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Humoral and Cell-Mediated Responses to a Ribosomal Preparation from *Streptococcus mutans*

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Streptococcus mutans 6715 ribosomes disrupted in a Braun homogenizer were isolated in sodium dodecyl sulfate by differential centrifugation. This preparation contained 80% RNA and 20% protein, and carbohydrate was not detected by phenol-sulfuric acid and methyl pentose assays. The sedimentation coefficient of the ribosomes was 70S. After dialysis in 0.01 M phosphate buffer containing 10^{-4} M $MgCl_2$, the ribosomes dissociated into 54S and 32S particles. Leukocytes from rabbits immunized intramuscularly with the ribosomal preparation showed transformation and migration indices of 13.0 and 0.71, which were significantly different ($P < 0.05$) from the respective indices of 0.9 and 0.98 in nonimmunized animals. Hyperimmune serum from these rabbits agglutinated representative Formalin-killed strains of all seven serotypes of *S. mutans*, inhibited adherence of live *S. mutans* 6715 to glass, and agglutinated *S. mutans* 6715 ribosomes adsorbed upon erythrocytes. These findings suggested that animals immunized with *S. mutans* ribosomes may be protected from caries caused by any of the seven serotypes of this organism.

Streptococcus mutans has been implicated as a major causative agent of dental caries in humans (25, 26; for review, see reference 30). The species has been divided into seven serologically distinct serotypes (*a* through *g*) (4, 35), most of which are capable of causing carious lesions in monoassociated animals (32). A number of different preparations of *S. mutans* have been studied as possible immunogens against the disease.

Lehner and colleagues demonstrated various cell-mediated responses such as delayed hypersensitivity, lymphocyte transformation, and macrophage inhibition associated with reduction of caries in animals vaccinated with a protein antigen from a serotype *c* organism (21–24). Furthermore, T-helper and suppressor cells active against *S. mutans* have been obtained from protected monkeys and mice injected subcutaneously with this immunogen (21–23). In addition, phagocytes from these animals showed increased ingestion of opsonized *S. mutans* (37, 38).

Previous studies by McGhee et al. (31) and Smith and colleagues (39) have shown that injection of whole, Formalin-killed *S. mutans* (31) or glucosyltransferase preparations (39) in the salivary gland region of rats (31, 39) or hamsters (39) resulted in the induction of specific salivary immunoglobulin A (IgA) and serum IgG antibodies and correlated with fewer carious lesions than were observed in unimmunized animals challenged with this organism.

Although injection of *S. mutans* whole cells or glucosyltransferase preparations may protect animals in certain instances, other studies indicated that their use in humans may not be desirable. Recent reports showed that antibodies from animals injected intravenously (i.v.) with *S. mutans* cross-reacted in vitro with human heart and muscle tissues (18, 44). Guggenheim et al. (15), Colman and Cohen (7), and Fukui et al. (13) have shown that glucosyltransferase preparations did not protect and may have actually increased the susceptibility of immunized animals to dental caries.

Ribosomal vaccines have been used successfully to protect rodents against many bacterial and fungal pathogens, including *Haemophilus influenzae* (27), *Histoplasma capsulatum* (12), *Mycobacterium tuberculosis* (47–50), *Pseudomonas aeruginosa* (14), *Salmonella typhimurium* (11, 19, 20), *Streptococcus pneumoniae* (41, 43), and *Streptococcus pyogenes* (36). In addition, the *P. aeruginosa*, *S. typhimurium*, *S. pneumoniae*, and *S. pyogenes* vaccines protected mice against challenge by strains or serotypes different from those used to obtain the ribosomal immunogens.

In an effort to exclude cell wall- and membrane-associated antigens that may cross-react

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with human tissues from a vaccine against dental caries, we prepared a ribosomal immunogen from *S. mutans* 6715 (serotype *g*) and injected it intramuscularly (i.m.) in rabbits. We report here results of experiments which show that the animals developed humoral and cell-mediated responses against *S. mutans* 6715 ribosomes and whole cells of all serotypes.

