INFECTION AND IMMUNITY, Feb. 1984, p. 497–501
0019-9567/84/020497-05$02.00/0

Vol. 43, No. 2

Specific Inhibition of Adsorption of *Actinomyces viscosus* T14V to Saliva-Treated Hydroxyapatite by Antibody Against Type 1 Fimbriae

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Received 8 August 1983/Accepted 21 October 1983

Specific antibodies and their Fab fragments were used to study adsorption of *Antinomyces viscosus* T14V to saliva-treated hydroxyapatite. Antibody against *A. viscosus* T14V blocked adsorption of the strain to saliva-treated hydroxyapatite, and this activity was removed after preincubation of the immune immunoglobulin G (IgG) with purified type 1 fimbriae in amounts near equivalence but not after preincubation with type 2 fimbriae in antigen excess. Specific IgG or Fab fragments against type 1 fimbriae inhibited adsorption of strain T14V to saliva-treated hydroxyapatite and promoted desorption of cells already attached to the apatite surface. Anti-type 2 IgG or Fab fragments did not possess these activities. These data demonstrate that type 1 but not type 2 fimbriae mediate adsorption of strain T14V to the saliva-treated hydroxyapatite surface.

Actinomyces viscosus, a gram-positive facultative rod found in human dental plaque, has been implicated in the etiology of gingivitis (15), periodontitis (23), and cemental caries (12). In addition, *A. viscosus* T14V has been shown to induce periodontal pathology and root caries when monoinfected in germfree rats, and in this animal model, virulence depends on the ability of strain T14V to colonize the teeth and gingival crevice (2). In recent years it has become evident that specific fimbriae on many gram-positive and gram-negative bacteria promote colonization of a host by mediating bacterial adsorption to specific host tissue receptors (1). With *A. viscosus* T14V, fimbriae defined by the VA1 antigen have been associated with in vitro adsorption of bacteria to saliva-treated hydroxyapatite (SHA) (21), an interaction that is not inhibited by lactose (22) or various simple sugars (T. T. Wheeler, Ph.D. dissertation, University of Florida, Gainesville, 1980). More recently, these fimbriae have been identified as one of two antigenically distinct types present on strain T14V (3, 5, 6, 19). Lactose-sensitive adherence of *A. viscosus* to certain oral streptococci (i.e., coaggregation [7]) and neuraminidase-treated erythrocytes (i.e., hemagglutination [10]) is mediated by the other type of fimbriae, initially designated Ag2 (4–6, 19). Thus, the two types of fimbriae, type 1 (VA1) and type 2 (Ag2), may promote distinct adsorptive interactions within the oral environment.

The present study was initiated to determine whether adsorption of strain T14V to SHA was associated exclusively with type 1 fimbriae or whether both type 1 and type 2 fimbriae contributed to this interaction. Rabbit immunoglobulin G (IgG) and Fab fragments monospecific for each type of fimbriae were examined for their abilities to inhibit adsorption of strain T14V to SHA. The results obtained indicate that adsorption to SHA is mediated by type 1 but not type 2 fimbriae.

MATERIALS AND METHODS

Bacterial strains. *A. viscosus* T14V was obtained from the culture collection at the University of Florida College of Dentistry, Gainesville. *A. viscosus* PK455 was kindly provided by P. E. Kolenbrander, National Institute of Dental Research, Bethesda, Md. Strain PK455, initially isolated as a spontaneous coaggregation-defective mutant of *A. viscosus* T14V (13), has been shown to possess type 1 but no type 2 fimbriae (6). All isolates were maintained as multiple frozen stocks and cultured in tryptic soy broth with glucose (Difco Laboratories, Detroit, Mich.) as described previously (22). For adsorption assays, strain T14V cells were cultured in tryptic soy broth with glucose supplemented with 10 μCi of [^{3}H]thymidine per ml (Schwarz/Mann, Orangeburg, N.Y.) (8).

Fimbrial antigens. Type 1 fimbriae were isolated from strains T14V and PK455 by methods described previously (19, 21). Briefly, fimbriae were removed from bacteria by passage of the cells through a French pressure cell (21) or a continuous-flow Sonifier cell disrupter (19). The removed fimbriae were sedimented for 24 h at 160,000 × *g* (21) or collected in fractions obtained at the void volume of an Agarose 5M column (Bio-Rad Laboratories, Richmond, Calif.) (19). Type 1 fimbriae were purified by repeated precipitation with 10% (wt/vol) ammonium sulfate (21). Type 2 fimbriae from strain T14V were separated from type 1 fimbriae and purified by procedures (4, 19) that included the use of an affinity column prepared with an anti-type 2 monoclonal antibody, protein 2A (5). The protein concentrations of all antigen preparations were determined by the method of Lowry et al. (16), using bovine serum albumin as a standard.

