Immunochemistry of the *Streptococcus mutans* BHT Cell Membrane: Detection of Determinants Cross-Reactive with Human Heart Tissue†

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Received 19 October 1984/Accepted 18 January 1985

Cell membranes of *Streptococcus mutans* BHT serotype *b* were prepared after glass bead disruption or mutanolysin digestion of whole cells. Immunoblot analyses of BHT membrane extracts revealed major polypeptides of 42,000, 46,000, 62,000, and 82,000 daltons, as well as several minor bands, to be reactive with rabbit anti-human heart immunoglobulins. Heart cross-reactive antigens have been reported in the cell walls and culture fluids of several *S. mutans* serotypes. This represents the first report of cell membrane-localized heart cross-reactive antigens in this oral pathogen. Positive enzyme-linked immunosorbent assay and immunoblot reactions were also obtained with heart tissue antigen and anti-BHT sera, indicating mutual cross-reactivity. The major cross-reactive component detected by immunoblotting of human heart extracts was a 69,000-dalton polypeptide.

A vaccine against *Streptococcus mutans*, the presumed etiologic agent of human dental caries, has been the subject of great interest for several years (4, 12). It has stimulated intensive research focused on the immunobiology of caries and on the immunoglobulin class, secretory immunoglobulin A (IgA), prevalent in the oral cavity. For a current review of this important area, see the article by McGhee and Michalek (14). Because beta-hemolytic streptococci are known to induce tissue cross-reactive antibodies in humans, there was concern when van de Rijn et al. (30) reported that rabbit antisera raised against various *S. mutans* serotypes cross-react with human heart (HH) and skeletal muscle tissue as detected by indirect immunofluorescence. These findings soon were confirmed by other investigators using both indirect immunofluorescence and crossed immunoelectrophoresis (8, 10, 19). It was clear that the development of a safe and effective anti-caries vaccine would require greater knowledge of key surface immunogens of *S. mutans*.

In recent years, a series of cell wall-localized proteins of *S. mutans* has been isolated, characterized, and tested in monkeys for abilities to protect against dental caries. Russell and collaborators have concentrated their studies on four proteins, A through D, present in cell walls and culture fluids of *S. mutans* Ingbrit. Antigen B provided excellent protection against dental caries in experimental animals (21) but was found to possess immunological cross-reactivity with a heart tissue protein (19) and, indeed, was identical with a cell wall component, antigen IF, found by Hughes et al. (10) to be heart cross-reactive. Antigen B is a 190,000-dalton glycoprotein found in cell walls and culture supernatants of all *S. mutans* serotypes except *b* (19, 20). Lehner and colleagues have studied a series of antigens they have designated I through IV. Russell and Lehner (18) reported the existence of the four antigens in culture supernatants and on cell walls of *S. mutans* serotypes, with a major component being a double antigen called I/II. This antigen is a 185,000-dalton moiety that is partially susceptible to common proteases, leading to the formation of antigens I and II (17). It has been detected in culture fluids and on cell walls of every serotype except *b* (17, 33). The double antigen I/II has been shown to be particularly effective in inducing immunologic protection against caries in monkeys.

The apparent similarity of antigens I/II, B, and IF led Forester et al. (9) to undertake a careful comparative study of these purified proteins. They found each of these antigens to be a 185,000-dalton glycoprotein. Double-diffusion serologic analyses with specific antiserum for each antigen indicated absolute immunologic identity. The authors concluded that I/II also is a heart cross-reactive antigen (HRA). Bergmeier and Lehner (1), however, found no significant titers of anti-HH antibodies in rabbits and rhesus monkeys immunized with purified I/II.

