with periodontitis (44). Taken together, these reports suggested that there may be specific distribution patterns in ethnically distinct population.

The serum IgG antibody titer to *A. actinomycetemcomitans* (10), especially serotype b (5, 37), has been reported to be elevated in the sera of localized juvenile periodontitis patients. Moreover, Gunsolley et al. (17) demonstrated a significant inverse relationship between antibody titers to *A. actinomycetemcomitans* Y4 and the number of teeth having slight or moderate attachment loss in aggressive periodontitis patients. These results suggest that the failure to mount a substantial antibody response to this organism leads to greater and more widespread periodontal destruction in generalized aggressive periodontitis subjects.

Antibody avidity is a measure of the net binding strength between multivalent antigen and polyclonal antibodies. It reflects the functional activity of the antibodies and increased relative avidity often occurs as a consequence of maturation of humoral response. Lopatin et al. (23) found elevated IgG antibody avidity to *Porphyromonas gingivalis* in adult periodontitis patients compared with healthy controls. On the other hand, Chen et al. (7) demonstrated that IgG avidity to *P. gingivalis* was lower in rapidly progressive periodontitis patients than in healthy controls and concluded that many generalized aggressive periodontitis patients do not produce protective levels of biologically functional antibody during the course of their natural infection. Whitney et al. (43) also reported that rapidly progressive periodontitis patients had lower avidity to *P. gingivalis*. Saito et al. (36) demonstrated that high-titer sera from patients without cultivable *A. actinomycetemcomitans* had higher avidity than sera from patients with high antibody titers and cultivable *A. actinomycetemcomitans*. These results suggest that high avidity antibody to *A. actinomycetemcomitans* may afford protection against *A. actinomycetemcomitans* infection.

Most previous studies on antibody responses against *A. actinomycetemcomitans* relied on using a single reference strain (usually serotype b) as the antigen in an Enzyme-Linked Immunosorbent Assay (ELISA) (9, 21). It may be crucial to use several serotypes of *A. actinomycetemcomitans* as antigens in the ELISA to ensure a high reliability of the results (42). Vilkuna-Rautiainen et al. (42) also reported that the sera of periodontitis patients particularly reacted with homologous *A. actinomycetemcomitans* isolated from the patients. The homologous *A. actinomycetemcomitans* could be replaced by the reference strain of the same serotype in ELISA. In the present study, we performed a systematic comparison of the serum IgG antibody responses (titer and avidity) to different serotypes of *A. actinomycetemcomitans* in patients with various forms of periodontitis and assessed the relationship between the presence of *A. actinomycetemcomitans* and serum IgG antibody titer and avidity in aggressive and chronic periodontitis. The purpose of our study was to determine whether a certain type of *A. actinomycetemcomitans* was associated with any specific form of periodontitis in a Japanese population.

Materials and methods

Study population

The study population included 74 periodontitis patients attending the Periodontics Clinic at Tokyo Medical & Dental University, and 18 periodontally healthy subjects. Informed consent was obtained from each subject. All subjects were evaluated by measuring clinical parameters such as probing depth, bleeding on probing and oral radiographs. The 74 patients included 46 with aggressive periodontitis (AgP) and 28 with chronic periodontitis (CP), who were diagnosed based on the workshop for classification in 1999 (12, 41). All patients had at least five teeth per quadrant. Each patient in CP group had at least two teeth with 5 mm or more pocket depth and radiographic evidence of bone loss. The AgP patients were further divided into those with localized ($n = 8$) and generalized ($n = 38$) forms. Generalized AgP patients had at least three teeth with 5 mm or more pocket depth and radiographic evidence of bone loss, other than molars and incisors. Healthy subjects showed no clinical or radiographic evidence of periodontal disease. All subjects were generally healthy and had not received any periodontal treatment during the previous 6 months or systemic antibiotic administration during the last 3 months.

Bacterial sampling and polymerase chain reaction (PCR) detection

Subgingival plaque samples were collected with a paper point from the deepest pockets in each quadrant in the periodontitis patients, and from the mesiobuccal surface of #16, #21, #36, and #41 of healthy subjects. Approximately 1 ml of unstimulated saliva was also collected from each individual in a sterile plastic tube. Probing pocket depth and bleeding on probing were measured and recorded for each tooth.

