INFECTION AND IMMUNITY, July 1994, p. 2707–2714
0019-9567/94/$04.00+0

Vol. 62, No. 7

Adenovirus Infection Enhances In Vitro Adherence of *Streptococcus pneumoniae*

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Received 18 January 1994/Returned for modification 28 February 1994/Accepted 12 April 1994

Viruses are thought to facilitate bacterial infections of the respiratory tract, but the mechanisms are poorly understood. The present study analyzed the effect of adenovirus on bacterial adherence to human respiratory tract epithelial cells. The human lung carcinoma cell line A549 was infected with adenovirus of types 1, 2, 3, 4, 5, and 9. At a multiplicity of infection of 75 particles per cell, cytopathic effects occurred in 75 to 100% of the cells within 48 h. The virus-infected cells were harvested at various times after infection and analyzed for the ability to bind strains of *Haemophilus influenzae* and *Streptococcus pneumoniae*. Adenovirus (types 1, 2, 3, and 5) commonly causing respiratory tract infections increased the binding of adherent *S. pneumoniae* strains to the cells. This effect was not seen for other adenovirus types. Adenovirus infection did not change the adherence of cells of poorly adhering strains of *S. pneumoniae* or *H. influenzae*. The increase in adherence of *S. pneumoniae* could be inhibited by the DNA synthesis inhibitor cytosine arabinofuranoside, which is known to block the late phase of the adenovirus infection. When electron microscopy was used, there was no evidence that virus particles bound directly to bacteria. Adherence was not affected by pretreatment of the cells with virus particles or viral proteins. This suggested that adenovirus infection upregulated receptors for *S. pneumoniae*. The increased attachment may be one mechanism by which viruses precondition the respiratory mucosa for bacterial infection.

The upper respiratory tract is an ecological niche for bacteria such as *Streptococcus pneumoniae* and *Haemophilus influenzae*. These species are carried by healthy individuals and especially by children (3). The same bacterial strain may, however, also cause infection. It is not clear how the transition from asymptomatic carriage to infection can occur.

Adherence facilitates the establishment of bacterial populations at mucosal surfaces. *H. influenzae* and *Pseudomonas aeruginosa* adhere to nasopharyngeal epithelial cells as well as respiratory tract mucins (8, 28, 29). Group A streptococci, *Neisseria meningitidis*, and *S. pneumoniae* adhere mainly to respiratory tract epithelial cells (8). No clear-cut correlation between in vitro adherence and virulence for the respiratory tract has been established (1). Nasopharyngeal isolates of *S. pneumoniae* from healthy carriers were found to adhere better to respiratory tract epithelial cells than isolates from the blood or cerebrospinal fluid of patients with pneumonia or meningitis. The in vitro adherence assays are, however, defective with regard to factors that influence adherence in vivo.

Viral infections of the upper respiratory tract predispose to bacterial superinfection. The mortality during influenza epidemics has been attributed to bacteria such as *Staphylococcus aureus*, *N. meningitidis*, and group A streptococci as well as *S. pneumoniae* and *H. influenzae* (12, 35). Both viral and bacterial agents can be isolated from the middle ear fluid of children with acute otitis media (AOM) (6, 7, 16, 19). Evidence for an association between viral and bacterial AOM was obtained by Heikkinen et al. (15), who showed that children vaccinated against influenza A during an influenza epidemic had a lower frequency of AOM than nonvaccinated children. In animals, inoculation with influenza virus predisposed to experimental bacterial AOM (13). Those studies suggested that viruses play an important role in preconditioning the respiratory mucosa for bacterial infections. The mechanisms of these effects are not known.

Previous studies have shown that influenza virus infections can influence bacterial adherence in vitro. Infection of MDCK cells with influenza virus increased the adhesion of *S. aureus*, *N. meningitidis*, and group A and group B streptococci (9–11, 20, 26, 27, 28). The possibility that other respiratory viruses can cause a similar change in bacterial adherence has not been examined. Adenoviruses, *H. influenzae*, and *S. pneumoniae* are common causes of respiratory tract infections, especially in young children. The aim of the present study was to investigate whether adenovirus infection could change the adherence of *S. pneumoniae* and *H. influenzae* to cultured human respiratory tract epithelial cells.

MATERIALS AND METHODS

Bacteria. Nineteen strains of *S. pneumoniae* and four strains of *H. influenzae* were used. Most strains were obtained from the Culture Collection of the University of Gothenburg (CCUG), Gothenburg, Sweden. They were selected on the basis of their known capacity to adhere to nasopharyngeal epithelial cells in vitro (1).

Eighteen *S. pneumoniae* strains were isolated from the nasopharynx of children with frequent episodes of AOM. Eight (CCUG 18377, CCUG 19566, CCUG 19773, CCUG 20125, CCUG 20347, CCUG 20719, CCUG 21375, and CCUG 21395) were highly adherent (mean, >45 bacteria per cell), and 10 (CCUG 19459, CCUG 19583, CCUG 19484, CCUG 19554, CCUG 19886, CCUG 20165, CCUG 20210, CCUG 20527, CCUG 20670, and CCUG 20734) were poorly adherent (mean, <10 bacteria per cell). One highly adherent strain (CCUG 10175) was isolated from a healthy carrier.

