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Tracheobronchial Mucin Receptor for *Pseudomonas aeruginosa*: Predominance of Amino Sugars in Binding Sites

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***Pseudomonas aeruginosa*, a common respiratory tract colonizer and pathogen, adheres to injured tracheal cells and to tracheobronchial mucin. These phenomena suggest that there are specific receptors for this organism in the respiratory tract. The receptor on injured tracheal cells contains *n*-acetylneuraminic acid as the principal sugar, but the structure of the receptor in mucin has not been described. Using a microtiter plate assay to study bacterial adherence to mucin, we have partially characterized the mucin receptor for *P. aeruginosa*. The receptor for both nonmucoid and mucoid strains is sensitive to periodate oxidation, suggesting that it is carbohydrate in nature, and the amino sugars *n*-acetylglucosamine and *n*-acetylneuraminic acid inhibited the adherence of both types of strains. Nonmucoid strains were more sensitive to inhibition by *n*-acetylneuraminic acid than to inhibition by *n*-acetylglucosamine, but the mucoid strains varied in their sensitivities to inhibition by each amino sugar. Preincubation of mucin with heat-inactivated influenza A virus (which binds to neuraminic acid) significantly reduced the adherence of *P. aeruginosa*. Treatment of mucin with *Clostridium perfringens* neuraminidase also reduced bacterial adherence significantly. Treatment of mucin with pronase did not affect adherence. Our results suggest that *n*-acetylglucosamine and *n*-acetylneuraminic acid are important constituents of the binding sites for *P. aeruginosa* on human tracheobronchial mucin.**

Mucus is thought to be a part of the respiratory host defense against invading microorganisms. It is presumably the first barrier that pathogens encounter in the tracheobronchial tree, and since it normally flows in a cephalad direction from the tracheobronchial tree due to ciliary movement, carrying particles, it may prevent organisms from reaching the lungs and establishing infection. Mucin, a glycoprotein constituent of mucus, is responsible for the rheological properties and the "stickiness" of mucus (14). Microorganisms may therefore be trapped in mucus because of this stickiness or because of a specific interaction between receptors in mucin and surface components (adhesins) of bacteria.

Using a microtiter plate assay, we showed that *Pseudomonas aeruginosa*, a bacterium that often chronically colonizes the respiratory tract in diseases such as cystic fibrosis and bronchiectasis in which tracheobronchial mucosal clearance is impaired, binds with greater affinity to human tracheobronchial mucin (HTBM) than do some members of the family *Enterobacteriaceae* (27). Others have shown that some diarrheagenic *Escherichia coli* strains bind well to colonic mucus (9), and that some oral bacteria bind to salivary mucins (7, 11). These findings, and the fact that different organisms colonize and infect different mucosal surfaces, suggest that specific interactions between certain bacteria and mucins in addition to those between bacteria and cells may determine the patterns of physiological and pathological colonization of mucosal surfaces. Such specific interactions with mucins may be similar to adhesin-receptor interactions that are responsible for bacterial adherence to epithelial cells (4).

In the studies described here, we present evidence that HTBM contains a receptor for *P. aeruginosa*, and that the receptor contains the aminosugars *n*-acetylglucosamine (GlcNAc) and *n*-acetylneuraminic acid (NANA).

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MATERIALS AND METHODS

Bacteria. *P. aeruginosa* R1, a nonmucoid sputum isolate, and *P. aeruginosa* M35, a mucoid isolate from the sputum of a patient with cystic fibrosis (donated by R. L. Boyd, University of Texas, San Antonio) were our representative "standard" strains. Our nonmucoid and mucoid strains have been described elsewhere (17, 20, 27). The bacteria were maintained on MacConkey agar (BBL Microbiology Systems, Cockeysville, Md.) at room temperature and subcultured every month. Bacteria grown overnight in Trypticase soy broth (BBL) to late log phase were pelleted by centrifugation at 10,000 × *g* for 15 min and washed three times with phosphate-buffered saline (PBS) (pH 7.0). They were then suspended in PBS and used in adherence assays.