Subgingival plaque and saliva samples were subjected to a 16S rRNA-based PCR detection method (3) to determine the presence of *A. actinomycetemcomitans*. Briefly, 50 μl of PCR reaction mixtures contained 5 μl sample, 5 μl 10 × PCR buffer, 1.25 unit of *Taq* DNA polymerase (Promega, Madison, WI), 0.2 mM dNTP (Pharmacia LKB, Piscataway, NJ), 1.0 μM primer, and 1.0 mM $MgCl_2$ for *A. actinomycetemcomitans*. The temperature profile included an initial step of 95°C for 2 min, followed by 36 cycles of 94°C for 30 s, 55°C for 1 min and 72°C for 2 min, and a final step at 72°C for 2 min. PCR products were analyzed by 1.0% agarose gel electrophoresis with 0.01 μg/ml ethidium bromide. The plaque samples were analyzed as individual site samples, and subjects were designated as positive for *A. actinomycetemcomitans* if they had at least one positive sample.

Serum samples

Peripheral blood samples were collected from the patients and healthy subjects. Each blood sample was centrifuged at 1500 *g* for 20 min. The serum was filtered through a sterile 0.45 μm diameter filter (Millex, Millipore Japan Ltd, Tokyo, Japan) and stored at − 20°C until analysis.

Preparation of microorganisms

A. actinomycetemcomitans strains ATCC 29523 (serotype a), ATCC 43717 (serotype a), ATCC 29522 (serotype b), Y4 (serotype b), ATCC 43719 (serotype c) and NCTC 9710 (serotype c) were employed in this study. All *A. actinomycetemcomitans* strains were grown in brain heart infusion (BHI) broth (Difco, Sparks, MD) supplemented with 1% (w/v) yeast extract at 37°C for 3 days in a 5% CO_2 atmosphere. The microorganisms were harvested by centrifugation, washed three times with distilled water and lyophilized.

Of the six strains, ATCC 43717, Y4 (corresponding to the strain ATCC 43718), ATCC 43719, ATCC 29522 and ATCC 29523 were obtained from the American Type Culture Collection (Rockville, MD) and NCTC 9710 was obtained from the National Collection of Type Cultures (London, UK).

Sonicated whole cell extracts of *A. actinomycetemcomitans* were prepared according to the method proposed by Naito et al. (29). Cells were suspended in saline (200 mg/ml, wet weight) and disrupted by sonication at 30-s intervals for a total sonication time of 3 min at maximum output on ice. The sonicated cell suspensions were centrifuged and the supernatants lyophilized.

Autoclaved extracts of *A. actinomycetemcomitans* were prepared for the crude purification of serotype-specific antigen, according to the method proposed by Amano et al. (1). Lyophilized cell suspension in saline was autoclaved at 120°C for 15 min. The autoclaved extracts were centrifuged at 10,000 *g* for 20 min to collect the supernatants and lyophilized.

Serum antibody titer measurement

Specific serum IgG titers to each *A. actinomycetemcomitans* strain were measured using a previously described ELISA method (19). Briefly, 96-well microtiter plates (EIA plate, Costar, Cambridge, MA) were coated with sonicated extracts (10 μg/ml) or autoclaved antigen (10 μg/ml) in carbonate buffer, and incubated for 2 h at 37°C. After blocking with 2% BSA in carbonate buffer, plates were washed three times with PBS-T (1 × PBS, 0.05% Tween 20, pH 7.2). Serially diluted serum (2^5–2^{14}, 100 μl per well) was added into each well in duplicate and the plates were incubated for 2 h at 37°C. Following incubation, the plates were washed again three times. Subsequently, 100 μl per well of Alkaline phosphatase-conjugated goat anti-human IgG (Sigma Chemical Co., St. Louis, MO) was added. Following incubation, plates were washed three times and developed with phosphate substrate (Sigma 104). The optical density (OD) at 405 nm for each well was then measured using a Microplate Reader (SOFT Max™). Calculation for the antibody titer was done according to the method of Ishikawa et al. (19). Absorbance was plotted against the dilution ratio. The logarithm of the dilution ratio, at which the regression curve (obtained with a cubic polynomial expression) intersected the line expressed by OD = 0.8, was adopted as an antibody titer for a given sample, so that the antibody titer was expressed as $\log_2$ of the dilution.

