INFECTION AND IMMUNITY, July 1988, p. 1800–1806
0019-9567/88/071800-07$02.00/0

Vol. 56, No. 7

Interaction of *Haemophilus influenzae* with Human Erythrocytes and Oropharyngeal Epithelial Cells Is Mediated by a Common Fimbrial Epitope

LOEK VAN ALPHEN,* NINA VAN DEN BERGHE,† AND LEA GEELEN-VAN DEN BROEK

Room L-162, Department of Medical Microbiology, University of Amsterdam, Meibergdreef 15, NL-1105 AZ Amsterdam, The Netherlands

Received 17 November 1987/Accepted 4 April 1988

The role of fimbriae in the adherence of *Haemophilus influenzae* to oropharyngeal epithelial cells and the hemagglutination (HA) of human Anton-positive erythrocytes was examined. HA of bacteria was lost after shearing. Fimbriae purified from the extracellular fluid caused HA and bound to oropharyngeal epithelial cells, as analyzed with immunoperoxidase staining, in a way which was similar to the adherence of bacteria to these cells: binding was over the entire surface of the cells and showed cell-to-cell variation. The specific role of fimbriae in HA and adherence was further examined by inhibition experiments with monoclonal antibodies elicited against the isolated fimbriae. These monoclonal antibodies bound along the entire length of the fimbriae, as seen by immunogold electron microscopy. The monoclonal antibodies and their Fab fragments inhibited HA (reduction in titer from 1:512 to 1:128 and 1:64, respectively) and inhibited the adherence of the homologous *H. influenzae* strain and of three of eight heterologous *H. influenzae* strains to oropharyngeal epithelial cells. These results indicate that fimbriae are involved in adherence and HA and that the binding site for the monoclonal antibodies on the fimbriae is not common on all strains.

Haemophilus influenzae type b is an important pathogen in childhood, causing the systemic infections meningitis, epiglottitis, cellulitis, pneumonia, arthritis, and sepsis. Acapsular strains cause mainly mucous membrane infections (sinusitis, otitis, nasopharyngitis, and chronic or acute bronchitis). Despite the availability of potent antibiotics, *H. influenzae* continues to produce significant morbidity and mortality. Recently developed vaccines can only protect against infections by *H. influenzae* type b (19).

Children who suffer from infections with *H. influenzae*, regardless of its anatomic location, invariably carry the bacterium in their nasopharynx, which is generally assumed to be the port of entry for the bacterium (19). Fimbriated isolates of *H. influenzae* adhere to nasopharyngeal epithelial cells (5, 9–11, 14–16). Those strains which adhere agglutinate human erythrocytes expressing the blood group Anton antigen (3, 22). Fimbriae of *H. influenzae* have been purified to homogeneity. The fimbriae are composed of subunits with a molecular weight of 23,000 (4). These cell organelles have been proposed as candidates for a vaccine against nontypeable *H. influenzae*. However, it has not been proven so far that fimbriae are essential for adherence and hemagglutination (HA).

In this study we found (i) that the fimbriae of *H. influenzae* are the cell fraction required for adherence and HA, (ii) that monoclonal antibodies (MAbs) specific for the fimbriae inhibit adherence and HA, and (iii) that purified fimbriae bind to epithelial cells and cause HA of Anton-positive erythrocytes.

MATERIALS AND METHODS

Bacterial strains and growth conditions. *H. influenzae* type b strains were isolated from the cerebrospinal fluid of patients with meningitis, and nontypeable strains were isolated from the sputum of patients with chronic bronchitis. *H. influenzae* type b strains 760705, 780100, 850348 (outer membrane protein [OMP] subtype 1, lipopolysaccharide [LPS] serotype 1, and biotype I), 770235 (OMP subtype 2, LPS serotype 2, and biotype I), and 770277 (OMP subtype 3, LPS serotype 10, and biotype II) were chosen as representative examples of invasive disease isolates in western Europe (20, 23). Strains 1481, 4.1, A840138, and A840220 were randomly chosen sputum isolates with different OMP compositions.

Bacteria were cultured on histidine-enriched brain heart infusion plates as described by Guerina et al. (4). Enrichment of the bacterial cultures for fimbriated bacteria was performed by the HA method described by Pichichero et al. (11), except that Ficoll was omitted and that settling of the erythrocytes with attached bacteria was repeated twice before plating. These enriched bacterial strains are indicated by the addition of f+. *H. influenzae* type b strain 770235f+ was obtained as a strongly agglutinating, heavily fimbriated variant of strain 770235, which was used in earlier experiments (21, 22). Its acapsular derivative strain 770235b^{0}f+ was selected on antiserum-containing agar plates as described before (25). For the isolation of fimbriae, bacteria of this strain were grown with shaking (120 rpm) in 8 liters of brain heart infusion broth supplemented with X and V factors (10 mg of each per liter).

HA and HA inhibition. Bacteria grown on histidine plates were suspended in 15 mM sodium phosphate–0.1 M NaCl (pH 7.4) (PBS) at a density of 10^{10} bacteria per ml. Fifty microliters was mixed with 50 μl of a 3% (vol/vol) suspension of normal erythrocytes from a healthy donor. HA was performed by a standard tube blood-grouping assay (22). The results were read with a dark-field microscope. Anton-negative erythrocytes were used as negative controls. The HA titer was determined in V-shaped microdilution plates with 50 μl of twofold dilutions of the bacterial suspensions in

* Corresponding author.
† Present address: Department of Biochemistry I, Erasmus University, Rotterdam, The Netherlands.

