Differential binding of *Haemophilus influenzae* to human tissues by fimbriae

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Summary. The hypothesis was investigated that tissue tropism of *Haemophilus influenzae* during colonisation and infection is associated with the ability of fimbriate bacteria to bind to the organs and cell types involved. *H. influenzae* type b with fimbriae (strain 770235f⁺) bound to several cell types, including ciliated columnar epithelial cells, pneumocytes, ependymal cells, glial cells, connective tissue fibroblasts, synovial cells, antigen-presenting cells, lymphocytes, erythrocytes and endothelial cells. Binding of *H. influenzae* to kidney, liver and conjunctiva cells was poor. Fimbriae-specific monoclonal antibody (MAb 6HE8) inhibited this binding. Some binding to endothelial cells and macrophages was also observed with non-fimbriate strains. This binding was not inhibited by MAb 6HE8. We conclude that in-vitro binding of fimbriate *H. influenzae* is mainly to those tissues and cells where *H. influenzae* is found during colonisation and infection. The data suggest that a shift to the non-fimbriate form is required for bacteria in the bloodstream to escape clearance mechanisms mediated by blood cells.

Introduction

Haemophilus influenzae is a normal inhabitant of the human nasopharynx and causes various infectious diseases, which are restricted to man. Capsulate strains with a serotype b polysaccharide cause mainly meningitis, epiglottitis, cellulitis, arthritis, pneumonia and sepsis; non-typable *H. influenzae* strains cause nasopharyngitis, pharyngitis, (chronic) bronchitis, otitis media and sinusitis.¹

Although the capsular polysaccharide of *H. influenzae* type b mainly determines the survival of the bacterium in body fluids,¹ the mechanisms responsible for tissue tropism of *H. influenzae* are not known. The ability of bacteria to adhere to specific host cells is regarded as a pre-requisite for the onset of infectious diseases.² Adherence may be an important mechanism in determining the organ and tissue specificity during infection. Both capsulate and non-capsulate strains of *H. influenzae* are able to adhere to oropharyngeal epithelial cells. This process is mediated mainly by fimbriae.^{3–10} Fimbriae of various bacterial species recognise different cell receptors,^{2,11} which have often a specific distribution,^{11–13} e.g., *Escherichia coli* causing neonatal meningitis binds through S-fimbriae to the luminal surfaces of the vascular endothelium and of the epithelium lining the choroid plexuses and brain

ventricles (ependyma).¹² The AnWj blood group antigen is the receptor for the fimbriae of *H. influenzae*.¹³ The receptors for the fimbriae on oropharyngeal epithelial cells and erythrocytes have a common genetic basis, but their chemical structures have not been resolved.¹⁴

To determine the significance of fimbriae-mediated adherence in relation to tissue tropism of *H. influenzae*, we analysed the binding of bacteria of the same *H. influenzae* strain, with and without fimbriae, to various human tissues involved in the pathogenesis of *H. influenzae* disease. For optimal morphological clarity the experiments were performed with sections of fixed tissues. The bacteria were stained by an immunoperoxidase method allowing concomitant observation of the bacteria and the tissue structures. The effect of fixation and pre-treatment of the tissues was analysed by comparison of the binding of *H. influenzae* to adenoid tissue sections with binding to fresh adenoid tissue, which is regarded as an appropriate in-vivo model for studying the first steps of *H. influenzae* infection.¹⁵

Materials and methods

Bacteria and growth conditions

H. influenzae type b strain 770235, isolated from cerebrospinal fluid, was used throughout the study. This strain has been described before^{10,13} and was

originally reported not to express fimbriae.¹⁰ After seven enrichment cycles by culturing human erythrocytes with attached bacteria, the strain became heavily fimbriated and was able to agglutinate human erythrocytes and to bind strongly to oropharyngeal epithelial cells.^{10,13,14} This fimbriate variant is designated as strain 770235f⁺.¹⁰ The presence of fimbriae on these bacteria was assessed by electronmicroscopy after negative staining with phosphotungstate. Bacteria were cultured overnight on heated blood ("chocolate") agar plates at 37°C in a CO₂ incubator and then resuspended to a density of 10⁹ cfu/ml in Dulbecco Modified Eagle Medium (DMEM; Gibco, Breda, The Netherlands) containing fetal calf serum (FCS) 10%, as described before.^{10,13}

