INFECTION AND IMMUNITY, Aug. 1985, p. 344–350
0019-9567/85/080344-07$02.00/0

Vol. 49, No. 2

Antibody Specificity and Antigen Characterization of Rat Monoclonal Antibodies against *Streptococcus mutans* Cell Wall-Associated Protein Antigens

FABIENNE ACKERMANS,[1] JEAN-P. KLEIN,[2]* FRANÇOISE CORMONT,[3] HERVÉ BAZIN,[3] JOËLLE A. OGIER,[2] ROBERT M. FRANK,[2] AND JOSÉ VREVEN[1]

Ecole de Médecine Dentaire,[1] and Immunologie Expérimentale, Ecole de Santé Publique,[3] 1200 Brussels, Belgium, and Unité de Recherches, Institut National de la Santé et de la Recherche Médicale, U. 157, Faculté de Chirurgie Dentaire, 68000 Strasbourg, France[2]

Received 15 October 1984/Accepted 10 May 1985

Monoclonal antibodies to *Streptococcus mutans* OMZ175 (serotype f) cell wall-associated antigens (wall-extracted antigens [WEA]) were derived from the fusion of Lou C plasmocytoma rat cells (IR 983 F) and spleen cells from Wistar R inbred rats immunized with WEA. Four cell lines producing monoclonal antibodies directed against a component of *S. mutans* WEA have been established. All four monoclonal antibodies reacted only with two antigens of WEA from *S. mutans* OMZ175 by Western blotting and immunoprecipitation techniques, enzyme-linked immunosorbent assay (ELISA), and competitive ELISA. Western blot analysis of WEA showed that the four monoclonal antibodies recognized two related cell wall-associated proteins with apparent molecular weights of 125,000 and 76,000. Immunoprecipitation of whole cells with the monoclonal antibodies confirmed the surface localization of the two antigens. The ELISA and competitive ELISA were used to analyze the distribution of the epitopes on seven *S. mutans* serotypes. All *S. mutans* serotypes were found to express the recognized epitopes; however, different reactivity patterns could be distinguished among the various strains tested, and the four monoclonal antibodies reacted only weakly with *S. mutans* serotypes d and g.

The components of *Streptococcus mutans* cell wall, which seem to play an important role in bacterial adherence or agglutination and dextran aggregation, are composed of two major classes of macromolecules, i.e., serotype antigens and wall-associated proteins (12, 16). The serotype antigens have been extensively studied by several investigators (12). Most recent studies have focused on the proteins which bind more or less strongly to the streptococcal cell wall. Several of these proteins were purified (8, 19, 20, 22, 25), and some of them were characterized as glucosyltransferases or dextran-binding proteins (11, 21). Until now, our own and other studies on the *S. mutans* cell wall-associated proteins have involved polyclonal antisera raised in animals, and it seemed difficult to establish a precise comparison between the various purified proteins.

Monoclonal antibodies, produced by the hybridoma technique of Köhler and Milstein (13), have been successfully used for bacterial serotyping and characterization of antigen. They have, for example, been used to analyze the lipopolysaccharides of several gram-negative bacteria (1, 10) and to characterize the cell surface proteins of various bacterial strains (6, 18, 27). This study was undertaken to obtain anti-cell wall protein monoclonal antibodies to facilitate the protein typing of *S. mutans* serotypes. Since monoclonal antibodies react with a single antigenic determinant or epitope, we did not, before the immunization of animals, extensively purify the streptococcal wall-extracted antigen (WEA) (24) which contains several proteins common to various *S. mutans* serotypes.

In the present paper, we report the production of four rat monoclonal antibodies against WEA antigen of *S. mutans* OMZ175 (serotype f) and describe the distribution of the recognized antigens in seven *S. mutans* serotypes.

* Corresponding author.

MATERIALS AND METHODS

Bacterial strains and antigen preparation. *S. mutans* strains E49 (serotype a), OMZ51 (serotype b), OMZ70 (serotype c), OMZ176 (serotype d), B-2 (serotype e), OMZ175 (f), and 6715 (serotype g) have been described previously (24). The reference strain was *S. mutans* OMZ175. Strains were maintained in brain heart infusion broth (Difco Laboratories, Detroit, Mich.).

Antigens were prepared from exponential-phase cultures as previously described (24). Briefly, wall-associated antigens (WEA) were obtained from washed bacterial pellets extracted with 0.5 M phosphate buffer (pH 6.0), which underwent shaking for 1 h at 4°C, after the inactivation of autolytic enzymes by heating the bacterial pellet at 60°C for 30 min (23). The crude fraction was chromatographed on a Bio-Gel P6 column (Bio-Rad Laboratories, Richmond, Calif.) and adjusted to a final protein concentration of 2 mg/ml.

Production of antibody-secreting hybridomas. Wistar R rats (8 to 10 weeks old) were intraperitoneally immunized with 50 μg of alum-precipitated *S. mutans* OMZ175 WEA fraction two times at 14-day intervals. Rats were tested for antibody production by double diffusion, and the positive serum was subsequently used as total anti-WEA serum. Seven months later, the rats were boosted intravenously with 100 μg of WEA in phosphate-buffered saline (PBS). Spleen cells were harvested 4 days later and fused with Lou rat nonsecreting IR 983F myeloma cells (3) in a cell ratio of 5:1. The fusion solution was 50% (wt/wt) polyethylene glycol 4000 (Merck, Darmstadt, West Germany). The fused cells were diluted in complete Dulbecco modified Eagle medium (GIBCO Laboratories, Grand Island, N.Y.) containing 5% calf fetal serum and 5% horse serum supplemented with 0.1 mM hypoxanthine, 0.4 mM aminopterin, and 0.015 mM thymidine and plated in 24- and 96-well tissue culture plates

(Nunc, Kamstrup, Denmark). Approximately 2 weeks after fusion, wells with growth of hybrid cells were tested for the presence of antibodies against *S. mutans* OMZ175 WEA antigen by indirect solid-phase radioimmunoassay. Positive clones were recloned by limiting the dilution at least two times to ensure monoclonality.

