INFECTION AND IMMUNITY, Dec. 1997, p. 5042–5051 Vol. 65, No. 12
0019-9567/97/$04.00+0

A Specific Cell Surface Antigen of *Streptococcus gordonii* Is Associated with Bacterial Hemagglutination and Adhesion to α2-3-Linked Sialic Acid-Containing Receptors

YUKIHIRO TAKAHASHI,[1]† ANN L. SANDBERG,[1] STEFAN RUHL,[1]‡
JACQUELINE MULLER,[2] AND JOHN O. CISAR[1]*

Oral Infection and Immunity Branch, National Institute of Dental Research, Bethesda, Maryland 20892,[1]
and Division of Viral Products, Center for Biologics Evaluation and Research,
Food and Drug Administration, Rockville, Maryland 20852[2]

Received 3 June 1997/Returned for modification 17 July 1997/Accepted 16 September 1997

A Ca^{2+}-independent lectin activity for α2-3-linked sialic acid-containing receptors is associated with *Streptococcus gordonii* DL1 (Challis) but not with a spontaneous mutant, strain D102, that specifically lacks hemagglutinating activity. Comparison of crossed-immunoelectrophoresis patterns of parent and mutant sonicated cell extracts identified a unique antigen (Hs antigen) in the parent cell extract that was purified by DEAE Sephacel column chromatography and by a wheat germ agglutinin (WGA) lectin affinity column. The purified antigen formed a single arc in crossed immunoelectrophoresis with anti-DL1 serum and migrated as a diffuse band above the 200-kDa marker in sodium dodecyl sulfate-polyacrylamide gel electrophoresis. Immunoelectron microscopy with specific anti-Hs antibody revealed labeling of structures in the fibrillar layer of strain DL1 and no labeling of fibrillar structures on strain D102. Rabbit anti-DL1 serum and anti-Hs Fab inhibited the hemagglutinating activity of strain DL1, and the inhibition was specifically neutralized by purified Hs antigen. Anti-Hs Fab did not inhibit the hemagglutinating activities of several heterologous *S. gordonii* strains; however, these bacteria were agglutinated by anti-Hs immunoglobulin G and also by WGA. In contrast, two *S. gordonii* strains that lacked hemagglutinating activity did not react with anti-Hs antibody or with WGA. These findings associate the sialic acid-binding lectin activity of *S. gordonii* DL1 with a specific fibrillar antigen, which is composed of protein and WGA reactive carbohydrate, and indicate that cross-reactive antigens occur on other strains of this species that possess hemagglutinating activity.

The sialic acid-reactive lectins of oral viridans group streptococci may contribute to microbial adhesion and colonization as well as to the clearance of bacteria from the oral cavity (22). The lectin activity of these bacteria was initially detected by aggregation of streptococci in the presence of saliva, an interaction that did not occur following sialidase treatment of high-molecular-weight salivary mucins and glycoproteins (30, 33). Subsequently, Gibbons and coworkers (20, 21) found that the interaction of streptococci with saliva-coated hydroxyapatite (SHA), an in vitro model of bacterial adhesion to the tooth surface, also was reduced by pretreatment of SHA with sialidase. In addition, adhesion of oral streptococci to erythrocytes (RBC) (25, 40) and to human polymorphonuclear leukocytes (29) is sensitive to sialidase treatment of these host cells. Potential receptor structures for streptococcal sialic acid binding lectins include O-linked oligosaccharides with α2-3-linked sialic acid termini in various glycoconjugates such as salivary mucins (40), secretory immunoglobulin A1 (IgA1) (45), and leukosialin on polymorphonuclear leukocytes (44).

Characteristic differences between streptococcal aggregation in the presence of saliva and streptococcal adhesion to SHA suggest that these interactions depend not only on different salivary macromolecules but also on different bacterial lectins (43). The most extensively studied sialic acid-binding streptococcal lectin is SspB (previously termed SSP-5), a 205-kDa cell surface protein of *Streptococcus gordonii* M5 (formerly *S. sanguis*) that is a member of the antigen I/II family (26). This protein mediates Ca^{2+}-dependent aggregation of strain M5 by a high-molecular-weight salivary glycoprotein (8–11). However, the involvement of SspB in other sialidase-sensitive interactions is unclear, since a fibrillar glycoprotein with a subunit molecular weight in excess of 300,000 and a nonglycosylated protein composed of disulfide-linked 96- and 70-kDa subunits were associated with saliva-induced aggregation of *S. sanguis* 12 (37, 38) and hemagglutinating activity of *Streptococcus mitis* KS32AR (39, 40), respectively.

The objective of the present study was to associate a specific bacterial cell surface antigen with sialidase-sensitive streptococcal adhesion. This adhesive property was exhibited by most *S. gordonii* as well as *S. sanguis* strains but, interestingly, not by *S. gordonii* M5, which lacked hemagglutinating activity and also failed to adhere to sialidase-sensitive receptors of SHA (25). These observations suggest that a cell surface component other than SspB, which occurs on strain M5 (11), mediates sialidase-sensitive adhesion of a number of oral streptococci. In the present study, a specific fibrillar antigen, which was composed of protein and carbohydrate, was associated with the hemagglutinating activity of *S. gordonii* DL1 (Challis). In addition, related antigens on other strains of *S. gordonii* that possess hemagglutinating activity were detected.

* Corresponding author. Mailing address: Building 30, Room 302, NIDR, NIH, Bethesda, MD 20892. Phone: (301) 496-1822. Fax: (301) 402-0396. E-mail: jcisar@yoda.nidr.nih.gov.

† Present address: Department of Microbiology, Faculty of Dentistry, Nippon Dental University, 1-9-20 Fujimi, Chiyoda-ku, Tokyo 102, Japan.

‡ Present address: Department of Operative Dentistry and Periodontology, Dental School, University of Regensburg, 93042 Regensburg, Germany.

MATERIALS AND METHODS

Bacteria and growth conditions. All strains of *S. gordonii*, *S. sanguis*, and *Streptococcus oralis* have been previously described (25, 27). In addition, two strains were generously obtained from other investigators: *S. gordonii* M5 from D. R. Demuth, University of Pennsylvania, Philadelphia, Pa., and *Streptococcus parasanguis* FW213 from P. M. Fives-Taylor, University of Vermont, Burlington, Vt. Streptococci were stored as frozen stocks and cultured overnight at 37°C in complex medium containing 0.5% tryptone, 0.5% yeast extract, 0.5% K_2HPO_4, 0.05% Tween 80, and 0.2% glucose (32).

Mutant isolation. Spontaneous mutant strain D102 was isolated following enrichment of *S. gordonii* DL1 (Challis) for bacteria that lacked hemagglutinating activity. Initially, *S. gordonii* DL1 from an overnight broth culture (10 ml) and canine RBC from citrated blood (NIH Animal Center, Poolesville, Md.) were washed and suspended in RPMI 1640 (Biofluids Inc., Rockville, Md.). Equal volumes (3 ml) of the bacterial cell (approximately 10^9/ml) and the RBC (2% by volume) suspension were incubated for 1 h in an ice bath with gentle mixing to promote hemagglutination. Agglutinated bacteria and RBC were allowed to settle, and the upper 1 ml of clear supernatant fluid containing nonadherent bacteria was transferred into 10 ml of broth for overnight growth at 37°C to obtain bacteria for the ensuing cycle of enrichment. The bacterial population obtained following three cycles of enrichment lacked hemagglutinating activity and was plated on brain heart infusion agar (Difco Laboratories, Detroit, Mich.). Of 20 colonies examined, 2 isolates exhibited reduced hemagglutinating activity and 18 were unreactive with canine RBC. One of the latter isolates (*S. gordonii* D102) was selected for further characterization.

Bacterial adhesion. Bacterium-mediated hemagglutination of canine or human type O RBC that were untreated or treated with type X neuraminidase from *Clostridium perfringens* (Sigma Chemical Co., St. Louis, Mo.) was performed in a total volume of 50 μl by mixing 25-μl aliquots of bacteria in serial twofold dilutions, starting with approximately 2×10^9/ml, with 25-μl aliquots of 0.5% RBC in round-bottom wells of polyvinyl microtitration plates (25). Bacteria tested for hemagglutinating activity were untreated or treated for 1 h at 37°C with 1 mg of pronase (Calbiochem, LaJolla, Calif.) (6) per ml or for 1 h at room temperature in the dark with 0.01 M $NaIO_4$ in sodium acetate buffer (0.02 M; pH 4.0) containing 0.15 M NaCl (35). Assays were performed in Tris-buffered saline (20 mM Tris-HCl [pH 7.4], 0.15 M NaCl, 0.1 mM $CaCl_2$, 0.02% NaN_3) containing 0.2% bovine serum albumin (fraction V; Sigma) (TBS-BSA) or in Ca^{2+}-free TBS-BSA containing 10 mM EGTA. The endpoints of titrations (i.e., the lowest concentration of bacteria that caused visible agglutination of RBC) were determined after overnight incubation at 4°C and gentle suspension of cells at room temperature.

