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NOTES

Available Immunoglobulin A Antibodies in Mouth Rinses and Implantation of *Streptococcus mutans*

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Immunoglobulin A (IgA) antibodies reacting with *Streptococcus mutans* were analyzed in mouth rinses from 38 adults. Antibody activity was determined by an enzyme-linked immunosorbent assay. The IgA antibody activity varied considerably in samples from different individuals. Of the 38 subjects, 12 volunteered in an implantation experiment and were challenged with streptomycin-resistant *S. mutans*. The results indicated that individuals with relatively high IgA antibody activity in mouth rinses more rapidly eliminated the challenge strain than subjects with low IgA antibody activity.

Many studies of the physiological effect of salivary antibodies have used parotid saliva (1, 3, 7, 11). For example, the antibody activity to *Streptococcus mutans* in parotid saliva has been compared with the result of implantation of the corresponding bacterial strain (C. Y. Bonta, R. Linzer, F. Emmings, R. T. Evans, and R. J. Genco, J. Dent. Res., p. 143, 1979.). This does not, however, properly reflect the in vivo situation in which foreign microorganisms or other antigenic material is introduced into the oral cavity.

Material introduced into the mouth will meet not only saliva from a single source but a mixture of secretions from different glands and gingival pocket fluid, the so-called oral fluid.

Components from the oral fluid will bind to surfaces in the mouth as well as material used to stimulate the salivary flow, e.g., paraffin wax.

In an attempt to mimic a more natural situation, we examined the available antibodies to *S. mutans* in mouth rinses. The effect of such antibodies on the implantation of *S. mutans* was also evaluated.

A total of 38 adults (men and women 22 to 59 years old) kept 3 ml of sodium phosphate-buffered saline (0.01 N [pH 7.1]) in the mouth for 5 min without swallowing. The expectorated volume varied between 3.1 and 7.5 ml (median value, 4.6 ml). The expectorate was heated at 56°C for 30 min and centrifuged at 12,500 × *g* for 20 min. Thereafter, the supernatant was analyzed for immunoglobulin A (IgA) antibodies by an enzyme-linked immunosorbent assay (2, 4, 5). Whole cells of *S. mutans* serotype *c* and *d* were used as antigens. The antibody activity was expressed as the average optical density (absorbance at 405 nm) for duplicate samples multiplied by 100/*t*, where *t* is the number of minutes of color development. The antibody activity as a percentage of a whole saliva pool from 16 subjects, collected by chewing on paraffin wax, is also given. As a negative control, parotid saliva from a hypogammaglobulinemic subject was used (5). Twelve subjects volunteered for the implantation studies. In mouth rinses collected just before challenge, six had a slightly higher and six a slightly lower antibody activity to *S. mutans* than the value of the reference pool. These subjects were challenged with a 5-ml suspension of a freshly isolated strain of *S. mutans*, serotype *d*. The suspension, which contained about 2×10^9 cells, was kept in the mouth for 2 min and then expectorated. The challenge strain had been made resistant to streptomycin by genetic transformation (the strain was kindly supplied by G. Westergren). Saliva samples were collected after 1, 2, 3, 5, and 7 days by chewing on a piece of paraffin wax. The CFU of *S. mutans* were enumerated after cultivation on mitis salivarius agar containing streptomycin (200 μg/ml) by the micromethod described by Westergren and Krasse (14). A value of $>10^3$ CFU after 1 day and recovery of the implanted strain after 3 and 5 days were considered as a positive result, whereas $<10^3$ CFU after 1 day and no implanted bacteria in the samples after 3 and 5 days were considered a negative result of the implantation.

In the supernatant of the mouth rinses from all

TABLE 1. IgA antibodies reacting with *S. mutans* in mouth rinses from 38 adults

Organism	Antibody activity[a]			% Activity[b]		
	Mean	Median	Range	Mean	Median	Range
S. mutans KPSK 2 serotype *c*	4.78 ± 2.2[c]	4.81	0.8–12.2	202	187	34–515
S. mutans B13 serotype *d*	2.56 ± 1.6[c]	2.41	0.4–9.6	238	200	37–892

[a] Antibody activity as measured by enzyme-linked immunosorbent assay. Expressed as mean absorbtion of duplicates × (100/*t*), where *t* is the time in minutes when the enzyme-substrate reaction was stopped.
[b] Percent activity in reference pool of whole saliva diluted 1:2.
[c] Mean ± standard deviation.

38 persons, a definite antibody activity to *S. mutans* was found (Table 1). However, the range of antibody activity varied 15-fold for serotype *c* and 24-fold for serotype *d*.

For comparison, we used a reference pool of whole saliva diluted 1:2. The mean value of the activity of the mouth rinses was 202, 238% of that of the whole saliva to *S. mutans* serotype *c* and *d*.

After challenge with the streptomycin-resistant *S. mutans* strain, 10 of the 12 subjects fulfilled the criteria for positive or negative implantation. Five of these belonged to the group with a relatively high antibody activity, and five belonged to the group with a low antibody activity (Table 2). Four of the persons in whom the implantation could be considered as positive had low antibody activity in the mouth rinses. In the five persons in whom the challenge strain was less successfully implanted, four had a relatively high antibody activity.

The large variation in antibody activity between different individuals can be explained by variations in saliva secretion rate, immune response, and absorption of antibodies. All these factors could, of course, be analyzed separately, but the important fact is that when foreign microorganisms are presented to a new host, a varying amount of antibodies is available in the mouth. This variation might be one of the explanations for the variability observed in implantation experiments with *S. mutans* (9, 10, 12, 13).

TABLE 2. Effect of antibody activity in mouth rinses on experimental implantation of *S. mutans* serotype *d*[a]

Implantation	Total no.	High[b]	Low[c]
Positive[d]	5	1	4
Negative[e]	5	4	1

[a] Mouth rinses were collected immediately before challenge.
[b] 110 to 210% of antibody activity in reference pool.
[c] 60 to 80% of antibody activity in reference pool.
[d] Greater than 10^3 CFU/ml of saliva after 1 day, and recovery of the challenge strain after 3 and 5 days.
[e] Less than 10^3 CFU/ml of saliva after 1 day, and no recovery of the challenge strain after 3 and 5 days.

This concept formed the basis for the implantation study, and the data obtained support the hypothesis that available IgA antibodies could influence the colonization of *S. mutans* in the oral cavity. They also are in accordance with observations made in an immunization study on human subjects in which a high antibody response in parotid saliva was concomitant with a low recovery of *S. mutans* after challenge (6).

It should be mentioned that, in spite of the fact that the streptomycin-resistant *S. mutans* strain was freshly isolated, these organisms were quickly eliminated. Thus, to solve the question of the degree of influence which specific IgA antibodies could have on *S. mutans* colonization, young children have to be examined. In such a population, in which *S. mutans* naturally becomes established in about every second child between 2 to 3 years of age (9), mouth rinses also would be more easily obtained than, e.g., parotid saliva.

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