Detection of Serum Antibodies of Oral Porphyromonas (Bacteroides) asaccharolyticus in Dogs: Relationship to Periodontal Disease

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KEY WORDS: periodontitis, Porphyromonas asaccharolyticus, serum antibody.

Several species of the genus Porphyromonas (formerly classified as Bacteroides) have been implicated as major pathogens in the progression of periodontal diseases in man and animals [1, 2, 5, 6]. Porphyromonas (Bacteroides) asaccharolyticus is a predominant isolate from some advancing lesions of periodontitis in dogs [2, 6]. Bacterial infections usually are associated with an immune response, and diagnosis is often aided by assessing the specific serum antibody response to the candidate pathogens. No information existed on the dog immune response to oral gram-negative organisms and particularly to P. asaccharolyticus in periodontal health and disease. The purpose of the present investigation was to measure the specific serum antibodies which were reactive with P. asaccharolyticus in dog with and without periodontal disease. The enzyme-linked immunosorbent assay (ELISA) was used for this purpose.

The experiments were performed on 40 dogs (mainly mongrel dogs). Twenty-one dogs, ranging 1 to 2 years old, had healthy gingiva and 18, ranging 1 to 6 years, had the gingiva with periodontal disease. A beagle dog was immunized with P. asaccharolyticus and the hyper immune serum was used for standard positive serum. The degree of periodontal disease was examined by the gingival index system as described previously [2]. Oral P. asaccharolyticus strain INU-1 was anaerobically grown in GAM broth for 48 h at 37°C. The cells were harvested by centrifugation at 10,000 g for 20 min at 4°C and washed 3 times with phosphate buffered saline (PBS, pH 7.4). The cells were sonicated for 20 min in an ice bath, and then centrifuged at 12,000 g for 20 min. The supernatant was collected, dialyzed and then lyophilized. The preparation was used as sonicated antigen (SA). Lipopolysaccharide (LPS) was extracted from the organisms and purified by the method as described previously [3].

Whole blood was collected from each subject and the serum was separated and stored in aliquots at −35°C. Specific antibodies of the IgG and IgM classes were measured using the ELISA method [4]. The SA was suspended in 0.1 M Na2CO3 buffer (pH 9.6) at a concentration of 50 μg/ml. The wells of the microtiter plate were sensitized with 200 μl of the antigen solution at 37°C for 2 h; 100 μl of 2% (wt/vol) bovine serum albumin in PBS was then added, and the plates were stored at 4°C until used. The LPS was suspended in distilled water at a concentration of 200 μg/ml. The well of the microtiter plate dried up at 37°C overnight; 100 μl of 2% bovine serum albumin in PBS was then added and the plates were stored at 4°C until used. Before testing, the wells were washed 3 times with 0.05% Tween 20-PBS. A 200 μl volume of test serum at the various dilution in 0.05% Tween 20-PBS supplemented with 0.5% BSA, and the plates were incubated for 2 h in a moistened chamber at 37°C. After the wells were washed as described above, 200 μl of goat peroxidase-conjugated anti-dog immunoglobulin diluted in 0.05% BSA was added to each well, and the plate was incubated at 37°C for 1 h. After washings, 100 μl of the substrate solution, 0.06% 5-aminosalicylic acid, was added to each well and incubated for 1 h at 37°C. After incubation, the enzymatic reaction was blocked by adding 20 μl of 0.2% NaN2 to each well and the absorbance was determined in a colorimeter. Results were obtained as ratios of antibody activity by relating the individual sera to the standard curve obtained with standard positive serum. The ratio values
obtained for determination of the IgG antibody activity, multiplied by 100, expressed the level of IgG-specific antibody as ELISA unit (EU) relative to the reference serum given the antibody value of 100 EU according to the method of Mouton et al. [4].

Preliminary checkerboard assays were conducted to identify the levels of both IgG and IgM antibodies to SA and LPS from *P. asaccharolyticus*. A titration curve for determination of the IgG antibody reactive with SA is presented in Fig. 1, by plotting the absorbances against the dilution. The computed equation defined each standard curve and used for all subsequent calculations. Other titration curves were obtained by similarly and used for experiments.

The levels of specific IgG antibody to SA detected in the various periodontal animals are presented in Fig. 2. A close correlation between the levels of serum antibody and gingival index was found (r=0.7745, P<0.01). The mean IgG antibody level in the periodontal disease group (64.5 EU) differed from that of the healthy gingiva group (17.5 EU) (Table 1). The mean level of IgG antibody to LPS in the disease group (10.3 EU) was slightly elevated; however, it was not significantly different from that in the healthy group. The IgM antibodies to SA and LPS were not detected in the ELISA system.

Microbiologic studies have identified *P. asaccharolyticus* as major constituents of the flora associated with periodontal disease in dog [2, 6]. These bacteriological findings are supported by the immunologic data presented in this study. Proliferation of *P. asaccharolyticus* must have taken place in order to stimulate and elevate the IgG antibody titers. Since IgM antibody titers

![Chart 1](image1.png)

Fig. 1. Standard curve for determination of IgG antibody to sonicated antigen of oral *P. asaccharolyticus* using the standard positive serum. Each point represents the absorbance value observed.

![Chart 2](image2.png)

Fig. 2. Correlation between level of serum IgG reactive with sonicated antigen and gingival index. Each point expresses the value of serum from dogs with (●) or without (○) gingival inflammation.

<table>
<thead>
<tr>
<th>Group of dog with</th>
<th>ELISA unit of</th>
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<tbody>
<tr>
<td></td>
<td>IgG against</td>
</tr>
<tr>
<td></td>
<td>SA</td>
</tr>
<tr>
<td>Periodontal disease</td>
<td>64.5±3.0a</td>
</tr>
<tr>
<td>Healthy gingiva</td>
<td>17.5±2.2</td>
</tr>
</tbody>
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a) Mean±SE.
b) Not detected.
c) P<0.01.
d) Not significant.

Table 1. Levels of serum immunoglobulins reactive with *P. asaccharolyticus* antigens
SERUM ANTIBODY TO ORAL P. ASCCHAROLYTICUS

Fig. 3. Correlation between level of serum IgG reactive with sonicated antigen and age of the dogs. Each point expresses the value of serum from dogs with (●) or without (○) gingival inflammation.

generally decrease rapidly after the cessation of an acute infection, the IgM antibodies to SA and LPS were not detected in the ELISA system.

There was, in addition, a positive correlation between the IgG antibody level and increase in age (Fig. 3, r=0.4721, P<0.01). However, the pattern was different from that between the antibody level and gingival index. Scatterings were observed among the antibody titers of the sera from dogs at 2.5 years old and younger. It was suggested that the development of normal or natural antibodies likely dependent on the presence of environmental antigenic challenge associated with presence of the bacteria. Highly colonization of P. asaccharolyticus in the gingival pockets could induce strong and consequent stimulation of antibody production.

The observation of antibody specific for oral P. asaccharolyticus in some periodontally healthy individuals suggests the antigen stimulation of this organisms. Actually, cultural study have shown presence of P. asaccharolyticus in oral floras of normal dog [2]. Prior infection may occur in young dogs with healthy gingiva.

We could detect only slight or no immunoglobulins to LPS. P. asaccharolyticus used in the present study was characteristically surrounded by capsular-like materials and fimbriae (unpublished data). Therefore, recognition of LPS of P. asaccharolyticus was not easy and the antibodies were low level in dog.

REFERENCES