All of the work reviewed above concerns cross-reactive antigens isolated from walls or culture supernatants of *S. mutans* serotypes, excepting serotype *b*. Our initial discovery of heart cross-reactivity in *S. mutans*, however, was made with strain BHT, a member of serotype *b* (30). Preliminary investigations failed to indicate the presence of HRAs in cell walls or culture supernatants of this organism (unpublished observations). In a preliminary communication, Siegel et al. (J. L. Siegel, P. T. Beede, and A. S. Bleiweis, Abstr. Annu. Meet. Am. Soc. Microbiol. 1982, K167, p. 164) reported the presence of HRAs in BHT membranes by using crossed immunoelectrophoresis. This finding was supported by the previous isolation and partial purification of an HRA from the membrane of *Streptococcus pyogenes* by van de Rijn et al. (31). In the present paper, we report further immunologic data obtained with enzyme-linked immunosorbent assays (ELISAs) and immunoblotting showing the presence in BHT membranes of polypeptides able to react with antibodies to HH tissue. Mutual cross-reactivity is demonstrated by the ability of anti-BHT immunoglobulins to react with a component of HH tissue.

(This work was presented in part previously [G. Y. Ayakawa and A. S. Bleiweis, Annu. Meet. Am. Soc. Microbiol. 1984, K31, p. 152].)

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† Florida Agricultural Experiment Station Journal Series no. 5918.
MATERIALS AND METHODS

Bacterial strains and culture conditions. S. mutans BHT, a serotype b strain, was used exclusively in the studies described below. Bacteria were grown in the chemically defined medium of Terleckijy et al. (27). Cultures were grown aerobically to late-exponential phase at 37°C and monitored turbidimetrically at 600 nm with a spectrophotometer (model 2600, Gilford Instrument Laboratories, Inc., Oberlin, Ohio).

Preparation of bacterial membranes. Cells were harvested by passing the culture through a Gyro-Testor (DeLaval Separator Co., Foughkeepsie, N.Y.) followed by centrifugation at 8,000 × g for 30 min at 4°C. Cells were washed once in 0.1 M phosphate-buffered saline (PBS) (pH 6.8), twice in 5 mM EDTA, and once in distilled water. Cells then were broken with glass beads in a Braun tissue homogenizer (Bronwill Scientific Inc., Rochester, N.Y.) by the method of Bleiweis et al. (3) or by lysis by the muration of enzyme mutanolyis (Miles Laboratories, Inc., Elkhard, Ind.) by the procedures of Siegel et al. (23). In all cases, 1 mM phenylmethylsulfonyl fluoride was added to broken cells to inhibit protease activity. Broken or lysed cells then were treated with DNase and RNAse (Sigma Chemical Co., St. Louis, Mo.) and washed as described previously (3). Crude membranes were purified by centrifugation through a one-step sucrose gradient (23) in a Beckman L3-50 ultracentrifuge at 143,000 × g for 90 min or 4°C. Membranes were collected, washed extensively to remove sucrose, and stored at −20°C. Siegel et al. (23) detected a minimal level of wall contamination of membranes prepared by this technique (0.6 to 1.3% rhamnose by weight). Cytoplasmic contamination was assessed by assaying membranes for the presence of glucokinase (EC 2.7.1.2) by the method of Porter and Chassy (15). Membranes were found to possess less than 1% of total glucokinase activity (data not shown).

Protein determination. Total protein contents of membranes, membrane extracts, and heart tissue preparations were determined by the method of Lowry et al. (13).

Preparation of immunogens. A protocol similar to that of Stinson et al. (26) was used for preparing BHT immunogen. Late-exponential phase cells were harvested as described above, washed twice in PBS and once in distilled water, and broken with glass beads. Cells then were suspended in PBS and heated at 70°C for 30 min. The suspension was adjusted to a protein concentration of 7 to 10 mg/ml and stored at −20°C.

HH tissue immunogen was prepared with atrial appendages excised from patients undergoing coronary bypass surgery. Patients were determined to be free of endocarditis-like infections. The tissue was placed in sterile PBS immediately upon removal from the patient and was frozen at −70°C within 4 h. Sterility was monitored by using blood agar plates. Atrial appendages from several donors were pooled, chopped into small pieces, blended in ice-cold PBS, and strained through sterile gauze to remove connective tissue. The HH tissue suspension was adjusted to 1 to 2 mg of protein per ml and stored at −20°C.

