RECEPTOR ANALOGS AND MONOCLONAL ANTIBODIES
THAT INHIBIT ADHERENCE OF *BORDETELLA PERTUSSIS*
TO HUMAN CILIATED RESPIRATORY EPITHELIAL CELLS

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Whooping cough is the prototype of diseases of the respiratory tract in which adherence plays a central role in the pathogenesis of infection. The bacteria, *Bordetella pertussis*, are noninvasive, remaining localized to the cilia of the respiratory mucosal surface throughout the 4–6 wk of disease. Such prolonged colonization restricted topographically to a single host cell type suggests a highly specific bacterial-host recognition system(s). Two secreted proteins of *B. pertussis* have been identified as adhesins for human ciliated cells: the filamentous hemagglutinin (FHA)[1] and pertussis toxin (PT) (1). Both bacterial surface-associated and cell-free adhesins have been shown to associate specifically with ciliated cells and promote the adherence of *B. pertussis* (1). *Streptococcus pneumoniae, Staphylococcus aureus*, and *Haemophilus influenzae* (2) also appear to be able to bind the *B. pertussis* adhesins to their surfaces, indicating that the interaction between bacterial surface and adhesin is not highly specific. Thus, the interaction that confers the species and topographical stringency to the natural disease must be inherent in the interaction between the adhesins and host cell receptor. Since the disease is virtually 100% contagious (3), the receptor must be a common feature of the highly differentiated human ciliary membrane. Early studies have suggested that the receptor is carbohydrate in nature by virtue of the staining characteristics of material spanning the space between ciliary and bacterial membranes and the sensitivity of adherence to treatment of ciliated cells with periodate (4). This study characterizes the chemical nature of receptors for *B. pertussis* adhesins. mAbs recognizing this receptor were then shown to interrupt adherence to human ciliated

[1] *Abbreviations used in this paper:* C/M, chloroform/methanol; CHO, Chinese hamster ovary; FHA, filamentous hemagglutinin; PT, pertussis toxin.

cells. This is the first demonstration of the potential contribution of anti-receptor antibodies to the therapy and prevention of respiratory disease.

Materials and Methods

Bacterial Strains and Cultivation Conditions.   Virulent strains Br (clinical isolate [4]) and BP338 (derivative of Tohama I [5]) were grown on Bordet-Gengou agar (lot 689592; Difco Laboratories Inc., Detroit, MI) containing 15% sheep blood and 1% glycerol for 48 h, were transferred to Stainer Scholte medium for 48 h, harvested into Medium 199S (M. A. Bioproducts, Walkersville, MD), and adjusted to a concentration of 5×10^9 *B. pertussis*/ml (1, 4). Then, 0.5 ml of the bacterial suspension was aliquoted into tubes containing one of the following as listed in Tables I and II: simple carbohydrates (Sigma Chemical Co., St. Louis, MO) at between 10^{-7} and 10^{-2} M, complex carbohydrates (Calbiochem-Behring Corp., La Jolla, CA) at between 0.005 and 2.5 mg/ml, mAbs (purified ascites fluid, C. A. Schoenenberger, Universitat Basel, Basel, Switzerland) at between 0.03 and 160 µg protein/ml, and lectins (Sigma Chemical Co.) at 250 µg/ml. Control organisms received no additions. All mixtures were incubated for 30 min in a stationary water bath at 37°C, passed three times through a 21-gauge needle, and then exposed to ciliated cells for the adherence assay.

Radiolabeled *B. pertussis* strain BP338 was prepared by transfer from Bordet Gengou agar to Stainer Scholte medium containing ^{35}S methionine (0.07 Ci/ml, New England Nuclear, Boston, MA) for 48 h.