PBS and 50 μl of the erythrocyte suspension. The plates were analyzed after 1 h of incubation at 37°C.

In the HA inhibition test 50 μl of twofold dilutions of the bacterial suspensions were preincubated in the microdilution plates for 30 min at 37°C with 20 μl of a solution containing MAbs against the fimbriae. Then, 50 μl of the 3% erythrocyte suspension was added, and the plates were incubated for 1 h at 37°C and read.

Adherence of *H. influenzae* to oropharyngeal epithelial cells. Adherence of *H. influenzae* to oropharyngeal epithelial cells was determined as described previously (21). Briefly, oropharyngeal epithelial cells (10^5 cells per ml) were incubated for 1 h at 37°C with 10^{10} bacteria per ml in Eagle-Hanks tissue culture medium. Unbound bacteria were removed by repeated washing and centrifugation. The washed epithelial cells were fixed on glass slides, the bacteria were immunostained with murine MAb 8BD9 directed against a common OMP (2) specific for *H. influenzae* and anti-mouse immunoglobulin G bound to horseradish peroxidase, and the stained bacteria were counted by light microscopy. Twenty cells were counted.

Cell fractionation and purification of fimbriae. Basically, the procedure described by Guerina et al. (4) was followed. Bacteria were harvested from the 8-liter liquid culture by centrifugation for 10 min at 10,000 × *g* and suspended in 80 ml of 0.1 M Tris hydrochloride (pH 8.0). The suspension was treated with a Sorvall omnimixer for 5 min at 0°C at maximum speed and centrifuged for 20 min at 8,000 × *g*. A saturated ammonium sulfate solution was added to the supernatant to a final concentration of 50% (vol/vol). After 16 h at 4°C the precipitate was collected by centrifugation, washed with 45% (wt/wt) $(NH_4)_2SO_4$, and suspended in 10 ml of 0.1 M Tris hydrochloride (pH 8.0). The solution was centrifuged for 1 h at 100,000 × *g* to remove membrane fragments. The supernatant was dialyzed against 10 mM NH_4HCO_3 and lyophilized. The purity of the fimbriae was analyzed under an electron microscope (Philips EM 310) after negative staining with 1% uranyl acetate in PBS for 1 min and by sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis after staining with Coomassie brilliant blue (8, 23) or silver nitrate (18).

Cell envelopes and lithium chloride-EDTA extracts of cells were prepared as described before (24).

Antibodies and Fab fragments. MAbs were raised against purified fimbriae. BALB/c mice were immunized weekly with 50 μg of purified fimbriae and 10^8 *H. influenzae* 770235b^{0}f+ organisms intraperitoneally. After 31 days the spleen was used for the isolation of lymphocytes. The lymphocytes were fused with myeloma cells of the NS-1 cell line in a ratio of 4:1 with polyethylene glycol as the fusion catalyst as described by Tam et al. (17). Hybridomas were cloned by two-step limiting dilution. Antibodies were supposed to be monoclonal if four individual wells containing only one clone produced antibodies with the same specificity. The culture supernatants were tested by the enzyme-linked immunosorbent assay (ELISA) with purified fimbriae, cell envelopes, purified LPS, and capsular polysaccharide as antigens (25). The ELISA was performed with protein A-horseradish peroxidase as a conjugate to select for protein A-reactive antibodies.

MAbs against OMPs and LPS were used as controls. They were obtained after fusion of spleen cells from mice injected weekly for 4 weeks intraperitoneally with outer membranes of *H. influenzae* type b strain 760705 (100 μg of protein in 1 ml of PBS). Outer membranes were prepared by the sarcosyl method (24). The protein concentration was determined by the method of Lowry et al. (7). The specificity of the MAbs was analyzed by the gel immunoradioassay (13).

Murine ascites containing MAbs was raised by injecting pristane-treated BALB/c mice intraperitoneally with 5×10^6 hybridomas. The subclass of the MAbs was determined by immunoprecipitation (Ouchterlony method). Rabbit anti-mouse immunoglobulin G subclass-specific antibodies were obtained from Dakopatts, Copenhagen, Denmark.

Fab fragments of MAbs were prepared as follows. Antibodies were isolated from 0.5 ml of ascites by protein A-Sepharose 4B affinity chromatography by the procedure described by the manufacturer (Pharmacia, Uppsala, Sweden). The immunoglobulins were eluted from the protein A-Sepharose column with 0.1 M glycine hydrochloride (pH 2.8), collected in vials containing 1 M Tris hydrochloride (pH 8.5), and desalted immediately on a Sephadex G-25 column which was eluted with PBS. Protein-containing fractions were analyzed for antibody activity by the ELISA as described above. Antibody-containing fractions were dialyzed against 10 mM NH_4HCO_3, lyophilized, and solubilized in 0.5 M sodium phosphate (pH 8.0) containing 10 mM cysteine and 2 mM EDTA to a final concentration of 2 mg/ml. The solution was incubated with papain (100 μg/ml) for 4 h at 37°C and dialyzed overnight against 10 mM sodium phos-

