INFECTION AND IMMUNITY, Oct. 1984, p. 42–47
0019-9567/84/100042-06$02.00/0

Vol. 46, No. 1

Lack of Cross-Reactivity of Antibodies to Ribosomal Preparations from *Streptococcus mutans* with Human Heart and Kidney Antigens

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Received 16 February 1984/Accepted 27 June 1984

Previous studies have suggested that sera from animals immunized with whole *Streptococcus mutans* cells may cross-react with human and monkey heart sarcolemmal tissues. In the present study, sera and saliva from rats and rabbits immunized peripherally with ribosomal preparations from *S. mutans* 6715 (serotype *g*) or GS-5 (serotype *c*) were examined for their ability to react with normal human heart sarcolemmal and kidney glomerular tissues by using enzyme-linked immunosorbent and immunofluorescence assays. The results showed that antibodies to serotype *g* and *c* ribosomal preparations do not react with either the human heart or renal antigens. Sera from mice immunized with human heart tissue and from a patient with a high anti-streptolysin O titer reacted strongly with human heart sarcolemmal and kidney glomerular tissues. These data indicated that ribosomal preparations from *S. mutans* lack the putative human heart cross-reactive determinant and suggest that the use of an *S. mutans* ribosomal vaccine against dental caries may not be pathogenic to human heart or renal tissues.

Streptococcus mutans has been implicated as the major etiological agent of dental caries (15, 16; for a review, see reference 17). Results from a number of studies suggested that rabbits immunized with *S. mutans* whole cells develop antibodies that cross-react with the sarcolemmal sheath of human and monkey heart tissue. Van de Rijn et al. (25, 26) in 1976, using an indirect immunofluorescence assay, were the first to show that sera from *S. mutans* whole cell-immunized rabbits reacted with human heart tissue. Feretti et al. (4), Stinson et al. (22, 23), and Hughes and co-workers (11, 12) confirmed these results by using a number of immunological techniques, including rocket immunoelectrophoresis, immunofluorescence, and radioimmunoassay. Stinson et al. (21) have also shown that extracted antigens from a number of strains and serotypes of *S. mutans* and *Streptococcus pyogenes* adsorb onto the sarcolemmal sheath of monkey cardiac muscle cells in vitro by means of specific receptor sites. In contrast, Bergmeier and Lehner (1) have recently shown that multiple immunizations of rhesus monkeys with *S. mutans* whole cells, cell walls, or surface protein antigens fail to induce antibodies that cross-react with human heart tissue antigens. A review of studies concerned with the ability of anti-streptococcal antibodies to cross-react with human tissues has been published (3). The conflicting reports on the ability of antisera against *S. mutans* to bind to human heart tissue and of antigens from *S. mutans* to bind directly to primate heart tissue obviously stress the need to eliminate the putative antigen(s) responsible for this cross-reactivity from any vaccine developed against dental caries.

We have recently isolated and characterized ribosomal preparations from representative strains of two serotypes of *S. mutans*. A ribosomal preparation from *S. mutans* 6715 (serotype *g*) has been shown to induce both humoral and cell-mediated immune responses in rats and rabbits (7, 9). Furthermore, injection of this ribosomal preparation into the salivary gland region of gnotobiotic rats results in the induction of salivary immunoglobulin A (IgA) antibody responses and protection from caries formation after challenge with virulent *S. mutans* (5, 18). The ribosomal preparation is contaminated with at least six cell wall or membrane proteins, including glucosyltransferase, and lipoteichoic acid (8). Antibodies induced to this preparation react with whole cells of the seven serotypes (*a* through *g*) of *S. mutans* (7, 9) and prevent adherence to glass surfaces, acid production, and growth of this organism in the presence of sucrose (6, 7). An *S. mutans* GS-5 (serotype *c*) ribosomal preparation has been found to be strongly immunogenic in rabbits and contains contaminating glucosyltransferase (unpublished observation). The present study shows that peripheral immunization of either rats or rabbits with ribosomal preparations from *S. mutans* serotype *g* and *c* strains leads to the production of specific anti-ribosome antibodies in sera and saliva that do not cross-react with normal human heart sarcolemmal or kidney glomerular antigens.

(Certain results of these studies were presented previously [R. L. Gregory, I. L. Schechmeister, J. O. Brubaker, C. T. Smedberg, S. M. Michalek, and J. R. McGhee, Annu. Meet. Am. Soc. Microbiol. 1984, E69, p. 159].)