Two *H. influenzae* strains (CCUG 20441 and CCUG 21594)

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TABLE 1. Adherence of *S. pneumoniae* and *H. influenzae* to human nasopharyngeal epithelial cells and to the A549 cell line

Bacterial strain	Capsular type	No. of adherent bacteria/cell[a]		
		NPH[b] cells	A549 cells	
			−[c]	+
S. pneumoniae				
CCUG 10175	19	75	65 (30–100)[d]	140 (80–250)*[e]
CCUG 18377	NT[f]	150	65 (40–90)	130 (70–210)*
CCUG 19566	6	45	12 (0–50)	45 (30–110)*
CCUG 19773	35	161	110 (50–150)	210 (120–250)*
CCUG 20125	6	47	30 (20–50)	52 (30–110)*
CCUG 20347	19	67	33 (20–50)	50 (40–120)*
CCUG 20719	6	39	36 (20–60)	71 (40–140)*
CCUG 21375	19	74	36 (10–60)	57 (40–110)*
CCUG 21395	6	85	46 (20–80)	65 (30–120)*
CCUG 19459	6	4	11 (0–30)	7 (0–20)
CCUG 19483	NT	2	8 (0–20)	8 (0–30)
CCUG 19484	6	7	0 (0)	0 (0)
CCUG 19554	22	2	1 (0–10)	1 (0–5)
CCUG 19886	6	5	3 (0–15)	4 (0–10)
CCUG 20165	6	10	10 (0–30)	9 (0–30)
CCUG 20210	19	2	3 (0–10)	4 (0–15)
CCUG 20527	19	0	0 (0)	0 (0)
CCUG 20670	15	4	11 (0–25)	7 (0–15)
CCUG 20734	11	2	11 (0–30)	10 (0–20)
H. influenzae				
CCUG 21594	NT	111	69 (40–130)	66 (40–140)
CCUG 20441	NT	66	79 (40–130)	67 (40–120)
770235f$^+$	b	133	8 (0–20)	8 (0–20)
770235f^0	b	2	2 (0–5)	1 (0–5)

[a] Mean of two experiments.
[b] Nasopharyngeal epithelial cells.
[c] −, uninfected A549 cells; +, A549 cells infected with adenovirus type 5.
[d] The means (and ranges) are indicated.
[e] *, $P < 0.001$ compared with uninfected A549 cells.
[f] NT, nontypeable.

were nontypeable nasopharyngeal isolates (HiNT) from children with AOM. An encapsulated and fimbriated type b (Hib) strain, 770235f$^+$, and its nonfimbriated mutant, 770235f^0, were kindly provided by L. van Alphen (Department of Medical Microbiology, University of Amsterdam, The Netherlands) (33).

The bacterial strains were stored lyophilized. For experiments, lyophils were transferred to blood agar plates (*S. pneumoniae*) or chocolate agar plates (*H. influenzae*) and incubated at 37°C overnight. *S. pneumoniae* was cultured for 9 h at 37°C in liquid growth medium (32). *H. influenzae* was cultured for 4 h at 37°C in liquid growth medium (5) in a shaking water bath. The bacteria were then harvested by centrifugation at 1,500 × *g* for 30 min and suspended in 0.15 M NaCl (*H. influenzae*) or in 0.15 M NaCl with 1% choline chloride (*S. pneumoniae*). The bacterial concentration was estimated by interference contrast microscopy (Ortolux II microscope with interference contrast equipment; TE Leitz, Wetzlar, Germany) in a Bürker chamber. This method was standardized by viable counts.

Virus isolation, typing, and culture. The adenoviruses were isolated and passaged by standard methods, using the human lung carcinoma cell line A549 (ATCC CCL 185). A549 cells are permissive for human adenovirus infection (18, 28). The A549 cells were grown in RPMI 1640 (Gibco-BRL, Life Technology Ltd., Paisley, Scotland, United Kingdom), supplemented with 10% fetal calf serum, 2 mM glutamine, and 50 μg of gentamicin per ml, in 25-cm^2 (50-ml) cell culture flasks (Nunc, Roskilde, Denmark). For maintenance, the same medium, but with 1% fetal calf serum was used. For passage of virus, A549 cells were grown in 175-cm^2 cell culture flasks (Nunc) until confluent. The medium was discarded, and the cell layer was washed twice with phosphate-buffered saline (PBS). The virus suspension was added to the flask and left to incubate for 1 h at 37°C in an atmosphere of 5% CO_2. The cell layer was washed with PBS, and maintenance medium was added. The cells were incubated at 37°C until the monolayer showed cytopathic effects (28). The cells were then detached by shaking the flask, harvested, and centrifuged at 1,700 × *g* for 15 min. Supernatant was removed to leave a remaining total volume of 5 ml. The pellet and remaining medium were frozen and thawed three times to lyse the cells. The suspension was centrifuged at 1,700 × *g* for 15 min, and the supernatant containing virus and soluble virus components was collected and stored at −70°C.

The adenovirus serotypes were confirmed by a neutralization test with rabbit polyclonal typing antisera on A549 cells (31).

Quantitation of virus. The concentration of virus was determined by using the lytic plaque assay (31). Briefly, A549 cells were grown on Falcon cell culture petri plates (60 by 15 mm; Becton Dickinson and Co., Paramus, N.J.) until confluent. The virus suspension was serially diluted in PBS from 10^{-1} to 10^{-7}/ml. Three hundred microliters of the 10^{-3} to 10^{-7} dilutions was added to the plates, and the plates were incubated for 1 h at 37°C in an atmosphere of 5% CO_2. Sterile Bacto Agar (1.8%; Difco Laboratories, Detroit, Mich.) was prepared and maintained at 45°C. Overlay medium containing 1 part of fetal calf serum, 4 parts of 2× minimal essential medium (Gibco-BRL), and 5 parts of melted agar was prepared at 45°C. The plates were then washed in PBS, overlaid with 5 ml of this medium, and left to solidify for 10 min. The plates were incubated at 37°C in a 5% CO_2 atmosphere for 5 days. After incubation, the plates were covered with melted agar solution supplemented with neutral red (0.01 g/100 ml) and left standing overnight at 37°C to develop. The number of lytic plaques was counted. The virus concentration was given as PFU per milliliter.