Adherence Assay.   The assay was performed as described previously using 0.5 ml of a suspension of human ciliated epithelial cells in Medium 199S (5×10^5 cells/ml) for each test condition (4). Respiratory epithelial cells were obtained by the brushing of apparently normal human trachea during bronchoscopy. For some assays, ciliated cells were pre-incubated for 30 min at 37°C with one of the above-mentioned potential inhibitors of adherence (carbohydrates, antibodies, or lectins) or with *Clostridium perfringens* neuraminidase (200 µg/ml; Sigma Chemical Co.). This treatment was followed by washing (250 g at 4°C), resuspension in 0.5 ml of Medium 199S, and finally, exposure to the bacteria. For all assays, organisms and ciliated cells were incubated together for 3 h at 37°C on a tumbler. Ciliated cells were then washed free of nonadherent bacteria over a 3-µm polycarbonate membrane filter (Nuclepore Corp., Pleasanton, CA), spread on a slide, and dried. Adherent bacteria were stained with a 1:40 dilution of bacterial fluorescent antibody (Difco Laboratories, Inc.) by a direct-antibody technique. Slides were read in a coded fashion by one of us (E. Tuomanen) (4). Results were expressed as the mean number of bacteria adherent to cilia on 25 cells.

Detection of Antibody or Adhesin Binding to B. pertussis and to Ciliated Cells.   For these assays, suspensions of bacteria or ciliated cells in Medium 199S were tested independently. 10^9 bacteria (0.5 ml) or 10^5 ciliated cells (0.5 ml) were incubated for 30 min with potential inhibitors of adherence listed in Tables I and II, then with FHA (50 µg/ml), PT (5 µg/ml) (courtesy of Dr. J. Cowell, Bureau of Biologics, Bethesda, MD), or no addition for 30 min. Samples were washed twice (bacteria: 10,000 g; cells: 250 g; both for 10 min at 4°C), then exposed to 10 µl of antibody to FHA or to PT (goat gamma globulin fraction as per reference 1, courtesy of Dr. J. Cowell, Bureau of Biologics, Bethesda, MD) for 30 min at 37°C. The bacteria or cells were then washed, resuspended in 20 µl of Medium 199S, spotted onto a microscope slide, and air dried. Antibody that bound to the bacteria or to the ciliated cells was detected by staining with a 1:20 dilution of fluorescein-conjugated antibody to goat Fc (Cappel Laboratories, Cochranville, PA). Slides were read in a coded fashion by one of us (E. Tuomanen).

Preparation of Glycoconjugate Extracts of Human Ciliary Membranes.   Glycoconjugates were extracted from the cilia of a 0.5 × 5-cm strip of human trachea by an adaptation of the method of Ramesha (6) for deciliation of tetrahymena. Ciliated cells were scraped from the cartilagenous support into 20 ml 20 mM Tris, 10 mM EDTA, 125 mM sucrose buffer, pH 7.2. Cells were washed free of debris (centrifuged at 300 g for 5 min) and resuspended in 80 mM acetate, 10 mM EDTA, 125 mM sucrose buffer, pH 6.8, to a final concentration of 10^6 cells/ml. After incubation for 5 min, 0.2 M $CaCl_2$ was added to a final concentration of 10 mM, the mixture was swirled for 10 min, and passed three times through an 18-gauge needle. This procedure releases ~70% of the cilia from cell bodies as determined by light microscopy. The mix-

ture was diluted in 3 vol of Tris, EDTA, sucrose buffer (as above), and whole deciliated cells were pelleted at 480 g for 5 min and stored in saline at 4°C. The supernatant fluid containing the cilia free of cell bodies was centrifuged at 18,000 g for 20 min, and the pellet was stored in saline at 4°C overnight.

