Proc. Natl. Acad. Sci. USA
Vol. 88, pp. 345–349, January 1991
Biochemistry

Pertactin, an Arg-Gly-Asp-containing *Bordetella pertussis* surface protein that promotes adherence of mammalian cells

(bacterial adhesin/Arg-Gly-Asp-binding site/bacterial mutant)

ELIZABETH LEININGER*†, MARK ROBERTS‡, JAMES G. KENIMER*, IAN G. CHARLES‡, NEIL FAIRWEATHER‡, PAVEL NOVOTNY‡, AND MICHAEL J. BRENNAN*

*Division of Bacterial Products, Center for Biologics Evaluation and Research, Food and Drug Administration, 8800 Rockville Pike, Bethesda, MD 20892; and ‡Department of Bacteriology, Wellcome Biotech, Beckenham, Kent BR3 3BS, United Kingdom

Communicated by Frederick C. Robbins, October 19, 1990

ABSTRACT **A 69-kDa protein has been identified on the surface of the Gram-negative pathogen *Bordetella pertussis* that can elicit a protective immune response in animal models. This protein is associated with virulent strains of *B. pertussis* but its function has remained unclear. In this report we demonstrate that purified preparations of the 69-kDa outer membrane protein can promote the attachment of Chinese hamster ovary (CHO) cells. The interaction between the mammalian cells and this protein can be specifically inhibited by an Arg-Gly-Asp (RGD)-containing synthetic peptide that is homologous with a region found in the 69-kDa protein sequence. These studies indicate that a specific cell binding site containing an Arg-Gly-Asp sequence may be involved in the interaction of this bacterial protein with mammalian cell surfaces. To further investigate the role of this protein as a bacterial adhesin, a mutant of *B. pertussis* W28 that does not express the 69-kDa protein was constructed using the plasmid vector pRTP1. This mutant was 30–40% less efficient at adhering to CHO cells and to human HeLa cells than was the parent strain. These data support a role for this 69-kDa outer membrane protein in the attachment of *B. pertussis* to mammalian cells. We propose the name "pertactin" for this protein.**

The adherence of *Bordetella pertussis*, the human pathogen that causes whooping cough, to cells in the human respiratory tract appears to involve a number of bacterial proteins that use various mechanisms of attachment. Filamentous hemagglutinin (FHA), a large filamentous protein that is secreted and also found on the surface of *B. pertussis*, has been reported to mediate adherence of the bacteria to ciliated (1) and nonciliated (2) human cells. A recent report has implicated an Arg-Gly-Asp (RGD) sequence in the mechanism of attachment of FHA to eukaryotic cells (3). Another putative *B. pertussis* adhesin, pertussis toxin, binds to cells through a lectin-like mechanism in which carbohydrate moieties on the host cells serve as receptors (4, 5) and evidence has been published demonstrating a role of the toxin in the adherence of *B. pertussis* to ciliated cells (1). Although a number of bacteria have been shown to utilize fimbrial proteins as adhesins (6), there is no evidence, to date, that suggests that fimbrae have a major role in the adherence of *B. pertussis* to mammalian cells.

A 69-kDa outer membrane protein has been identified (7) as a nonfimbrial protein found on all virulent strains of *B. pertussis*. Its expression is modulated by the "vir locus" (8), which controls expression of other *B. pertussis* virulence-associated proteins such as FHA, pertussis toxin, fimbriae, and adenylate cyclase toxin (9). The 69-kDa protein is a protective antigen in animal models for disease caused by *Bordetellae* spp. and induces an immune response in animals (10), including the production of agglutinating antibodies (7). A number of human T-cell clones reactive with *B. pertussis* have also been shown to be directed against this protein (11). Although some evidence suggests that the 69-kDa protein may be associated with the adenylate cyclase toxin of *B. pertussis* (10, 12), to our knowledge, no function for this protein has been described.

The gene that encodes the 69-kDa protein has been identified (13) and DNA-sequence analysis predicts the presence of two RGD sequences, one at residues 225–227 and the second at residues 665–667 in the putative mature protein. The sequence RGD has been identified as the cell-attachment site of various mammalian adhesion proteins such as fibronectin (14), vitronectin (15), fibrinogen, and von Willebrand factor (16). In this report we present evidence that Chinese hamster ovary (CHO) cells adhere to purified preparations of the 69-kDa outer membrane protein of *B. pertussis* and that this interaction involves one of the RGD-containing sites on the protein. Also, studies using a mutant strain of *B. pertussis* that specifically lacks the 69-kDa protein indicate that the 69-kDa protein plays a role in the attachment of the bacteria to mammalian cells. We propose the name "pertactin" [per (pertussis) and tactin (from *tactus* [L], to touch)] for this 69-kDa protein.

