Humoral Immunity to Commensal Oral Bacteria: Quantitation, Specificity and Avidity of Serum IgG and IgM Antibodies Reactive with Actinobacillus actinomycetemcomitans in Children

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Abstract: The levels, specificity and avidities of serum IgM and IgG antibodies reactive with Actinobacillus actinomycetemcomitans (Aa) serotypes a, b and c were determined in periodontally healthy (PH) children and compared with subjects with localized juvenile periodontitis (LJP). All PH children exhibited IgM and IgG Aa-reactive antibodies whether or not Aa was detected subgingivally but the antibodies were not specific for Aa. In contrast, LJP sera contained high concentrations of IgM and IgG antibodies reactive with Aa that were largely specific for this bacterium. IgM and IgG antibodies in both PH and LJP subjects were of low avidity. With one exception, the avidities of IgG anti-Aa antibodies were significantly greater than those of IgM antibodies in both PH and LJP subjects. However, although the LJP subjects had as much as 115-fold more Aa-reactive IgG antibody than did the PH subjects the avidities of their IgG antibodies were no greater than those of the PH group. The induction by the host of low-avidity antibodies, that are ineffective in immune elimination, may be a reason why commensal bacteria persist at mucosal surfaces and why persons with LJP fail to eliminate Aa from their periodontal pockets.

Key words: Actinobacillus actinomycetemcomitans, Antibodies, Avidity

It remains unclear whether periodontopathic bacteria are true exogenous pathogens or opportunistically pathogenic commensal oral bacteria (4, 6, 16, 32, 36). The microbiology of periodontal diseases shares characteristics with mixed anaerobic infections (33), in that both are polymicrobial with the bacterial composition changing during the evolution of the infection. There are considerable data that implicate Actinobacillus actinomycetemcomitans (Aa) in the etiology and pathogenesis of localized juvenile periodontitis (LJP) (8, 28, 36-38). A number of studies have reported that patients with LJP have elevated levels of serum and gingival crevicular fluid antibodies reactive with Aa compared to periodontally healthy (PH) subjects (9-12, 15). The presence of elevated serum anti-Aa antibodies has been suggested as a means to diagnose LJP (15). The majority of PH subjects possess Aa-reactive serum antibodies also, and Aa can be isolated from PH individuals (27). However, the specificity of Aa-reactive antibodies in PH and LJP subjects and the role they play in host defense remain unclear.

All effector functions of antibody involved in protection against bacterial infections are dependent not only on the isotype, quantity and specificity of antibody but on antibody avidity (3, 5, 14). The purpose of this study was to examine the prevalence of Aa in PH children and measure the levels, specificity and avidity of their Aa-reactive serum antibodies. These data were then compared with data obtained from a small number of LJP subjects in an attempt to understand the role of antibody in the regulation of commensal oral bacteria, some of which are opportunistically pathogenic.

Materials and Methods

Sera. Serum was collected from fifty PH children aged 6–14 years (mean age 9.4 years) who attended the

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Abbreviations: Aa, Actinobacillus actinomycetemcomitans; ATCC, American Type Culture Collection; ELISA, enzyme-linked immunosorbent assay; γ, heavy chain of immunoglobulin G; Ha, Haemophilus aphrophilus; Hp, Haemophilus paraphrophilus; HRP, horseradish peroxidase; IgG, immunoglobulin G; IgM, immunoglobulin M; LJP, localized juvenile periodontitis; μ, heavy chain of immunoglobulin M; MW, molecular weight; PBS, phosphate-buffered saline; PH, periodontally healthy.
Department of Pediatric Dentistry or who sought acute care at the Emergency Room of Children’s Hospital National Medical Center, Washington, D.C., U.S.A. Details of these patients have been published elsewhere (1). No subject had received antibiotics for the previous two months before sampling. Serum samples from five age-matched subjects with Aa-associated localized juvenile periodontitis (LJP) were obtained from Dr. Joseph Zambon, Department of Oral Biology, S.U.N.Y. at Buffalo, Buffalo, N.Y., U.S.A.

