Precipitating Antibody against Lipopolysaccharide of *Haemophilus actinomycetemcomitans* in Human Serum

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Approximately 6% of 50 tested human sera possessed precipitating antibody against lipopolysaccharide from *Haemophilus actinomycetemcomitans* (*Actinobacillus actinomycetemcomitans*).

Elevation of humoral immune responses to *Haemophilus actinomycetemcomitans* (*Actinobacillus actinomycetemcomitans*) in patients with juvenile periodontitis has been demonstrated (1, 2, 6, 14, 16). During studies of humoral immune responses to this organism (9, 10, 12), we found a specific antibody in human sera that was reactive against lipopolysaccharide (LPS) from *H. actinomycetemcomitans*. A micro-enzyme-linked immunosorbent assay was used to measure the specific immunoglobulin G levels against sonicated antigens from *H. actinomycetemcomitans* Y4 and ATCC 29522. The details of the assay methods were described previously (9, 10). All measurements were performed in duplicate. All sera studied were also subjected to immunodiffusion to determine whether they possessed immunoprecipitating antibody against soluble antigens from *H. actinomycetemcomitans*.

*H. actinomycetemcomitans* Y4, ATCC 29522, ATCC 29523, and ATCC 29524 were cultured in a diffusate broth medium (11) in an anaerobic chamber for 4 days. Harvested cells were washed with phosphate-buffered saline (pH 7.2), suspended in phosphate-buffered saline at a concentration of 100 mg (wt weight) per ml, and subjected to an ultrasonicator with a tapered tip working at an output of 100 W (model 5213; Ohtake Works, Tokyo, Japan). The sonication was carried out at 0°C until more than 95% breakage of cells had occurred; this level of breakage was determined by phase-contrast microscopy. The supernatants were obtained by centrifugation at 12,000 × g for 20 min. The sonicated antigens from the four strains were mixed to detect the precipitating antibody in human sera. Agarose (1%) dissolved in Veronal buffer (Winthrop Laboratories, Div. Sterling Drug Co., New York; pI = 0.03, pH 8.6) was used for double immunodiffusion and immunoelectrophoresis. The results of the screening for antibody are shown in Fig. 1. All rabbit antisera to whole cells of *H. actinomycetemcomitans* Y4, ATCC 29522, ATCC 29523, and ATCC 29524 formed several precipitin lines with the mixed sonicated antigen. Two sera (Fig. 1, wells 12 and 18) from patients with adult periodontitis and one serum (well C) from a periodontally healthy adult formed distinct precipitin lines against the mixed sonicate supernatants of the four strains. Sera from patients with localized juvenile periodontitis did not react with the antigens. No elevated immunoglobulin G antibody levels in the sera were noted, as had been reported previously (10). The average micro-enzyme-linked immunosorbent assay titers of sera from patients with adult periodontitis and from healthy groups were $29.8 \pm 1.5$ and $28.03 \pm 0.72$, respectively. The three sera which formed precipitin lines possessed significantly high titers to the sonicated antigen. The titers of sera from wells 12 (KT), 18 (FO), and C (NA) were $2^{13.22}$, $2^{11.9^*}$, and $2^{13.31}$, respectively. The donors had not had any incidents of systemic bacterial infection during the 5 years before the blood collection. It is reasonable to presume that the immune responses had been induced by *H. actinomycetemcomitans* harbored in periodontal pockets.

The effect of periodontal treatment of the two adult periodontitis patients on the immune response to the organism was determined. No significant changes were found in sera from these two patients 14 months after the first blood collection. One serum (Fig. 1, well C) donor was a young dental hygienist with no history of periodontal disease, periocoronitis, tonsillitis, empyema, or actinomycosis. Because there is no other obvious source, we concluded that the microbial flora from subgingival plaque should be studied in the future to clarify the source of the antigenic stimulation.