MATERIALS AND METHODS

Bacteria. *S. mutans* 6715 (serotype *g*) and GS-5 (serotype *c*) (kindly provided by Ronald J. Gibbons, Forsyth Dental Center, Boston, Mass.) were used in these studies. They were originally isolated from human carious lesions. *S. mutans* 6715 and GS-5 were grown in 3-liter batches of 2% tryptic peptone broth and 2% Todd Hewitt broth, respectively (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 (7). The flasks were inoculated with 150 ml of an 18- to 24-h culture of *S. mutans* and incubated without shaking at 37°C

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for 5 h in 5% CO_2 and 95% air. The cells were harvested by centrifugation (10,000 × *g*; 10 min), washed three times with 0.01 M phosphate buffer (pH 7.4) containing 0.01 M $MgCl_2$, and frozen at −80°C.

Preparation of ribosomes. Ribosomes were prepared by a modification (5) of the procedure of Youmans and Youmans (27). Briefly, the frozen *S. mutans* 6715 and GS-5 cells were thawed rapidly at 37°C, and 1 g was suspended with 1 g of microglass beads (0.17 to 0.18 mm) in 1 ml of 0.01 M phosphate buffer (pH 7.4)–0.01 M $MgCl_2$ containing 3 μg of DNase (Sigma Chemical Co., St. Louis, Mo.) per ml. The cells were then disrupted by shaking for three 2-min cycles in a Braun homogenizer (B. Braun Co., Melsungen, Germany), after which intact cells and cellular debris were removed by two low-speed centrifugations (27,000 × *g* and 47,000 × *g*, for 10 min each). The ribosomes in the supernatants were harvested by centrifugation at 250,000 × *g* for 2.5 h and then subjected to five successive washes in 0.01 M phosphate buffer (pH 7.4)–0.01 M $MgCl_2$ at 250,000 × *g* for 2.5 h each, followed by two 20-min centrifugations at 47,000 × *g* and finally were filtered through sterile 0.45-μm membrane filters (Millipore Corp., Bedford, Mass.). The ribosomal preparations were then adjusted to 1 mg/ml on the basis of protein content by the Lowry method (2) with bovine serum albumin as a standard. Inoculation of the preparations on mitis salivarius agar (Difco) did not reveal the presence of viable *S. mutans* cells.

Immunization of animals. Rats and rabbits were immunized with ribosomal preparations and sera and saliva were obtained as reported previously (5, 7). Briefly, three rabbits were immunized with the 6715 ribosomal preparation by 12 injections (the first 3 injections were intramusular with Freund complete adjuvant [Difco]; the others were injected intravenously without adjuvant) with a total of 4.2 mg of ribosomal protein. Three rabbits were immunized with the GS-5 preparation by five subcutaneous injections with a total of 3.2 mg of ribosomal protein in incomplete Freund adjuvant (Difco). Seven rats were immunized with the 6715 ribosomal preparation by five injections in the salivary gland region with a total of 0.7 mg of ribosomal protein (the first two injections were without adjuvant; the others were in complete Freund adjuvant). Blood was collected 8 days after the last immunization from rabbits and rats by cardiac puncture and allowed to clot for 4 h at room temperature and 4°C overnight. Serum was separated after centrifugation (5,000 × *g*, 10 min), and individual samples were stored at −20°C until used. Saliva was collected at the same time from rats after injection with pilocarpine (0.75 mg/100 g of body weight) while under sodium pentobarbital sedation. The rats were placed in the horizontal position, and approximately 1.0 ml of whole saliva was collected with the aid of a capillary pipette from each animal over a 20-min interval. The individual saliva samples were clarified by centrifugation (2,800 × *g*, 30 min) and stored at −20°C until used.

Preparation of heart and kidney tissue. Human heart sarcolemma and renal cortex (glomeruli) were obtained at autopsy (less than 4 h after death) from a previously healthy 26-year-old man who died of trauma. The donor had no clinical history or anatomical evidence of rheumatic heart or renal disease. A 1-g piece of each tissue was washed extensively in saline to remove extraneous erythrocytes, homogenized in a tissue homogenizer, resuspended in 0.1 M carbonate buffer (pH 9.6) to an optical density of 0.500 at 660 nm, and stored at −20°C until used in the enzyme-linked immunosorbent assay (ELISA) described below. Because of large amounts of innate alkaline phosphatase (13), the kidney tissue was placed in a boiling water bath for 10 min toinactivate endogenous enzyme before use in the ELISA.