Quantitation of adherence. Our method for studying adherence has been described elsewhere (27). Briefly, wells of a 96-well microtiter plate were siliconized and then coated with HTBM by leaving mucin solutions in the wells overnight. Excess HTBM was removed by washing the wells before the addition of bacteria. Bacterial suspensions (100 μl) of known concentration between 5×10^6 and 5×10^7 CFU/ml were added to wells, and the plates were incubated at 37°C for 30 min. The wells were washed 15 times with PBS to remove unbound bacteria, and bacteria adherent to wells were desorbed by adding a 0.5% solution of Triton X-100 in PBS and quantitated by plating a known dilution of this solution on MacConkey agar plates. A set of siliconized wells served as a control for nonspecific "background" binding in each experiment. Only the results of experiments with little or no background binding were considered valid. All experiments were done at least three times.

Sodium metaperiodate treatment of HTBM. Sodium metaperiodate (Sigma Chemical Co., St. Louis, Mo.) was added to a solution of HTBM (2 μg of mucin protein per ml) to yield a concentration of 100 mM and kept in the dark at room temperature for 4 h to allow oxidation to take place. The reaction was stopped by exposing the solution to light. The

solution was then diluted to yield a concentration of 1 μg of mucin protein per ml. Triplicate sets of wells were coated with equal concentrations of either HTBM or periodate-treated HTBM and washed, and adherence of the standard *P. aeruginosa* strains to the coated wells was tested. To show that periodate-treated mucin did bind to the wells, we coated wells separately with ^{125}I-labeled mucin and ^{125}I-labeled periodate-treated mucin, washed them, and measured the amounts of radioactivity bound to each well. We found that periodate did not affect the binding of the radiolabeled mucin.

Effect of monosaccharides and amino sugars on adherence. Six sugars were used in these studies. Of these, L-(−)-fucose, D-(+)-galactose, *n*-acetylgalactosamine (GalNAc), GlcNac, and NANA are the sugars found in HTBM, whereas D-(+)-mannose is not a constituent of mucin (21). All were purchased from Sigma.

The bacterial suspensions were prepared in PBS. Equal volumes of the suspensions were mixed with either PBS or 200 mM sugar solutions in PBS to prepare solutions containing equal concentrations of bacteria in either PBS or 100 mM sugar solutions. Solutions of NANA prepared in PBS had a pH of ca. 2.0 which was lethal for the bacteria. Therefore, bacterial inocula were prepared in solutions of NANA dissolved in Tris-hydrochloride (Sigma) and buffered to a pH of 6.9, which ensured the viability of the bacteria. (When tested, bacterial adherence was the same whether bacterial suspensions were made in PBS or Tris-hydrochloride, and thus Tris-hydrochloride alone had no effect on adherence). Samples (100 μl) of equal concentrations of bacteria in PBS or 100 mM sugar solutions were added to triplicate sets of mucin-coated wells, and bacterial adherence to each set was determined. We also examined the effect of 1 and 10 mM GlcNAc and 1 and 10 mM NANA in similar studies, since these sugars markedly inhibited adherence when used at 100 mM concentrations.

To determine that the sugars inhibited adherence by binding to bacterial adhesins rather than to mucin receptors, bacteria were preincubated in PBS or 100 mM sugar solutions for 60 min, pelleted by centrifugation, washed three times, and resuspended in PBS to desired concentrations for use in adherence assays.

To determine whether the sugars inhibited adherence by binding to the receptors in HTBM rather than to the adhesins of the bacteria, mucin-coated wells were preincubated with 100 mM sugar solutions and the sugars were washed off before bacteria were added in adherence assays.