Binding of virus particles to bacteria. Suspensions of *S. pneumoniae* CCUG 10175 and CCUG 18377 (5 × 10^7 bacteria per ml) were mixed with equal volumes of adenovirus type 5 in suspension (6 × 10^9 PFU/ml), incubated for 30 min at 37°C, and inspected by light microscopy. The mixtures were washed three times in PBS with centrifugation at 1,500 × *g* for 20 min. Each sample was stained with phosphotungstic acid (1%, pH 6.5; Taab Laboratory Equipment Ltd., Berkshire, United Kingdom), put on a carbon grid, and inspected in a transmission electron microscope.

Viral supernatants. The virus suspensions (3 × 10^9 to 6 × 10^9 PFU/ml) were centrifuged in 300-μl tubes at 100,000 × *g* for 30 min in an Airfuge (Beckman Instruments, Inc., Fullerton, Calif.). The supernatants were collected. A549 cells were preincubated for 1 h with 100 μl of the supernatant and then used for adherence testing.

Assays for bacterial adherence. Adherence was tested by using exfoliated human nasopharyngeal epithelial cells or A549 cells. Human nasopharyngeal epithelial cells were obtained from healthy donors by scraping the pharyngeal wall behind the soft palate with a cotton-tipped wooden swab. The cells were detached by gentle stirring in 0.15 M NaCl, washed twice by centrifugation at 500 × *g* with resuspension in NaCl, and diluted in 0.15 M NaCl to a concentration of 10^5 cells per ml.

The A549 cells were grown to confluency as described above.

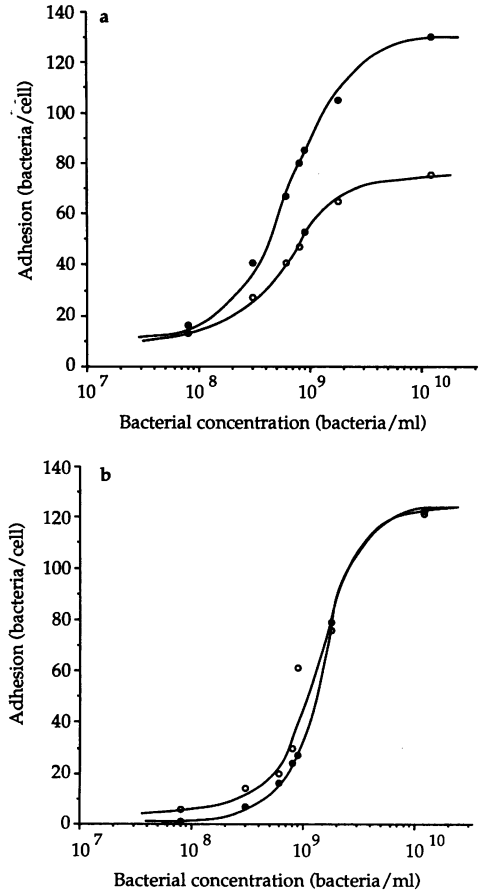

FIG. 1. Adherence with different concentrations of *S. pneumoniae* CCUG 10175 (a) and *H. influenzae* CCUG 21594 (b) to uninfected A549 cells (○) and to A549 cells infected with adenovirus type 5 (●).

At time zero, the medium was discarded and new medium containing virus or control medium without virus was applied to the monolayer and left to incubate for 1 h at 37°C. The cells were washed in PBS, and maintenance medium was added. The cells were cultured at 37°C in 5% CO_2 and inspected daily for the appearance of CPE. The cells were harvested for adherence testing, trypsin treated for about 10 min at room temperature, and centrifuged at 500 × *g* for 10 min. The cells were washed twice by resuspension in 0.15 M NaCl and centrifuged at 500 × *g* for 10 min. The cells were then suspended in 0.15 M NaCl to a final concentration of 5×10^5 cells per ml, as counted in a Bürker chamber.

The bacteria were used at a concentration of 2×10^9/ml, diluted in 0.15 M NaCl (*H. influenzae*) or in 0.15 M with 1% choline chloride (*S. pneumoniae*) unless otherwise stated. The bacteria (50 μl) were mixed with 50 μl of cell suspension in a 3-ml plastic tube. Mixtures of cells and *S. pneumoniae* were centrifuged at 500 × *g* for 3 min and incubated at 37°C for 30 min. Mixtures of cells and *H. influenzae* were incubated at 37°C for 30 min with shaking. After incubation, unbound bacteria were eliminated by repeated cycles of centrifugation (500 × *g*, 15 min) and resuspension in NaCl (*H. influenzae*) or NaCl plus 1% choline chloride (*S. pneumoniae*) (1, 2).

The washed mixtures were applied to microscope slides, fixed with pure methanol, and stained with the May-Grünwald reagents (Merck AG, Darmstadt, Germany). The number of attached bacteria was determined by visual count at 800× magnification in a Nikon microscope (Nikon, Tokyo, Japan).