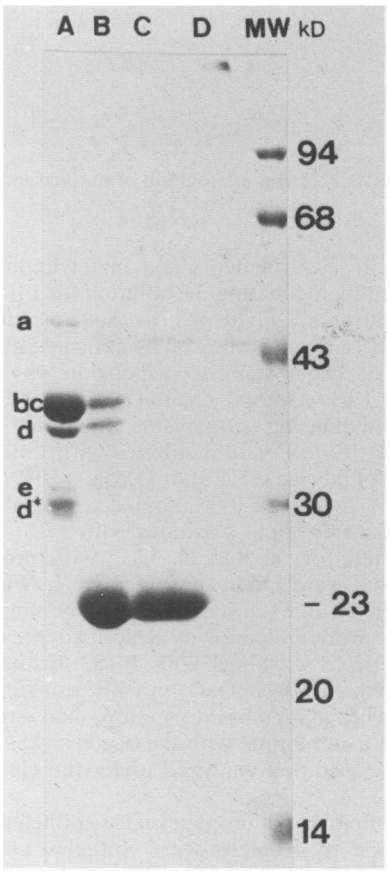

FIG. 1. SDS-polyacrylamide gel electrophoresis of cell fractions of *H. influenzae* 770235f+. A, Cell envelopes; B, membrane vesicles from the supernatant obtained after centrifugation of sheared bacteria; C and D, purified fimbriae. All samples were boiled in SDS before they were applied to the gel, except for D. Note that the fimbriae did not disintegrate into subunits without boiling (D). Molecular mass markers (in kilodaltons [kD]) are indicated on the right (lane MW); OMP designations are on the left.

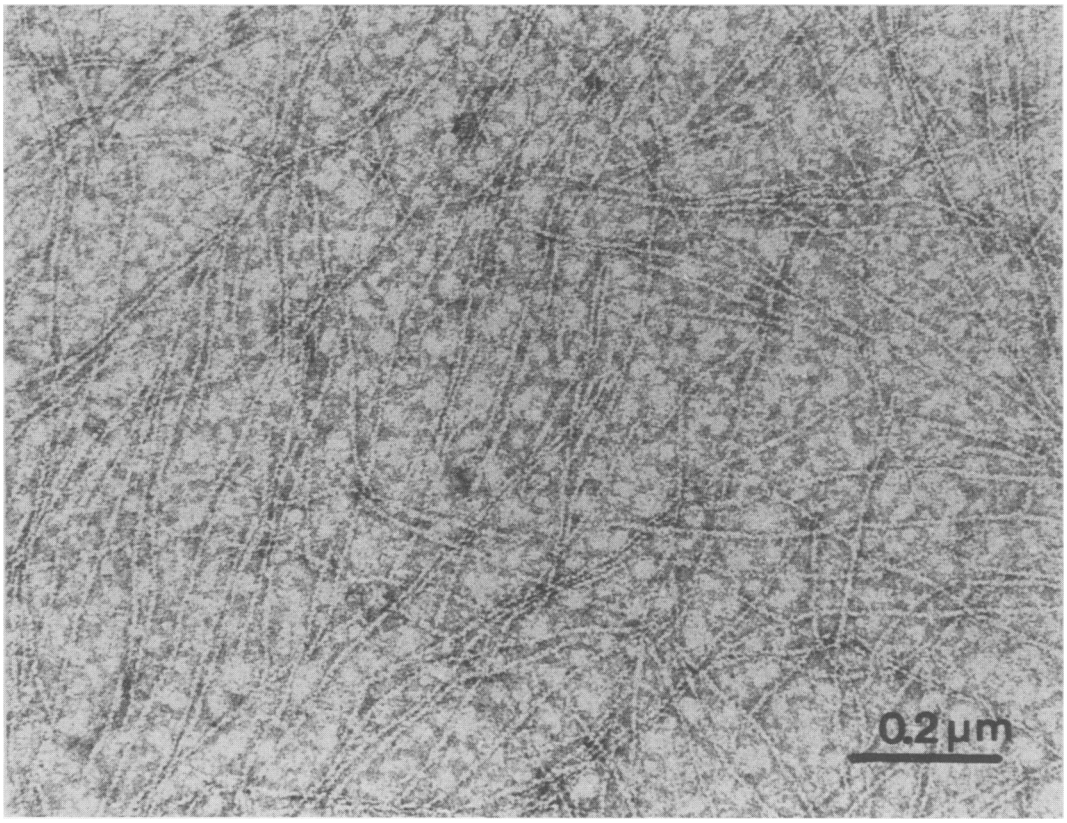

FIG. 2. Electron micrograph of purified fimbriae of *H. influenzae* 770235f+ after negative staining with uranyl acetate.

phate (pH 8.0). Fc fragments and intact immunoglobulins were removed by incubating the solution for 1 h at 37°C with protein A-bearing *Staphylococcus aureus* at a final concentration of 10% (vol/vol), followed by centrifugation for 3 min at 10,000 × *g*. The *S. aureus* suspension was prepared as described by Dirks-Go and Zanen (1).

Immunogold labeling. Organisms of strain 770235f+ or 770235 (1.5×10^7/ml) were incubated with 1:10 dilutions of MAbs against fimbriae (6HE8) or OMPs (8BD9, for control) in PBS–0.05% Tween 20–0.5% bovine serum albumin for 30 min at 37°C. After triple washings with PBS, the bacteria were incubated for 30 min at 37°C with protein A-gold spheres (which were kindly provided by J. Weel) in PBS–0.05% Tween 20–0.5% bovine serum albumin. Protein A-gold spheres were prepared as described previously (12). After more triple washings with PBS, the bacteria were counterstained with 0.2% OsO_4 in PBS, transferred onto an agar plate with a membrane on top, and stored at 4°C overnight. The membrane with the bacteria was then caught on a Formvar grid and analyzed under the electron microscope.