Antisera and immunological methods. Rabbit antiserum against *A. viscosus* T14V was prepared by multiple subcutaneous and intravenous injections of washed bacterial cells. The immunization schedule was similar to that described previously (21). Antiserum against type 1 fimbriae of strain T14V was prepared by immunization with the purified antigen (2 mg) in Freund incomplete adjuvant (Difco) administered subcutaneously in multiple spots. After 50 to 60 days, the animals each received a booster injection of 2 mg of type 1 fimbriae intravenously. Antiserum was obtained 7 days after the final booster injection. The production of antiserum

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against affinity-purified type 2 fimbriae has been described previously (4). To prepare immune rabbit IgG, serum proteins precipitated with 50% saturated ammonium sulfate were dissolved and dialyzed against 0.02 M sodium phosphate buffer (pH 8.0) and applied to a column of DEAE-cellulose (De-52; Whatman, Inc., Clifton, N.J.). The peak of IgG that passed through the column was collected.

To establish specificity, the immune IgG against each fimbrial component was titrated by enzyme-linked immunosorbent assay (ELISA), using microELISA plates (Immulon I; Dynatech Laboratories, Inc., Alexandria, Va.) coated with 10 μg of purified type 1 or type 2 fimbriae per ml. The detailed procedures used for ELISA have been described previously (4, 19). The results of such titrations showed that the immune IgG against each type of fimbriae also contained detectable antibody against the other type of fimbriae (4). The contaminating antibody in each preparation was specifically removed by precipitation at equivalence with the corresponding purified fimbriae to produce monospecific reagents (Table 1).

Fab and Fc fragments were obtained by the digestion of monospecific rabbit IgG with 2% (wt/wt) papain (Boehringer Mannheim Biochemicals, Indianapolis, Ind.) for 2 h at pH 5.6. The fragments were isolated from the digest by gel filtration chromatography (Sephacryl S200; Pharmacia Fine Chemicals, Piscataway, N.J.) and separated from each other by carboxymethyl cellulose (CM-52, Whatman) column chromatography (18).

Adsorption assays. Preparation of SHA (BDH, Poole, England) was by methods described previously (8). Whole paraffin-stimulated saliva from one donor (female; age, 24 years) was collected, clarified, and used as described previously (11). Tritium-labeled bacteria (final concentration, 4×10^6 cells per ml) were added to 300-μl-capacity microfuge tubes (Fisher Scientific Co., Pittsburgh, Pa.) containing 10 mg of SHA, incubated for 90 min at room temperature, and then assayed by scintillation counting to determine the number of cells adsorbed (22). Under these conditions, ca. 80 to 90% of the added cells adsorbed to the SHA surface.

Adsorption inhibition assays were performed by including antigen-specific IgG or Fab fragment preparations in the cell-SHA reaction mixture (9, 21). Strain T14V cells suspended at a concentration of 8×10^6 per ml were diluted with an equal volume of adsorption buffer containing various concentrations of anti-type 1 or anti-type 2 IgG or Fab fragments. Controls consisted of cells mixed with normal rabbit IgG, Fab fragments, or adsorption buffer alone. In other experiments IgG or Fab fragment preparations were incubated for 30 min with purified fimbriae of each type and then assayed for adsorption inhibition activity. Percent inhibition of adsorption was calculated by the following formula: % inhibition of adsorption = [1 − (adsorption in the presence of antigen-specific IgG or Fab fragments/adsorption in the presence of normal IgG or Fab fragments)] × 100.

The ability of specific antibody to cause desorption of strain T14V cells from SHA also was tested. Ten milligrams SHA was incubated with 4×10^6 cells per ml for 90 min. After incubation, SHA was rinsed two times with adsorption buffer to remove nonadherent cells. The washes were pooled with the supernatant and assayed by scintillation counting. SHA with adsorbed cells was transferred to a fresh test tube. Desorption was accomplished by incubating SHA in 25 μg of the various IgG or Fab fragment preparations per ml. After 60 min the supernatant was removed, and a fresh IgG or Fab fragment preparation was added to the tube for an additional 60 min. Supernatants from each 60-min incubation as well as the 10 mg of SHA were assayed separately by scintillation counting to determine the percentage of cells which had been desorbed. Quenching by the 10 mg of SHA was corrected by counting known quantities of ^{3}H-labeled cells adsorbed to the 10 mg of SHA (8).