The remainder of the heart and kidney tissues was cut into small pieces, (1 by 2 cm), flash frozen in liquid N_2, and stored at −80°C. Thin sections (4 μm) of the tissues were cut with a Tissue-Tek II microtome-cryostat (Lab-Tek Products, Naperville, Ill.), placed on precleaned glass microscope slides, and stored at −20°C until used in the immunofluorescence assay described below.

Adsorption of antibodies to *S. mutans* ribosomal preparations with heart and kidney tissues. Rabbit serum and rat serum and saliva from animals immunized with *S. mutans* ribosomal preparations were adsorbed with samples of human sarcolemmal and glomerular tissues in phosphate-buffered saline (pH 7.0) at room temperature. Briefly, heart and kidney tissues in phosphate-buffered saline were centrifuged (10,000 × *g*, 15 min), and the pellets were suspended in 2 ml of serum (or saliva). The suspensions were incubated at room temperature for 10 min and centrifuged, and the adsorptions were repeated four additional times. As a control, an equal volume of serum from rabbits immunized with the *S. mutans* 6715 or GS-5 ribosomal preparations was mixed with 6715 or GS-5 ribosomes (1 mg of ribosomal protein per ml) and incubated (10 min, room temperature). The levels of antibodies to the ribosomal preparations in the adsorbed samples were determined by the ELISA as described previously (5, 10).

Analysis of cross-reactivity between antibodies raised against *S. mutans* 6715 and GS-5 ribosomes and human heart and kidney antigens with ELISA and immunofluorescence. The extent of cross-reactivity was examined with ELISA as described previously (5, 10) and with an indirect immunofluorescence assay as described below. Positive controls included (i) a mouse antiserum prepared by intraperitoneally injecting mice twice at weekly intervals with solubilized human heart tissue in Freund complete adjuvant and (ii) serum from a patient who had an elevated anti-streptolysin O (ASO) titer (1:320).

(i) ELISA. Individual wells of Linbro flat-bottom EIA plates (Flow Laboratories, McLean, Va.) were coated with antigens by adding 100 μl of the heart or boiled kidney ELISA tissue suspensions described above or 100 ng of ribosomal RNA per ml and incubating for 3 h at 37°C and overnight at 4°C. Unbound antigens were removed by washing wells three times with saline containing 0.05% Tween 20 (Sigma; Tween-saline), 200 μl of 1% bovine serum albumin (Sigma) in Tween-saline was added to each well, and plates were incubated at room temperature for 1.5 h to block unreacted sites. After washing, 1:100 and 1:4 dilutions of sera and saliva, respectively, in Tween-saline were added to the wells (100 μl), and plates were incubated for 1 h at 37°C and then washed three times. Alkaline phosphatase (Sigma)-labeled rabbit IgG anti-rat γ, anti-rat α, anti-mouse γ, anti-human γ, or goat anti-rabbit γ heavy-chain reagent (10, 14, 19) was added (100 μl) to the appropriate wells. After incubation for 3 h at 37°C and overnight at 4°C, wells were washed three times with Tween-saline, and the reaction was developed for 1.5 h with 100 μl of 1 mg of *p*-nitrophenylphosphate (Sigma) per ml in 10% diethanolamine (Sigma) buffer (pH 9.6). The absorbance was read at 405 nm in a Titertek Multiskan photometer (Flow).

(ii) Immunofluorescence assay. Microscope slides with thin sections (4 μm) of sarcolemmal and glomerular tissues were covered with 50 μl of various dilutions (serum, 1:2 to 1:50; saliva, 1:2 to 1:10) of rat serum and saliva and rabbit serum and allowed to react for 30 min at 25°C in a moist chamber.

TABLE 1. Lack of cross-reactivity of antibodies to *S. mutans* ribosomal preparations with soluble human antigens by ELISA

Source of serum or salivary antibodies[a]	Immunogen	Absorbance (405 nm)[b]	
		Heart sarcolemma	Kidney glomeruli
None	None	0.017 ± 0.001	0.054 ± 0.004
Normal rabbit serum	None (4)[c]	0.036 ± 0.008	0.059 ± 0.007
Immune rabbit serum	6715 ribosomes (3)	0.042 ± 0.003	0.047 ± 0.005
	GS-5 ribosomes (3)	0.037 ± 0.009	0.034 ± 0.009
Normal rat serum	None (6)	0.009 ± 0.002	0.037 ± 0.017
Immune rat serum	6715 ribosomes (7)	0.008 ± 0.002	0.033 ± 0.011
Normal rat saliva	None (6)	0.010 ± 0.002	0.014 ± 0.003
Immune rat saliva	6715 ribosomes (7)	0.009 ± 0.001	0.015 ± 0.002
Immune mouse serum	Sarcolemma (4)	1.180 ± 0.065	0.867 ± 0.018
Human serum (1:320 anti-streptolysin O titer)	None (1)	2.103 ± 0.009	2.021 ± 0.097