(Certain results of these studies were presented at the 81st and 82nd Annual Meetings of the American Society for Microbiology, 1 through 6 March 1981 and 7 through 12 March 1982 in Dallas, Tex., and Atlanta, Ga., respectively.)

MATERIALS AND METHODS

Animals. New Zealand white female rabbits (1.8 to 3.6 kg) were obtained from Lomax Small Animal Farms, Opdyke, Ill. The rabbits were housed individually and given Purina laboratory chow and water ad libitum.

Organisms. *S. mutans* strain 6715, serotype *g* (kindly provided by R. J. Gibbons, Forsyth Dental Center, Boston, Mass.) was used in these studies. (It was originally isolated from a human carious lesion and was subsequently shown to be cariogenic for rats and hamsters.) Other strains of *S. mutans* (Table 1) used in this study were obtained from various sources. *Escherichia coli* was obtained from our stock culture collection. *S. mutans* 6715 was grown in 3-liter batches of 2% tryptic peptone broth (Difco Laboratories, Detroit, Mich.) containing 1% glucose, 0.8% NaCl, 0.5% yeast extract (Difco), 0.1% K_2CO_3, 0.05% KCl, and 0.05% Na_2HPO_4 in 6-liter Erlenmeyer flasks. *E. coli* was grown in 3 liter batches of 3% tryptic soy broth (Difco) containing 1% glucose in 6-liter Erlenmeyer flasks. Flasks containing either *S. mutans* 6715 or *E. coli* were inoculated with 150 ml of an 18 to 24-h culture of the appropriate organism and incubated for 5 h. All incubations described in this study, unless stated otherwise, were at 37°C in 5% CO_2 and 95% air. After incubation, cells were harvested by centrifugation (10,000 × *g* for 10 min), washed three times with 0.01 M phosphate buffer (pH 7.0) containing 0.01 M $MgCl_2$ (PMB), and frozen at −75°C.

Preparation of ribosomes. Ribosomes were prepared by a modification of the procedures of Youmans and Youmans (49). Briefly, the pelleted frozen *S. mutans* 6715 and *E. coli* cells were thawed rapidly at 37°C, and 1 g was suspended with 1 g of microglass beads in 1 ml of a solution containing 10^{-4} M phosphate buffer (pH 7.0), 0.44 M sucrose, 0.25% sodium dodecyl sulfate, 3 × 10^{-2} M $MgCl_2$, and 3 μg of DNase (Sigma Chemical Co., St. Louis, Mo.) per ml. The cells were then broken by shaking for three 2-min cycles in a Braun homogenizer (B. Braun Co., Melsungen, Germany), and the unbroken cells and cellular debris were removed by two low-speed centrifugations (27,000 × *g* and 47,000 × *g* for 10 min). The ribosomes in the supernatant were harvested by centrifugation at 250,000 × *g* for 2.5 h, and the ribosomal pellet was resuspended in PMB at a wet weight concentration of 100 mg/ml. An equal volume of PMB containing 0.5% sodium dodecyl sulfate was added, gently rotated by hand for 20 min at room temperature, and placed in an ice bath overnight. Precipitated sodium dodecyl sulfate was removed by two 15-min low-speed centrifugations at 37,000 × *g*, and the ribosomes in the supernatant were harvested as described above. The ribosomal preparations were then subjected to five successive washes in PMB at 250,000 × *g* for 2.5 h each, two 20-min low-speed centrifugations at 47,000 × *g*, and filtration through sterile 0.45-μm membrane filters (Millipore Filter Corp., Bedford, Mass.). These preparations were standardized on the basis of protein content. Inoculation of the preparations on appropriate media did not reveal the presence of viable organisms.

Chemical and physical analyses of ribosomal preparations. The amount of RNA and DNA in the ribosomes was measured by the orcinol (46) and diphenylamine (10) methods, respectively, using D-ribose and 2-deoxy-D-ribose as standards. The amount of protein, carbohydrate, and methyl pentose was determined by the Campbell et al. (5), phenol-sulfuric acid (1), and Ashwell (2) procedures, respectively, with bovine serum albumin, glucose, and rhamnose as standards.

