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

BY ELAINE TUOMANEN,* HARRY TOWBIN,† GUNTER ROSENFELDER,† DIETMAR BRAUN,† GORAN LARSON,§ GUNNAR C. HANSSON,† AND ROSS HILL†

From the *Laboratory of Microbiology, The Rockefeller University, New York, New York 10021; †CIBA-GEIGY Ltd., CH-4002, Basel, Switzerland; ‡Department of Clinical Chemistry, University of Gothenburg, 413 45 Gothenburg, Sweden; the §Department of Medical Biochemistry, University of Gothenburg, 400 33 Gothenburg, Sweden; and the †Pulmonary Division, State University of New York Health Science Center at Brooklyn, Brooklyn, New York, 11203

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) 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...
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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 $5 \times 10^8 B. pertussis$/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 $10^{-1}$ and $10^{-3}$ 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, Universität 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 35S 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 x 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. 10^5 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 x 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 cartilaginous 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, pH 8.3 (Tris-EDTA), for ~2 min. This suspension was centrifuged at 48,000 g 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 × 10⁴ 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 Leα pentaglycosylceramide) (13, 14) were dried and exposed to 0.5% (wt/vol) polyisobutyl methacrylate saturated in diethyl ether. The plate was washed 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 10⁸ radiolabeled B. pertussis; (b) 50 μl/ml FHA or 20 μg/ml PT; or (c) 1:1,000 dilution of antiacarbohydrate 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 (a) 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 (10⁻¹ 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, 10⁻³ 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-acetylgalcosamine 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 fucosyl-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.
| Carbohydrate/lectin | Carbohydrate specificity | Mean number of bacteria per cell | Percent of control binding |
|---------------------|-------------------------|---------------------------------|---------------------------|
| Simple carbohydrate (10^{-5} 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-acetylglucosamine | 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→4GlcNAc | 0.2 | 4 |
| | Human Chorionic Gonadotropin | Galβ1→3GlcNAc | 6.1 | 111 |
| | IgA | Galβ1→3GlcNAc | 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→3GlcNAc | 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-acetylglactosamine, and Glc = glucose.
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 radiolabeled 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

| Antibody       | Antigen specificity | Isotype | Anti-adherence activity | Binding to lung epithelium | Binding to ciliated cells |
|----------------|--------------------|---------|-------------------------|---------------------------|---------------------------|
| 1018569-4      | A, Le<sup>b</sup>  | IgG3    | +                       | +                         | Cilia and cell body       |
| 1021510-69     | Le<sup>+</sup>     | IgM     | -                       | -                         | None                      |
| 1022525-17     | Le<sup>a</sup>     | IgG2b   | +                       | ++                        | Cilia and cell body       |
| 1022523-24     | Le<sup>a</sup>     | IgG2b   | +                       | +                         | Cilia                     |
| 1018519-11     | Le<sup>a</sup>     | IgG1    | -                       | +                         | Cilia                     |
| 1022523-24     | Le<sup>a</sup>     | IgG3    | -                       | +                         | None                      |
| 1018565-4      | U                  | IgM     | -                       | -                         | None                      |
| 1000593-1-3    | U                  | IgM     | -                       | -                         | None                      |
| 1021511-28     | U                  | IgG2a   | +                       | +                         | None                      |
| 1022567-11     | Le<sup>x</sup>     | IgM     | -                       | -                         | None                      |
| 1022566-58     | Nonfucosylated     | IgG     | -                       | -                         | None                      |

* A = [GalNAcα1→3] Gal [α1→2Fucβ1→3Galβ1→3GlcNAc; Le<sup>a</sup> = Galβ1→3GlcNAc [4-1aFuc]; Le<sup>b</sup> = [Fucα1→2]Galβ1→3GlcNAc [4-1aFuc] [3-1aFuc]; Le<sup>+</sup> = [Fucα1→2]Galβ1→4GlcNAc [3-1aFuc]; U = specificity not related to A, B, H, or Lewis antigens; GSL = glycosphingolipid.

† (+) Block adherence (0 BP/cell with 10<sup>10</sup> inoculum in standard assay); (-) normal adherence (≥3 BP/cell with 10<sup>10</sup> 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-Lea (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.

The ability of antibodies to block adherence of whole organisms and purified adhesins to cilia.

As shown in Table II, a series of antibodies recognizing the Lewis a (Lea), Leb, or A blood group antigens were effective in blocking adherence of B. pertussis to cilia. Upon titration of blocking activity, the IC50 for the Lea-specific antibodies 23-24 and 19-11 was the lowest, 0.03 μg/ml, while that for the a Leb-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 Propionibacterium 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 Leb antigens, were effective antibody inhibitors of adherence. These antibodies bound to ciliated cells and, in the case of the most effective antibody (anti-Leb), 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 non-ciliated 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, lactose, lactose, and complex carbohydrates containing lactose eliminated adherence when preincubated with the bacteria. 10^-2 M lactose 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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References

1. Tuomanen, E., and A. Weiss. 1985. Characterization of two adhesins of Bordetella pertussis for human ciliated respiratory epithelial cells. J. Infect. Dis. 152:118.

2. Tuomanen, E. 1986. Piracy of adhesins: attachment of superinfecting pathogens to respiratory cilia by secreted adhesins of Bordetella pertussis. Infect. Immun. 54:905.

3. Broome, C. V., and D. W. Fraser. 1981. From the Centers for Disease Control - Pertussis in the United States, 1979: a look at vaccine efficacy. J. Infect. Dis. 144:187.