The ciliary membrane was extracted by an adaptation of the method of Adoutte et al. (7). The ciliary membrane was removed by vortexing the ciliary pellet in freshly prepared 1 mM Tris, 0.1 mM EDTA, pH 8.3 (Tris-EDTA), for ~2 min. This suspension was centrifuged at 48,000 g_{max} for 30 min to recover all particulate material. The pellet obtained was suspended in 10 mM Tris, pH 8.0, to a concentration of ~5-10 mg protein ml^{-1}, and 0.2-0.3 ml was layered on a sucrose step gradient consisting of 0.7 ml of 66% (wt/wt) sucrose overlaid with 1.7 ml each of 55 and 45% sucrose and 0.7 ml of 20% sucrose in 10 mM Tris, pH 8.0. The gradient was poured and left at 4°C for ~1 h before being used. The gradient was centrifuged in an SW 50.1 rotor (Beckman Instruments, Inc., Palo Alto, CA) at 4.5 × 10^4 rpm for 1.5 h at 4°C to separate the different fractions. Ciliary membranes banded within the 45% sucrose layer, incompletely demembranated cilia banded within the 55% layer, and axonemes banded at the interface of the 55 and 66% layers of sucrose. The bands were collected from the gradient, checked for purity by phase-contrast microscopy, and washed by centrifugation in 20-30 vol of 10 mM Tris, pH 8.0, before gel electrophoresis or lipid extraction.

Fractions enriched in ciliary and cell body membranes were extracted for both glycolipids and glycoproteins. Fractions were extracted three times in chloroform/methanol (C/M) 2:1. The protein precipitate was reserved for later examination, while the lipid extract was evaporated, re-extracted in C/M, and evaporated under a stream of nitrogen. Extracts were subjected to a Folch partition in six parts C/M to one part water (8). The upper phase was harvested as the glycolipid fraction and repartitioned twice in chloroform/methanol/0.1 KCl (1:2:10). Salt was removed by passage through a Sep-Pak C18 cartridge (Waters Associates, Milford, MA) by washing with water. Glycolipids were eluted from the cartridge with methanol and C/M. The eluate was evaporated under nitrogen, resuspended, and separated by TLC carried out on high performance thin layer chromatography plates precoated with Silica gel 60 (Merck & Co., Rahway, NJ), and developed in chloroform/methanol/water (60:40:9 by vol) (9).

The protein precipitates of the cilia and cell body fractions were suspended in sample buffer containing 2-ME and subjected to gel electrophoresis in SDS according to the method of Laemmli (10) (3.5% stacking gel, 10% separating gel). Proteins were then electrophoretically transferred to nitrocellulose by the method of Towbin et al. (11) in Tris-glycine-0.01% SDS transfer buffer. Nitrocellulose strips containing ciliary and cell body glycoproteins were incubated with a 1:1,000 dilution of mAb for 3 h, the peroxidase conjugated anti-mouse Fc fragment (Cappel Laboratories), and finally peroxidase substrate.

Binding of Bacteria, Isolated Adhesins, or Antibodies to Chromatographed Glycosphingolipids.   An adaptation of the Magnani technique (12) as described by Hansson et al. (13) was used. TLC plates containing either ciliary or cell body glycolipids, total neutral glycolipids from dog and rat intestine, human meconium, or glycosphingolipid standards (lactosylceramide, globotriaosylceramide, and Le^a pentaglycosylceramide) (13, 14) were dried and exposed to 0.5% (wt/vol) polyisobutyl methacrylate saturated in diethyl ether. The plate was dried thoroughly and sprayed with 1% BSA in PBS, pH 7.3, incubated for 1 h at room temperature, rinsed with PBS, and incubated for 2 h with one of the following in 1% BSA in 20 ml PBS: (a) a suspension of 10^8 radiolabeled *B. pertussis*; (b) 50 μl/ml FHA or 20 μg/ml PT; or (c) 1:1,000 dilution of anticarbohydrate antibody. The plate was washed upside down and in experiment (a) was dried and autoradiographed using XAR-5 X-ray film (Kodak) for 24 h. For experiment (b), plates were incubated with 1% BSA in PBS and 1:1,000 goat anti-FHA or anti-PT antibody for 3 h. The plate was then rinsed with PBS, and samples from experiments (b) and (c) were incubated for 3 h with 1:100 peroxidase-conjugated anti-goat Fc or anti-mouse Fc antibody, respectively (Cappel Laboratories), were rinsed again, and were exposed to freshly prepared peroxidase substrate (9, 13).

