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# Lectin-Dependent Attachment of *Actinomyces naeslundii* to Receptors on Epithelial Cells

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**The adherence of *Actinomyces naeslundii* WVU45 to monolayer cultures of human epithelial cell lines was mediated by the lactose-sensitive fimbriae (type 2) of strain WVU45. The attachment of *Actinomyces viscosus* T14V, which has both types 1 and 2 fimbriae, was approximately half that of *A. naeslundii*, and only minimal attachment of *A. naeslundii* and *A. viscosus* mutants lacking type 2 fimbriae was detected. The adherence of strain WVU45 was enhanced two- to threefold by neuraminidase treatment of the epithelial cells. The Fab fragments of antibodies which recognize the type 2 fimbriae inhibited the adherence of *A. naeslundii* WVU45 to the epithelial cells. The bacterial interaction with epithelial cells was inhibited by lactose, methyl-β-D-galactoside, and *N*-acetyl-D-galactosamine, but not by methyl-α-D-galactoside, cellobiose, *N*-acetyl-D-glucosamine, L-fucose, or D-mannose. To further characterize the epithelial cell receptors for the bacterial lectin, we utilized several plant and invertebrate lectins as potential inhibitors of bacterial adherence. Lectins from *Bauhinia purpurea* and *Arachis hypogaea* which recognize *N*-acetyl-D-galactosamine, D-galactose, and D-galactose-β-(1→3)-*N*-acetyl-D-galactosamine inhibited bacterial attachment, and binding of these lectins to epithelial cells was enhanced by the addition of neuraminidase. Lectins reacting with α-linked D-galactose, α-linked *N*-acetyl-D-galactosamine, D-mannose, or sialic acid were not inhibitory. Under similar assay conditions, adherence of a mannose-sensitive strain of *Escherichia coli* was inhibited by concanavalin A but not by the lectin from *Bauhinia purpurea*. These results indicate that certain plant lectins have specificities similar to that of the actinomyces fimbrial lectin and are, therefore, useful probes for identifying receptors on epithelial cells for certain bacteria.**

The initial event in host infection by many bacteria is their attachment to mammalian cells via specific recognition structures. Colonization of the oral cavity by *Actinomyces naeslundii* may be attributable to such interactions since typical strains of these bacteria are associated with epithelial surfaces (10, 11, 22). Fimbriae have been detected on *A. naeslundii* and have been implicated in its attachment to buccal epithelial cells (13). As demonstrated in the accompanying paper (5), only lactose-sensitive fimbriae are present on a typical strain of *A. naeslundii*, WVU45. These fimbriae are antigenically related to the type 2 fimbriae of *Actinomyces viscosus* T14V that mediate lactose-sensitive attachment to streptococci and neuraminidase-treated human erythrocytes (2, 3, 9, 20). The type 2 fimbriae are clearly distinguishable by immunochemical criteria from the type 1 fimbriae of *A. viscosus* T14V which interact with saliva-treated hydroxyapatite and are postulated to be the adhesins for bacterial colonization of tooth surfaces (8, 24).

The present studies were designed to investigate the involvement of type 2 fimbriae in adherence of actinomyces to epithelial cells and, further, to identify the structures on epithelial cells which interact with these bacteria. These investigations have utilized human cell lines of epithelial origin to maintain a stable cell population from which this receptor can eventually be isolated in the absence of previously attached bacteria. Partial characterization of this receptor has been approached not only by examination of the inhibitory effects of certain saccharides on bacterial attachment but also by the utilization of plant lectins of defined specificity as inhibitors of this process.

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## MATERIALS AND METHODS

**Microorganisms.** *A. naeslundii* WVU45 and *A. viscosus* T14V were obtained and grown in complex media as previously described (6, 9). The selection and characterization of *A. naeslundii* WVU45M, a mutant lacking fimbriae, is described in the accompanying report (5). Mutants of *A. viscosus* T14V lacking type 1 ($1^- 2^+$), type 2 ($1^+ 2^-$), or both ($1^- 2^-$) fimbriae were selected by their lack of agglutination with antisera to type 1, type 2, or both types 1 and 2 fimbriae (J. O. Cisar, manuscript in preparation), and the absence of specific fimbriae was verified by methods similar to those previously described (4). *Escherichia coli* B (ATCC e11303) was purchased from the American Type Culture Collection, Bethesda, Md., and grown in tryptic soy broth. Bacteria were radiolabeled for 48 h in growth media containing 0.01 mCi of [*methyl*-$^3$H]thymidine per ml (specific activity, 6 Ci/mmol; Schwarz/Mann, Orangeburg, N.Y.).