MATERIALS AND METHODS

Materials. The 69-kDa surface protein pertactin was purified from *B. pertussis* CN2992 as described (12) and is free of all other surface components such as FHA, pertussis toxin, lipooligosaccharide, and fimbriae, as determined by SDS/PAGE and Western blot analysis with monoclonal antibodies. Monoclonal antibodies BPE3 and BPD8 [which react with pertactin (7)], monoclonal antibody BPG10 [which reacts with lipooligosaccharide A (17)], and the anti-fimbrial monoclonal antibody BPF2 (18) have been described. Monoclonal antibody MO8-X3C, directed against FHA, will be described elsewhere (E.L., P. G. Probst, and J.G.K., unpublished data). Human fibronectin was purchased from Collaborative Research.

Peptides. The following five peptides, corresponding to RGD-containing sequences in fibronectin or pertactin, were synthesized using an Applied Biosystems model 430A peptide synthesizer: (*i*) a hexapeptide from the fibronectin sequence with the amino acid sequence GRGDSP and (*ii*) the corresponding control peptide GRGESP; (*iii*) a 14-amino acid peptide corresponding to the sequence containing the first pertactin RGD, ATIRRGDALAGGAC (termed P1), and (*iv*) the corresponding control peptide ATIRRGEALAGGAC

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviations: RGD, Arg-Gly-Asp; FHA, filamentous hemagglutinin; BSA, bovine serum albumin.

†To whom reprint requests should be addressed.

(termed P3); and (*v*) a 14-amino acid peptide corresponding to the sequence containing the second pertactin RGD, AGYTRGDRGFTGDC (termed P2). Purity of the peptides was verified by HPLC. The carboxyl-terminal cysteine residue on peptides P1, P2, and P3 is not part of the native sequence and was added to facilitate conjugation and immobilization of the peptide. These peptides had no visible effects on the mammalian cells during the course of the inhibition assay, as determined by staining and visual examination of the cells under the microscope.

Cell Attachment Assays. CHO cells were cultured in Ham's F12 medium (S & S Media, Rockville, MD) supplemented with 2 mM glutamine, gentamicin (Flow Laboratories) at 20 μg/ml, and 10% (vol/vol) fetal calf serum (HyClone). The cells were passaged and labeled overnight with [^{3}H]thymidine (New England Nuclear; 6.7 Ci/mmol; 1 Ci = 37 GBq) at 25 μCi per 75-cm^2 flask to a specific activity of 1–2 cpm per cell. The cell attachment assay is a modification of the method described by Ruoslahti *et al.* (19). In brief, 96-well microtiter plates (Costar 3590) were coated with various concentrations of pertactin or fibronectin (60 μl per well) in Dulbecco's phosphate-buffered saline (DPBS) diluted 1:4 with deionized water for 3 hr at room temperature and blocked with 1% bovine serum albumin (BSA; Sigma, A7638) in DPBS for 1 hr. Then 100 μl of attachment buffer [Ham's F12 medium/2 mM glutamine/gentamicin (20 μg/ml)/BSA (2 mg/ml)] was added to each well, followed by 100 μl of [^{3}H]thymidine-labeled CHO cells at 5×10^5 cells per ml. The plates were incubated for 60 min at 37°C and then the wells were washed by gently aspirating the attachment buffer and adding 100 μl of DPBS. Cells that remained attached after two washings were solubilized with 100 μl of 10% (wt/vol) SDS and radioactivity was measured in a Beckman liquid scintillation counter. In selected assays attached cells were visualized after fixation with 3% (wt/vol) paraformaldehyde and staining with toluidine blue.

Inhibition Assays. Peptide or monoclonal antibody inhibition of CHO cell attachment to pertactin or fibronectin was assessed by addition of the peptides or monoclonal antibody purified from ascites fluid as described (7) to the microtiter plate assay wells immediately prior to the addition of the [^{3}H]thymidine-labeled CHO cells. All other aspects of the assay were performed as described above.