Minimum concentrations of antibody for inhibition of bacterium-mediated hemagglutination were determined in round-bottom wells of polyvinyl microtitration plates in a total volume of 75 μl. Serial twofold dilutions of immunoglobulin (25-μl aliquots) were mixed for 30 min at room temperature with 25-μl aliquots of bacterial cell suspensions containing two hemagglutinating units (approximately 1.5×10^6 streptococci) prior to the addition of 25 μl of canine RBC (0.75%), and hemagglutination was determined as described above.

Minimum concentrations of different antigen preparations that neutralized antibody inhibition of bacterial hemagglutination in a total volume of 100 μl were determined. Serial twofold dilutions of soluble antigen (25-μl aliquots) were incubated for 30 min at room temperature with 25-μl aliquots of antibody at a concentration that was four times that required for inhibition of bacterial hemagglutination. Bacterial cell suspension (25 μl containing two hemagglutinating units) was then added to each well, the wells were incubated for an additional 30 min with gentle mixing prior to the addition of canine RBC (25 μl of 1% RBC), and hemagglutination was assessed.

Coaggregation between different streptococci in the presence or absence of 10 mM *N*-acetyl-D-galactosamine (GalNAc; Sigma) was quantified by a spectrophotometric assay similar to that described previously (34). Coaggregation of streptococci with actinomyces and aggregation of streptococci with proline-rich protein-coated latex beads were performed as previously described (25).

Adhesion of NHS-LC-Biotin (Pierce, Rockford, Ill.)-labeled streptococci to glycoconjugates immobilized on nitrocellulose (45) was performed in the presence of either 0.1 mM $CaCl_2$ or 10 mM EGTA. Membranes were spotted with 1-μl volumes containing 1 μg of fetuin, asialofetuin, human serum albumin (HSA; Sigma), or neoglycoproteins prepared by conjugation of reduced oligosaccharides to HSA through an acetylphenylenediamine spacer (Accurate Chemical and Scientific Corp., Westbury, N.Y.). Membranes spotted with glycoconjugates were incubated overnight at 4°C with biotin-labeled bacteria, washed to remove nonadherent bacteria, incubated with avidin-D-alkaline phosphatase (Vector Laboratories, Inc., Burlingame, Calif.), washed, and developed for 5 min with 5-bromo-4-chloro-3-indolyl phosphate–nitroblue tetrazolium chloride (ImmunoPure; NBT/BCIP Substrate kit; Pierce) to detect adherent bacteria.

Antigen extraction and fractionation. *S. gordonii* DL1 and D102 were harvested from 6-liter overnight cultures, washed with TBS (pH 7.8), and suspended in 120 ml of buffer. Washed streptococci were sonicated in a Sonifier Cell Disruptor 350 (Branson Ultrasonics Co., Danbury, Conn.) for 5 min at 0°C under conditions similar to those described previously for removal of fimbriae from actinomyces (5). Unbroken cells and cellular debris were removed by centrifugation, and supernatants were passed through membrane filters (pore size, 0.22 μm) to obtain crude soluble antigens. Each filtered extract was dialyzed against starting buffer (20 mM Tris-HCl [pH 7.8] containing 0.1 mM $CaCl_2$ and 0.02% NaN_3) and applied to a column of DEAE Sephacel (1.6 by 25 cm; Pharmacia LKB Biotechnology, Uppsala, Sweden). Each column was rinsed with 250 ml of starting buffer and eluted with a 400-ml linear gradient of 0 to 800 mM NaCl in starting buffer. Pooled material from the DL1 sonic extract was further fractionated by lectin affinity column chromatography performed at 4°C with a column (7 by 25 mm) of succinyl wheat germ agglutinin (WGA)-Sepharose (EY Laboratories, Inc., San Mateo, Calif.).

Streptococci were also extracted in either barbital buffer (9) or 0.3 M lithium 3,5-diiodosalicylate (LIS) (40). In addition, spent culture supernatants were filtered to remove bacteria, concentrated by ultrafiltration above a low-binding cellulose membrane (cutoff, 10,000 molecular weight; Millipore Corp., Bedford, Mass.), and dialyzed.

The amounts of protein in antigen solutions and column fractions were determined by the bicinchoninic acid (BCA) Protein Assay Reagent (Pierce) following the enhanced protocol of the manufacturer, with BSA as the standard.

Production of antisera. Rabbit antiserum R202 against *S. gordonii* DL1 was prepared by repeated intravenous injections of bacteria following a schedule similar to that described previously (41). Bacteria for immunization were cultured in complex medium that was ultrafiltrated to remove macromolecules. Anti-DL1 was obtained 1 week after a series of 18 injections of washed bacterial cells (0.25 to 1.0 ml of approximately 2×10^9/ml) administered over 6 weeks.

Rabbit antiserum R206 was produced against the DL1 antigen of interest by immunization with immunoprecipitate from rocket line immunoelectrophoresis plates (1). Three plates were prepared, each with 300 μl of DEAE Sephacel pool 1 of the DL1 sonic extract (50 μg of protein/ml) and 300 μl of anti-DL1 serum. The plates were soaked in cold 0.15 M NaCl to remove unprecipitated protein, and the specific immunoprecipitate was excised, suspended in 0.8 ml of 0.15 M NaCl, emulsified with an equal volume of Freund's complete adjuvant (Sigma), and administered in multiple subcutaneous sites. Anti-immunoprecipitate serum was obtained 1 week after five subsequent injections of antigen in incomplete adjuvant administered at biweekly intervals.

Rabbit antiserum R208 was prepared by immunization with pool C from the succinyl WGA affinity column. Pool C protein (50 μg) in complete adjuvant was injected subcutaneously on day 1, and the same amount of protein in incomplete adjuvant on days 14 and 42. Anti-pool C serum was obtained 1 week after the last injection. For absorption, antiserum (200 μl) was incubated overnight at 4°C with washed *S. gordonii* D102 (approximately 1.5×10^{10} bacteria) in a total volume of 1 ml of TBS and bacteria were removed by centrifugation. The serum was absorbed a total of three times and subsequently passed through a membrane filter.

Rabbit antiserum against SspB of *S. gordonii* M5 (8) was kindly provided by D. R. Demuth, University of Pennsylvania, Philadelphia, Pa., and rabbit IgG against SsaB of *S. sanguis* 12 (19) was a gift from N. Ganeshkumar, Forsyth Dental Center, Boston, Mass.

Immunological methods. Rabbit IgG was purified from serum by ammonium sulfate precipitation and anion-exchange column chromatography (7). Fab was prepared by papain digestion of IgG and purified by CM52 (Whatman, Inc., Clifton, N.J.) column chromatography as previously described (42). Immunoglobulin and Fab concentrations were determined by using the BCA protein reagent with rabbit IgG as the standard.

Crossed immunoelectrophoresis, rocket line immunoelectrophoresis, and fused rocket immunoelectrophoresis (1) were performed with a Multiphore apparatus (Pharmacia) cooled to 4°C. After overnight electrophoresis (1 V/cm) of antigen into antibody, plates were placed in a humid chamber and incubated for 1 day at 4°C to enhance immunoprecipitation, soaked 1 day at 4°C in 0.15 M NaCl to remove unprecipitated protein, dried, and stained with Coomassie brilliant blue R-250 (Bio-Rad Laboratories, Richmond, Calif.) by the procedure described by Fairbanks et al. (15).

Bacterial agglutination by antibody or WGA was assayed by using a standard protocol (3) in round-bottom wells of polyvinyl microtitration plates. Serial twofold dilutions of antibody or lectin (25-μl aliquots) were incubated with approximately 2.5×10^7 streptococci in a total volume of 50 μl of TBS-BSA for 1 h at room temperature and overnight at 4°C. The endpoints of titrations were judged by the settling patterns of bacteria.

SDS-PAGE. Samples for sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) (28) were boiled for 10 min in reducing sample buffer and were applied to 8 to 16% polyacrylamide gradient gels (Novex, San Diego, Calif.). Proteins in gels either were stained with Coomassie brilliant blue R-250 (15) or with silver (Daiichi Silver Stain-II; Daiichi Pure Chemicals Co., Ltd., Tokyo, Japan) or, alternatively, were transferred (48) to nitrocellulose membranes in 12 mM Tris-HCl (pH 8.3)–96 mM glycine with 6 V/cm for 18 h at 4°C. Transferred proteins were detected with a DIG Protein Detection kit (Boehringer Mannheim Co., Indianapolis, Ind.), which labels both free amino and sulfhydryl groups. Transferred carbohydrate was oxidized by a 30-min incubation in 10 mM sodium periodate in 0.1 M sodium acetate buffer (pH 5.5) (2) and then labeled for 1 h in 100 μg of biotin-LC-hydrazide (Pierce) per ml. The labeled membrane was washed with phosphate-buffered saline (PBS; 0.15 M NaCl, 0.02 M sodium phosphate [pH 7.2]), blocked for 1 h in TBS (without NaN_3) containing 3% BSA, incubated with avidin-D-horseradish peroxidase (Vector) (5 μg/ml