RESULTS

Before IgG and Fab fragment preparations were used in studies of bacterial adsorption, each preparation was evaluated for its specific reactivity with purified fimbriae of each type. The anti-T14V IgG contained antibody against type 1 and type 2 fimbriae, with 50 and 38 μg of IgG per ml, respectively, required for equivalent optical densities in each ELISA (Table 1). Low concentrations (ca. 4 μg/ml) of monospecific anti-type 1 IgG and Fab fragments reacted to give an optical density of 1.0 in ELISA for antibody against type 1 fimbriae, whereas 300 μg of these Ig preparations per ml did not react above background when ELISA plates were coated with type 2 fimbriae. Similarly, anti-type 2 IgG and Fab fragments were active in ELISA for antibody against type 2 but not type 1 fimbriae.

Antibody against T14V cells effectively blocked adsorption of strain T14V to SHA, with 50 μg of IgG per ml giving 87% inhibition. To establish specificity, the anti-T14V IgG was incubated with various amounts of purified type 1 or type 2 fimbriae before assays were done to determine the inhibition of adsorption (Fig. 1). Preincubation with as little as 2.5 μg of type 1 fimbriae per ml completely abolished the ability of antibody to prevent adsorption. This ratio of fimbriae to IgG removed ca. 80% of the anti-type 1 activity detected by ELISA (data not shown), and all remaining activity was lost in the presence of higher antigen concentrations. In contrast, the adsorption inhibition effect of anti-T14V IgG was not altered by preincubation with type 2 fimbriae at 50 μg/ml, a concentration at least twice that needed to completely abolish the anti-type 2 activity detected by ELISA (data not shown).

Preparations of monospecific IgG and Fab fragments against each fimbrial component also were compared for their effects on adsorption of strain T14V to SHA (Fig. 2). Approximately 2.5 μg of anti-type 1 IgG per ml and ca. 5 μg of anti-type 1 Fab fragments per ml gave 50% inhibition, and at 10 μg/ml, both gave maximum values of inhibition, similar to those achieved with much higher concentrations of anti-T14V IgG. In contrast, anti-type 2 IgG and Fab fragments exhibited no inhibitory activity even when 500 μg/ml was present in the adsorption assay.

Anti-type 1 IgG and Fab fragment were found to promote desorption of strain T14V from SHA. Thus, two 1-h incubations in the presence of either preparation removed ca. 75% or more of the cells initially adsorbed (Table 2). In contrast, less than 15% desorption occurred over the same period in

TABLE 1. ELISA of IgG and Fab fragment preparations against type 1 and type 2 fimbriae

Antibody specificity	Antibody activity[a] in ELISA with purified fimbriae	
	Type 1	Type 2
Anti-T14V IgG	50.0	38.0
Anti-type 1 IgG	4.5	>300
Anti-type 1 Fab fragments	4.3	>300
Anti-type 2 IgG	>300	8.3
Anti-type 2 Fab fragments	>300	7.3

[a] Activity is expressed as the IgG or Fab fragment concentration (micrograms per milliliter) required for an optical density of 1.0 in ELISA.