The adherence was given as the mean number of bacteria per cell for 40 cells counted per sample.

Effects of DNA synthesis inhibition on adherence to virus-infected cells. A549 cells were grown to confluency. The medium was discarded. Virus was applied to the monolayer at a concentration of 2×10^8 PFU/ml and left to incubate for 1 h at 37°C. The cells were washed in PBS, and 10 ml of maintenance medium containing 50 μg of cytosine arabinofuranoside per ml of medium (Sigma) was added. The cells were cultured at 37°C in 5% CO_2 for 48 h before adherence testing.

Statistics. The adhesion to uninfected cells and that to cells infected with different adenovirus serotypes were compared by using the Mann-Whitney U test. A P value of <0.05 was considered significant.

TABLE 2. Adherence of *S. pneumoniae* CCUG 10175 to A549 cells infected with different adenovirus types

Adenovirus		No. of adherent bacteria/cell [mean (range)][a]		P
Serotype	Subgroup	−[b]	+	
3	B	75 (50–100)	166 (100–300)	<0.003
1	C	76 (40–110)	200 (110–300)	<0.003
2	C	70 (40–100)	134 (90–250)	<0.003
5	C	72 (40–110)	139 (100–250)	<0.003
9	D	66 (40–100)	70 (40–100)	NS[c]
4	E	66 (40–100)	79 (40–120)	<0.003

[a] All values are means of two experiments except for those for adenovirus serotype 5, subgroup C, which are means of five experiments.
[b] −, uninfected; +, infected with adenovirus.
[c] NS, not significant ($P = 0.070$).

RESULTS

Adherence of *S. pneumoniae* and *H. influenzae* to nasopharyngeal epithelial cells and to the A549 cell line. A549 cells and human nasopharyngeal epithelial cells were incubated with 18 different strains of *S. pneumoniae*. Nine of these strains attached to the nasopharyngeal epithelial cells as defined by a mean adherence of ≥30 bacteria per cell. The mean adherence of this group of strains was 91 bacteria per cell (range, 39 to 161 bacteria per cell) (Table 1). These nine *S. pneumoniae* strains were found to bind to the A549 cells with a mean adherence of 48 bacteria per cell (range, 12 to 110 bacteria per cell). The remaining 10 *S. pneumoniae* strains attached poorly to the nasopharyngeal epithelial cells (mean, 4 bacteria per cell; range, 0 to 10 bacteria per cell) and to the A549 cells (mean, 6 bacteria per cell; range, 0 to 11 bacteria per cell). These results demonstrated that the A549 cell line expressed receptors for attaching *S. pneumoniae* and that the degree of attachment to A549 cells was similar to that for nontransformed human nasopharyngeal epithelial cells.

The adherence of *H. influenzae* to A549 cells and nasopharyngeal epithelial cells is also shown in Table 1. The four *H. influenzae* strains varied in adherence to the nasopharyngeal epithelial cells. The fimbriated, encapsulated *H. influenzae* type b strain 770235f$^+$ adhered in high numbers to the human nasopharyngeal epithelial cells (mean, 133 bacteria per cell). The isogenic, nonfimbriated mutant *H. influenzae* 770235f^0 attached poorly (mean, 2 bacteria per cell). Neither strain attached to the A549 cells (means, 8 and 2 bacteria per cell, respectively). The nontypeable *H. influenzae* strains CCUG

