Relative Avidity of Serum Immunoglobulin G Antibody for the Fimbria Antigen of Actinobacillus actinomycetemcomitans in Patients with Adult Periodontitis

ATSUSHI SAITO,1* YASUO HOSAKA,1 TANEAKI NAKAGAWA,1 SATORU YAMADA,1 AND KATSUJI OKUDA2

Department of Periodontics1 and Department of Microbiology,2 Tokyo Dental College, 1-2-2 Masago, Mihama-ku, Chiba 261, Japan

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The relative avidity of serum immunoglobulin G antibody for the fimbria antigen of Actinobacillus actinomycetemcomitans was assessed by diethylamine dissociation enzyme-linked immunosorbent assay in patients with adult periodontitis. High-titer sera from patients not harboring A. actinomycetemcomitans had significantly higher avidities for the fimbria antigen than did high-titer sera from patients with A. actinomycetemcomitans in their periodontal pockets. The elicited antibodies against the fimbria antigen may afford protection against A. actinomycetemcomitans infection.

Actinobacillus actinomycetemcomitans is considered to be associated with various forms of human periodontal disease (18). The microorganism has a variety of cell surface components such as fimbriae, serotype-specific polysaccharide antigen, and lipopolysaccharide (1, 13, 19). These cell surface components may play important roles in colonization and pathogenicity. Antifimbrial antibodies are considered to inhibit the colonization of many bacterial species (14), and fimbriae of periodontopathic bacteria such as Porphyromonas gingivalis (6) and A. actinomycetemcomitans may constitute good candidates for vaccines to prevent these colonizations.

The sum of the attractive and repulsive forces between a multivalent antibody and multivalent antigen is termed the functional avidity (17). Protective efficacy against infection is thought to be a function of such antibody avidity (10). The purpose of the present study was to gain information about the relative avidity of serum immunoglobulin G (IgG) antibody elicited against fimbria antigen of A. actinomycetemcomitans. Forty-two patients (mean age, 45 years; age range, 22 to 66 years) with clinical diagnoses of adult periodontitis were selected for this study. Patients who met the clinical selection criteria but had used antibiotics in the previous 6 months, suffered from systemic diseases, or refused to participate in the study were excluded. Blood samples were collected from each subject by venipuncture. Serum from clotted venous blood was harvested by centrifugation and stored at −70°C. Serum samples from 10 subjects ranging in age from 24 to 29 years, who were judged to be periodontally healthy by clinical examination, were also obtained. Subgingival plaque samples were obtained from at least two sites by using paper points, with the deepest probing depth per individual. The samples were transferred to 1 ml of reduced transport fluid (16) and dispersed in a Vortex mixer for 15 s at the maximum setting. The sample was serially diluted 10-fold for culture study. An aliquot of 0.1 ml was plated on TSBV medium (15) for detection of A. actinomycetemcomitans and incubated under 90% air–10% CO2, for 3 days. Bacteria on TSBV medium were identified by colony morphology, staining characteristics, biochemical tests, the catalase test, and the enzymatic activity test by the API-ZYM System (Biomerieux, Marcy l’Etoile, France). A. actinomycetemcomitans was recovered from 24 patients. If the initial sampling showed a patient who was not harboring A. actinomycetemcomitans, sampling from four additional sites with probing depths of over 5 mm was performed for possible detection of the organism to minimize false-negative results. None of them were subsequently found to harbor A. actinomycetemcomitans in their periodontal pockets.