**Mammalian cells.** The human cell lines KB (ATCC CCL 17), Intestine 407 (ATCC CCL 6), HEp-2 (ATCC CCL 23), RPMI 2650 (ATCC CCL 30), and Gin-1 (ATCC CRL 1292) were purchased from the American Type Culture Collection. The human skin fibroblasts (ATCC CRL 1475) were a gift from Pat Van Story Lewis, National Institute of Dental Research, Bethesda, Md. All cells were routinely grown in Dulbecco modified essential medium (HEM Research Inc., Bethesda, Md.) supplemented with 5% fetal calf serum (Flow Laboratories, Inc., McLean, Va.), 100 U of penicillin per ml, and 100 μg of streptomycin per ml and were maintained in a 5% $CO_2$ atmosphere at 37°C.

**Antibodies.** Antisera from rabbits hyperimmunized with *A.*

*naeslundii* WVU45 or the mutant of WVU45 (WVU45M) as well as monospecific antisera against the *A. naeslundii* fimbriae have been described elsewhere (5). The IgG fractions were obtained, and Fab fragments were prepared by papain digestion and isolated by gel filtration and ion exchange chromatography (8, 20).

**Lectins and saccharides.** Lectins from *Arachis hypogaea* (peanut) and *Ricinus communis* (RCA-120) were purchased from Miles Laboratories, Inc., Elkhart, Ind. Concanavalin A (ConA) was from Calbiochem-Behring, La Jolla, Calif., and *Erythrina cristagalli* agglutinin was a gift from Nathan Sharon, Weizmann Institute, Rehovoth, Israel. The lectins from *Bauhinia purpurea*, *Griffonia simplicifolia*, *Dolichos biflorus*, *Glycine max*, and *Limulus polyhemus* were purchased from E-Y Laboratories Inc., San Mateo, Calif., and were of the purest grade available. Lectins were radiolabeled by reductive methylation with 1 mM [$^3$H]formaldehyde (specific activity, 85 mCi/mmol; New England Nuclear Corp., Boston, Mass.) as described by Jentoft and Dearborn (14). Lactose (0.1 M) was added to the lectins from *Bauhinia purpurea*, *Arachis hypogaea*, and *Erythrina cristagalli*, and mannose (0.1 M) was added to ConA to protect the sugar binding sites during labeling.

Saccharides were obtained from Sigma Chemical Co., St. Louis, Mo.

**Attachment assay.** Twenty-four well cluster plates (Costar, Cambridge, Mass.) were seeded with $7 \times 10^4$ mammalian cells per well, and 48 h later confluent cultures ($2.5 \times 10^5$ cells per well) were used for the assay. Only confluent cultures were utilized to minimize the nonspecific adsorption of bacteria to the polystyrene surface. The cells were washed three times with Hanks balanced salt solution, and 0.75 ml of attachment buffer (Hanks balanced salt solution containing 0.2 mg of $CaCl_2$ per ml, 0.2 mg of $MgSO_4$ per ml, and 0.2% bovine serum albumin) was added to each well. Neuraminidase from *Clostridium perfringens* (type X; Sigma Chemical Co.) was included in the attachment buffer as indicated in the text. Radiolabeled bacteria were washed three times with Hanks balanced salt solution and suspended in attachment buffer to a concentration of ca. $8 \times 10^8$ bacteria per ml as determined with a Petroff-Hausser chamber. A sample of 0.25 ml was added to each well and incubated at 37°C with gentle rocking for the indicated time periods.

After the incubation period, the wells were washed three times with attachment buffer, and bound material was solubilized in 0.5 ml of a solution containing 1% sodium dodecyl sulfate, 8 M urea, and 1 M NaCl for 30 min at 37°C. This material and a subsequent 0.5-ml wash with the solubilization buffer were suspended in Ultrafluor (National Diagnostics Inc., Somerville, N.J.) and counted in a Beckman LS 9000 scintillation counter. In all assays, additional wells containing the various test materials were treated with trypsin (0.1% for 10 min); trypan blue was added, and the cell number and viability were determined. Attachment data are presented as the number of bacteria per mammalian cell calculated from the specific activity of the bacteria and the number of mammalian cells per well, and each value represents the arithmetic mean of three wells.