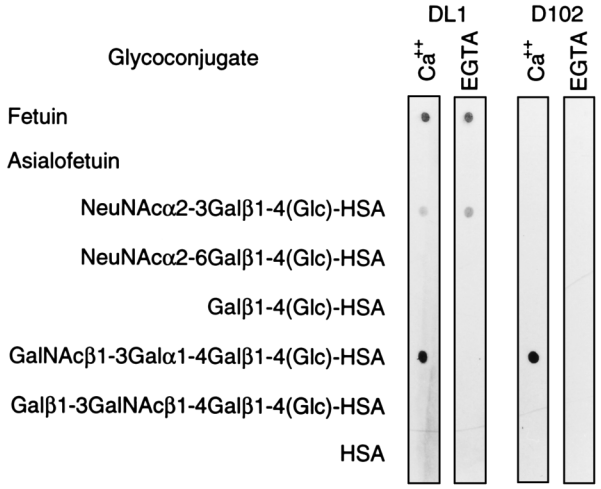

FIG. 1. Bacterial adhesion of *S. gordonii* DL1 and hemagglutination-negative mutant strain D102 to immobilized glycoconjugates. Nitrocellulose membranes were spotted with each glycoconjugate (1 μg), blocked with BSA, incubated with biotinylated bacteria in the presence of either 0.1 mM $CaCl_2$ (Ca^{2+}) or 10 mM EGTA (EGTA), and washed to remove unbound bacteria. Bound bacteria were detected with alkaline phosphatase-conjugated avidin.

in the same buffer containing 0.05% Tween 20), washed, and developed with 4-chloro-1-naphthol (Bio-Rad).

Transfers for Western blotting (48) were blocked with azide-free TBS containing 2% skim milk (TBS-milk), incubated with primary antibody and subsequently with horseradish peroxidase-conjugated goat anti-rabbit IgG (Bio-Rad Laboratories) in TBS-milk, washed, and developed with 4-chloro-1-naphthol.

Transfers for lectin overlays (23) were blocked at room temperature in azide-free TBS containing 2% polyvinyl alcohol (type II; low molecular weight; Sigma), 0.1% Tween 20, 1 mM $CaCl_2$, 1 mM $MgCl_2$, and 1 mM $MnCl_2$. They were then incubated at room temperature with biotinylated WGA (WGA-biotin; EY Laboratories) and subsequently with avidin-D-horseradish peroxidase (5 μg/ml) in the same buffer, washed, and developed with 4-chloro-1-naphthol.

Electron microscopy. Cells (1.5×10^9) of *S. gordonii* DL1 or D102 were washed with Hanks' balanced salt solution (HBSS; BioWhittaker, Inc., Walkersville, Md.) and incubated for 2 h at room temperature with 1 ml of primary antibody (10 μg of control or immune IgG per ml in HBSS containing 0.5% BSA). After washing with HBSS–0.5% BSA, samples were suspended in 250 μl of the same buffer and treated for 1 h at room temperature with 250 μl of a 10-nm colloidal gold-conjugated secondary antibody (goat anti-rabbit IgG [H+L]; AuroProbe EM; Amersham Life Science Inc., Arlington Heights, Ill.). After thorough washing, the cells were fixed overnight at 4°C with 2% glutaraldehyde–2% paraformaldehyde in 0.1 M sodium cacodylate buffer (pH 7.3), dehydrated with graded alcohols, and embedded in epoxy resin. Thin sections were stained with uranyl acetate and lead citrate. Bacteria and their association with the colloidal gold particles were examined with a Zeiss EM 912 Omega electron microscope.

RESULTS

Hemagglutinating activity of *S. gordonii* DL1 and adhesive properties of a spontaneous hemagglutination-negative mutant. Bacterium-mediated hemagglutination occurred rapidly when *S. gordonii* DL1 was mixed with RBC, and hemagglutination was completely abolished by sialidase treatment of RBC or protease treatment of streptococci. A slight reduction of hemagglutinating activity (i.e., twofold) was noted following periodate treatment of streptococci to oxidize surface carbohydrate. Significantly, the hemagglutinating activity of strain DL1 was identical in TBS containing either 0.1 mM Ca^{2+} or 10 mM EGTA, indicating that bacterial hemagglutination was not Ca^{2+} dependent.

Sequential enrichment of *S. gordonii* DL1 for bacteria that did not hemagglutinate canine RBC resulted in the isolation of spontaneous mutant strain D102. The parent and mutant aggregated to the same extent with proline-rich protein 1-coated latex beads and coaggregated with actinomyces. In addition, coaggregation between strains DL1 and *S. oralis* 34 was similar to that between strains D102 and 34 and was significantly reduced in the presence of 10 mM GalNAc (results not shown). Thus, the difference in adhesion properties of the parent and mutant strains was limited to hemagglutinating activity.

Structurally defined glycoconjugates were used to assess the specificity of bacterial adhesion. Biotinylated strain DL1 was adherent to immobilized fetuin and NeuNAcα2-3Galβ1-4(Glc)-HSA in the presence of 0.1 mM Ca^{2+} or 10 mM EGTA but failed to adhere to asialofetuin, NeuNAcα2-6Galβ1-4(Glc)-HSA, or Galβ1-4(Glc)-HSA in the presence or absence of 0.1 mM Ca^{2+} (Fig. 1). Mutant strain D102 did not bind to any of these glycoconjugates. Both strains were adherent to GalNAcβ1-3Galα1-4Galβ1-4(Glc)-HSA but only in the presence of Ca^{2+}. Neither strain bound to Galβ1-3GalNAcβ1-4Galβ1-4(Glc)-HSA. Collectively, these findings associate a Ca^{2+}-independent lectin activity for α2-3-linked sialic acid-containing receptors with the hemagglutinating activity of parent strain DL1.

Antigenic comparison of parent and mutant strains. Various procedures were utilized in attempts to extract the hemagglutinating activity from strain DL1 including sonication (5) or extraction with either 0.3 M LIS (40) or barbital buffer (9). The extracts obtained by these procedures lacked soluble hemagglutinating activity. However, reduced hemagglutinating activity of treated bacteria was noted following sonication of streptococci. Further evidence for the sonic extraction of a streptococcal cell surface structure associated with adhesion was obtained by crossed immunoelectrophoresis of DL1 and D102 sonic extracts with anti-DL1 serum (Fig. 2A and B, respectively). A number of shared antigens were present in the extracts of these bacteria. In addition, the DL1 extract contained a unique antigen (indicated by the arrow in Fig. 2A),

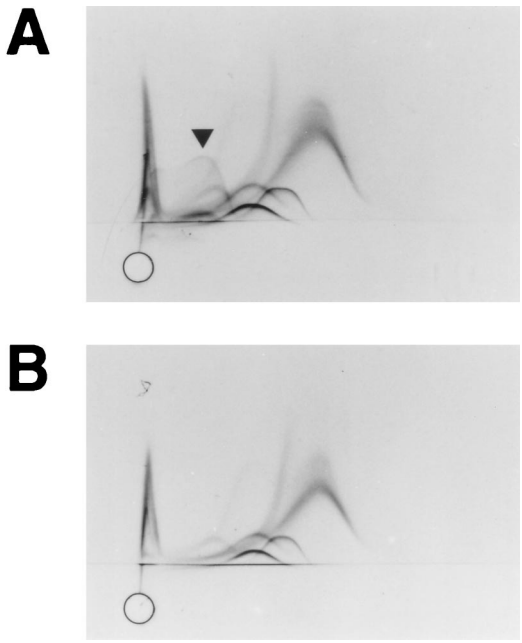

FIG. 2. Identification of Hs antigen by crossed immunoelectrophoresis with antiserum (1/20) against *S. gordonii* DL1 in the agarose gel. Antigen wells contained sonic extract (50 μg of protein) of strain DL1 (A) or sonic extract (50 μg of protein) of mutant strain D102 (B). The unique precipitin arc of the Hs antigen is identified by an arrow in panel A. Anode is to the right for electrophoresis in the first dimension.

