Ability of *Streptococcus mutans* and a Glucosyltransferase-Defective Mutant to Colonize Rodents and Attach to Hydroxyapatite Surfaces

W. B. CLARK,† L. L. BAMMANN, AND R. J. GIBBON*

Forsyth Dental Center and Harvard School of Dental Medicine, Boston, Massachusetts 02115

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A mutant of *Streptococcus mutans* forming little or no cell-associated glucosyltransferase attached to the teeth of rats and to saliva-treated hydroxyapatite comparably to its parent.

Synthesis of extracellular glucans from sucrose via cell-associated glucosyltransferase has been associated with the development of dental plaques in experimental animals and with the formation of deposits on solid surfaces in vitro by *Streptococcus mutans* (10, 15). These observations have frequently been interpreted as indicating that glucan synthesis played an important role in the attachment of *S. mutans* to smooth surfaces. However, van Houte et al. (18) demonstrated that all of 11 strains of *S. mutans*, representative of the five serological groups delineated by Bratthall (1), could establish and persistently colonize the teeth of rats fed diets with no detectable sucrose. In addition, the minimum infective dose for 9 of 11 strains studied was similar for rats fed diets containing either glucose or sucrose. In these experiments, colonization of the teeth of animals fed either diet occurred mainly in fissures and not on smooth tooth surfaces. These studies suggested that glucan synthesis was not required for attachment and implantation of most strains of *S. mutans*, at least in the fissures of rodents. We recently observed that glucan synthesis from sucrose was essential for, or enhanced, attachment of *S. mutans* cells to saliva-treated hydroxyapatite (HA) surfaces in vitro, or to the smooth surfaces of human teeth in vivo, thus extending these observations (3).

A mutant of *S. mutans* strain LM7 was recently isolated by Donkersloot et al. (4) which possessed only low levels of cell-associated glucosyltransferase activity relative to the parent strain. The present investigation compared the ability of this mutant and the parent strain to implant and colonize conventional rats fed sucrose-containing diets. Furthermore, because teeth are covered by an acquired pellicle comprised of selectively adsorbed salivary components (10, 12), the ability of the mutant and parent strain to attach to saliva-treated HA was also studied.

*S. mutans* strain LM7 and its mutant, strain A17, were generously provided by J. A. Donkersloot (National Institute for Dental Research, Bethesda, Md). Strain LM7 contains extrachromosomal DNA and appears to be similar to the original isolate (7); strain A17 also contains extrachromosomal DNA (4). The cell-associated glucosyltransferase activity of the two strains was compared in the present study. Both were grown in a Trypticase-salts medium (8) containing 0.2% glucose (TGB) supplemented with 0.01% sucrose (TGB+S) to promote binding of glucosyltransferase to the surface of the streptococci (17). Samples of overnight cultures and twice-washed organisms were assayed for glucosyltransferase activity by using [14C]glucose-labeled sucrose as described by Germaine et al. (6). Sodium fluoride (0.15%) was included in reaction mixtures to inhibit glycolysis and glycosyl synthesis (20). Unstandardized whole cultures of strain A17 contained 31% as much total glucosyltransferase activity as cultures of strain LM7; however, standardized, washed A17 cells possessed less than 3.0% of the activity of parental cells (Table 1). This observation confirms the findings of Donkersloot et al. (4). When strain A17 was propagated in 2% sucrose broth, it formed little macroscopically evident adherent growth on the walls of culture vessels. Washed cells of this organism agglutinated within 2 h after addition of dextran (molecular weight, 2 × 106; Pharmacia Fine Chemicals, Inc.) but not upon addition of sucrose when tested as previously described (8) (Table 1). This further indicates that the mutant cells possess little or no cell-associated glucosyltransferase.