Antibody inhibition was examined by incubating bacteria with Fab fragments for 10 min and washing the bacteria twice with attachment buffer before their addition to mammalian cell cultures.

Saccharide inhibition was assayed in cultures of KB cells incubated for 30 min in the presence of bacteria and saccharides diluted in attachment buffer.

The effects of lectins on adherence were assessed by incubating the epithelial cell monolayers with the lectins diluted in attachment buffer for 60 min at 37°C before the addition of bacteria.

Bacteria that had adhered to KB cells cultured in 25-cm$^2$ flasks (Falcon no. 3013; Becton Dickinson Labware, Oxnard, Calif.) were processed in situ for electron microscopic examination. The samples were fixed with Karnovsky fixative containing 1% tannic acid, post fixed in 0.1 M sodium cacodylate buffer (pH 7.3) containing 1% osmium tetroxide, dehydrated in ethanol, and infiltrated with Epon (7; M. J. Karnovsky, J. Cell Biol. **27**:137A, 1965). After polymerization, portions of the flask were sectioned and stained with lead citrate (21).

**Lectin binding assay.** Binding of $^3$H-lectins, diluted 1:10 with unlabeled lectin at the concentrations indicated in the text, to KB epithelial cells was assayed in 96 well tissue culture plates. The amount of radioactivity bound after 90 min of incubation and the specific activity of each lectin were used to determine the micrograms of lectin bound per $10^5$ cells.

## RESULTS

**Attachment of actinomyces to human cell lines.** *A. naeslundii* WVU45 attached to monolayer cultures of human KB oral epithelial cells (Fig. 1A). Adherence of *A. naeslundii* to this epithelial cell line was increased two- to threefold by the addition of $2 \times 10^{-4}$ U of neuraminidase per well. This enhancement was observed if the epithelial cells were preincubated with neuraminidase and washed or if the neuraminidase was present during attachment but not if the bacteria were pretreated with the enzyme. To standardize the assays, we performed all additional adherence experiments in the presence of exogenous neuraminidase. Similar results were obtained with other human cell lines of epithelial origin, including Intestine 407, HEp-2, and RPMI 2650. Under these assay conditions, bacterial attachment continued to increase over a 4-h incubation period. Electron microscopic observations suggested that this may be due to increased bacteria-

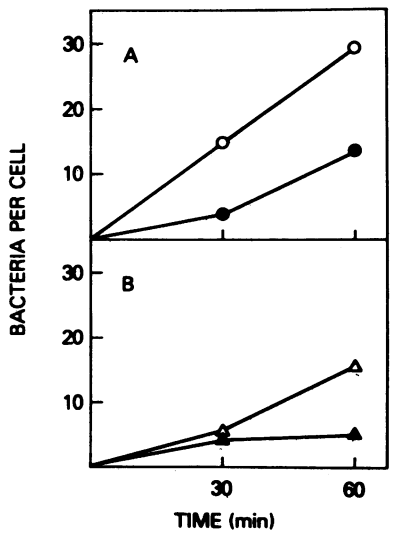


FIG. 1. Adherence of *A. naeslundii* WVU45 to monolayer cultures of human cells. [$^3$H]thymidine-labeled *A. naeslundii* ($8.6 \times 10^4$ cpm per well) were incubated at 37°C with KB human epithelial cells (A) or ATCC CRL 1475 human skin fibroblasts (B) in the absence (●, ▲) or presence (○, △) of $2 \times 10^{-4}$ U of neuraminidase per well. Data represent the arithmetic mean of triplicate determinations.