Immunization procedures. Subcutaneous immunization of rabbits with HH tissue was performed as follows. New Zealand white rabbits (female) were injected subcutaneously with 1 ml of HH antigen emulsified with 1 ml of Freund incomplete adjuvant (Difco Laboratories, Detroit, Mich.). Animals were injected once weekly for 3 weeks and bled from the marginal ear vein during week 4. After a 1-week rest, the regimen was repeated. Intravenous immunization with BHT wall-membrane complexes was performed as outlined by van de Rijn and Bleiweis (29). Antibody production was monitored with the Ouchterlony double immunodiffusion technique. Preimmune sera always were collected before immunization.

ELISA. ELISA was performed by using a modification of the basic technique of Voller et al. (32). Antigens (bacterial membrane suspensions or heart tissue homogenates) were diluted in 0.1 M carbonate-bicarbonate buffer (pH 9.6). Wells of polystyrene microplates (Linbro E.I.A. Microtitration Plate; Flow Laboratories, McLean, Va.) were coated with 100 μl of appropriate dilutions of the antigen per well. Plates were covered and incubated at 4°C overnight. The wells were then washed with PBS-Tween buffer (0.15 M PBS [pH 7.4] containing 0.05% Tween 20) by using a Skatron Multiplate Washer (Skatron Inc., Sterling, Va.). A 200-μl amount of PBS-gelatin (PBS containing 0.25% gelatin) was added to each well, and the plates were incubated for 2 h at room temperature or at 4°C overnight. After the wells were washed with PBS-Tween, 100 μl of primary antiserum (diluted in PBS-gelatin) was added per well. Plates were then incubated at room temperature for 1 to 2 h. They were washed with PBS-Tween, and 100 μl of a peroxidase-conjugated IgG fraction of goat anti-rabbit IgG (Cappel Laboratories, Cochranville, Pa.) diluted 1:1,000 in PBS-gelatin was added to each well. Plates were again incubated for 1 h at room temperature and washed with PBS-Tween. Finally, 200 μl of freshly prepared substrate solution (3.5 mg of o-phenylenediamine [Sigma] and 4 μl of 30% H2O2 per 10 ml of 0.1 M citrate buffer [pH 4.5]) was added to each well. Plates were incubated in the dark at room temperature for 20 to 30 min, and reactions were then read with a Titertek Multiskan photometer (Flow Laboratories) at 492 nm. Wells containing only substrate solution were included as a blank. Wells containing no primary antiserum (conjugate controls) were run to assess background levels of reactivity. Preimmune sera were also routinely tested. Reactions were run two to three times, and means and standard deviations were calculated for absorbance values at each serum dilution. The t-test was employed to assess the significance of differences between immune and preimmune sera.

Preparation of antigens for immunoblotting studies. Bacterial antigens used for immunoblots consisted of membranes from BHT cells grown to late-exponential phase in chemically defined medium. Our previous studies (7) of the chemical composition and sodium dodecyl sulfate-polyacylamide gel electrophoresis (SDS-PAGE) profiles of the BHT membrane had indicated that polypeptide patterns remained essentially constant throughout the growth cycle. Thus, late-exponential phase cells were chosen for study to generate the highest yield of actively growing cells.

HH tissue antigen was prepared as follows. Pooled atrial appendage material from several donors was chopped into small pieces and homogenized in PBS. The homogenate was centrifuged, and the pellet was extracted in PBS containing 1% SDS for 30 min at room temperature. The suspension then was homogenized on ice for several minutes with a Dounce homogenizer. This preparation was centrifuged at 10,000 × g for 30 min to remove connective tissue and other large debris. The supernatant then was subjected to centrifugation at 30,000 × g for 45 min. The final supernatant served as antigen for immunoblotting studies.