Antibody avidity measurement

Serum IgG avidity was determined by estimating the dissociation of antigen–antibody binding by increasing the concentration of ammonium thiocyanate (0–8 M) (24, 28, 31). The microtiter plates were coated with sonicated extracts, and patient serum was added in duplicate at a dilution of 1/400. After the incubation plates were washed with PBS, the wells were treated with increasing concentrations of ammonium thiocyanate (0–8.0 M). The plates were incubated at 37°C for 60 min, washed three times and then alkaline phosphatase-conjugated goat anti-human IgG (Sigma Chemical Co.) was added into the wells. Following incubation, plates were washed three times with PBS, and p-nitrophenyl-phosphate (Sigma Chemical Co.) was added as the substrate. The OD at 405 nm for each well was then measured using a Microplate Reader (SOFT Max™). Percentage of antibody binding was calculated for each concentration [(OD of thiocyanate treated well/OD of control well) × 100] of thiocyanate. The avidity was expressed by the calculation of thiocyanate concentration required to inhibit 50% of the bound antibody (ID_{50}).

Inhibition assay to assess cross-reactivity of *Haemophilus aphrophilus* with *A. actinomycetemcomitans* strains

Microtiter plates were coated with sonicated extracts of *A. actinomycetemcomitans* strain as described above. High- and low-responding sera were selected (42). After washing, serial dilutions (2^5–2^{14}) of test serum were mixed with sonicated antigen of *H. aphrophilus* (10 μg/ml) or the same sonicated *A. actinomycetemcomitans* (10 μg/ml) and incubated for 2 h at 37°C. After washing, 1 : 1000 diluted alkaline phosphatase-conjugated goat anti-human IgG (Sigma Chemical Co.) was added and incubated for 1 h at 37°C. Finally, phosphate substrate (Sigma 104) was added and OD measured at 405 nm. The amount of dilution ratio (x-axis) and percentage of OD (y-axis) were plotted. The OD of the control (without absorption) was used as 100% (40).

Cross-reactivity among *A. actinomycetemcomitans* serotypes a, b, and c

Microtiter plates were coated with sonicated extracts of *A. actinomycetemcomitans* as described above. Test sera which had high IgG titers only to a single serotype were selected. After washing, serial dilutions (2^5–2^{14}) of test serum were mixed with autoclaved antigens of *A. actinomycetemcomitans* (a–c, 10 μg/ml) and incubated for 2 h at 37°C. After washing, 1 : 1000 diluted alkaline phosphatase-conjugated goat anti-human IgG (Sigma Chemical Co.) was added and incubated for 1 h at 37°C. Finally, phosphate substrate (Sigma 104) was added and OD measured at 405 nm. The amount of dilution ratio (x-axis) and a percentage of OD (y-axis) were plotted. The OD of the control (without any inhibitors) was used as 100% (40).

Statistical analysis

The ANOVA and t-test were employed to compare the mean titers and avidities in the four study groups and to determine the differences between antibody titers and avidities of *A. actinomycetemcomitans*-positive and -negative patients. Correlation between antibody titer and avidity was evaluated by Pearson's rank correlation test.

Results

IgG antibody titers against *A. actinomycetemcomitans*

The IgG titers to sonicated whole cell extracts of each *A. actinomycetemcomitans* strain in periodontitis patients and healthy subjects are shown in Fig. 1. The IgG titers to *A. actinomycetemcomitans* serotype c strains (ATCC 43719, NCTC 9710) were significantly higher in CP patients (10.6 ± 2.22; 9.47 ± 2.17), than those in healthy subjects (8.84 ± 1.01;