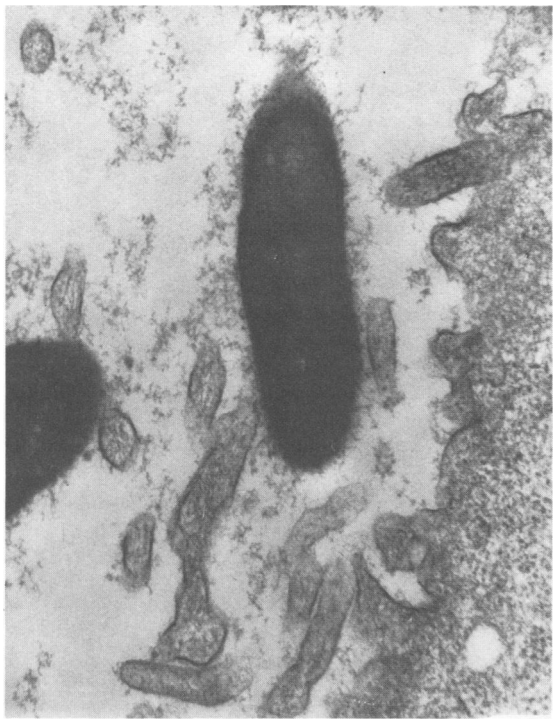

FIG. 2. Adherence of *A. naeslundii* WVU45 to KB epithelial cells. The bacterial fimbriae appear to interact with the microvilli of KB epithelial cells (×36,000).

bacteria interactions as well as bacteria-epithelial cell interactions (unpublished observations).

Although bacterial attachment to human fibroblasts was observed (Fig. 1B), it was consistently less than attachment to epithelial cells. Adherence to fibroblasts was also enhanced in the presence of neuraminidase. Identical results were obtained with ATCC CRL 1475 and Gin-1 fibroblasts.

**Identification of the *A. naeslundii* adhesin for human epithelial cell lines.** Observations at the ultrastructural level demonstrated that the bacteria interacted primarily with the microvillar extensions of the epithelial cells and that this interaction was mediated by bacterial fimbriae (Fig. 2). Support for this interpretation was provided by the finding that *A. naeslundii* WVU45M, a mutant which lacks type 2 fimbriae, adhered minimally to KB cells (Fig. 3A). Further evidence for the function of type 2 fimbriae in attachment to epithelial cells was obtained by using *A. viscosus* T14V, which possesses type 2 fimbriae as well as fimbriae implicated in attachment to saliva-treated hydroxyapatite (type 1), and mutants of *A. viscosus* which lack type 1, type 2, or both fimbriae (Fig. 3B). The parent strain and the mutant lacking only type 1 fimbriae attached to the epithelial cell monolayers. This adherence was approximately half that of *A. naeslundii* WVU45, which possesses only the type 2 fimbriae. The minimal levels of attachment of *A. viscosus* mutants lacking the type 2 fimbriae were similar to that of the *A. naeslundii* mutant which lacks fimbriae.

Additional support for the interaction of the *A. naeslundii* fimbriae with the epithelial cells was provided by the finding that Fab fragments of antibodies reactive with the type 2 fimbriae inhibited attachment. The Fab fragments of antibodies obtained from serum of a rabbit hyperimmunized with *A. naeslundii* WVU45 were almost completely inhibitory at a protein concentration of 25 μg/ml (Fig. 4). In

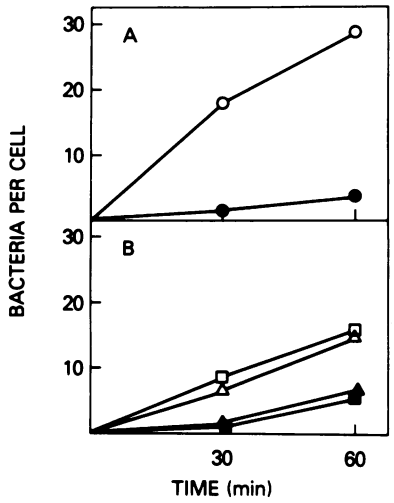


FIG. 3. Attachment of parent and mutant actinomyces strains to KB epithelial cells. (A) Adherence of *A. naeslundii* WVU45 ($4.9 \times 10^4$ cpm per well) (○) and *A. naeslundii* WVU45M, a mutant lacking type 2 fimbriae ($7.4 \times 10^4$ cpm per well) (●). Cultures contained $1.2 \times 10^8$ bacteria, $2.5 \times 10^5$ KB cells, and $3 \times 10^{-3}$ U of neuraminidase per well. Data represent the arithmetic mean of triplicate determinations. (B) Adherence of *A. viscosus* T14V ($9.6 \times 10^4$ cpm per well) (△) and the *A. viscosus* mutants lacking type 1 fimbriae ($9.9 \times 10^4$ cpm per well) (□), type 2 fimbriae ($9.8 \times 10^4$ cpm per well) (▲), or both fimbriae ($1 \times 10^5$ cpm per well) (■) was determined as described in (A).

contrast, the Fab fragments of antibodies derived from antisera raised against the mutant *A. naeslundii*, WVU45M, (50 μg/ml) had no effect on bacterial attachment. The anti-*A. naeslundii* WVU45 antiserum differed from the anti-*A. naeslundii* WVU45M antiserum in that the former contained an antibody population which recognized the fimbriae on the parent strain (5).