Fimbria antigen was prepared from A. actinomycetemcomitans 310-a by the method of Inoue et al. (4). Harvested cells were suspended in 0.15 M ethanalamine-HCl buffer (pH 10.5) and homogenized for 30 min in a blender (Polytron; Kinematica GmbH, Lucerne, Switzerland) in an ice bath. Intact cells and cell debris were removed by centrifugation at 10,000 × g for 20 min and then at 25,000 × g for 30 min. A saturated solution of (NH4)2SO4 was added dropwise to the supernatant until a final concentration of 10% was reached. The precipitate (crude fimbriae) was purified to near homogeneity by being dissolved in 0.5% deoxycholate–0.7% n-octyl-β-D-glucopyranoside. These detergents selectively dissolved 30- and <18-kDa proteins. The purified fimbriae were negatively stained with 0.2% sodium phosphotungstic acid (NaPT [pH 7]) by the method of Preus et al. (11) and subjected to electron-microscopic examination (H-600; Hitachi, Tokyo, Japan). Extracted fimbriae were seen to be composed only of fibrous components similar to the fimbriae isolated by Inoue et al. (4). Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) analysis of this material clearly showed a band at 54 kDa. This 54-kDa antigen corresponds to the fimbrial subunit protein reported by Inoue et al. (4).

Serum IgG titers against the purified antigen were assessed by an enzyme-linked immunosorbent assay (ELISA) with peroxidase-conjugated goat anti-human IgG as described by Nakagawa et al. (9). The antibody levels were expressed as ELISA units by the method of Murayama et al. (8). The treatment of serum with a mild protein denaturant,
A. actinomycetemcomitans

Controls (n = 10) 116.5 ± 81.2 72.1 ± 18.0
A. actinomycetemcomitans negative (n = 18) 677.4 ± 167.2* 59.6 ± 8.2*
A. actinomycetemcomitans positive (n = 24) 454.1 ± 111.3* 48.6 ± 7.1*

* P < 0.05 compared with controls.

diethylamine, was an adaptation of the method described by Chen et al. (2). The standard ELISA was modified in the following manner. Serum dilutions were made in duplicate rows, and diethylamine in phosphate-buffered saline plus 0.05% Tween 20 was added to each well of one row in each set to a final concentration of 0.33 M/liter. End point titers with and without diethylamine were then measured and calculated as in the standard ELISA. The avidity index (AI) was calculated as follows: AI = (titer with DEA/titer without DEA) × 100. The values for IgG titers and avidities obtained by ELISA with the purified antigens are shown in Table 1. Differences between the groups for titer or avidity data were determined by using Wilcoxon’s rank sum test. The sera from patients had statistically significantly higher IgG titers against A. actinomycetemcomitans 310-a fimbria antigen than did sera from periodontally healthy individuals, regardless of the presence of A. actinomycetemcomitans in the periodontal pockets. The specificity of the elicited IgG titer was confirmed by immunoblot analysis (Fig. 1). Patient sera with elevated IgG titers against the fimbria antigen recognized an approximately 54-kDa antigen, which corresponds to the main band of the fimbria antigen seen in a silver-stained SDS-PAGE pattern (Fig. 1, gel). No statistically significant differences were observed between patients with cultivable A. actinomycetemcomitans and patients without A. actinomycetemcomitans for titers against the fimbria antigen. The patient sera showed significantly lower avidities for the fimbria antigen than did sera from periodontally normal individuals. The frequency of A. actinomycetemcomitans culture-positive patients with elevated levels of serum antibodies against the fimbria antigen was 50% (12 of 24 patients), whereas that of A. actinomycetemcomitans culture-negative patients was 33% (6 of 18 patients). Avidities of sera with elevated antibody levels were compared in terms of the presence of cultivable A. actinomycetemcomitans. Sera from A. actinomycetemcomitans culture-negative patients with elevated IgG titers against the fimbria antigen had significantly (P < 0.01) higher avidities for the fimbria antigen than did sera from A. actinomycetemcomitans culture-positive patients with elevated titers (AI, 76.2 ± 10.2 and 45.2 ± 6.3, respectively). The relationships between titers and avidities between the groups were assessed by Spearman’s rank correlation coefficient. A statistically significant negative correlation was noted between titers and avidities for the fimbria antigen in periodontally normal individuals (r = −0.888; P < 0.05) and patients with cultivable A. actinomycetemcomitans (r = −0.436; P < 0.05). For patients without cultivable A. actinomycetemcomitans, however, a significantly positive correlation (r = 0.612; P < 0.05) existed between titers and avidities for the fimbria antigen.

