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Model for Studying Bacterial Adherence to Epithelial Cells Infected with Viruses

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Measles infection decreased adherence of staphylococci, streptococci, and pneumococci to cultured epithelial cells, whereas adenovirus had no effect. Rhinovirus increased staphylococcal and streptococcal adherence. Influenza A increased or decreased staphylococcal adherence at different times after infection.

There is a well-recognized association between infection of the respiratory tract by viruses and subsequent bacterial colonization (7, 12) and infection (5, 12). For instance, bacterial pneumonias associated with measles or influenza pose serious and potentially fatal problems to the host (5, 12).

Many postulates for this relationship between viral and bacterial infections have been forwarded, including various defects in cellular immunity, macrophage function, and mucociliary transport (2, 6, 8–10). Since attachment of pathogenic bacteria to mucosal surfaces is the first step in many infections (14), it also seems possible that viral infection of epithelial cells may render them more susceptible to bacterial adherence. Indeed, Fainstein et al. have recently demonstrated increased adherence of some bacterial species to pharyngeal cells obtained from individuals with naturally acquired acute respiratory illness and from volunteers experimentally infected with influenza virus (3). Sanford et al., using a line of stable canine kidney cells, have recently demonstrated that influenza A infection can affect the adherence of some species of streptococci (16).

In most of our studies, we used Detroit 562 cells (CCL 138), a pharyngeal epithelial tissue culture cell line derived from a patient with nasopharyngeal carcinoma. The adherence of *Streptococcus pyogenes* to this cell line has been determined by Bartelt and Duncan to compare favorably with streptococcal adherence to suspensions of oral epithelial cells (1). We also used three HeLa cell lines. HeLa cell line 229 is the parental cell line used to produce the clonal HeLa cell line K-11, which is persistently infected with the Edmonston strain of measles virus (15). HeLa line K is a clonal line of HeLa cells selected for their increased susceptibility to human rhinoviruses. All cell lines were propagated in Eagle minimal essential medium supplemented with 10% heat-inactivated fetal bovine serum, penicillin (100 U/ml), streptomycin (100 μg/ml), and amphotericin B (5 μg/ml). Standard trypsinization procedures with the use of 0.01% Enzar T (Reheis) and 10^{-3} M ethylenediaminetetraacetic acid in calcium and magnesium-free phosphate-buffered saline were used to obtain cell suspensions for culture. Stock cells were maintained in plastic cell culture containers. Cultures for the viral infection and adherence assay were on 12-mm, circular, no. 1 coverslips in one-dram vials (Fischer) at a cell concentration of 100,000 cells per ml of medium.

Adenovirus type 2 and the Edmonston strain of measles virus stocks were prepared in HeLa 229 cell monolayers incubated at 35°C. Influenza A (NWS) virus stocks were prepared in embryonated hens' eggs, with the allantoic fluids being harvested after 48 h of incubation at 35°C. Rhinovirus type 1A stocks were prepared in HeLa line K cell monolayers incubated at 33°C. The fluids from infected cell cultures with moderate to marked cytopathic effects were pooled for each virus to obtain stock suspensions, which were stored at −70°C. Infectivity titers by established methods were as follows: adenovirus, $10^{5.0}$ 50% tissue culture doses per ml; influenza virus, $10^{7.5}$ plaque-forming units per ml (18); measles virus, $10^{6.0}$ plaque-forming units per ml (13); and rhinovirus, $10^{7.0}$ plaque-forming units per ml (4). Detroit 562 cells were infected with measles, influenza A, and adenovirus at a multiplicity of infection of 0.1. After 1 h of absorption at 25°C, the infected cell monolayers were washed twice in Hanks balanced salt solution, fed with 1 ml of 2% fetal bovine serum–98% minimal essential medium, and incubated at either 33 or 37°C until approximately 50% of the cells showed cytopathic effect. Infection was confirmed by the direct fluorescent antibody method (11) and by hemadsorption.

One strain each of *S. pyogenes*, *Staphylococcus aureus*, and *Streptococcus pneumoniae* was studied. The organisms were grown overnight in brain heart infusion broth, washed three times in phosphate-buffered saline, and passed

through a 25-gauge needle to disrupt chains and clumps. The suspensions were then adjusted to the following concentrations in phosphate-buffered saline, using a spectrophotometer: *S. pyogenes*, 2×10^7/ml; *S. pneumoniae*, 1×10^8/ml; and *S. aureus*, 3×10^8/ml (17).

When infection of the light monolayer of epithelial cells was evident by cytopathic affect, the cells were washed gently five times with phosphate-buffered saline. The adherence assay was performed with 0.5 ml of the bacterial suspension, which was incubated with the cells for 35 min at 37°C with periodic shaking. The unattached bacteria were removed by six washes with phosphate-buffered saline. The cells were then air dried, fixed with absolute methanol, and stained with Giemsa. Each cover slip with its adherent cells was then removed from the vial, mounted on a glass slide, coded, and examined microscopically. The number of bacteria adherent to 100 consecutive epithelial cells was counted, and the mean was calculated. Adherence studies were performed a minimum of nine times for each virus studied, with the studies being divided so that they were performed on at least two separate days after infection.