Detection of A. actinomycetemcomitans. Aa was detected and semi-quantitated in subgingival plaque obtained with paper points from all four first permanent molar teeth using DNA hybridization (25) with a species-specific probe (BioTechnica Diagnostics, Cambridge, Mass., U.S.A.).

Bacteria. A. actinomycetemcomitans ATCC 43717 (serotype a), ATCC 43718 (serotype b), ATCC 43719 (serotype c), Haemophilus aphrophilus ATCC 33389 (Ha) and Haemophilus parainfluenzae (Haemophilus paraphrophilus) ATCC 7901 (22) (Hp) were grown in the ultrafilterate (10,000 MW) of brain heart infusion broth (Difco Labs., Detroit, Mich., U.S.A.) containing cysteine HCl and menadione in an atmosphere of 5% CO₂ in air at 37 °C for 72 hr. Fildes enrichment medium (Remel Labs., Lenexa, Kan., U.S.A.) was added for growth of Ha and Hp. The cells were harvested by centrifugation, washed twice in PBS and killed with formalin.

Quantitation of anti-A. actinomycetemcomitans IgG and IgM antibodies. IgG and IgM antibodies reactive with Aa serotypes a, b and c, and IgG antibodies reactive with Ha and Hp were quantitated in the serum samples by a direct enzyme-linked immunosorbent assay (ELISA) using whole bacterial cells as the solid phase. Total IgG and IgM in the serum samples were quantitated by a direct sandwich ELISA. Both antibacterial antibody and immunoglobulin quantitation ELISA assays employed the same horseradish peroxidase (HRP) conjugated anti-isotype antibodies. The absorbance values derived from measurement of Aa antibodies were interpolated into the IgG and IgM standard curves to convert absorbance into micrograms of antibody. Preliminary checkerboard titrations were performed to determine the optimal dilutions of the serum samples that fell in the IgG and IgM standard curves and to ensure that the slopes of the antibody and immunoglobulin quantitation curves were parallel. Polystyrene microtiter plates (Immulon I, Dynatech Labs., Chantilly, Va., U.S.A.) were coated overnight with whole bacterial cells at 10 µg dry wt/ml or with 1 µg/ml of the IgG fraction of rabbit anti-human μ or γ. The wells were blocked with 0.1% bovine serum albumin in 0.067 m phosphate-buffered saline (PBS), pH 8.0. Replicate samples of sera diluted in PBS-Tween 20 (0.1%) were added in parallel to the anti-human μ-, anti-human γ- and bacteria-coated wells and were incubated at ambient temperature for 1 hr. Following aspiration of the sera, the wells were washed three times with PBS-Tween and incubated for an additional hour with horseradish peroxidase-conjugated (HRP) rabbit anti-human μ or anti-human γ. After aspiration of the conjugate and washing, 0.1 mg/ml o-phenylene-diamine dihydrochloride (Sigma Chemical Co., St. Louis, Mo., U.S.A.) in 0.1 m citrate-phosphate buffer, pH 4.5, containing 0.012% H₂O₂ was added as substrate. Optical density was measured at 450 nm. In order to control for plate-to-plate variation and to serve as a positive control several dilutions of a hyperimmune rabbit, anti-Aa antisera were included on every plate. Bound rabbit antibodies were detected using the IgG fraction of swine anti-rabbit immunoglobulins conjugated with HRP. Wells in which PBS-Tween replaced sample served as negative controls. The range of the IgG standard curve was 0 to 100 ng/ml and the IgM curve 0 to 200 ng/ml. The data were fitted to a straight line using linear regression. The coefficients of determination (R²) always exceeded 0.90. All antibodies were obtained from Dako Corp., Carpinteria, Calif., U.S.A.

Absorption of sera. Ten randomly selected PH sera and all five LJP sera were diluted 1:50 in PBS-Tween and aliquots were mixed with equal volumes of packed, washed formalin-killed bacterial cells of Aa serotypes a, b, and c, Ha and Hp at 37 °C for 2 hr and overnight at 4 °C, respectively. After absorption, the bacteria were removed by centrifugation and anti-bacterial antibody in absorbed and unabsorbed aliquots of the sera were assayed in parallel by ELISA.