Fresh adenoid tissue

Fresh adenoid tissue was obtained by adenectomy from children (1–6 years) with hyperplastic adenoids related to repeated upper respiratory tract infections or otitis media. Adenectomy was decided on clinical indications in infection-free periods. The adenoid tissue was collected in DMEM containing FCS 10%, washed twice with DMEM and cut into pieces of about 1 cm³. Tissues were discarded if *H. influenzae* was detected immunochemically in sections. Adenoids from five individuals were further processed, as indicated below.

Post-mortem material

Material taken at autopsy from tissues without disease, or biopsies of human tissues, were selected for examination. Only those specimens were used which were fixed within 5 h of death and that did not show any macroscopic degeneration.

Preparation of tissue sections

Pieces of tissue (10 × 15 × 3 mm³) were fixed for 1 h at 22°C in formaldehyde 4% w/v buffered with phosphate-buffered saline (PBS) and were dehydrated by subsequent immersion, successively, in 70%, 80%, 96% and 100% ethanol, each step for 1 h, followed by toluene for 1 h. The dehydrated tissues were embedded in paraffin, unless otherwise specified, and then cut into 3-µm sections. The sections were transferred to poly-L-lysine-coated glass slides and were incubated with human plasma protein for 4 h at 37°C, to improve adhesion, and stored subsequently if necessary. The sections were deparaffinised in xylol and rehydrated with ethanol-water mixtures.

Preparation of nasopharyngeal epithelial cells

Freshly obtained oropharyngeal epithelial cells from a healthy adult (10⁵/ml) were incubated for 1 h at room temperature either with PBS or—in the same way as tissue sections—with formaldehyde, ethanol-

water, toluene, xylol, pepsin in HCl (see ahead), or HCl alone, followed by triple washing with PBS as previously described.¹⁰

Adherence of bacteria to fresh adenoid tissue

Pieces of tissue were immersed in 2 ml of DMEM containing FCS 10% and 2 × 10⁵ cfu of bacteria and incubated for 30 min at 37°C in a waterbath and subsequently in a CO₂ incubator for various lengths of time. Binding-inhibition experiments with monoclonal antibodies (MAbs) were performed to analyse the role of fimbriae in the binding of *H. influenzae* to these cells. An *H. influenzae* suspension of 2 × 10⁵ cfu/ml was pre-incubated for 30 min at 37°C with an equal volume of culture supernate from murine hybridoma cell line 6HE8, which produces anti-fimbriae antibodies.¹⁰ The pre-incubated bacteria were incubated with the tissue sections in the same way as for fresh bacterial suspensions. MAb 7BB1, specific for a heterologous LPS of *H. influenzae* type b (LPS-10), was used as a control.^{10,16} The two MAbs did not agglutinate the bacteria, nor did they bind to the Fc receptor on the tissue cells.¹⁰ After incubation, the tissue was rinsed five times with PBS, fixed with formaldehyde 4%, and sections were cut.

Adherence of bacteria to tissue sections

Immediately after deparaffination, the sections were pre-incubated for 15 min at 37°C with pepsin 0.5% in diluted HCl, pH 2.0, and then with normal swine serum 10% for 15 min at 22°C to reduce non-specific binding (see *Results*). Pre-incubated sections were overlaid with bacterial suspensions, prepared as described above (10⁹ cfu/ml), incubated for 1 h in a humid atmosphere at 37°C and washed three times with PBS containing Tween-20 0.05%. The fimbriae-dependence of the binding of *H. influenzae* to tissue sections was analysed in inhibition experiments with MAbs 6HE8 and 7BB1, as described for fresh adenoid tissue.

Adherence of bacteria to oropharyngeal epithelial cells

Glass slide smears of oropharyngeal epithelial cells,¹³ either untreated or treated (as above) with formaldehyde, ethanol-water mixtures, toluene, xylol, pepsin in HCl, or HCl alone, were incubated with bacterial suspensions in the same way as the tissue sections.

