INFECTION AND IMMUNITY, July 1984, p. 286–289
0019-9567/84/070286-04$02.00/0

Vol. 45, No. 1

Function of Anti-*Streptococcus mutans* Antibodies: Anti-Ribosomal Antibodies Inhibit Acid Production, Growth, and Glucose Phosphotransferase Activity

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Received 21 February 1984/Accepted 6 April 1984

Antibodies induced in sera and saliva of rats and rabbits immunized with ribosomal preparations from *Streptococcus mutans* 6715 inhibited transport of glucose by the phosphotransferase system by >60%, acid production from sucrose by >95%, and growth of the homologous *S. mutans* by >59%. Inhibition of growth and acid production by immune sera and saliva were abrogated by prior adsorption with *S. mutans* 6715 whole cells, glucosyltransferase, lipoteichoic acid, or α1-6 or α1-3 dextran. These results indicate that antibodies induced to an *S. mutans* ribosomal preparation react with cell surface determinants and suggest that the antibodies inhibit sucrose-induced acid formation and growth of virulent *S. mutans* by neutralizing the glucose-phosphotransferase system.

Streptococcus mutans has been implicated as the major etiological agent of dental caries (6, 7; for review, see reference 8). Recently, it was reported that ribosomal preparations from *S. mutans* induced humoral and cell-mediated immune responses in rabbits (2) and rats (4). Other studies have shown that *S. mutans* ribosomal preparations protect gnotobiotic rats from homologous *S. mutans*-induced dental caries (1, 9). Antibodies to the ribosomal preparations agglutinated *S. mutans* whole cells from all seven serotypes and inhibited the adherence of viable cells to a glass surface when grown in the presence of sucrose (2). These antibodies were also shown to cross-react with glucosytransferase (GTF), lipoteichoic acid (LTA), and several cell surface proteins (3). Preliminary data indicate no cross-reactivity between antibodies to *S. mutans* ribosomes and human heart sarcolemmal or renal glomerular antigens.

The pathogenesis of dental caries involves *S. mutans*: (i) attachment to tooth surfaces; (ii) growth; and (iii) acid production by use of caries-promoting foods such as sucrose. The formation of carious lesions can be circumvented, therefore, if any one of these stages is interrupted. We have previously shown that antibodies to *S. mutans* ribosomal preparations can reduce adherence of viable organisms to an attachment surface model (i.e., glass surface) (2). In the present study, we examined the ability of these antibodies to inhibit growth of the homologous bacterium and acid production from sucrose by the organism.

S. mutans 6715 (serotype *g*) ribosomal preparations were made by disrupting the whole cells in a homogenizer (B. Braun Co., Melsungen, Federal Republic of Germany) and purified by using differential centrifugation (2). The sera and saliva used were obtained from rats and rabbits immunized with the ribosomal preparations and were shown to contain specific immunoglobulin G (IgG) and IgA antibodies, respectively (1–4). Dilutions of filter-sterilized, pooled rat or rabbit serum and saliva samples were made in sterile 3% brain heart infusion broth (3 ml; BHI; Difco Laboratories, Detroit, Mich.) containing 1% sucrose in polystyrene tubes (no. 2058, Becton Dickinson and Co., Oxnard, Calif.). After inoculation (100 μl) with an 18-h culture of *S. mutans* 6715, the reaction mixtures were incubated for 24 h at 37°C in an atmosphere of 5% CO_2 in air. The pH and the amount of growth of each culture were determined with a Corning pH meter (model 130 with an Ag-AgCl electrode; Fisher Scientific Co., St. Louis, Mo.) and by measuring absorbance at 660 nm (Spectronic 20 spectrophotometer; Fisher), respectively.

Rat serum and saliva and rabbit serum from animals immunized with *S. mutans* 6715 ribosomes or whole cells inhibited acid production of viable *S. mutans* when compared with the effects of normal rat serum and saliva and normal rabbit serum (Table 1). Optimal inhibition was observed with 1:300 dilutions of pooled serum or saliva from rats immunized with ribosomes which reduced the amount of acid production by 99.3 and 99.7%, respectively, and with a 1:3,000 dilution of pooled immune rabbit serum which inhibited acid production by 99.3%. Serum and saliva from rats immunized with *S. mutans* ribosomal preparations inhibited acid production to a greater extent than did antibodies from animals immunized with *S. mutans* whole cells. Sera and saliva from animals immunized with ribosomes or whole cells also inhibited growth of *S. mutans* when compared with normal sera and saliva (Table 2). The optimal dilutions (and percent reduction) of sera and saliva causing maximum inhibition of growth of *S. mutans* was 1:300 for rat serum (59.0%) and saliva (75.6%) and 1:3,000 for rabbit serum (74.0%). In contrast to the rat serum, saliva from rats immunized with ribosomes did not inhibit growth of *S. mutans* as well as did saliva from rats injected with whole cells. These results were confirmed by plating the cultures onto mitis salivarius agar (Difco). The numbers of viable *S. mutans* were highly correlated ($r = 0.930$) with the absorbance of the cultures at 660 nm (data not shown). In all cases, the numbers of *S. mutans* were higher than or equal to the numbers of *S. mutans* originally inoculated into the cultures. This finding suggests that inhibition of growth was caused by a bacteriostatic rather than a bactericidal mechanism.