For sucrose gradient analysis, 0.5 ml of the ribosomal preparation containing 0.8 mg of RNA was layered over 5 ml of a 5, 10, 15, and 20% discontinuous, stepwise sucrose gradient, prepared in 0.02 M Tris-hydrochloride buffer (pH 7.8) containing either 10^{-2} or 10^{-4} M $MgCl_2$. The gradient was centrifuged for 70 min at 243,000 × *g*, and fractions were collected by upward displacement with mineral oil. The absorbance of each fraction at 260 nm was measured in a Gilford spectrophotometer. The sedimentation coefficients of *S. mutans* 6715 ribosomes and their subunits were determined by the method of Martin and Ames (29) with ribosomes and subunits from *E. coli* as 70S, 50S, and 30S markers.

An optical density scan between 215 and 300 nm as well as 260 nm/280 nm and 260 nm/235 nm ratios were done on the *S. mutans* ribosomal preparation to detect cell wall or membrane contamination (3, 43).

Immunization of rabbits with *S. mutans* 6715 ribosomes. Ribosomes were suspended in PMB (0.4 mg of ribosomal protein per ml) and emulsified in an equal volume of Freund complete adjuvant. To obtain hyperimmune serum, three rabbits were injected with 0.25 ml i.m. in each of four different sites in the hind legs for a total of 0.2 mg of protein. Injections were repeated twice at weekly intervals with the same concentration of ribosomes in incomplete Freund adjuvant. Subsequent injections consisted of 1 ml of the ribosomes (0.4 mg of protein per ml) without adjuvant, given i.v. on days 53, 55, 57, 63, 65, 67, 75, 203, and 205. Control rabbits were injected with PMB with the same immunization schedule. Leukocytes, sera, and salivas from all animals were collected on days 0, 6, 12, 42, 51, 77, 81, and 210. Whole blood, collected by cardiac puncture, was allowed to clot at 25°C for 1 h and then at 4°C overnight, and sera were separated by centrifugation. The leukocytes were separated from erythrocytes (see below for preparation) and used immediately. Salivation was induced with 2 mg of pilocarpine injected i.v., and saliva was collected as it dripped from the lower lip. All sera and salivas were heated at 56°C for 30 min, the salivas were clarified for 10 min at 10,000 × *g*, and both sera and salivas were stored at −20°C until needed.

Evaluation of immune response. Leukocytes, sera, and salivas were used to establish the extent of the

immune responses of the rabbits. The antibody responses in serum and saliva were determined by inhibition of adherence (IA) of live *S. mutans* 6715 to glass surfaces (33), microbial agglutination (MA) (42) of various Formalin-killed *S. mutans* strains, and passive hemagglutination (PHA) (6) of 6715 ribosomes.

The extent of the cell-mediated response was measured by lymphocyte transformation (LT) (34), determined by tritiated thymidine uptake and agarose droplet migration inhibition (MI) (16).

Assay of the inhibition of adherence of live *S. mutans* 6715 to glass. Serum and saliva samples were serially diluted in saline, and 500 μl was placed in sterile glass test tubes. Equal volumes of 12% tryptic soy broth and 4% sucrose were mixed, and 500 μl was added to each tube. Controls included: (i) a 100% adherent tube containing 250 μl of tryptic soy broth, 500 μl of normal saline, and 250 μl of 4% sucrose; and (ii) a nonadherent tube containing 250 μl of tryptic soy broth and 750 μl of saline. Fifty microliters of a 24-h tryptic soy broth culture of *S. mutans* 6715 was added to all tubes, which were mixed and incubated at a 30° angle for 36 h. Bacteria which did not adhere to the glass walls of the tubes were removed by pouring off the medium and gently washing the tubes twice with 500-μl volumes of saline. The washes containing nonadhered bacteria were pooled and washed once in saline. Three milliliters of 0.5 N NaOH was added both to tubes containing adherent and nonadherent bacteria. The cells were mixed, and the turbidities were measured at 540 nm. The titer was defined as the reciprocal of the serum or saliva dilution at which 50% of the bacteria were adherent.