Staining of bacteria in tissue sections

Incubated tissue sections were stained immunochemically with MAbs to detect specifically *H. influenzae*. In this procedure, the sections were incubated for 1 h at room temperature with murine ascites fluid (diluted 1 in 500 in PBS) raised with hybridoma

cell lines producing MAbs directed, respectively, to outer-membrane protein P6 (MAb 8BD9), outer-membrane protein a (MAb 5HA5) and LPS (MAb 3DA11). The production and specificity of these MAbs have been described before.¹⁰ The sections were rinsed with PBS for 5 min and incubated for 20 min with H₂O₂ 0.03% in methanol at 4°C to reduce endogenous peroxidase activity. After another wash with PBS, rabbit anti-mouse immunoglobulin coupled to horse-radish peroxidase (Dako, Copenhagen, Denmark) was added at a dilution of 1 in 50 in PBS with pooled human serum 10% v/v, and the slides were incubated for 30 min at room temperature. The staining was amplified by a subsequent incubation with swine anti-rabbit serum coupled to horse-radish peroxidase (Dako), diluted 1 in 100 in PBS with human pooled serum, for 30 min. After washing with PBS, sections were stained with diaminobenzidine 0.5% in 0.05 M Tris-HCl, pH 7.6, containing H₂O₂ 0.03%. The reaction was allowed to proceed for 15 min and was then stopped by washing with distilled water.

Sections that were not incubated with bacteria were included as controls in all series to allow assessment of the tissue morphology and detection of background staining. Bacterial suspensions fixed to glass slides with methanol for 5 min were included as positive controls for the staining procedures. Cells were

counterstained with haematoxylin and the sections were examined by light microscopy.

Results

Binding of H. influenzae to fresh adenoid tissue

After incubation of fresh adenoids with the fimbriate *H. influenzae* strain 770235f⁺, followed by fixation, paraffin embedding and sectioning, immunocytochemically stained rods were observed on columnar epithelial cells and their cilia, in the epithelial cell layer and under the epithelial cells near lymphocytes and macrophages, and in vessels (figs. 1A, B). We observed small differences in the degree of binding between adenoid tissues from different donors. These differences, in the numbers of stained particles, were only quantitative and were within the binding categories indicated in table I. Binding of fimbriate bacteria was inhibited by the anti-fimbriae MAb 6HE8 and not by MAb 7BB1 specific for LPS, except for binding to endothelium, macrophages and fibroblasts. Binding inhibited specifically by MAb 6HE8 has been shown to be fimbriae-specific.^{3,5,7,13} Non-fimbriate bacteria did not bind to cells of fresh adenoid tissue. These data show that *H. influenzae* with fimbriae binds to some cell types of adenoid tissue before

Fig. 1. Comparison of adherence of fimbriate *H. influenzae* strain 770235f⁺ to fresh adenoids with that obtained with sections of various tissues of the air passages of the lower respiratory tract. A, B, strong adherence when incubated with fresh adenoid followed by fixation, embedding and sectioning; C, strong adherence when incubated with paraffin sections of fixed adenoid; strong adherence to tissue sections of, D, bronchus and, E, alveoli incubated with the same fimbriate bacteria (EP, epithelium; C, ciliated cells). The bacteria were immunostained after incubation with a mixture of MAbs 8BD9, 5HA5 and 3DA11, except in B. The bacteria in B were incubated with MAb 6HE8 (anti-fimbriae). Bar, 0.01 mm.

Table I. Effect of MAbs on the adherence of *H. influenzae* strain 770235f⁺ to paraffin-embedded sections of human tissue

Tissue from (organ)	Degree of binding		
	untreated	in the presence of MAb	
		6HE8	7BB1
Cerebrum	++	—	++
Upper respiratory tract	++	—	++
Lung	++	—	++
Joint	+	—	+
Eye	—	ND	ND
Spleen	++	—	++
Lymph node	++	—	++
Liver	—	—	+
Kidney	—	—	—
Blood vessels	++	—	++
Connective tissue	++	—	++

++, Strong to abundant binding (most of the cells bound several bacteria); +, sparse but significant binding (bacteria were seen on at least 10% of the cells); —, no binding (bacteria were rarely found on these cells). Representative examples are shown in the figs.

penetration into the tissue. Bacteria without fimbriae did not interact with adenoid cells. Fixation and embedding after incubation of fresh tissue with bacteria did not result in disappearance of stained rods, indicating that the bacteria and the outer-membrane antigens recognised by the MAbs in the immunoperoxidase test appeared to be preserved during the procedures (figs. 1A, B).