S. mutans whole cells (10^9/ml), ribosomes (1 mg/ml), GTF (1 mg/ml), LTA (1 mg/ml), or an equal mixture (1 mg/ml) of α1-6 and α1-3 dextrans (dextrans from *Leuconostoc mesenteroides* B512F [95% 1-6 branched linkages] and B1355

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TABLE 1. Inhibition of *S. mutans* acid production from sucrose with serum and salivary antibodies from rats and rabbits immunized with *S. mutans* ribosomes or whole cells[a]

Source of antibodies	Immunogen	Serum or saliva dilution	pH	
			Expt 1	Expt 2
None[b]	None	None	4.206	3.996
Rat serum				
Normal	None	1:30	4.022	4.000
Immune	Ribosomes	1:30	5.985	6.005
Immune	Ribosomes	1:300	6.180	6.232
Immune	Ribosomes	1:3,000	5.108	5.392
Immune	Whole cells	1:30	4.992	5.000
Immune	Whole cells	1:300	5.215	5.345
Rat saliva				
Normal	None	1:30	4.005	3.995
Immune	Ribosomes	1:30	6.055	6.227
Immune	Ribosomes	1:300	6.187	6.499
Immune	Ribosomes	1:3,000	5.040	5.985
Immune	Whole cells	1:30	5.053	5.447
Immune	Whole cells	1:300	5.145	5.205
Rabbit serum				
Normal	None	1:30	4.058	4.005
Immune	Ribosomes	1:30	5.005	5.028
Immune	Ribosomes	1:300	6.030	6.110
Immune	Ribosomes	1:3,000	6.034	6.145

[a] Three milliliters of BHI broth containing 1% sucrose and various dilutions of pooled serum and saliva samples were inoculated with *S. mutans* 6715 and incubated for 24 h; the pH of each culture was then determined. Values are reported for two experiments. The pH of uninoculated BHI broth containing 1% sucrose was 6.500. Serum and saliva samples had previously been shown to have major antibody activities in the IgG and IgA classes, respectively.
[b] BHI broth contained 1% sucrose (without serum or saliva) and was inoculated with *S. mutans* 6715 as described above.

[fraction S; 35% 1-3 branched linkages] were kindly donated by M. E. Slodki, Northern Regional Research Center, U.S. Department of Agriculture, Peoria, Ill.) were used in adsorption studies. *S. mutans* whole cells, ribosomes, GTF, and LTA were prepared as described previously (1–3), and a detailed description of their compositions has been reported previously (3). Briefly, an equal volume (1 ml) of immune serum or saliva was mixed with the appropriate antigen, incubated for 10 min at room temperature, and centrifuged at 20,000 × *g* for whole cells and 120,000 × *g* for soluble antigens to remove antigen-antibody complexes. The supernatants were removed and tested for antibody activity to the

TABLE 2. Inhibition of growth of *S. mutans* with serum and salivary antibodies from rats and rabbits immunized with *S. mutans* ribosomes or whole cells[a]

Source of antibodies	Immunogen	Serum or saliva dilution	Absorbance at 660 nm	
			Expt 1	Expt 2
None[b]	None	None	0.760	0.800
Rat serum				
Normal	None	1:30	0.540	0.585
Immune	Ribosomes	1:30	0.510	0.500
Immune	Ribosomes	1:300	0.310	0.240
Immune	Ribosomes	1:3,000	0.310	0.280
Immune	Whole cells	1:30	0.540	0.540
Immune	Whole cells	1:300	0.250	0.280
Rat saliva				
Normal	None	1:30	0.700	0.800
Immune	Ribosomes	1:30	0.240	0.230
Immune	Ribosomes	1:300	0.220	0.195
Immune	Ribosomes	1:3,000	0.345	0.295
Immune	Whole cells	1:30	0.220	0.260
Immune	Whole cells	1:300	0.140	0.180
Rabbit serum				
Normal	None	1:30	0.705	0.730
Immune	Ribosomes	1:30	0.330	0.317
Immune	Ribosomes	1:300	0.250	0.230
Immune	Ribosomes	1:3,000	0.230	0.190

[a] See Table 1, footnote *a*. Absorbance of each culture was read at 660 nm; uninoculated BHI broth containing 1% sucrose served as the blank.
[b] See Table 1, footnote *b*.