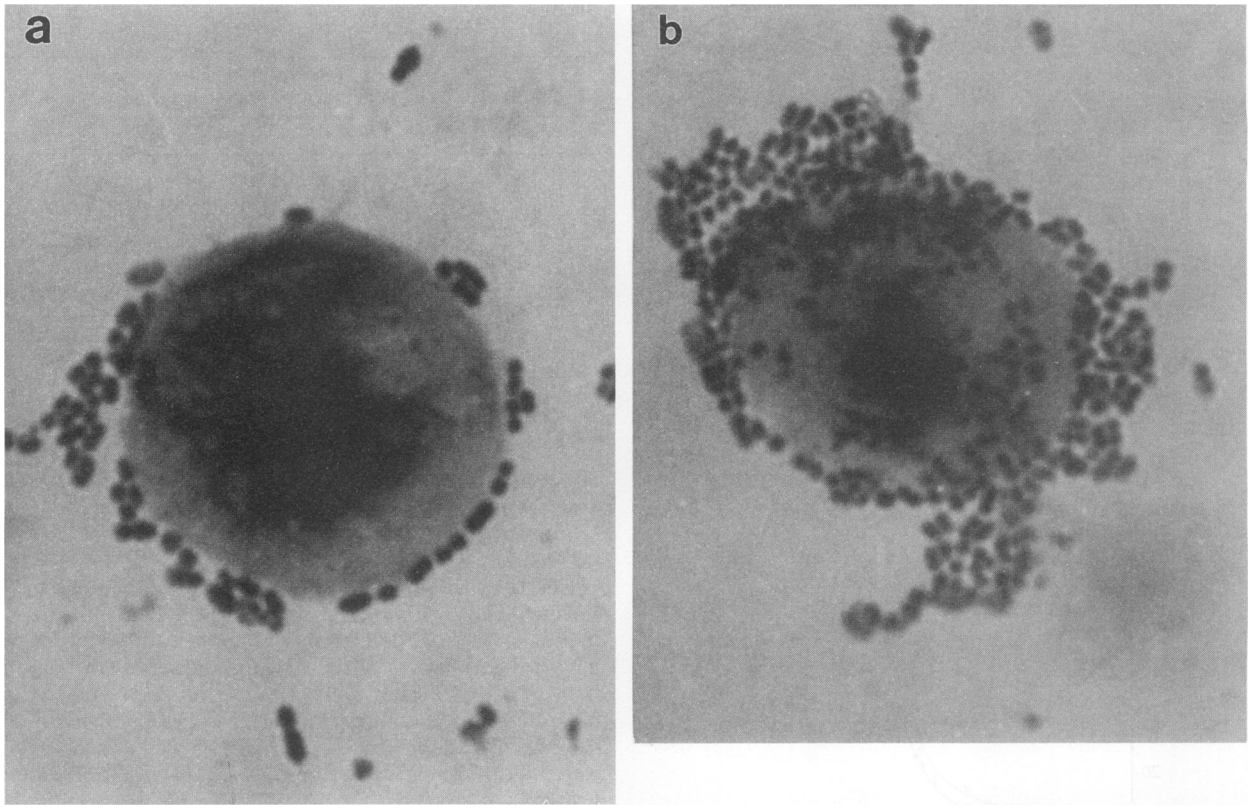

FIG. 2. Examples of adherence of *S. pneumoniae* CCUG 10175 to an uninfected A549 cell (a), an A549 cell infected with adenovirus type 5 (b), and an A549 cell infected with adenovirus type 9 (c).

20441 and CCUG 21594 attached to the nasopharyngeal cells (means, 66 and 111 bacteria per cell, respectively) and to A549 cells (means, 79 and 69 bacteria per cell, respectively). This demonstrated that the A549 cell line did not express receptors for the fimbria-associated adhesins of *H. influenzae* type b but expressed receptors for the adhesins of nontypeable *H. influenzae* strains that predominate in the upper respiratory tract.

Adherence to adenovirus-infected A549 cells. The A549 cells were infected with 2×10^8 PFU of adenovirus type 5 (multiplicity of infection [MOI], 75). The cells were left until microscopic examination showed cytopathic changes in 75 to 100% of the cells (usually after 48 h). At this time, adenovirus-infected and control cells were harvested and used to test the adherence of *S. pneumoniae* and *H. influenzae.*

The adherence of *S. pneumoniae* to A549 cells was increased after infection with adenovirus type 5 (Table 1). The mean adherence increased from 48 to 91 bacteria per cell for the highly adherent *S. pneumoniae* strains (mean increase, 100%; range, 41 to 275%). There was no effect of virus infection on the adherence of the 10 poorly adhering *S. pneumoniae* strains. Furthermore, there was no increase in adherence of the *H. influenzae* strains to the virus-infected cells.

The influence of bacterial concentration on adherence to virus-infected and control A549 cells is shown in Fig. 1. Adherence of *S. pneumoniae* was detected at 10^8 bacteria per ml and reached a maximum at 2×10^9 bacteria per ml. The threshold bacterial concentrations required for adherence were similar for control and virus-infected cells as were the concentrations required for maximum adherence. The adherence to the virus-infected cells of *S. pneumoniae* CCUG 10175 was higher than that to control cells at all concentrations that were $>10^8$ bacteria per ml.

The adherence of *H. influenzae* CCUG 21594 to the A549 cells was detected at 2×10^8 bacteria per ml and reached a maximum at 2×10^9 bacteria per ml. There was no difference in the adherence to virus-infected and control cells at any bacterial concentration.

Adherence in relation to adenovirus type. The A549 cells were infected with 2×10^8 PFU of adenovirus of types 1, 2, 3, 4, 5, and 9. Each adenovirus type established an infection in the A549 cells as demonstrated by the cytopathic changes after 48 h. The infected cells were harvested and used for adherence testing with *S. pneumoniae* 10175 at a concentration of 2×10^9 bacteria per ml.

There was a distinct increase in adherence to cells infected with adenovirus types 1, 2, 3, and 5 (mean increase, 111%; range, 92 to 140%; $P < 0.001$) (Table 2). There was a moderate increase in adherence to cells infected with adenovirus type 4 (mean increase, 25%; $P < 0.001$) but no change in adherence to cells infected with adenovirus type 9 (mean increase, 9%). The adherence pattern is shown in Fig. 2 for an uninfected cell (Fig. 2a) and for cells infected with adenovirus type 5 (Fig. 2b) or type 9 (Fig. 2c). The levels of adherence to uninfected cells and cells infected with adenovirus type 9 were similar, while the adherence to cells infected with adenovirus type 5 was increased. The influence of bacterial concentration on adherence to cells infected with adenovirus types 1, 3, 4, and 9 is shown in Fig. 3. Infection with adenovirus types 1 and 3 did not change the threshold bacterial concentration required for adherence but increased the attachment at all concentrations