Expression of fimbriae during interaction of H. influenzae strain 770235f⁺ with fresh adenoid. The use of fresh adenoidal tissue allowed us to investigate whether *H. influenzae* cells that had penetrated through the epithelial cells to the submucosa still expressed fimbriae. Sections of fresh tissue which had been incubated with bacteria, and then fixed, embedded and sliced, were incubated either with MAbs against outer-membrane components for immunochemical staining of the bacteria or, in parallel sections, with MAb 6HE8, in each case followed by immunoperoxidase staining. The *H. influenzae* cells reacted with both MAbs, indicating the presence of fimbriae on the bacteria in the submucosa. Stained bacteria were observed in the tight junctions of epithelial cells (fig. 1B).

Binding of H. influenzae to tissue sections of adenoid

Preliminary experiments with fixed and sectioned adenoid tissue showed that binding of the fimbriate *H. influenzae* strain 770235f⁺ and its non-fimbriate variant strain 770235 varied extensively with most cell types of adenoid tissue. This binding was hardly diminished in the presence of MAb 6HE8, which is specific for the fimbriae, indicating that the majority of the binding was not fimbriae-dependent and was

most likely a non-specific interaction (sticking of bacteria). Enrichment of the incubation medium with Triton-X 100 1%, Tween-20 1%, FCS 10%, or bovine milk powder (ELK; Campina, Eindhoven, The Netherlands) 1% which prevent non-specific binding, resulted in a slight reduction in the overall binding. Pre-incubation of the adenoid tissue sections with pepsin, after adjustment of the pH to 2.0 by addition of HCl (the pH at which the enzyme is active), but not pre-incubation with HCl alone, resulted in a strong reduction of the binding of *H. influenzae* without fimbriae to most of the cell types tested. Binding to endothelium, fibroblasts in connective tissue and tissue macrophages did not disappear after such pre-treatment (fig. 1C). Specific binding of fimbriate bacteria (i.e., binding which could be inhibited by MAb 6HE8 but not by MAb 7BB1) was noted, after such pre-treatment, to the same cells as those to which fimbriate *H. influenzae* bound in fresh adenoid tissue (figs. 2B, C, D and table I). The viability of the bacteria, as determined by the numbers of cfu, was not affected by any of these pre-treatments.

Binding of H. influenzae to oropharyngeal epithelial cells

Since a rather harsh treatment of the tissue sections was required to avoid non-specific binding, we examined the effect of fixation, embedding and pre-treatment on the adherence of *H. influenzae*. Oropharyngeal epithelial cells obtained from scrapings from the oropharynx of healthy humans are well-suited for this purpose.^{3-7, 9, 10, 13, 14} Neither pre-treatment of these cells with formalin, followed by pepsin + HCl, HCl alone, toluene, xylol or ethanol, for 1 h at room temperature, nor the effect of Triton-X100, Tween-20, ELK and FCS, appreciably influenced the binding of *H. influenzae* strain 770235f⁺ to these cells. The effects of pre-treatments on the adherence of these bacteria to oropharyngeal epithelial cells are shown in fig. 3(A-G). These results are in agreement with results obtained earlier.^{10, 13, 14}

The combined results obtained with fresh adenoids, sections of adenoids, and oropharyngeal epithelial cells show that the binding characteristics of fimbriate *H. influenzae* to unfixed, intact adenoids and fixed, embedded, sectioned and pre-treated tissues were similar, indicating that the binding sites specific for *H. influenzae* with fimbriae were not inactivated during the fixation, embedding and pre-treatment steps. Subsequently, we pre-incubated all tissue sections with pepsin and included Tween-20 in the washing buffers, as described in *Materials and methods*, before they were incubated with bacteria.