Infection of Detroit 562 cells with measles resulted in significantly decreased attachment of all three bacterial strains (Table 1). By using the fluorescent antibody technique to detect which cells were infected, it was determined that 50% of the uninfected Detroit 562 cells had adherent *S. pyogenes*, compared with only 5% of the cells in which both the cell membrane and the cytoplasm fluoresced. Adherence of all three bacterial strains to HeLa K-11 (infected) cells was significantly decreased compared with adherence to HeLa 229 (uninfected) cells, confirming the inhibitory effect of measles virus on bacterial adherence. Adherence of all three bacterial strains to adenovirus-infected Detroit 562 cells was decreased, but in no instance was this decrease statistically significant. HeLa line K cells infected with rhinovirus 1A demonstrated increased adherence of all three bacterial strains tested, the results with *S. pyogenes* and *S. aureus* being statistically significant. Adherence tests performed 3 to 7 days after influenza A inoculation of Detroit 562 cells revealed increased adherence of all three strains tested, the increased adherence of *S. aureus* being statistically significant. Adherence tests performed on day 9 post-inoculation, however, revealed decreased bacterial adherence involving all three strains, but only that with *S. aureus* was statistically significant (Fig. 1). These results could not be explained by a difference in cell density

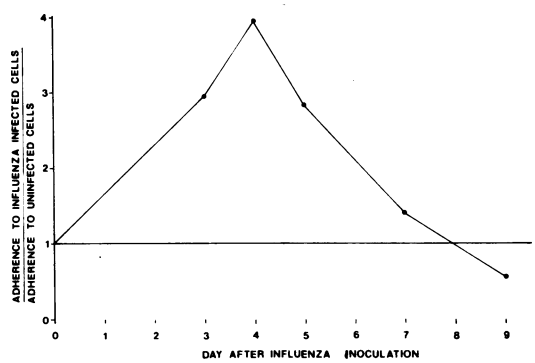

FIG. 1. *Effect of influenza infection of Detroit 562 cells on adherence of S. aureus. A time course study is shown. Infection initially resulted in increased adherence which subsequently decreased until adherence was inhibited.*

TABLE 1. *Effect of virus infection on adherence of bacteria to tissue culture cells*

Cell line	Virus	Day after infection	Mean adherence (bacteria per cell) ± standard error of the mean		
			Streptococcus[a]	*Staphylococcus*[a]	Pneumococcus[a]
Detroit 562	Uninfected	5 and 7	4.2 ± 0.3	2.1 ± 0.2	1.3 ± 0.4
	Measles		2.9 ± 0.1 ($p < 0.01$)	1.1 ± 0.1 ($p = 0.001$)	0.9 ± 0.1 ($p < 0.05$)
HeLa line 229	Uninfected	5 and 9	10.0 ± 1.0	7.1 ± 0.1	0.5 ± 0.1
HeLa K-11	Measles		5.7 ± 0.7 ($p < 0.01$)	2.2 ± 0.2 ($p < 0.001$)	0.2 ± 0.1 ($p < 0.02$)
Detroit 562	Uninfected	2 through 6 (daily)	2.0 ± 0.3	1.8 ± 0.1	0.3 ± 0.1
	Adenovirus		1.6 ± 0.2 (NS)	1.6 ± 0.1 (NS)	0.1 ± 0.03 (NS)
HeLa line K	Uninfected	5 and 9	3.8 ± 0.1	2.8 ± 0.5	0.6 ± 0.1
	Rhinovirus		8.5 ± 0.7 ($p < 0.0001$)	4.6 ± 0.4 ($p < 0.01$)	0.7 ± 0.1 (NS)
Detroit 562	Uninfected	3, 4, 5, and 7	3.1 ± 0.4	1.2 ± 0.3	1.0 ± 0.2
	Influenza A		3.8 ± 0.4 (NS)	2.3 ± 0.5 ($p < 0.005$)	1.1 ± 0.1 (NS)
Detroit 562	Uninfected	9	2.9 ± 0.2	3.0 ± 0.2	0.9 ± 0.2
	Influenza A		2.6 ± 0.2 (NS)	1.9 ± 0.2 ($p < 0.005$)	0.6 ± 0.1 (NS)

[a] The significance of difference, using Student's two-tailed *t*-test, is given within parentheses. NS, Not significant.