The implantation and colonization of the strains were compared in conventional rats. Overnight cultures grown in Todd-Hewitt broth

† Present address: College of Dentistry, University of Florida, Gainesville, FL 32610.
(Difco Laboratories) were centrifuged, and the streptococci were suspended in fresh medium at a concentration of approximately $10^{9}$ cells per ml. Dilutions in fresh medium were then prepared to yield suspensions ranging from $10^{4}$ to $10^{8}$ organisms per ml. The actual number of colony-forming units (CFU) present was determined by plating appropriate dilutions on mitis-salivarius bacitracin agar (11). The plates were incubated in an atmosphere of 80% N$_2$, 10% H$_2$, and 10% CO$_2$ for 48 h at 35°C, and the number of CFU was determined with the aid of a dissecting microscope.

Before the experiment, conventional female Sprague-Dawley rats and their 8-day-old litters (Charles River Breeding Laboratories, Wilmington, Mass.) were fed diet 2000 (13) for two weeks. Oral and fecal samples were collected three times from each mother and from representative pups at 5-day intervals; these were streaked on mitis-salivarius agar (Difco) and on mitis-salivarius bacitracin agar to determine whether the animals harbored S. mutans.

Twenty-one-day-old weanling rats which were free of detectable levels of S. mutans were caged separately and continued on diet 2000. One day later, 0.1 ml of a suspension of strain LM7 or A17 was placed into the mouths of the animals with a pipette. The rats were sacrificed in a CO$_2$ chamber 9 days later, and the six upper molar teeth were extracted. These were ground in glass tissue grinders, and suspensions were prepared as described by van Houte et al. (19). Samples of the suspensions were plated on mitis-salivarius bacitracin medium to determine the number of CFU of S. mutans present on the teeth of each rat. Uninoculated rats maintained under similar conditions were used as controls; they remained free of S. mutans throughout the experiment.

Both S. mutans strain LM7 and strain A17 established in all rats inoculated with $10^7$, $10^8$, or $10^9$ CFU (Table 2). The minimum dose at which some animals in each group became colonized and some did not appear to be approximately $10^{9}$ CFU for both strains (Table 2). The minimum dose of strain A17 required to infect rats fed a glucose diet free of detectable sucrose was also between $10^4$ to $10^5$ CFU (data not shown). Thus, there appears to be little difference between S. mutans cells which possess high or low levels of cell-associated glucosyltransferase to attach and initiate colonization of the teeth of rats.

In general, higher numbers of S. mutans were recovered from the teeth of animals infected with strain LM7 than from rats infected with the glucosyltransferase-defective mutant. These differences were most pronounced in animals infected with small inocula (Table 2), and they are consistent with the suggestion that glucan synthesis by cell-associated glucosyltransferase promotes the accumulation of S. mutans cells subsequent to their attachment to teeth (3, 8, 10, 18). The observations are also consistent with the report that mutants of S. mutans which produce elevated levels of glucosyltransferase can be recovered in higher numbers from the teeth of infected animals and induce more severe dental caries than parental strains possessing lower levels of enzyme (14).

The ability of S. mutans strain LM7 and A17 to attach to saliva-treated HA was also compared. In addition, TGB and TGB+S-grown cells of S. mutans 6715 were also studied; previous studies have shown that TGB-grown 6715 cells contain little or no cell-associated glucosyltransferase whereas TGB+S-grown 6715 cells contain high levels of this enzyme (17). For these studies, paraffin-stimulated whole saliva was collected in a container chilled over ice from one human donor (W.C.); it was heat inactivated at 60°C for 30 min and clarified by centrifugation.

### Table 1. Comparative properties of S. mutans strain LM7 and mutant strain A17

<table>
<thead>
<tr>
<th>S. mutans strain</th>
<th>Cell-associated glucosyltransferase activity$^*$</th>
<th>Macrococci-</th>
<th>Agglutination after addition of:</th>
<th>Saccharose Dextran</th>
</tr>
</thead>
<tbody>
<tr>
<td>LM7</td>
<td>49.2</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>A17</td>
<td>1.2</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
</tbody>
</table>

$^*$ Nanograms of glucan synthesized per hour per microgram of dry cell weight.