Avidity of IgG and IgM A. actinomycetemcomitans-reactive antibodies. Avidity was measured by chaotrope dissociation ELISA as described previously (23). Briefly, 96-well polystyrene microtiter plates were coated overnight with Aa and blocked as described above. Blocks of ten replicate Aa-coated wells were charged with each serum sample or a hyperimmune rabbit anti-Aa serum diluted in 0.1% Tween 20 in PBS. A set of duplicate wells received PBS alone as a negative control. After incubation for 1 hr, the samples and controls were removed and the wells thoroughly washed with PBS-Tween. Duplicate wells of each block were then incubated with sodium thiocyanate in PBS at concentrations of 1.0, 2.0, 3.0, and 4.0 m for 1 hr. Following washing to remove the chaotrope, HRP-conjugated rabbit anti-human μ or γ was added to the wells for 1 hr to detect bound human antibody. HRP-conjugated swine anti-rabbit Ig was used to detect bound rabbit antibodies. After aspiration of the HRP conjugate and washing, the plates were developed as described above. The
absorbance values at 450 nm were plotted against the molarity of NaSCN and fitted to a straight line by linear regression. If the coefficient of determination ($R^2$) for any sample set fell below 0.90, the assay was repeated. The avidity index (ID$_{50}$) was defined as the molarity of NaSCN required to reduce the absorbance of buffer control wells by 50%. Preliminary experiments in which the Aa-coated wells were incubated with 4.0 M NaSCN confirmed that the chaotrope did not remove bacteria from the wells. The ID$_{50}$ of the Aa-reactive antibodies from the LJP and PH subjects were compared with the ID$_{50}$ of hyperimmune rabbit anti-Aa sera and anti-tetanus toxoid antibodies from human subjects receiving booster immunization.

Statistical analysis. The Shapiro-Wilk’s statistic $W$ (26) was used to test the hypothesis that the concentration and avidity data values approximated those of a random sample from a normal distribution. Student’s $t$-test for equal and unequal variances was used to test mean concentration and avidity differences by source of population, immunoglobulin isotype and Aa serotype. Simple linear regression was employed for separate source-Ig combinations to determine the amount of variation in avidity accounted for by concentration. Statistical differences at $P \leq 0.05$ were considered to be significant.

Results

A total of 200 subgingival plaque samples from the four first permanent molars in the 50 PH children were

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**Fig. 1.** Concentrations of IgM and IgG antibodies in periodontally healthy (PH) children reactive with *A. actinomycetemcomitans* serotypes $a$, $b$ and $c$ (open circles) versus the number of subgingival sites positive for *A. actinomycetemcomitans*. Concentrations of *A. actinomycetemcomitans*-reactive IgM and IgG antibodies in subjects with localized juvenile periodontitis (LJP) (closed triangles).
probed for the presence of Aa. The sensitivity of the DNA probe was 10^3 bacteria. Aa was detected in at least one site from half of the PH subjects. None of the subjects harbored Aa at all four sites sampled.

The concentrations of IgM and IgG antibodies reactive with Aa serotypes a, b and c in the PH and LJP subjects are shown in Fig. 1. The data are displayed to show the antibody concentrations of those PH subjects in whom A. actinomycetemcomitans was not detected at any of the four sampled sites and of PH subjects harboring Aa in one or more of the four sampled sites. All PH and LJP subjects harbored IgM antibodies reactive with Aa serotypes a, b and c, however, in the PH subjects, there was no relationship between the number of sites positive for Aa and antibody concentration. Indeed, the PH subjects in whom Aa could not be detected at any of the sampled sites had concentrations of Aa-reactive antibodies no different to those PH subjects that harbored Aa at one or more of the sampled sites. The subjects with LJP had significantly higher concentrations of IgM antibodies reactive with Aa serotype a than the PH children (P=0.04), but this was not the case for the concentrations of IgM antibodies reactive with Aa serotype b (P=0.08) and Aa serotype c (P=0.09).

