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Antibodies that Bind to Fimbriae Block Adhesion of *Streptococcus sanguis* to Saliva-Coated Hydroxyapatite

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Antibodies raised against a fimbriated, adhesive strain of *Streptococcus sanguis* (FW213) were found to block the adhesion of this organism to saliva-coated hydroxyapatite. Antibodies were made specific for adhesion antigens by (i) adsorption with isogenic, nonadhesive mutants (for rabbit polyclonal adsorbed antibody) or (ii) selection based on nonreactivity with two nonadhesive mutants (for monoclonal antibody). Rabbit antibody raised against isogenic, nonfimbriated nonadhesive mutants served as a control for antibodies present, but not related to fimbriation. Adsorbed antibody and monoclonal antibody were shown to be specific for fimbriae (antigen 1), since both antibodies (i) could be seen by immune electron microscopy to bind to 3.6-nm fimbriae, (ii) reacted only with the fimbriated parent and not the mutants in a whole bacterial cell enzyme-linked immunosorbent assay, and (iii) could immunoprecipitate fimbriae from fimbrial extracts of FW213. Antibodies isolated from preimmune and mutant sera did not react with fimbriae in any of the above assays. Only adsorbed antibody and monoclonal antibody were capable of blocking the adhesion of FW213 to saliva-coated hydroxyapatite. Adsorbed antibody, purified to immunoglobulin G (IgG), was an effective inhibitor of adhesion without causing interfering cellular aggregation. Monoclonal IgG, papain-cleaved to Fab fragments to prohibit cell-to-cell cross-linking, was also a potent inhibitor of *S. sanguis* FW213 adhesion. Both IgG from mutant sera and Fab fragments from normal mouse IgG could not be shown to block adhesion. These data further support the hypothesis that *S. sanguis* fimbriae are involved in adhesion.

The ability of organisms to adhere to various body and cell surfaces has been recognized as a major step in the pathogenesis of many organisms. Dental caries and periodontal disease occur only after bacterial colonization and the formation of dental plaque (15). It has long been recognized that *Streptococcus sanguis* is the first organism to adhere to the teeth (3) and, as such, initiates dental plaque formation.

Little is known about the molecular basis of the adhesion of *S. sanguis* to the teeth or to the in vitro tooth model, saliva-coated, spheroidal hydroxyapatite (SC-SHA). Various researchers have proposed lectin-like interactions (25), hydrophobic interactions (11, 26), specific protein interactions (8, 21), and combinations of the three (12, 24).

This study was undertaken to provide an immunological analysis of *S. sanguis* adhesion. This approach has proven vital in understanding the adhesion of other organisms. With both polyclonal and monoclonal antisera it was determined that fimbriae are involved in the adhesion of *S. sanguis* to SC-SHA.

MATERIALS AND METHODS

Abbreviations. The following abbreviations were used. Saliva-coated spheroidal hydroxyapatite (SC-SHA); phosphate-buffered saline, 0.05M, pH 7.4 (PBS); phosphate buffer, 0.067 M, pH 6.0 (adhesion buffer); immunoglobulin G (IgG); polyclonal immunoglobulin fraction (PAb); PAb adsorbed with a mutant (AdAb); purified monoclonal antibody (MAb); papain-cleaved IgG (Fab).

Bacterial strains. *S. sanguis* FW213 (obtained from R. Cole, National Institutes of Health) was the primary organism used in this study. In addition, isogenic mutants of *S. sanguis* FW213, nonadhesive to SC-SHA, were isolated by J. Larson, University of Vermont (strain VT507), and D. Thompson, University of Vermont (strain VT321). All strains were kept frozen at −70°C, and portions were removed weekly for use.

Media, reagents, and supplies. The following sources were used: Amersham Corp., Arlington Heights, Ill., for [^{3}H]-thymidine; BBL Microbiology Systems, Cockeysville, Md., for Trypticase soy broth; BDH Chemicals Ltd., Poole, England, for spheroidal hydroxyapatite beads; Bio-Rad Laboratories, Richmond, Calif., for acrylamide, sodium dodecyl sulfate, *N,N'*-methylene-bis-acrylamide, glycine, ammonium persulfate, and Coomassie brilliant blue R250; Cappel Laboratories, Cochranville, Pa., and Dynatech Diagnostics, Windham, Maine, for alkaline phosphatase-conjugated, affinity-purified goat anti-mouse and goat anti-rabbit IgG. Difco Laboratories, Detroit, Mich., for Todd-Hewitt broth and tryptose blood agar base; FMC Corp., Bioproducts, Rockland, Maine, for gelbond film; Krutulis, Inc., Bridgeport, N.Y., for defibrinated sheep's blood; LKB, Gaithersburg, Md., for agarose-M; New England Nuclear Corp., Boston, Mass., for Aquasol-2; Nuclepore Corp., Pleasanton, Calif., for polycarbonate membrane filter; Sigma Chemical Co., St. Louis, Mo., for agar, barbital, citric acid, diethanolamine, EDTA, iodoacetamide, and Tris; Ted Pella Inc., Tustin, Calif., for phosphotungstic acid, 200-mesh copper grids, carbon grating replica, and Formvar resin; Whatman Ltd., Clifton, N.J., for carboxy-methyl cellulose 52 and diethylaminoethyl cellulose 52; Worthington Diagnostics, Freehold, N.J., for papain.