Binding of bacteria to tissue sections of various organs

A major difference was observed between the binding of *H. influenzae* strain 770235f⁺ and that of

Fig. 2. Adherence of *H. influenzae* strain 770235f⁺ to paraffin-embedded sections of adenoid. **A**, abundant adherence to epithelium, especially the ciliated cells; **B**, no adherence after pre-incubation of the bacteria with fimbriae-specific MAb 6HE8; **C**, sparse binding to lymphoid tissue after inhibition with MAb 6HE8; in contrast to, **D**, strong binding with control MAb 7BB1, which did not inhibit binding. Bar, 20 μ m. Bacteria were stained as in fig. 1.

strain 770235 to various tissues. These results are summarised in tables I and II. *H. influenzae* strain 770235 (without fimbriae) was found only on vascular endothelial cells, especially of the capillaries (fig. 4A), and on macrophages from various sites of the human body. This binding was not inhibited by anti-fimbriae MAb 6HE8. Fimbriae-specific binding of *H. influenzae* strain 770235f⁺ (i.e., binding which was inhibited by MAb 6HE8 but not by MAb 7BB1) was clearly observed in sections of brain, lung, joints (especially the synovial membrane), adenoid and other pharyngeal tissues, but only weakly in those of liver, kidney and the eye (table I). However, the various cell types in these tissues showed different degrees of binding (table II).

In the brain, binding was predominantly to glial cells (astrocytes and oligodendrocytes) and to the connective tissue surrounding the blood vessels, especially those of the large sinuses. Many bacteria were seen on ependyma of the choroid plexus and on the ciliated ependyma of the ventricles. The bacteria were seen on both sides—ventricular and stromal (fig. 5).

In the lower respiratory tract, fimbriate bacteria were found in similar numbers on alveolar macrophages, type II pneumocytes and in the bronchi on epithelial cells, especially the ciliated ones (fig. 1D, E). In the upper respiratory tract, binding to the

epithelial cells was observed in the same way as described for the bronchi and adenoids, and also to the fibroblasts in the subepithelial connective tissue. The normal respiratory tract is lined by pseudostratified ciliated columnar, cuboidal, and squamous epithelial cells. Strain 770235f⁺ bound only to the ciliated columnar cells and hardly at all to the cuboidal or squamous epithelial cells. Bacteria appeared to be trapped in the respiratory mucus when this was present on the epithelial cell layer. This was seen most clearly in cryosections of fresh adenoid, in which many *H. influenzae* cells were seen in the slimy mucus material on the surface of the ciliated and squamous epithelium.

In the knee joint, fimbriate bacteria were found in association with synovial cells and on the fibroblasts of the underlying connective tissue. Bacteria were found on the cartilage of the joint only if the surface was damaged. Binding of fimbriate bacteria to connective tissue appeared to be a general phenomenon, since this was also observed with connective tissue in the respiratory tract and the connective tissue surrounding the vena saphena magna and the cerebral vessels.

The binding of *H. influenzae* to the lymphoid tissues of adenoid, spleen and lymph nodes showed marked similarities. Bacteria with fimbriae were found on monocytes, macrophages, follicle centre cells, dendrit-

Fig. 3. Effect of various pretreatments of oropharyngeal epithelial cells on the adherence of *H. influenzae* strain 770235f⁺ to these cells. **A**, methanol-fixed oropharyngeal epithelial cells obtained from scrapings; note that the bacteria did not bind to all cells. A four-fold enlargement of part of an epithelial cell with bacteria is shown in the insert to show the bacterial morphology. **B–G**, adherence to oropharyngeal epithelial cells pretreated with various agents: **B**, formalin; **C**, ethanol 70%; **D**, toluene; **E**, xylol; **F**, pepsin-HCl; and **G**, HCl alone. Bar, 20 μ m; all have the same enlargement. EP, epithelium; M, surrounding medium.

ic reticulum cells and lymphocytes. Only the binding to macrophages was not inhibited by anti-fimbrial antibodies.

