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Role of Carbohydrate Recognition Domains of Pertussis Toxin in Adherence of *Bordetella pertussis* to Human Macrophages

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Pertussis toxin (PT) and filamentous hemagglutinin can each mediate the association of *Bordetella pertussis* with human macrophages. Adherence via filamentous hemagglutinin leads to integrin-mediated entry and survival of the bacteria within the human cell. We determined the contribution of PT to bacterial adherence to human macrophages. Plating macrophages on wells coated with recombinant PT subunit 2 (S2) or S3 decreased PT-dependent bacterial binding by >60%; S1, S4, and S5 were ineffective. S3-dependent adherence was reduced 63% ± 8% by sialic acid, while S2-dependent adherence was reduced 53% ± 11% by galactose. Loss of the carbohydrate recognition properties of S2 by deletion of residues 40 to 54 or site-specific mutations at Asn-93, His-47, or Arg-50 eliminated the ability of the subunit protein to competitively inhibit bacterial binding. Peptides corresponding to residues 28 to 45 of S2 and S3 competitively inhibited adherence. Treatment of macrophages with antibodies to Le^a or Le^x but not CD14, CD15, CD18, or HLA interfered with PT-mediated binding. Exposure of the macrophages to the B oligomer, S2, or S3 increased binding to the CD11b/CD18 integrin. These results indicate that the carbohydrate recognition domains of both S2 and S3 participate in adherence of *B. pertussis* to human macrophages. The PT receptor(s), as yet unidentified, appears to carry the Le^a or Le^x determinants and is functionally capable of modulating integrin-mediated binding to the macrophage.

The pentameric B oligomer of pertussis toxin (PT) promotes the adherence of *Bordetella pertussis* to several eukaryotic cells (9, 18) and confers cell recognition properties to the toxin (2, 15, 19, 21). Since a wide range of eukaryotic cells, both primary and tissue culture cell lines, is sensitive to the toxin and intoxication is blocked by loss of sialylated carbohydrates (2, 21), the B oligomer is thought to contain a binding site for a common sialylated cell surface glycoconjugate. In contrast, nonsialylated receptors appear to be involved in rapid B-oligomer effects on T cells (10) and when the B oligomer serves as a bacterial adhesin for ciliated cells and leukocytes (9, 17). This dual specificity is consistent with recent evidence suggesting that subunit 2 (S2) binds galactose-containing glycoconjugates (15) and recognizes cilia (15) and T cells (10); the 80% homologous S3 recognizes sialylated glycoconjugates such as those on monocytes (15), a process which can result in intoxication by ADP ribosylation of G proteins (2). The carbohydrate recognition domains of the homologous S2 and S3 have been suggested to include the regions of residues 37 to 54 (13–15), and chimeric recombinant subunits of S2 or S3 containing swapped cassettes of this region switch carbohydrate and cellular targeting (15). The carbohydrate recognition domains of S2 and S3 structurally resemble the C-type lectins of eukaryotic adherence systems (15).

B. pertussis enters and persists within cultured macrophages in vitro and alveolar macrophages in vivo (16). Both PT and the large, nonfimbrial adhesin filamentous hemagglutinin (FHA) can independently mediate the initial interaction of the bacteria with macrophages (9). FHA mediates bacterial uptake into the macrophage in a process involving recognition of the integrin CD11b/CD18 (9, 16), but the ligand-receptor interaction for PT-bound bacteria is unknown. We demonstrate here that S2 and S3 mediate PT-dependent bacterial binding to macrophages through their carbohydrate recognition domains. The receptors for S2 and S3 may be Le^a and Le^x determinants present on a number of macrophage surface molecules.

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MATERIALS AND METHODS

Bacterial strains. *B. pertussis* BP101 (*fhaB*Δ101) is a BP536 derivative which contains an in-frame 2.4-kb deletion within the FHA structural gene, *fhaB* (9). This strain expresses and excretes a truncated FHA protein which is missing both the predicted Arg-Gly-Asp (RGD) sequence at positions 1097 to 1099 and the carbohydrate-binding domain. Bacteria, grown for 3 days at 37°C on Bordet Gengou agar supplemented with 15% sheep blood, were harvested and suspended in phosphate-buffered saline (PBS), washed, and adjusted to a density of 2×10^8 CFU/ml. For some experiments, bacteria were fluoresceinated as described previously (22, 23).