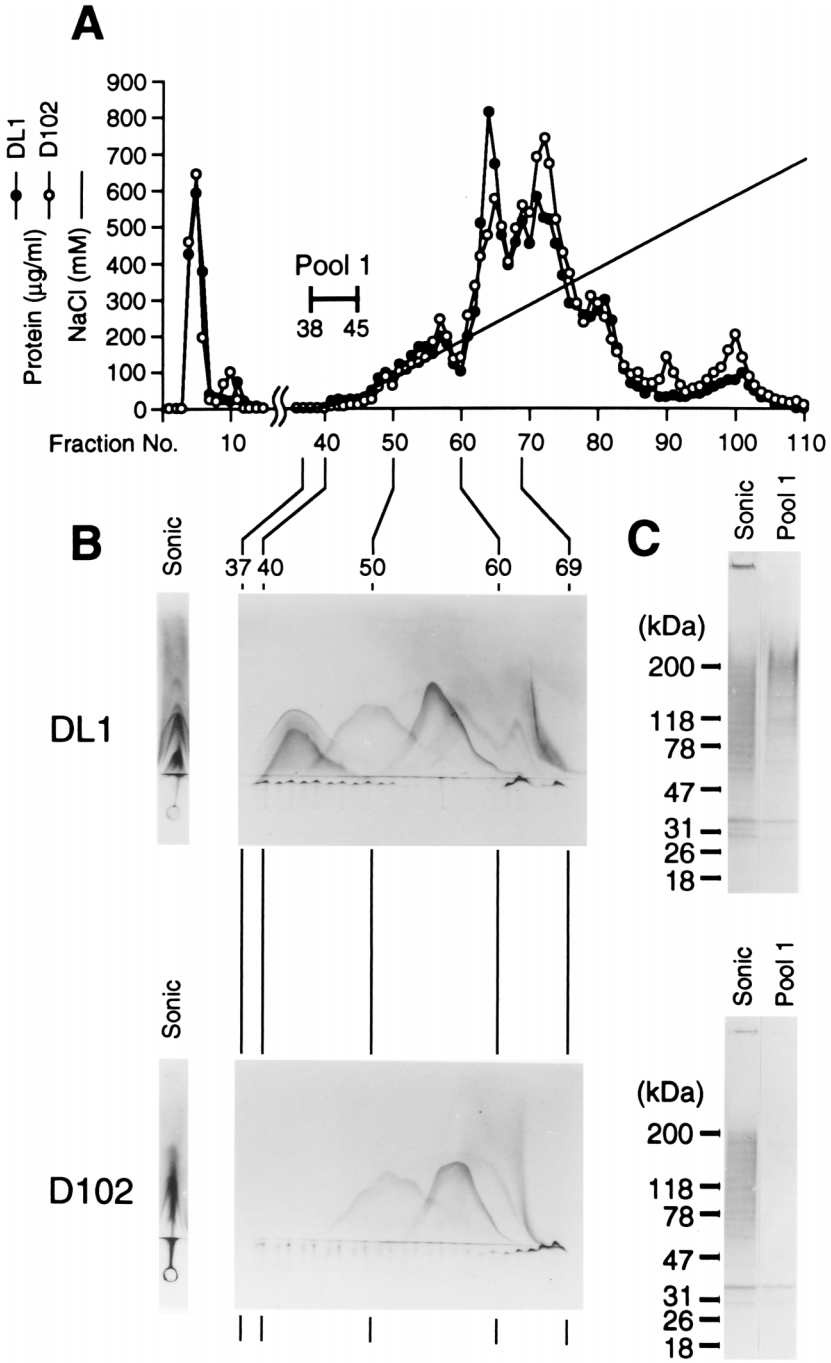

FIG. 3. Identification of the Hs antigen by comparative DEAE Sephacel column chromatography of *S. gordonii* DL1 and D102 sonic extracts. (A) Superimposed chromatograms of strain DL1 and D102 sonic extracts showing the locations of pool 1 fractions; (B) rocket immunoelectrophoresis of strain DL1 and D102 sonic extracts (Sonic [20 μg of protein]) and fused rocket immunoelectrophoresis of DEAE Sephacel fractions (4 μl of each) from DL1 and D102 sonic extracts (upper and lower plates, respectively) with anti-DL1 serum (1/20); (C) Western blots of strains DL1 and D102 sonic extracts (Sonic [50 μg of protein]) and pool 1 (1.5 μg of protein) (upper and lower blots, respectively) with anti-DL1 serum (1/1,000).

which we have designated as the Hs antigen because of its association with streptococcal hemagglutinating activity. A dense precipitin arc, which was also unique to the DL1 pattern, was noted below the Hs antigen, near the boundary of the antibody-containing gel.

Sonic extracts of strains DL1 and D102 were separated by DEAE Sephacel column chromatography (Fig. 3A), and the fractions were analyzed for reactions with anti-DL1 serum by fused rocket immunoelectrophoresis (Fig. 3B). The Hs antigen in the DL1 sonic extract emerged in fractions collected at the beginning of the salt gradient (i.e., fractions 38 to 45 containing from 5 to 50 mM NaCl). Two nonoverlapping precipitin arcs were observed in these fractions, both of which were absent from the D102 pattern. Following concentration of the DL1 pool 1 fractions and membrane filtration, the height of the slowly migrating precipitin arc was reduced, presumably due to

aggregation of high-molecular-weight material (results not shown).

Western blotting of pool 1 from strain DL1 with anti-DL1 serum revealed a diffuse band in the 200-kDa region of the transfer as well as a 35-kDa antigen (Fig. 3C). Only the latter component was detected in pool 1 of mutant strain D102. Thus, the Hs antigen was identified in the high-molecular-weight region of Western blots.

Fractions from ion-exchange chromatography of parent and mutant sonic extracts were also spotted on nitrocellulose and overlaid with a rabbit antibody against SspB (SSP-5) of *S. gordonii* M5. The cross-reactive antigen of strain DL1 was detected from fractions 48 to 58 of the DL1 and D102 chromatograms but not in pool 1, thereby indicating separation of the Hs antigen from SspB (results not shown).

Isolation of Hs antigen and production of a specific antibody. An important observation made during the present study was the reaction of WGA, a GlcNAc binding lectin, with the Hs antigen. This reaction, which was initially detected by WGA-mediated agglutination of strain DL1 but not D102, was utilized to further purify the Hs antigen. Pool 1 material (900 μg of protein) from the DL1 sonic extract was applied to a column of succinyl WGA-agarose. The column was washed with buffer to obtain fractions that were either not retained (pool A) or weakly retained (pool B) prior to elution with 100 mM GlcNAc (pool C) and subsequently with 1.2 M NaCl (pool D) as indicated in Fig. 4A. Each pool was concentrated and examined by Western blotting with antiserum against immunoprecipitated Hs antigen (i.e., the sharp upper arc of the pool 1 immunoelectrophoretic pattern of strain DL1 shown in Fig. 3B). The Hs antigen was barely visible with 50 μg of crude sonic extract protein but was observed as two broad bands with 1.5 μg of pool 1 protein from the DL1 sonic extract (Fig. 4B). Material that eluted from the WGA affinity column migrated with the lower band. The reaction of anti-immunoprecipitate serum, although weak, indicated enrichment of the Hs antigen in the GlcNAc eluate (pool C). Thus, the immunoreactive band in the 200-kDa region of the transfer observed with 0.5 μg of pool C protein was at least as strong as that observed with 2.5 μg of pool B protein and considerably stronger than that observed with 4 μg of pool A protein. The reaction of WGA-biotin on an identical transfer was localized to the upper band of the pool 1 pattern observed with anti-immunoprecipitate serum (Fig. 4B). WGA-biotin-reactive material was not detected in pools A, B, or D from succinyl WGA Sepharose but was detected in pool C as two faintly stained bands. Thus, a substantial fraction of the soluble Hs antigen in pool 1 of the DL1 sonic extract appeared to be WGA reactive.

Isolation of the Hs antigen was also monitored by SDS-PAGE and silver staining. A band in the region of the Hs antigen (i.e., above the 200-kDa marker) was not observed in the sonic extract of strain DL1, even when the lane was overloaded as was intentionally done in Fig. 5. However, a faint band in this region was noted with DEAE Sephacel pool 1 of the DL1 sonic extract and also with pool C of the succinyl WGA affinity column. Although this band was stained with silver, it was not stained with Coomassie brilliant blue (results not shown). Pool C also contained material that did not enter the SDS-polyacrylamide gel and only one additional band of lower molecular mass at 37 kDa. When pool C was transferred to nitrocellulose and overlaid with rabbit antibody against DL1 cells (Fig. 5), a broad zone of high-molecular-mass material was detected, but no bands below the 118-kDa marker were detected. Material in pool C was weakly labeled with digoxigenin-containing reagents for detection of free-amino or sulfhydryl groups (Fig. 5). Stronger labeling of transferred pool C was noted with biotin hydrazide following periodate oxidation indicating the presence of carbohydrate (Fig. 5).