The immunoglobulin class and subclass of the monoclonal antibodies were determined by immunodiffusion precipitation with culture supernatants of the monoclonal antibody-producing hybridomas and subclass-specific antisera against rat immunoglobulin G1 (IgG1), IgG2a, IgG2b, IgG2c, IgA, IgM, IgE, and IgD (prepared as described in references 4 and 5).

Production of ascitic fluid and antibody purification. Hybridoma cells of monoclonal origin were injected intraperitoneally into (Wistar R/Lou C) F1 hybrid rats, ascites fluid was collected, and the immunoglobulin was purified.

Each IgM monoclonal antibody was precipitated from clarified ascites fluid by dialysis against 0.1 M Tris-hydrochloride buffer (pH 8) (48 h, 4°C). Radial immunodiffusion of the supernatants revealed a 100% removal of the antibody from each ascite. The protein was dissolved in PBS (pH 7.2), applied to a column of Bio-Gel A 5m (90 by 2.6 cm) (Bio-Rad Laboratories), and eluted with PBS. The IgM peak was recovered, adjusted to a final protein concentration of 4 mg/ml, and stored at −80°C.

Each monoclonal antibody was evaluated for purity by immunoelectrophoresis against whole anti-rat serum. The electrophoretic pattern revealed a minor contamination with protein α2 macroglobulin.

Antibody detection. Antibody production by the hybrid cells was assessed by indirect solid-phase radioimmunoassay. Briefly, a WEA fraction from *S. mutans* OMZ175 was diluted to 50 μg of protein per ml in 0.1 M carbonate buffer (pH 9.6), and 50 μl of the solution was added to each well of flexible microtiter plates (Dynatech Laboratories, Inc., Dynatech Corp., Alexandria, Va.). The plates were incubated for 2 h at room temperature, followed by an overnight incubation at 4°C. The plates were then washed with PBS containing 0.05% Tween (PBS-T) and incubated for 1 h at room temperature with 4% bovine serum albumin in PBS-T to bind the residual groups. After the plates were washed with PBS-T, 50 μl of the hybridoma culture supernatant was added to the wells, and the plate was incubated for 1 h at room temperature. The plates were washed again with PBS-T, 50 μl of ^{125}I-labeled mouse IgG1 monoclonal antibodies against rat kappa light chains (MARK-1) (3a) was added to each well, and the plates were incubated at room temperature for 1 h. The plates were washed again and counted in a gamma scintillation counter. 5% calf fetal serum–5% horse serum supplemented with hypoxanthine-aminopterin-thymidine was used as control, and samples which assayed 10-fold over background were considered positive.

ELISA and competitive binding assays. The antigenic cross-reactivity of the four monoclonal antibodies with various *S. mutans* serotypes was established (i) by directly measuring with enzyme-linked immunosorbent assay (ELISA) the binding of the antibodies of the various strains and (ii) by a standard competitive assay.

In the first procedure, flat-bottomed polystyrene microtiter plates (Nunc) were coated with identical amounts (50 μg of protein per ml) of WEA fractions from seven *S. mutans* serotypes under the conditions used for indirect solid-phase radioimmunoassay. Serial dilutions in PBS-T of the purified antibody were added to the wells (50 μl).

Antibody binding was detected with MARK-1–alkaline phosphatase conjugate (MARK-1–AP) at a 1:500 dilution with *p*-nitrophenyl phosphate as the substrate (17). The absorbance at 405 nm was read in a Multiskan reader (Flow Laboratories, Inc., McLean, Va.) after incubation of the plates for 1 h at 25°C. Normal rat IgM was used as the control. The minimum concentration of purified monoclonal antibody required for saturation of *S. mutans* OMZ175 WEA was determined for each of the four antibodies.

In the competitive ELISA procedure, 50 μl of the predetermined saturating amount of purified antibody was incubated (1 h at 25°C) with 50 μl of PBS-T containing constant amounts of WEA from various *S. mutans* serotypes or from an unrelated *Bacillus subtilis* strain (H. Monteil, Institute of Bacteriology, Strasbourg). Samples (50 μl) were then transferred to a microtiter plate coated with *S. mutans* OMZ175 WEA, and hybridoma binding was determined by ELISA as described above. Incubation of the monoclonal antibodies was always performed with at least four different concentrations of antigens. The results are expressed as percent inhibition of binding of the monoclonal antibodies to the homologous antigen. Percent inhibition was calculated as [(OD of the control − OD of the competitor)/OD of the control] × 100, where OD is the optical density. Wells in which no WEA antigen was added were controls.

Epitope analysis by competitive ELISA. Various amounts of purified antibody in PBS were mixed with an equal volume of PBS containing the predetermined saturating concentration of AP-labeled antibody. Fifty-microliter samples of the reaction mixture were added to *S. mutans* OMZ175-coated microtiter plates and incubated 1 h at 25°C. The amount of AP-labeled antibody bound was determined by ELISA, and the results are expressed as the percent inhibition of binding of AP-labeled antibody as described above.