The mean concentrations of IgG Aa-reactive antibodies in the PH subjects were between a fifth to a half that of IgM Aa-reactive antibodies for all three serotypes. These differences in concentrations between the IgM and the IgG Aa-reactive antibodies in PH subjects were statistically significant for all serotypes (P=0.0001). In contrast, LJP subjects exhibited concentrations of IgG Aa-reactive antibodies approximately 3- to 14-fold greater than their IgM Aa-reactive antibodies. However, the small sample size and the large standard deviations in this group precluded these differences from reaching statistical significance: serotype a (P=0.14), serotype b (P=0.06), and serotype c (P=0.06). When compared to PH subjects, the concentrations of IgG Aa-reactive antibodies in the LJP subjects were 35-fold greater for serotype a (P=0.05), 115-fold greater for serotype b (P=0.05) and 57-fold higher for serotype c (P=0.05).

Absorption studies revealed that IgG Aa-reactive antibodies in the PH sera were extensively cross-reactive with all three serotypes of Aa and with the two commensal Haemophilus species examined (Table 1). In contrast, IgG antibodies in the LJP sera were more specific for Aa (Table 2). Absorption of PH sera with Aa serotype c completely removed IgG antibodies reactive with serotypes a and b. Absorption with Aa serotype b removed all IgG antibodies reactive with serotype c and over 85% of IgG antibodies reactive with serotype a. Absorption with Aa serotype a removed approximately three-quarters of IgG antibodies reactive with serotype b and with serotype c. Absorption of the PH sera with either Ha or Hp depleted IgG antibodies reactive with all three serotypes of Aa by approximately 59–70%. However, combined absorption with both Ha and Hp did not absorb more antibody than did Ha or Hp alone. In marked contrast to the results obtained with PH sera, absorption of LJP sera with Aa serotype c removed only 28.8% of IgG antibody reactive with Aa serotype b. Absorption with Aa serotype a removed only 28.1% of serotype b and 26.8% of serotype c-reactive antibodies. However, Aa serotype b was equally effective in absorbing serotype a and serotype c-reactive IgG antibodies from LJP sera as from PH sera. Individually, Ha and Hp

<table>
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<tr>
<th>Antigen</th>
<th>Aa (a)</th>
<th>Aa (b)</th>
<th>Aa (c)</th>
<th>Ha</th>
<th>Hp</th>
<th>Ha + Hp</th>
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<tr>
<td>Aa (a)</td>
<td>100</td>
<td>85.1±9.8</td>
<td>100</td>
<td>69.3±7.5</td>
<td>59.6±9.8</td>
<td>66.6±10.0</td>
</tr>
<tr>
<td>Aa (b)</td>
<td>72.7±9.1</td>
<td>100</td>
<td>99.3±7.7</td>
<td>59.2±6.8</td>
<td>59.5±8.1</td>
<td>60.5±7.6</td>
</tr>
<tr>
<td>Aa (c)</td>
<td>74.0±8.8</td>
<td>100</td>
<td>100</td>
<td>57.9±6.6</td>
<td>60.4±8.8</td>
<td>58.6±8.4</td>
</tr>
</tbody>
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Table 1. Effect of absorption with A. actinomycetemcomitans (Aa) serotypes a, b and c, H. aphrophilus (Ha) and H. paraphrophilus (Hp) on serum IgG antibody reactive with Aa serotypes a, b and c in periodontally healthy (PH) children

| Percent reduction of IgG antibody activitya in PH sera
<p>|</p>
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<tr>
<td>Aa (a)</td>
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<tr>
<td>Aa (a)</td>
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<td>Aa (b)</td>
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<td>Aa (c)</td>
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Table 2. Effect of absorption with A. actinomycetemcomitans (Aa) serotypes a, b and c, H. aphrophilus (Ha) and H. paraphrophilus (Hp) on serum IgG antibody reactive with Aa serotypes a, b and c in subjects with localized juvenile periodontitis (LJP)

<p>| Percent reduction of IgG antibody activitya in LJP sera |</p>
<table>
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<th>absorbed with:</th>
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<td>Aa (a)</td>
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<td>Aa (a)</td>
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<td>Aa (b)</td>
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<td>Aa (c)</td>
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a Mean ± standard error of the mean; b n=10.