Adherence of bacteria to human macrophages. Human monocytes were purified from buffy coats on Percoll gradients and cultured in 12.5% human serum in Teflon beakers as described previously (22). Macrophages were harvested from Teflon beakers after 5 to 10 days and suspended at 0.5×10^6 to 1.0×10^6/ml in PBS containing 3 mM glucose, 0.5 mg of human serum albumin (Armour Pharmaceutia, Kankakee, Ill.) per ml, and 0.3 U/ml aprotinin. To determine the components of PT recognizing the macrophage, Terasaki tissue culture plates (Miles Laboratories Inc., Naperville, Ill.) were coated by a 60-min incubation at 20°C (23) with one of the following: human serum albumin (1 mg/ml); purified

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TABLE 1. Ability of antibodies against macrophage surface determinants to interfere with binding of PT-coated erythrocytes to human macrophages[a]

Specificity	Antibody	Reference or source	% of control erythrocytes bound
Anti-CD11a	TS1/22	11	111 ± 12
Anti-CD11b	OKM9	23	101 ± 13
Anti-CD11c	L29	5	83 ± 9
Anti-CD18	IB4	23	90 ± 9
Anti-CD14	3C10	20	111 ± 13
Anti-HLA	W6/32	1	92 ± 8
Anti-CD64 (FcRI)	22	M. Fanger	92 ± 12
Anti-CD15 (Le^x)	7C3	8	101 ± 9
Anti-Le^x	1021-62-71	17	58 ± 8
Anti-Le^a	1022-25-17	17	96 ± 10
Anti-Le^a	1022-75-67	17	47 ± 12
Anti-Le^y	1021-10-69	17	122 ± 10
Anti-short GSL[b]	1022-56-58	17	112 ± 13

[a] Macrophages were plated in plastic wells precoated with antibodies to sequester receptors under the cells. The availability of receptors for PT on the apical surface of the cell was determined by the ability to bind PT-coated erythrocytes. Values are the means from three experiments testing each antibody in duplicate. In the absence of antibody, 577 ± 23 PT-coated erythrocytes were bound per 100 macrophages.

[b] GSL, short chain glycosphingolipids, nonfucosylated.

PT or B oligomer (100 to 0.01 μg/ml; List Biological Laboratories, Inc., Campbell, Calif.); or recombinant proteins S1, S2, S3, S4, and S5, chimeric S2 containing the sialic acid-binding region of S3 (S2/2S3), or chimeric S3 containing the lactose-binding region of S2 (S3/2S2) (15). Recombinant proteins were used as insoluble inclusion body preparations adjusted to a concentration of 50 μg of the desired subunit protein per ml (15). These inclusion body forms of S2 and S3 have been shown to assemble into a B oligomer after processing in vitro, and this reassembled oligomer retains the following properties of native B oligomer: binding to fetuin, hemagglutination activity, lymphocyte mitogenicity, and protective activity in animal models of infection (3a). Monoclonal antibodies (MAbs) (50 μg/ml; Table 1) against leukocyte antigens or cell surface carbohydrates were used to coat the plates for assays directed at determining the leukocyte receptors for PT.

After coating, the surfaces were washed, 5 μl of macrophage suspension was added per well, and the cells were allowed to spread at 37°C for 45 min. Binding of macrophages to coated surfaces has been shown to lead to movement of receptors (Fc, complement, mannose, and CD14, etc.) in the plane of the membrane to trap the bound receptor under the macrophage (for examples, see references 7 and 23). Attachment of *B. pertussis* to the apical surface of the adherent macrophages was determined by adding 2×10^5 fluoroscein-labelled bacteria per well and incubating them at 37°C for 30 min (9, 23). For some competitive inhibition assays, macrophages plated on albumin were exposed to bacteria in the presence of solutions containing PT, B oligomer, synthetic peptide RALTVAELRGSGDLQEYL (residues 28 to 45 of S2) or RALTVAELRGNAELQEYL (residues 28 to 45 of S3) (courtesy of T. Jones, Amgen Boulder, Boulder, Colo.), recombinant subunits, or 16 distinct, S2-derived mutant recombinant proteins with amino acid substitutions or deletions in residues 40 to 54 as described previously (15) (Fig. 1). Alternatively, competition assays were performed in the presence of isotonic buffers containing up to 50 mM D-galactose, lactose, sialic acid,