Binding of fimbriae to oropharyngeal epithelial cells. Binding of fimbriae to oropharyngeal epithelial cells was performed exactly as described for binding of bacteria to these cells, except that the bacterial suspension was replaced by a solution of fimbriae in 2 mM Tris hydrochloride (pH 7.8) (1 mg/ml). Inhibition of the binding by MAb 6HE8 was determined after preincubation of a solution containing 2 mg of fimbriae per ml and an equal volume of MAb 6HE8 for 30 min at 37°C. Fimbriae bound to epithelial cells were detected with MAb 6HE8 by the immunoperoxidase staining technique described above. This MAb did not agglutinate the fimbriae, as seen by phase-contrast microscopy.

RESULTS

Cell fractions involved in HA. Organisms of well-adhering and strongly hemagglutinating *H. influenzae* 770235f+ were fractionated to analyze whether fimbriae are the essential cell organelles for HA. The HA titer of the bacteria was 1:512. After shearing of the bacteria a reduction of the HA titer to 1:16 was observed, indicating that the components causing HA had been released from the bacteria into the extracellular fluid. The extracellular solution was fractionated. The protein composition of these fractions is shown in Fig. 1; a membrane fraction and a fimbrial fraction were obtained. Analysis of purified fimbriae by SDS-polyacrylamide gel electrophoresis showed that they contained one protein band of 23,000 daltons. Examination of the fimbriae by transmission electron microscopy after negative staining with uranyl acetate revealed flexible rods (Fig. 2) similar to those described by Guerina et al. (4). LPS could not be detected in the purified fimbria preparations if 10-µg quantities of fimbriae were analyzed by SDS-polyacrylamide gel electrophoresis, followed by staining with silver nitrate reagent. Under these conditions 10 ng of LPS would have been detected (18). The recovery of fimbriae after the purification was 10 mg. About half of the fimbriae appeared in the membrane fraction after ultracentrifugation. They were bound to membrane blebs, as determined by electron microscopy. The isolated fimbriae agglutinated erythrocytes from Anton-positive donors to a dilution of 0.1 mg/ml, but 5

TABLE 1. Specificity of MAbs against fimbriae, as analyzed by the ELISA

MAb[a]	ELISA reading (optical density) after coating of plates with:			
	Fimbriae	Cell envelopes	LPS	bPS
6HE8, 6CC1, 6AE4, or 6HB12	>2	0.1	0.1	0.1
3BD11	0.2	>2	>2	0.1
8BD9	0.1	>2	0.1	0.1
K1-022	0.1	0.1	0.1	>2

[a] All MAbs were used as ascites fluids in a 1:1,000 dilution. MAbs 6HE8, 6CC1, 6AE4, and 6HB12 were isolated as antifimbrial MAbs; MAb 3BD11 was an anti-LPS MAb; MAb 8BD9 was an anti-OMP P6 MAb; and K1-022 was a polyclonal anti-capsular type b polysaccharide (bPS) antiserum from rabbit (25).

mg of fimbriae per ml did not agglutinate Anton-negative erythrocytes. The membrane fraction also agglutinated erythrocytes. This result was due to the presence of fimbriae, since the HA could be inhibited by MAbs against the fimbriae (see below).

Cell fractions of bacteria which did not carry fimbriae (strain 770235) did not agglutinate Anton-positive or -negative erythrocytes.

Specificity of MAbs against the fimbriae. Four hybridomas which reacted with purified fimbriae in the ELISA were obtained from different wells of the fusion plates. The antibodies produced by these cells did not react with cell envelopes, LPS, or capsular polysaccharide of the homologous strain (Table 1). Clones 6HE8, 6CC1, and 6AE4 produced immunoglobulin G3 antibodies and may therefore be identical. Clone 6HB12 was immunoglobulin G2b. Further characterization of these MAbs with immunogold labeling showed that they all reacted with fimbriae on *H. influenzae* 770235f+. This result is illustrated for MAb 6HE8 in Fig. 3. Gold spheres were observed along the side and on top of the fimbriae, indicating that the epitope recognized by these antibodies was distributed over the entire structure. The MAbs did not react with nonfimbriated bacteria (strain 770235), and MAbs directed against an OMP of gonococci (kindly provided by J. P. M. van Putten) did not react, indicating that the background reaction was negligible. MAb 8BD9 (anti-outer membrane protein P6) reacted with the surfaces of strains 770235f+ and 770235 but not with fimbriae.

Inhibition of HA by MAbs against the fimbriae. Results of HA inhibition tests are summarized in Table 2. MAb 6HE8 reduced the HA titer from 1:512 to 1:128 when Anton-positive erythrocytes were used. MAb 8BD9, specific for an OMP (16,000 daltons), MAb 1DA12, directed against LPS, and culture supernatant from a nonproducing clone, 1DD1, did not inhibit HA. Limited autoagglutination was observed with Anton-negative erythrocytes (titer, 1:8). Therefore, we prepared Fab fragments of MAb 6HE8 and of MAb 8BD9 as a control. The Fab fragments of MAb 6HE8 reduced the HA titer of Anton-positive erythrocytes to 1:64. Autoagglutination of Anton-negative erythrocytes in the presence of these Fab fragments was no longer seen. Fab fragments of MAb 8BD9 did not inhibit HA. MAb 6HE8 also inhibited HA by the membrane fraction of *H. influenzae* 770235f+, indicating that these extracts contained fimbriae as an agglutinin (Table 2).