Table 2. Effect of absorption with A. actinomycetemcomitans (Aa) serotypes a, b and c, H. aphrophilus (Ha) and H. paraphrophilus (Hp) on serum IgG antibody reactive with Aa serotypes a, b and c in subjects with localized juvenile periodontitis (LJP)
absorbed less than 4% of IgG antibodies reactive with either $Aa$ serotype $b$ or $Aa$ serotype $c$. $Ha$ absorbed 12% and $Hp$ 20%, approximately, of antibodies reactive with $Aa$ serotype $a$. However, in distinction to the finding with PH sera, absorption of LJP sera with $Ha$ and $Hp$ was additive.

Low-avidity IgM and IgG antibodies reactive with $Aa$ serotypes $a$, $b$ and $c$ were present in both the PH and LJP subjects. The mean avidities of IgM $Aa$-reactive antibodies in the serum samples of the PH children and the subjects with LJP were remarkably similar for all serotypes. There also were no significant differences between the mean avidities of the IgG $Aa$-reactive antibodies in the PH children and the LJP subjects (Table 3).

The mean avidities of IgG $Aa$-reactive antibodies in the PH subjects were significantly higher than those of IgM antibodies for all $Aa$ serotypes ($P=0.0001$). For the LJP subjects, only the avidities of IgG antibodies reactive with $Aa$ serotypes $b$ ($P=0.028$) and $c$ ($P=0.032$) were significantly higher than those for IgM.

Associations between antibody concentration ($\mu$g/ml) and antibody avidity (SCN$^-$ molarity) were examined by regression analysis (Table 4). For the PH children there were no statistically significant relationships between avidity and concentration of IgG or IgM antibodies reactive with $Aa$ serotype $a$, $b$ or $c$, with the single exception of IgM antibodies reactive with $Aa$ serotype $a$ ($P=0.003$). For IgM antibodies reactive with $Aa$ serotype $a$, there was a fairly strong correlation between the avidity and concentration ($\rho=0.45$), but concentration accounted for little of the variation in avidity (adjusted $R^2=0.18$).

For LJP subjects, there was no relationship between concentration and avidity for IgG antibodies reactive with $Aa$ serotype $a$. Although the relationship between concentration and avidity of IgG antibodies reactive with $Aa$ serotype $b$ did not reach statistical significance ($P=0.06$), there was a very strong correlation between avidity and concentration ($\rho=0.87$) with concentration accounting for much of the variation in avidity (adjusted $R^2=0.67$). For $Aa$ serotype $c$, the relationship between concentration and avidity was highly significant ($P=0.012$). In addition, there was a very strong correlation between avidity and concentration ($\rho=0.95$) with concentration again accounting for much of the variation in avidity (adjusted $R^2=0.84$).

Table 3. Comparison of avidities of IgM and IgG antibodies reactive with $A. actinomycetemcomitans$ ($Aa$) serotypes $a$, $b$ and $c$ in periodontally healthy (PH) and localized juvenile periodontitis (LJP) subjects

<table>
<thead>
<tr>
<th>Serotype</th>
<th>Source</th>
<th>IgM (mean ± S.E.)</th>
<th>IgG (mean ± S.E.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>$Aa$ ($a$)</td>
<td>PH</td>
<td>1.85 (±0.013)</td>
<td>2.01* (±0.030)</td>
</tr>
<tr>
<td></td>
<td>LJP</td>
<td>1.96 (±0.044)</td>
<td>1.90 (±0.11)</td>
</tr>
<tr>
<td>$Aa$ ($b$)</td>
<td>PH</td>
<td>1.82 (±0.0076)</td>
<td>2.24* (±0.051)</td>
</tr>
<tr>
<td></td>
<td>LJP</td>
<td>1.87 (±0.034)</td>
<td>2.30* (±0.13)</td>
</tr>
<tr>
<td>$Aa$ ($c$)</td>
<td>PH</td>
<td>1.85 (±0.0084)</td>
<td>2.07* (±0.035)</td>
</tr>
<tr>
<td></td>
<td>LJP</td>
<td>1.86 (±0.027)</td>
<td>2.32* (±0.14)</td>
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* $IgG>IgM P=0.0001$; ** $IgG>IgM P=0.028$; *** $IgG>IgM P=0.032$.