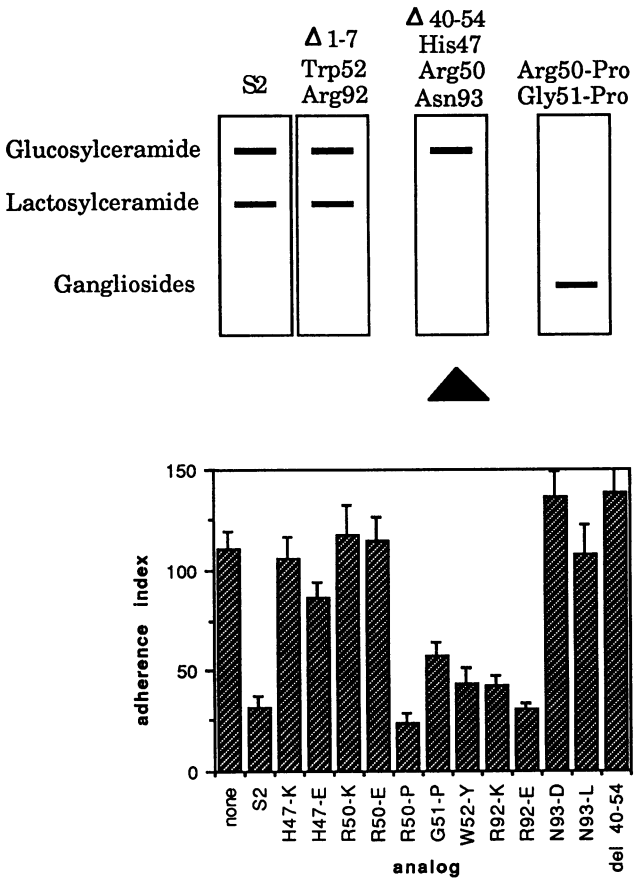

FIG. 1. Inhibition of bacterial binding by PT subunit analogs. (Top) The known carbohydrate recognition properties of S2 and its analogs created by site-specific mutagenesis are illustrated by dark bands representing binding to thin-layer-chromatographed glycolipids named on the left (15). Analogs are grouped according to similar binding patterns; each amino acid designation above the box represents the residue at which a mutation or deletion (triangle) was made. In some cases, a single residue was changed to several different alternative residues: residues 47, 50, and 92 were each changed to lysine and glutamic acid, and residue 93 was changed to aspartic acid and leucine. (Bottom) To quantitiate inhibition, analogs (50 μg/ml) were added together with BP101 to wells containing adherent macrophages. The activities of analogs are compared with that of S2 to block BP101 binding to macrophages (analogs are named by a single-letter code for the normal amino acid with its residue number followed by the code for the residue as changed in the analog). The arrowhead indicates the group of mutations which greatly reduced inhibitory activity.

lacto-*N*-fucopentose, 2'-fucosyllactose, 6'-sialyllactose, 2'-sialyllactose, or lacto-*N*-tetraose (Calbiochem, La Jolla, Calif.).

The attachment of bacteria was scored as an attachment index defined as the mean number of bacteria on 100 macrophages enumerated by fluorescence microscopy. Some values were expressed as a percentage of binding by the control BP101. All assays were performed in triplicate. The total number of bacteria (intracellular and extracellular) was quantified by fixing preparations with methanol and staining with fluorescein-labelled anti-*B. pertussis* antibody (16). For some assays, bacteria were replaced by C3bi-coated erythrocytes prepared as described previously (22); such cells are known to be specific ligands for CD11b/CD18.

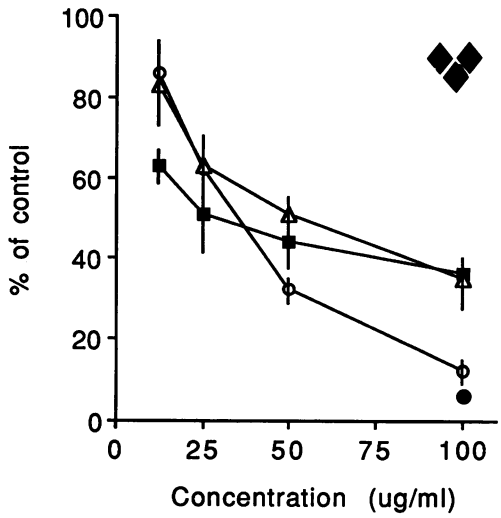

FIG. 2. Inhibition of binding of BP101 to macrophages by PT and its recombinant subunits. The proteins were adsorbed to tissue culture substrates, macrophages were added, and binding of BP101 to the adherent macrophages was measured. S1, S4, and S5 (diamonds) were tested only at the highest concentration and showed no effect. S2 and S3, added together at 50 or 100 μg/ml each (closed circle), showed inhibition equivalent to that of purified PT (open circles). Recombinant S2 (triangles) and S3 (squares) demonstrated intermediate inhibitory capability. Values are the means ± standard deviations for four experiments testing each condition in triplicate. In 15 control wells, 168 ± 44 BP101 cells were bound per 100 macrophages.