SDS-PAGE. SDS-PAGE was accomplished by using the discontinuous buffer system of Laemmli (11). A 5% acrylamide stacking gel and a 10% acrylamide resolving gel were used in these studies. Samples (either BHT membrane
suspensions or heart tissue extracts) were diluted 1:1 with 2× concentrated sample buffer (7) and heated at 100°C for 5 min before application to the gel. After sample application, electrophoresis was begun at 20 mA of constant current per slab until the bromophenol blue tracking dye reached the interface between the stacking and resolving gels, at which time the current was increased to 30 mA per slab. SDS-PAGE runs were conducted at 15°C with constant cooling by a circulating water bath. Electrophoresis was continued until the dye migrated to within 10 to 20 mm of the bottom of the gel (4 to 5 h). Molecular weights of polypeptides were estimated by using protein standards of known molecular weights (Dalton Mark VII; Sigma).

Transfer of polypeptides from SDS-PAGE gel to nitrocellulose (blotting). Transfer of polypeptides to nitrocellulose essentially followed the protocol of Towbin et al. (28) with some modifications. The Trans-Blot electrophoretic transfer apparatus (Bio-Rad Laboratories, Richmond, Calif.), in conjunction with a Bio-Rad model 160/1.6 high-current power supply, was used in these experiments. Blotting was performed at 30 V constant voltage overnight at 15°C. Alternatively, transfers occasionally were carried out at 60 V for 3 h at 4°C. Nitrocellulose (pore size, 0.45 μm), was obtained from Bio-Rad. Nitrocellulose strips were stained with 0.1% naphthol blue black in 45% methanol–2% acetic acid and destained in 90% methanol–2% acetic acid (5) to monitor the efficiency of polypeptide transfer.

Immunologic detection of antigenic polypeptides on nitrocellulose blots. Unstained, blotted nitrocellulose strips were immersed in blocking buffer (0.01 M PBS [pH 7.2] containing 0.3% [vol/vol] Tween 20 [Sigma]) for at least 1 h at 37°C to saturate any remaining protein adsorption sites. Strips which were not immediately used were stored in blocking buffer at 4°C. After removal of the blocking buffer, strips were incubated overnight in primary antisera diluted in blocking buffer. Strips treated with preimmune sera served as controls. This incubation, as well as all subsequent steps, was performed at room temperature with continuous rotation on an orbital shaker (Bellco Glass, Inc., Vineland, N.J.). Strips then were washed four times (10 min each wash) in blocking buffer. They were then incubated for 2 h in a peroxidase-conjugated IgG fraction of goat anti-rabbit IgG (heavy and light chain specific; Cappel) diluted 1:1,000 in blocking buffer. The strips then were washed twice in blocking buffer and twice with Tris-buffered saline (50 mM Tris-HCl [pH 7.4] containing 150 mM NaCl). Finally, they were immersed in 40 ml of Tris-buffered saline, to which 8 ml of a 3-mg/ml solution of 4-chloro-1-naphthol (Sigma) prepared in methanol was added. The peroxidase reaction then was initiated by adding 48 μl of a 30% H₂O₂ solution. Positive reactions were determined by the appearance of bluish black bands. Reactions were allowed to run for 20 to 30 min. Strips were washed in distilled water and stored in the dark.

RESULTS

Demonstration of cross-reactive antigens in S. mutans BHT membranes by ELISA. Figure 1 presents ELISA data obtained with BHT membrane antigen by using anti-BHT serum (Fig. 1A) and anti-HH sera (Fig. 1B and C). As expected, the homologous reaction (Fig. 1A) was very strong, with significant immune reactivity down to a 1:64,000 dilution. The heterologous reactions (Fig. 1B and C) were much weaker, requiring higher concentrations of antibodies to attain similar levels of reactivity. Figure 1B depicts the reaction obtained with an anti-HH serum kindly provided by

FIG. 1. ELISA with S. mutans BHT membrane antigen. Wells of microtiter plates were coated with whole BHT membranes (20 μg of protein per ml). Wells were reacted with indicated dilutions of: (A), anti-S. mutans BHT serum (rabbit 203); (B), anti-HH serum (rabbit 224); (C), anti-HH serum (rabbit 196). Antigen-antibody reactions were visualized with peroxidase-conjugated goat anti-rabbit IgG. Means (± standard deviations) of two to three runs were plotted. Conjugate control values: (A and B), 0.075 ± 0.023 (n = 6); (C), 0.071 ± 0.010 (n = 4).