Effect of lectins on adherence. All lectins were purchased from Sigma. Lectins from *Dolichos biflorus* (horse gram) which binds to GalNAc, *Triticum vulgaris* (wheat germ) which binds to GlcNAc, and *Ulex uropeus* (gorse) which binds to L-fucose were used in these studies. A lectin binding specifically to NANA alone was unavailable. Since galactose did not markedly inhibit adherence, and the lectin that binds to galactose (from *Ricinus communis*, castor bean) is extremely toxic, we tested only the three lectins mentioned. The lectins were used in assays similar to those described for the sugars. Lectin solutions (500 μg/ml) were added to mucin-coated wells and washed off before adherence was tested in one group of experiments, and were mixed with the bacteria and added to the wells in other studies of competitive inhibition.

Effect of heat-inactivated influenza virus. Heat-inactivated influenza virus A/Port Chalmers/73 (H_3N_2) was donated by Parker Small, University of Florida, Gainesville. Suspensions containing 1,000 or 10,000 hemagglutinating units of virus in PBS were added to mucin-coated wells for 30 min. The wells were then washed, and adherence of the standard strains was assayed. A set of mucin-coated wells not exposed to the virus served as a control. We confirmed that the heat-inactivated virus did not release sialic acid from HTBM as tested by the thiobarbituric acid assay (28).

Neuraminidase treatment of mucin. *Clostridium perfringens* neuraminidase type V (Sigma) was used to treat mucin in a series of experiments. HTBM was treated with neuraminidase (2 U of enzyme per μg of mucin protein) for 1 h at 37°C in 50 mM sodium acetate solution (pH 4.9). The reaction was stopped by readjustment of the pH to 7.0. Wells were coated with equal concentrations of untreated and neuraminidase-treated HTBM. Adherence assays were done as described above.

Protease treatment of mucin. The naked peptide region of HTBM is more extensively degraded by pronase than by other proteases (8, 22). Wells were coated with HTBM treated with pronase at 0.2 and 1.0 U of pronase (Calbiochem-Behring, San Diego, Calif.) per μg of mucin in 2 mM calcium acetate buffer at 37°C for 24 h. HTBM in 2 mM calcium acetate buffer alone was used to coat control wells. The calcium acetate buffer itself had no effect on adherence of *P. aeruginosa*. Adherence assays were performed as in other experiments.

Statistical analysis. From the colony counts, the mean number of bacteria adherent per well ± one standard deviation was calculated. A one-tailed Student's *t* test was used to analyze the significance of differences between control and test results.

RESULTS

Effect of periodate treatment of HTBM on adherence. Sodium metaperiodate oxidizes vicinal hydroxyl groups on carbohydrates. HTBM is structurally comprised of oligosaccharide chains surrounding a polypeptide core (14). The oligosaccharide chains are therefore more exposed than the peptide core and thus are more likely to contain the receptors for bacteria. Periodate treatment of HTBM abolished the adherence of the mucoid *P. aeruginosa* M35 and reduced the adherence of the nonmucoid *P. aeruginosa* R1 by 93% (Table 1), suggesting that the binding sites for these bacteria were in the carbohydrate portion of the HTBM molecule.

Effect of sugars on adherence. Since bacterial adherence to HTBM was reduced by periodate treatment, it seemed that the sugars present in the oligosaccharide chains were likely candidates as the receptor. The five sugars found in HTBM and D-(+)-mannose, a sugar not found in HTBM, were used in competitive inhibition experiments. D-(+)-mannose had no effect on adherence (data not shown). Of the sugars found in HTBM, 100 mM solutions of GlcNAc and NANA reduced adherence more markedly than the same concentrations of

TABLE 1. Effect of periodate treatment of HTBM on the adherence of *P. aeruginosa* to HTBM

Strain	No. of adherent bacteria per well (mean ± SD)		% Reduction	P^a
	Control	Treated HTBM		
P. aeruginosa R1[b]	2,267 ± 356	167 ± 121	93	<0.001
P. aeruginosa M35[c]	2,867 ± 446	0	100	<0.001

[a] Determined by Student's *t* test.
[b] Nonmucoid strain.
[c] Mucoid strain.