Alternatively, 10^8 erythrocytes were coated with PT by exposure to 0.5 or 0.05 μg of PT in saline for 30 min at 37°C. In some assays, PT-coated erythrocytes were incubated for 30 min with 50 μg of anti-PT MAbs directed to S2 (MAb 9G8) or S3 (MAb 7E10) per ml (12).

RESULTS

Identification of subunits involved in recognition of macrophages. BP101, a strain deficient in FHA-mediated adherence to macrophages, was used to quantitate PT-dependent bacterial binding. When macrophages were plated on wells coated with native PT or B oligomer to down modulate receptors for these proteins under the cells, binding of fluorescent BP101 to the apical surface of the cell decreased by 90% in a dose-dependent manner (Fig. 2). Plating of macrophages on recombinant S2 or S3 (at or above 50 μg/ml) reduced bacterial binding to a plateau value of 40 to 50% of the control value; S1, S4, and S5 were ineffective. A combination of S2 and S3 at 50 or 100 μg/ml each reduced adherence by >90%, a value similar to the maximum observed with PT. These results suggest that S2 and S3 bind molecules on the surface of macrophages that are independent of one another and that the receptors participate in adhesion of BP101. The results are consistent with but do not directly prove the suggestion that these receptors are mobile in the plane of the membrane.

Residues 28 to 54 of S2 and S3 direct binding to macrophages. The regions of the homologous S2 and S3 which were important to bacterial recognition of macrophages were identified by testing the efficacy of 16 site-specific mutant proteins (Fig. 1) to competitively inhibit binding of BP101 when added in solution together with the bacteria. All but seven subunit analogs inhibited bacterial binding by up to 90%; inhibitory activity was lost by deletion of residues 40 to 54 or by two site-specific mutations each at Asn-93, Arg-50, or His-47. The common feature of these mutations was that each eliminated the ability of the subunit to bind both gangliosides and lactosylceramide (15) (Fig. 1). Preservation of the ability to recognize one or the other carbohydrate was sufficient to maintain the ability of the subunit analog to competitively inhibit bacterial binding to macrophages. The importance of this region was further indicated by the ability of synthetic peptides derived from residues 28 to 45 of S2 or S3 to competitively inhibit bacterial binding to macrophages by 59% ± 12% and 68% ± 9%, respectively.

TABLE 2. Inhibition of binding of PT-coated erythrocytes to macrophages by carbohydrates or antisubunit antibodies[a]

Inhibitor	% of control E-PT bound with[b]:		
	No antibody	Anti-S3	Anti-S2
None	100	100	100
Anti-S3	34 ± 5		
Anti-S2	47 ± 11		
Galactose	47 ± 11	42 ± 17	84 ± 6
Sialic acid	37 ± 8	84 ± 9	36 ± 2
Dextran sulfate	87 ± 9	112 ± 23	98 ± 7
Lacto-*N*-fucosylpentose	116 ± 16		
Lacto-*N*-tetraose	108 ± 15		
2′-Fucosyllactose	55 ± 12		
6′-Sialyllactose	38 ± 8		
3′-Sialyllactose	58 ± 9		

[a] To achieve subunit-specific binding of PT-coated erythrocytes (E-PT), anti-S2 or anti-S3 antibodies were incubated with the PT-treated erythrocytes to mask the cognate epitope. Anti-S4 was used as a control and altered the control binding by <5%. Washed erythrocytes were incubated with the macrophages in the presence of 50 mM carbohydrate.

[b] Values are means ± standard deviations for three experiments testing each condition at least in triplicate. To facilitate comparison of inhibitory activity down a column, values were calculated based on 100% at the top of each column. For the "No antibody" column, 100% represents the mean from 15 control wells counted (109 ± 15 erythrocytes bound per 100 macrophages). For the anti-S3 and anti-S2 columns, 100% represents values (shown in the "No antibody" column) of 34 ± 5 and 47 ± 11 erythrocytes bound per 100 macrophages for anti-S2 and anti-S3, respectively. Values are not directly comparable across columns.