Microbial agglutination assay of various Formalin-killed *S. mutans* strains. *S. mutans* cultures (5 h) were washed twice in sterile 0.01 M phosphate-buffered saline (PBS; pH 7.2) and suspended in PBS containing 0.5% Formalin, and the cells were killed by incubating the suspension for 48 h at room temperature. The cells were then washed twice and suspended to an optical density of 0.75 at 650 nm in PBS. Serial dilutions of sera and salivas from immunized and control rabbits were made in PBS containing 0.01 M EDTA and 1 mg of bovine serum albumin per ml. Fifty microliters of each dilution was placed in U-shaped wells of a microtiter plate (Linbro Chemical Co., New Haven, Conn.). Fifty microliters of the killed *S.mutans* cells was added to each well, and the plate was incubated for 2 h at 37°C and then for 18 to 24 h at 4°C. After incubation, the wells were examined for agglutination.

PHA assay. A fresh 2% (vol/vol) rabbit erythrocyte suspension washed three times with PBS was mixed with an equal volume of PBS containing 0.05 mg of tannic acid per ml and incubated for 30 min with occasional mixing. The cells were then washed once and suspended to 2% in PBS. One milliliter of the cells, 4 ml of PBS, and 1 ml of the *S. mutans* 6715 ribosomal preparation, diluted to 0.2 mg of protein per ml, were mixed and incubated for 10 min at room temperature with occasional mixing. The cells were then washed and resuspended to 2% in PBS containing 1% normal rabbit serum. Sera and salivas were serially diluted with PBS containing 1% normal rabbit serum, and 25-μl samples were placed in U-shaped wells of a microtiter plate (Linbro). Twenty-five microliters of the coated erythrocytes was added to each well, the plate was incubated for 1 to 2 h at room temperature, and then the wells were examined for agglutination. All sera and salivas were adsorbed three times with untreated erythrocytes before use.

Lymphocyte transformation and migration inhibition. A mixture of equal volumes of whole rabbit blood and Alseiver solution was added to an equivalent amount of 3% dextran (molecular weight 175,000) in saline and placed for 1 h at a 30° angle in an ice bath. The leukocyte-rich supernatant was removed, centrifuged for 15 min at 200 × *g*, washed twice in Hanks saline, and suspended to a concentration of 10^6 cells per ml in RPMI 1640 medium (Microbiological Associates, Bethesda, Md.) supplemented with 100 U of penicillin and 100 μg of streptomycin per ml and 100 mM of L-glutamine (RM). Every preparation contained >95% viable cells as determined by trypan blue dye exclusion. Stimulated cultures were obtained by placing 200 μl of previously determined, optimal concentrations of either concanavalin A (Sigma; 0.25 g/ml) or *S. mutans* 6715 ribosomal protein (100 μg/ml) in flat-bottom wells of a microtiter plate (Linbro) with RM. Unstimulated cultures were prepared in a similar manner, but with RM alone. After 3 days of incubation, 20 μl containing 1 μCi of [^{3}H]thymidine (1 mCi/ml; 18.5 Ci/mmol; New England Nuclear Corp., Boston, Mass.) was added to each well, and the plate was incubated for 18 h. The cells were harvested on glass fiber filters with a multiple automated sample harvester (Microbiological Associates, Bethesda, Md.), and the incorporated [^{3}H]thymidine was measured in a scintillation counter. The transformation index was calculated by determining the ratio between the stimulated and unstimulated culture counts.

The migration inhibition assay was done as described below. Droplets containing 1 μl of a mixture of washed leukocytes in RM, 15% fetal calf serum (KC Biological, Inc., Lenexa, Kans.), and 0.8% agarose (Sea Plaque Agarose; Microbiological Associates) were placed in the centers of flat-bottom wells of a microtiter plate (Linbro) and kept for 15 min at 4°C to solidify the agarose droplets. Stimulated and unstimulated cultures were prepared by adding 200 μl RM containing an optimal concentration of ribosomal protein (100 μg/ml) and RM alone, respectively, to the wells. The plate was incubated for 48 h when the diameter of the leukocyte migration was measured. The migration index was calculated by determining the ratio between the diameters of the leukocyte migration in the stimulated and unstimulated cultures.