### Table 2. Colonization of rats inoculated with S. mutans strain LM7 and glucosyltransferase-defective mutant strain A17

<table>
<thead>
<tr>
<th>S. mutans strain</th>
<th>Inoculum (CFU)</th>
<th>No. of rats infected/ no. of rats inoculated</th>
<th>Mean recovery (CFU) $\times 10^3$ per upper molar teeth (range)</th>
</tr>
</thead>
<tbody>
<tr>
<td>LM7</td>
<td>$3.6 \times 10^4$</td>
<td>5/5</td>
<td>23.0 (6.0-36.0)</td>
</tr>
<tr>
<td></td>
<td>$3.6 \times 10^5$</td>
<td>5/5</td>
<td>3.8 (5.8-8.4)</td>
</tr>
<tr>
<td></td>
<td>$3.6 \times 10^6$</td>
<td>5/5</td>
<td>14.0 (2.2-32.7)</td>
</tr>
<tr>
<td></td>
<td>$4.7 \times 10^7$</td>
<td>2/3</td>
<td>6.8 (6.3-7.4)</td>
</tr>
<tr>
<td></td>
<td>$4.7 \times 10^8$</td>
<td>1/3</td>
<td>9.3</td>
</tr>
<tr>
<td>A17</td>
<td>$4.0 \times 10^4$</td>
<td>5/5</td>
<td>6.1 (0.5-13.2)</td>
</tr>
<tr>
<td></td>
<td>$4.0 \times 10^5$</td>
<td>5/5</td>
<td>3.9 (0.2-10.8)</td>
</tr>
<tr>
<td></td>
<td>$4.0 \times 10^6$</td>
<td>5/5</td>
<td>1.2 (0.07-3.7)</td>
</tr>
<tr>
<td></td>
<td>$9.8 \times 10^4$</td>
<td>2/3</td>
<td>0.2 (0.002-0.3)</td>
</tr>
<tr>
<td></td>
<td>$9.8 \times 10^5$</td>
<td>2/3</td>
<td>0.05 (0.009-0.01)</td>
</tr>
</tbody>
</table>
(9) Whole rat saliva was obtained from the mothers used in the experiments described above. The animals were injected intraperitoneally with 33 to 66 mg of ketamine hydrochloride (Bristol Laboratories) per kg of body weight and 10 mg of pilocarpine nitrate (ICN Pharmaceuticals, Inc.) per kg of body weight. The stimulated saliva was collected in containers chilled over ice; saliva from four rats was pooled, heat inactivated, and clarified by centrifugation. The adsorption of [3H]thymidine-labeled streptococci to spheroidal HA beads pretreated with the respective saliva samples was determined as previously described (2).

Somewhat higher numbers of mutant A17 cells adsorbed to HA treated with either clarified human saliva or with rat saliva as compared with parental strain LM7 (Table 3). Similarly, TGB-grown 6715 cells tended to adsorb in higher numbers to HA treated with human saliva than TGB+S-grown cells. Thus, streptococcal cells possessing low levels of glucosyltransferase activity due to a genetic defect, or because of physiological manipulation, attached at least as well or better to saliva-treated HA surfaces than corresponding organisms with high levels of enzyme. In fact, the presence of high levels of cell-associated glucosyltransferase appeared to impair attachment to some degree. Glucosyltransferase molecules are thought to exist in the form of aggregates (5, 17); therefore, such aggregates and/or associated glucan molecules may sterically interfere with streptococcal binding to adsorbed salivary components comprising the HA pellicle. These observations are not in agreement with a recent report that higher percentages of sucrose-grown S. mutans cells adsorb to HA or saliva-treated HA than glucose-grown organisms (16). However, this study used finely powdered HA of very high surface area which is difficult to quantitatively separate from unadsorbed bacteria; in addition, because S. mutans cells grow as aggregates in sucrose broth, it seems likely that some streptococcal clumps would settle out along with the HA powder during the 30-min sedimentation period used for separating adsorbed from nonadsorbed organisms.

Collectively, both the in vivo and in vitro data obtained in the present study provide further support for the contention that neither glucosyltransferase nor glucan synthesis from sucrose is required for, or promotes, initial attachment of S. mutans cells to saliva-treated HA surfaces or to teeth.

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