Involvement of fimbriae in the adherence of *H. influenzae* to oropharyngeal epithelial cells. Involvement of fimbriae in the adherence of *H. influenzae* to oropharyngeal epithelial cells was investigated by performing adherence inhibition experiments with MAbs against the fimbriae and by studying the binding of purified fimbriae to the epithelial cells (see below).

H. influenzae 770235f+ adhered readily to oropharyngeal epithelial cells. In a typical experiment with 5×10^9 bacteria per ml, more than 200 bacteria bound per cell (Fig. 4A). Preincubation of the same bacterial suspension with MAb 6HE8 in a parallel experiment resulted in a reduction to less than five bacteria bound per cell (Fig. 4B). Agglutinated bacteria were seen between the cells. Fab fragments from MAb 6HE8 also inhibited adherence by 70% but did not agglutinate the bacteria. Control ascites 1DD1 from a nonproducing clone and ascites and Fab fragments from MAb 8BD9 did not reduce adherence; more than 200 bacteria bound per epithelial cell in all cases.

Binding of isolated fimbriae to epithelial cells. An obvious way of testing whether fimbriae bind to cells is by measuring the amount of radioactive fimbriae which bind to these cells after incubation. However, we were not able to label the fimbriae of *H. influenzae* with ^{125}I by the chloramine-T method, although protein A from *S. aureus* bound 1 atom of ^{125}I per molecule under these conditions. Therefore, immunoperoxidase staining with MAb 6HE8 was used for the detection of bound fimbriae (Fig. 4). Oropharyngeal epithelial cells stained brown after incubation with fimbriae (Fig. 4C). A minority of the cells did not stain. This variation in staining reflects the variation in the adherence of bacteria to epithelial cells, since bacteria also did not bind to about 10% of the cells. The lack of binding of fimbriae to some cells shows that the interaction with cells is not aspecific. Aspecific binding could further be excluded by inhibition experiments. None of the cells stained after preincubation of the fimbriae with MAb 6HE8 (Fig. 4D). An MAb against an OMP (MAb 8BD9) did not inhibit binding, and MAb 6HE8 did not stain the epithelial cells by itself.

Presence of binding sites for MAb 6HE8 on different strains. Inhibition of the adherence of other *H. influenzae* strains to oropharyngeal epithelial cells by MAb 6HE8 is summarized in Table 3. MAb 6HE8 inhibited the adherence of two of five *H. influenzae* type b strains (from cerebrospinal fluid) completely and of one strain (strain 770277f+) partly. The adherence of one of four nontypeable strains obtained from sputum was also inhibited. These results indicate limited strain specificity.

TABLE 2. Influence of MAbs on HA by fimbriated *H. influenzae* 770235f+ and its nonfimbriated variant 770235

Erythrocyte blood group (Anton)	Strain	Preincubation with:	HA titer
Positive	770235f+	Nothing	1:512
Positive	770235	Nothing	<1
Negative	770235f+	Nothing	<1
Positive	770235f+	MAb 6HE8[a]	1:128
Positive	770235f+	MAb 8BD9[a]	1:512
Negative	770235	MAb 6HE8	1:8
Positive	770235f+	Fab 6HE8	1:64
Positive	770235f+	Fab 8BD9	1:512
Negative	770235	Fab 6HE8	<1
Positive	770235	Fab 6HE8	<1

[a] MAb 6HE8 is specific for fimbriae; MAb 8BD9 is specific for an OMP with a molecular weight of 16,000.

