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Adherence and *Streptococcus mutans* Infection: In Vitro Study with Saliva from Noninfected and Infected Preschool Children

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An in vitro adherence experiment was designed to mimic the transmission of *Streptococcus mutans* from mother to child to test the hypothesis that differences in initial adherence reflect differences in susceptibility to infection. The data show that the pretreatment of *S. mutans* cells with the saliva of the mother in a mother-child pair and the pretreatment of spheroidal hydroxyapatite with that of the child may result in combinations which counteract or foster the initial adherence to a varying extent. The findings indicate that such combinations may determine the risk of *S. mutans* infection.

Streptococcus mutans is acquired early in life after the eruption of the first tooth (2, 3, 5, 6, 8). The mother is likely to be one major source of infection for the infant (1, 3, 14, 16, 18; N. Masuda, T. Shimamoto, S. Sobue, and S. Hamada, J. Dent. Res. special issue A AADR, abstr. no. 784, **59**:463, 1980). The child is not always infected, however, even though the mother is carrying *S. mutans* in high numbers in the saliva (16).

The initial adherence of oral bacteria is a selective process (12), partly influenced by salivary components (13, 17). To test the hypothesis that differences in initial adherence reflect differences in susceptibility to *S. mutans* infection, we examined the attachment of *S. mutans* cells that had been pretreated with saliva from a mother to spheroidal hydroxyapatite (SHA) that had been pretreated with saliva from her child.

A total of 9 noninfected and 14 *S. mutans*-infected 6-year-old children and their highly *S. mutans*-infected mothers (approximately 10^6 colony-forming units per ml of saliva) were selected for the study. Children with no detectable *S. mutans* in two samples were considered noninfected. The number of *S. mutans* in the saliva of the infected children varied between 4×10^3 and 4×10^6 colony-forming units per ml (24). All mothers and all infected children harbored *S. mutans* serotype c, which was confirmed by immunofluorescent identification (4).

We collected paraffin-stimulated whole saliva (a minumum of 1 ml from the child and 3 to 4 ml from the mother). The saliva was diluted 1:2 in 0.005 M potassium phosphate buffer containing 0.154 M NaCl (pH 7) (PBS) and centrifuged for 30 min at 17,000 × *g* at 4°C. The clarified whole saliva was either used immediately or stored at 4°C until used the next day.

Adherence assays were performed according to Clark et al. (9) with some modifications. *S. mutans* strain KPSK-2 cells were grown in a dialyzed tryptose-yeast extract medium (7) supplemented with [*methyl*-^{3}H]thymidine (The Radiochemical Centre Ltd., Amersham, England) at a concentration of 10 μCi of [^{3}H]thymidine per ml. Five milliliters of a suspension of ^{3}H-labeled cells was centrifuged, and the pellet was suspended in and incubated with 4.5 ml of clarified whole saliva from the mother. ^{3}H-labeled cells suspended in 4.5 ml of PBS served as controls in some experiments. This permitted the evaluation of the influence of saliva as the suspending medium on adherence. A 40-mg amount of SHA was incubated in polystyrol tubes (70 by 11 mm; Labassco, Gothenburg, Sweden) and slowly inverted for 60 min at 22°C with 0.5 ml of clarified whole saliva from the child. As a control, SHA was incubated and suspended in 0.5 ml of PBS. A 0.5-ml amount of the ^{3}H-labeled KPSK-2-saliva suspension was then mixed with the SHA pretreated with and suspended in either 0.5 ml of the child's saliva or 0.5 ml of PBS. The mixtures were inverted as described above, and the SHA was allowed to settle for 60 s. The supernatant fluid was discarded, and the SHA was washed twice with 2.5 ml of PBS. The washed SHA was dissolved in 0.2 ml of 6 M HCl to facilitate transfer to a scintillation vial. After transfer of the sample to the vial, the tube was rinsed twice with a total of 1.0 ml of buffer, which was also added to the vial. The radioactivity was monitored in a 1215 Rackbeta liquid

scintillation counter (Wallac; LKB Instruments, Sweden). All assays were performed in duplicate.

Adherence was expressed as a percentage of the activity of the control, i.e., adherence to SHA pretreated with and suspended in PBS instead of saliva; or, where the influence of pretreating and suspending the cells in saliva was assessed, adherence was expressed as a percentage of the adherence of cells pretreated and suspended in PBS instead of saliva from the mother. Batches of cells labeled on different occasions were used. As the effect of labeling varied between different batches, the data are presented as described above, i.e., as percentages of adherence relative to PBS controls. The number of cells used in the experiments varied between 6×10^8 and 9×10^8 cells/ml.

The error of the method was examined from duplicate samples. In 55 such samples, the mean difference was 112.0 cpm, and the error of the mean was ±17.4 or ±15.5%.

To assess the reproducibility of the assay, experiments were performed using saliva from three individuals (subjects 1, 2, and 3) on three occasions. Saliva from subject 1 was always used to pretreat the ^{3}H-labeled *S. mutans* cells. SHA was pretreated with saliva from each of the subjects, and as a control the SHA was pretreated with PBS. The relative adherence of cells pretreated with saliva from subject 1 to SHA pretreated with saliva from the different subjects is shown in Table 1. On all three occasions, relative adherence was greatest to SHA pretreated with saliva from subject 2 and was least to SHA pretreated with saliva from subject 3. An activity varying between 1.3 and 4.9% of the total input was recovered from the SHA.

