Salivary Immunoglobulin A and Serum Antibodies to *Streptococcus mutans* Ribosomal Preparations in Dental Caries-Free and Caries-Susceptible Human Subjects

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Caries-free subjects or individuals with low caries susceptibility exhibited significantly higher \( P < 0.001 \) levels of naturally occurring salivary immunoglobulin A (IgA) and serum IgG, IgA, and IgM antibodies to a *Streptococcus mutans* ribosomal preparation than subjects with high caries susceptibility. Absorption of saliva and serum samples with *S. mutans* ribosomal preparations, but not with other *S. mutans* antigens or with *Escherichia coli* and *Neisseria gonorrhoeae* ribosomal preparations, removed the antibody activity. Absorption with *Streptococcus sanguis* ribosomes and NH\(_4\)Cl-washed *S. mutans* ribosomes partially removed the anti-*S. mutans* ribosome antibody activity. These results provide evidence that naturally occurring salivary and serum antibodies to the *S. mutans* ribosomal preparation correlate with susceptibility to dental caries.

*Streptococcus mutans* has been implicated as the principal causative agent of human dental caries (17, 18). A number of studies in experimental animals have shown that parenteral or oral immunization with *S. mutans* whole cells, cell surface components, or extracellular products inhibits *S. mutans* colonization and subsequent dental caries formation (see reference 21 for review). Several laboratories have shown that salivary immunoglobulin A (IgA) and serum antibodies are important in preventing dental caries in humans and animals; however, some studies show the protective effects of salivary IgA antibodies (1, 5, 7, 15, 20, 23, 29), whereas others support serum antibody-mediated control of carious lesions (3, 4, 14). Nevertheless, studies in humans have shown that oral immunization with *S. mutans* whole cells results in the induction of salivary IgA antibody responses (9, 22) and a reduction in the number of *S. mutans* in dental plaque (9).

Recently, we found that local injection of gnotobiotic rats in the vicinity of the parotid and submandibular salivary glands with a ribosomal preparation of *S. mutans* 6715 (serotype g) resulted in significantly lower numbers of carious lesions, lower numbers of plaque-adherent *S. mutans*, and higher levels of specific salivary IgA antibodies in immunized animals than in control rats after challenge with the homologous cariogenic *S. mutans* (8, 24). The present investigation determined the levels of salivary and serum antibodies reacting with *S. mutans* ribosomal preparations in caries-free (CF) individuals or in subjects with high or low caries susceptibility (HCS or LCS, respectively [15]) in order to determine whether a correlation exists between the level of natural anti-*S. mutans* ribosomal antibodies and the severity of dental caries in humans.

*S. mutans* 6715, originally obtained from Robert J. Fitzgerald (Dental Research Unit, Miami Veterans Administration Medical Center, Miami, Fla.), was used to prepare ribosomes. This strain was isolated from a human carious lesion and has been shown to be cariogenic in our gnotobiotic rat caries model (8). Ribosomal preparations were purified from early-log-phase cultures of *S. mutans* 6715, *Streptococcus sanguis* ATCC 10556 (kindly provided by Sam Rosen, Ohio State University, Columbus, Ohio), and *Escherichia coli* ATCC 25922 cells (obtained from the American Type Culture Collection, Rockville, Md.) by procedures previously described (8, 10). Briefly, cells were disrupted in a Braun homogenizer (B. Braun Co., Mel- sungen, Federal Republic of Germany), and the ribosomes were purified by differential centrifugation. The purity of the preparation was assessed by the relative absorbances at 260, 235, and 280 nm and by determining levels of RNA, protein, and total neutral hexose as previously described (8, 10). The ribosomal preparation from *Neisseria gonorrhoeae* was kindly provided by Morris D. Cooper (Department of Medical Microbiology and Immunology, Southern Illinois University School of Medicine, Springfield).