Raney et al. (12) and Gunsolley et al. (3) showed that the severity of lesions of localized juvenile periodontitis or severe periodontitis in patients with high antibody levels against A. actinomycetemcomitans is limited compared with that of patients with low antibody titers, thus emphasizing the protective role of anti-A. actinomycetemcomitans antibody. In contrast, the elevation of antibody level may merely reflect the fact that the organism was once involved in the pathogenesis and progression of periodontitis (7) or may indicate that the destruction of periodontal tissue is rapid and severe (5). In the present study, the presence of specific IgG antibodies against the fimbria antigen of A. actinomycetemcomitans was observed. Although no statistically significant difference was found, the patients without cultivable A. actinomycetemcomitans showed higher serum IgG titers against the fimbria antigen than did those with cultivable A. actinomycetemcomitans. This leads us to the hypothesis that the specific IgG titer to the fimbria antigen elicited by initial contact with A. actinomycetemcomitans played a role in clearing the organisms from the periodontal pockets of these patients. However, the patients harboring A. actinomycetemcomitans in their periodontal pockets also had significantly higher serum IgG titers against the fimbria antigen than did periodontally healthy individuals, and the frequencies of patients who showed elevated levels of antibody against the fimbria antigen were greater than those without A. actinomycetemcomitans.

Periodontally healthy individuals had significantly higher avidities for the fimbria antigens than did the patient group. Chen et al. (2) reported similar results with P. gingivalis antigens and stated that individuals who are resistant to early-onset periodontitis may be able to mount a protective humoral immune response on subclinical infection by P. gingivalis. In the present study, significantly higher values for avidity for the fimbria antigen were observed in high-titer patients without cultivable A. actinomycetemcomitans than in high-titer patients with cultivable A. actinomycetemcomitans. Our preliminary experiments with A. actinomycetemcomitans lipopolysaccharide revealed no statistically significant difference in avidity in terms of the presence of A. actinomycetemcomitans in the periodontal pockets. This may be due to differences in the epitope density on the antigens. The data support the notion that the humoral antibodies elicited were directed at different antigens and

TABLE 1. Serum IgG titers and avidities for the fimbria antigen of A. actinomycetemcomitans

<table>
<thead>
<tr>
<th>Subjects</th>
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* P < 0.05 compared with controls.

FIG. 1. Immunoblot analysis of the fimbria antigen. The fimbria antigen (10 μg) was resolved by SDS-PAGE and transferred to a polyvinylidene difluoride membrane. Lanes 1 and 2: Representative immunoblots of the purified fimbria antigen and patient sera with elevated IgG titers against the fimbria antigen. The gel is a silver-stained SDS-PAGE pattern of the fimbria antigen. Lanes: 1, molecular mass markers; 2, crude fimbriae; 3, purified fimbriae.
that the difference between protective and nonprotective antibodies is the avidity that the antibody demonstrates for the epitope recognized on the surface of the bacteria. Furthermore, for patients without cultivable *A. actinomyctemcomitans*, a significantly positive correlation was observed between the titers and avidities, whereas the correlation was negative for other groups. In other words, for periodontally healthy individuals or patients harboring *A. actinomyctemcomitans*, a higher titer is accompanied by a higher avidity. Thus, the patients not harboring *A. actinomyctemcomitans* in the present study appeared to be capable of mounting a biologically significant humoral immune response to *A. actinomyctemcomitans* fimbriae.

In summary, the presence of specific and biologically functional serum IgG antibodies to the fimbria antigen of *A. actinomyctemcomitans* was confirmed in patients with adult periodontitis. The elicited antibodies against the fimbria antigen may afford protection against continued infection by *A. actinomyctemcomitans*.

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REFERENCES