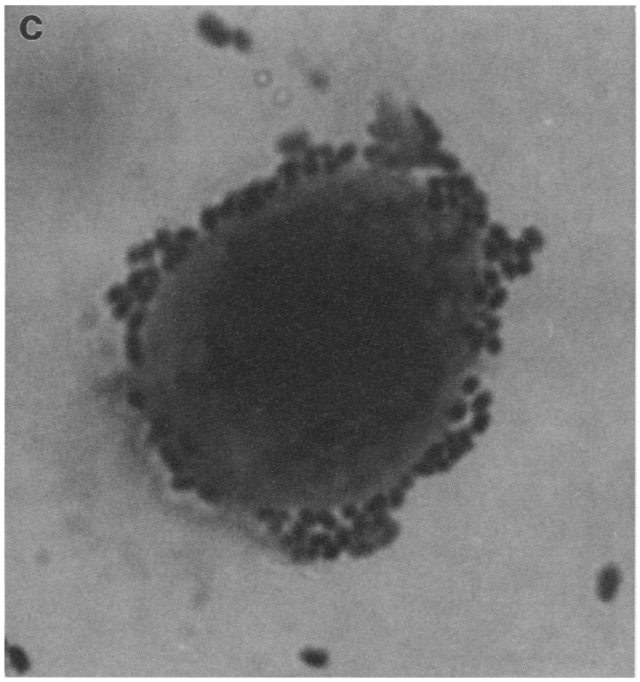

FIG. 2—*Continued.*

that were $>2 \times 10^8$ bacteria per ml. This clear effect was not seen for adherence to cells infected with adenovirus types 4 and 9, regardless of bacterial concentration.

Effect of virus concentration. A549 cells were infected with two concentrations of adenovirus type 5 (MOIs of 75 and 25) and used for adherence testing. The higher dose of virus caused cytopathic changes in 100% of the cells after 48 h and an increase in adherence of *S. pneumoniae* 10175 to most or all of the cells (mean, 133 bacteria per cell; range, 110 to 200 bacteria per cell). The lower dose of virus caused cytopathic changes in about 50% of the cells after 48 h and also less of an increase in adherence (mean, 104 bacteria per cell; range, 55 to 200 bacteria per cell). Both cells with high numbers of attaching *S. pneumoniae* and cells with low numbers of attaching *S. pneumoniae* were present. The mean adherence of *S. pneumoniae* to uninfected cells was 74 bacteria per cell (range, 50 to 110 bacteria per cell) (Fig. 4).

Binding of virus particles to *S. pneumoniae*. We examined the ability of bacteria to bind directly to adenovirus particles. Suspensions of *S. pneumoniae* CCUG 10175 or *S. pneumoniae* CCUG 18377 were mixed with suspensions of adenovirus type 5, incubated for 30 min at 37°C, and inspected by light microscopy. No aggregation of the bacteria was observed. The mixtures were washed, fixed, and stained for transmission electron microscopy. There were few virus particles in the suspension, and none of them were in close contact with the bacteria.

Effect of viral products on the A549 cells. Adenoviruses release toxic components, predominantly pentons, which can be harvested from the culture supernatants (24, 25). We examined the possibility that pentons might condition the A549 cells for bacterial adherence. A549 cells were preincubated for 30 min at 37°C with virus-free supernatants from cells infected with adenovirus types 2, 4, 5, and 9. The cells were subsequently used for adherence testing (Table 3). This pretreatment had no effect on adherence.

Adherence in relation to the stage in the virus infectious cycle. A549 cells were incubated with adenovirus type 5 (2×10^8 PFU). Cells were harvested after 30 min, 2 h, 6 h, 24 h, and 48 h and used to test the adherence of *S. pneumoniae* CCUG 10175. The results are shown in Fig. 5. The increase in adherence to the virus-infected cells occurred only after 24 h. Adherence at 24 h and 48 h was significantly higher than the adherence to uninfected A549 cells (means, 125 and 72 bacteria per cell, respectively, after 48 h).

TABLE 3. Adherence of *S. pneumoniae* CCUG 10175 and *H. influenzae* CCUG 21594 to A549 cells pretreated with supernatants from cells infected with adenovirus types 2, 4, 5, and 9

Origin of supernatant	No. of adherent bacteria/cell [mean (range)]	
	Strain 10175	Strain 21594
Control	57 (30–100)	38 (20–70)
Adeno[a] 2	47 (30–90)	34 (20–60)
Adeno 4	52 (30–100)	32 (15–60)
Adeno 5	44 (30–100)	34 (15–60)
Adeno 9	42 (25–90)	30 (15–70)

[a] Adeno, adenovirus.

Inhibition of DNA synthesis blocks the increase in adhesion. The A549 cells were infected with 2×10^8 PFU of adenovirus type 5. Two hours postinfection, the DNA synthesis inhibitor cytosine arabinofuranoside (50 μg/ml of medium) was added to infected and uninfected cells. The cells were incubated until microscopic examination showed cytopathic changes in 75 to 100% of the virus-infected control cells. At this time, the infected and drug-treated cells showed no cytopathic changes. The cells were harvested and used for adherence testing. There was no significant difference in adherence to uninfected control cells and drug-treated control cells (mean adherences, 74 and 75 bacteria per cell, respectively) (Table 4). The adherence to the infected cells increased significantly compared with that of the uninfected control cells (mean adherences, 142 and 74 bacteria per cell, respectively; $P < 0.001$). This effect was reversed by the drug. There was no difference in adherence to the virus-infected and drug-treated cells compared with adherence to the uninfected control (mean adherences, 73 and 74 bacteria per cell, respectively).

DISCUSSION

Viruses change the surface properties of infected cells. These changes may be caused by the expression of virus-encoded molecules on the cell surface or by the modification of endogenous cell surface structures. The virus-induced cell surface changes may, in turn, alter the interactions of the infected cell with adjacent stromal cells, with cells from the lymphoid system, with soluble mediators such as growth factors and cytokines, and with bacteria. Adenovirus infection has been shown to downregulate the expression of epidermal growth factor and tumor necrosis factor receptors as well as HLA class I antigens (17, 21, 34).