Antibody specificity assay. The specificities of the four monoclonal antibodies were determined by immunodiffusion, Western blot analysis, and immunoprecipitation. Double diffusion experiments were performed with *S. mutans* OMZ175 WEA fraction and purified antibodies in 1% agarose with barbital buffer (pH 7.2).

Molecular weight markers and WEA fractions were electrophoresed in 10% polyacrylamide gels in presence of sodium dodecyl sulfate (sodium dodecyl sulfate-polyacrylamide gel electrophoresis [SDS-PAGE]) by the method of Laemmli (14). After electrophoresis the proteins were electrophoretically transferred to nitrocellulose paper (BA 85, Schleicher & Schüll LPCR, Strasbourg, France) as described by Towbin et al. (26). An immunoperoxidase assay was then performed on a nitrocellulose sheet (7). The blots were first soaked with 4% bovine serum albumin in PBS-T for 1 h at room temperature, then rinsed in PBS-T five times over a 30-min period, and were finally incubated 1 h at room temperature with rat control IgM, rat anti-total WEA serum, or purified monoclonal antibody diluted in PBS-T. The nitrocellulose sheets were washed again and soaked in MARK-1–peroxidase conjugate for 1 h at room temperature and then washed in PBS-T as described above. The blots were then shaken in a 0.2 mg/ml solution containing 3,3′-diaminobenzidine (Sigma Chemical Co., St. Louis, Mo.) and 0.001% H_2O_2 in PBS-T. Molecular weight markers were stained separately in 0.1% amido black in 10% acetic acid.

Immunoprecipitation was carried out by incubating whole *S. mutans* OMZ175 cells with either monoclonal antibodies or rat anti-WEA serum. After being washed, the bacteria were extracted with 1% Triton X-100, and the immune

complexes were recovered by incubating the extract with MARK-1–Sepharose CL-4B beads. The immunoprecipitates were submitted to SDS-PAGE and then transferred to nitrocellulose paper as before. The antigens were detected by incubating the sheets with rat horseradish peroxidase-labeled anti-WEA globulin, followed by enzyme substrate. This procedure has been described in detail elsewhere (16).

Labeling of antibodies. MARK-1 was coupled to ^{125}I by the chloramine-T method of Greenwood et al. (9). MARK-1, monoclonal antibodies, and rat total serum phosphatase alkaline or peroxidase conjugates were prepared according to the procedure of Avrameas (2). MARK-1 was coupled to CnBr-activated Sepharose CL-4B (Pharmacia Fine Chemicals, Inc., Piscataway, N.J.) as described by the manufacturers.

Chemical modification of the WEA fraction. Pronase (B grade [Calbiochem-Behring, La Jolla, Calif.], 0.1 ml of a 200 μg/ml solution in 0.05 M Tris-hydrochloride buffer [pH 7.5]) was added to 0.1 ml (1 mg of protein per ml) of the WEA fraction from *S. mutans* OMZ175. The mixture was incubated for 4 h at 37°C. After incubation, the sample was heated to 100°C for 5 min to inactive the enzyme, freeze-dried and reconstituted in SDS gel electrophoresis sample buffer, electrophoresed, blotted, and then reacted with monoclonal antibodies as described above. Untreated samples and enzyme alone were run in parallel.

A 0.1-ml WEA fraction (1 mg/ml) was incubated with 0.1 ml of sodium metaperiodate (0.05 M in 0.05 M acetate buffer [pH 5.0]) overnight at 4°C in the dark. After exhaustive dialysis against distilled water at 4°C, the sample was freeze-dried, reconstituted in SDS sample buffer, and used to generate electrophoretic blots. A buffer control without periodate was run in parallel.

RESULTS

Isolation of monoclonal antibodies. From the fusion of nonsecreting IR 983 F myeloma cells with primed spleen cells, 311 wells were tested for anti-*S. mutans* OMZ175 WEA antibody. Thirty-five supernatants were strongly positive in the indirect solid-phase radioimmunoassay against the immunizing WEA fraction. Cloning of the positive cultures resulted in the recovery of 11 stable antibody-secreting hybridomas. Of these, four were chosen for detailed study. The selected hybridomas and their corresponding antibodies were named LOSM1, LOSM2, LOSM3, and LOSM4.

Each one of the monoclonal antibodies gave a single precipitin line in Ouchterlony gel diffusion analysis against antisera specific for only one of the eight rat immunoglobulin isotypes tested, and all of them were identified as IgM(κ). The hybridomas were grown in vivo to produce large amounts of ascitic fluids rich in specific antibodies (5 to 20 mg/ml). Each antibody was purified before its use in subsequent experiments.

Characterization of the monoclonal antibodies. The purified monoclonal antibody from all four hybridoma lines reacted with the *S. mutans* OMZ175 WEA fraction but to various extents (Fig. 1). Antibody LOSM4 yielded a strong reaction with the WEA fraction (Fig. 1d); antibodies LOSM1 and LOSM2 exhibited lower specificity against the antigen (Fig. 1a and b), whereas antibody LOSM3 showed a reduced activity (Fig. 1c). When antibodies were present in amounts greater than required for saturation, the ELISA showed differences in the maximum absorbance achieved, suggesting differences in the avidity of binding of the monoclonal antibodies to their epitopes.

Antigen identification. The reactions between the WEA fraction and each monoclonal antibody or rat anti-WEA serum were examined by immunodiffusion (Fig. 2). The monoclonal antibodies precipitated with the same antigen of WEA components, whereas total rat antiserum reacted with this antigen and with additional ones.