[a] Normal and immune rabbit, rat, mouse, and human sera were diluted 1:100 in Tween-saline. Normal and immune rat saliva were diluted 1:4 in Tween-saline.
[b] As determined by the procedure described in the text with either heart or boiled kidney tissues as coating antigens. Values indicate means ± standard errors of the mean of triplicate determinations per sample.
[c] Numbers within parentheses indicate the numbers of animals.

The slides were washed three times with phosphate-buffered saline, and rabbit IgG anti-rat γ, anti-rat α, anti-mouse γ, or anti-human γ heavy-chain reagent (10, 14, 19) was added (50 μl) to the appropriate tissue sections. After incubation for 30 min at 25°C in a humid chamber, the slides were washed three times with phosphate-buffered saline, and rhodamine isothiocyanate (Hyland Laboratories, Costa Mesa, Calif.)-labeled goat anti-rabbit γ heavy chain reagent (10, 14, 19) was added (50 μl) and allowed to react for 30 min at 25°C. The slides were washed as before, mounted under a cover slip with Elvinol, and observed with a Leitz Orthoplan fluorescence microscope and vertical Ploem illumination (Wetzler, West Germany). A value of 0 to 4+ was assigned to each sample relative to the amount of fluorescence observed.

Statistics. Antibody activities were statistically reduced by computing means and standard errors of the mean. Values of serum and saliva samples were expressed as the mean ± standard error of the mean of the absorbance at 405 nm and corrected for background absorbance without any serum or saliva. The values represent the means of triplicate determinations per sample.

RESULTS

Characterization of ribosomal preparations and antisera. The composition of the *S. mutans* ribosomal preparations has been described earlier (5, 7, 8). Briefly, the 6715 ribosomal antigen used to immunize rabbits was prepared with sodium dodecyl sulfate (27) and contained 80% RNA and 20% protein. In addition, six cell wall- or membrane-associated proteins, D-lactate dehydrogenase (a membrane-associated enzyme), glucosyltransferase, and lipoteichoic acid were found in this preparation (8). The *S. mutans* 6715 ribosomal antigen used to immunize rats was prepared without sodium dodecyl sulfate (5) and contained 55.0% RNA and 45.0% protein, clearly indicating that incorporation of sodium dodecyl sulfate into the purification protocol results in significantly less protein. This preparation was not analyzed for cell wall or membrane components, but was found to induce protection in gnotobiotic rats from *S. mutans*-induced carious lesions after challenge with the homologous, virulent bacterium (5, 18). The *S. mutans* GS-5 ribosomal preparation used to immunize rabbits was made with sodium dodecyl sulfate and was found to contain 61.0% RNA, 36.1% protein, 2.9% carbohydrate, and a small, but undefined, amount of glucosyltransferase (unpublished observation). All three of the *S. mutans* ribosomal preparations induced good antibody responses to the ribosomes in rabbits and rats (5, 7; see below).

Analysis of cross-reactivity between antibodies to *S. mutans* ribosomal preparations and human heart and kidney tissues. Two different types of immunological assays were used to examine whether antibodies to *S. mutans* ribosomal preparations cross-reacted with human antigens. The first, ELISA, is a very sensitive technique and detects as little as 12.5 ng of antibody protein (10) in some systems. The second assay, indirect immunofluorescence, will allow detection of cross-reactive antibody and the subcellular region where binding occurs.

(i) ELISA. Pooled serum from rabbits immunized with the *S. mutans* ribosomal preparations did not react with either human heart sarcolemmal or kidney glomerular tissues when compared with pooled, normal rabbit serum (Table 1). Pooled antiserum from rabbits immunized with the *S. mutans* 6715 ribosomal preparation exhibited the same background activity as pooled serum from animals injected with the GS-5 ribosomal preparation. In contrast, immune mouse serum and high-titered ASO human serum bound strongly to the human antigens (Table 1). These antisera were reactive with human tissues out to dilutions of 1:8,000 and 1:2,048, respectively (data not shown).