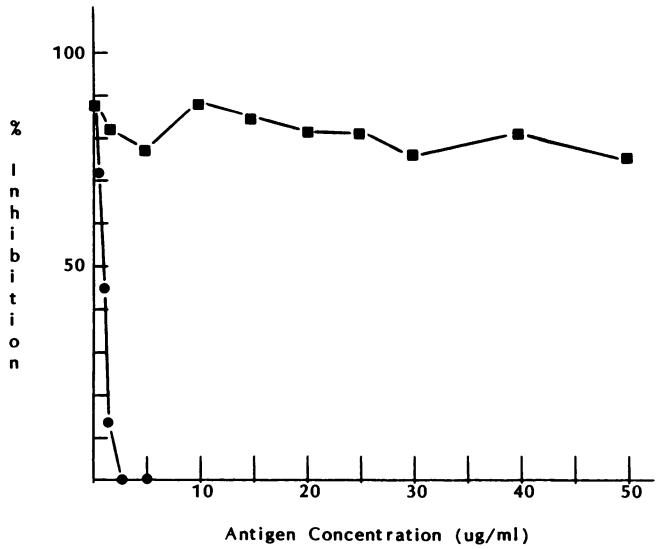

FIG. 1. Removal of adsorption inhibition activity from anti-T14V IgG by preincubation with various quantities of type 1 (●) or type 2 (■) fimbriae. Absorbed antibody was then mixed with strain T14V cells and incubated with SHA. The final concentration of antibody was 50 μg/ml. The final concentration of antigen used to absorb the antibody is shown. In the presence of normal IgG or buffer alone, 88% of the available cells (4×10^6 cells per ml) adsorbed to the SHA surface. In the presence of 50 μg of nonabsorbed anti-T14V IgG per ml, 13% of the available cells adsorbed to SHA. Data are expressed as percent inhibition of adsorption relative to inhibition by normal IgG.

the presence of anti-type 2 IgG or Fab fragments, normal IgG, or buffer alone.

DISCUSSION

This study clearly associates the type 1 fimbriae of *A. viscosus* T14V with bacterial adsorption to SHA. In extending the previous finding that antibody against T14V cells inhibits this interaction (21), the present results demonstrate that this effect is lost after preincubation of immune IgG with purified type 1 fimbriae in amounts near equivalence but not with type 2 fimbriae in antigen excess (Fig. 1). Therefore, antibodies directed against bacterial surface components other than type 1 fimbriae do not block adsorption of T14V cells to SHA. Further evidence of specificity was obtained from the inhibition of bacterial adherence by monospecific anti-type 1 but not anti-type 2 IgG or Fab fragments (Fig. 2). Anti-type 1 Fab fragments exhibited inhibitory properties quite similar to those of the undigested antibody. Therefore, this effect resulted directly from the binding of antibody to type 1 fimbriae and not from bacterial agglutination or cross-linking of surface structures on individual cells (14). Finally, the ability of anti-type 1 IgG to cause desorption of attached bacteria (Table 2) clearly suggests that type 1 fimbriae participate in the firm attachment of strain T14V cells to SHA. Collectively, these findings from experiments performed in vitro favor the hypothesis that adherence of *A. viscosus* strains to the salivary pellicle of teeth represents a specific function of type 1 fimbriae.

Certain recent observations suggest that only a fraction of the total antibody against type 1 fimbriae is active in blocking the interaction of bacteria with SHA. Thus, different monospecific antisera, though similar in anti-type 1 activity as determined by ELISA, have been found to differ