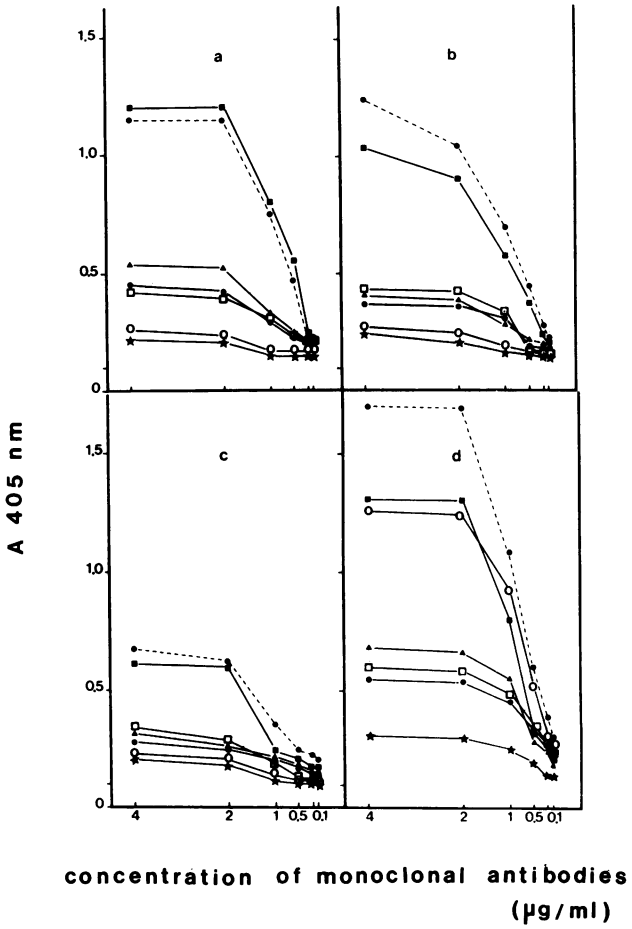

FIG. 1. ELISA of the four purified monoclonal antibodies diluted in PBS—LOSM1 (a), LOSM2 (b), LOSM3 (c), and LOSM4 (d)—to the homologous WEA fraction from *S. mutans* serotype f (● - - - - - ●) and the heterologous WEA fraction from *S. mutans* serotypes a (●), b (■), c (▲), d (○), e (□), and g (★). A 405 nm, Absorbance at 405 nm.

Immunoblotting was implemented to determine the molecule-specific binding of the monoclonal antibodies. SDS-PAGE of the WEA fraction from *S. mutans* OMZ175, stained with Coomassie blue, revealed at least 20 bands ranging in molecular weights from 135,000 to 30,000 (Fig. 3h); a quite identical pattern was obtained by Western blot analysis with rat anti-WEA serum (Fig. 3a). Attempts at immunoblotting with antibodies LOSM1, LOSM2, LOSM3, and LOSM4 showed that all four monoclonal antibodies were directed against two molecules with apparent molecular weights of 125,000 and 76,000 (Fig. 3b to e). Moreover, antigenic components did not react with control IgM followed by labeled MARK-1. The protease sensitivity of the reactive antigens was evident upon digestion of WEA with pronase (Fig. 3f), which completely abolished the reactivity of the antigens with the monoclonal antibodies on immunoblots. However, when the WEA fraction was submitted to periodate oxidation before electrophoretic transfer to

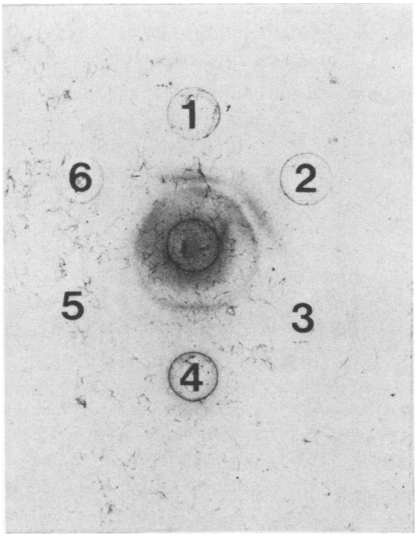

FIG. 2. Precipitation of each monoclonal antibody with an antigen of *S. mutans* OMZ175 WEA fraction. Immunodiffusion wells 1 and 2 contained rat antiserum against *S. mutans* WEA, and wells 3 to 6 contained purified monoclonal antibodies, each at 5 mg/ml (LOSM1, LOSM2, LOSM3, and LOSM4, respectively). Antigen well contained *S. mutans* OMZ175 WEA at 0.5 mg/ml.

nitrocellulose paper, only the low-molecular-weight (76K) component was revealed by the four monoclonal antibodies (Fig. 3g). This was also confirmed by SDS-PAGE of periodate-treated WEA, directly stained with Coomassie blue, which showed the disappearance of the 125K component. These results suggest the presence of carbohydrate moieties in the 125K antigen.