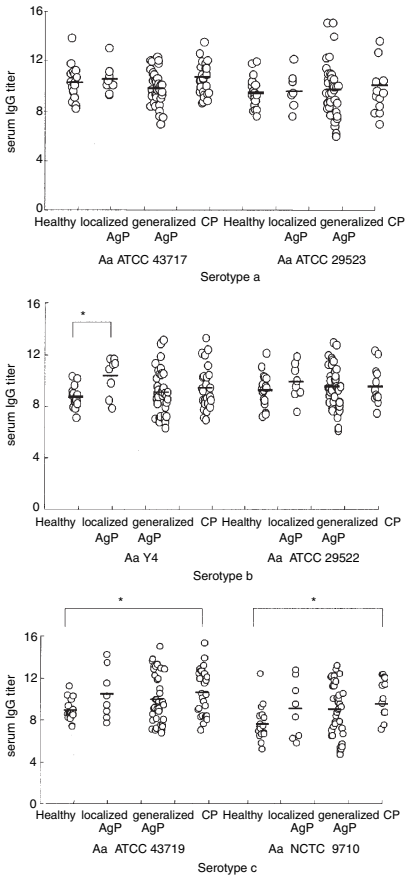

Fig. 1. Serum IgG titers to sonicated whole cell extracts of *A. actinomycetemcomitans* strains in periodontitis patients and healthy subjects. The black line expresses the mean titer of each group. The stars indicate the statistically significant differences between the groups (**P* < 0.05).

7.61 ± 1.63), respectively. This trend was also found in localized and generalized AgP patients, although the values failed to reach statistical significance. The localized AgP patients exhibited significantly higher IgG titers only to *A. actinomycetemcomitans* Y4 (serotype b) when compared to healthy subjects ($P = 0.033$). Neither the CP ($n = 28$) nor generalized AgP ($n = 38$) group showed significant difference in IgG titers to any serotype b strain from healthy subjects. No difference was found in IgG titers to *A. actinomycetemcomitans* serotype a strains (ATCC 43717, ATCC 29523) among the four study groups.

IgG antibody titers in *A. actinomycetemcomitans*-positive and -negative subjects

A. actinomycetemcomitans was detected from patients with CP (25%) and generalized AgP (24%), but was not found in localized AgP patients (0%) or healthy subjects (0%) (Table 1). The serum IgG titers to different *A. actinomycetemcomitans* strains in *A. actinomycetemcomitans*-positive and -negative patients are summarized in Table 2. In the CP group, the IgG titers to ATCC 43719 (serotype c) in the *A. actinomycetemcomitans*-positive patients (n = 7) were significantly higher than those of *A. actinomycetemcomitans*-negative patients ($P < 0.001$). In the generalized AgP group, *A. actinomycetemcomitans*-positive patients (n = 9) showed significantly elevated IgG titers not only to ATCC 43719 (serotype c) but also to ATCC 29523 (serotype a) compared with *A. actinomycetemcomitans*-negative patients ($P = 0.002$ and $P = 0.002$, respectively).

Antibody avidity in *A. actinomycetemcomitans*-positive and -negative subjects

Table 3 displays the serum IgG avidity to different *A. actinomycetemcomitans* strains in *A. actinomycetemcomitans*-positive and -negative subjects. *A. actinomycetemcomitans*-positive patients exhibited significantly higher IgG avidity to ATCC 43719 (serotype c) than *A. actinomycetemcomitans*-negative patients in both the generalized AgP and CP groups ($P = 0.01$ and $P < 0.01$, respectively). In addition, *A. actinomycetemcomitans*-positive patients with generalized AgP showed lower IgG avidity to ATCC 43719 (serotype c) than those with CP; however, the difference was not significant.

Correlation between antibody titer and avidity

Figure 2 illustrates the relationship between IgG avidity and titer to *A. actinomycetemcomitans* strains in *A. actinomycetemcomitans*-positive patients. A significant relationship between antibody avidity and titer to ATCC 43719 (serotype c) was found in generalized AgP ($r = 0.88$, $P = 0.002$) and CP patients ($r = 0.79$, $P = 0.04$).

Absorption test

The effect of possible cross-reactions of *A. actinomycetemcomitans* with *H. aphrophilus* is shown in Fig. 3A. Anti *A. actinomycetemcomitans* was inhibited by the absorption with homologous *A. actinomycetemcomitans* strain dose-dependently, while anti *A. actinomycetemcomitans* response was not affected by *H. aphrophilus*.