The liver and kidney, both used as control organs, bound *H. influenzae* very poorly. In the liver, some bacteria were found, after incubation with *H. influenzae* strain 770235f⁺, in sinusoidal blood vessels, probably attached to endothelium. In the kidney, bacteria were bound to the endothelium of the glomeruli and in very small numbers to the mesangium and the luminal site of the tubuli.

Binding of the fimbriate bacteria to erythrocytes was always seen, wherever these cells were found (fig. 4B). This is consistent with the known ability of these bacteria to cause haemagglutination by the blood-group AnWj antigen.¹⁴

Discussion

The experiments described here were designed to test the hypothesis that tissue tropism of *H. influenzae* during colonisation and infection is determined by the presence of specific binding sites for the fimbriae of these bacteria. Tissue sections were incubated with bacteria, followed by immunochemical labelling of the bacteria with immunoperoxidase reagents and examination by light microscopy. Compared with immunofluorescence, this technique has the advantage that it permits the simultaneous visualisation of bacteria and cells, which is important for the identification of the binding sites in the tissues.

The ability of fimbriate *H. influenzae* to bind to various cells differed from those of the same strain

Table II. Binding of *H. influenzae*, with and without fimbriae, to various cell types

Cell type	Source tissue	Binding of <i>H. influenzae</i> strains	
		770235f ⁺	770235
Epithelium			
squamous, cubic	Pharynx	—	—
pseudostratified columnar	Epiglottis	++	—
ciliated columnar	Rhinopharynx, nasopharynx	++	—
	adenoid, bronchi		
conjunctival	Eye	—	—
glomerular, distal and proximal tubulus	Kidney	+/-	—
Ependyma (+ cilia)	Ventricles	++	—
	Choroid plexus	++	—
Pneumocytes type II	Alveoli	++	—
Endothelium	Cerebral sinuses, vena saphena magna, liver, sinusoid	+	+
	capillaries	++	+
Fibroblasts	Connective tissue underlining epithelia and surrounding vessels,	++	++
Lymphocytes, monocytes, tissue macrophages, including type II	Upper and lower respiratory tract, RES, spleen, brain	++	++
pneumocytes, follicle centre cells, dendritic reticulum cells			
Glial cells and macrophage-like microglial cells	Cerebrum	++	+

++ , + , — , see table I.

without fimbriae. The best way to show specificity of binding is by inhibition experiments with isolated receptor structures or by specific antibodies. However, the receptor for fimbriate and non-fimbriate *H. influenzae* is not known. MAbs specifically inhibiting fimbriae-mediated adherence were included in this study, but nothing is known about the bacterial and host components involved in fimbriae-independent binding. Therefore it is not at present possible to draw conclusions about the specificity of the fimbriae-independent binding.

The fimbriae appeared to be involved in most of the binding of fimbriate *H. influenzae* to some organs and specialised tissues (tables I and II), since most of the binding was inhibited by MAbs specific for the fimbriae. The residual binding was also seen with non-fimbriate bacteria. Fimbriae-mediated binding was not affected by treatment of the tissues with toluene and pepsin, indicating that the receptor for the fimbriae on the various cell types is probably not a lipid or a protein. This is in agreement with earlier results which suggested that the receptor is probably part of the sugar chain of a glycolipid or a glycoprotein.¹³ The tissues involved in fimbriae-dependent binding include the pharynx, the site colonised by *H. influenzae* in the carrier state, and also organs and tissues involved in respiratory tract infections (rhinopharynx, nasopharynx, bronchi and alveoli). Binding

was predominantly to ciliated columnar epithelial cells, to the connective tissue underlying the epithelia and surrounding vessels, and to cells of the immune system. In this respect it is important to note that *H. influenzae* has been found in the epithelium and the subepithelial tissues of the bronchi in patients with chronic bronchitis¹⁷ and that the bacteria have been shown to penetrate cultured human adenoid tissue in a similar fashion.¹⁵ Binding to ciliated epithelium was not observed by Farley *et al.* or Loeb *et al.*^{8,15} Trapping of the bacteria between the cilia is unlikely, since these authors showed that this binding was inhibited specifically by MAbs to the fimbriae and since this type of binding was not observed with non-fimbriate bacteria. We observed also that the bacteria were trapped in the mucus layer overlying the epithelial cells, a common observations in sputum preparations taken during respiratory tract infections. The binding of *H. influenzae* to mucus and connective tissue was striking. Both these materials are rich in glucosaminoglycans. It would be interesting to see whether the receptor for the fimbriae is part of such molecules. Other explanations for the differences in the observed binding to ciliated cells may lie in the differences in methodology used in the previously published studies^{8,15} and in our current studies for the analysis of the interaction between bacteria and cells.