Immunoelectrophoresis was carried out to clarify the specificity of the immunoprecipitating antibody. An immunoelectrophoretic pattern of soluble antigens from *H. actinomycetemcomitans* Y4 against rabbit antisera and serum from a patient with adult periodontitis (KT) is shown in Fig. 2. LPS was extracted by the hot phenol-water method (18) as previously described (7). Electrophoresis of the sonicated antigen and LPS was carried out at 2 mA/cm for 50 min; then rabbit antiserum and serum from a patient with adult periodontitis were placed in the upper and lower troughs, respectively. Sonicated antigen formed several precipitin lines with rabbit serum (Fig. 2). Serum from this adult periodontitis patient formed a clear line with sonicate antigen and an arcine line with LPS.

An immunodiffusion between the sonicated antigen or LPS and rabbit antiserum or human serum is shown in Fig. 3. Sera from the two adult periodontitis patients (KT and FO) and one healthy adult (NA) formed identical precipitin lines against LPS. The heating of *H. actinomycetemcomitans* LPS to 100°C for 60 min did not alter the immunodiffusion reaction. None of these three sera formed precipitin lines with LPS preparations from *Bacteroides gingivalis* 381, *Eikenella corrodens* FDC1073, *Fusobacterium nucleatum* ATCC 25586, *Escherichia coli* 0111:B4, or *Salmonella minnesota* ATCC 9700. These results indicate that the three sera possessed an antibody specific against the LPS of *H. actinomycetemcomitans*.

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FIG. 1. Immunodiffusion reactions between the mixed sonicated antigen of *H. actinomycetemcomitans* and human serum or rabbit antiserum. Wells: Y4, 29522, 29523, and 29524, rabbit antiserum to each respective strain of *H. actinomycetemcomitans*; 1 through 32, sera from patients with adult periodontitis; JP1 and JP2, sera from patients with juvenile periodontitis; A through P sera from periodontally healthy adults. The troughs contained mixed sonicated antigen from *H. actinomycetemcomitans* Y4, ATCC 29522, ATCC 29523, and ATCC 29524.

When viable cells of *H. actinomycetemcomitans* Y4 and ATCC 29522 were exposed to the three fresh sera, no significant decrease in viable number was detected. These results did not contradict the previous findings of Sundqvist and Johansson (15). The present results indicate that antibody to LPS did not enhance complement-mediated bactericidal activity. Sera from patients with juvenile periodontitis have been reported to inhibit the leukotoxicity of *H. actinomycetemcomitans* in vitro (8, 17). In the present study, no distinct immune response was found in two sera obtained from patients with juvenile periodontitis. Further study is needed to clarify whether some sera from other patients with juvenile periodontitis react with the LPS of *H. actinomycetemcomitans*.

Recently, it has been demonstrated that the tissue-invading bacteria in human periodontal disease is *H. actinomycetemcomitans* (13; L. A. Cristersson, B. Albini, J. J. Zambon, J. Slots, and R. J. Genco, Abstr. J. Dent. Res. 62:198, 1983). It has also been reported that LPS from *H. actinomycetemcomitans* probably occurs freely in the external environments (3-5) and that membranous vesicles can be shed from cells. Hammond and Stevens (3) indicated that *H. actinomycetemcomitans* contained free endotoxin in small vesicles, which could allow endotoxins and other pharma-

FIG. 2. Immunelectrophoretic pattern of sonicated antigen and LPS of *H. actinomycetemcomitans* reacted with rabbit serum and serum from a patient with adult periodontitis. The upper and lower wells contained LPS, and the center well contained sonicated antigen.

FIG. 3. Immunodiffusion pattern between the sonicated antigens or LPS of *H. actinomycetemcomitans* Y4 and rabbit antiserum or human serum.
coloically active toxins of bacterial origin to gain access into periodontal tissue. It is possible that the antibody to LPS is induced mainly by the penetration of this type of free endotoxin through the periodontal tissue.

LITERATURE CITED