Healthy individuals were screened for the number of decayed, missing, and filled surfaces (DMFS) and the number of unfilled and active lesions by using a fine-tipped dental explorer, radiographs, and transillumination. Volunteers who had no detectable DMFS were classified as CF; those with fewer than three DMFS and two or fewer small, unfilled lesions not extending into the dentine were designated as LCS subjects. Volunteers who had a DMFS score of greater than 10 and at least 5 relatively large, unfilled, smooth surface lesions with at least one lesion extending into the dentine represented HCS individuals. A total of 11 CF, 9 LCS, and 24 HCS subjects were recruited for these studies. The CF group was composed of 7 males and 4 females and ranged in age from 18 to 60 years. The LCS group consisted of 6 males and 3 females (22 to 40 years old), and the HCS group included 13 males and 11 females (19 to 44 years old). *S. mutans* serotype c was detected in whole saliva samples (>1,000 CFU/ml) from all volunteers. Plaque and gingival indices were obtained using the method described by Löe (16). Unstimulated parotid saliva samples were collected by using plastic intraoral cups (28) and clarified by centrifugation (8,000 × g; 15 min). Blood specimens were obtained by venipuncture and collected in glass tubes. Serum was separated from the clot by centrifugation (5,000 × g; 10 min). The
saliva and serum samples were stored at −20°C until assayed for antibody activity in an enzyme-linked immunosorbent assay (ELISA) as described below.

In one series of experiments, saliva and serum samples from five CF subjects were pooled, and 1-ml portions were mixed with equal volumes of S. mutans 6715 whole cells (Aco of 3.0, representing approximately 2 × 10⁸ CFU/mL), glucosyltransferase (GTF; 1 mg/ml [11]), lipoteichoic acid (LTA; 1 mg/ml [11]). S. mutans serotype c (Guy’s strain) surface antigen I/II (1 mg/ml [27], kindly provided by Michael W. Russell, Department of Microbiology, University of Alabama at Birmingham), or S. mutans 6715, S. sanguis, E. coli, or N. gonorrhoeae ribosomal preparations (1 mg/ml) and incubated with occasional mixing at 37°C for 3 h and overnight at 4°C. The samples were clarified by centrifugation (15 min at 10,000 × g for S. mutans whole cells or 2 h at 40,000 × g for the soluble antigens) and stored at −20°C until assayed for antibody activity by ELISA. A determinant cross-reactive with surface antigen I/II has previously been shown to be present on the cell surface of S. mutans 6715 (25). Furthermore, Bozza et al. (2) reported that the GTF responsible for insoluble-glucan synthesis in S. mutans was localized at the cell surface.

The ELISA technique used has been described previously (7, 24). A 100-μl portion of the ribosomal preparation (100 ng of RNA/ml) was added to wells of flat-bottom polystyrene microtiter plates (EIA; Flow Laboratories, Inc., McLean, Va.). The plates were incubated for 3 h at 37°C and overnight at 4°C and then washed three times with 0.9% NaCl containing 0.05% Tween 20 (TWEEN-saline) to remove unbound antigen. After the final wash, 10 μg of human serum albumin (globulin free; Sigma Chemical Co., St. Louis, Mo.) per ml was added to each well (200 μl), and the plates were incubated at 25°C for 1.5 h. The plates were washed, and then optimal dilutions of human parotid saliva (1:4) or serum (1:100) were added to each well (100 μl) in triplicate and incubated at 37°C for 1 h. The plates were washed, and rabbit anti-human IgA, IgG, or IgM heavy-chain-specific reagents (Melo Laboratories, Inc., Springfield, Va.), adsorbed with κ and λ light chains to remove anti-light-chain activity, were added to appropriate wells (100 μl) and incubated at 37°C for 1 h. The plates were washed, and alkaline phosphatase (Sigma)-labeled goat anti-rabbit IgG heavy-chain reagent (Behring Diagnostics, Div. of American Hoechst Corp., Somerville, N.J.) was added to each well (100 μl). After incubation at 37°C for 3 h and then at 4°C overnight, the plates were washed, and alkaline phosphatase substrate (p-nitrophenyl phosphate; Sigma), dissolved in 10% diethanolamine buffer (1 mg/ml, pH 9.8), was added to each well (100 μl) and reacted at 25°C for 1.5 h. The amount of color which developed was measured at 405 nm in the microtiter plate with a Titertek Multiskan photometer (Flow Laboratories). The data were reduced by computing the means and standard errors of the mean of the absorbances of triplicate determinations per sample and logarithmically transformed as previously described (7, 26), using a reference saliva and serum, and are expressed as ELISA units (EU). The results were analyzed by analysis of variance and the paired t test, and comparisons were made among sample groups to determine correlation coefficients.

The S. mutans ribosomal preparation consisted of 55% RNA and 45% protein, and therefore was similar to those previously described (8, 11). The 260/235-nm and 260/280-nm absorption ratios of this preparation were 1.42 and 1.85, respectively, indicating a lack of membrane contamination. However, the ribosomal preparation did contain minute amounts of GTF, LTA, other surface proteins, and carbohydrates as described previously (11; R. L. Gregory, manuscript in preparation).