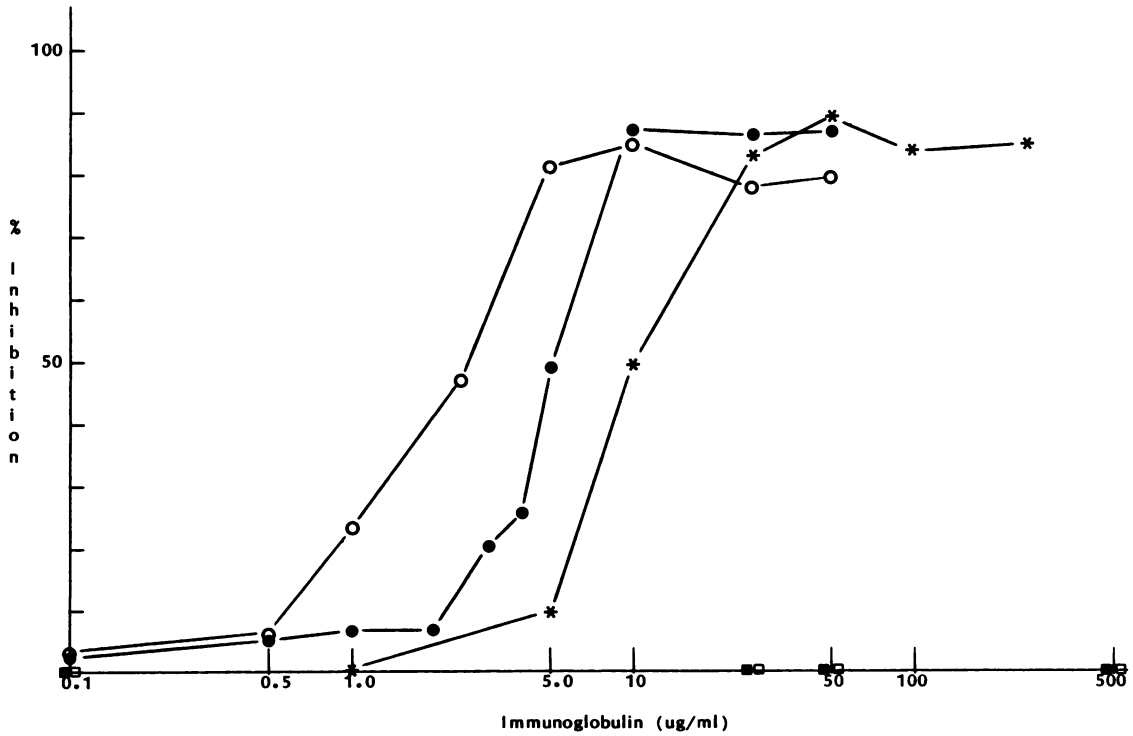

FIG. 2. Titration of adsorption inhibition activity for anti-type 1 IgG (○), anti-type 1 Fab fragments (●), anti-T14V IgG (*), anti-type 2 IgG (■), anti-type 2 Fab fragments (□). In the presence of buffer alone or normal IgG in buffer, 85% of the available 4×10^6 cells per ml adsorbed to the SHA surface. Data are expressed as percent inhibition of adsorption relative to inhibition by normal IgG.

TABLE 2. Specific desorption of strain T14V cells from SHA by anti-type 1 IgG

Antibody specificity	% of cells desorbed from SHA ± SE[a]	
	h 1	h 2
Anti-type 1 IgG	64.4 ± 9.7	10.6 ± 4.1
Anti-type 1 Fab fragment	64.4 ± 9.3	21.6 ± 2.1
Anti-type 2 IgG	6.8 ± 1.3	5.6 ± 1.5
Anti-type 2 Fab fragment	6.7 ± 0.4	1.9 ± 0.1
Normal IgG	7.8 ± 2.6	6.1 ± 1.4
Buffer	7.5 ± 3.2	4.7 ± 0.7

[a] Number of cells initially adsorbed to 10 mg of SHA ± the standard error was (3.3 ± 0.1) × 10^6.

significantly in their abilities to inhibit adsorption, and certain monoclonal antibodies specific for type 1 fimbriae have failed to inhibit adsorption even when tested in combinations (unpublished data). These results may indicate that the fimbrial domains for adsorption are not closely associated with certain principal epitopes of type 1 fimbriae. Studies are in progress to isolate the fraction of antibody that blocks adsorption and to determine whether the fraction binds to the fimbrial region or subcomponent directly involved in the interaction with SHA.

The apparent involvement of type 1 but not type 2 fimbriae in adsorption of strain T14V to SHA provides a further indication that the two structures are distinct in their functional properties. Previous studies (4–6, 19) have shown that lactose-sensitive adherence of *A. viscosus* represents an exclusive function of type 2 fimbriae. Consequently, the lectin activity that mediates coaggregation of *A. viscosus* strains with certain plaque streptococci appears not to participate in the attachment of bacteria to the acquired salivary pellicle, a possibility consistent with the failure of lactose to inhibit adsorption of strain T14V to SHA (22). The presence of functionally different fimbriae on *A. viscosus* suggests certain similarities in the adherence properties of this organism and certain uropathogenic *Escherichia coli* strains. For example, mannose-resistant fimbriae on *E. coli* O6:K2 H1 C124 are thought to mediate adsorption of that bacteria to uroepithelial surfaces, whereas other fimbriae which are mannose sensitive seem more important in the colonization of the large intestine (17). Similarly, type 1 fimbriae of *A. viscosus* strains appear to be the principal structures for the lactose-resistant attachment of the bacteria to the acquired salivary pellicle, leaving type 2 fimbriae free for interactions with other plaque bacteria such as *Streptococcus sanguis*. The presence of both fimbriae on the same bacterium may therefore help to explain the appearance of *A. viscosus* among the early colonizers of a cleaned tooth surface and the ability of *A. viscosus* to increase significantly in number as the plaque matures (20). Possession of two or more mechanisms for attaching to and colonizing on the tooth surface increases the probability that the bacteria will persist in colonizing the host even in the presence of potent host defenses.

ACKNOWLEDGMENTS

This work was supported by Public Health Service grant DE-05429 from the National Institute of Dental Research. W.B.C. was supported by Research Career Development Award DE00115 from the National Institute of Dental Research. Additional support was provided by the College of Dentistry, University of Florida, Gainesville.

We acknowledge the expert technical assistance of Marcia D. Lane and Shelley Curl. We thank Ann L. Sandberg for her critical advice on the preparation of this manuscript. We also thank M. J. Owen for typing this manuscript.

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