Growth conditions. *S. sanguis* cells were inoculated from a weekly stock plate onto a plate containing tryptose blood agar base with 5% defibrinated sheep blood and incubated for 12 to 15 h at 36°C in 5% CO_2. The cells were harvested into Todd-Hewitt broth to a concentration of approximately 1.5×10^8 cells per ml, incubated aerobically at 36°C with gentle agitation, and assayed turbidimetrically until an optical density equivalent to 5.5×10^8 bacteria per ml was

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reached. The cells were washed three times by centrifugation at 12,000 × *g* for 15 min at 4°C with PBS. *S. sanguis* cells for adsorption experiments were grown on 33- by 23-cm plates containing Trypticase soy agar for 12 to 13 h at 36°C in 5% CO_2. The cells were harvested and washed three times by centrifugation.

Polyclonal antibody production. Rabbits were injected subcutaneously with 10^9 live *S. sanguis* cells in sterile saline. After 1 to 2 months, the rabbits were boosted intravenously three times per week with live bacteria for a period of 2 weeks. The inoculum size for boosting was increased from 10^8 cells to 10^9 cells over the 2-week period. The animals were then bled by cardiac puncture, and the sera were harvested.

Adsorption of rabbit polyclonal antisera. Polyclonal antisera were enriched for imunoglobulins by ammonium sulfate precipitation as described under antibody purification. The immunoglobulin fraction from the polyclonal antisera (PAb) was enriched for adhesion antibody by adsorbing the PAb eight times with approximately 0.4 g (wet weight) of cells from a nonadhesive mutant at 36°C with the 4th and 8th adsorption at 4°C. The mutant cells and PAb were rocked for 30 min and then centrifuged at 12,000 × *g* for 15 min, and the pellet was discarded. The adsorbed supernatant (AdAb) was used directly or further purified to IgG.

Monoclonal antibody production and selection. The production of monoclonal antibody was based on techniques developed by Kohler and Milstein (19) and modified by Gefter et al. (10). Briefly, mice were immunized on days 0, 35, and 140 with live *S. sanguis* cells. The spleen cells were removed and fused with myeloma line x63Ag8.653 (18) by using polyethylene glycol 1000. The resulting hybridoma colonies were screened in a whole bacterial cell enzyme-linked immunosorbent assay (bactELISA) for antibody reactive with *S. sanguis* FW213 and nonreactive with two isogenic nonadhesive mutants (VT321, VT507). These cells were cloned by limiting dilution on a rat thymocyte feeder layer, retested for antibody reactivity, and then expanded and injected into pristane-primed mice for ascites fluid development. Ascites fluid containing normal mouse immunoglobulins was obtained by injecting pristane-primed mice with the parental myeloma line x63Ag8.653.

Antibody purification. The antibodies used in this study were purified by several different techniques. Polyclonal antisera were partially purified by serial ammonium sulfate precipitation at 50, 40, and 33% saturation, each time reserving the pellet, followed by dialysis against PBS. Protein concentration was determined using a Bio-Rad protein assay based on the method of Bradford (2) with bovine gamma globulin as a standard. The immunoglobulin fraction (PAb) was further purified by ion-exchange chromatography on a DE52 column equilibrated with 0.0175 M phosphate buffer (pH 7.0). The breakthrough fraction was collected and found to contain IgG by both 12.5% sodium dodecyl sulfate–polyacrylamide gel electrophoresis (20) and immunoelectrophoresis against goat anti-rabbit IgG (16). Antibody-containing ascites fluid was partially purified by ammonium sulfate precipitation at 45% saturation. MAb, designated as F51, was prepared by cycles of cold precipitation in PBS. Immunochemical analyses showed that the preparation was homogeneous IgG3. Nonimmune ascites fluid was dialyzed into 0.02 M Tris hydrochloride (pH 8.0) and further purified by ion-exchange chromatography with DE52 with a KCl gradient of 0 to 0.2 M. The IgG-containing peak was confirmed by its reaction with rabbit anti-mouse IgG in an ouchterlony diffusion assay (27). Protein concentrations of purified IgGs were calculated by UV absorbance at 280 nm with an extinction coefficient of 14.3.

Fab production. Papain-cleaved IgG (Fab) fragments were prepared from purified IgG as originally described by Porter (28). A final concentration of 2% papain was added to a solution of 5 to 10 mg of IgG in 0.2 M cysteine. The mixture was incubated for 2 h at 37°C. Proteolysis was ended, and sulfhydryl groups were alkylated by raising the pH to 8 to 9 with 1 M Tris base, followed by the addition of iodoacetamide (final concentration, 0.025 M) and a 15-min incubation at 37°C. The extent of cleavage was monitored by 12.5% sodium dodecyl sulfate–polyacrylamide gel electrophoresis.

bactELISA. The bactELISA has been described previously (6).

Immune electron microscopy. *S. sanguis* cells grown and washed as described above were suspended to 5.4 × 10^8 bacteria per ml in PBS. Immunoglobulin fractions of AdAb, MAb, and nonimmune antibody were diluted in PBS and mixed with 1 ml of the *S. sanguis* cell suspension. The bacteria-antibody mixture was incubated for 60 min at 4°C then centrifuged at 12,000 × *g* for 15 min. The pellet was thoroughly drained and then suspended in 0.1 ml of PBS. One drop of the antibody-coated bacteria was mixed with a drop of 3% phosphotungstic acid and applied to a Formvar-coated copper specimen grid. After 1 min, the excess fluid was blotted away; the grid was dried and then examined in a Phillips 300 electron microscope at 60 kV. Calibration was performed with a waffled carbon grating replica.