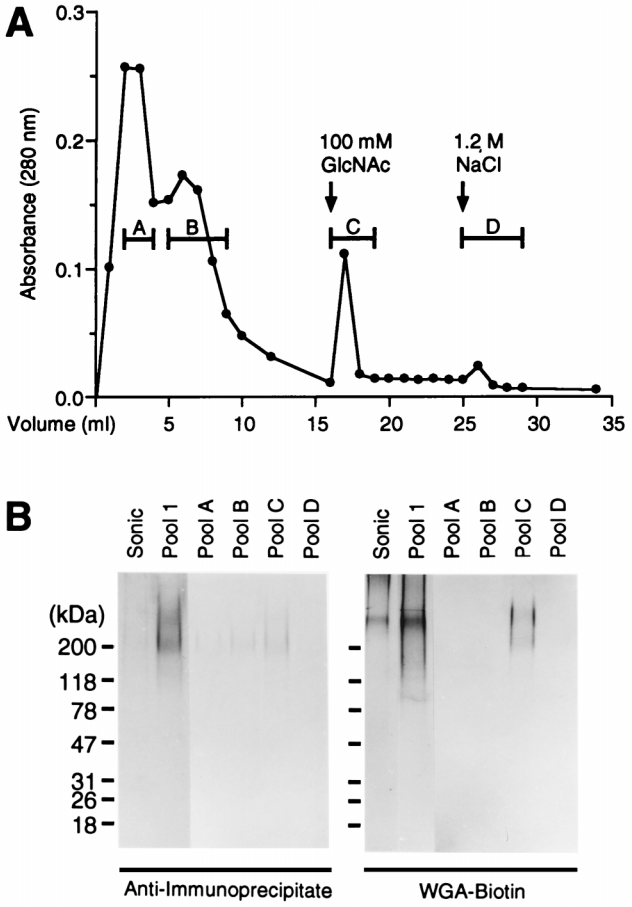

FIG. 4. Isolation of the Hs antigen by succinyl WGA affinity column chromatography of pool 1 material (900 μg of protein) from the DL1 sonic extract. (A) Chromatogram showing elution of material from a 1.0-ml lectin affinity column with TBS (pools A and B), 100 mM GlcNAc (pool C), and 1.2 M NaCl (pool D); (B) Western blot with anti-immunoprecipitate (1/100) and lectin blot with WGA-biotin (10 μg/ml) of strain DL1 sonic extract (Sonic [50 μg of protein]), DEAE Sephacel pool 1 of DL1 sonic extract (pool 1 [1.5 μg of protein]), and pooled fractions from a succinyl WGA affinity column (4 μg of pool A, 2.5 μg of pool B, 0.5 μg of pool C, and 0.2 μg of pool D).

Pool C material from the succinyl WGA affinity column formed a single precipitin arc in crossed immunoelectrophoresis with antiserum against strain DL1 (Fig. 6A) in a position similar to that indicated by the arrow in Fig. 1. Moreover, a rabbit antiserum prepared against pool C material precipitated only one antigen from the sonic extract of strain DL1 (Fig. 6B) and, as expected, none from the sonic extract of strain D102 (results not shown). While these reactions indicate the presence of antibody against the Hs antigen, the sensitivity of immunoprecipitation is not sufficient to preclude the presence of other antibodies against additional bacterial antigens. Consequently, Western blotting of crude antigen preparations was also performed with a high concentration of anti-pool C serum (i.e., 1/100 dilution). Under these conditions, reactions were seen with a number of bands other than the Hs antigen in the sonic extracts and culture supernatants of strains DL1 and D102 (Fig. 7). However, all contaminating antibodies were removed by absorption of anti-pool C serum with washed D102 cells. The antibody in absorbed serum reacted weakly in the 200-kDa region upon the transfer of DL1 sonic extract but not

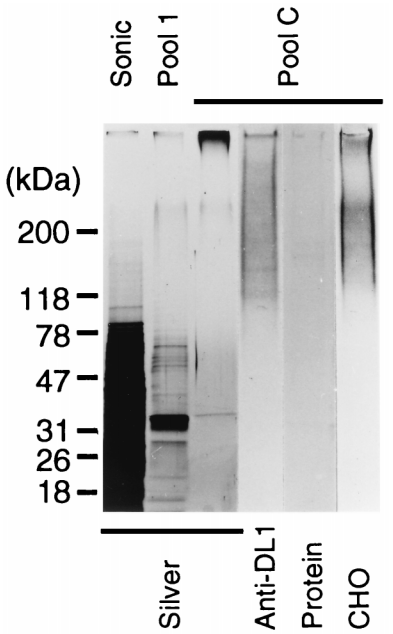

FIG. 5. SDS-PAGE and silver staining (Silver) of DL1 sonic extract (Sonic [5 μg of protein]), DEAE Sephacel pool 1 of DL1 sonic extract (pool 1 [1.5 μg of protein]), and GlcNAc eluate of a succinyl WGA affinity column (pool C [5 μg of protein]). Separated pool C material (5 μg of protein) was also transferred to nitrocellulose to detect Hs antigen by Western blotting with antiserum (1/1,000) against *S. gordonii* DL1 (Anti-DL1), labeling of free amino and sulfhydryl groups with digoxigenin-containing protein detection reagents (Protein), or labeling of periodate oxidized carbohydrate (CHO) with biotin-LC-hydrazide.

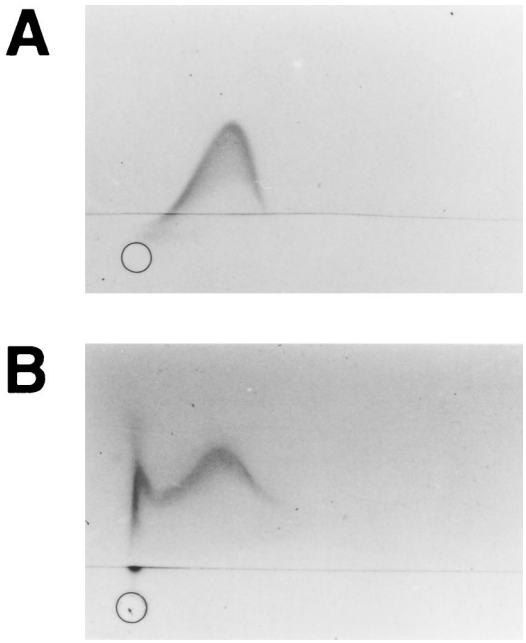

FIG. 6. Detection of Hs antigen by crossed immunoelectrophoresis. (A) Antiserum (1/20) against *S. gordonii* DL1 in the agarose gel and pool C (0.15 μg of protein) from succinyl WGA affinity chromatography in the antigen well; (B) antiserum (1/10) against pool C in the agarose gel and sonic extract of strain DL1 (100 μg of protein) in the antigen well. Anode is to the right for electrophoresis in the first dimension.

upon the transfer of D102 sonic extract (Fig. 7). Hs antigen was also detected as a faint band above the 200-kDa marker upon the transfer of DL1 culture supernatant but not upon the transfer of D102 culture supernatant. That the band from culture supernatant appeared slightly above the Hs antigen observed in DL1 sonic extract raises the possibility of antigen degradation during sonic extraction. These observations also document the specificity of the antibody used in subsequent experiments and clearly illustrate the difficulty of detecting the Hs antigen by Western blotting.

Electron microscopy. The Hs antigen was localized on the surface of *S. gordonii* DL1 by immunoelectron microscopy utilizing absorbed anti-pool C IgG (Fig. 8). Immunogold labeling of the distal portions of fibrillar structures on the surface of strain DL1 but no labeling of morphologically similar structures on the surface of strain D102 was observed. Neither strain DL1 nor D102 was labeled when preimmune serum was used as the primary antibody.

Specific inhibition of hemagglutination by anti-Hs antibodies. Absorbed anti-pool C immune IgG and Fab at concentrations of as little as 1 and 2 μg/ml, respectively, inhibited the hemagglutinating activity of strain DL1, whereas preimmune IgG or Fab was inactive at concentrations of as much as 170 μg/ml. In comparable assays, anti-DL1 serum at dilutions of as much as 1/9,600 also inhibited bacterial hemagglutination, but preimmune serum at a dilution of 1/6 did not. The specificity of inhibition was evident from further experiments in which antigen-containing solutions from different stages of the Hs antigen purification scheme were preincubated with Fab or anti-DL1 serum to neutralize the antibody that inhibited bacterium-mediated hemagglutination. Both antibodies were neutralized by low concentrations of pool C and by progressively greater concentrations of pool 1 and sonic extract of strain DL1 (Table 1). In contrast, antigen preparations from strain D102 lacked neutralizing activity. These findings indicate that antibodies directed against the Hs antigen inhibit the hemagglutinating activity of strain DL1, whereas antibodies directed against the surface antigens of strain D102 are either far less active or inactive as inhibitors of adhesion.

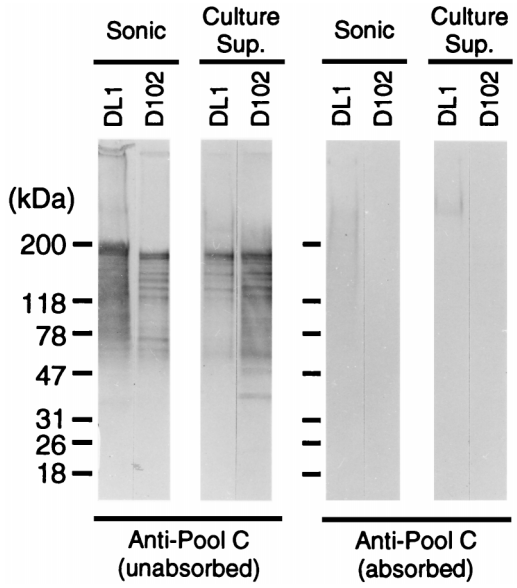

FIG. 7. Specificity of anti-pool C (unabsorbed) and anti-pool C (absorbed) for Hs antigen in DL1 and D102 sonic extracts (Sonic [50 μg of protein]) or culture supernatants (Culture Sup. [10 μg of protein]) evaluated by Western blotting with 1/100 dilutions of either unabsorbed and absorbed antisera.