TABLE 2. Effect of sugars[a] on adherence of *P. aeruginosa* to HTBM

Sugar	Mean % inhibition of adherence compared with control (no. of experiments)	
	P. aeruginosa R1[b]	*P. aeruginosa* M35[c]
Fucose	16 (3)	33 (3)
Galactose	23 (4)	24 (3)
GalNAc	38 (4)	29 (3)
GlcNAc	68 (4)	79 (3)
NANA	83 (4)	77 (3)

[a] All sugar solutions were 100 mM.
[b] Nonmucoid strain.
[c] Mucoid strain.

L-(−)-fucose, D-(+)-galactose, and GalNAc (Table 2). Both NANA and GlcNAc inhibited adherence when bacteria were preincubated in 100 mM solutions of these sugars, pelleted, washed, and tested in adherence assays (Table 3). This provided evidence that the sugars inhibited adherence by binding to the bacterial adhesins rather than to the receptors in HTBM. To further confirm that these two amino sugars inhibited adherence by reacting with the bacterial adhesins rather than by reacting with the receptor in HTBM, we preincubated mucin-coated wells with 100 mM solutions of these sugars, washed the solutions off, and then tested adherence. There was no inhibition of adherence in these experiments (data not shown), again suggesting that the amino sugars inhibited adherence by reacting with the bacterial adhesins rather than with the receptor in mucin.

Since GlcNAc and NANA inhibited adherence of *P. aeruginosa* R1 and M35 to HTBM, we studied the effects of these two amino sugars on the adherence of two other nonmucoid and two other mucoid *P. aeruginosa* strains. Adherence of all strains was significantly inhibited by 100 mM solutions of both GlcNAc and NANA (Table 4). At lower sugar concentrations, these two sugars varied in their ability to inhibit the adherence of different strains. Whereas 10 mM NANA significantly inhibited the adherence of all the nonmucoid strains, 10 mM GlcNAc reduced the adherence of only one of these strains, and even for this strain the reduction was minimal. On the other hand, the mucoid strains varied in their sensitivity to inhibition by 10 mM concentrations of these sugars. *P. aeruginosa* strain M35 was sensitive only to GlcNAc, strain 2192 was sensitive only to NANA, and strain 258 was not sensitive to 10 mM concentrations of either sugar. Neither sugar inhibited adherence at 1 mM.

Effect of lectins on adherence. To ascertain which sugars were contained on the receptors in HTBM, we preincubated mucin-coated wells with lectins or added lectins with bacteria, and studied adherence. If a particular sugar constituted an important portion of the binding site in the receptor on HTBM for *P. aeruginosa*, the lectin specific for that sugar ought to reduce adherence. None of the three lectins used at concentrations of 500 μg/ml consistently inhibited or enhanced adherence (data not shown). However, effects of lectin solutions at higher concentrations could not be studied since such solutions could not be prepared.

Effect of heat-inactivated influenza A virus on adherence. Since our studies with sugars indicated that NANA was an important sugar in the HTBM receptor for *P. aeruginosa*, then influenza A virus that binds to NANA (26) ought to reduce the adherence of *P. aeruginosa* to HTBM if the mucin was first exposed to this virus. The virus was heat inactivated to ensure the destruction of viral neuraminidase, which otherwise could have modified HTBM by cleaving off NANA. Preincubation of mucin-coated wells with influenza A virus markedly reduced the adherence of both nonmucoid and mucoid strains of *P. aeruginosa* in a dose-dependent fashion (Table 5), adding further support to our evidence for the presence of NANA as an important sugar in the binding site on HTBM for *P. aeruginosa*.