Tissues that are involved in generalised infections

Fig. 4. Adherence of *H. influenzae* to blood vessels. **A**, strong adherence of *H. influenzae* strain 770235 to the endothelium of a blood vessel in the lung; **B**, strong adherence of strain 770235f⁺ to a small blood vessel in the brain, in which the bacteria are found on erythrocytes. Bar, 20 μ m. V, blood vessel; RBC, red blood cell. Arrows indicate some of the bacteria adhering to the vessel walls and erythrocytes.

of *H. influenzae* appeared to contain binding sites for fimbriate *H. influenzae*. These included the ependyma of the choroid plexus and the ventricles, on the stromal and ventricular side during meningitis. It is probable that bacteria pass close to these cells when they enter the cerebrospinal fluid from the bloodstream. Binding to the stroma of the cerebrum may indicate that the encephalitis which is sometimes observed after meningitis is also associated with the presence of fimbriae. Similar binding characteristics were found by Parkkinen *et al.*¹² for *E. coli* from neonatal meningitis which express mainly S-fimbriae, which recognise terminal neuraminyl 2-3 galactosides as receptor.¹⁸ Epiglottitis may be explained as an inflammation

following binding by fimbriate bacteria, since the bacteria bound to the epithelial cells and the underlying tissue.^{4,6}

The binding of fimbriate bacteria to macrophages, lymphocytes and monocytes suggests that bacteria with fimbriae are recognised by cells of the immune system, thereby probably stimulating clearance of the bacteria. In this case, a non-fimbriate phase may be an advantage for the bacteria which penetrate via the bloodstream into target organs. Fresh isolates from blood cultures are, indeed, much less fimbriate than, for example, strains from the throat or sputum.^{4,7} Based on this clearance mechanism, the survival out of an intranasal inoculum of 10^5 bacteria of one

Fig. 5. Adherence of fimbriate *H. influenzae* to brain sections. **A**, strong adherence of *H. influenzae* strain 770235f⁺ to brain ependyma cells of the choroid plexus; **B**, strong adherence to the ventricles. Bar, 20 μ m. Some bacteria are indicated by arrows.

organism in the blood stream in an animal model for invasive *H. influenzae* disease¹⁹ may reflect the natural rate of one non-fimbriate bacterium to c. 10^5 fimbriate cells. Subsequent phase variation to the fimbriate form should be assumed, if adherence to the blood brain barrier is essential for penetration of the bacteria into the cerebrospinal fluid. Whether bacteria which attach to the walls of blood vessels or the spinal cord during infection are fimbriate is not known.

In conclusion, our experiments indicate that fimbriate *H. influenzae* cells have the ability to interact with the surface epithelium and with some cells in various tissues where *H. influenzae* is found during colonisation and infection. Furthermore, fimbriae could be detected on bacteria after penetration of *H. influenzae* to below the epithelial cell layer of fresh adenoid tissue (fig. 1B). These results suggest that fimbriae are a pre-requisite for the tissue tropism of

the organism. The absence of fimbriate bacteria on the blood cells in the vessels of fresh adenoid and the direct binding of fimbriate bacteria to these cells in tissue sections fit well with the notion that this type of binding probably contributes to the clearance of bacteria from the circulation. Consequently, phase variation in the expression of fimbriae may be an important mechanism for the bacteria to direct themselves to those cells that they can colonise.

Identification and localisation of the receptor molecules for the fimbriae on these tissues in health and disease may contribute to a better understanding of the pathogenetic mechanisms of *H. influenzae* infections.

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