Pooled sera and saliva from rats immunized with the 6715 ribosomal preparation did not react with the heart or kidney antigens when compared with pooled, normal rat serum and saliva. The ELISA antibody levels with the rat serum and saliva were somewhat lower than those with the rabbit serum (Table 1).

The ELISA antibody levels obtained when human kidney glomerular preparations were used as the coating antigen were slightly higher than those with sarcolemmal antigens (Table 1). The reason for the higher activity is that human kidney tissue contains large amounts of endogenous alkaline phosphatase (13), and the tissue has to be boiled to inactivate the enzyme activity; however, in all cases the levels obtained with normal serum or saliva was equal to or greater than the levels with immune serum or saliva. These results show that serum or saliva from animals immunized with *S. mutans* ribosomal preparations does not cross-react with human heart or kidney tissues. It should be pointed out that individual sera from immunized rabbits and sera and salivas

TABLE 2. Level of antibodies to *S. mutans* ribosomal preparations in serum and saliva adsorbed with human heart and kidney antigens[a]

Source of serum or salivary antibodies	Immunogen	Adsorbent	Absorbance (405 nm)[b]
Immune rabbit serum	6715 ribosomes (3)[c]	None	1.190 ± 0.087
		Heart	1.167 ± 0.109
		Kidney	1.205 ± 0.098
		6715 ribosomes[d]	0.032 ± 0.009
	GS-5 ribosomes (3)	None	0.797 ± 0.054[e]
		Heart	0.789 ± 0.043
		Kidney	0.793 ± 0.067
		GS-5 ribosomes[d]	0.043 ± 0.003
Immune rat serum	6715 ribosomes (7)	None	0.213 ± 0.029
		Heart	0.227 ± 0.032
		Kidney	0.210 ± 0.019
		6715 ribosomes	0.017 ± 0.002
Immune rat saliva	6715 ribosomes (7)	None	0.187 ± 0.013
		Heart	0.195 ± 0.021
		Kidney	0.199 ± 0.023
		6715 ribosomes	0.011 ± 0.001

[a] Serum (1:100) and saliva (1:4) from immunized animals were incubated with heart or kidney tissues (10 min, room temperature). Suspensions were centrifuged (15 min, 10,000 × *g*), and supernatants were adsorbed four additional times.
[b] As determined by the procedure described in the text with the *S. mutans* 6715 ribosomal preparation as the coating antigen. Values indicate means ± standard errors of the mean of triplicate determinations per sample.
[c] Numbers within parentheses indicate the numbers of animals.
[d] Adsorbance control containing immune serum or saliva adsorbed with 6715 or GS-5 ribosomes (10 min, room temperature).
[e] An *S. mutans* GS-5 ribosomal preparation was used as the coating antigen.

from immunized rats gave antibody titers to the ribosomes within 2 standard deviations of the pooled samples and did not exhibit tissue reactivity.

After adsorbing the rabbit serum and rat serum and saliva with either heart or kidney tissues, the antibody levels to the ribosomal preparations were determined (Table 2). Adsorption did not reduce the antibody activities to *S. mutans* ribosomes of any serum or saliva. However, adsorption of antisera to the *S. mutans* 6715 or GS-5 ribosomal preparations with the homologous antigen completely removed antibody activity.

TABLE 3. Indirect immunofluorescence of human heart and kidney tissues with antibodies to *S. mutans* ribosomal preparations[a]

Source of serum or salivary antibodies	Immunogen	Relative fluorescence[b]	
		Heart sarcolemma	Kidney glomeruli
None	None	0	0
Normal rabbit serum	None (4)[c]	±	±
Immune rabbit serum	6715 ribosomes (3)	±	±
	GS-5 ribosomes (3)	±	±
Normal rat serum	None (6)	±	±
Immune rat serum	6715 ribosomes (7)	±	±
Normal rat saliva	None (6)	±	±
Immune rat saliva	6715 ribosomes (7)	±	±
Immune mouse serum	Sarcolemma (4)	4+	3+
Human serum (high anti-streptolysin O titer)	None (1)	3+	1+

[a] As determined by procedure in the text with either heart or kidney tissue sections and serum diluted 1:10 and saliva diluted 1:2 in phosphate-buffered saline.
[b] Values of 0 to 4+ were assigned to each sample relative to the amount of fluorescence observed: 0, no reaction; ±, only lipofuscin deposits were seen; 1+, weak reaction; 2+, moderate reaction; 3+, strong reaction; 4+, very strong reaction.
[c] Numbers within parentheses indicate the numbers of animals.