Carbohydrate specificity of S2- and S3-dependent recognition of macrophages. To confirm that the carbohydrate recognition properties of regions 28 to 54 of S2 and S3 and not other properties of these regions or the bacteria themselves were responsible for PT-mediated adherence to macrophages, PT-coated erythrocytes were substituted in the adherence assay and inhibition of binding of these particles to macrophages by soluble carbohydrates was tested. Preliminary fluorescence-activated cell sorter analysis demonstrated that PT remained associated with erythrocytes in the presence of 50 mM galactose or sialic acid (data not shown), indicating that any decreased binding of PT-coated erythrocytes could not be attributed to loss of PT. The presence of galactose, 2′-fucosyllactose, sialic acid, 3′-sialyllactose, or 6′-sialyllactose inhibited binding of the PT-coated particles, while dextran sulfate, lacto-*N*-fucosylpentaose, or lacto-*N*-tetraose did not (Table 2). To localize carbohydrate inhibition to individual subunits, 50 μg of antibodies to S2 (MAb 9G8) or S3 (MAb 7E10) per ml was incubated with the PT-coated erythrocytes to mask the cognate subunit and adherence was compared to control levels in the absence of antibody; anti-S4 MAb 6G6 served as a control and did not reduce adherence under any circumstances. S3-dependent

adherence, measured in the presence of antibody to S2, was reduced by sialic acid; conversely, S2-dependent adherence, measured in the presence of antibody to S3, was reduced by galactose (Table 2).

Receptors for PT on macrophages. Plating of macrophages on surfaces coated with a variety of antibodies to leukocyte surface determinants is known to sequester these receptors under the cells, leaving the apical surface depleted of receptor (see, for example, references 7, 22, and 23). Several antibodies were assayed for the ability to down modulate receptors necessary for binding of PT-coated erythrocytes to macrophages. Coating of the well surface with antibodies to HLA, CD18, or CD14 decreased binding by <10%. In contrast, one of two antibodies each to the Le^a or Le^x blood group determinants decreased adherence to 47% ± 6% or 58% ± 9% of the control level, respectively (Table 1). These results suggest that the structures recognized by PT are borne by molecules that express the Le^a or Le^x fucosylated polylactosamines.

Although the receptors for PT were not identified by these antibody capture studies, it was noted that the ligation of these receptors affected the amount of adherence mediated by the second bacterial adhesin, FHA. It is known that the interaction between FHA and CD11b/CD18 leads to bacterial uptake and survival in macrophages (16). The effect of treatment of macrophages with PT on CD11b/CD18-dependent adherence was determined. Incubation of macrophages with PT, B oligomer, S2, or S3 enhanced CD11b/CD18-dependent binding as measured by increased adherence of C3bi-coated erythrocytes (Fig. 3); adherence of immunoglobulin G-coated erythrocytes was not affected (data not shown). Similarly, exposure of macrophages to synthetic peptides (1.5 mg/ml) derived from residues 28 to 45 of S2 or S3 enhanced CD11b/CD18-dependent binding (adherence index of 3,171 ± 210 or 3,979 ± 560, respectively). This effect of PT or its subcomponents was also apparent for the adherence of the FHA-bearing bacterium BPTOX6, while adherence of the FHA-deficient bacterium BP101 was not affected (Fig. 3).