Bacteria. *B. pertussis* was grown as described (7) using Bordet–Gengou agar medium (Difco) containing 15% (vol/vol) defibrinated rabbit blood. This growth was used to inoculate flasks containing liquid Cohen–Wheeler medium (S & S Media) and the culture was continued for 24–36 hr with constant agitation. For attachment experiments, bacteria were radiolabeled for 36 hr in Cohen–Wheeler medium containing L-[^{35}S]methionine (Amersham; 1160 Ci/mmol) at 25 μCi/ml to a specific activity of $\approx 10^{-4}$ cpm per organism.

Construction and Characterization of a *B. pertussis* Mutant. A streptomycin-resistant strain (BBC8) was derived from *B. pertussis* W28 (from the Wellcome Biotech culture collection) and a mutant specifically lacking pertactin (BBC9) was generated from strain BBC8 by homologous recombination using the suicide vector pRTP1 (20) carrying a kanamycin-resistance gene and flanking regions of the pertactin gene. The kanamycin-resistant clone BBC9 was selected and demonstrated differences in its Southern blot profile for the pertactin gene were compared to the parent strain. Results of Southern blot analysis indicate that the kanamycin-resistance gene has been inserted into the structural gene of pertactin altering its expression.

Outer membrane preparations of the bacteria were analyzed on Western blots using specific monoclonal antibodies by the following methods. Bacteria were collected by centrifugation (5000 × *g* for 30 min) and were resuspended in 0.1 M Tricine (pH 8) containing 4 M urea and stored at −70°C overnight. The 4 M urea cell extracts were centrifuged at 12,000 × *g* for 10 min and the supernatant protein concentrations were determined using the BCA assay (Pierce). Extract samples were separated on 4–20% polyacrylamide gels containing SDS (Integrated Separation Systems–Enprotech, Hyde Park, MA) and proteins were electroblotted to an Immobilon membrane (Millipore) by the method of Towbin *et al.* (21). The membranes were incubated in DPBS containing 0.1% BRIJ 35 (Sigma) for 1 hr at room temperature and monoclonal antibodies were added at a dilution of 1:1000 for at least 2 hr. The immunoblots were developed as described (7) using alkaline phosphatase-conjugated goat anti-mouse antibody (Southern Biotechnology Associates, Birmingham, AL) and ProtoBlot (Promega Biotec).

A microagglutination assay of intact bacteria (22) using the monoclonal antibody BPE3, directed against pertactin, demonstrated that the parent strain BBC8 was agglutinated by the antibody but not BBC9, indicating that pertactin is not expressed on the surface of BBC9. The amounts of pertussis toxin were determined by the CHO cell clustering assay (4) and found to be the same in supernatants obtained after bacteria cultures were resuspended to the same cell density. The microagglutination assay of intact bacteria (22) using monoclonal antibodies BPG10 and BPF2 showed that both strains expressed similar amounts of lipooligosaccharide A and fimbriae 2. Strains BBC8 and BBC9 expressed similar amounts of FHA as determined by goose erythrocyte hemagglutination using intact bacteria.

Bacterial Attachment Assay. The attachment assay was performed as described by Urisu *et al.* (2) with the following alterations. Confluent monolayers of CHO (ATCC CCL61) or HeLa (ATCC CCL2) cells in 96-well tissue culture plates (Costar) were washed three times with Hanks' balanced salt solution (Flow Laboratories) containing calcium, magnesium, and 0.2% BSA. ^{35}S-labeled *B. pertussis* cells (40,000 cpm per well) were added to the wells and incubated for various periods of time at room temperature with gentle shaking. The wells were washed three times with 200 μl of the same buffer and 100 μl of 10% (wt/vol) SDS was added to each well to solubilize the attached cells. Radioactivity in the samples was measured in a Beckman scintillation counter after dissolving in Hydrofluor (National Diagnostics, Manville, NJ). The results shown are the means of triplicate determinations. Wells were scanned using phase optics to verify that the bacteria were bound to intact cell monolayers.

RESULTS

Adherence of CHO Cells to the Bacterial Protein Pertactin. The adherence of CHO cells to pertactin-coated microwells was compared with CHO cell adherence to wells coated with the known mammalian cell adhesin fibronectin (Fig. 1). CHO cells adhered to both pertactin-coated (Fig. 1*a*) and fibronectin-coated (Fig. 1*b*) substrates. The interaction of cells with fibronectin results not only in the attachment of cells but also in spreading of those cells on the surfaces coated with fibronectin, a function thought to be related to cell migration (23). The extent of cell spreading on fibronectin was more pronounced than that observed on pertactin. The attachment of CHO cells to pertactin-coated surfaces was dose dependent (Fig. 2*a*) and was half-maximal at ≈10 μg/ml. Attachment to fibronectin was half-maximal when wells were coated with 5 μg/ml (Fig. 2*b*). CHO cell binding to pertactin was found to be specific since attachment was inhibited by anti-pertactin monoclonal antibodies (Fig. 2*a*), whereas CHO cell attachment to fibronectin was unaffected by these antibodies (Fig. 2*b*).