Chinese Hamster Ovary (CHO) Cell Assay.   The CHO cell assay was performed as described (15). Confluent monolayers, grown in flat-bottomed microtiter plates, were exposed in duplicate to 5 μl of mAbs (Table II), anti-FHA, or anti-PT for 1 h. One well of each pair was

exposed to 30 ng/ml PT for 30 min. The other well was washed and incubated with 5 µl fluorescein-conjugated anti-Fc fragment for 1 h. Wells were washed and cellular morphology determined by light microscopy at 6 and 24 h. Fluorescence was assessed qualitatively to determine binding of antibodies to the cell surface.

Results

The Chemical Nature of the Receptor.   The minimal receptor unit was defined using competition assays in which several types of agents were used to interfere with *B. pertussis* adherence to human ciliated cells. Inhibition of adherence was quantitated upon addition of simple or complex carbohydrates, lectins, or anti-carbohydrate antibodies. All sugars were tested by pre-incubating the bacteria or the ciliated cells for 30 min at 37°C with 10^{-2} M sugar. The sugar was then removed and adherence was assessed after 3 h of co-incubation of the bacteria and ciliated cells. Inhibition of adherence was found only if the simple or complex carbohydrates were preincubated with the bacteria, but not with the ciliated cell (Table I). At high concentrations $(10^{-2}$ M), mannose was found to agglutinate the bacteria, causing an apparent enhancement of adherence. Comparison of the ability of simple sugars of the six lectin classes or sialic acid to inhibit adherence indicated that galactose was the single most effective monosaccharide competitive inhibitor. When a dose-response curve was constructed between 10^{-7} and 10^{-2} M, 50% inhibition of binding occurred at 10^{-5} M galactose. When galactose was added to washed ciliated cells to which bacteria had been previously allowed to adhere, $\geq 10^{-2}$ M galactose was able to elute the adherent bacteria from ciliary tufts within 30 min. No change in the number of adherent bacteria occurred in the presence of the other monosaccharides in Table I.

Since the pattern of competitive inhibition by monosaccharides does not necessarily predict the structure of a receptor (16), the ability of a library of complex carbohydrates containing galactose and N-acetylated amino sugars to inhibit adherence was tested. Many complex carbohydrates containing galactose in β1-4 linkage blocked adherence (Table I). Lactose was a highly effective competitive inhibitor of adherence. Breast milk, the carbohydrates of which are >90% lactose, decreased binding to 2% of control values, while breast milk devoid of lactose did not inhibit (91% of control binding). Lactosamines with or without sialic acid also blocked adherence. However, N-acetylgalactosamine-containing compounds with a β1-3 backbone linkage did not block adherence. Removal of sialic acid from ciliated cells by neuraminidase did not decrease adherence.

The importance of the galactose-N-acetylglucosamine moiety to adherence of *B. pertussis* to cilia was further demonstrated by testing lectins for their ability to engage the human cell receptor and block adherence. The lectins shown in Table I were incubated with bacteria or ciliated cells, and after washing, bacterial adherence was tested. Two of the seven lectins tested decreased adherence; inhibition occurred only when the ciliated cell and not when the bacterium was incubated with the lectin. The specificity of the most effective antiadherence lectin, Lotus tetragonolobus, was fucose-α1-2-galactose-β1-4-N-acetylglucosamine. Reduced, but detectable, activity was found for peanut agglutinin (recognizes fucosylated galactose β1-3 N-acetylgalactosamine). Thus, a common receptor structure containing part of the lactose structure could be proposed from the studies with the library of simple and complex carbohydrates and the lectins.