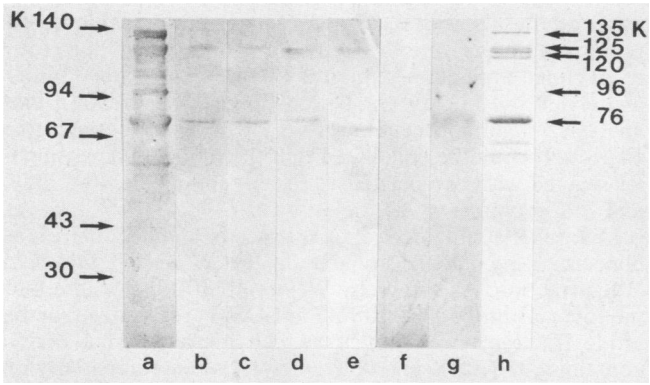

FIG. 3. Western blot analysis of electrophoretically resolved *S. mutans* OMZ175 WEA fractions (lanes a to e) and WEA fractions treated with pronase (f) or $NaIO_4$ (g) before electrophoresis. The electrophoretic blots were treated with rat anti-WEA serum (lane a) or monoclonal antibodies LOSM2 (lane b), LOSM1 (lane c), LOSM3 (lane d), and LOSM4 (lanes e to g), followed by peroxidase-conjugated MARK-1 and the addition of substrate. Coomassie blue staining of the WEA fraction on polyacrylamide gel (lane h). The arrows on the left indicate the position of the molecular weight markers, and the arrows on the right indicate the molecular weight of the major antigens contained in the WEA fraction. Molecular weight standards are represented by the following: lactate dehydrogenase, 140; phosphorylase b, 94; bovine serum albumin, 67; ovalbumin, 43; and carbonic anhydrase, 30.

Monoclonal antibodies LOSM1 to LOSM4 used in the Western blot study were also used for immunoprecipitation experiments. When whole *S. mutans* OMZ175 cells were incubated with the four monoclonal antibodies extracted with Triton X-100 and antigen-antibody complexes precipitated with MARK-1–Sepharose, 125K and 76K antigens were present in the immunoprecipitates (Fig. 4a to d). Thus, these results confirm the ability of the monoclonal antibodies to bind to the native antigens as well as the surface localization of the antigens. No immunoprecipitation of the surface antigens was seen with *S. mutans* incubated with rat nonimmune IgM molecules.

Epitope analysis. The distribution of the antigenic determinants on 125K and 76K proteins was studied in a competitive ELISA procedure by using the four IgM monoclonal antibodies labeled with AP. ELISA had shown that antibody concentrations of 2 μg/ml corresponded to saturating levels for each monoclonal antibody (Fig. 1a to d). Controls showed that 90 to 98% of AP-labeled antibodies could be displaced by 20 μg/ml concentrations of homologous unlabeled antibodies (Fig. 5a to d).

Notable differences were seen in the ability of heterologous antibodies to inhibit binding (Fig. 5a to d). AP-labeled LOSM1 binding was 90% inhibited by a 20 μg/ml concentration of LOSM1 antibody and was 70, 55, and 35% inhibited by monoclonal antibodies LOSM4, LOSM2, and LOSM3, respectively (Fig. 5a). The binding of AP-labeled LOSM2 antibody was completely inhibited by homologous antibody; there was a 85% inhibition by antibodies LOSM3 and LOSM4 and 75% inhibition by antibody LOSM1 (Fig. 5b). When LOSM3 was the labeled antibody, 88, 80, and 70% inhibitory activity was obtained with monoclonal antibodies LOSM2, LOSM4, and LOSM1, respectively (Fig. 5c). On the other hand, antibodies LOSM1, LOSM2, and LOSM3 exhibited almost the same inhibitory effect on AP-labeled LOSM4 binding as did the homologous antibody (Fig. 5d).

Interaction of the monoclonal antibodies with WEA fractions from various *S. mutans* serotypes. Monoclonal antibodies specific for 125K and 76K proteins of *S. mutans* serotype f were tested by ELISA for binding to WEA fractions of the

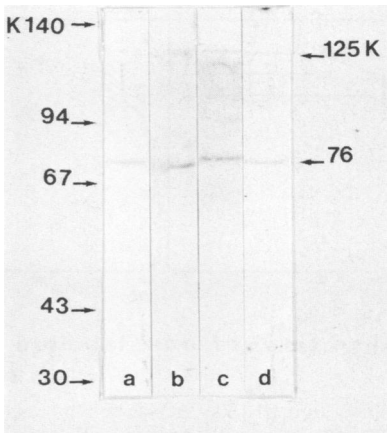

FIG. 4. Western blot analysis of *S. mutans* OZM175 antigens obtained after immunoprecipitation with monoclonal antibodies LOSM1 (lane a), LOSM2 (lane b), LOSM3 (lane c), and LOSM4 (lane d). Arrows on the left indicate the position of molecular weight markers, and arrows on the right indicate the position of the 125K and 76K antigens. Molecular weight standards are the same as described in the legend to Fig. 3.

six other *S. mutans* serotypes. Results for LOSM1, LOSM2, LOSM3, and LOSM4 are shown in Fig. 1a to d. Monoclonal antibody LOSM1 showed a quite identical reactivity with WEA from *S. mutans* serotypes f and b, reduced activity against WEA from serotypes c, e, and a, and weak binding to serotypes d and g (Fig. 1a). Monoclonal antibodies LOSM2 and LOSM3 exhibited the same specificity as did LOSM1 against the various *S. mutans* serotypes; however, antibody LOSM3 showed a lower binding to the recognized epitopes, as suggested by the shape of the ELISA curves. On the other hand, LOSM4 reacted strongly to WEA from serotypes b and d, to a lesser extent to serotypes c, e, and a, and weakly with serotype g. Thus, the epitopes recognized by the four monoclonal antibodies on 125K and 76K proteins from serotype f are apparently present in all of these *S. mutans* strains.