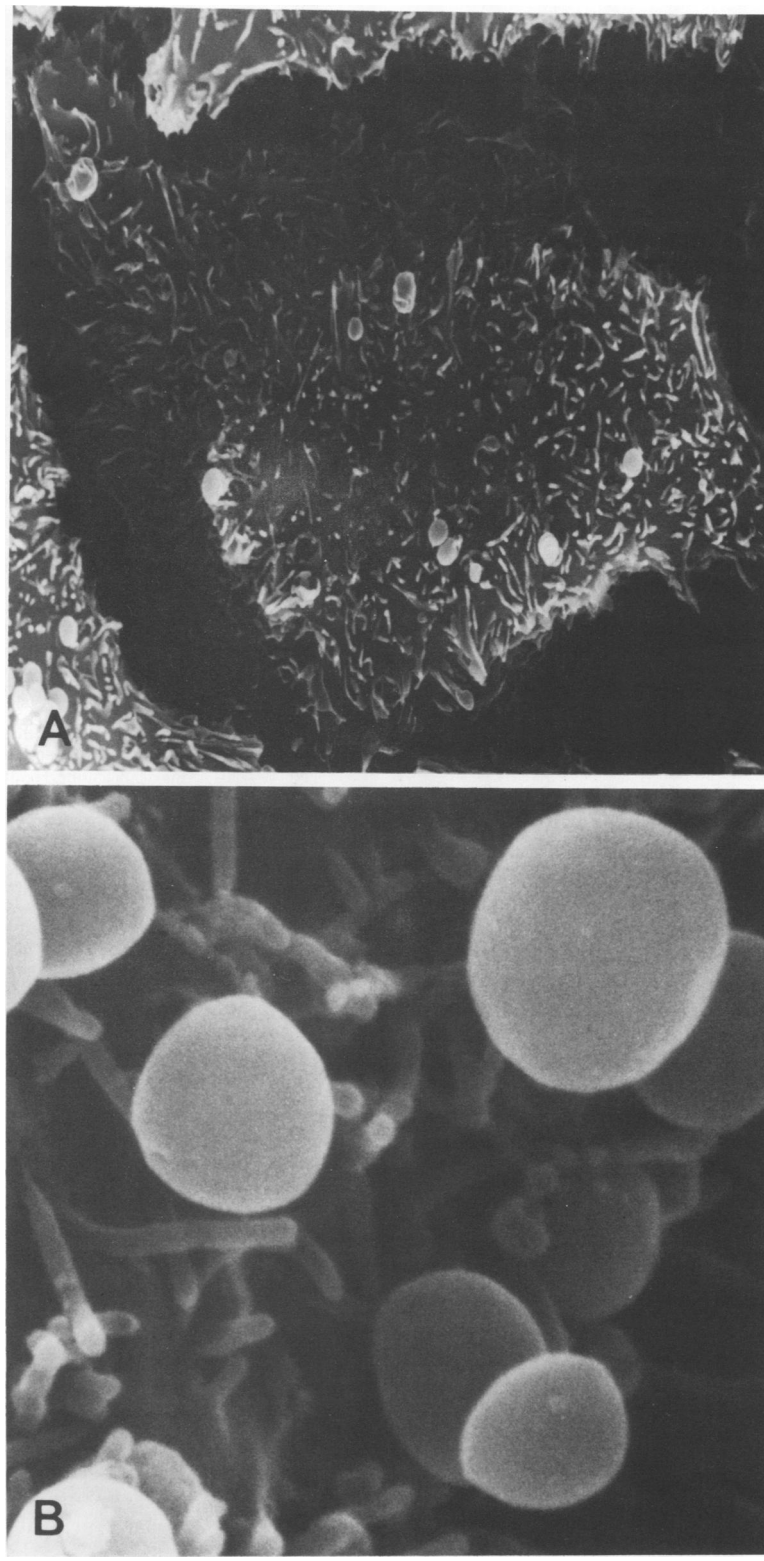

FIG. 2. *(A) Photomicrograph of Detroit 562 cell with adherent S. aureus (×3,000). (B) Surface of Detroit 562 cells. Organisms appear to be associated with microvilli on the cell surface (×30,000).*

or viability between control and infected cells on any day.

Cell surfaces of the Detroit 562 and HeLa K lines were studied by scanning electron microscopy. Both demonstrated microvilli projecting from the cell surface, the microvilli on the HeLa cell line being longer than on the Detroit 562 cells. Bacteria appeared to be associated with these microvilli and in many cases were clearly attached to them. No changes on the cell surface, as demonstrated by scanning electron microscopy, could be detected as a result of infection with any of the viruses (Fig. 2).

These studies do not indicate the nature of the cell surface receptor that is altered by viral infection. However, Bartelt and Duncan have shown that mild treatment of uninfected cells with trypsin reduces bacterial adherence, thus suggesting that a cell surface protein is the receptor for bacteria (1). Sanford and co-workers have reported that influenza A infection of MDCK cells promotes bacterial adherence which can be blocked with antiinfluenza antibody, thus indicating that viral proteins may influence adherence (16). The fact that in this study the effect of each viral infection was similar for all three strains of bacteria tested suggests that viral infections may induce generalized changes of the cell surface rather than only specific changes of isolated receptors. Alternatively, all three bacterial species may adhere to the same receptor on the epithelial cell surface.

It is intriguing to hypothesize that the increased bacterial adherence to cells infected with influenza A and rhinovirus may play a role in the pathogenesis of bacterial infections associated with diseases produced by these viruses.

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