TABLE I

Antiadherence Activity of Carbohydrates, Glycoconjugates, and Lectins

Carbohydrate/lectin (maximum concentration)	Carbohydrate specificity	Mean number of bacteria per cell	Percent of control binding
Simple carbohydrate (10^{-2} M)			
D-Glucose		6.1	111
D-Galactose		0.3	5
L-Fucose		5.8	105
D-Mannose		9.7	176
D-N-acetylglucosamine		5.9	107
D-N-acetylgalactosamine		6.2	113
Sialic acid		6.3	114
Control		5.5	100
Glycoconjugates (2.5 mg/ml)			
Lactose	Galβ1→4Glc	0.3	5
Lactoferrin	Galβ1→4GlcNAc(Man)	0.6	11
Fetuin	NANA→Galβ1→4GlcNAc(Man)	0.7	13
Fibronectin	NANA→Galβ1→4GlcNAc(Man)	0.6	11
Pneumovac	Galβ1→4G1cNAc	0.2	4
Human Chorionic Gonadotropin	Galβ1→3GalNAc	6.1	111
IgA	Galβ1→3GalNAc	5.8	105
Breast milk oligosaccharides	Galβ1→4Glc	0.1	2
Breast milk oligosaccharides-lactose	Galβ1→3GlcNAc	5.0	91
Lectin (250 μg/ml)			
Peanut agglutinin	Galβ1→3GalNAc	2.1	38
Lotus tetragonolobus	Fuc(α1→2)Galβ1→3GlcNAc	0.1	2
BS II	D-N-acetylglucosamine	4.9	89
Con A	D-Mannose, D-glucose, D-N-acetylglucosamine	5.4	98
PPA	Terminal D-galactose	5.9	107
Wheat germ	D-N-acetylglucosamine	5.8	105
Ulex EA	L-Fucose	6.2	113

* Gal = galactose, GlcNAc = N-acetylglucosamine, Man = mannose, NANA = sialic acid, Fuc = fucose, GalNAc = N-acetylgalactosamine, and Glc = glucose.
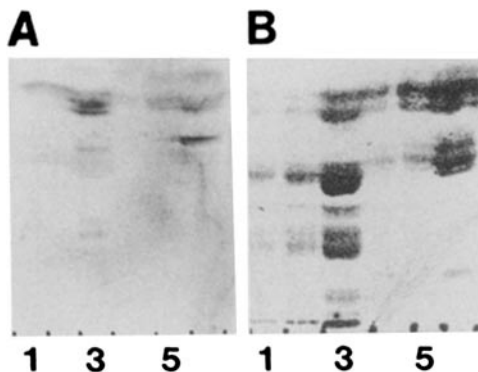
A   **B**

FIGURE 1.   Pattern of binding of whole *B. pertussis* to natural glycolipids. All panels schematically represent binding to the same set of glycolipids. (Lanes 1–3) Total non-acid glycosphingolipids of dog intestine at 1, 2, and 10 µl, respectively. (Lanes 4–6) Purified lactosyl- and globotriaosyl-ceramide at 1, 2, and 4 and 1, 2, and 10 µl respectively. (*A*) Radiolabeled *B. pertussis* whole organisms; (*B*) Anisaldehyde detection of all glycolipids.

1   3   5   1   3   5

Direct Determination of the Binding of Whole Bacteria and Purified Adhesins to Ciliary and Natural Carbohydrates.   Glycolipids were separated by TLC and the ability of radio-labeled whole bacteria, purified adhesins, and anti-carbohydrate antibodies to bind to the plate was tested. Bound whole bacteria were detected by autoradiography in the region of the TLC plate corresponding to lactosylceramide and a species of triglycosylceramide (Fig. 1). The purified adhesins FHA and PT, either alone or in combination, bound to the same carbohydrate species as the whole bacteria.