**Properties of the receptor for *A. naeslundii* on epithelial cell lines.** Attachment of *A. naeslundii* to KB cells was inhibited most effectively by β-linked galactosides (Table 1). The most

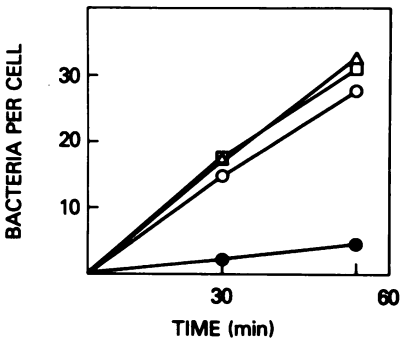


FIG. 4. The effects of Fab fragments of antibodies which recognize *A. naeslundii* type 2 fimbriae on bacterial attachment to KB epithelial cells. Radiolabeled *A. naeslundii* ($2 \times 10^8$ bacteria per ml) were preincubated for 10 min with 25 μg of Fab fragments of antibodies against *A. naeslundii* WVU45 (●) per ml, 50 μg of Fab fragments against *A. naeslundii* WVU45M, a mutant lacking type 2 fimbriae (○) per ml, 25 μg of Fab fragments of IgG from preimmune rabbit serum (□) per ml, or buffer (△). Adherence of *A. naeslundii* ($3.5 \times 10^4$ cpm per well) to KB epithelial cells ($2.5 \times 10^5$ cells per well) was performed in the presence of $3 \times 10^{-3}$ U of neuraminidase per well. Data represent the arithmetic mean of triplicate determinations.

TABLE 1. Inhibition of bacterial attachment to epithelial cells by saccharides[a]

| Saccharide | Concn for 50% inhibition (mmol) | |
|---|---|---|
| | *A. naeslundii* | *E. coli* |
| Methyl-β-D-galactoside | 2 | >100 |
| Lactose | 5 | >100 |
| D-Galactose | 8 | >100 |
| *N*-Acetyl-D-galactosamine | 9 | >100 |
| Methyl-α-D-galactoside | >100 | >100 |
| Cellobiose | >100 | >50 |
| *N*-Acetyl-D-glucosamine | >100 | >100 |
| L-Fucose | >100 | >100 |
| Methyl-α-D-mannoside | >100 | 1 |

[a] KB cells were incubated with saccharides and $2 \times 10^8$ radiolabeled bacteria for 30 min in the presence of $3 \times 10^{-3}$ U of neuraminidase. In the absence of inhibitors, 20 *A. naeslundii* cells and 22 *E. coli* cells attached per KB cell.

potent inhibitor of attachment was methyl-β-D-galactoside, whereas its α anomer had no effect on adherence at concentrations up to 100 mM. Lactose, D-galactose, and *N*-acetyl-D-galactosamine were also inhibitory, whereas 50% inhibition was not achieved by 100 mM cellobiose, *N*-acetyl-D-glucosamine, L-fucose, or methyl-α-D-mannoside. In addition to its inhibitory properties, lactose also reversed the attachment of bacteria which had been incubated with KB cells for 30 min before the addition of the sugar at a final concentration of 50 mM (data not shown). The specificity of these interactions was established in similar studies utilizing a mannose-sensitive strain of *E. coli*. Attachment of radiolabeled *E. coli* to KB cells was inhibited 50% by 1 mM methyl-α-D-mannoside, whereas the β-galactosides had no effect on adherence of this organism (Table 1).

Since the bacterial fimbriae which mediate attachment to the epithelial cells have lectin activity, a variety of plant and invertebrate lectins of known specificity were screened as potential inhibitors of attachment. Lectins from *Bauhinia purpurea* and *Arachis hypogaea* (peanut), which have specificities for *N*-acetyl-D-galactosamine, D-galactose, and D-galactose-β(1→3)-*N*-acetyl-D-galactosamine, were the most effective inhibitors of *A. naeslundii* attachment to KB cell monolayers (Table 2). They inhibited attachment by 79 and 52%, respectively, at 75 μg of lectin per ml. The lectin from *Ricinus communis* (RCA-120) inhibited adherence by 26%, whereas little or no inhibition was obtained by ConA or with the lectins from *Glycine max*, *Griffonia simplicifolia*, *Dolichos biflorus*, *Erythrina crystagalli*, and *Limulus polyhemus*. Under similar assay conditions, *E. coli* attachment to the KB cells was inhibited 57% in the presence of 75 μg per ml of the mannose-reactive lectin, ConA, whereas the lectin from *Bauhinia purpurea* had no effect on adherence (Table 2).