RESULTS

Characterization of ribosomes. The ribosomal preparation from *S. mutans* 6715 contained 80% RNA and 20% protein, but tests for DNA, carbohydrate, and methyl pentose were negative. The absence of carbohydrate and methyl pentose (8), as well as spectral analyses showing 260 nm/280 nm and 260 nm/235 nm ratios of 1.78 and 1.38, respectively, suggested the absence of cell wall or membrane contamination. However, other more sensitive assays, such as two-dimensional gel electrophoresis, immunoprecipitation, and immunoadsorption revealed a small, but definite, contamination with at least six cell wall

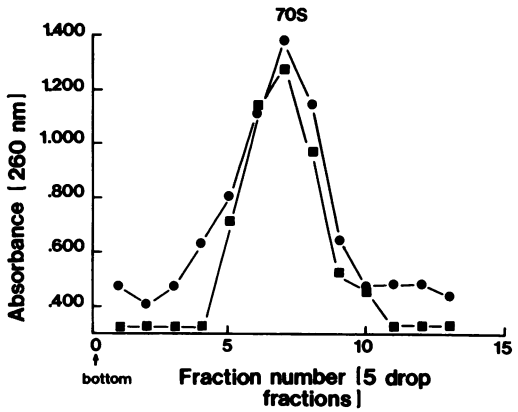

FIG. 1. Absorbance pattern of 5 to 20% sucrose density gradient fractions of *S. mutans* 6715 ribosomes (●) and *E. coli* ribosomes (■) after centrifugation for 70 min at 243,000 × *g*.

proteins, possibly derived from the peptidoglycan, one membrane-associated enzyme, lactate dehydrogenase, and lipoteichoic acid (manuscript submitted). In addition, the optical density scan showed a minimum absorbance at 235 nm and a maximum of 260 nm, which correlated with values for RNA.

Density gradient centrifugation. The results of sucrose density gradient analyses of the ribosomal preparations are shown in Fig. 1 and 2. *S. mutans* 6715 and *E. coli* ribosomes sedimented as a single peak (Fig. 1). The sedimentation coefficient of *S. mutans* 6715 ribosomes was 70S with *E. coli* ribosomes as a 70S marker. *S. mutans* 6715 ribosomes dissociated into 54S and 32S subunits (Fig. 2) after dialysis against 0.01 M phosphate buffer (pH 7.4) containing 10^{-4} M $MgCl_2$ with *E. coli* 50S and 30S subunits as markers.

Immunogenicity of ribosomes. The results of the humoral response to injection of ribosomes are shown in Fig. 3. The peak titer for all assays was on day 81. The highest PHA titer was 10,240, whereas the highest MA and IA titers against *S. mutans* 6715 were 512 and 256, respectively. Antiserum from day 210 agglutinated representative strains from all seven serotypes of *S. mutans* (Table 1). The highest MA titers (2,048) were obtained against serotypes *a* (strains AHT and E-49) and *f* (OMZ-175), as well as a strain of serotype *c* isolated from a wild rat (8S1). The lowest titers (64 to 128) were observed against serotypes *b* (BHT and FA-1) and *c* (Ingbritt, 10449, and KPSK2). Normal rabbit serum had titers less than or equal to 1 against any of the strains. All of the salivas and the sera from rabbits injected with PMB had titers below 2 in all of the assays.

The results of the LT and MI assays are shown in Fig. 4 and 5, respectively. The peak indices for the LT and MI assays were 13.0 on day 12 and 0.71 on day 6, respectively. Lymphocytes from animals inoculated with PMB did not react against the ribosomes.