Activities of the Anti-carbohydrate mAbs in Blocking Adherence.   mAbs to human cell surface glycosphingolipids with carbohydrate composition related to lactosamine were tested for three properties expected of antibodies recognizing the *B. pertussis* receptor: (*a*) the ability to bind to human ciliary tufts but not ciliated cell bodies; (*b*) the ability

TABLE II

Antiadherence Activity of Anticarbohydrate mAbs[‡]

Antibody	Antigen specificity*	Isotype	Anti-adherence activity[‡]	Binding to lung epithelium[§]	Binding to ciliated cells
1018S69-4	A, Le^b	IgG3	+	+	Cilia and cell body
1021S10-69	Le^y	IgM	+	+ +	None
1022S25-17	Le^a	IgG2b	+	+ +	Cilia and cell body
1022S23-24	Le^a	IgG2b	+	+ +	Cilia
1018S19-11	Le^a	IgG1	+	+ +	Cilia
1022S75-67	Le^a	IgG3	+	+ +	None
1018S5-3	Le^{ab}	IgM	–	+	None
1018S56-4	U	IgM	–	–	None
1000S93-1-3	U	IgM	–	–	None
1021S11-28	U	IgG2a	–	+	None
1021S62-71	Le^x	IgM	–	–	None
1022S56-58	Nonfucosylated short chain GSL	IgG	–	–	None

* A = [GalNAcα1→3] Gal [α1←2Fuc]β1→3GlcNAc; Le^a = Galβ1→3GlcNAc [4-1αFuc]; Le^b = [Fucα1→2]Galβ1→3GlcNAc [4-1αFuc]; Le^x = galβ1→4GlcNAc [3-1αFuc]; Le^y = [Fucα1→2]Galβ1→4GlcNAc [3←1αFuc]; U = specificity not related to A, B, H, or Lewis antigens; GSL = glycosphingolipid.

‡ (+) Block adherence (0 BP/cell with 10^{10} inoculum in standard assay); (–) normal adherence (>5 BP/cell with 10^{10} inoculum in standard assay).

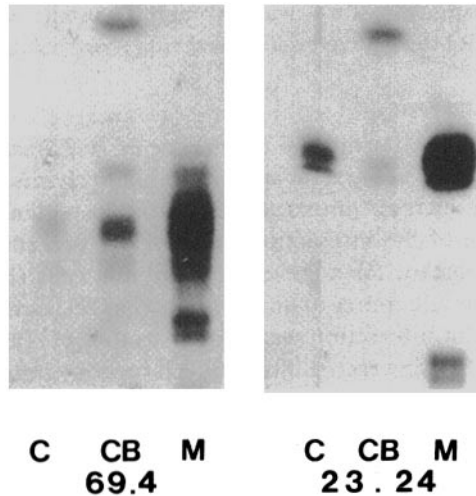§ Fluorescence: – = negative, + = detectable, + + = bright.

FIGURE 2. Binding of antiadherence antibodies to chromatographically separated glycolipids of human cilia and ciliated cell bodies. Anti-A (69.4, poor antiadherence activity) and anti-Le^a (23-24, good antiadherence activity) antibodies were incubated with duplicate thin layer chromatograms containing glycolipids extracted from cilia (C), deciliated cell body (CB), or meconium (M). Antibody binding was detected by overlaying peroxidase conjugated anti–mouse Fc antibody followed by peroxidase substrate.

C   CB   M
69.4

C   CB   M
23 . 24

to bind to extracts of human ciliary membranes; and (c) the ability to block adherence of whole organisms and purified adhesins to cilia.