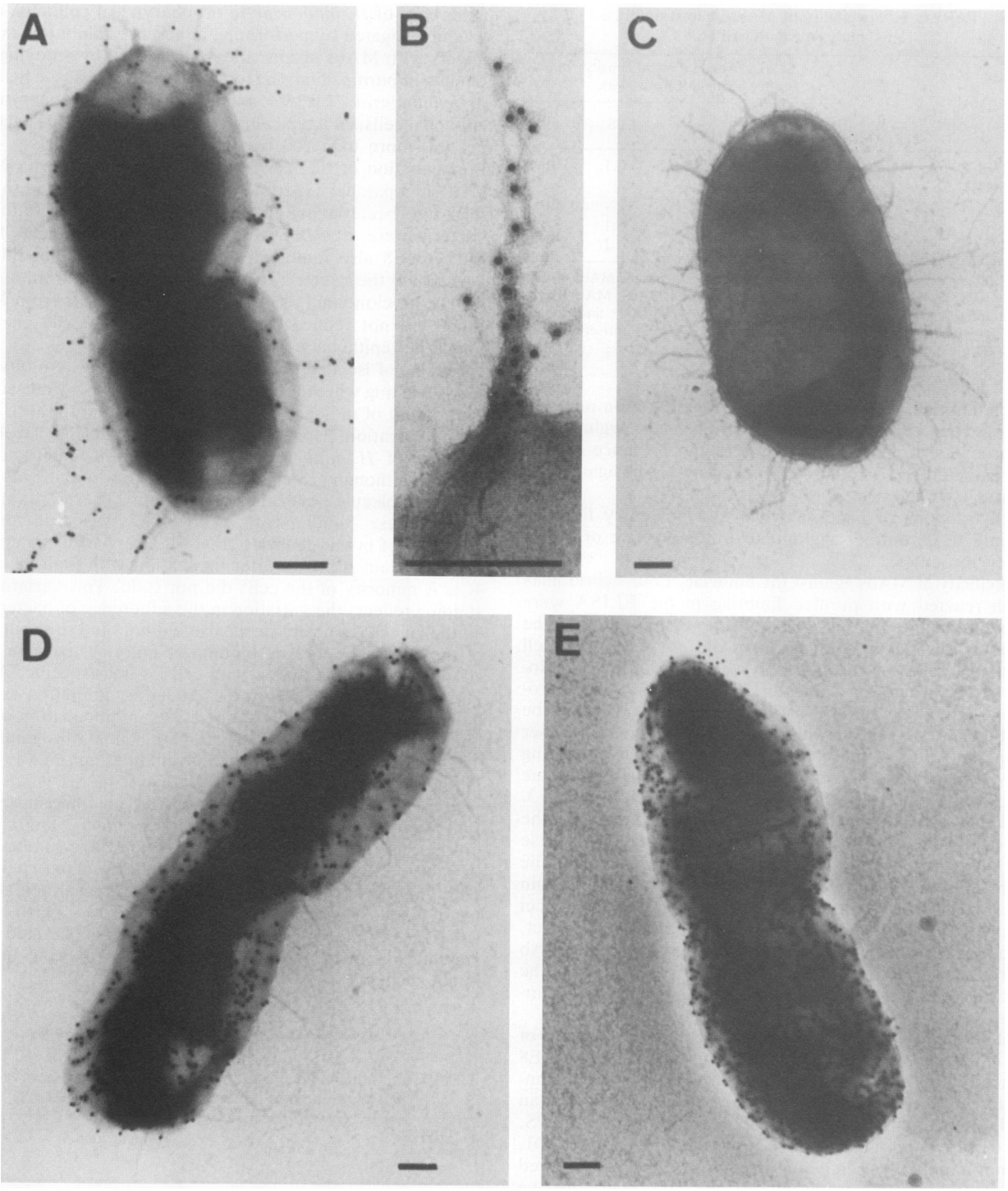

FIG. 3. Immunogold labeling of *H. influenzae* 770235f+ (A, B, and D) and 770235 (E) with an MAb against the fimbriae (MAb 6HE8) (A and B) or outer membrane protein P6 (MAb 8BD9) (D and E) and protein A-gold. Bacteria of strain 770235f+ without gold spheres are included for comparison (C). The bacteria were counterstained with uranyl acetate. Bars, 0.2 μm.

DISCUSSION

Various investigators have shown that the adherence of *H. influenzae* to oropharyngeal epithelial cells is caused by strains carrying fimbriae and that adherence parallels HA (3, 5, 9–11, 14–16). In addition, the ability of an *H. influenzae* strain to hemagglutinate could be transferred by transformation to a nonhemagglutinating strain, which thereby acquired fimbriae (16). These data suggest that fimbriae mediate adherence of the organism but do not exclude the possibility that other bacterial components are expressed concomitantly, as has been shown for the expression of genes