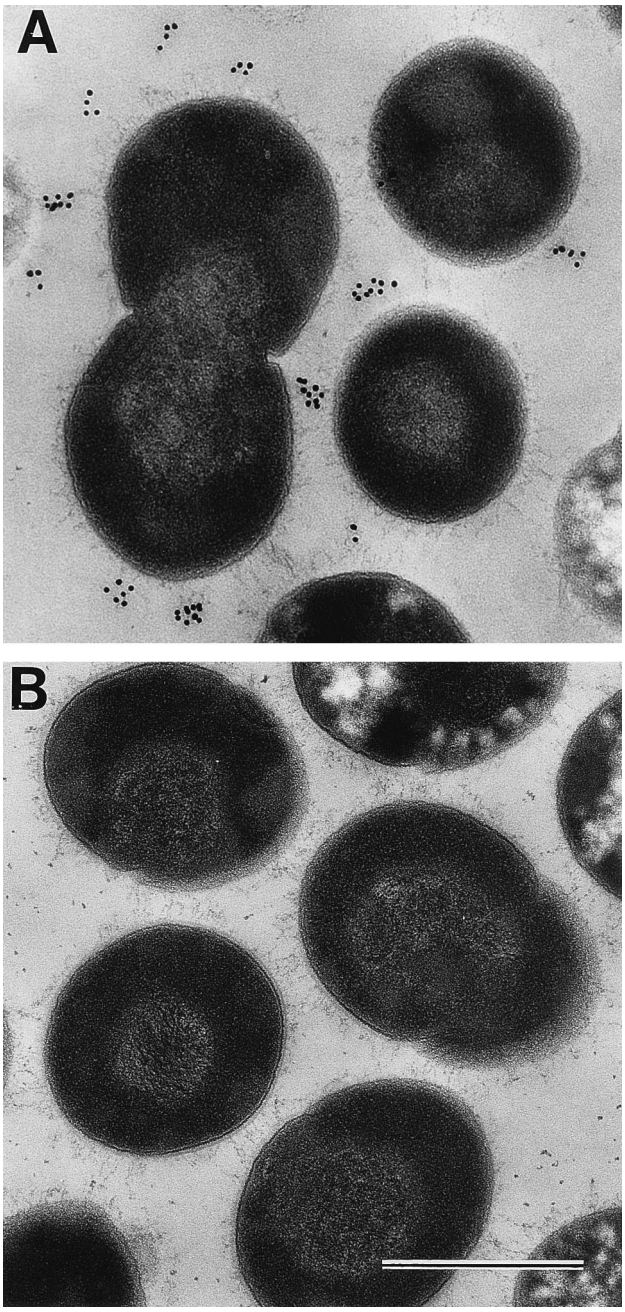

FIG. 8. Electron micrographs of thin sections. (A) *S. gordonii* DL1; (B) *S. gordonii* D102. Bacteria were incubated with antibody against pool C that was preabsorbed with strain D102, washed and treated with goat anti-rabbit IgG on 10-nm gold particles, fixed, and sectioned. Bar, 0.5 μm.

Detection of Hs-like antigens on heterologous strains. In addition to *S. gordonii* DL1, other viridans group streptococci were examined for the presence of Hs-like antigens. Initially, anti-pool C Fab was tested for inhibition of bacterium-mediated hemagglutination with *S. gordonii* 38, SK6, SK184, SK9, and SK12, but no inhibition was observed. These five strains (Table 2) and eight others with hemagglutinating activity (25) (not included in Table 2) were, however, agglutinated by anti-pool C immune IgG. The concentrations of antibody required for these heterologous reactions ranged from 10 to 1,000 that required for agglutination of strain DL1. These reactions were strongest with strains SK184, SK12, and SK9, intermediate with the eight *S. gordonii* strains not listed in Table 2, and weakest with strains 38 and SK6. In contrast, *S. gordonii* M5 and 10558, which lack hemagglutinating activity (25), did not react with anti-pool C IgG. Moreover, this antibody agglutinated *S. sanguis* 10556, the type strain of this species, but did not agglutinate strain SK1, a hemagglutination-negative variant of strain 10556 (25). Anti-pool C IgG did not, however, agglutinate *S. oralis* 34, which has hemagglutinating activity for

TABLE 1. Neutralization of antibodies that inhibit bacterium-mediated hemagglutination by different antigen preparations

Antigen preparation	Concn (μg of protein/ml) of antigen preparation for neutralization of antibody[a]	
	Anti-DL1 serum (1/1,600)[b]	Anti-pool C Fab (16 μg/ml)[b]
Sonic extract of DL1	63	125
Sonic extract of D102	>1,000	>1,000
Pool 1 of DL1	1	4
Pool 1 of D102	>31	>31
Pool C	0.25	1

[a] Neutralization of the antibody or Fab that inhibits bacterium-mediated agglutination of canine RBC (25 μl of 1% cell suspension) by 6.3×10^6 DL1 cells in a total volume of 100 μl.
[b] Dilution contained four times more antibody than what was required for inhibition of strain DL1 hemagglutinating activity.

TABLE 2. Bacterial hemagglutinating activities of *S. gordonii* and other viridans group streptococcal strains and reactions of these bacteria with anti-Hs antibody and WGA detected by bacterial agglutination

Species and biovar[b]	Strain	Hemagglutinating activity[c]	Bacterial agglutination with[a]:	
			Anti-Hs IgG (μg/ml)[d]	WGA (μg/ml)
S. gordonii				
1	38	+	250	63
	SK6	+	250	0.25
2	SK184	+	4	2
	DL1	+	0.25	0.13
	D102	−	>250	>250
	M5	−	>250	>250
	10558	−	>250	>250
3	SK9	+	4	1
	SK12	+	2	1
S. sanguis 1	10556	+	16	1
	SK1	−	>250	>250
S. oralis	34	+	>250	>250
S. parasanguis	FW213	−[e]	>250	250

[a] Minimal concentration of IgG or WGA for agglutination of streptococci (2.5×10^7) in a total volume of 50 μl.
[b] Biovars based on biochemical and serological differences between strains (27).
[c] Bacterium-mediated hemagglutinating activities summarized from previous determinations with human, goat, canine, or guinea pig RBC (25).
[d] IgG fraction of anti-pool C serum absorbed with strain D102.
[e] Strain FW213 was negative for hemagglutination of canine RBC.

goat RBC (25), and also failed to agglutinate *S. parasanguis* FW213, which lacks hemagglutinating activity for canine RBC but has a glycosylated fimbrial structure that mediates adhesion of this strain to SHA (13, 14, 16).

Like anti-pool C IgG, WGA reacted with all *S. gordonii* strains that possess hemagglutinating activity, but not with strains D102, M5, and 10558, which are hemagglutination negative. The minimum concentrations of WGA for bacterial agglutination was approximately 60 μg/ml for strain 38 and from 0.1 to 2 μg/ml for all other *S. gordonii* strains with hemagglutinating activity, six of which are listed in Table 2. WGA also reacted with *S. sanguis* 10556, a strain that hemagglutinated, but not with SK1, the hemagglutination-negative variant. Thus, the reactions of WGA with these bacteria were closely correlated with the reactions of anti-Hs antibody and with the expression of sialic acid binding lectin activity detected by bacterial hemagglutination.

DISCUSSION

The present results indicate that the adhesion of *S. gordonii* DL1 to sialic acid-containing receptors on RBC is specifically inhibited by antibody directed against a single antigenic component in the outer fibrillar layer of the bacterial surface. This component, which has been designated the Hs antigen, was identified by an antigenic difference in crossed immunoelectrophoresis between the undenatured cell surface structures removed by sonication of strain DL1 and a spontaneous hemagglutination-negative mutant. Mutant strain D102 lacked one parental antigen and was negative for adhesion to the NeuNAcα2-3Gal termini of immobilized glycoproteins and neoglycoproteins but was indistinguishable from parent strain DL1 in other adhesive properties. Antibody against the Hs antigen reacted with a high-molecular-weight component composed of protein and WGA-reactive carbohydrate that was not easily detected by Western blotting or silver staining. The reactions of both anti-Hs antibody and WGA with several other *S. gordonii* and *S. sanguis* strains also suggested the presence of Hs-like antigens on strains of these species that possessed hemagglutinating activity but not on strains that were negative for this adhesive property. Considered together, these findings suggest that adhesion of these bacteria to sialic acid-containing receptors depends on a common type of cell surface structure which differs antigenically among strains.