As shown in Table II, a series of antibodies recognizing the Lewis a (Le^a), Le^b, or A blood group antigens were effective in blocking adherence of B. pertussis to cilia. Upon titration of blocking activity, the IC$_{50}$ for the Le^a-specific antibodies 23-24 and 19-11 was the lowest, 0.03 µg/ml, while that for the a Le^b-specific antibody 69-4 was 0.5 µg/ml. Antibodies 23-24 and 69-4 were chosen for further study. The localization of binding of antibodies 69-4 and 23-24 to ciliated cells, the latter being the more effective antiadherence antibody, was different. Antibody 23-24 bound to cilia and not to ciliated cell bodies as detected by fluorescein-labeled anti-Fc antibody. The same antibody also bound to a doublet of glycolipids in extracts of ciliary but less to cell body cytoplasmic membrane (Fig. 2); antibody 69-4 bound to cell body–related glycolipids. Antibody 23-24 also bound to a single ciliary glycoprotein (not shown); 69-4 did not bind. The anti-adherence antibody 23-24 bound to as little as 1 ng of Lewis a antigen on thin layer chromatograms. Antibody 23-24 did not crossreact with the Lewis b antigen, the H antigen (the A and B precursor), or structural isomers of the Lewis a antigen in the TLC assay.

In addition to the ability to block adherence of whole B. pertussis to cilia, mAbs were tested for the ability to alter adhesin binding to intact ciliated cells as well as for the phenomenon of enhancement of adherence. Purified adhesins have been shown to localize to the ciliary membrane (1, 17, 18). When preincubated with antibodies 23-24, 19-11, and to a lesser extent 69-4, ciliated cells could no longer capture purified adhesins from the medium as detected by immunofluorescence. The capture of adhesins from the medium is known to enhance the binding of virulent B. pertussis to the adhesin-coated ciliated cell (1). Antibody 23-24 blocked this enhancement phenomenon.

Since PT is an adhesin in the human ciliated cell assay in vitro and antibody 23-24 blocked its association with cilia, we tested if the antibody blocked binding of PT to its specific receptor on CHO cells. Even at high concentrations of antibody (5

mg/ml protein), a low concentration (30 ng/ml) of PT was still able to intoxicate CHO cells as indicated by the development of the characteristic clustering morphology.

Discussion

The development of an in vitro assay quantitating the adherence of *B. pertussis* to human ciliated cells has led to the elucidation of several aspects of the mechanism by which this interaction is sustained for such an unusually long time in patients with whooping cough. The bacterial side of the interaction involves two secreted proteins, FHA and PT (1). Both are important for adherence to ciliated cells (1), while FHA alone appears to be sufficient for adherence to nonciliated cells (19). Early evidence suggested that the *B. pertussis*–cilia interaction was similar to that of classical mucosal pathogens, in that bacterial proteins recognized host cell carbohydrates (4). This report describes the nature of a family of host cell receptors on human ciliated cells that interact with both adhesins.

Several lines of evidence indicate that part of the lactose moiety is the minimal receptor unit for *B. pertussis* on human cilia. Carbohydrates that mimicked the receptor and blocked adherence when preincubated with the organism included galactose, galactose β1-4-glucose, and complex lactosamines. Substitution with sialic acid or mannose did not alter antiadherence activity. Breast milk, in which the carbohydrate is >90% lactose, also was an inhibitor of adherence. Alveolar glycoprotein also contains similar moieties, suggesting endogenous competitive inhibitors of adherence occur in the distal lung where organisms are only rarely found in natural disease (17). Importantly, the incubation of ciliated cells bearing adherent bacteria with high concentrations of galactose caused elution of the adherent *B. pertussis*. This suggests that receptor analogs may be candidates for adjuncts to the therapy of *B. pertussis*.