This study demonstrated that adenovirus infection increased the attachment of *S. pneumoniae* to a human respiratory tract cell line (A549). The increase in adherence was limited to pneumococcal strains with high adhesive capacities. Virus infection had no effect on poorly adhering pneumococcal strains. Representative adenoviruses that commonly cause respiratory tract infections (types 1, 2, 3, and 5) increased pneumococcal adherence, while adenovirus type 9, which is not usually associated with respiratory tract infection, did not. The

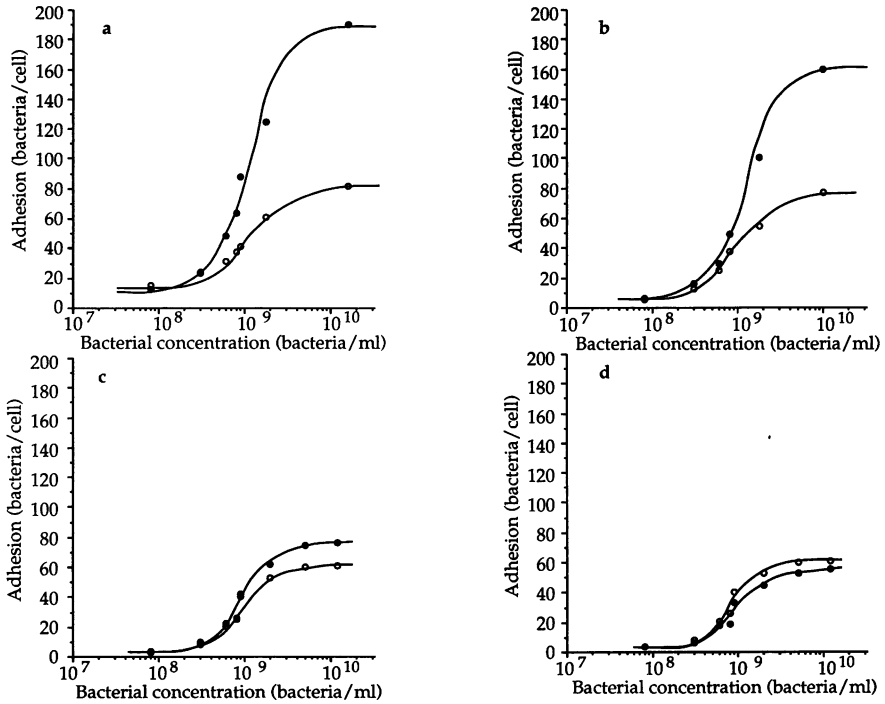

FIG. 3. Adherence with different concentrations of *S. pneumoniae* CCUG 10175 to uninfected A549 cells (○) and to A549 cells infected with different adenoviruses (●). The different adenovirus types used for infection were type 1 (a), type 3 (b), type 4 (c), and type 9 (d).

results suggested that adenovirus infections may increase the susceptibility of the respiratory tract mucosa to bacterial infection by modulating the expression of receptors for *S. pneumoniae*.

In this study, we selected the A549 cells as a model on the basis of their known permissiveness for adenovirus infection (30). Infection caused cytopathic effects in 75 to 100% of the cells after 48 h. The cytopathic effects occurred earlier when the virus was used at a higher MOI. The A549 cells were also found to be a useful model to study adherence of *S. pneumoniae*. For each pneumococcal isolate, the degree of attachment to the A549 cells was similar to the adherence to human nasopharyngeal epithelial cells. Thus, poorly adhering strains (<10 bacteria per cell) bound in low numbers to both cell types while highly adherent strains bound in greater numbers to both cell types. Adenovirus infection increased the binding of highly adherent *S. pneumoniae* strains but not of poorly adhering *S. pneumoniae*.

The A549 cells differed from the nasopharyngeal epithelial cells with regard to the adherence of *H. influenzae*. The encapsulated and fimbriated *H. influenzae* type b strain attached to human nasopharyngeal epithelial cells but not to the A549 cells. The nontypeable, highly adhering *H. influenzae* strains attached in similar numbers to the A549 cells and to human nasopharyngeal epithelial cells. The A549 cells therefore appeared appropriate for the study of the adhesins expressed by the nontypeable *H. influenzae* strains but not for studies of the fimbrial adhesin expressed by the encapsulated strains. Adenovirus infection had no effect on the adherence of encapsulated nontypeable *H. influenzae* strains. Other groups have studied the effects of influenza A virus and respiratory syncytial virus and did not find any change in the adherence of *H. influenzae* (4, 22, 23).

The abilities of adenoviruses to upregulate receptor expression differed by the adenovirus type. The classification of human adenoviruses, based on DNA homology (14), assigns types 1, 2, and 5 to the same subgroup (i.e., C). Adenoviruses of this subgroup had the strongest effect on the adherence of *S. pneumoniae* in this study. Adenovirus type 3 of subgroup B caused a similar increase in adherence. Adenovirus type 4 of subgroup E had little effect on the adherence, and type 9 in subgroup D had no effect.