The LCS group had significantly lower (P < 0.001) DMFS and unfilled lesions than the HCS population (Table 1). The LCS group had DMFS and unfilled-lesion medians of 2 and 1, respectively, while the HCS subjects had median DMFS and unfilled-lesion scores of 51.5 and 16.5, respectively. The unfilled lesions observed in the LCS group were small (<0.25 mm), whereas the HCS volunteers had larger lesions (>0.50 mm). Plaque indices were 0.66 ± 0.09, 0.69 ± 0.11, and 1.25 ± 0.08 in the CF, LCS, and HCS subjects, respectively, whereas gingival indices were 0.68 ± 0.09, 0.70 ± 0.11, and 1.20 ± 0.08.

The CF and LCS subjects had significantly higher (P < 0.001) levels of salivary IgA antibodies to the S. mutans ribosomal preparation than did the HCS individuals (Table 1). The median level of salivary IgA antibodies was 70.4 EU in CF subjects, 74.2 EU in LCS subjects, and 28.8 EU in HCS subjects. The salivary IgA antibody levels of all subjects correlated with the numbers of DMFS and unfilled lesions (r = −0.341 and −0.272, respectively).

The CF and LCS subjects had significantly higher (P < 0.001) levels of serum IgA, IgG, and IgM antibodies to the S. mutans ribosomal preparation than did the HCS individuals, although a large variation in antibody levels was seen in all three groups (Table 2). The serum antibody levels correlated with the numbers of DMFS (r = −0.385, IgA; −0.286, IgG; and −0.394, IgM). These results indicate that the levels of salivary and serum antibodies to the S. mutans ribosomal preparation are significantly higher in CF and LCS subjects than in HCS individuals.

Absorption of pooled parotid saliva samples from CF subjects with the S. mutans 6715 ribosomal preparation significantly depleted antibody activity (P < 0.01), whereas adsorption with S. mutans 6715 whole cells, GTF, LTA, S. mutans Guy's strain surface antigen I/II, or ribosomes from E. coli and N. gonorrhoeae had no significant effect on the
antibody activity to the *S. mutans* ribosomal preparation (*P* > 0.05) (Table 3). On the other hand, absorption of saliva with *S. sanguis* ribosomes or NH₄Cl-washed *S. mutans* ribosomes partially depleted antibody activity to *S. mutans* 6715 ribosomes by approximately 30 and 40%, respectively. NH₄Cl reportedly removes cell wall or membrane components adhered to ribosomes (13). Similar results were obtained with pooled serum samples from CF subjects (data not shown). These results indicate that the salivary and serum antibodies to *S. mutans* ribosomal preparations from the CF subjects were specific for this antigen, although some cross-reactivity with ribosomes from *S. sanguis* and *S. mutans* cell wall or membrane determinants were noted.

In the present study, we surveyed parotid saliva and serum samples from 11 CF, 9 LCS, and 24 HCS subjects for levels of naturally occurring antibodies to the *S. mutans* ribosomal preparation. Our results indicate that most adults have antibody activity to the *S. mutans* ribosomal preparation, although CF and LCS subjects have significantly higher levels of salivary and serum antibodies than HCS individuals (*P* < 0.001). The levels of salivary and serum antibodies from all subjects correlated with the number of DMFS and unfilled lesions.

Adsortion of saliva and sera from CF subjects with *S. mutans* 6715 whole cells, GTF, LTA, surface antigen I/II, or *E. coli* or *N. gonorrhoeae* ribosomes did not significantly affect the level of antibodies to the *S. mutans* ribosomal preparation; however, antibody activity was completely removed by absorption of saliva and sera with *S. mutans* ribosomes and only partially removed by absorption with *S. sanguis* ribosomes. These results suggest that the antibodies were specific for *S. mutans* ribosomes and for common determinants of the two streptococcal ribosomal preparations.