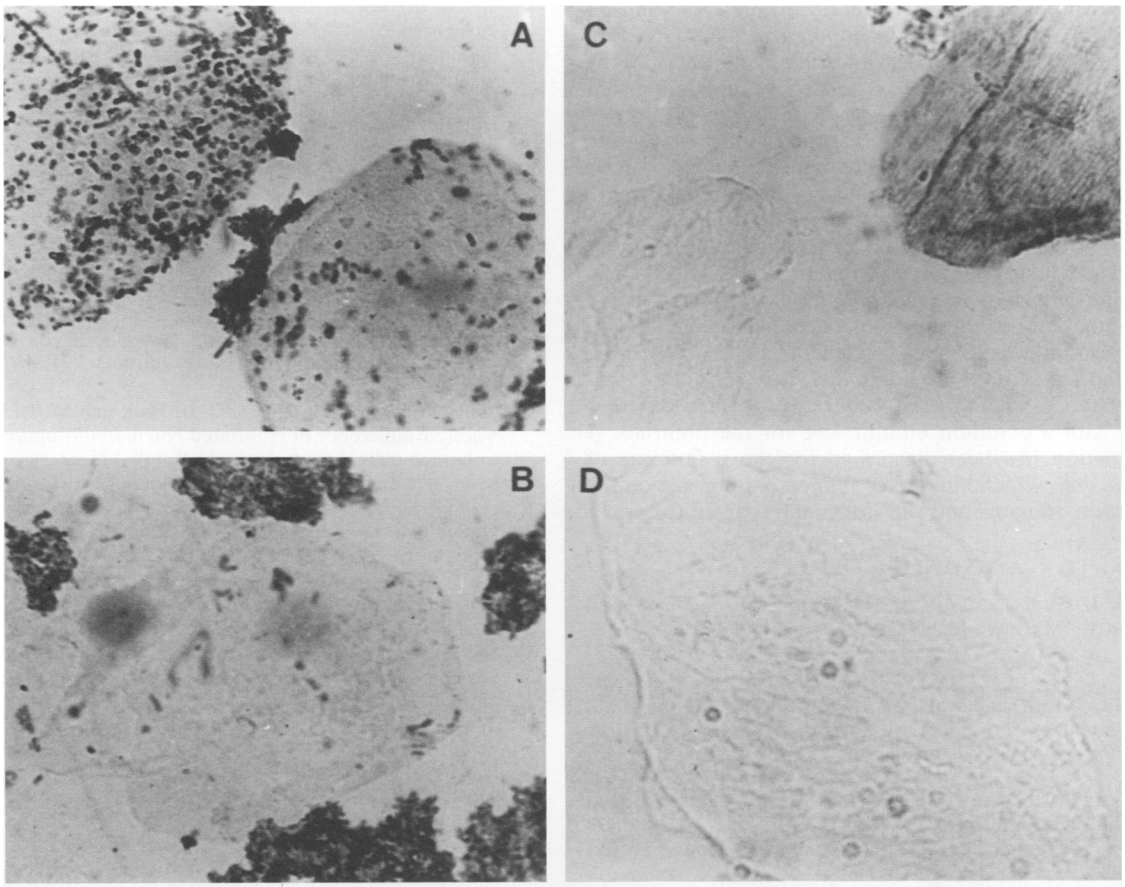

FIG. 4. Binding of *H. influenzae* 770235f+ in the absence (A) or presence (B) of MAb 6HE8 and binding of isolated fimbriae in the absence of (C) or after preincubation with (D) MAb 6HE8 to oropharyngeal epithelial cells. The bound bacteria and fimbriae were visualized by subsequent incubation with an MAb against the fimbriae (MAb 6HE8) and immunoperoxidase staining. The cells were counterstained with methylene blue.

involved in the synthesis of LPS after transformation of DNA coding for the capsular type b polysaccharide (26). Our experiments have shown that fimbriae indeed mediate adherence and HA. This conclusion is based on the following observations. (i) Isolated fimbriae (Fig. 1 and 2) caused HA and bound to epithelial cells (Fig. 4C). (ii) Murine MAbs which reacted in the ELISA and, after immunogold labeling, reacted specifically with fimbriae (Fig. 3) and the Fab fragments of these MAbs inhibited HA and adherence to oropharyngeal epithelial cells of fimbria-carrying *H. influenzae* (Table 2 and Fig. 4B). These HA reactions were concluded to be specific, since HA was only observed with Anton-positive erythrocytes. HA and adherence were not observed with *H. influenzae* lacking fimbriae. Not only fimbriae but also membrane fragments of bacteria with fimbriae caused HA. This result could be explained by the presence of fimbriae in these fragments, as HA was inhibited by antibodies against the fimbriae.

TABLE 3. Effect of MAbs against the fimbriae of *H. influenzae* 770235f+ on the adherence of various *H. influenzae* strains

Strain	Capsular type	Adherence[a]	
		Without MAb 6HE8	With MAb 6HE8
770235f+	b	+++	−
770235	b	−	−
760705f+	b	++	−
770277f+	b	++	+
780100f+	b	+	+
850348f+	b	+	+
1481f+	NT[b]	+++	+++
A840138f+	NT	++	++
A840220f+	NT	++	−
4.1f+	NT	+++	+++

[a] Adherence was expressed as +++ if more than 200 bacteria bound per epithelial cell, as ++ for 50 to 200 bacteria, as + for 5 to 50 bacteria, and as − for less than 5 bacteria.

[b] NT, Nontypeable.

The binding site for the MAbs against the fimbriae appeared to be distributed over the entire length of the polymer (Fig. 3), suggesting that the binding site is part of the fimbrial subunit. These MAbs reacted only with part of the *H. influenzae* strains used in this study. This may mean that various strains of *H. influenzae* have different binding sites on their fimbriae. However, this explanation is unlikely, since all strains agglutinated Anton-positive erythrocytes and not Anton-negative ones and since this HA was inhibited by anti-Anton serum (22). A more likely explanation for the difference in the reactivity of MAb 6HE8 with various strains is that this MAb reacted partly with the conserved binding site for the receptor on the fimbriae and partly with a variable sequence on the fimbrial subunit. In this respect it

is important to note that Guerina et al. (4) have shown that fimbriae from some strains of *H. influenzae* have a strong serological relationship and that fimbriae from other strains have a low, but significant, cross-reactivity, indicating that all these fimbriae share at least some common epitopes.

In conclusion, we have shown that the binding site on the fimbriae of *H. influenzae* which is recognized by MAb 6HE8 interacts with the epithelial cell and erythrocyte receptors. This result strongly suggests that the receptor has the same structure in both cell types. We have previously shown that the molecule which contains the receptor site on erythrocytes is different from the receptor molecule on epithelial cells, since these molecules are expressed independently and since they are immunologically not identical (21). When the previous and actual data are combined, it is very likely that the erythrocyte and epithelial cell receptors are different molecules with a common binding site for the fimbriae. A similar situation has been described for the p fimbriae of *Escherichia coli*, which bind to a common binding site on the P blood group antigens and the Forssman antigen (6).

ACKNOWLEDGMENTS

We thank J. Weel and J. P. M. van Putten for immunological reagents and W. Maris for technical assistance.