Ferritin-labeled immune electron microscopy. *S. sanguis* cells were treated as described above. However, after antibody coating, the bacteria were washed three times with PBS to remove nonbound antibody. A ferritin-conjugated goat anti-mouse IgG was diluted 1:100 in PBS, and the bacteria were suspended in 1 ml of this diluted conjugate. After 30 min of incubation at room temperature, the bacteria were washed three times in PBS, stained, and examined as described above.

Preparation of antigen extracts. Streptococcal surface antigens were removed from *S. sanguis* FW213 (adhesive, fimbriated parent) and VT321 (an isogenic, nonadhesive, nonfimbriated mutant [9]) by high-speed pulse blending in a Sorvall omnimixer (16,000 rpm, 20 times for 1 min, 4°C) in 3 M potassium iodide. Potassium iodide, a chaotropic agent (17), was used to prevent the hydrophobic fimbriae from pelleting with the cells. Cells were removed by centrifugation at 12,000 × *g* for 15 min, and the bacterial wet weight was determined. The supernatant fluid containing the fimbriae was dialyzed to remove potassium iodide and concentrated by lyophilization. To maintain in the extracts the relative proportion of antigens found in the parent and mutants, the lyophilized material was suspended in sterile, distilled water to a concentration equivalent to 3 g (wet weight) of cells per ml. Nonsoluble material was removed by a 3-min spin in a microcentrifuge, model 235B (Fisher Scientific Co., Pittsburgh, Pa.). Further purification of the fimbrial antigen was accomplished by isopycnic centrifugation in 3.5 M potassium iodide for 16 h at 58,000 rpm in a Beckman L2-65B ultracentrifuge fitted with a type 65 fixed-angle rotor (Beckman Instruments, Inc., Palo Alto, Calif.). Only the visible band from FW213 at a buoyant density of 1.42 g per ml contained free fimbriae and was reactive with both AdAb and MAb (unpublished data).

Immunoelectrophoresis of antigen extracts. Antigens present in cell extracts were separated electrophoretically by crossed immunoelectrophoresis (5) in 12 ml of 1% agarose (Tris-barbiturate buffer [pH 8.6]; ionic strength, 0.02), poured

onto an 8.4- by 9.4-cm glass plate. A 5-μl sample of antigen extract was placed into a well and electrophoresed for 1.5 h at 10 V/cm on a cooled (4°C) LKB Multiphor apparatus (LKB Products, Bromma, Sweden). The gel was trimmed to isolate the agarose strip containing the antigens and transferred to a 7- by 8.4-cm Gelbond film. For the second dimension of electrophoresis, 150 μl of PAb was added to 6 ml of molten 1% agarose (cooled to 50°C) and poured onto the remaining area of the Gelbond film, forming a connection with the agarose strip containing the separated antigens. Electrophoresis was performed at 2 V/cm for 16 to 25 h. To prepare the gel for staining, it was pressed twice for 3 min with several layers of filter paper and paper towels under a flat weight and then washed for at least 45 min in several changes of 0.1 M NaCl. The gel was pressed once more and then dried at 50°C. Staining was done for 5 min in 1% Coomassie blue in 45% ethanol–10% glacial acetic acid. Destaining was done in the same solution, without Coomassie blue.

Immunoprecipitation of fimbriae. (i) Blended extracts. A 10-μl sample of MAb, AdAb, or PBS was added to 5 μl of blended strain FW213 extract, incubated at 37°C for 15 min and loaded onto a crossed immunoelectrophoresis gel for separation and identification. Only unbound antigen is able to move through the 1% agarose gel.

(ii) Gradient fractions. A 5-μl sample of MAb was added to 2 μl of the dialyzed band of 1.42 g/ml obtained from isopycnic centrifugation of blended extracts of *S. sanguis* FW213 and VT321. These preparations were viewed by phase microscopy in a Zeiss Photomicroscope III (Atlantex and Zieler Instrument Corp., Dedham, Mass.) at ×800. Material without antibody and antibody alone served as controls.

Adhesion of *S. sanguis* to SC-SHA. (i) Clarification of saliva. To minimize batch-to-batch variability of adhesion factors in saliva, large pools (ca. 3.0 liters) of paraffin-stimulated saliva from over 200 volunteers were collected on ice. The saliva was clarified by centrifugation at 17,000 × *g* for 10 min at 4°C. The supernatant fluid was heated to 60°C for 30 min to inactivate enzymes that destroy the adhesion factors (14). Sodium azide was added to a final concentration of 0.05%, and the clarified saliva was frozen in 10-ml portions at −30°C.

(ii) Hydroxyapatite. Spherodial hydroxyapatite beads were suspended in 0.067 M, pH 6.0 phosphate buffer (adhesion buffer) and settled for 5 min. The supernatant fluid containing the fine particles was aspirated. This washing was repeated four times. The remaining hydroxyapatite beads were resuspended in adhesion buffer and captured on a 12-μm polycarbonate membrane filter. The beads were allowed to dry and were distributed in 40-mg portions.

(iii) Preparation of SC-SHA. One milliliter of pooled, clarified saliva was added to tubes containing 40 mg of hydroxyapatite beads and incubated with rocking sufficient to keep the beads in suspension for 1 h at 37°C. The beads were allowed to settle, and the supernatant fluid was removed and discarded. The beads were washed three times in adhesion buffer.