The adenovirus types that commonly cause respiratory tract infections (types 1, 2, 3, 4, and 5) caused the highest increase

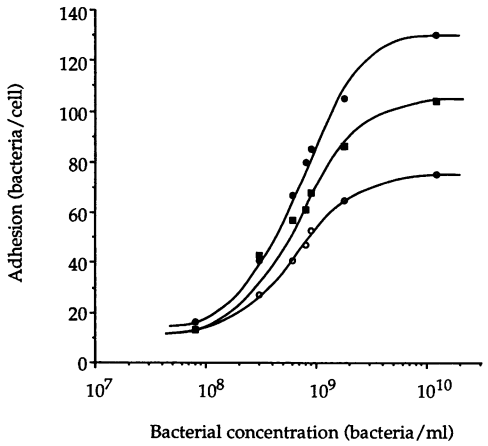

FIG. 4. Adherence with different concentrations of *S. pneumoniae* CCUG 10175 to uninfected A549 cells (○), A549 cells infected with a low dose of adenovirus type 5 (MOI, 25) (■), and A549 cells infected with a high dose of adenovirus type 5 (MOI, 75) (●).

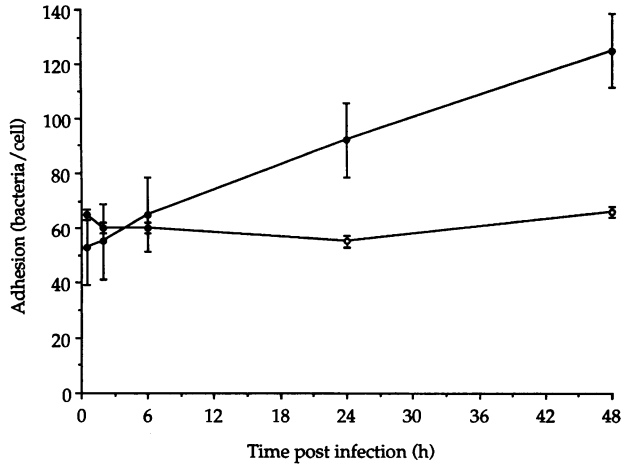

FIG. 5. Adherence of *S. pneumoniae* CCUG 10175 to A549 cells at different times postinfection with adenovirus type 5 (MOI, 75) (●) and to uninfected A549 cells (○).

in pneumococcal adherence. Adenovirus type 9 has not been shown to cause a defined infection or to have affinity for the respiratory tract (14). Adenovirus type 4 can cause acute respiratory disease in military recruits but is not as common in children with respiratory illness as types 1, 2, 3, and 5. Neither type 9 nor type 4 had a pronounced effect on adherence. The results suggested that the effects on adherence of the different adenovirus types may correlate with a tendency to cause respiratory tract infection, especially in children.

The effects of viruses on bacterial adherence have been studied by using influenza virus-infected MDCK cells as a model. Sanford et al. observed that group B streptococci bound to MDCK cells only after infection with influenza A virus (26). Group B streptococci were shown to bind directly to the influenza virus particles. Elbein et al. suggested that group B streptococci interacted with viral glycoproteins, most probably one of the two coat proteins hemagglutinin and neuraminidase (11). Kostyukova et al. observed an increased adherence of *N. meningitidis* to HEp-2 cells infected with influenza A virus (20). Electron microscopy showed binding of pili to the virus particles. Sanford et al. (26, 27) and Davison and Sanford (9, 10) showed increased adherence of group A streptococci and *S. aureus* to influenza virus-infected MDCK cells. Unlike group B streptococci, these bacteria did not interact directly with influenza virions.

In contrast to the influenza virus system, there was no evidence that pneumococci bound directly to adenovirus particles or to viral products outside the infected cells. There was no binding of *S. pneumoniae* 10175 to adenovirus type 5 or aggregation of viral and bacterial particles as determined by electron microscopy. Furthermore, pretreatment of bacteria with supernatants from virus-infected cells did not influence the adhesion. The increase in adherence seen after virus infection occurred at the same time as the cytopathic changes in the cell monolayer. We elected to examine this association by using cytosine arabinofuranoside, a drug that interferes with DNA replication. This cytosine analog inhibited the late phase of the infectious cycle and the cytopathic effects. There was no increase in adherence to virus-infected cells that had been treated with cytosine arabinofuranoside, suggesting that the cellular changes leading to increased adherence required DNA replication. The effect may have been mediated by one or more late gene products or by an early gene product accumulating late in the infectious cycle.

Adenoviruses are a common cause of respiratory tract infections, especially in children. The results of the present study suggest that adenovirus infection may change the interaction of the respiratory tract epithelium with *S. pneumoniae*. The in vivo relevance of this phenomenon remains to be investigated.

TABLE 4. Cytosine arabinofuranoside (CAF) inhibition of the adenovirus-induced increase in adherence of *S. pneumoniae* CCUG 10175 to A549 cells

Cell treatment	No. of adherent bacteria/cell [mean (range)]		*P*
	−[a]	+	
Medium	74 (55–90)	142 (100–220)	<0.001
CAF (50 μg/ml)	75 (60–100)	73 (50–90)	NS[b]

[a] −, uninfected cells; +, cells infected with adenovirus type 5.
[b] NS, not significant ($P = 0.83$).

ACKNOWLEDGMENTS

We gratefully acknowledge the contributions of Anette Cederberg, Karin Annersten, Ingrid Lindström, Monica Öberg, and Jarl Ekstrand. *H. influenzae* 770235f$^+$ and 770235f^0 were kindly provided by L. van Alphen.

This study was supported by grants from the Swedish Medical Research Council, the Swedish Agency for Research in Developing Countries, the Medical Faculty, University of Lund, the Royal Physiographical Society of Lund, and the Crawford and Österlund foundations.

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