Inhibition of Cell Attachment by RGD-Containing Peptides. Cloning and sequencing of pertactin (13) has revealed that it contains RGD sequences. Fig. 3 depicts the predicted relative

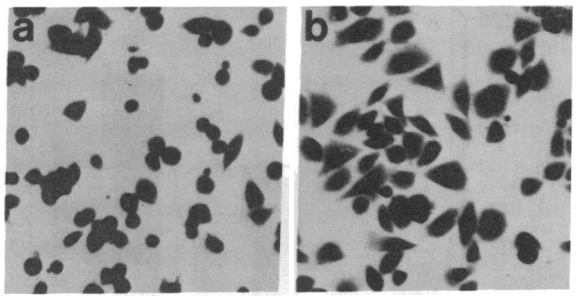

FIG. 1. Attachment of CHO cells to pertactin and to fibronectin. Wells were coated with 60 μl of pertactin (30 μg/ml) (*a*) or fibronectin (10 μg/ml) (*b*) and blocked with BSA. Attached cells were fixed with 3% paraformaldehyde and stained with toluidine blue. Very few cells adhered to control wells coated with BSA alone. (×13.)

position of the two RGD sequences within the pertactin molecule and also shows the sequences of the synthetic pertactin peptides that were used in inhibition studies. An RGD-containing hexapeptide derived from the fibronectin sequence and the corresponding RGE-containing control hexapeptide were also synthesized and used in the inhibition studies. The P1 pertactin peptide (which corresponds to the amino-terminal RGD sequence) and the fibronectin hexapeptide GRGDSP inhibited the attachment of CHO cells to pertactin (Fig. 4*a*). A 4-fold decrease in attachment occurred

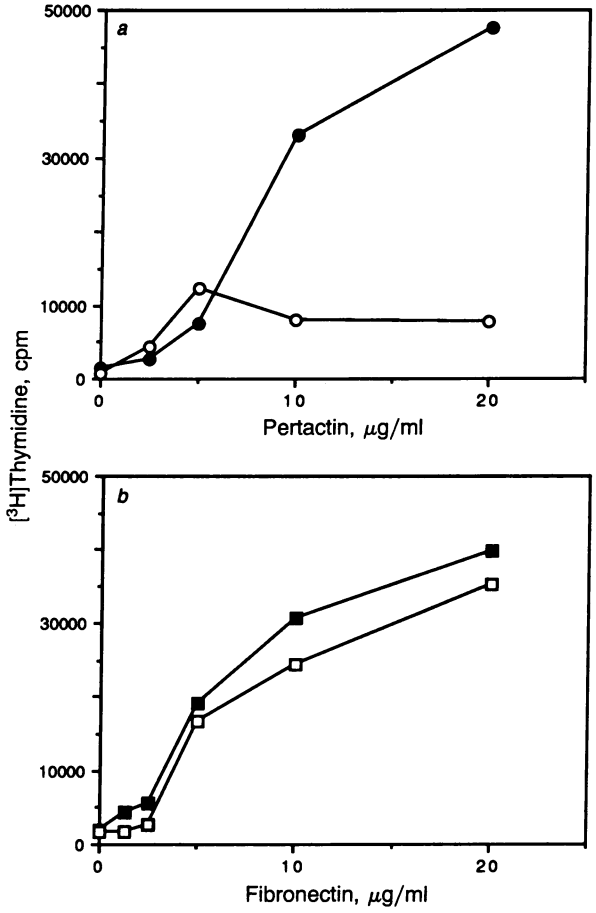

FIG. 2. Dose–response curves of CHO cell attachment to pertactin and to fibronectin and inhibition of attachment by anti-pertactin monoclonal antibodies. Wells were coated with 60 μl of pertactin (*a*) or fibronectin (*b*) at the concentrations shown. CHO cell attachment was measured in the presence (open symbols) or absence (solid symbols) of anti-pertactin monoclonal antibodies BPE3 (100 μg) and BPE8 (100 μg). Data points in cpm are the mean of duplicate wells.