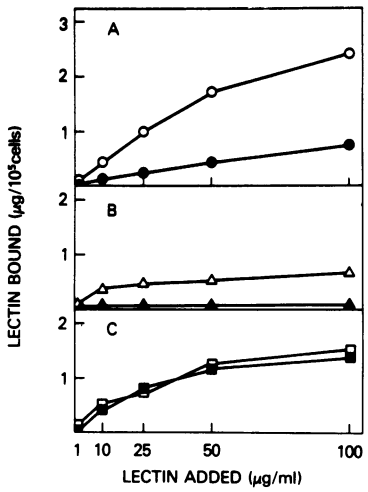


FIG. 5. Effect of neuraminidase on the binding of lectins from *Bauhinia purpurea* and peanut and ConA to KB epithelial cells. Radiolabeled lectins from *Bauhinia purpurea* ($1.8 \times 10^4$ cpm per μg) and peanut ($5.6 \times 10^5$ cpm per μg) and ConA ($6.2 \times 10^4$ cpm/μg) were diluted 1:10 with unlabeled lectin and incubated with KB epithelial cells ($10^5$ cells per well) in 96 well plates for 90 min at 37°C in the presence (○, △, □) or absence (●, ▲, ■) of $6 \times 10^{-4}$ U of neuraminidase per well. Data represent the average of bound radioactivity in three wells. (A) *Bauhinia purpurea* lectin; (B) peanut lectin; (C) ConA.

Binding of *Bauhinia purpurea* lectin to the KB cell monolayer was increased two- to threefold in the presence of neuraminidase, whereas the binding of the peanut lectin was strictly dependent upon the presence of neuraminidase (Fig. 5). These lectins did not bind to *A. naeslundii* (data not shown). ConA, which had no effect on actinomyces attachment, bound to the epithelial cells, and the amount of lectin bound was similar in the presence or absence of neuraminidase. These results indicate that the plant lectins from *Bauhinia purpurea* and *Arachis hypogaea* (peanut) have specificities similar to that of the actinomyces type 2 fimbriae.

TABLE 2. Inhibition of bacterial attachment to epithelial cells by plant lectins[a]

| Bacterium | Lectin | Lectin specificity[b] | % Inhibition at concn: | |
|---|---|---|---|---|
| | | | 25 μg/ml | 75 μg/ml |
| *A. naeslundii* | *Bauhinia purpurea* | D-GalNAc, D-Gal | 65 | 79 |
| | *Arachis hypogaea* | D-Gal-β(1→3)-GalNAc | 46 | 52 |
| | *Ricinus communis* | β-D-Gal | 21 | 26 |
| | *Griffonia simplicifolia* | α-D-Gal | 0 | 0 |
| | *Glycine max* | α-D-GalNAc | 0 | 4 |
| | *Dolichos biflorus* | α-D-GalNAc | 0 | 0 |
| | *Erythrina cristagalli* | D-Gal-β(1→4)GlcNAc | 0 | 5 |
| | *Limulus polyhemus* | Sialic Acid | 0 | 0 |
| | ConA | α-D-Man, α-D-Glc | 0 | 0 |
| *E. coli* | ConA | α-D-Man, α-D-Glc | 52 | 57 |
| | *Bauhinia purpurea* | D-GalNAc, D-Gal | 0 | 0 |

[a] KB cells were preincubated with lectins for 60 min in the presence of $3 \times 10^{-3}$ U of neuraminidase, and $2 \times 10^8$ radiolabeled bacteria were then added for 30 min.

[b] Abbreviations: Gal, galactose; Glc, glucose; Man, mannose; GalNAc, *N*-acetylgalactosamine; GlcNAc, *N*-acetylglucosamine.