DISCUSSION

Immunization of rabbits with *S. mutans* 6715 ribosomes elicited both humoral and cell-mediated responses. Serum antibodies against ribosomes were apparent 7 days after the first injection of the ribosomal preparation, as shown by the PHA results. The PHA titer increased to the maximal level at 81 days, where it persisted for at least 129 days. Serum antibodies against whole *S. mutans* 6715 cells or components of whole *S. mutans* 6715 cells, as measured by the MA and IA assays, were apparent 12 days after the first injection of the ribosomal preparation. These titers dropped during the next 30 days and then peaked at 81 days, thereafter dropping to low levels at 210 days, even after two additional injections at days 203 and 205. The reason for this decline is not known. However, since the latter titers were lower and took longer to develop than the PHA titer against ribosomes, it must be remembered that the antigen used in both MA and IA assays was whole *S. mutans* 6715 cells. The results obtained here may be explained by contamination of the ribosomal immunogen with low levels of cell wall and membrane components. In addition, since the MA and perhaps the IA measured mostly IgM antibodies, it is conceivable that the vaccine stimulated predominantly IgG antibodies at day 210 which were not measurable using these assays. Because the animals were immunized i.m. in the hind legs

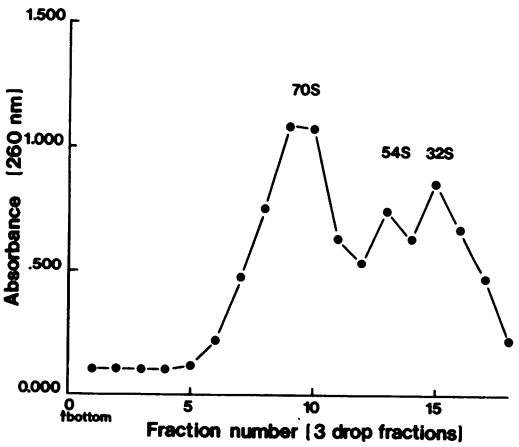

FIG. 2. Absorbance pattern of 5 to 20% sucrose density gradient fractions of *S. mutans* 6715 ribosomes dialyzed against 10^{-4} M $MgCl_2$ buffer after centrifugation for 70 min at 243,000 × *g*.

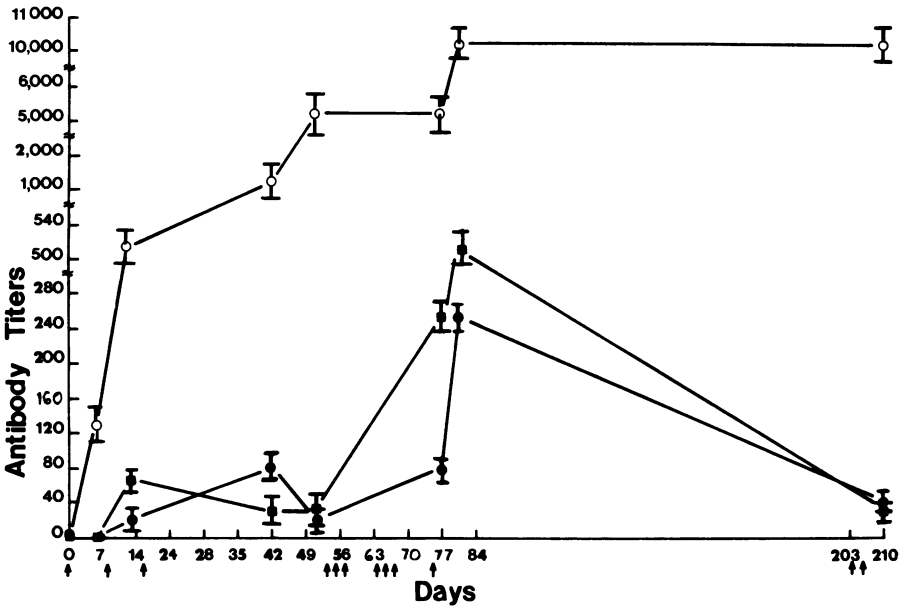

FIG. 3. Humoral immune response of rabbits injected with *S. mutans* 6715 ribosomes. Arrows indicate day of immunization. The first three injections were i.m. with Freund adjuvant; the others were i.v. without adjuvant. Symbols: ○, passive hemagglutination of *S. mutans* 6715 ribosome-coated erythrocytes; ■, microbial agglutination of *S. mutans* 6715 cells; ●, inhibition of adherence of *S. mutans* 6715 cells to glass. Each point represents results obtained from three rabbits. Vertical bars represent ±1 standard deviation.

and not in the salivary gland region, salivary antibodies were not detected.