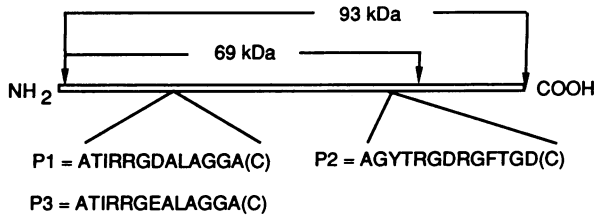

FIG. 3. Relative positions of the pertactin RGD sequences and composition of the synthetic peptides. The 93-kDa precursor protein predicted from molecular cloning of the pertactin gene (13) and the putative cleavage region for the formation of the 69-kDa protein are shown. Peptide P1 extends from amino acids 221 to 233 and peptide P2 from amino acids 661 to 673 of the mature 69-kDa protein. Peptide P3 is identical to peptide P1 with the exception that the aspartic acid (D) has been changed to a glutamic acid (E).

within the initial concentration range of 0.05–0.1 mM for the P1 and GRGDSP peptides. The specificity of this interaction is supported by the finding that a single conservative amino acid change in either of these inhibitory peptides resulted in a dramatic loss of inhibitory activity (see Fig. 4*a*, peptides P3 and GRGESP). The inhibition of adherence of CHO cells to pertactin at 0.1 mM peptide was statistically significant for P1 compared to P3 ($P < 0.02$) and for GRGDSP compared to GRGESP ($P < 0.03$) by using the Student's *t* test. The P2

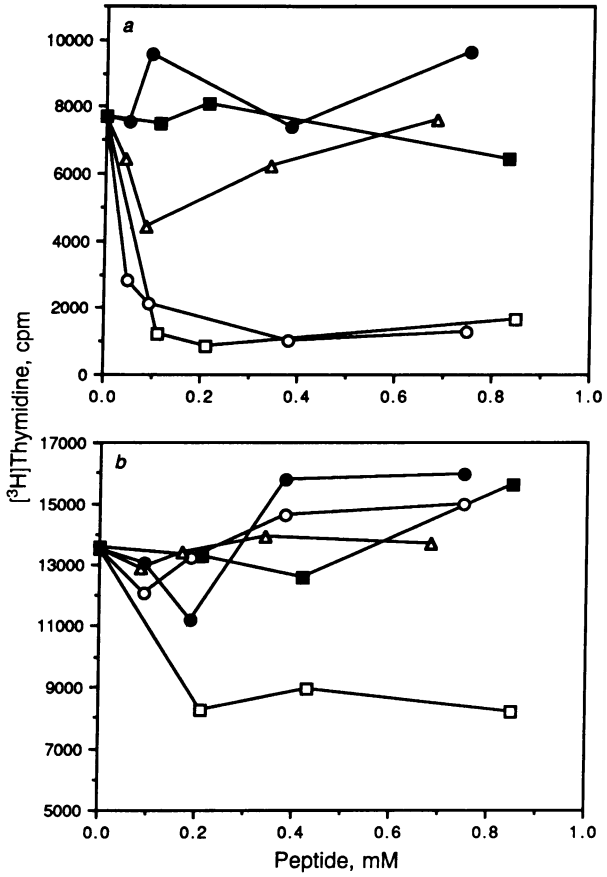

FIG. 4. Inhibition of CHO cell attachment by synthetic peptides. Peptides were added at various concentrations to wells coated with pertactin (20 μg/ml) (*a*) or fibronectin (10 μg/ml) (*b*) followed by the addition of [3H]thymidine-labeled CHO cells. The peptides used were the fibronectin sequence GRGDSP (open squares) and its control peptide GRGESP (solid squares) and the pertactin peptides P1 (open circles), P2 (open triangles), and P3 (solid circles). Data points are the mean of duplicate wells. Standard deviations were all within 18% of the mean. These results have been verified in duplicate assays.

pertactin peptide (which corresponds to the carboxyl-terminal RGD sequence) demonstrated some inhibition at low concentrations but the effect was not dose dependent (Fig. 4*a*). The fibronectin peptide GRGDSP specifically inhibited the adherence of CHO cells to fibronectin (Fig. 4*b*) whereas neither of the two pertactin RGD-containing peptides nor the two control RGE-containing peptides were able to inhibit attachment.