(ii) Indirect immunofluorescence. Serum from immunized rabbits did not bind to heart and kidney tissues when compared with pooled, normal rabbit serum (Table 3). In addition, rabbit antiserum from animals immunized with the *S. mutans* 6715 ribosomal preparation exhibited the same background activity to these tissues as serum from rabbits injected with the GS-5 ribosomal preparation.

Sera and saliva from rats immunized with the 6715 preparation bound to neither heart nor kidney tissues when compared with pooled, normal rat serum or saliva. These results confirm the ELISA data described above and show that immunization of animals with *S. mutans* ribosomal preparations does not lead to induction of antibodies that cross-react with human tissues. Furthermore, sections of human heart adsorbed serum antibodies from mice immunized with human sarcolemma, but not serum antibodies from rabbits injected with the *S. mutans* 6715 ribosomal preparation (data not shown). Fluorescent staining with the mouse serum (and the high-titered ASO human serum) was concentrated at the sarcolemmal sheath, indicating a selective affinity for this antigen. Brightly fluorescent foci caused by deposits of lipofuscin pigment are present in unstained heart and renal tissues and increase in intensity and number of foci with the age of the donor (24). Identical results were obtained with tissues from a 73-year-old man, except for the increased deposits of lipofuscin. In addition, tissues from a 32-year-old man provided the same information. These results provide further evidence that antibodies elicited by *S. mutans* ribosomal preparations do not cross-react with human sarcolemmal and glomerular tissues.

DISCUSSION

Immunization of rabbits and rats with *S. mutans* ribosomal preparations did not result in the production of antibodies that cross-react with either human sarcolemmal or glomerular antigens by ELISA or immunofluorescence. It was unlikely that the assays lacked sensitivity to the human tissues, since a high degree of binding to heart and kidney tissues by ELISA and immunofluorescence was found with

positive control serum from mice immunized with heart tissue and a high-titered ASO human serum.

The *S. mutans* 6715 ribosomal preparation was used to immunize rabbits to induce a hyperimmune response. The immunization protocol consisted of injecting the animals three times intramuscularly with Freund complete adjuvant and nine times intravenously with a total of 4.2 mg of ribosomal protein. The hyperimmune serum had a bacterial agglutination titer of 512 against *S. mutans* 6715 whole cells and a passive hemagglutination titer of over 10,000 against a 6715 ribosomal preparation (7). Since the 6715 ribosomal preparation induces protection in gnotobiotic rats against *S. mutans*-induced carious lesions with locally injected doses as low as 0.7 mg of ribosomal protein (5), it is significant that the 6715 ribosomal preparation failed to induce antibodies to human heart and kidney antigens when rabbits were injected 12 times with up to 4.2 mg of 6715 ribosomal protein. In this regard, rabbits immunized with the GS-5 ribosomal preparation were injected five times with a total of 3.2 mg of ribosomal protein, consequently, no antibodies were induced against the human tissues. These results suggest that the preparations are nontoxic in these animals and that their use as a vaccine to prevent carious lesions in humans will not lead to cardiac or renal damage. This is a significant consideration in the development of such vaccines for use in humans.

The results of this investigation should also be considered in the context of our human studies on naturally occurring antibodies to *S. mutans* ribosomal preparations. We have examined 45 healthy subjects for salivary and serum antibodies to the 6715 ribosomal preparation, and all had salivary IgA and serum IgG antibodies to this antigen (R. L. Gregory, S. J. Filler, S. M. Michalek, and J. R. McGhee, manuscript submitted for publication). It is unlikely that *S. mutans* ribosomal preparations would induce antibodies cross-reactive with heart tissue and that normal healthy subjects should have such antibodies.

A number of possibilities exist for the reported cross-reactivity between human heart tissue and antibodies to streptococci: (i) the presence of cross-reactive antigens in broth media used for preparation of streptococcal vaccines; (ii) anti-immunoglobulins in the sera react with immunoglobulin present in the tissue or with tissue IgG Fc receptors; and (iii) the presence of naturally occurring antibodies directed against heterophile antigens present on both human tissues and streptococci. We have tried to circumvent the first problem by not growing *S. mutans* in brain heart infusion broth (Difco), which may contribute cross-reactive antigens to the streptococcal vaccines. In addition, exhaustive adsorption of antisera with human sarcolemma and glomeruli did not remove antibody activity to the *S. mutans* ribosomal preparation. This indicates that the antibodies were specific for the immunogen and provides further evidence for the lack of heart cross-reactivity with antibodies to *S. mutans* ribosomal preparations.