Table 4. Relationship between avidity and concentration for IgM and IgG antibodies reactive with $A. actinomycetemcomitans$ ($Aa$) serotypes $a$, $b$ and $c$ in periodontally healthy (PH) and localized juvenile periodontitis (LJP) subjects

<table>
<thead>
<tr>
<th>Serotype</th>
<th>IgG</th>
<th>Source</th>
<th>IgM</th>
<th>Source</th>
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<tbody>
<tr>
<td>$Aa$ ($a$)</td>
<td>PH</td>
<td>ns$^{*}$</td>
<td>LJP</td>
<td>ns</td>
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<tr>
<td></td>
<td></td>
<td>$P=0.003$</td>
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<td>$P=0.02$</td>
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<td></td>
<td></td>
<td>$R^2=0.18$</td>
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<td>$R^2=0.84$</td>
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<tr>
<td>$Aa$ ($b$)</td>
<td>PH</td>
<td>ns</td>
<td>LJP</td>
<td>ns</td>
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<td></td>
<td>$P=0.06$</td>
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<td>$R^2=0.67$</td>
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<td></td>
<td></td>
<td>$\rho=0.87$</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$Aa$ ($c$)</td>
<td>PH</td>
<td>ns</td>
<td>LJP</td>
<td>ns</td>
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<tr>
<td></td>
<td></td>
<td>$P=0.012$</td>
<td></td>
<td>$R^2=0.88$</td>
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<td></td>
<td></td>
<td>$\rho=0.95$</td>
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ns = not significantly different.

### Discussion

The prevalence of $Aa$ in the subgingival dental plaque of the PH children determined by DNA-DNA hybridization was much greater than the frequency of isolation reported in PH children and young adults using culture techniques (4, 6, 16, 37, 38). This no doubt reflects the greater sensitivity of the DNA probes compared with culture (19, 25). It is likely that the isolation frequency of $Aa$ from the PH subjects would have been even greater had additional subgingival sites and/or other oral sites, such as the dorsum of the tongue, been sampled (4). This high isolation frequency of $Aa$ in the PH subjects strongly suggests that $Aa$ is indigenous to the oral cavity of these children.

All of the PH children possessed naturally occurring antibodies reactive with $Aa$ serotypes $a$, $b$ and $c$ in both

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the IgM and IgG isotypes, irrespective of whether or not Aa was detected at one or more of the four sampled sites. This observation suggests that either the PH subjects in whom Aa could not be detected at the sampled sites harbored Aa elsewhere in the oral cavity or that the naturally occurring Aa-reactive antibodies were induced by closely related indigenous bacteria, such as Ha and/or Hp that share antigens with Aa. Indeed, absorption of PH sera from subjects who harbored and who failed to harbor Aa at the sampled sites with the commensal haemophili, Ha and Hp, essentially depleted their sera of Aa-reactive antibodies. This result indicates that the Aa-reactive antibodies were directed against epitopes common to Aa, Ha and Hp and that little, if any, were Aa-specific. The finding that, in PH subjects, the concentration of Aa-reactive antibodies in the IgM isotype was 2- to 5-fold greater than that in the IgG isotype may indicate that these children are manifesting a primary immune response to these bacteria or that the immune response is largely directed to T-independent antigens.