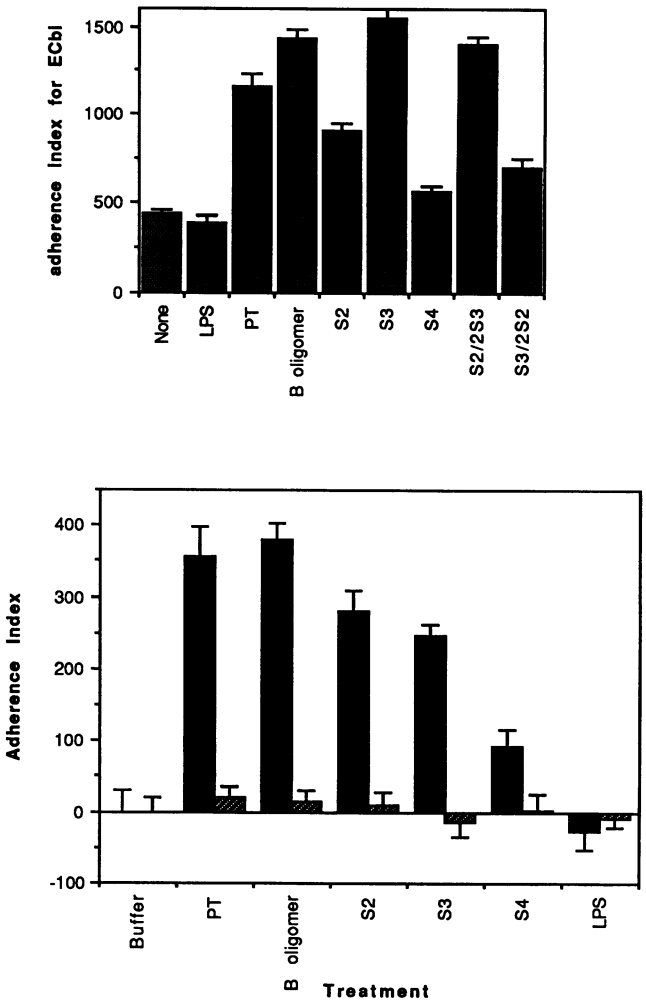

FIG. 3. Enhanced binding to CD11b/CD18 upon treatment of macrophages with PT or its subunits. Macrophages adherent to Terasaki plates were treated with 50 μg of the indicated bacterial components per ml for 30 min and washed. Lipopolysaccharide (LPS) was used as a control at a concentration of 10 μg/ml in order to mimic the residual LPS determined to contaminate the recombinant bacterial preparations. (Top) Macrophages were incubated with C3bi-coated erythrocytes and washed, and the adherence was quantitated visually. (Bottom) Macrophages were incubated with *B. pertussis* BPTOX6 (a strain deficient in PT; solid bars) and BP101 (a strain without functional FHA; hatched bars), and the total number of fluorescent intra- and extracellular bacteria was determined. Direct comparison with wild-type bacteria was not performed since both PT and FHA independently mediate full adherence of bacteria to the macrophages. Values were standardized to zero in buffer. For both panels, the means ± standard deviations from triplicate wells were calculated and data shown are representative of three experiments. Values for BPTOX6 PT, B oligomer, S2, and S3 were each statistically greater than those for S4 or LPS at the $P < 0.01$ level by the Student t test.

DISCUSSION

It has been demonstrated that *B. pertussis* associates with macrophages in vitro and in vivo (9, 16). One adherence mechanism involves the recognition of the leukocyte-restricted integrin CD11b/CD18 by FHA (16). The present studies suggest that bacteria are also bound to macrophages in a carbohydrate-dependent manner by PT. PT-dependent binding of bacteria to respiratory cells, including macrophages, would be a logical target for elimination by agents designed to prevent or treat disease.

PT binds to a wide variety of eukaryotic cells, recognizes several glycoproteins and glycolipids, and produces a number of distinct biological effects. Effects which appear to involve recognition of nonsialylated glycoconjugates include adherence to cilia (16, 17) and rapid elevation of intracellular calcium and phosphotidyl inositol in T cells (10). On the other hand, intoxication of cells by ADP ribosylation appears to involve recognition of sialylated receptors (2, 4, 19, 21). The studies reported here suggest that both of these binding activities can be shown to play a role in adherence of bacteria to human macrophages, a cell type relevant to the process of bacterial elimination in vivo. A number of studies now suggest that it is S2 that mediates recognition of nonsialylated determinants while S3 preferentially binds sialylated determinants (2, 4, 13, 15, 17, 19, 21). Our results indicate that these carbohydrate specificities also apply to the macrophage.

With the production of the B oligomer and its subunits in recombinant inclusion body form (3), the binding properties of the subunits can be further dissected. Although the conformation of the subunit proteins in these preparations is unknown, the preparations are of interest because functional

B oligomer has been assembled from them and they demonstrate binding to carbohydrates consistent with the binding properties of the holotoxin (15). This suggests that until such time as purified material documented to be folded properly is available, these inclusion body forms can serve as useful indicators of properties of the B oligomer and its subunits. Results of competitive inhibition studies using recombinant subunits as presented here indicate that both S3 and S2 mediate PT-dependent bacterial attachment to human macrophages. The fact that their effects were additive suggests that each subunit interacts with a different receptor(s).

The interaction of S3 with macrophages was preferentially inhibited by sialylated glycoconjugates, while that of S2 was blocked by galactose. These findings are consistent with studies of the differential carbohydrate recognition properties of the recombinant subunits (15), native S2-S4 and S3-S4 dimers (21), and antibody mapping studies (13, 14). Using site-directed mutations to create a panel of subunit analogs, the region from 40 to 54 and specifically residues His-47, Arg-50, and Asn-93 were shown to affect the ability of S2 to inhibit PT-dependent attachment in solution. The effects of specific mutagenic changes can support but not prove participation of a region in the function of a protein. It is possible that the loss of ability of a mutant protein to inhibit attachment in this assay is due to an effect of the mutation on the protein structure rather than on the binding properties. We believe this explanation to be less likely since mutations to two different residues were made at each site and both had equivalent effects. Moreover, peptides spanning residues 28 to 45 of each subunit also showed competitive inhibitory activity. These results are consistent with our recent studies indicating the location and essential structural features of the carbohydrate recognition domains of S2 and S3 in other assay systems (15). This concordance supports the conclusion that the eukaryotic-like, C-type lectin domains of these bacterial proteins play an important role in the interaction of *B. pertussis* with human leukocytes.