Immunization with *S. mutans* has been proposed for the prevention of human carious lesions (20). The possibility of incorporating into such a preparation antigens that cross-react with human tissues must be addressed before such a vaccine could be used in humans. Monoclonal antibodies may be useful to delineate the putative cross-reactive antigen(s) so that these determinants can be eliminated from a vaccine against dental caries. We are currently examining monoclonal antibodies to various components of *S. mutans* for activity against human sarcolemmal and glomerular antigens. Preliminary results indicate that monoclonal antibodies to *S. mutans* that cross-react with the putative human heart antigen(s) do not react with the *S. mutans* 6715 ribosomal preparation.

Ribosomal preparations from *S. mutans* 6715 have now been shown to induce protection from dental caries in gnotobiotic rats after challenge with the homologous, virulent *S. mutans* (5, 18). Antibodies raised to this antigen cross-react with whole *S. mutans* cells from all seven serotypes of this species and inhibit acid production, growth, and adherence of *S. mutans* in sucrose broth (6, 7, 9). We are currently examining whether oral immunization of rats with the 6715 ribosomal preparation incorporated into liposomes protects the animals from carious lesions after challenge with a virulent strain of *S. mutans*. Preliminary results indicate that as little as 12.5 μg of ribosomal protein in liposomes protects rats from caries, reduces the number of *S. mutans* in dental plaque, and induces salivary IgA antibodies to the ribosomal preparation that do not cross-react with human tissues. The present investigation shows that *S. mutans* ribosomal preparations do not stimulate antibodies that cross-react with human tissues and may not be pathogenic for cardiac or renal tissues. These results provide further support for the continued investigation of this antigen in an anticaries vaccine.

ACKNOWLEDGMENTS

We are grateful to Thurman Richardson for assistance in obtaining heart and kidney tissues, Jiri Mestecky for advice, and Betty Wells for preparing the manuscript.

This work was supported by Public Health Service grants DE 04217, CA 13148, AM 07069, DE 07026, and DE 02670 and contracts DE 02426 and DE 42551 from the National Institutes of Health. R.L.G. is supported by Research 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.

LITERATURE CITED

1. **Bergmeier, L. A., and T. Lehner.** 1983. Lack of antibodies to human heart tissue in sera of rhesus monkeys immunized with *Streptococcus mutans* antigens and comparative study with rabbit antisera. Infect. Immun. **40**:1075–1082.
2. **Campbell, D. H., J. S. Garvey, N. E. Cremer, and D. H. Sussdorf.** 1970. Lowry (Folin-Ciocalteu) method, p. 76–79. *In* P. Cederborg (ed.), Methods in immunology, 2nd ed. W. A. Benjamin, Inc., New York.
3. **Christensen, P., C. Schalén, and S. E. Holm.** 1979. Reevaluation of experiments intended to demonstrate immunological cross-reactions between mammalian tissues and streptococci. Prog. Allergy **26**:1–41.
4. **Feretti, J., M. Humphrey, M. Begay, and C. Shea.** 1979. Two-dimensional immunoelectrophoresis of *Streptococcus mutans* antigens: immunological cross-reactions, p. 224–225. *In* M. T. Parker (ed.), Pathogenic streptococci. Reedbooks, LtD., Windsor, Berks, England.
5. **Gregory, R. L., S. M. Michalek, I. L. Shechmeister, and J. R. McGhee.** 1983. Effective immunity to dental caries: protection of gnotobiotic rats by local immunization with a ribosomal preparation from *Streptococcus mutans*. Microbiol. Immunol. **27**:787–800.
6. **Gregory, R. L., S. M. Michalek, I. L. Shechmeister, and J. R. McGhee.** 1984. Function of anti-*Streptococcus mutans* antibodies: anti-ribosomal antibodies inhibit acid production, growth and glucose-phosphotransferase activity. Infect. Immun. **45**:286–289.
7. **Gregory, R. L., and I. L. Shechmeister.** 1982. Humoral and cell-mediated responses to a ribosomal preparation from *Streptococcus mutans*. Infect. Immun. **38**:1094–1101.
8. **Gregory, R. L., and I. L. Shechmeister.** 1983. Analysis of cell

wall and membrane contamination of ribosomal preparations from *Streptococcus mutans*. Infect. Immun. **39**:807–816.