M. W. Hughes, Wellcome Research Laboratories, Beckenham, England (rabbit 224). There did not appear to be a great deal of difference between preimmune and immune sera, although the mean absorbance values shown for the immune serum were significantly higher (P < 0.05) than those obtained for the preimmune serum. The results ob-
preimmune reaction was negative for rabbit 224, whereas the preimmune serum from rabbit 196 displayed a few faintly reactive bands. The presence of polypeptides reacting with preimmune sera was not surprising, based on the ELISA results (Fig. 1). It is important to recognize that these two anti-HH sera originated from two different laboratories and yet demonstrated qualitatively identical immunoreactivities with BHT membrane antigen, despite the fact that different immunogen preparations and immunization schedules were used. These reactions clearly demonstrated the existence of membrane-associated HRAs in S. mutans BHT, further buttressing the ELISA (Fig. 1B and C) data.

Demonstration of cross-reactive antigens in HH tissue by ELISA. ELISA data obtained with HH antigen are illustrated in Fig. 3. Figure 3A depicts the homologous reaction with anti-HH serum (rabbit 224), and Fig. 3B and C show the reactions obtained with anti-BHT sera (rabbits 202 and 203). The latter two sera displayed very different reaction patterns. Serum from rabbit 202 (Fig. 3B) apparently possessed a great deal of preimmune reactivity against HH tissue. The source of this reactivity is unknown, but it may be attributable to stimulation of the rabbit immune system by HRAs present in streptococci of the normal flora or to the presence of autoantibodies to rabbit heart tissue which cross-react with HH determinants. Other investigators (6, 8) have also observed the presence of anti-HH antibodies in presumably normal rabbit preimmune sera. Despite the appearance of the curves (Fig. 3B), mean absorbance values for the immune serum from rabbit 202 are significantly higher (P < 0.05, except for the 1:50 dilution) than the preimmune values. A much more convincing cross-reaction was observed with serum from rabbit 203 (Fig. 3C). Strikingly less preimmune reactivity was apparent in this animal, although there was a linear response with the preimmune serum at the three highest concentrations tested, suggesting a specific low-level immune reaction.

Demonstration of cross-reactive antigens in HH tissue by Immunoblotting. A series of protein blots of SDS-extracted HH tissue is presented in Fig. 4. The homologous reaction (rabbit 196) displays the expected multitude of reactive polypeptides. The heterologous reaction with anti-BHT serum (rabbit 202) shows several cross-reactive polypeptides, with a sharply defined major band at 69,000 daltons, two other major bands at 54,000 and 32,000 daltons, and a cluster of polypeptides between 40,000 and 50,000 daltons. There is also a minor band at approximately 68,000 daltons. It should be pointed out that the lower-molecular-weight bands did not react consistently by immunoblotting; however, the higher-molecular-weight polypeptides (69,000 and 68,000) were always seen. Reactions with these higher-molecular-weight polypeptides were also observed with serum from rabbit 203 (data not shown). Preimmune reactivity was also seen with serum from rabbit 202, particularly with the 54,000-dalton polypeptide, and was seen very faintly with the 69,000-dalton band and several bands in the 40,000- to 50,000-dalton range. This would certainly be expected, based on the ELISA data (Fig. 3B). Preimmune reactions were a particular problem with several of our anti-BHT sera and may have obscured other cross-reactive polypeptides. Thus, the data shown here probably represent the minimum number of cross-reactive polypeptides, since the exquisite sensitivity of the immunoblotting technique made a very cautious interpretation of the results necessary. However, these results, along with the ELISA (Fig. 3B and C) data above, provide evidence supporting the existence of moieties in HH tissue which cross-react with S. mutans BHT antigens.
FIG. 3. ELISA with HH antigen. Wells of microtiter plates were coated with HH homogenate (21.8 μg of protein per ml). Wells were reacted with indicated dilutions of: (A), anti-HH serum (rabbit 224); (B), anti-S. mutans BHT serum (rabbit 202); and (C), anti-S. mutans BHT serum (rabbit 203). Antigen-antibody reactions were visualized with peroxidase-conjugated goat anti-rabbit IgG (1:1,000). Means (± standard deviations) of two to three runs were plotted. Conjugate control values: (A), 0.224 ± 0.024 (n = 6); (B and C), 0.135 ± 0.024 (n = 6).