Cell-mediated responses, as exhibited by the LT and MI indices, were apparent 12 and 7 days, respectively, after the first injection. Both indices declined after the third injection on day 14 to a minimal level at 26 days. The LT index then increased, after the seven i.v. injections, to slightly less than its maximal level at 77 days.

The results of the MA assay indicated that the *S. mutans* 6715 ribosomal preparation contained determinants in common with cell surface antigens of other *S. mutans* strains. Data not presented here suggested that the cross-reactive determinants were components of the cell wall or membrane (or both) of *S. mutans* 6715. Experiments are in progress to establish the nature of the cross-agglutinating determinant. Preliminary studies indicate that antibodies against *S. mutans* 6715 ribosomes do not cross-react with human heart tissue antigens, suggesting that such cross-reactive antigens are not present in the ribosomal preparation. This implies that the use of a ribosomal vaccine from *S. mutans* in humans would stimulate antibodies that would not cause undesirable cardiac pathology, but would cross-agglutinate other serotypes of this organism and hopefully protect those individuals from dental caries caused by any of the seven serotypes of *S. mutans*.

The results presented here parallel those characteristics reported for ribosomal preparations from other procaryotic organisms in that the biochemical content, sedimentation coefficients, biophysical appearance, and ability of an antiserum raised against one strain or serotype of an organism to cross-react with other serotypes are

TABLE 1. Agglutination of various strains of *S. mutans* with anti-*S. mutans* 6715 ribosome serum

Serotype	Strain	MA titer
a	AHT	2,048
a	E-49	2,048
b	BHT	128
b	FA-1	64
c	V318	1,024
c	V310	512
c	GS-5	256
c	Ingbritt	128
c	10449	128
c	KPSK2	64
c	8S1	2,048
d	SL1	512
d	B13	256
d	O1	64
e	LM7	256
f	OMZ-175	2,048
g	KIR	512
g	6715	512

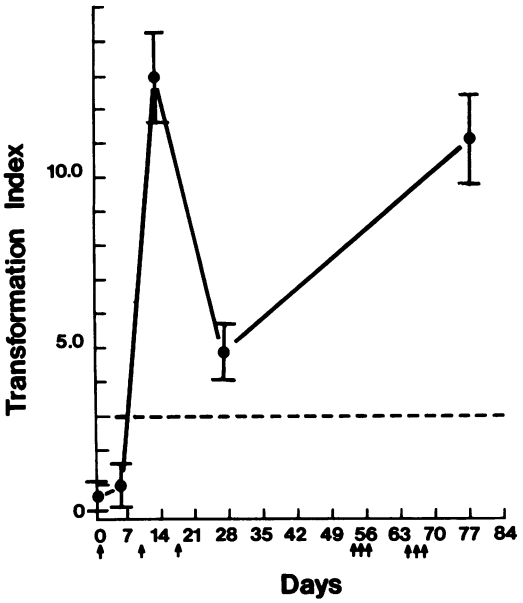

FIG. 4. LT response of animals injected with *S. mutans* 6715 ribosomes. LT responses in animals injected with PMB were <1.0. Arrows indicate day of immunization. The first three injections were i.m. with Freund adjuvant; the others were i.v. without adjuvant. Transformation was considered significant when the transformation index was above the dotted line (>3.0). (An index of 3.0 was 2 standard deviations above a normal cell index.) Each point represents results obtained from three rabbits. Vertical bars represent ±1 standard deviation.

similar. Cell wall or membrane material was not detected with the assays done in this study, although more sensitive procedures have revealed the presence of a number of such materials in the ribosomal preparation (manuscript submitted). Most of the ribosomal preparations from other organisms have been found to contain such cellular contaminants. Although we attempted to remove these materials from our preparation with such techniques as washing with SDS, differential centrifugation, and filtration, we were unsuccessful in this endeavor. It has been reported that much of the immunological or protective ability (or both) of ribosomal preparations could be ascribed to cell wall or membrane contaminants, and it is conceivable that our preparation is similar in this regard.