Effect of neuraminidase and pronase treatment on adherence to HTBM. Lastly, to add more support to the evidence for NANA as part of the binding site on HTBM, we tested the adherence of *P. aeruginosa* to neuraminidase-treated HTBM. Adherence of both the nonmucoid and mucoid *P.*

TABLE 3. Adherence of sugar-treated *P. aeruginosa* to HTBM[a]

Strain	Mean % inhibition of adherence	
	GlcNAc	NANA
R1[b]	42 (3)[c]	44 (2)
M35[d]	40 (3)	56 (3)

[a] Bacteria were preincubated in 100 mM sugar solutions, pelleted, washed, and suspended in PBS, then used in adherence assays.
[b] Nonmucoid strain.
[c] Parentheses indicate the number of experiments done (each in triplicate).
[d] Mucoid strain.

TABLE 4. Effect of NANA and GlcNAc on adherence of *P. aeruginosa* to HTBM[a]

Strain	Sugar	% Reduction of control[b]	
		10 mM sugar	100 mM sugar
Nonmucoid			
R1	NANA	62 ($P < 0.001$)	86 ($P < 0.001$)
	GlcNAc	3 (NS)	54 ($P < 0.001$)
NM2192	NANA	46 ($P < 0.05$)	77 ($P < 0.001$)
	GlcNAc	20 (NS)	80 ($P < 0.001$)
T2A	NANA	61 ($P < 0.001$)	77 ($P < 0.001$)
	GlcNAc	25 ($P < 0.001$)	55 ($P < 0.001$)
Mucoid			
M35	NANA	13 (NS)	97 ($P < 0.02$)
	GlcNAc	98 ($P < 0.01$)	99 ($P < 0.01$)
2192	NANA	71 ($P < 0.01$)	84 ($P < 0.001$)
	GlcNAc	4 (NS)	89 ($P < 0.001$)
258	NANA	0	84 ($P < 0.001$)
	GlcNAc	0	41 ($P < 0.001$)

[a] Results of one representative experiment.
[b] *P* values were determined by Student's *t* test from raw data (not shown). NS, Not statistically significant.

TABLE 5. Effect of heat-inactivated influenza A virus on adherence of *P. aeruginosa* to HTBM

Strain	Dose of virus[a]	No. of adherent bacteria per well (mean ± SD)	% Reduction	P[b]
P. aeruginosa R1[c]	0	15,067 ± 4,180		
	1,000	4,850 ± 2,151	68	<0.001
	10,000	1,650 ± 957	89	<0.001
P. aeruginosa M35[d]	0	36,933 ± 759		
	1,000	13,660 ± 10,773	63	<0.02
	10,000	500 ± 141	99	<0.001

[a] Hemagglutinating units added per well.
[b] Determined by Student's *t* test.
[c] Nonmucoid strain.
[d] Mucoid strain.

TABLE 6. Effect of neuraminidase treatment of HTBM on adherence of *P. aeruginosa*

Strain	No. of adherent bacteria per well (mean ± SD)		% Reduction	P^a
	Control	Treated HTBM		
P. aeruginosa R1[b]	45,150 ± 2,462	13,250 ± 547	71	<0.001
P. aeruginosa M35[c]	49,600 ± 12,866	15,317 ± 4,547	69	<0.001

[a] Determined by Student's *t* test.
[b] Nonmucoid strain.
[c] Mucoid strain.

aeruginosa strains to HTBM was reduced significantly by treating HTBM with neuraminidase (Table 6), thus confirming the importance of NANA in the binding site on HTBM. We should note that the neuraminidase preparation contained trace amounts of protease activity. However, this protease has not been characterized. To rule out an effect of protease, we tested the effects of pronase on adherence. Adherence of both the nonmucoid and mucoid *P. aeruginosa* was unaffected by treatment of HTBM with pronase (data not shown).

DISCUSSION

These results provide evidence for the existence of a receptor on HTBM for both mucoid and nonmucoid *P. aeruginosa*.