(iv) Adhesion assay. The adhesion assay used was a modification of that developed by Gibbons et al. (13). Broth-grown log-phase FW213 cells were labeled in Todd-Hewitt broth with 2.0 μCi of [^{3}H]thymidine per ml. Labeled cells were centrifuged and washed three times with adhesion buffer. Cell suspensions, ranging from 5×10^8 to 5×10^9 cells per ml, were made in adhesion buffer and sonicated for 15 s at 95 W in a Bronson sonifier with an ultrasonic cuphorn. A 100-μl sample was removed from the concentration of 10^9 cells per ml to determine the specific activity of the cells. Samples (1 ml) of each cell concentration were added to duplicate tubes of SC-SHA and incubated for 1 h at 37°C with gentle rocking. The beads were allowed to settle, and 200 μl of the supernatant fluid was removed to determine the free cell concentration. The beads were then washed three times in adhesion buffer and transferred to scintillation vials to determine the number of cells bound to SC-SHA. All samples were counted in a Beckman scintillation counter (model LS 7500). The number of bound cells was plotted against the number of free cells.

TABLE 1. Characterization of ammonium sulfate-precipitated polyclonal antiserum by bactELISA

Antibody specificity	Antibody activity[a] in bactELISA with *S. sanguis* (μg/ml)		
	FW213	VT321	VT507
Preimmune	>256.0	>256.0	>256.0
FW213	0.5	1.0	1.0
FW213/VT321[b] (AdAb)	2.0	>256.0	>256.0
FW213/VT507[b] (AdAb)	16.0	>256.0	>256.0
VT321	1.0	0.5	1.0
VT507	2.0	4.0	2.0

[a] Activity is expressed as the protein concentration required to give an optical density of 0.5 in the bactELISA.
[b] Antibody was raised against the parent strain and absorbed with the mutant.

(v) Adhesion blocking assay. The effects of various antibody preparations on the adhesion of *S. sanguis* FW213 to SC-SHA was done by using the adhesion protocol described above with an additional step. After the *S. sanguis* cells were labeled with [^{3}H]thymidine and washed, they were suspended in adhesion buffer, or adhesion buffer plus antibody, and rocked for 1 h at 37°C. The cells were washed two times in adhesion buffer, suspended, and then added to the SC-SHA for the adhesion phase of the assay. The extent of blocking was calculated by comparing the number of antibody-coated cells that bound to SC-SHA with the number of buffer-coated cells.

Agglutination assay. *S. sanguis* FW213 cells were grown in Todd-Hewitt broth to a concentration of 2.8×10^8 bacteria per ml. Portions of 1 ml were placed in test tubes, and mixed with MAb. The antibody concentrations used ranged from 0.3 to 300 μg/ml. The bacteria-antibody mixtures were rocked for 1 h at 37°C and then examined by phase microscopy, as described above, for the presence of antibody-induced agglutinates. Controls included bacteria in PBS alone and bacteria mixed with normal mouse immunoglobulin.

RESULTS

Characterization of antibody. (i) Adsorbed antibody. Extensive adsorption of FW213 PAb with either mutant decreased the bactELISA reactivity of the sera for the mutant from control levels to that seen for preimmune serum (Table 1). These sera continued to show reactivity against the parent strain, although at a slightly diminished level. Serum adsorbed with one mutant had the same decreased reactivity against the second mutant as it did with itself. Sera raised against either nonadhesive mutant reacted equally well with the parent. These sera served as controls for antibody present, but not related to adhesion.

(ii) Monoclonal antibody. The monoclonal antibody-secret-

TABLE 2. Characterization of monoclonal Fab fragments by bactELISA

Fab source	Fab activity[a] in bactELISA with *S. sanguis* (μg/ml)	
	FW213	VT321
Normal mouse IgG	>60	>60
Monoclonal IgG (MAb)	3	>300

[a] Activity is expressed as the protein concentration required to give an optical density of 0.3 in the bactELISA.

ing line used in this study, F51, will be described completely in a separate paper (manuscript in preparation). Briefly, in the bactELISA, MAb reacted specifically with the adhesive parent strain, FW213, and was nonreactive with the two isogenic, nonadhesive mutants. The Fab fragments of MAb maintained high-titer, specific reactivity toward *S. sanguis* FW213 in the bactELISA. The MAb Fab fragments were strongly reactive at 3 μg/ml against *S. sanguis* FW213 (Table 2), whereas even concentrations of >300 μg/ml failed to show reactivity against strain VT321. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis of these fragments revealed no uncleaved IgG. The fragments were also tested for their functional ability to agglutinate *S. sanguis* FW213 cells. Uncleaved MAb was a potent agglutinator of these cells (Fig. 1A). After papain cleavage, the MAb was unable to agglutinate the FW213 cells (Fig. 1B) at any concentrations between 0.3 and 300 μg/ml.

Immune electron microscopy. AdAb coated fimbriae on intact *S. sanguis* (Fig. 2A) and fimbriae free in suspension (Fig. 2B). The antibody-coated fimbriae, both attached and free from the cell, exhibited a constant width of 3.6 nm (standard deviation, 0.45 nm). Neither preimmune nor mutant strain-directed antibodies were able to label fimbriae (Fig. 2C). The MAb coated the peritrichous fimbriae of *S. sanguis* FW213 (Fig. 3A), as determined with ferritin-labeled immune electron microscopy, but reacted with no structures on the mutants (Fig. 3B). Normal mouse immunoglobulins were used as a control to demonstrate that the ferritin conjugate did not nonspecifically bind to fimbriae (Fig. 3C).