FIG. 4. Immunoblots of HH tissue antigens. HH tissue polypeptides were resolved by SDS-PAGE by using 10% gels and electrophoretically transferred to nitrocellulose sheets. Lane S indicates a blot (60 μg of protein) stained for protein with naphthol blue black. Remaining blots (20 μg of protein) were reacted with antisera prepared against HH tissue (rabbit 196, 1:50 dilution) and S. mutans BHT (rabbit 202, 1:500 dilution). A 1:500 dilution of the heterologous serum was used because of the presence of a mottled background at lower dilutions which obscured the reactive polypeptides. Antigen-antibody reactions were visualized with peroxidase-conjugated goat anti-rabbit IgG (1:1,000). Lanes P illustrate reactions with preimmune sera, and lanes I show results obtained with immune sera. Molecular weights of cross-reactive polypeptides are indicated at right; KD, kilodaltons.

DISCUSSION

The studies presented here confirm and extend the findings of van de Rijn et al. (30) regarding the presence of HRAs in the cytoplasmic membrane of S. mutans BHT and also demonstrate the existence of mutually cross-reactive antigens associated with HH tissue. Much attention (9, 19, 20) has been focused in recent years on the wall-associated and extracellular HRAs of S. mutans, and the cell membrane has been all but ignored. Similarly, there has been a dearth of information in the literature concerning the molecular characterization of the cross-reactive heart tissue antigen. In this investigation, we applied the sensitive immunochemical techniques of ELISA and immunoblotting to study of this problem.

In any investigation concerned with the detection of immunological cross-reactivity between microbial and mam-
malian antigens, the possibility of artifactual positive results must always be considered. We have taken several precautions to eliminate or at least minimize the occurrence of artifacts.

First, bacteria were cultured in a chemically defined medium to avoid the possibility of contamination of membranes by components of complex growth media, e.g., peptides derived from beef heart tissue, a constituent of the commonly used Todd-Hewitt broth. Stinson and Jones (25) demonstrated that such peptides with molecular weights in excess of 12,000 adsorb readily to the surface of S. mutans BHT and related strains. Furthermore, these peptides were found to be able to induce heart cross-reactive antibodies in rabbits. Therefore, it was imperative to use a chemically defined medium to eliminate the possibility of foreign antigens in microbial membrane extracts displaying cross-reactive properties with anti-HH sera.

Another important factor to be considered when investigating possible immunologic cross-reactions is the presence of Fc receptor sites on the bacterial surface. Fc receptor sites are well-recognized components of the streptococcal cell surface (16) and allow the nonspecific attachment of immunoglobulin molecules to such cells. If present in membrane extracts, such receptor proteins would mimic tissue cross-reactive antigens. Fortunately, Shea and Ferretti (22) failed to detect Fc reactivity in S. mutans BHT. As a precaution, however, we submitted our BHT strain to M. D. P. Boyle, University of Florida, for detection of Fc receptors by his recently published method (16) for measuring direct uptake of immunoglobulins by whole bacteria. Boyle (personal communication) confirmed the findings of Shea and Ferretti (22).