Although carbohydrate determinants are important to the binding of PT to macrophages, the identity of the molecules bearing these determinants remains unknown. Individually removing the numerically most abundant molecules on the leukocyte surface, HLA, CD11b/CD18, and CD14, did not decrease adherence of PT-coated erythrocytes, a ligand chosen in lieu of whole bacteria in order to maximize specificity for PT. However, it remains possible that PT subunits bind more than one of these leukocyte surface molecules by shared determinants. This is suggested by results with anticarbohydrate antibodies. Down modulation of glycoconjugates sharing the Lewis a or x blood group determinants (fucosylated polylactosamines) decreased adherence by half; this effect was seen for only one of two antibodies against each of these determinants and suggests that a subset of molecules containing these determinants may function as the true receptors. These results can be interpreted to indicate that PT can recognize a variety of molecules sharing Lewis carbohydrate determinants. Two such receptors on T cells may be the 70- and 43-kDa proteins described by Clark and Armstrong (4) and Rogers et al. (10), respectively.

Although PT and FHA can each independently mediate adherence of *B. pertussis* to macrophages (9), PT was found to affect FHA-dependent adherence. Ligation of carbohydrates on the macrophage surface by PT increased binding of particles or bacteria to CD11b/CD18, the receptor for FHA. This effect was localized to the B oligomer and thus was not attributable to cellular intoxication by PT. This cooperative effect between adhesins could result from either of two mechanisms. The receptors for FHA and PT might be topographically close to each other such that binding of FHA on a particle (bacterium or erythrocyte, in these studies) to its receptor brings the particle into proximity with PT on its receptor and promotes cooperative binding. Alternatively, the PT receptors may communicate with the integrin through intracellular signalling events. Rapid signalling within T cells has been shown to occur as a function of the B oligomer (10). This is particularly interesting in light of the recent recognition of the kinship of the B oligomer to the eukaryotic C-type lectin family (15). A subgroup of these lectins, the selectins, contribute to leukocyte transmigration across endothelia and have recently been demonstrated to upregulate integrins on the surfaces of polymorphonuclear leukocytes (6). Recent evidence from our lab suggests that PT S2 and S3 have structural and functional features of the selectins (16a). In this context, it is reasonable to speculate that *B. pertussis* may have co-opted a natural system of communication between the selectin and integrin families of adhesion molecules to optimize attachment to and entry into the human macrophage.

Regardless of the exact mechanism of cross talk between the PT receptor(s) and the integrin, the fact that binding of one adhesin may modulate subsequent binding by a second adhesin illustrates that eukaryotic cell surfaces respond and change as a consequence of interacting with adhesins. Ligation of receptors by cell-free adhesins (e.g., fimbriae and other molecules) must occur frequently in natural disease and underlines the importance of examining adhesins as biological response modifiers with activities beyond simple tissue tropism and colonization.

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REFERENCES

1. **Barnstable, C., W. Bodmer, G. Brown, G. Falgre, C. Milstein, A. Williams, and A. Siegler.** 1978. Production of monoclonal antibodies to group A erythrocytes, HLA and other human cell surface antigens—new tools for genetic analysis. Cell **14**:9–20.
2. **Brennan, M., J. David, J. Kenimer, and C. Manclark.** 1988. Lectin-like binding of pertussis toxin to a 165-kilodalton Chinese hamster ovary cell glycoprotein. J. Biol. Chem. **263**:4895–4899.
3. **Burnette, N., V. Mar, W. Cieplak, C. Morris, K. Kaljot, K. Marchitto, R. Sachdev, C. Locht, and J. Keith.** 1988. Direct expression of Bordetella pertussis toxin subunits to high levels in Escherichia coli. Biotechnology **6**:699–706.

3a. **Burnette, W. N.** Infect. Immun., in press.

4. **Clark, C. G., and G. D. Armstrong.** 1990. Lymphocyte receptors for pertussis toxin. Infect. Immun. **58**:3840–3846.
5. **Lanier, L., M. Arnaout, R. Schwarting, N. Warner, and G. Ross.** 1985. p150,95, third member of the LFA-1/CR3 polypeptide family identified by anti-Leu M5 monoclonal antibody. Eur. J. Immunol. **15**:713–718.
6. **Lo, S. K., S. Lee, R. Ramos, R. Lobb, M. Rosa, R. G. Chi, and S. D. Wright.** 1991. Endothelial-leukocyte adhesion molecule a stimulates the adhesive activity of leukocyte integrin CR3 (CD11b/CD18, Mac-1, alpha m beta 2) on human neutrophils. J. Exp. Med. **173**:1493–1500.
7. **Michl, J., M. Pieczonka, J. Unkeless, G. Bell, and S. Silverstein.** 1983. Fc receptor modulation in mononuclear phagocytes main-

tained on immobilized immune complexes occurs by diffusion of the receptor molecule. J. Exp. Med. **157:**2121–2139.