Immunoelectrophoresis of antigen extracts. Immunoelectrophoretic analyses of *S. sanguis* FW213 blended extracts revealed that there were three distinct antigens recognized by the PAb made in response to the whole cell (Fig. 4A). For convenience, these antigens were labeled Ag1, Ag2, and Ag3 in the order they migrated from the origin. Similar analyses of the nonadhesive, nonfimbriated mutant, VT321, revealed that there were only two distinct antigens recognized by PAb (Fig. 4B). These data clearly demonstrate that there is only one antigenic difference between the parent strain and its isogenic mutant; that difference is Ag1. Since VT321 resulted from a single mutation, and the only observable difference in VT321 is a loss of fimbriae (9), these data imply that Ag1 is fimbriae. Similar analysis of nonfimbriated VT507 also demonstrated a loss of Ag1. In addition, immunoprecipitation of the FW213 blended extracts with AdAb or MAb resulted in the loss of only Ag1 from the antigen profile (Fig. 4C).

Immunoprecipitation of fimbriae. To demonstrate further that the MAb is specific for fimbriae, the MAb was used to immunoprecipitate free fimbriae that banded at a density of 1.42 g/ml in isopynic centrifugation. When fimbriae were immunoprecipitated they formed large, visible aggregates. When viewed by phase microscopy at ×800, these aggregates appeared as refractile large mats (Fig. 5A). Electron microscopy of these mats revealed the 3.6-nm fimbriae previously demonstrated with AdAb (Fig. 2B). The corresponding antibody-treated VT321 fraction (Fig. 5B) and the uncentrifuged VT321 extract, as well as the FW213 fraction without antibody and the antibody alone, showed no aggregates when viewed by phase microscopy. Analyses with AdAb gave similar results.

Adhesion-blocking ability of antibody. (i) Adsorbed antibody. Purified IgG from *S. sanguis* FW213 AdAb was capable of blocking the adhesion of FW213 to SC-SHA. At an IgG concentration of 100 μg/ml, adhesion was inhibited by greater than 90% (Table 3). Mutant VT507-directed IgG, with a bactELISA titer slightly higher than that of the AdAb, was unable to block adhesion at a concentration of 400 μg/ml. Antibody directed against *S. sanguis* FW213 formed small agglutinates (less than 10 cells in size) during incubation in the adhesion assay. Significantly, IgG directed against the nonadhesive mutants formed agglutinates equivalent in size (or larger) to those seen with AdAb IgG, yet adhesion was not blocked.

(ii) Monoclonal antibody. MAb cleaved to Fab fragments was found to be a potent and specific inhibitor of *S. sanguis* FW213 adhesion to SC-SHA. When these fragments, at concentrations from 40 to 250 μg/ml, were added to *S. sanguis* FW213, the adhesion of these cells was reduced 86 to 98% compared with untreated cells (Table 4). When normal mouse immunoglobulins, also cleaved to Fab fragments, were used in place of MAb, adhesion was decreased by only 0 to 16%, indicating that the blocking was specific for the MAb.

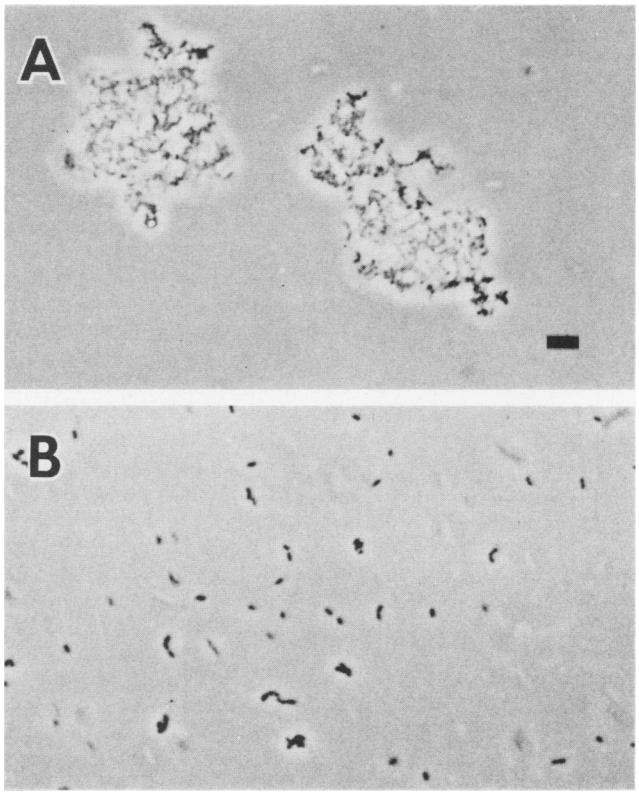

FIG. 1. Agglutination of *S. sanguis* FW213 after a 1-h incubation at 37°C with (A) MAb (1 μg) or (B) MAb Fab digest (300 μg). Bar, 5 μm.