Figure 3B illustrates the possible cross-reactions of *A. actinomycetemcomitans* serotypes a, b, and c. Test sera which had elevated titer only to sonicated extract of serotype a, b or c were selected. IgG response to sonicated extract of

Table 1. Clinical parameters and microbiological data

	Chronic periodontitis	Aggressive periodontitis		Healthy
		Generalized	Localized	
No. of subjects	28	38	8	18
Age*	54.9 ± 6.8	27.0 ± 5.8	22.8 ± 5.6	26.3 ± 3
PD > 4 mm* (%)	43.2 ± 26.1	73.0 ± 21.7**	21.9 ± 11.5	0
BOP* (% of sites)	21.7 ± 22.7	38.9 ± 27.1	5.9 ± 3.5	0
Aa detection rate (%)	25%	24%	0	0

Aa, *A. actinomycetemcomitans*.
*Mean ± SD.
**Significantly higher than those in CP and localized AgP.

Table 2. Relationship between presence of *A. actinomycetemcomitans* and IgG titers to sonicated whole cell of *A. actinomycetemcomitans* strains

Aa strains	Subjects	IgG titers (mean ± SD)		*P*-value*
		Aa-positive	*Aa*-negative	
ATCC 29523 (serotype a)	CP	9.94 ± 1.92 (n = 7)	10.09 ± 2.35 (n = 21)	
	Generalized AgP	11.51 ± 1.69 (n = 9)	9.6 ± 1.59 (n = 29)	$P = 0.002$
	Localized AgP	–	9.5 ± 1.46 (n = 8)	
	Healthy	–	9.39 ± 1.26 (n = 18)	
Y4 (serotype b)	CP	9.94 ± 1.62 (n = 7)	9.17 ± 1.63 (n = 21)	
	Generalized AgP	9.85 ± 1.54 (n = 9)	8.76 ± 1.66 (n = 29)	
	Localized AgP	–	10.3 ± 1.48 (n = 8)	
	Healthy	–	8.66 ± 0.79 (n = 18)	
ATCC 43719 (serotype c)	CP	13.04 ± 1.26 (n = 7)	9.78 ± 1.83 (n = 21)	$P < 0.001$
	Generalized AgP	12.02 ± 1.87 (n = 9)	9.28 ± 2.18 (n = 29)	$P = 0.002$
	Localized AgP	–	10.4 ± 2.41 (n = 8)	
	Healthy	–	8.84 ± 1.01 (n = 18)	

**Aa*-positive vs. *Aa*-negative.

Table 3. Relationship between presence of *A. actinomycetemcomitans* and IgG avidity to sonicated whole cell of *A. actinomycetemcomitans* strains

Aa strains	Subjects	Antibody avidity (mean ± SD) *Aa*-positive	*Aa*-negative	*P*-value*
ATCC 29523	CP	2.40 ± 0.63 (n = 7)	2.59 ± 0.54 (n = 21)	
(serotype a)	Generalized AgP	2.41 ± 0.60 (n = 9)	2.41 ± 0.6 (n = 29)	
	Localized AgP	–	2.45 ± 0.18 (n = 8)	
	Healthy	–	2.33 ± 0.22 (n = 18)	
Y4 (serotype b)	CP	1.63 ± 0.91 (n = 7)	1.51 ± 0.63 (n = 21)	
	Generalized AgP	1.48 ± 0.89 (n = 9)	1.51 ± 0.5 (n = 29)	
	Localized AgP	–	1.59 ± 0.66 (n = 8)	
	Healthy	–	1.01 ± 0.37 (n = 18)	
ATCC 43719	CP	3.27 ± 0.51 (n = 7)	2.04 ± 0.93 (n = 21)	$P < 0.01$
(serotype c)	Generalized AgP	2.87 ± 1.16 (n = 9)	1.72 ± 1.11 (n = 29)	$P = 0.01$
	Localized AgP	–	2.07 ± 1.54 (n = 8)	
	Healthy	–	1.21 ± 0.64 (n = 18)	

**Aa*-positive vs. *Aa*-negative.

A. actinomycetemcomitans ATCC 29523 (serotype a) was reduced by the absorption of autoclaved homologous serotype a antigen but was not affected by serotypes b and c. IgG response to sonicated extracts of *A. actinomycetemcomitans* Y4 (serotype b) or ATCC 43719 (serotype c) was also inhibited only by the absorption of homologous autoclaved serotype antigen and not by the other antigens.