The LJP subjects had higher concentrations of IgM and, in particular, IgG Aa-reactive antibodies than the PH subjects. Additionally, these antibodies appeared to be specific for Aa in that little Aa-reactive antibody could be removed by absorption with the commensal haemophili. These findings are in accordance with the results of a study by Genco et al (15). Moreover, the results of cross-absorption of LJP sera with Aa serotypes a, b and c and the observation that the greatest fold increase in IgG antibody was against serotype b strongly suggest that the LJP subjects are infected with serotype b (38). While some IgG antibody was cross-reactive with all three serotypes of Aa (10), much appeared to be specific for serotype b and may be directed against the serotype carbohydrate that resides in the polysaccharide moiety of the lipopolysaccharide (7, 21, 27, 28).

Whether Aa-reactive antibodies are protective, or whether they contribute to the pathogenesis of LJP remains unclear. Serum from LJP patients infected with Aa lacks bactericidal IgG antibody against this bacterium (35). Although anti-leukotoxic and opsonic activity have been reported (31, 35), antibodies in low-titer sera from patients with rapidly progressive periodontitis were significantly less effective at opsonizing Aa than antibodies present in low-titer sera from periodontally healthy subjects (29). As the effector functions of these antibodies are dependent on their functional affinity (30), the measurement of the avidity of the polyclonal Aa antibody response may provide insight into the potential role of antibody in LJP. In this context, it is interesting to note that the avidity of IgM and IgG antibodies reactive with Aa were low in both PH and LJP subjects.

Similar findings have been reported for the oral anaerobic Gram-negative rod, Bacteroides (Porphyromonas) gingivalis (20, 34). Depending on serotype, the concentrations of IgG Aa-reactive antibodies were between 35- and 115-fold higher in the sera of the LJP patients than in the sera of the PH children, indicating that the LJP subjects had mounted an active humoral immune response to this bacterium (10). Furthermore, there was a correlation between Aa-reactive antibody concentration and avidity in the LJP subjects, perhaps indicating affinity maturation. This was also suggested by the finding that the bivalent IgG Aa-reactive antibodies were significantly more avid than the potentially decavalent IgM Aa-reactive antibodies, indicating that the IgG antibodies had greater intrinsic affinity (17). Nonetheless, with the single exception of IgM antibodies reactive with serotype a, the avidities of the IgM and IgG Aa-reactive antibodies from the LJP subjects were not significantly greater than those of the PH subjects. Therefore, it can be construed that, in LJP subjects with low-titer Aa-reactive antibodies, the avidity of these antibodies was, in fact, lower than those of PH children.

The use of whole bacterial cells to determine antibody avidity yields a measurement of the average avidity of all populations of antibodies reactive with cell surface antigens. As such, it is possible that high-avidity antibodies reactive with individual antigens may be masked. However, Sims et al (27) reported that 72-90% of the total antibody-binding activity of high-titer sera from LJP patients bound the serotype-specific surface antigen. Furthermore, the avidity of IgG fimbria-reactive antibodies in serum of subjects with adult periodontitis who harbored Aa was significantly lower than the avidity of IgG fimbria-reactive antibodies in the serum of periodontally healthy subjects (24). A limitation of the chaotrope dissociation ELISA to measure avidity is the lack of high and low avidity standard antibodies that can be used to unequivocally rank ID50 values.

The high prevalence of Aa in these and other PH children (4) supports the contention that this bacterium is an oral commensal and not an extrinsic pathogen. As such, the host is likely to be exposed to a chronic antigen load. In contrast to acute infection that induces increased levels of antibody with increased affinity (30), chronic exposure to antigen or a high antigen dose may result in low-affinity antibody (2, 18). Thus, the constant association of a bacterium with its host, as is the case with the normal bacterial flora, might be expected to give rise to low-avidity antibody. The presence of low-avidity antibody has been associated with a greater severity and chronicity of disease as a result of impairment in the elimination of antigen and tissue localization of antigen-antibody complexes (5, 18). These consequences could
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Contribute to the immune pathology of periodontal disease as well as to other chronic infections. Moreover, the induction of low-avidity antibodies to commensal bacteria that are ineffective in immune elimination could, in part, explain how these microorganisms are able to avoid elimination by the host humoral immune system.

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References