Sodium metaperiodate which oxidizes adjacent hydroxyl groups on carbohydrates reduced the adherence of nonmucoid *P. aeruginosa* and abolished that of mucoid *P. aeruginosa*. These findings suggested that carbohydrates were essential components of the binding sites on the receptors in HTBM for *P. aeruginosa*. In competitive inhibition experiments with sugars, D-(+)-mannose, a sugar not found in HTBM, had no effect on adherence. Of the five sugars found in HTBM, only the amino sugars GlcNAc and NANA were able to markedly inhibit adherence. Preincubating bacteria in sugar solutions first inhibited adherence, whereas treating mucin-coated wells first with sugars did not, suggesting that the sugars inhibited adherence in competitive inhibition studies by interacting with the adhesins on the bacteria and not with the receptors on HTBM. The degree of inhibition seen in experiments in which bacteria were pretreated with sugars was less than that seen in competitive inhibition assays. We are uncertain about the reason for these differences. It is possible that the binding of individual sugars to bacterial adhesins may not be as "tight" as that of a complex oligosaccharide receptor and thus a sugar may be washed off easily. We were unable to inhibit adherence with lectins that bind to L-fucose, GalNAc, and GlcNAc. However, concentrations of 10 to 100 mM GlcNAc were required to inhibit adherence of *P. aeruginosa* in our sugar inhibition studies, and since one molecule of the lectin from wheat germ agglutinin binds to one molecule of GlcNAc (10), it would theoretically require a 10 to 100 mM solution of lectin from wheat germ agglutinin to inhibit adherence. However, the maximum lectin concentration that could be prepared was 20 μM (500 μg/ml). Therefore, our inability to inhibit adherence with this concentration of the lectin does not contradict the evidence provided by sugar-inhibition studies for the role of GlcNAc as a component of the binding site. In addition, lectins are known to be multivalent (3), and their subunits also form aggregates (13). Our results, therefore, could also be seen if the lectin were to bind to the sugar in the mucin, on one hand, and to a sugar on the cell surface of the bacteria on the other. Since no purified lectin specific for NANA alone was available, we tested the ability of heat-inactivated influenza virus to inhibit adherence because this virus binds to NANA (26). The dose-dependent inhibition of adherence of both nonmucoid and mucoid *P. aeruginosa* by this virus added further evidence for the presence of NANA in the binding site on HTBM. In addition, treatment of HTBM with neuraminidase also reduced the adherence of both bacterial strains, again strengthening the evidence for the presence of NANA in the binding site.

Our data suggest that the binding sites on HTBM for both nonmucoid and mucoid *P. aeruginosa* contain GlcNAc and NANA. Earlier studies from this laboratory showed that NANA is part of the receptor on injured murine tracheal cells for nonmucoid and mucoid *P. aeruginosa* (19). A more recent report presented evidence that free pili inhibited the binding of nonmucoid strains to injured tracheal cells (20), but not the mucoid strains, suggesting that the receptor sites for nonmucoid and mucoid strains on cells may differ somewhat, although both types of receptors may contain NANA. In the present study, the nonmucoid strains were uniformly more sensitive to NANA than to GlcNAc, suggesting a common receptor in which either NANA is a more important sugar in the binding site or NANA is more accessible. In addition, there were differences not only between the nonmucoid and mucoid strains, but also among the mucoid strains themselves. The mucoid exopolysaccharide, comprised of mannuronic and guluronic acids (6), is probably the adhesin that mediates binding of mucoid strains to injured mouse tracheal cells (18). The mucoid exopolysaccharide of two of the mucoid strains used, strains 258 and 2192, differs in the ratios of the two uronic acids (17), and the structure of the exopolysaccharide from strain M35 is unknown. If the exopolysaccharide is also the adhesin that binds to HTBM, then the differences in its composition between strains may explain the differences in sensitivities to the amino sugars used.

It is of interest teleologically that NANA is involved in adherence in this system since it is involved in the adherence of other respiratory pathogens. *Mycoplasma pneumoniae* (24) and, presumably, influenza viruses bind to NANA on tracheal cells (26). In addition, *P. aeruginosa* binds to NANA on injured murine tracheal cells (19). It is probably not coincidental that these three organisms are tracheobronchial pathogens.