LITERATURE CITED

1. **Dirks-Go, S. I. S., and H. C. Zanen.** 1978. Latex agglutination, counterimmunoelectrophoresis, and protein A co-agglutination in diagnosis of bacterial meningitis. J. Clin. Pathol. **31**:1167–1171.
2. **Groeneveld, K., L. van Alphen, N. J. Geelen-van den Broek, P. P. Eijk, R. J. van Ketel, and H. C. Zanen.** 1987. Detection of *Haemophilus influenzae* with monoclonal antibody. Lancet **i**:441–442.
3. **Guerina, N. G., S. Langermann, H. W. Clegg, T. W. Kessler, D. A. Goldmann, and J. R. Gilsdorf.** 1982. Adherence of piliated *Haemophilus influenzae* to human oropharyngeal cells. J. Infect. Dis. **146**:564.
4. **Guerina, N. G., S. Langermann, G. K. Schoolnik, T. W. Kessler, and D. A. Goldmann.** 1985. Purification and characterization of *Haemophilus influenzae* pili, and their structural and serological relatedness to *Escherichia coli* p and mannose-sensitive pili. J. Exp. Med. **161**:145–159.
5. **Lampe, R. M., E. O. Mason, Jr., S. L. Kaplan, C. L. Umstead, M. D. Yow, and R. D. Feigin.** 1982. Adherence of *Haemophilus influenzae* to buccal epithelial cells. Infect. Immun. **35**:166–172.
6. **Leffler, H., and C. Svanborg-Eden.** 1981. Glycolipid receptors for uropathogenic *Escherichia coli* on human erythrocytes and uroepithelial cells. Infect. Immun. **34**:920–929.
7. **Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall.** 1951. Protein measurement with the Folin phenol reagent. J. Biol. Chem. **193**:265–275.
8. **Lugtenberg, B., J. Meijers, R. Peters, P. van der Hoek, and L. van Alphen.** 1975. Electrophoretic resolution of "the major outer membrane protein" of *Escherichia coli* K-12 into four bands. FEBS Lett. **58**:254–258.
9. **Mason, E. O., Jr., S. L. Kaplan, B. L. Wiedermann, E. P. Norrod, and W. A. Stenback.** 1985. Frequency and properties of naturally occurring adherent piliated strains of *Haemophilus influenzae* type b. Infect. Immun. **49**:98–103.
10. **Pichichero, M. E.** 1984. Adherence of *Haemophilus influenzae* to human buccal and pharyngeal epithelial cells: relationship to piliation. J. Med. Microbiol. **18**:107–116.
11. **Pichichero, M. E., M. Loeb, P. Anderson, and D. H. Smith.** 1982. Do pili play a role in the pathogenesis of *Haemophilus influenzae* type b? Lancet **ii**:960–962.
12. **Poolman, J. T., F. B. Wientjes, C. T. P. Hopman, and H. C. Zanen.** 1985. Influence of the length of lipopolysaccharide molecules on the surface exposure of class 1 and 2 outer membrane proteins of *Neisseria meningitidis* 2996 (B:2b:P1.2), p. 562–571. *In* G. K. Schoolnik (ed.), The pathogenic neisseriae. American Society for Microbiology, Washington, D.C.
13. **Poolman, J. T., and H. C. Zanen.** 1980. Detection of antibody activity in human sera against meningococcal cell wall antigens using a gel-immuno-radio-assay (GIRA). FEMS Microbiol. Lett. **7**:293–296.
14. **Sable, N. S., E. M. Connor, C. B. Hall, and M. R. Loeb.** 1985. Variable adherence of fimbriated *Haemophilis influenzae* type b to human cells. Infect. Immun. **48**:119–123.
15. **Scott, S. S., and D. C. Old.** 1981. Mannose-resistant and eluting (MRE) haemagglutinins, fimbriae and surface structure in strains of *Haemophilus*. FEMS Microbiol. Lett. **10**:235–240.
16. **Stull, T. L., P. M. Mendelman, J. E. Haas, M. A. Schoenborn, K. D. Mack, and A. L. Smith.** 1984. Characterization of *Haemophilus influenzae* type b fimbriae. Infect. Immun. **46**:787–796.
17. **Tam, M. R., T. M. Buchanan, E. G. Sandstrom, K. K. Holmes, J. S. Knapp, A. W. Siadek, and R. C. Nowinski.** 1982. Serological classification of *Neisseria gonorrhoeae* with monoclonal antibodies. Infect. Immun. **36**:1042–1053.
18. **Tsai, C. M., and C. E. Frasch.** 1982. A sensitive silver stain for detecting lipopolysaccharides in polyacrylamide gels. Anal. Biochem. **119**:115–119.
19. **Turk, D. C.** 1985. The pathogenicity of *Haemophilus influenzae*. J. Med. Microbiol. **18**:1–16.
20. **van Alphen, L., L. Geelen, K. Jonsdottir, A. K. Takala, H. Kayhty, and H. C. Zanen.** 1987. Distinct geographic distribution of subtypes of *Haemophilus influenzae* type b in Western Europe. J. Infect. Dis. **156**:216–218.
21. **van Alphen, L., J. Poole, L. Geelen, and H. C. Zanen.** 1987. The erythrocyte and epithelial cell receptors for *Haemophilus influenzae* are expressed independently. Infect. Immun. **55**:2355–2358.
22. **van Alphen, L., J. Poole, and M. Overbeeke.** 1986. The Anton blood group antigen is the erythrocyte receptor for *Haemophilus influenzae*. FEMS Microbiol. Lett. **37**:69–71.
23. **van Alphen, L., T. Riemens, J. Poolman, C. Hopman, and H. C. Zanen.** Homogeneity of cell envelope protein subtypes, lipopolysaccharide serotypes, and biotypes among *Haemophilus influenzae* type b from patients with meningitis in The Netherlands. J. Infect. Dis. **148**:75–80.
24. **van Alphen, L., T. Riemens, J. Poolman, and H. C. Zanen.** 1983. Characteristics of major outer membrane proteins of *Haemophilus influenzae*. J. Bacteriol. **155**:878–885.
25. **van Alphen, L., T. Riemens, and H. C. Zanen.** 1986. The capsular polysaccharide of *Haemophilus influenzae* type b prevents killing by complement and antibodies against outer membrane protein *a*. FEMS Microbiol. Lett. **34**:273–276.
26. **Zwahlen, A., L. G. Rubin, and E. R. Moxon.** 1986. Contribution of lipopolysaccharide to pathogenicity of *Haemophilus influenzae*: comparative virulence of genetically-related strains in rats. Microb. Pathogenesis **1**:465–473.