Previous results suggested that sialic acid-binding adhesins, which are detectable by bacterial hemagglutination, are present on most strains of *S. sanguis* and *S. gordonii* and a majority of *S. oralis* strains (25). Streptococci with hemagglutinating activity were generally more adherent to SHA than to sialidase-treated SHA, although the contribution of sialic acid-binding lectins was masked with certain strains by the presence of other adhesins for proline-rich proteins. Surprisingly, *S. gordonii* M5, a well-studied strain (9, 11, 12), was negative for bacterial hemagglutination (25), thereby suggesting that this adhesive interaction may not be mediated by SspA or SspB (formerly SSP-5), the surface proteins that mediate sialic acid-inhibitable aggregation of strain M5 in the presence of salivary agglutinin, an interaction that is Ca^{2+} dependent (11, 12). This possibility was supported in the current investigation by the demonstration that the hemagglutinating activity of strain DL1 does not require Ca^{2+} and also by the association of this activity with the Hs antigen which was separated from the homolog of SspB by anion-exchange column chromatography of the strain DL1 sonic extract. A contribution of SspB to bacterial hemagglutination was previously suggested from a fourfold reduction of bacterial hemagglutinating activity observed following insertional inactivation of the SspA and SspB genes (10). However, a partial reduction of hemagglutinating activity could also result from a pleiotropic effect of the inactivated SspA and SspB genes on the cell surface exposure of another component such as the Hs antigen. Once the genes for Hs antigen are identified, it will be important to determine whether insertional inactivation of this locus results in a complete or partial loss of sialic acid-binding lectin activity.

The properties of the Hs antigen of strain DL1 are also distinct from those of the hemagglutinin of *S. mitis* KS32AR (39, 40). LIS extraction of the latter strain yielded soluble hemagglutinating activity that was attributed to disulfide-linked proteins with molecular masses of 96 and 70 kDa. In contrast, soluble hemagglutinating activity was not detected with the LIS or sonic extracts of strain DL1 or with the purified Hs antigen. In previous studies, isolated actinomyces type 2 fimbriae also lacked agglutinating activity; however, this activity was detected with fimbriae in immune complexes formed with type 2 fimbria-specific monoclonal antibodies or with type 2 fimbriae adsorbed on latex beads (4). Both procedures restore multivalence and thereby permit detection of low-affinity lectin binding. Similar experiments performed with purified Hs antigen in the present investigation failed to detect hemagglutinating activity (results not shown), perhaps because the antibodies used to form immune complexes inhibited the putative lectin activity of Hs antigen or because of inadequate adsorption of soluble Hs antigen to latex beads. Clearly, unequivocal identification of the Hs antigen as the cell surface structure that mediates adhesion to sialic acid-containing receptors must ultimately depend on the detection of specific binding, either with the antigen purified from streptococci or the corresponding cloned protein isolated from *Escherichia coli*. This detection may require the use of additional molecular cross-linking strategies, including chemical approaches similar to that used in studies of a sponge aggregation factor (36). Consideration of such approaches in the current investigation was limited by the small amount of purified Hs antigen.

Short peritrichous fibrils, similar to those on *S. gordonii* DL1, were previously observed on two strains of *S. sanguis*, each with hemagglutinating activity, but not on four strains of *S. mutans* that had smooth cell surfaces and were negative for bacterial hemagglutination (24). Subsequently, adhesion of *S. sanguis* 12 to sialidase-sensitive receptors of SHA was suggested to depend on a fibrillar glycoprotein that migrated above the 200-kDa marker in SDS-PAGE (37, 38). This structure may well be similar to that of the Hs antigen of strain DL1. The nature of the association between protein and carbohydrate in these structures is unclear. Resistance of the strain 12 fibrillar glycoprotein to digestion with either lysozyme or mutanolysin suggested that the carbohydrate moiety of this structure was not peptidoglycan (37). The Hs antigen of strain DL1 is also not degraded by these enzymes (results not shown), but a substantial fraction reacted with WGA, a GlcNAc-binding plant lectin that reacts with peptidoglycan (31). Consequently, it is not clear whether the Hs antigens of viridans group streptococci are true glycoproteins or, alternatively, proteins covalently linked through peptidoglycan-related structures (46) that are resistant to lysozyme and mutanolysin digestion. The detection of Hs antigen that was not bound by the succinyl WGA affinity column in addition to that which was bound (i.e., pool C) may be explained by association of a protein moiety with various amounts of exposed carbohydrate. Whether such heterogeneity is characteristic of the Hs antigen or was introduced by sonic extraction of the corresponding cell surface structure remains to be determined.

Adhesion of *S. parasanguis* FW213 to SHA was inhibited by

a monoclonal antibody that reacted with fimbriae on the bacterial surface and with a diffuse band that migrated near the 200-kDa marker in SDS-PAGE (13, 14). Recent studies indicate that the fimbrial antigen of strain FW213 is glycosylated (16) and thereby suggest a possible similarity between this component and the Hs antigen of strain DL1. Strain FW213 was agglutinated by a high concentration of WGA (Table 2) but, unlike DL1, lacked hemagglutinating activity, at least with canine RBC. It has been suggested that a 35-kDa protein, FimA, is associated with FW213 fimbriae based on the detection of this protein with a rabbit antibody against strain FW213 that was absorbed with a nonadherent mutant lacking the fimbrial antigen (17). In the current study, the 35-kDa DL1 homolog of FimA, which was detected by Western blotting with anti-SsaB (19), was present in pool 1 from the DL1 and D102 sonic extracts as well as in fractions collected from other regions of the DEAE Sephacel column chromatograms (results not shown). In addition, anti-DL1 serum, which contains antibody against the FimA homolog, did not detect this component in pool C (Fig. 5). Thus, this 35-kDa protein, which is a membrane lipoprotein (18), is not specifically associated with the Hs antigen. We suspect that the extraction of bacterial cell surface antigens by methods such as sonication results in release of membrane components which are then carried in small amounts through the steps of Hs antigen purification. Consequently, the production of rabbit antibodies in the present study that react with low-molecular-weight bands in Western blotting following immunization with purified Hs antigen (i.e., pool C from the succinyl WGA affinity column) most likely represents a response to contaminating proteins rather than to minor structural components of the Hs antigen.

The fimbriae and shorter fibrillar structures that occur on *S. gordonii* and related oral species are likely sites of specific adhesins. The association of sialic acid-reactive lectin activity with a specific fibrillar antigen of strain DL1 raises the possibility that other adhesins such as those for proline-rich protein and GalNAc receptors (25) are also associated with distinct components of the fibrillar layer. To our knowledge, streptococcal surface structures such as the Hs antigen, which appear to be composed of covalently linked protein and carbohydrate, have not been dissociated to subunits nor have the corresponding genes been cloned. In this regard, a protein that reacts strongly with specific anti-Hs antibody has recently been identified by cloning and expression of a streptococcal gene in *E. coli* (47). Characterization of the cloned gene, which is in progress, should clarify the relationship of the Hs antigen to other streptococcal cell surface proteins (26) and also provide a molecular basis for further studies to define the sialic acid-binding adhesins of oral viridans group streptococci.

ACKNOWLEDGMENTS

We thank Michael J. Brennan, Kiyoshi Konishi, and Catherine Whittaker for their helpful comments during preparation and review of the manuscript.