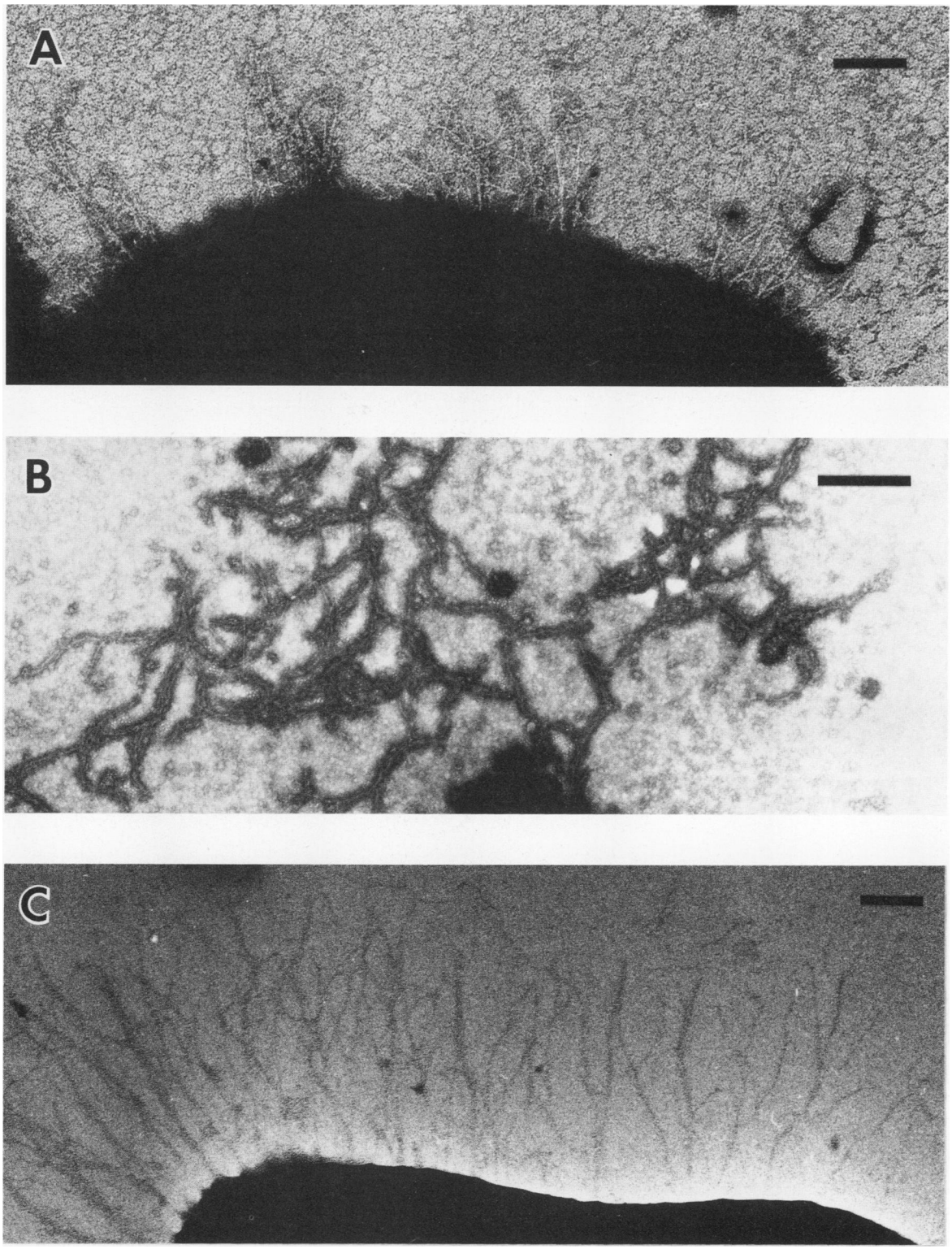

FIG. 2. Immune electron micrograph of *S. sanguis* FW213 negatively stained with 1.5% phosphotungstic acid with (A) adsorbed FW213 antiserum, (B) free fimbriae with adsorbed FW213 antiserum, or (C) preimmune serum. Bars, 0.1 μm.

DISCUSSION

The antibodies used in this study were raised against the adhesive parent and made specific for adhesion antigens by (i) adsorptions with isogenic, nonadhesive mutants (for AdAb) or (ii) selection based on nonreactivity with the nonadhesive mutants (for MAb). The nonadhesive mutants had originally been selected solely for their inability to