Our studies also suggest that GlcNAc is contained in the HTBM receptor for *P. aeruginosa*. This amino sugar has been implicated as a binding site on HeLa cells (5), L cells (12), and McCoy cells (25) for some strains of the genus *Chlamydia*, and on pharyngeal cells for *Streptococcus pneumoniae* (1). Although both GlcNAc and NANA may comprise the binding sites on the receptor in HTBM for *P. aeruginosa* as part of a complex oligosaccharide, it is possible that a configurational similarity between these two sugars (15) is the reason for these observations. Studies with oligosaccharide chains of known structure prepared from mucin should provide more information on these relationships. Since lipids are also found in mucus, the role of glycolipids in adherence needs to be examined because they also possess oligosaccharide side chains.

The findings reported here support the existence of specific receptor-adhesin interactions between HTBM and *P. aeruginosa*. Studying these interactions in depth will increase the understanding of the mechanisms of chronic

colonization of the respiratory tract by *P. aeruginosa*, and may also increase knowledge about tissue tropisms of pathogens. In addition, characterizing the receptor and adhesin may permit the development of new methods to control or prevent chronic colonization, e.g., by immunizing against bacterial adhesins (16, 23), or using receptor analogs (2). These considerations are obviously important in diseases in which impaired clearance of mucus is associated with colonization of the respiratory tract by *P. aeruginosa*, the most striking example of such diseases being cystic fibrosis.