It has been shown previously that antibodies to ribosomes from many species of bacteria cross-react immunologically, which suggests that the genetic coding for ribosomal proteins is highly conserved (6). In addition, Lynn et al. (19) have shown a large degree of cross-reactivity between ribosomes from *Haemophilus influenzae*, *Streptococcus pneumoniae*, and *Pseudomonas aeruginosa*. Therefore, it is possible that infection with other related bacteria may induce antibodies which cross-react with *S. mutans* ribosomes. In this regard, Gregory and Shechmeister (12) have shown that *S. mutans* and *E. coli* ribosomes cross-react immunologically in rabbits and may contain at least six identical proteins. However, in the present study, the absorption of saliva and serum samples from the human volunteers with *E. coli* (or *N. gonorrhoeae*) ribosomes resulted in only a slight reduction in the level of antibodies to *S. mutans* ribosomes. Thus, the antibodies were not directed to those determinants cross-reactive between *S. mutans* and *E. coli* ribosomes, but were directed to unique antigens in the *S. mutans* ribosomal preparation. However, absorption of saliva from CF subjects with *S. sanguis* ribosomes partially removed anti-*S. mutans* ribosome antibodies, indicating that there is cross-reactivity between the antigens of *S. mutans* and *S. sanguis* ribosomes. Further absorption of the pooled saliva sample with *S. sanguis* ribosomes did not remove additional antibody activity to *S. mutans* ribosomes (data not shown). Further studies are needed to determine whether children with lower levels of salivary or serum antibodies to *S. mutans* ribosomes are at a higher risk of developing carious lesions than those with higher levels of anti-*S. mutans* ribosome antibodies.

We are indebted to George A. Kentros for the use of the University Hospital General Practice Dental Clinic. We thank Gloria Richardson for expert technical assistance and Betty Wells and Betty Couch for secretarial support. These studies were carried out under complete supervision and with informed consent and were approved by the Human Use Committee of the University of Alabama at Birmingham.

This work was supported in part by United States Public Health Service grants DE 04217, AM 07069, DE 05358, DE 02670, DE 07026, and contract DE 42591. R.L.G. is supported by New Investigator Research Award DE 07318 from the National Institute of Dental Research (NIDR). S.J.F. is supported by Nutrition Training Fellowship Award DE 07020 from the NIDR. S.M.M. is a recipient of Research Career Development Award DE 00092 from the NIDR.

**LITERATURE CITED**


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**TABLE 2. Serum IgA, IgG, and IgM levels of anti-*S. mutans* 6715 ribosome antibody from CF, LCS, and HCS subjects**

<table>
<thead>
<tr>
<th>Subjects</th>
<th>Serum level (EU/ml) of anti-<em>S. mutans</em> ribosome antibody:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>IgA</td>
</tr>
<tr>
<td>CF</td>
<td>507.9</td>
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<tr>
<td>LCS</td>
<td>567.7</td>
</tr>
<tr>
<td>HCS</td>
<td>191.6</td>
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</tbody>
</table>

* Data after logarithmic transformation are expressed in EU per milliliter of serum. Antibody levels were determined in the sera from 11 CF, 9 LCS, and 24 HCS subjects.

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**TABLE 3. Specificity of pooled parotid salivary IgA antibodies to *S. mutans* ribosomes from CF subjects**

<table>
<thead>
<tr>
<th>Immunoadsorbent</th>
<th>Level of salivary IgA anti-<em>S. mutans</em> 6715 ribosome antibodies (mean EU/ml ± SEM)</th>
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<tbody>
<tr>
<td>None</td>
<td>171.0 ± 30.4</td>
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<tr>
<td><em>S. mutans</em> 6715</td>
<td></td>
</tr>
<tr>
<td>Whole cells</td>
<td>168.3 ± 29.7</td>
</tr>
<tr>
<td>GTF</td>
<td>153.7 ± 30.6</td>
</tr>
<tr>
<td>LTA</td>
<td>156.3 ± 31.2</td>
</tr>
<tr>
<td><em>S. mutans</em> Guy's strain surface antigen I/II</td>
<td>145.3 ± 21.7</td>
</tr>
<tr>
<td><em>E. coli</em> ribosomes</td>
<td>126.5 ± 19.3</td>
</tr>
<tr>
<td><em>N. gonorrhoeae</em> ribosomes</td>
<td>142.0 ± 27.9</td>
</tr>
<tr>
<td><em>S. sanguis</em> ribosomes</td>
<td>75.2 ± 16.8</td>
</tr>
<tr>
<td><em>S. mutans</em> 6715 ribosomes (NH₄Cl washed)</td>
<td>103.8 ± 17.3</td>
</tr>
<tr>
<td><em>S. mutans</em> 6715 ribosomes</td>
<td>14.3 ± 2.4</td>
</tr>
</tbody>
</table>

* Pooled parotid saliva samples from five CF subjects were adsorbed with an equal volume of the indicated immunoadsorbent for 3 h at 37°C and overnight at 4°C. See the text for details.

* Data after logarithmic transformation are expressed in mean ELISA units per milliliter of parotid saliva from CF subjects.