## DISCUSSION

These studies implicate the specific involvement of lactose-sensitive fimbriae in the attachment of actinomyces to human epithelial cells. *A. naeslundii* WVU45, on which only type 2 fimbriae are present, adhered to monolayers of the epithelial cells, whereas a mutant lacking the type 2 fimbriae adhered minimally. Mutants of *A. viscosus* T14V selected for their absence of type 2 fimbriae also attached poorly. In contrast, *A. viscosus* T14V, which has both type 1 and type 2 fimbriae on its surface and an *A. viscosus* mutant with only the type 2 fimbriae, adhered to the epithelial cells, although to a lesser extent than did *A. naeslundii*.

The requirement for type 2 fimbriae in the interaction of actinomyces with epithelial cells was supported by the finding that Fab fragments of antibodies reactive with *A. naeslundii* WVU45 inhibited attachment, whereas the Fab fragments of antibodies raised against the nonfimbriated mutant *A. naeslundii* WVU45M did not. The antiserum reactive with the parent strain detected the fimbrial antigen which was not recognized by the antimutant antiserum (5). Monospecific antibodies against the *A. naeslundii* fimbriae have also been produced (5). However, the Fab fragments of these antibodies failed to inhibit attachment (unpublished data). This finding, in conjunction with the inability of these Fab fragments to block bacterial attachment to neuraminidase-treated erythrocytes (unpublished data), indicates that the fragments react with epitopes which are not closely associated with the carbohydrate-binding sites of the fimbriae.

Studies on the coaggregation of actinomyces with streptococci have shown that the type 2 fimbriae also mediate lactose-sensitive attachment of the actinomyces to specific strains of streptococci (3, 4, 20). The participation of this lectin associated with the type 2 fimbriae in the adherence of *A. naeslundii* to epithelial cells was established by inhibition of adherence by β-linked galactosides. The finding that methyl-α-D-galactoside did not inhibit attachment, whereas methyl-β-D-galactoside was the most potent inhibitor, suggests that the β-glycosidic linkage is important for lectin recognition. The similarities between these findings and the inhibition of coaggregation of *A. naeslundii* WVU45 with *Streptococcus sanguis* 34 by saccharides (2) imply that the receptors on epithelial cells and certain oral streptococci may be structurally related. These receptors clearly differ from the epithelial cell receptor for *E. coli* strain B. This bacterium was included in the present study as a specificity control and is representative of strains which have previously been shown to interact with mannose-containing receptors (18).

The attachment of the actinomyces was significantly enhanced by treatment of epithelial cells with neuraminidase, a procedure that unmasks the receptors for actinomyces on human erythrocytes (9). The removal of sialic acid by neuraminidase may be an important prerequisite for adherence of actinomyces since neuraminidase has, in fact, been found in the supernatants of cultures of these bacteria as well as on the surface of the actinomyces (9, 12, 23). This bacterial attachment mechanism bears a striking resemblance to the recognition of asialoglycoproteins by other cell-associated lectins (1).

To further characterize bacteria-epithelial cell adherence, we used plant and invertebrate lectins of defined carbohydrate specificity as potential inhibitors of bacterial attachment. Lectins from *B. purpurea* and *A. hypogaea* (peanut), which recognize *N*-acetyl-D-galactosamine, D-galactose (25), and D-galactose-β(1→3)-*N*-acetyl-D-galactosamine (15), were found to be the most effective inhibitors of attachment. Binding of these two lectins to the epithelial cells was enhanced by neuraminidase. The lectin from *R. communis* (RCA-120), which recognizes β-linked galactose, was a more effective inhibitor than lectins which recognize α-linked galactose and α-linked *N*-acetyl-D-galactosamine, a finding which supports the concept that the β anomer of the galactoside is present in the receptor. Although the lectin from *Erythrina crystagalli*, which is specific for D-galactose-β(1→4)-*N*-acetyl-D-glucosamine (16), bound to epithelial cells (data not shown), it had no effect on bacterial adherence. This suggests that the saccharide in the penultimate position as well as its glycosidic linkage participates in the bacterial lectin combining site as has been demonstrated for the peanut lectin (17, 19).

In these studies plant lectins have proven to be useful as probes for identifying epithelial cell receptors for the actinomyces lectin since the lectins from *Bauhinia purpurea* and peanut interact with the same or similar receptors as does the actinomyces lectin. It is anticipated that these plant lectins will prove to be extremely useful in the purification and further characterization of the epithelial cell glycoconjugates which serve as receptors for actinomyces.

## ACKNOWLEDGMENTS

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