9. **Gregory, R. L., and I. L. Shechmeister.** 1983. Comparative study of salivary and serum antibody, and cell-mediated responses to local injection of *Streptococcus mutans* ribosomes and whole cells. Ann. N.Y. Acad. Sci. **409**:825–827.
10. **Hamada, S., S. M. Michalek, M. Torii, I. Morisaki, and J. R. McGhee.** 1983. An enzyme-linked immunosorbent assay (ELISA) for quantitation of antibodies to *Streptococcus mutans* surface antigens. Mol. Immunol. **20**:453–464.
11. **Hughes, M.** 1979. Cross-reactions of *Streptococcus mutans* with heart tissue antigens, p. 222–224. *In* M. T. Parker (ed.), Pathogenic streptococci. Reedbooks, LtD., Windsor, Berks, England.
12. **Hughes, M., S. Machardy, A. Sheppard, and N. Woods.** 1980. Evidence for an immunological relationship between *Streptococcus mutans* and human cardiac tissues. Infect. Immun. **27**:576–588.
13. **Kachmar, J. F.** 1970. Enzymes: serum alkaline phosphatase, p. 394. *In* N. W. Tietz (ed.), Fundamentals of clinical chemistry. The W. B. Saunders Co., Philadelphia.
14. **Kiyono, H., S. M. Michalek, L. M. Mosteller, M. Torii, S. Hamada, and J. R. McGhee.** 1982. Enhancement of murine responses to orally administered haptenated *Streptococcus mutans*. Scand. J. Immunol. **16**:455–463.
15. **Loesche, W. J., J. Rowan, L. H. Straffon, and P. J. Loos.** 1975. Association of *Streptococcus mutans* with human dental decay. Infect. Immun. **11**:1252–1260.
16. **Loesche, W. J., and L. H. Straffon.** 1979. Longitudinal investigation of the role of *Streptococcus mutans* in human tissue decay. Infect. Immun. **26**:498–507.
17. **McGhee, J. R., and S. M. Michalek.** 1981. Immunobiology of dental caries: microbial aspects and local immunity. Annu. Rev. Microbiol. **35**:595–638.
18. **Michalek, S. M., I. Morisaki, R. L. Gregory, H. Kiyono, S. Hamada, and J. R. McGhee.** 1983. Oral adjuvants enhance IgA responses to *Streptococcus mutans*. Mol. Immunol. **20**: 1009–1018.
19. **Morisaki, I., S. M. Michalek, C. C. Harmon, M. Torii, S. Hamada, and J. R. McGhee.** 1983. Effective immunity to dental caries: enhancement of salivary anti-*Streptococcus mutans* antibody responses with oral adjuvants. Infect. Immun. **40**:577–591.
20. **Scherp, H. W.** 1971. Dental caries: prospects for prevention. Science **173**:1199–1205.
21. **Stinson, M. W., R. J. Nisengard, and E. J. Bergey.** 1980. Binding of streptococcal antigens to muscle tissue *in vitro*. Infect. Immun. **27**:604–613.
22. **Stinson, M. W., R. J. Nisengard, M. E. Neiders, and B. Albini.** 1983. Serology and tissue lesions in rabbits immunized with *Streptococcus mutans*. J. Immunol. **131**:3021–3027.
23. **Stinson, M. W., R. J. Nisengard, L. Pelonero, and A. J. Drinnan.** 1979. Immunological cross-reactivity of oral non-streptococcal bacteria with mammalian tissue. Infect. Immun. **24**:532–538.
24. **Strehler, B. L., D. D. Mark, A. S. Mildvan, and M. V. Gee.** 1959. Rate and magnitude of age pigment accumulation in the human myocardium. J. Gerontol. **14**:430–439.
25. **Van de Rijn, I., A. S. Bleiweis, and J. B. Zabriskie.** 1976. Antigens in *Streptococcus mutans* cross-reactive with human heart tissue. J. Dent. Res. **55**:C59–C64.
26. **Van de Rijn, I., and J. B. Zabriskie.** 1976. Immunological relationship between *Streptococcus mutans* and human myocardium, p. 187–194. *In* W. H. Bowen, R. J. Genco, and T. C. O'Brien (ed.), Immunological aspects of dental caries (a special supplement to Immunology Abstracts), Information Retrieval, Inc., London, England.
27. **Youmans, A. S., and G. P. Youmans.** 1969. Factors affecting immunogenic activity of mycobacterial ribosomal and ribonucleic acid preparations. J. Bacteriol. **99**:42–50.