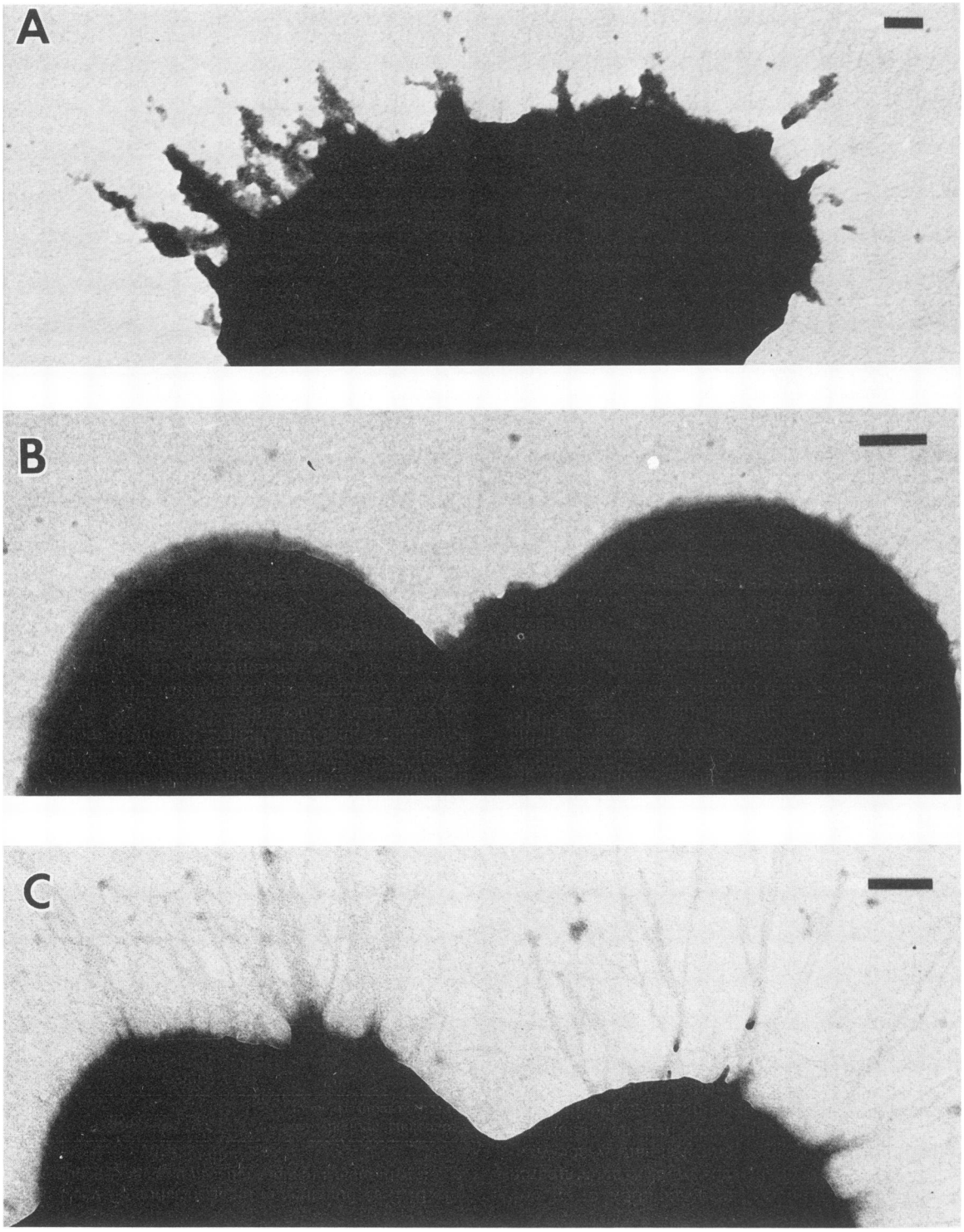

FIG. 3. Ferritin-labeled immune electron micrograph of *S. sanguis* (A) FW213 with MAb. (B) VT321 with MAb. (C) FW213 with normal mouse immunoglobulins. Bars, 0.1 μm.

adhere to SC-SHA (8, 9). This approach favored the identification of any surface structures involved in the adhesion of *S. sanguis* FW213 to the salivary pellicle. This is important since several groups of researchers have proposed that *S. sanguis* adhesion involves multiple types of bonds with differing affinities (12, 24). However, the analyses of these mutants indicated that only the fimbriae of *S. sanguis* play an important role in the adhesion of this organism to SC-SHA (9).

This study was undertaken to locate and identify addi-

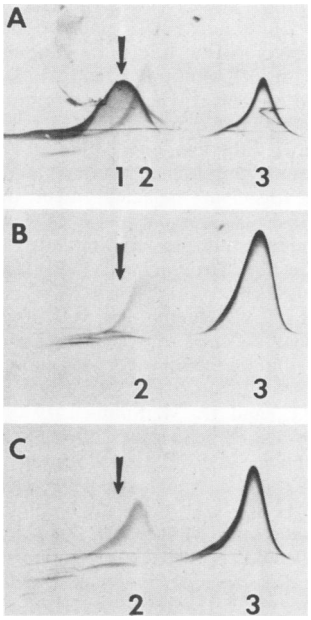

FIG. 4. Crossed immunoelectrophoresis of blended *S. sanguis* extracts run against polyclonal antisera raised in rabbits to whole *S. sanguis* FW213 cells. The anode is to the right and to the top of each panel. Ag1, Ag2, and Ag3 are labeled in the order they migrated from the origin. The antigen extracts separated in the first dimension were obtained from (A) FW213, (B) VT321, or (C) FW213 immunoprecipitated with MAb. The arrows indicate the presence (A) and absence (B and C) of Ag1.

tional antigens or surface structures involved in the adhesion of *S. sanguis* FW213 to SC-SHA. Crossed immunoelectrophoretic analyses demonstrated three major antigens associated with *S. sanguis* FW213 blended extracts. Similar analyses of the isogenic nonadhesive mutants used in this study revealed a single antigenic difference from the parent: a lack of Ag1. Since partially purified fimbriae, when examined by crossed immunoelectrophoresis, migrate in the same manner as Ag1 in blended extracts (data not shown), these data suggest that Ag1 and fimbriae are the same. Hence no additional antigens were found.

Both the AdAb and MAb used in this study were found to be specific for fimbriae. The evidence was fourfold. (i) Both AdAb and MAb could be seen by immune electron microscopy to bind to the 3.6-nm fimbriae on intact cells and free in suspension. These antibodies did not recognize any structures on the mutants. (ii) Both AdAb and MAb reacted with the fimbriated parent, but not the mutants, in a bactELISA assay. (iii) Both AdAb and MAb were observed by phase microscopy to precipitate the fimbriae from partially purified

TABLE 3. Adhesion blocking ability of adsorbed purified polyclonal IgG

IgG source	% Adhesion to SC-SHA at the following IgG concn (μg/ml)[a]:				
	400	100	50	12	0
Preimmune	92	89	95	83	100
FW213/VT507[b] (AdAb)	8	6	32	117	100
VT507	100				

[a] Protein concentration was determined by optical density at 280 nm and an extinction coefficient of 14.3.