IgG antibody titer to *A. actinomycetemcomitans* serotype antigen.

Table 4 shows the IgG antibody titer against autoclaved serotype antigens of *A. actinomycetemcomitans*. *A. actinomycetemcomitans*-positive patients showed significantly higher IgG titers to serotype c than *A. actinomycetemcomitans*-negative patients in the CP and generalized AgP groups ($P < 0.001$, $P = 0.013$). In the generalized AgP group, *A. actinomycetemcomitans*-positive patients (n = 9) also showed significantly elevated IgG titers to serotype b compared with *A. actinomycetemcomitans*-negative patients ($P = 0.037$).

Discussion

The present study examined the systemic antibody responses (titer and avidity) to *A. actinomycetemcomitans* serotype (a, b, and c) strains in periodontally healthy subjects and patients with various types of periodontitis. Our results showed higher IgG titers and avidities to *A. actinomycetemcomitans* serotype c in generalized AgP and CP patients compared with healthy subjects. Moreover, the antibody avidity was found to be positively correlated with antibody titer in *A. actinomycetemcomitans*-positive patients (both generalized AgP and CP). These results indicate that *A. actinomycetemcomitans* serotype c may be mainly associated with generalized AgP and CP.

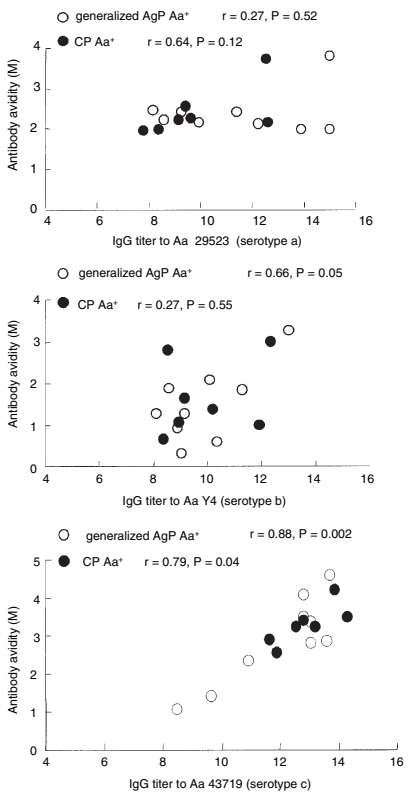

Fig. 2. Correlation between antibody avidities and titers to sonicated whole cell extracts of *A. actinomycetemcomitans* strains in *A. actinomycetemcomitans*-positive patients. Correlation coefficient (r) represents the relationship between IgG titers and avidities.