To confirm the presence of a common epitope in the various serotypes, we reinvestigated the above results by a competitive ELISA analysis. Increasing amounts of WEA fractions from *S. mutans* serotypes were preincubated with adequately diluted monoclonal antibodies and then tested on homologous strains for remaining binding activity. A nearly complete inhibition of binding for the four monoclonal antibodies was achieved with the homologous WEA fraction at a protein concentration higher than 25 μg/ml.

The results for heterologous competition are shown in Table 1, and the data presented correspond to the inhibition of binding obtained with an identical amount of WEA (25 μg of protein per ml). In the solid-phase competitive assay, the crude WEA fraction from serotypes a, b, c, and e is able to inhibit the binding of the four monoclonal antibodies to immobilized WEA from *S. mutans* serotype f by >50%. WEA from serotype d showed only an inhibitory effect for antibody LOSM4, whereas WEA from serotype g had little effect on the binding of the four monoclonal antibodies to homologous WEA. On the other hand, the four monoclonal antibodies did not react with an unrelated *B. subtilis* strain.

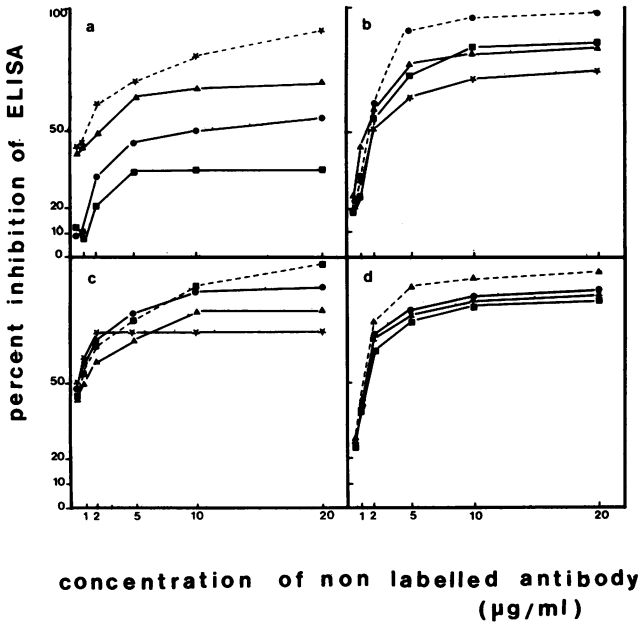

FIG. 5. Competitive ELISA. AP-labeled monoclonal antibody preparations were added simultaneously with a dilution of nonlabeled monoclonal antibody to WEA-coated wells. Labeled antibodies were LOSM1 (a), LOSM2 (b), LOSM3 (c), and LOSM4 (d). The purified antibodies used to inhibit the binding of conjugates were LOSM1 (★), LOSM2 (●), LOSM3 (■), and LOSM4 (▲). Broken lines indicate homologous competition in each case. Controls containing rat IgM without anti-WEA activity showed less than 10% inhibition of binding.

TABLE 1. ELISA inhibition assays of the four monoclonal antibodies binding to *S. mutans* OMZ175 WEA with WEA antigens from seven different *S. mutans* serotypes

Serotype	% Inhibition of binding of monoclonal antibodies to S. mutans OMZ175 WEA[a]			
	LOSM1	LOSM2	LOSM3	LOSM4
f	91	87	86	94
a	54	47	52	59
b	60	83	60	89
c	74	79	76	73
d	20	21	21	74
e	68	79	75	72
g	10	0	0	23

[a] Monoclonal antibodies were used at concentrations required for maximal binding in ELISA with *S. mutans* OMZ175 WEA as the solid phase. Inhibition experiments were performed in triplicate with at least four different concentrations of heterologous antigens. For ease of comparison, data from only one quantity of WEA are shown (25 μg of protein).

These data confirm the results shown above for direct ELISA and suggest that WEA from serotype d bears a common epitope only recognized by monoclonal antibody LOSM4. The low inhibitory effect of WEA from serotype g could be due either to the fact that serotype g expresses slightly cross-reactive epitopes or that crude WEA fraction from serotype g contains very weak amounts of 125K and 76K proteins.

DISCUSSION

We report here the successful production and partial characterization of four IgM monoclonal antibodies directed against cell wall proteins from *S. mutans* serotype f and describe the distribution of the recognized antigenic determinants among the *S. mutans* serotypes. The four monoclonal antibodies exhibited unique immunoreactivities as defined by four different procedures, i.e., Western blot analysis, immunoprecipitation, ELISA, and competitive ELISA. It could be concluded that the monoclonal antibodies reacted with two partially different epitopes on the 125K and 76K proteins.

The results obtained above lead to some conclusions concerning recognized antigens in the *S. mutans* OMZ175 WEA fraction. As shown by Western blot transfer, the four monoclonal antibodies LOSM1 to LOSM4 recognized, in the crude fraction, two antigens which migrated in a region containing the 125K and 76K antigens when compared with SDS-PAGE profiles or with blots revealed with rat anti-WEA serum of identical extracts (16). The precipitation of WEA antigens with the monoclonal antibodies could be explained by the existence of an aggregated form of 125K and 76K antigens in the crude WEA extract which could be considered as a component with repetitive units. Indeed, preliminary data indicate that purified 76K protein exists as an aggregate with a molecular weight of more than 300,000 in nondenaturing conditions (unpublished data). The four monoclonal antibodies are also able to react with the native form of both antigens, as assessed by immunoprecipitation and ELISA. The 125K and 76K antigens are surface located, as shown by the ability of the monoclonal antibodies to

immunoprecipitate them. The protein or protein-associated nature of both antigens was demonstrated by their pronase sensitivity.