The relative adherence of *S. mutans* cells pretreated with saliva from mothers to SHA pretreated with saliva from their infected children had a higher mean value than the adherence to SHA pretreated with saliva from their noninfected children (Fig. 1). The difference between the groups is statistically significant (Student's t test, $0.05 > P > 0.01$).

For some of the mother-child pairs, an additional adherence assay was performed to evaluate the influence of pretreating the cells with saliva. Here *S. mutans* cells pretreated with PBS were used as a control. In most cases adherence to SHA was reduced when the cells were pretreated with saliva. Adherence of saliva-treated cells to SHA pretreated with saliva from noninfected children varied between 23 and 46% of that of the cells pretreated with PBS. When the SHA was pretreated with saliva from infected children, the corresponding figures were between 48 and 142% (Table 2). Table 2 also shows that the relative adherence values for noninfected siblings were lower than those of their infected siblings. With increasing percentage of adherence, an increasing level of infection was generally found, and the child with the highest relative adherence value (142%) had the highest level of *S. mutans* in saliva ($3{,}000 \times 10^3$ per ml of saliva).

The results show that in young children the initial adherence of *S. mutans* to the tooth surface might influence the probability of infection.

TABLE 1. *Adherence of ^{3}H-labeled KPSK-2 cells pretreated with saliva to SHA pretreated with saliva*[a]

Subject	% Adherence on sampling occasion:			CV[b] (%)
	I	II	III	
1	53	50	45	8.2
2	82	88	70	11.5
3	32	40	27	19.8

[a] The *S. mutans* cells were on all occasions pretreated with saliva from subject 1. The adherence is expressed as a percentage of the activity of hydroxyapatite pretreated with PBS.

[b] CV, Coefficient of variation.

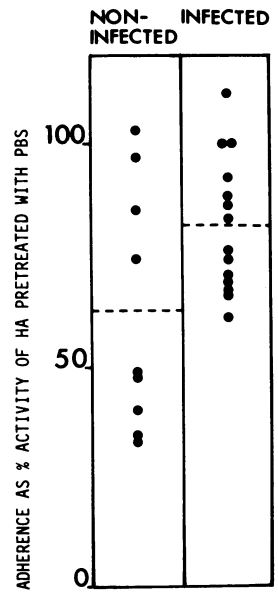

FIG. 1. *Adherence of ^{3}H-labeled KPSK-2 cells pretreated with saliva from noninfected or infected children. Each dot represents the result of one experiment using saliva from a single mother-child pair. The adherence is expressed as a percentage of the activity for SHA pretreated with PBS. The mean is indicated by the dotted line. The difference between the two groups is statistically significant (Student's t test, $t = 2.14$; $0.05 > P > 0.01$).*

TABLE 2. *Relative adherence of ^{3}H-labeled KPSK-2 cells pretreated with saliva from the mother to SHA pretreated with saliva from noninfected or infected children*[a]

Noninfected	Infected	
% Adherence	% Adherence	No. of *S. mutans* per ml of saliva × 10^3
23	54	7
31	77	15
46	108	272
37	48	358
36	142	3,000

[a] Adherence is expressed as a percentage of the activity of cells pretreated with PBS. □ indicates siblings.

Certain individuals may be prone to develop caries because strains of caries-inducing streptococci adhere more avidly to their teeth than to the teeth of other individuals. Thus the findings in this study seem to parallel those on adherence to pharyngeal cells of group A streptococci associated with rheumatic fever (20).

Low adherence was observed in some of the assays in which saliva from children of highly infected mothers was used to pretreat the SHA. These children were not infected (Fig. 1), an observation in agreement with our hypothesis. The hypothesis is also supported by the finding of higher adherence when saliva from infected children was used. High adherence was also found, however, when saliva from some of the noninfected children was used. This might indicate that these individuals were susceptible to *S. mutans* infection, but that other factors had prevented colonization. Such factors could be a low sucrose intake (11, 15) or a competing flora (21-23) or both. Furthermore, the children were 6 years old, and by this time salivary composition both in mother and in child might differ from that at the time when the chance of *S. mutans* infection in the young child seems to be the greatest, i.e., when the primary teeth erupt. This age group was selected, however, because of difficulties in obtaining enough saliva for the experiment from younger children.

In most cases the adherence of cells pretreated with saliva was impaired when the SHA was pretreated with saliva. This is in accordance with other in vitro studies (9, 10). It is also in agreement with the in vivo observation that a lower number of *S. mutans* cells adhere to a pellicle-coated tooth surface than to a pellicle-free surface (19). Table 2 shows that the pretreatment of the bacteria usually gives a variable reduction in adherence in comparison with the adherence of cells pretreated with PBS. In some cases, however, pretreatment of the SHA or the bacteria with saliva gave an increased relative adherence (Fig. 1 and Table 2). Thus the data clearly indicate that pretreatment of the cells with the saliva of one person and pretreatment of SHA with that of another may result in combinations which counteract or foster the adherence to a varying extent. Our data indicate that such combinations may determine the risk of *S. mutans* infection. If the data are confirmed and suitable methods can be developed, the knowledge could be used as a diagnostic tool to identify children who are susceptible to *S. mutans* infection for special preventive measures.

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