REFERENCES

1. **Axelsen, N. H., J. Krøll, and B. Weeke.** 1973. A manual of quantitative immunoelectrophoresis: methods and applications. Scand. J. Immunol. **2**(Suppl. 1)**:**15–83.
2. **Bayer, E. A., H. Ben-Hur, and M. Wilchek.** 1988. Biocytin hydrazide—a selective label for sialic acids, galactose, and other sugars in glycoconjugates using avidin-biotin technology. Anal. Biochem. **170:**271–281.
3. **Campbell, D. H., J. S. Garvey, N. E. Cremer, and D. H. Sussdorf.** 1964. Methods in immunology. W. A. Benjamin, Inc., New York, N.Y.
4. **Cisar, J. O.** 1986. Fimbrial lectins of the oral actinomyces, p. 183–196. *In* D. Mirelman (ed.), Microbial lectins and agglutinins. John Wiley & Sons, Inc., New York, N.Y.
5. **Cisar, J. O., E. L. Barsumian, S. H. Curl, A. E. Vatter, A. L. Sandberg, and R. P. Siraganian.** 1981. Detection and localization of a lectin on *Actinomyces viscosus* T14V by monoclonal antibodies. J. Immunol. **127:**1318–1322.
6. **Cisar, J. O., P. E. Kolenbrander, and F. C. McIntire.** 1979. Specificity of coaggregation reactions between human oral streptococci and strains of *Actinomyces viscosus* or *Actinomyces naeslundii*. Infect. Immun. **24:**742–752.
7. **Clark, W. B., T. T. Wheeler, and J. O. Cisar.** 1984. Specific inhibition of adsorption of *Actinomyces viscosus* T14V to saliva-treated hydroxyapatite by antibody against type 1 fimbriae. Infect. Immun. **43:**497–501.
8. **Demuth, D. R., P. Berthold, P. S. Leboy, E. E. Golub, C. A. Davis, and D. Malamud.** 1989. Saliva-mediated aggregation of *Enterococcus faecalis* transformed with a *Streptococcus sanguis* gene encoding the SSP-5 surface antigen. Infect. Immun. **57:**1470–1475.
9. **Demuth, D. R., C. A. Davis, A. M. Corner, R. J. Lamont, P. S. Leboy, and D. Malamud.** 1988. Cloning and expression of a *Streptococcus sanguis* surface antigen that interacts with a human salivary agglutinin. Infect. Immun. **56:** 2484–2490.
10. **Demuth, D. R., Y. Duan, W. Brooks, A. R. Holmes, R. McNab, and H. F. Jenkinson.** 1996. Tandem genes encode cell-surface polypeptides SspA and SspB which mediate adhesion of the oral bacterium *Streptococcus gordonii* to human and bacterial receptors. Mol. Microbiol. **20:**403–413.
11. **Demuth, D. R., E. E. Golub, and D. Malamud.** 1990. Streptococcal-host interactions: structural and functional analysis of a *Streptococcus sanguis* receptor for a human salivary glycoprotein. J. Biol. Chem. **265:**7120–7126.
12. **Duan, Y., E. Fisher, D. Malamud, E. Golub, and D. R. Demuth.** 1994. Calcium-binding properties of SSP-5, the *Streptococcus gordonii* M5 receptor for salivary agglutinin. Infect. Immun. **62:**5220–5226.
13. **Elder, B. L., and P. Fives-Taylor.** 1986. Characterization of monoclonal antibodies specific for adhesion: isolation of an adhesin of *Streptococcus sanguis* FW213. Infect. Immun. **54:**421–427.
14. **Fachon-Kalweit, S., B. L. Elder, and P. Fives-Taylor.** 1985. Antibodies that bind to fimbriae block adhesion of *Streptococcus sanguis* to saliva-coated hydroxyapatite. Infect. Immun. **48:**617–624.
15. **Fairbanks, G., T. L. Steck, and D. F. H. Wallach.** 1971. Electrophoretic analysis of the major polypeptides of the human erythrocyte membrane. Biochemistry **10:**2606–2617.
16. **Fives-Taylor, P., M. Ladha, and K. Mintz.** 1995. Purification of a high molecular weight adhesin of *Streptococcus parasanguis*, abstr. 1516. J. Dent. Res. **74:**201.
17. **Fives-Taylor, P. M., F. L. Macrina, T. J. Pritchard, and S. S. Peene.** 1987. Expression of *Streptococcus sanguis* antigens in *Escherichia coli*: cloning of a structural gene for adhesion fimbriae. Infect. Immun. **55:**123–128.
18. **Ganeshkumar, N., N. Arora, and P. E. Kolenbrander.** 1993. Saliva-binding protein (SsaB) from *Streptococcus sanguis* 12 is a lipoprotein. J. Bacteriol. **175:**572–574.
19. **Ganeshkumar, N., M. Song, and B. C. McBride.** 1988. Cloning of a *Streptococcus sanguis* adhesin which mediates binding to saliva-coated hydroxyapatite. Infect. Immun. **56:**1150–1157.
20. **Gibbons, R. J., I. Etherden, and E. C. Moreno.** 1983. Association of neuraminidase-sensitive receptors and putative hydrophobic interactions with high-affinity binding sites for *Streptococcus sanguis* C5 in salivary pellicles. Infect. Immun. **42:**1006–1012.
21. **Gibbons, R. J., I. Etherden, and E. C. Moreno.** 1985. Contribution of stereochemical interactions in the adhesion of *Streptococcus sanguis* C5 to experimental pellicles. J. Dent. Res. **64:**96–101.
22. **Gibbons, R. J., and J. van Houte.** 1980. Bacterial adherence and the formation of dental plaques, p. 61–104. *In* E. H. Beachey (ed.), Bacterial adherence: receptors and recognition, series B, vol. 6. Chapman and Hall, London, United Kingdom.
23. **Hawkes, R.** 1982. Identification of concanavalin A-binding proteins after sodium dodecyl sulfate-gel electrophoresis and protein blotting. Anal. Biochem. **123:**143–146.
24. **Hogg, S. D., P. S. Handley, and G. Embery.** 1981. Surface fibrils may be responsible for the salivary glycoprotein-mediated aggregation of the oral bacterium *Streptococcus sanguis*. Arch. Oral Biol. **26:**945–949.
25. **Hsu, S. D., J. O. Cisar, A. L. Sandberg, and M. Kilian.** 1994. Adhesive properties of viridans streptococcal species. Microb. Ecol. Health Dis. **7:**125–137.
26. **Jenkinson, H. F., and D. R. Demuth.** 1997. Structure, function and immunogenicity of streptococcal antigen I/II polypeptides. Mol. Microbiol. **23:** 183–190.
27. **Kilian, M., L. Mikkelsen, and J. Henrichsen.** 1989. Taxonomic study of viridans streptococci: description of *Streptococcus gordonii* sp. nov. and emended descriptions of *Streptococcus sanguis* (White and Niven 1946), *Streptococcus oralis* (Bridge and Sneath 1982), and *Streptococcus mitis* (Andrewes and Horder 1906). Int. J. Syst. Bacteriol. **39:**471–484.
28. **Laemmli, U. K.** 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature **227:**680–685.
29. **Lee, S.-Y., J. O. Cisar, and A. L. Sandberg.** Unpublished data.
30. **Levine, M. J., M. C. Herzberg, M. S. Levine, S. A. Ellison, M. W. Stinson, H. C. Li, and T. Van Dyke.** 1978. Specificity of salivary-bacterial interactions: role of terminal sialic acid residues in the interaction of salivary glycopro-

teins with *Streptococcus sanguis* and *Streptococcus mutans*. Infect. Immun. **19:**107–115.
31. **Lotan, R., N. Sharon, and D. Mirelman.** 1975. Interaction of wheat-germ agglutinin with bacterial cells and cell-wall polymers. Eur. J. Biochem. **55:** 257–262.
32. **Maryanski, J. H., and C. L. Wittenberger.** 1975. Mannitol transport in *Streptococcus mutans*. J. Bacteriol. **124:**1475–1481.
33. **McBride, B. C., and M. T. Gisslow.** 1977. Role of sialic acid in saliva-induced aggregation of *Streptococcus sanguis*. Infect. Immun. **18:**35–40.
34. **McIntire, F. C., L. K. Crosby, and A. E. Vatter.** 1982. Inhibitors of coaggregation between *Actinomyces viscosus* T14V and *Streptococcus sanguis* 34: β-galactosides, related sugars, and anionic amphipathic compounds. Infect. Immun. **36:**371–378.
35. **McIntire, F. C., A. E. Vatter, J. Baros, and J. Arnold.** 1978. Mechanism of coaggregation between *Actinomyces viscosus* T14V and *Streptococcus sanguis* 34. Infect. Immun. **21:**978–988.
36. **Misevic, G. N., and M. M. Burger.** 1986. Reconstitution of high cell binding affinity of a marine sponge aggregation factor by cross-linking of small low affinity fragments into a large polyvalent polymer. J. Biol. Chem. **261:**2853–2859.
37. **Morris, E. J., N. Ganeshkumar, M. Song, and B. C. McBride.** 1987. Identification and preliminary characterization of a *Streptococcus sanguis* fibrillar glycoprotein. J. Bacteriol. **169:**164–171.
38. **Morris, E. J., and B. C. McBride.** 1984. Adherence of *Streptococcus sanguis* to saliva-coated hydroxyapatite: evidence for two binding sites. Infect. Immun. **43:**656–663.
39. **Murray, P. A., M. J. Levine, M. S. Reddy, L. A. Tabak, and E. J. Bergey.** 1986. Preparation of a sialic acid-binding protein from *Streptococcus mitis* KS32AR. Infect. Immun. **53:**359–365.
40. **Murray, P. A., M. J. Levine, L. A. Tabak, and M. S. Reddy.** 1982. Specificity of salivary-bacterial interactions. II. Evidence for a lectin on *Streptococcus sanguis* with specificity for a NeuAcα2,3Galβ1,3GalNAc sequence. Biochem. Biophys. Res. Commun. **106:**390–396.
41. **Osterland, C. K., E. J. Miller, W. W. Karakawa, and R. M. Krause.** 1966. Characteristics of streptococcal group-specific antibody isolated from hyperimmune rabbits. J. Exp. Med. **123:**599–614.
42. **Porter, R. R.** 1959. The hydrolysis of rabbit γ-globulin and antibodies with crystalline papain. Biochem. J. **73:**119–126.
43. **Rosan, B., D. Malamud, B. Appelbaum, and E. Golub.** 1982. Characteristic differences between saliva-dependent aggregation and adhesion of streptococci. Infect. Immun. **35:**86–90.
44. **Ruhl, S., J. O. Cisar, and A. L. Sandberg.** Leukosialin and leukocyte common antigen: polymorphonuclear leukocyte and HL-60 cell receptors for streptococcal and actinomyces lectins. Submitted for publication.
45. **Ruhl, S., A. L. Sandberg, M. F. Cole, and J. O. Cisar.** 1996. Recognition of immunoglobulin A1 by oral actinomyces and streptococcal lectins. Infect. Immun. **64:**5421–5424.
46. **Schneewind, O., A. Fowler, and K. F. Faull.** 1995. Structure of the cell wall anchor of surface proteins in *Staphylococcus aureus*. Science **268:**103–106.
47. **Takahashi, Y.** Unpublished data.
48. **Towbin, H., T. Staehelin, and J. Gordon.** 1979. Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: procedure and some applications. Proc. Natl. Acad. Sci. USA **76:**4350–4354.

Editor: J. R. McGhee