The fact that the four monoclonal antibodies recognized common epitopes present on two antigens with very different molecular weights could be due to the existence of a dimeric form (125K) of 76K protein, to degradation of protein 125K, or to the presence of common epitopes in two unrelated antigens. Degradation products have been observed by Russell et al. for purified protein I/II from *S. mutans* serotype c which splits into three different components (A, B, and C) after SDS-PAGE (19) or for purified protein B from *S. mutans* serotype c which gives multiple bands in Western blot analysis with corresponding antiserum, a problem which is probably due to the degradation of antigen B (23). The disappearance of 125K protein in gels or blots after periodate treatment of the crude WEA fraction suggests either that, in the initial extract, the 76K component could exist as a protein carbohydrate complex or that 125K antigen, which is a glycoprotein (16), is destroyed by periodate. However, at the present time we do not have enough data allowing us to choose between the three hypothesis, and further investigations would be necessary to clarify this point.

The distribution of the epitopes recognized by the four monoclonal antibodies on *S. mutans* OMZ175 125K and 76K cell surface proteins was investigated by using AP-labeled monoclonal antibody in a competitive ELISA procedure. The results of epitope analysis suggest the existence of at least two partially different antigenic determinants on 125K and 76K proteins that are recognized by monoclonal antibody LOSM1 and by both antibodies LOSM2 and LOSM3. On the other hand, antibody LOSM4 seems to recognize a common part of the two epitopes.

We observed in ELISA significant differences in the interactions of the four monoclonal antibodies with cell wall proteins from various *S. mutans* serotypes. Monoclonal antibody LOSM4 detected the cross-reactive epitope on 125K and 76K proteins in all *S. mutans* serotypes, with the exception of serotype g which showed an appreciably weaker cross-reaction in ELISA. The epitope distribution in the six *S. mutans* serotypes was also confirmed by competitive ELISA. In contrast to the cross-reactive antibody LOSM4, monoclonal antibodies LOSM2 and LOSM3 reacted strongly with serotype b and significantly with serotypes c and e, the two latter serotypes being correlated to serotype f on the basis of their polysaccharide determinants. The cross-reactivity of both monoclonal antibodies was very weak with *S. mutans* serotypes d and g when compared with homologous serotype f. The epitope recognized by monoclonal antibody LOSM1 was apparently restricted to serotypes a, b, c, and e and, to a lesser extent, to the other serotypes. The epitope distribution observed among the various *S. mutans* serotypes with monoclonal antibodies LOSM4, LOSM2, LOSM3, and LOSM1 by competitive ELISA confirms the results obtained by direct ELISA. The quantitative differences in the reactivities of the four monoclonal antibodies against the wall-associated proteins from various *S. mutans* serotypes could also result from (i) various amounts of proteins in the various antigenic preparations, (ii) similar but not identical determinants, (iii) alterations of the antigenic sites giving rise to different antigen-antibody affinities, or (iv) different affinities of the monoclonal antibody for the different serotypes of *S. mutans*.

In conclusion, we have reported the production of four monoclonal antibodies specific for at least two different antigenic determinants present on 125K and 76K proteins from *S. mutans* serotype f. Data obtained by Western blot and immunoprecipitation showed that the four monoclonal antibodies are able to recognize both SDS-denaturated and native epitopes on 125K and 76K proteins. Using these monoclonal antibodies, we have demonstrated cross-reactivity and conservation of the specific epitopes among the various *S. mutans* serotypes. The detection of such epitopes is not surprising and confirms earlier observations of cross-reactivities among extracellular proteins in *S. mutans* serotypes, obtained with conventional antisera (16, 19, 23). Furthermore, the ability of all four monoclonal antibodies to bind to whole cells of *S. mutans* and previous data obtained by immunoprecipitation with polyclonal antiserum (16) suggest strongly that the antigenic determinants are surface located. These antibodies should be useful in studying the streptococcal surface proteins.

ACKNOWLEDGMENTS

We thank M. Schöller for helpful contributions to the paper. We also thank C. Genart, F. Nisol, T. Goffart, J. P. Kints, and A. L. Pini for excellent technical assistance and J. Kempler for preparation of the manuscript.

LITERATURE CITED

1. **Apicella, M. A., K. M. Bennet, C. A. Hermerath, and D. E. Roberts.** 1981. Monoclonal antibody analysis of lipopolysaccharide from *Neisseria gonorrhoeae* and *Neisseria meningitidis*. Infect. Immun. **34**:751–756.
2. **Avrameas, S., and B. Guilbert.** 1971. A method for quantitative determination of cellular immunoglobulins by enzyme labelled antibodies. Eur. J. Immunol. **1**:394–396.
3. **Bazin, H.** 1982. Production of rat monoclonal antibodies with the LOU rat non secreting IR 983 F myeloma cell-line. Prot. Biol. Fluids **29**:615–658.

3a. **Bazin, H., L. M. Xhuderbise, G. Burtonboy, A. M. Lebacq, L. Declerq, and F. Cormont.** 1984. Rat monoclonal antibodies. I. Rapid efficient purification for in vitro culture supernatant. J. Immunol. Methods **71**:9–16.