Ribosomal vaccines from other procaryotic organisms have been reported to stimulate antibodies and cell-mediated responses against the ribosomes (for review, see reference 9). Our results show that good immune responses could be obtained using *S. mutans* ribosomes when administered with Freund adjuvant. We have also found that conventional and gnotobiotic rats injected with ribosomes in the salivary gland region with or without Freund adjuvant developed strong immunological responses against the particles (R. L. Gregory and I. L. Shechmeister, Ann. N.Y. Acad. Sci., in press).

Although the precise chemical definition of the immunogenic principle in our preparation was not definitively established, some indirect evidence exists. Treatment of the ribosomes with protease before injection reduced the PHA titer with untreated ribosomes as the assay antigen by 10-fold, whereas treatment with RNase did not reduce the titer. This suggests that ribosomal protein acted as the immunogenic moiety in *S. mutans* ribosomes. Support for a number of possibilities for the immunogenic principle exists in the literature. Youmans and Youmans (50) and Venneman (45) provided evidence that RNA was the effective immunogen, whereas Johnson and Swendsen (19, 20, 41) demonstrated that ribosomal protein acts as the principle immunogen. Bigley and co-workers (28, 40) considered an RNA-protein complex to be the important moiety. However, Eisenstein (11) and Hoops et al. (17) indicated that an exogenous contaminant, perhaps lipopolysaccharide, is the immunogenic principle. However, the absolute identification of the immunogenic moiety, although it is of basic interest, is not essential for the practical application of ribosomal vaccines. The important properties to

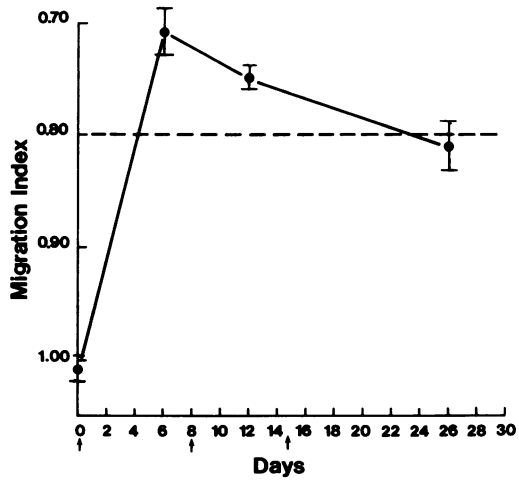

FIG. 5. MI response of animals injected with *S. mutans* 6715 ribosomes. MI responses in animals injected with PMB were >0.95. Arrows indicate day of i.m. immunization with Freund adjuvant. Inhibition was considered significant when the migration index was above the dotted line (<0.80). (An index of 0.80 was 2 standard deviations above a normal cell index.) Each point represents results obtained from three rabbits. Vertical bars represent ±1 standard deviation.

consider for a *S. mutans* ribosomal vaccine would be the following: (i) ability to induce protection by immunization, (ii) ability to provide cross-serotype protection, and (iii) lack of toxicity. We report here, for the first time, that immunization with an *S. mutans* ribosomal vaccine induces humoral and cellular responses that react with all seven serotypes of the organism. Furthermore, we have also determined that antibodies produced against this immunogen do not cross-react with human tissues, indicating a lack of toxicity. In addition, although we have not reported here the ability of this preparation to protect against dental caries, preliminary data indicate that a high level of protection against this disease is obtained in immunized gnotobiotic rats (data to be reported in subsequent publications). Thus, overall, the *S. mutans* ribosomal vaccine appears to be a very promising approach in developing an effective and safe anti-caries vaccine.

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