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LITERATURE CITED

1. **Andersson, B., J. Dahmén, T. Frejd, H. Leffler, G. Magnusson, G. Noori, and C. S. Edén.** 1983. Identification of an active disaccharide unit of a glycoconjugate receptor for pneumococci attaching to human pharyngeal epithelial cells. J. Exp. Med. **158**:559–570.
2. **Aronson, M., O. Medalia, L. Schori, D. Mirelman, N. Sharon, and I. Ofek.** 1979. Prevention of colonization of the urinary tract of mice with *Escherichia coli* by blocking of bacterial adherence with methyl α D-mannopyranoside. J. Infect. Dis. **139**:329–332.
3. **Barondes, S. H.** 1981. Lectins: their multiple endogenous cellular functions. Annu. Rev. Biochem. **50**:207–231.
4. **Beachey, E. H.** 1981. Bacterial adherence: adhesin-receptor interactions mediating the attachment of bacteria to mucosal surfaces. J. Infect. Dis. **143**:325–345.
5. **Bose, S. K., G. B. Smith, and R. G. Paul.** 1983. Influence of lectins, hexoses, and neuraminidase on the association of purified elementary bodies of *Chlamydia trachomatis* UW-31 with HeLa cells. Infect. Immun. **40**:1060–1067.
6. **Carlson, D. M., and L. W. Matthews.** 1966. Polyuronic acids produced by *Pseudomonas aeruginosa*. Biochemistry **5**:2817–2822.
7. **Gibbons, R. J., and J. van Houte.** 1980. Bacterial adherence and the formation of dental plaques, p. 61–104. *In* E. H. Beachey (ed.), Bacterial adherence. Receptors and recognition, series B, vol. 6. Chapman and Hall, London.
8. **Havez, R., P. Roussel, P. Degand, and G. Biserte.** 1967. Étude des structures fibrillaires de la sécrétion bronchique humaine. Clin. Chim. Acta **17**:281–295.
9. **Laux, D. C., E. F. McSweegan, and P. S. Cohen.** 1984. Adhesion of enterotoxigenic *Escherichia coli* to immobilized intestinal mucosal preparations: a model for adhesion to mucosal surface components. J. Microbiol. Methods **2**:27–39.
10. **LeVine, D., M. J. Kaplan, and P. J. Greenaway.** 1972. The purification and characterization of wheat-germ agglutinin. Biochem. J. **129**:847–856.
11. **Levine, M. J., M. C. Herzberg, M. S. Levine, S. A. Ellison, M. W. Stinson, H. C. Li, and T. Van Dyke.** 1978. Specificity of salivary-bacterial interactions: role of terminal sialic acid residues in the interaction of salivary glycoproteins with *Streptococcus sanguis* and *Streptococcus mutans*. Infect. Immun. **19**:107–115.
12. **Levy, N. J.** 1979. Wheat germ agglutinin blockage of chlamydial attachment sites: antagonism by *N*-acetyl-D-glucosamine. Infect. Immun. **25**:946–953.
13. **Lis, H., and N. Sharon.** 1973. The biochemistry of plant lectins (phytohemagglutinins). Annu. Rev. Biochem. **42**:541–574.
14. **Lopez-Vidriero, M. T.** 1981. Airway mucus. Production and composition. Chest **80**(Suppl.):799–804.
15. **Monsigny, M., A.-C. Roche, C. Sene, R. Maget-Dana, and F. Delmotte.** 1980. Sugar-lectin interactions: how does wheat-germ agglutinin bind sialoglycoconjugates? Eur. J. Biochem. **104**:147–153.
16. **Nagy, B., H. W. Moon, R. E. Isaacson, C.-C. To, and C. C. Brinton.** 1978. Immunization of suckling pigs against enteric enterotoxigenic *Escherichia coli* infection by vaccinating dams with purified pili. Infect. Immun. **21**:269–274.
17. **Pier, G. B., W. J. Matthews, Jr., and D. D. Eardley.** 1983. Immunochemical characterization of the mucoid exopolysaccharide of *Pseudomonas aeruginosa*. J. Infect. Dis. **147**:494–503.
18. **Ramphal, R., and G. B. Pier.** 1985. Role of *Pseudomonas aeruginosa* mucoid exopolysaccharide in adherence to tracheal cells. Infect. Immun. **47**:1–4.
19. **Ramphal, R., and M. Pyle.** 1983. Evidence for mucins and sialic acid as receptors for *Pseudomonas aeruginosa* in the lower respiratory tract. Infect. Immun. **41**:339–344.
20. **Ramphal, R., J. C. Sadoff, M. Pyle, and J. D. Silipigni.** 1984. Role of pili in the adherence of *Pseudomonas aeruginosa* to injured tracheal epithelium. Infect. Immun. **44**:38–40.
21. **Roberts, G. P.** 1974. Isolation and characterisation of glycoproteins from sputum. Eur. J. Biochem. **50**:265–280.
22. **Roberts, G. P.** 1976. The role of disulfide bonds in maintaining the gel structure of bronchial mucus. Arch. Biochem. Biophys. **173**:528–537.
23. **Rutter, J. M., and G. W. Jones.** 1973. Protection against enteric disease caused by *Escherichia coli*—a model for vaccination with a virulence determinant? Nature (London) **242**:531–532.
24. **Sobeslavsky, O., B. Prescott, and R. M. Chanock.** 1968. Adsorption of *Mycoplasma pneumoniae* to neuraminic acid receptors of various cells and possible role in virulence. J. Bacteriol. **96**:695–705.
25. **Söderlund, G., and E. Kihlström.** 1983. Attachment and internalization of a *Chlamydia trachomatis* lymphogranuloma venereum strain by McCoy cells: kinetics of infectivity and effect of lectins and carbohydrates. Infect. Immun. **42**:930–935.
26. **Sweet, C., and H. Smith.** 1980. Pathogenicity of influenza virus. Microbiol. Rev. **44**:303–330.
27. **Vishwanath, S., and R. Ramphal.** 1984. Adherence of *Pseudomonas aeruginosa* to human tracheobronchial mucin. Infect. Immun. **45**:197–202.
28. **Warren, L.** 1959. The thiobarbituric acid assay of sialic acids. J. Biol. Chem. **234**:1971–1975.