TABLE 3. Specificity of the inhibition of *S. mutans* acid production from sucrose and growth by purified serum IgG and salivary IgA antibodies from rats and rabbits immunized with *S. mutans* ribosomes[a]

Source of antibodies	Immunogen	*S. mutans* antigen used as an immunosorbent	pH (% inhibition of acid production)[b]	Absorbance at 660 nm (% reduction of growth)[c]
None[d]	None	None	4.265	0.730
Rat serum				
Normal	None	None	4.105 (control)	0.760 (control)
Immune	Ribosomes	None	5.078 (89.3)	0.550 (27.6)
Immune	Ribosomes	Ribosomes	4.760 (77.8)	0.530 (30.3)
Immune	Ribosomes	GTF	4.272 (31.8)	0.420 (44.7)
Immune	Ribosomes	LTA	4.233 (25.5)	0.240 (68.4)
Immune	Ribosomes	α1-6 and α1-3 dextrans	4.233 (25.5)	0.260 (65.8)
Immune	Ribosomes	Whole cells	4.070 (−8.3)[e]	0.800 (−5.3)[e]
Eluted[f]	Ribosomes	Whole cells	4.692 (74.1)	0.200 (73.7)
Rat saliva				
Normal	None	None	4.145 (control)	0.750 (control)
Immune	Ribosomes	None	5.972 (98.5)	0.250 (66.7)
Immune	Ribosomes	Ribosomes	4.725 (73.7)	0.260 (65.3)
Immune	Ribosomes	GTF	4.285 (27.5)	0.160 (78.7)
Immune	Ribosomes	LTA	4.291 (28.5)	0.320 (57.3)
Immune	Ribosomes	α1-6 and α1-3 dextrans	4.211 (14.1)	0.305 (59.3)
Immune	Ribosomes	Whole cells	4.225 (16.8)	0.180 (76.0)
Eluted[f]	Ribosomes	Whole cells	4.517 (57.5)	0.290 (61.3)
Rabbit serum				
Normal	None	None	4.135 (control)	0.790 (control)
Immune	Ribosomes	None	5.321 (93.5)	0.287 (63.7)
Immune	Ribosomes	Ribosomes	4.625 (67.7)	0.560 (29.1)
Immune	Ribosomes	GTF	4.325 (35.5)	0.360 (54.4)
Immune	Ribosomes	LTA	4.612 (66.7)	0.520 (34.2)
Immune	Ribosomes	α1-6 and α1-3 dextrans	4.312 (33.4)	0.350 (55.7)
Immune	Ribosomes	Whole cells	4.305 (32.5)	0.450 (43.0)
Eluted[f]	Ribosomes	Whole cells	4.707 (73.3)	0.330 (58.2)

[a] See Table 1, footnote *a*. Purified rat serum and saliva antibodies and purified rabbit serum antibodies were used at 1:30 and 1:300 dilutions, respectively.
[b] Percent inhibition of acid production from control which consisted of unadsorbed normal rat or rabbit serum or saliva samples is within parentheses. pH values were converted to arithmetic numbers before determining percent inhibition.
[c] Percent reduction of growth from control which consisted of unadsorbed normal rat or rabbit serum or saliva samples is within parentheses.
[d] See Table 1, footnote *b*.
[e] Immune rat serum adsorbed with whole cells had slightly increased acid production and growth.
[f] Sera and saliva from immunized animals were incubated with *S. mutans* (10 min at room temperature). After washing the cells with borate-saline (0.1 M; pH 8.2), specific anti-*S. mutans* antibodies were eluted with glycine-hydrochloride (0.1 M; pH 2.8) and neutralized with borate-saline.

appropriate antigen, using the enzyme-linked immunosorbent assay to ensure that the adsorption was complete (data not shown).

Adsorption of immune rat serum and saliva with GTF, LTA, dextran, or whole cells, but not ribosomes, removed antibodies that inhibited acid production by *S. mutans*, whereas adsorption of serum with whole cells or saliva with LTA or dextran eliminated antibody activity which inhibited growth of *S. mutans* (Table 3). The serum IgG and salivary anti-*S. mutans* antibodies eluted from the whole cells inhibited both acid production and growth. In contrast, prior adsorption of immune rabbit serum with GTF, dextran, or whole cells, and to a lesser extent with ribosomes and LTA, removed antibodies which inhibited *S. mutans* acid production and growth. *S. mutans* acid production and, to a lesser degree, growth was inhibited by rabbit serum IgG anti-*S. mutans* antibodies eluted from the whole cells. From these results it appears that antibodies to either GTF or dextran inhibit *S. mutans* acid production and growth. GTF is associated with the cell wall of whole *S. mutans* cells and is also present in low amounts in *S. mutans* 6715 ribosomal preparations (3). Although the low GTF concentration in the ribosomal preparation was sufficient to induce antibodies to GTF after repeated injections of the ribosomal preparation in rats and rabbits, an inadequate amount was present to remove the anti-GTF antibodies in the immune sera or saliva after adsorption. It is possible that the antibodies bind to either GTF or GTF-associated glucan and result in the inhibition of GTF enzyme activity, which could conceivably prevent sucrose from being utilized by the cell as an energy and carbon source and thereby inhibit both acid production and growth. We are currently investigating this possibility by using monoclonal antibodies to *S. mutans* cell surface determinants.