4. **Bazin, J., A. Beckers, G. Urbain-Vansanten, R. Pauwels, C. Bruyns, A. F. Tickin, B. Platteau, and J. Urbain.** 1978. Transplantable IgD immunoglobulin secreting tumors in rats. J. Immunol. **121**:2077–2082.
5. **Bazin, M., A. Beckers, and P. Querinjejean.** 1974. Three classes and four subclasses of rat immunoglobulins: IgM, IgA, IgE, and IgG, IgG2a, IgG2b, IgG2c. Eur. J. Immunol. **4**:44–48.
6. **Cisar, J. O., E. L. Barsumian, S. H. Curl, A. E. Vatter, A. N. Sandberg, and R. P. Siracanian.** 1981. Detection and localization of a lectin on *Actinomyces viscosus* T1 4V by monoclonal antibodies. J. Immunol. **127**:1318–1322.
7. **De Blas, A. L., and H. M. Cherwinski.** 1983. Detection of antigens on nitrocellulose paper immunoblots with monoclonal antibodies. Anal. Biochem. **133**:214–219.
8. **Forester, H., N. Hunter, and K. W. Knox.** 1983. Characteristics of high molecular weight extracellular protein of *Streptococcus mutans*. J. Gen. Microbiol. **129**:2779–2788.
9. **Greenwood, F. W., W. M. Hunter, and J. S. Glover.** 1963. The preparation of ^{131}I labelled human growth hormone of high specific activity. Biochem. J. **89**:114–123.
10. **Gustafsson, B., A. Rosén, and T. Holme.** 1982. Monoclonal antibodies against *Vibrio cholerae* lipopolysaccharide. Infect. Immun. **38**:449–454.
11. **Hamada, S.** 1983. Role of glucosyltransferase and glucan in bacterial aggregation and adherence to smooth surfaces. Chem. Sens. (Special suppl.):31–38.
12. **Hamada, S., and H. D. Slade.** 1980. Biology, immunology, and cariogenicity of *Streptococcus mutans*. Microbiol. Rev. **44**:331–384.

13. **Köhler, G., and C. Milstein.** 1975. Continuous cultures of fused cells secreting antibody of predefined specificity. Nature (London) **256**:495–497.
14. **Laemmli, U. K.** 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature (London) **227**:680–685.
15. **McGhee, J. R., and S. M. Michalek.** 1981. Immunobiology of dental caries: microbial aspects of local immunity. Annu. Rev. Microbiol. **35**:595–638.
16. **Ogier, J. A., J. P. Klein, P. Sommer, and R. M. Frank.** 1984. Identification and preliminary characterization of saliva-interacting surface antigens of *Streptococcus mutans* by immunoblotting, ligand blotting, and immunoprecipitation. Infect. Immun. **45**:107–112.
17. **Ono, E., M. Naiki, and R. Yanagawa.** 1982. Production and characterization of monoclonal antibodies to lipopolysaccharide antigen of *Leptospira interrogans* serovar Kremastos and Canicola. Zentralbl. Bakteriol. Parasitenkd. Infektionskr. Hyg. Abt. 1 Orig. Reihe A **252**:414–424.
18. **Robertson, S. M., C. F. Frisch, P. A. Gulig, J. R. Kettman, K. H. Johnston, and E. J. Hansen.** 1982. Monoclonal antibodies directed against a cell surface-exposed outer membrane protein of *Haemophilus influenzae* type b. Infect. Immun. **36**:80–88.
19. **Russell, M. W., L. A. Bergmeier, E. D. Zanders, and T. Lehner.** 1980. Protein antigens of *Streptococcus mutans*: purification and properties of a double antigen and its protease-resistant component. Infect. Immun. **28**:486–493.
20. **Russell, M. W., E. D. Zanders, L. A. Bergmeier, and T. Lehner.** 1980. Affinity purification and characterization of protease-susceptible antigen I of *Streptococcus mutans*. Infect. Immun. **29**:999–1006.
21. **Russell, R. R. B.** 1979. Glucan-binding proteins of *Streptococcus mutans* serotype c. J. Gen. Microbiol. **112**:197–201.
22. **Russell, R. R. B.** 1979. Wall associated protein antigens of *Streptococcus mutans*. J. Gen. Microbiol. **114**:109–115.
23. **Russell, R. R. B., S. L. Peach, G. Colman, and B. Cohen.** 1983. Antibody responses to antigens of *Streptococcus mutans* in monkeys (Macaca fascicularis) immunized against dental caries. J. Gen. Microbiol. **129**:865–875.
24. **Schöller, M., J. P. Klein, and R. M. Frank.** 1981. Common antigens of streptococcal and non-streptococcal oral bacteria: immunochemical studies of extracellular and cell-wall-associated antigens from *Streptococcus sanguis*, *Streptococcus mutans*, *Lactobacillus salivarius*, and *Actinomyces viscosus*. Infect. Immun. **31**:52–60.
25. **Schöller, M., J. P. Klein, P. Sommer, and R. M. Frank.** 1983. Common antigens of streptococcal and nonstreptoccal oral bacteria: characterization of wall-associated protein and comparison with extracellular protein antigen. Infect. Immun. **40**:1186–1191.
26. **Towbin, H., T. Staehelin, and J. Gordon.** 1979. Electrophoretic transfer of proteins form polyacrylamide gels to nitrocellulose sheets: procedures and source applications. Proc. Natl. Acad. Sci. U.S.A. **76**:4350–4354.
27. **Virji, M., J. E. Heckels, and P. J. Watt.** 1983. Antigenic cross reactivity of Neisseria pili: investigations with type and species-specific monoclonal antibodies. J. Gen. Microbiol. **129**:2761–2768.