Masking of the receptor on cilia was achieved by preincubation of ciliated cells within lectins or anti-carbohydrate mAbs recognizing the galactose-glucose structure. Reagents directed against the β1-4 (and, to a lesser extent, the β1-3) configuration were effective inhibitors. A wide variety of saccharide substitutions (fucosylation, *N*-acetylation, etc.), was acceptable among inhibitors; such permissiveness has been shown previously for *Proprionobacterium granulosum* and *Escherichia coli* adherence to nonciliated cells (20). This suggested that the core structure of many blood group antigens such as A, B, H, and Lewis could serve as a receptor for *B. pertussis*, a fact that would explain why virtually all people are susceptible to the disease. In fact, mAbs against the Lewis a (Gal B1-3GlcNAc[4-1-α-Fuc]) and, to a lesser extent, A or Le^b antigens, were effective antibody inhibitors of adherence. These antibodies bound to ciliated cells and, in the case of the most effective antibody (anti-Le^a), bound to cilia and not ciliated cell bodies. This is consistent with the topographic restriction of the bacteria to ciliary tufts during natural infection. Interestingly, several pneumococcal polysaccharides carry the lactose moiety and block adherence, suggesting that anti-capsular antibody (particularly to types 14 and 19) may affect the adherence of *B. pertussis* (17). It remains to be shown if prophylaxis of contacts might be achieved by anti-receptor antibodies or by receptor analogs.

Bordetella demonstrate remarkable species specificity for the target host. Differences in adherence have been clearly shown to parallel the natural pattern of disease (18), suggesting receptors may differ between infected hosts. Studies by Plotkin and

Bemis (21) suggest that N-acetylglucosamine is critical to the receptor of *B. bronchiseptica* in the hamster. In contrast, the receptor in swine has been characterized as a ganglioside (22). It is possible that this discrepancy represents a similarity to the situation found for *Vibrio cholera*, which adheres primarily to the ganglioside GM1 but less efficiently to fucosylated nonacidic carbohydrates (23, 24). In the case of *B. pertussis*, gangliosides are capable of inhibiting FHA-dependent adherence to nonciliated tissue culture cells (J. Cowell, Bureau of Biologics, Bethesda, MD, personal communication). Despite extensive effort, however, we were not able to demonstrate a primary role for sialic acid–containing compounds in adherence of *B. pertussis* to human cilia either by competition studies or by treatment of cells with neuraminidase. Both FHA and PT, alone or in combination, adhered to purified lactose-containing glycolipids on TLC plates, suggesting that this receptor can interact with both adhesins. Since adherence to cilia involves two adhesins, FHA and PT, while that to nonciliated cells occurs with FHA alone, it is possible that the binding specificity of PT masks the ganglioside specificity of FHA when human cells are the target or that two carbohydrate specificities exist. We suggest that the latter is more likely for two reasons. Fetuin and haptoglobin not only block adherence but also are known to bind to PT sufficiently well so as to serve as ligands for purification of the toxin by affinity chromatography (25, 26). Fetuin does not, however, block the binding to and intoxication of CHO cells by PT (27). This is consistent with our finding that although the antiadherence mAbs bound to CHO cells, they did not prevent intoxication. PT is a structurally complex toxin, and these findings indicate that the PT binding site promoting adherence of the whole organism to cilia is distinct from that associated with ADP-ribosylating activity. This does not rule out the possibility that binding at one site may facilitate binding at the other, since the association of structurally similar glycolipids and glycoproteins as part of a complex linked to intracellular signaling systems has been described (28).

Summary

The adherence of *Bordetella pertussis* to human respiratory cilia is critical to the pathogenesis of whooping cough. To explore the development of agents that could interrupt adherence, the structure of the receptor on the ciliary surface was investigated. Using an in vitro adherence assay to human ciliated epithelial cells, galactose, lactose, and complex carbohydrates containing lactose eliminated adherence when preincubated with the bacteria. 10^{-2} M galactose eluted adherent bacteria from cilia. *B. pertussis* and its two purified adhesins bound specifically to natural lactose-containing glycolipids in a TLC assay. mAbs to eukaryotic glycoconjugates with specificity for substituted galactose-glucose moieties blocked adherence when preincubated with ciliated cells. The carbohydrates that serve as receptors for *B. pertussis* on human cilia are galactose-glucose-containing glycolipids. Receptor analogs and anti-receptor antibodies effectively block adherence of *B. pertussis* to cilia and thus should be considered candidates for therapeutic intervention against disease.

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