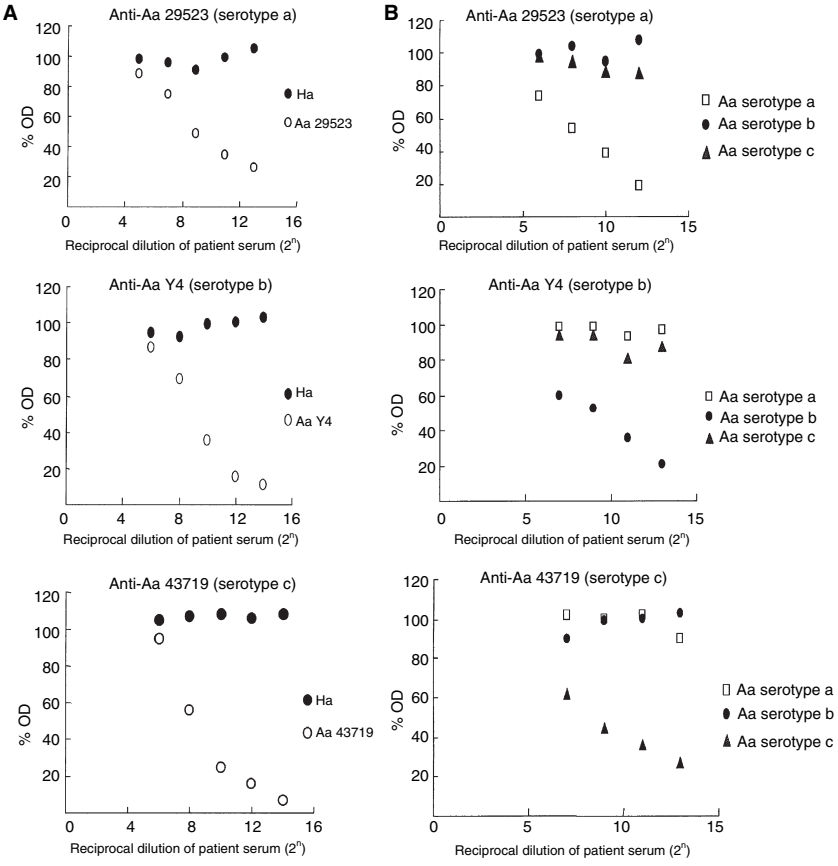

Fig. 3. Cross-reactivity of *A. actinomycetemcomitans* strains with *H. aphrophilus* and among *A. actinomycetemcomitans* serotypes. A) Effect of the absorption with *H. aphrophilus* on the IgG titer to sonicated extracts of *A. actinomycetemcomitans* strains. B) Effect of the absorption with autoclaved serotype antigens on IgG antibody to sonicated extracts of *A. actinomycetemcomitans*.

Table 4. Serum IgG titers to autoclaved serotype antigen of *A. actinomycetemcomitans*

Aa strains	Subjects	IgG titers (mean ± SD)		*P*-value*
		Aa-positive	*Aa*-negative	
Serotype a (ATCC 29523)	CP	10.63 ± 2.27 (n = 7)	10.36 ± 2.82 (n = 21)	
	Generalized AgP	11.18 ± 3.32 (n = 9)	8.98 ± 1.88 (n = 29)	
	Localized AgP	–	9.27 ± 1.77 (n = 8)	
	Healthy	–	8.16 ± 1.85 (n = 18)	
Serotype b (Y4)	CP	9.22 ± 2.02 (n = 7)	8.29 ± 1.58 (n = 21)	
	Generalized AgP	9.54 ± 2.12 (n = 9)	7.79 ± 2.13 (n = 29)	$P = 0.037$
	Localized AgP	–	8.87 ± 1.98 (n = 8)	
	Healthy	–	5.96 ± 1.19 ($n = 18$)	
Serotype c (ATCC 43719)	CP	10.16 ± 0.95 (n = 7)	7.03 ± 1.8 (n = 21)	$P < 0.001$
	Generalized AgP	9.42 ± 1.32 (n = 9)	6.96 ± 2.69 (n = 29)	$P = 0.013$
	Localized AgP	–	8.08 ± 3.01 (n = 8)	
	Healthy	–	5.82 ± 1.45 (n = 18)	

* *Aa*-positive vs. *Aa*-negative.

We tested the effect of possible cross-reactions with *H. aphrophilus* (Fig. 3A). There was little difference in the IgG titers between the absorbed and control (no inhibition) sera, suggesting that the majority of these antibodies were *A. actinomycetemcomitans*-specific. This result is in accordance with previous studies (13, 42).

Previous findings suggested that the pathogenic properties of *A. actinomycetemcomitans* isolates may differ (4, 30, 33, 38, 45). Therefore, we detected IgG antibody responses to sonicated antigens of two strains of each *A. actinomycetemcomitans* serotype and found the same trend in IgG responses to the same two serotype strains and difference among serotypes. Our absorption test indicated that IgG titers to sonicated extracts mainly resulted from antibody reactivity with the serotype-specific polysaccharide antigen (Fig. 3B). We further determined the level of IgG titers to *A. actinomycetemcomitans* using autoclaved serotype antigen and found similar results as when using sonicated extracts, confirming that the IgG response to serotype c was elevated in *A. actinomycetemcomitans*-positive patients with generalized AgP and CP.