Examination of preimmune sera was mandatory to check for the presence of antibodies directed against streptococcal membrane components or HH tissue in normal rabbits before immunization. Natural antibodies against S. mutans would certainly not be unexpected in rabbits, as these organisms are part of the normal oral flora. The presence of anti-HH antibodies in such animals, however, would be more difficult to account for. Two possible explanations have already been suggested above, namely, the production of antibodies against HRAs of normal oral streptococci and the production of autoantibodies against rabbit heart tissue which cross-react with HH tissue. Nevertheless, the increased reactivity of immune versus preimmune sera by ELISA (Fig. 1 and 3) and by immunoblotting (Fig. 2 and 4) strongly supports the idea that a specific cross-reactive antibody response is generated upon immunization.

Great care was exercised to secure fresh, sterile HH tissue (atrial appendages) directly from the surgical table to prevent contamination with microbial antigens introduced from the environment. It is still possible, however, that reactions displayed in Fig. 3B and C and 4 are not due to a constitutive human antigen but instead to an adherent microbial antigen present in the subject before the operative procedure. Donors were free of endocarditis and other obvious infections immediately before surgery, but their medical histories may not have disclosed prior infections by invasive streptococci. The work of Stinson and Bergey (24) suggests that small S. pyogenes polypeptides of 18,000 to 20,000 daltons can bind to cardiac tissues with great avidity under their experimental conditions. If the reactions reported here were due to such external antigens, we would be dealing with immune complex formation rather than with true tissue cross-reactivity. It should be pointed out, however, that Hughes et al. (M. Hughes, S. M. MacHardy, and A. J. Sheppard, J. Dent. Res. 60(B):1191, 1981) have obtained data showing that anti-S. mutans sera react with heart tissue from germfree rats which had never been exposed to streptococci. This indicates that antigens cross-reactive with S. mutans are constitutive tissue components in that mammalian system.

Thus, the weight of the evidence reported in this communication strongly suggests that HRAs reside in the cytoplasmic membrane of S. mutans BHT and that mutually cross-reactive molecules exist in association with HH tissue. The relevance of the microbial antigens to pathogenesis is not evident, especially since S. mutans usually is noninvasive, but the possible hazards which would be presented if these factors were included in anti-caries vaccines for humans cannot be underestimated (2). Whether the mammalian antigen is a constitutive tissue component or an adherent microbial antigen may be debatable at this point, but it is also debatable whether there would be any consequent difference to a host receiving an inappropriately formulated vaccine. The distinction between an autoimmune reaction and immune complex disease would be a subtle one indeed.

Still unclear is the relationship between the S. mutans BHT membrane antigens described herein and the wall-associated and extracellular HRAs found in other strains of S. mutans. Work is currently in progress in our laboratory in an attempt to establish whether such a relationship exists. We have obtained preliminary data with monospecific antisera prepared against antigen B (kindly provided by R. R. B. Russell, Royal College of Surgeons, Downe, England) and antigen PI (courtesy of K. W. Knox and H. Forester, Institute of Dental Research, Sydney, Australia) which indicate that the BHT membrane HRAs cross-react with these well-characterized wall and extracellular antigens (unpublished observations). Further work along these lines is in progress.

In summary, we have demonstrated that HRAs are found in membranes of S. mutans BHT and that mutually cross-reactive antigens exist in association with HH tissue. Further immunohistochemical studies of S. mutans membranes are warranted to ascertain whether membrane-localized HRAs can be found in other strains. Comparisons with membranes of other streptococcal species clearly are required to detect this important antigen, if present, in species more invasive than the etiologic agent of dental caries. Of particular interest would be a comparative study of the S. mutans membrane HRA with the well-characterized membrane HRAs of S. pyogenes (31).

ACKNOWLEDGMENTS

This work was supported by Public Health Service grant DE06051 from the National Institute of Dental Research and by grant BC-01440 from the Florida Agricultural Experiment Station.

We thank Thomas Bartley and M. Arthur Nesmith for generous gifts of HH tissue, Patti Beede for technical assistance, and William McArthur for the use of his equipment and facilities for ELISA.

LITERATURE CITED