These results could also be explained by antibody-mediated inhibition of the cell-associated glucose-phosphotransferase system (PTS) which would prevent transport of glucose across the cell membrane of *S. mutans*. To test this hypothesis, we assayed the effect of antibody on glucose-PTS activity by the method of Kornberg and Reeves (5). Immune sera and saliva were incubated with toluene-treated whole *S. mutans* 6715 cells for 30 min at 30°C before the addition of the glucose substrate. Immune sera and saliva raised to the *S. mutans* ribosomal preparation and whole cells inhibited glucose-PTS activity by >60% (Table 4). Antiserum to whole cells inhibited the enzyme activity to a greater degree than did antiserum to the ribosomal preparation. Adsorption of antiserum with whole *S. mutans* cells abrogated glucose-

TABLE 4. Inhibition of the glucose-PTS of *S. mutans* with serum IgG and salivary IgA antibodies from rats and rabbits immunized with *S. mutans* ribosomes or whole cells

Source of antibodies	Immunogen	Serum or saliva dilution	Glucose-PTS activity[a]	% Inhibition of PTS activity[b]
None	None	None	41.5	0
Rat serum				
Normal	None	1:7.5	38.2	7.9
Immune	Ribosomes	1:7.5	12.8	69.1
Immune	Ribosomes	1:15	18.8	54.8
Immune	Ribosomes	1:30	23.6	43.2
Immune	Ribosomes	1:300	32.3	22.1
Rat saliva				
Normal	None	1:7.5	38.0	8.4
Immune	Ribosomes	1:7.5	14.4	65.3
Immune	Ribosomes	1:15	20.8	49.9
Immune	Ribosomes	1:30	25.4	38.8
Immune	Ribosomes	1:300	33.6	19.1
Rabbit serum				
Normal	None	1:7.5	36.1	12.9
Immune	Ribosomes	1:7.5	16.1	61.1
Immune	Ribosomes	1:15	26.1	37.2
Immune	Ribosomes	1:30	28.0	32.5
Immune	Ribosomes	1:300	27.1	34.6
Immune	Whole cells	1:7.5	8.9	78.6
Immune	Whole cells	1:15	23.1	44.4
Immune	Whole cells	1:30	25.4	38.7
Immune	Whole cells	1:300	33.3	19.7

[a] Activities were determined by using toluenized *S. mutans* cells (see text for details) and are expressed as micromoles of NADH oxidized per milligram (dry weight) of cells per minute.

[b] Percent inhibition of glucose-PTS activity as compared with that of control without antibody.

PTS inhibition. These data indicate that antibody inhibition of the glucose-PTS could contribute to the reduction in acid production and growth of *S. mutans*.

These results also indicate that the ability of antibodies induced by an *S. mutans* ribosomal vaccine to inhibit acid production does not correlate with their capacity to prevent growth of the organism and suggest that distinct mechanisms exist for antibody-mediated inhibition of *S. mutans* acid production and growth. Inhibition of the PTS may be responsible for reduction of acid production or growth or both. More studies are needed to elucidate the exact mechanisms involved in these inhibitions. Furthermore, because serum IgG and salivary IgA antibodies exhibited similar inhibition of *S. mutans* acid production and growth, we are currently investigating the effects of purified human salivary IgA and mouse monoclonal IgA, IgG, and IgM anti-*S. mutans* antibodies on these activities.

S. mutans ribosomal preparations may prove to be a beneficial agent for the reduction of the number of carious lesions for the following reasons: (i) they induce strong salivary, serum, and cell-mediated immune responses; (ii) antibodies to the preparations cross-react with whole cells of the seven serotypes of *S. mutans* but not with human heart or kidney tissues; (iii) these antibodies prevent the adherence to a glass surface, acid production, and growth of this organism in the presence of the caries-promoting agent, sucrose; and (iv) immunized rats are highly protected from *S. mutans*-induced dental caries.

We are indebted to Betty Wells for editorial assistance.

This work was supported, in part, by Public Health Service grants DE 04217, CA 13148, DE 02670, DE 07026, and Contract DE 02426 from the National Institutes of Health. R.L.G. is supported by Postdoctoral Fellowship Award DE 05358 from the National Institute of Dental Research. S.M.M. is a recipient of Research Career Development Award DE 00092 from the National Institute of Dental Research.

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