4. Tuomanen, E. I., and J. O. Hendley. 1983. Adherence of Bordetella pertussis to human respiratory epithelial cells. J. Infect. Dis. 148:125.

5. Weiss, A. A., E. L. Hewlett, G. A. Myers, and S. Falkow. 1983. Tn5-induced mutations affecting virulence factors of Bordetella pertussis. Infect. Immun. 42:33.

6. Ramesha, C. S., and G. A. Thompson, Jr. 1982. Changes in the lipid composition and physical properties of Tetrahymena ciliary membranes following low-temperature acclimation. Biochemistry. 21:3612.

7. Adoutte, A., R. Ramanathan, R. M. Lewis, R. R. Dute, K.-Y. Ling, C. Kung, and D. L. Nelson. 1980. Biochemical studies of the excitable membrane of Paramecium tetraurelia. III. Proteins of cilia and ciliary membranes. J. Cell Biol. 84:717.

8. Folch, J., M. Lees, and G. G. Sloane-Stanley. 1957. A simple method for the isolation and purification of total lipides from animal tissues. J. Biol. Chem. 226:497.

9. Towbin, H., C. Schoenenberger, R. Ball, D. G. Braun, and G. Rosenfelder. 1984. Glycosphingolipid-blotting: an immunological detection procedure after separation by thin layer chromatography. J. Immunol. Methods. 72:471.

10. Laemmli, U. K. 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature (Lond.). 227:680.

11. Towbin, H., T. Stachelin, and J. Gordon. 1979. Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: procedure and some applications. Proc. Natl. Acad. Sci. USA. 76:4350.

12. Magnani, J. L., M. Brockhaus, D. F. Smith, and V. Ginsburg. 1982. Detection of glycolipid ligands by direct binding of carbohydrate-binding proteins to thin-layer chromatograms. Methods Enzymol. 83:235.

13. Hansson, G. C., K.-A. Karlsson, G. Larson, N. Stromberg, and J. Thurin. 1985. Carbohydrate-specific adhesion of bacteria to thin-layer chromatograms. A rationalized approach to the study of host cell glycolipid receptors. Anal. Biochem. 146:158.

14. Karlsson, K.-A., and G. Larson. 1981. Molecular characterization of cell surface antigens of fetal tissue. J. Biol. Chem. 256:3512.

15. Hewlett, E. L., K. T. Sauer, G. A. Myers, J. L. Cowell, and R. L. Guerrant. 1983. Induction of a novel morphological response in Chinese hamster ovary cells by pertussis toxin. Infect. Immun. 40:1198.

16. Jones, G. W. 1980. The adhesive properties of Vibrio cholerae and other Vibrio species. In Receptors and recognition. Series B. Vol. 6. Bacterial adherence. E. H. Beachey, editor. Chapman & Hall, New York. 220–249.

17. Tuomanen, E. 1986. Adherence of Bordetella pertussis to human cilia: implications for disease prevention and therapy. In Microbiology 1986. D. Schlessinger, editor. American Society for Microbiology, Washington, DC. 59–64.

18. Tuomanen, E. 1988. Bordetella pertussis adhesins. In Pathogenesis and Immunity in Pertussis, A. C. Wardlaw, and R. Parson, editors. John Wiley & Sons Ltd., Sussex, England. In press.

19. Urisu, A., J. L. Cowell, and C. R. Manclark. 1986. Filamentous hemagglutinin has a major role in mediating adherence of Bordetella pertussis to human WiDr cells. Infect. Immun. 52:695.
20. Karlsson, K.-A. 1986. Animal glycolipids as attachment sites for microbes. *Chem. Phys. Lipids.* 42:153.
21. Plotkin, B. J., and D. A. Bemis. 1984. Adherence of *Bordetella bronchiseptica* to hamster lung fibroblasts. *Infect. Immun.* 46:697.
22. Ishikawa, H., and Y. Isayama. 1987. Evidence for sialyl glycoconjugates as receptors for *Bordetella bronchiseptica* on swine nasal mucosa. *Infect. Immun.* 55:1607.
23. Cuatrecasas, P. 1973. Interaction of *Vibrio cholerae* enterotoxin with cell membranes. *Biochemistry.* 12:3547.
24. Jones, G. W., and R. Freter. 1976. Adhesive properties of *Vibrio cholerae*: nature of the interaction with isolated rabbit brush border membranes and human erythrocytes. *Infect. Immun.* 14:240.
25. Irons, L. L., and A. P. MacLennan. 1979. Isolation of the lymphocytosis promoting factor-hemagglutinin of *Bordetella pertussis* by affinity chromatography. *Biochim. Biophys. Acta.* 580:175.
26. Sekura, R., F. Fish, C. Manclark, B. Meade, and Y. Zhang. 1983. Pertussis toxin. Affinity purification of a new ADP-ribosyltransferase. *J. Biol. Chem.* 258:14647.
27. Armstrong, G. D., and M. S. Peppler. 1987. Maintenance of biological activity of pertussis toxin radiiodinated while bound to fetuin-agarose. *Infect. Immun.* 55:1294.
28. Feizi, T., and R. A. Childs. 1985. Carbohydrate structures of glycoproteins and glycolipids as differentiation antigens, tumour-associated antigens and components of receptor systems. *TIBS (Trans. Bio. Chem. Sci.)* 1985:24.