[b] Antibody was raised against the parent strain and adsorbed with the mutant.

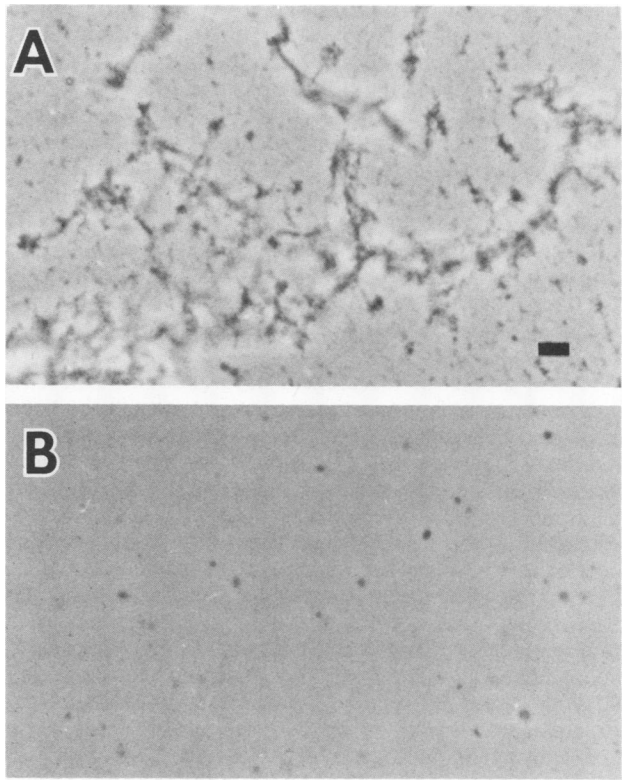

FIG. 5. Phase micrograph of immunoprecipitate formed by reacting MAb with *S. sanguis* (A) FW213 fimbriae isolated from isopynic centrifugation in potassium iodide (band at 1.42 g/ml) or (B) VT321 from the corresponding fraction at 1.42 g/ml. Bar, 5 μm.

fimbrial extracts of FW213. These antibodies did not immunoprecipitate any material from mutant extracts prepared in the same manner. (iv) When blended extracts of FW213 were immunoprecipitated with AdAb or MAb and analyzed by crossed immunoelectrophoresis, only Ag1 was missing.

We and other investigators (4, 23) studying bacterial adhesion utilized antibody in a monovalent form, after cleavage of the intact IgG to yield Fab fragments. In this manner, adhesion blocking by antibody is not a function of cell or fimbrial agglutination. Liljemark et al. (22) have found that agglutination of *S. sanguis* has a positive effect on adhesion when the aggregates are small (four to eight cells), but that the formation of large agglutinates decreases cell adhesion to SC-SHA. In this study, intact AdAb IgG, an effective inhibitor of *S. sanguis* FW213 adhesion, was only a weak agglutinator of *S. sanguis* FW213. The agglutinates formed were in the size range found by Liljemark et al. to have no effect, or even increase adhesion to SC-SHA. Antiserum directed against the nonadhesive mutant VT507 was seen to form agglutinates of FW213 equal to or even

TABLE 4. Adhesion-blocking ability of monoclonal Fab fragments

Fab source	% Adhesion to SC-SHA at the following Fab digest concn (μg/ml)[a]:			
	250	80	40	0
Normal mouse IgG	84	118	143	100
Monoclonal IgG (MAb)	2	5	14	100

[a] Protein concentration was determined by optical density at 280 nm.

larger in size than those produced by FW213 antiserum, yet was unable to inhibit the adhesion of *S. sanguis* FW213 to SC-SHA. This confirmed that adhesion blocking was due to specific antibody coating, rather than the effects of nonspecific agglutination. As opposed to the PAb, MAb was a potent agglutinating agent in its intact form, and Fab production was essential to assess its adhesion-blocking capabilities. The ability of this Fab fimbria-directed MAb to also block adhesion confirmed the results obtained with the AdAb and further substantiated the hypothesis that *S. sanguis* fimbriae are involved in adhesion. These data support the observations of other investigators that fimbriae often play a major role in the adhesion of many microorganisms (1, 8, 11, 29).

Several groups of researchers used antibodies that label fimbriae to identify a function for these organelles. Clark et al. (4) used blocking antibody to demonstrate two types of fimbriae on *Actinomyces viscosus* T14V, only one of which was involved in adhesion to SC-SHA. Mett et al. (23) found that fimbria-specific antibodies of *Escherichia coli* SS142 were able to both prevent and reverse the adhesion of this organism to human cells. Even commericial human immune serum globulin has been found to contain anti-fimbrial antibodies effective in reducing the adhesion of several urinary tract pathogens to bladder epithelial cells (7). Thus the finding that antibodies of *S. sanguis* that bind to fimbriae block the adhesion of this organism to SC-SHA indicates that this organism may share adhesion mechanisms in common with other pathogenic organisms. It also identifies a structure on *S. sanguis* that deserves further study. In our laboratory we have begun studies in this area.

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