Previous studies reported variations in the distribution of *A. actinomycetemcomitans* serotypes in different geographic/ethnic populations (4, 8, 27, 34, 35, 45). A recent report from Switzerland (16) demonstrated broad serotype diversity in a young ethnographically heterogeneous periodontitis group. Celenligil & Ebersole (6) previously reported that Turkish patients with localized juvenile periodontitis exhibited significantly increased antibody levels to *A. actinomycetemcomitans* serotypes c (ATCC 33384, SUNYa B67) and a (ATCC 29523, SUNYa B75), while antibody levels to *A. actinomycetemcomitans* Y4 and JP2 (serotype b) were significantly higher in localized juvenile periodontitis patients in the United States. In the present study, we identified a significant increase in serum antibody to *A. actinomycetemcomitans* Y4 in localized AgP patients, while generalized AgP and CP patients showed higher titers to serotype c than healthy subjects in a Japanese population. These data collectively suggest that considerable variation exists in the systemic antibody titers to *A. actinomycetemcomitans* among different populations. Ishikawa et al. (18) reported that *P. gingivalis* is associated with AgP as well as CP in Japanese population. Yoshida et al. (45) indicated that *A. actinomycetemcomitans* serotype c was detected more frequently in *P. gingivalis*-positive sites in Japanese adults. A recent study from our laboratory showed that most *A. actinomycetemcomitans*-positive sites harbored *P. gingivalis* (39). Thus, *A. actinomycetemcomitans* serotype c may have the ability to adapt to a *P. gingivalis*-positive microenvironment. However, the detection rate of *A. actinomycetemcomitans* was low in the present study. Methods of bacterial sampling and identification affect the results of bacterial analysis and we carefully considered and selected the PCR method to reduce the risk of underestimation. In this study *A. actinomycetemcomitans* was not commonly found in localized or generalized aggressive periodontitis, as found in North America and Europe (4, 38, 46), perhaps due to racial and geographic differences in the study populations.

The elevated antibody against *A. actinomycetemcomitans* serotype b (Y4) in localized AgP is in accordance with earlier studies (10, 22, 27). Ranney et al. (32) and Gunsolley et al. (17) showed that lesions of AgP in patients with high antibody titers against *A. actinomycetemcomitans* were less severe compared to those in patients with low antibody titers, thus emphasizing the protective role of anti-*A. actinomycetemcomitans* antibody. Lack of *A. actinomycetemcomitans*-positive subjects in the localized AgP group might be due to the elimination of *A. actinomycetemcomitans* by a robust antibody response, although the small sample size ($n = 8$) makes it difficult to verify this. We observed that the *A. actinomycetemcomitans*-positive patients showed higher serum IgG titers to serotype c than *A. actinomycetemcomitans*-negative patients in the CP and generalized AgP groups, implying that those patients could produce IgG antibody against *A. actinomycetemcomitans* serotype c, but that the antibody could not eliminate this organism. These results suggest that high IgG responses against *A. actinomycetemcomitans* may eliminate *A. actinomycetemcomitans* serotype b, but not serotype c.

Antibody avidity, the net binding strength between multivalent antigen and polyclonal antibodies, has been investigated in a number of infectious diseases, both in relation to antibody titer and in terms of disease susceptibility and progression (15, 20, 25). Chen et al. (7) showed that one-third of the rapidly progressive periodontitis patients in their study produced antibodies reactive with *P. gingivalis*, but the antibodies were of low avidity and thus presumably did not eliminate this infection. Whitney et al. (43) also suggested that in rapidly progressive periodontitis patients, low avidity antibodies to *P. gingivalis* may be ineffective in clearing this organism. Our results showed that *A. actinomycetemcomitans*-positive patients exhibited significantly higher IgG antibody avidity to serotype c (ATCC 43719) compared to *A. actinomycetemcomitans*-negative patients in both generalized AgP and CP groups. These results indicate that generalized AgP patients could undergo affinity maturation in response to *A. actinomycetemcomitans*. Moreover, a significantly positive correlation was observed between the titers and avidities to serotype c (ATCC 43719), indicating that antibody production was accompanied by the affinity maturation (increased avidity). However, *A. actinomycetemcomitans*-positive patients with generalized AgP showed lower IgG avidities than those with CP, suggesting that patients with generalized AgP could not produce strong avidity antibody compared to patients with CP.

In conclusion, the quality and quantity of serum antibody responses to *A. actinomycetemcomitans* serotypes a, b, and c are different in chronic and aggressive

periodontitis. *A. actinomycetemcomitans* serotype c may play a significant role in chronic and generalized aggressive periodontitis, while *A. actinomycetemcomitans* serotype b (Y4) may be associated with localized aggressive periodontitis patients in a Japanese population.

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