8. **Nauseef, W. M., R. K. Root, S. L. Newman, and H. L. Malech.** 1983. Inhibition of zymosan activation of human neutrophil oxidative metabolism by a mouse monoclonal antibody. Blood **62:**635–644.
9. **Relman, D., E. Tuomanen, S. Falkow, D. T. Golenbock, K. Saukkonen, and S. D. Wright.** 1990. Recognition of a bacterial adhesin by an integrin—macrophage CR3 (alpha-m-beta-2, CD11b/CD18) binds filamentous hemagglutinin of *Bordetella pertussis*. Cell **61:**1375–1382.
10. **Rogers, T., S. Corey, and P. Rosoff.** 1990. Identification of a 43-kilodalton human T lymphocyte membrane protein as a receptor for pertussis toxin. J. Immunol. **145:**678–683.
11. **Sanchez-Madrid, F., A. Krensky, C. Ware, E. Robbins, J. Strominger, S. Burakoff, and T. Springer.** 1982. Three distinct antigens associated with human T-lymphocyte-mediated cytolysis: LGA-1, LFA-2, and LFA-3. Proc. Natl. Acad. Sci. USA **79:**7489–7493.
12. **Sato, H., Y. Sato, A. Ito, and I. Oshishi.** 1987. Effect of monoclonal antibody to pertussis toxin on toxin activity. Infect. Immun. **55:**909–915.
13. **Schmidt, M., and W. Schmidt.** 1989. Inhibition of pertussis toxin binding to model receptors by antipeptide antibodies directed at an antigenic domain of the S2 subunit. Infect. Immun. **57:**3828–3833.
14. **Schmidt, W., and M. Schmidt.** 1989. Mapping of linear B-cell epitopes of the S2 subunit of pertussis toxin. Infect. Immun. **57:**438–445.
15. **Saukkonen, K., W. N. Burnette, V. Mar, H. R. Masure, and E. Tuomanen.** 1992. Pertussis toxin has eukaryotic-like carbohydrate recognition domains. Proc. Natl. Acad. Sci. USA **89:**118–122.
16. **Saukkonen, K., C. Cabellos, M. Burroughs, S. Prasad, and E. Tuomanen.** 1991. The role of the interaction between *Bordetella pertussis* and macrophages in pulmonary colonization. J. Exp. Med. **173:**1143–1149.

16a. **Tuomanen, E., and W. N. Burnette.** Submitted for publication.

17. **Tuomanen, E., H. Towbin, G. Rosenfelder, D. Braun, G. Hansson, G. Larson, and R. Hill.** 1988. Receptor analogs and monoclonal antibodies which inhibit adherence of Bordetella pertussis to human ciliated respiratory epithelial cells. J. Exp. Med. **168:**267–277.
18. **Tuomanen, E., and A. Weiss.** 1985. Characterization of two adhesions of Bordetella pertussis for human ciliated respiratory epithelial cells. J. Infect. Dis. **153:**118–125.
19. **Tyrell, G., M. Peppler, R. Bonnah, C. Clark, P. Chong, and G. Armstrong.** 1989. Lectinlike properties of pertussis toxin. Infect. Immun. **57:**1854–1857.
20. **Van Voorhis, W. C., R. M. Steinman, L. Sair, J. Luban, M. Witmer, S. Koide, and Z. Cohn.** 1983. Specific antimononuclear phagocyte monoclonal antibodies. J. Exp. Med. **158:**126–145.
21. **Witvliet, M., D. Burns, M. Brennan, J. Poolman, and C. Manclark.** 1989. Binding of pertussis toxin to eucaryotic cells and glycoproteins. Infect. Immun. **57:**3324–3330.
22. **Wright, S., and S. C. Silverstein.** 1982. Tumor-promoting phorbol esters stimulate C3b and C3bi receptor-mediated phagocytosis in cultured human monocytes. J. Exp. Med. **156:**1149–1164.
23. **Wright, S. D., P. E. Rao, W. C. Van Voorhis, L. S. Craigmyle, K. Iida, M. A. Talle, E. F. Westberg, G. Goldstein, and S. Silverstein.** 1983. Identification of the C3bi receptor of human monocytes and macrophages by using monoclonal antibodies. Proc. Natl. Acad. Sci. USA **80:**5699–5703.