Attachment of Bacteria to Mammalian Cells. To examine the putative role of pertactin in promoting interactions of *B. pertussis* with mammalian cells, BBC9, a mutant that does not express pertactin, was constructed in the virulent *B. pertussis* W28, which was selected to be streptomycin-resistant (BBC8). The lack of expression of pertactin by BBC9 was verified by Western blots using the monoclonal antibody BPE3, directed against pertactin (Fig. 5, lane 1). Strain BBC8 produced pertactin (Fig. 5, lane 2) and strains BBC9 and BBC8 expressed the *B. pertussis* adhesin FHA, as demonstrated by Western blots using the monoclonal antibody MO8-X3C (Fig. 5, lanes 3 and 4). This antibody recognizes a 220-kDa band and another band at ≈98 kDa that is thought to be a degradation product of the mature FHA protein. The expression of other bacterial antigens such as pertussis toxin, fimbriae 2, and lipooligosaccharide A was shown to be the same for both the mutant and the parent strain. The adherence of the mutant strain to CHO cells was ≈34% less than that of the parent strain after a 1-hr incubation period and 30% less after 2 hr (Fig. 6*a*). When the abilities of the mutant and parent strains to bind to monolayers of human HeLa cells were compared the adherence of the mutant was found to be 34% less than that of the parent at 1 hr of incubation and 47% less at 2 hr (Fig. 6*b*). Pertactin mutants constructed in another *B. pertussis* strain, CN2992, gave similar results (data not shown).

DISCUSSION

We have demonstrated that the *B. pertussis* outer membrane protein, pertactin, is capable of functioning as an adhesin for CHO cells and that the attachment of mammalian cells to purified pertactin involves the participation of an RGD-containing sequence in the pertactin molecule. The RGD sequence functions as a cell attachment site on various mammalian attachment proteins (23), on proteins produced by parasites (24), and on viruses (25). An RGD sequence found in the *B. pertussis* protein FHA has been shown to be involved in the interaction of the bacteria with macrophages (26). Although the pertactin protein apparently contains two RGD sequences, the differences observed in the inhibitory abilities of the pertactin RGD peptides P1 and P2 strongly suggest that the amino-terminal RGD triplet, and not the carboxyl-terminal RGD triplet, is part of the cell binding site. These results also indicate that the amino acids flanking an RGD sequence may affect its ability to function as part of a cell attachment site, as has been suggested (27). The finding that peptide P1 inhibited adherence of CHO cells to pertactin, but not to fibronectin, suggests that there may be different receptors on CHO cells for these two attachment proteins. The ability of the fibronectin RGD hexapeptide to inhibit CHO cell attachment to both fibronectin and pertactin may be due to its ability to interact with a broader range of RGD receptors on the cell surface due to the absence of specificity introduced by flanking amino acids.

The role of pertactin as a bacterial adhesin is additionally supported by our results that demonstrate that a *B. pertussis* mutant that does not express pertactin (BBC9) but does express other virulence-associated proteins, such as FHA, pertussis toxin, and fimbriae, adheres less well than the parent wild-type strain (BBC8) to both CHO and HeLa cells. The 30–40% decrease in bacterial adherence observed for

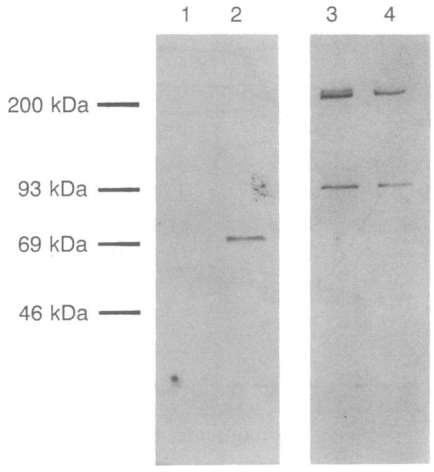

FIG. 5. Western blot of extracts of *B. pertussis* BBC8 and BBC9. Urea extracts (15 μg of protein per lane) of the *B. pertussis* BBC9 pertactin-minus mutant strain (lanes 1 and 3) and BBC8 parent strain (lanes 2 and 4) were separated on a 4–20% polyacrylamide gradient gel containing SDS and transferred to Immobilon filters. Proteins were detected using the anti-pertactin monoclonal antibody BPE3 (lanes 1 and 2) and the anti-FHA monoclonal antibody MO8-X3C (lanes 3 and 4). Positions of molecular mass standards are shown to the left.

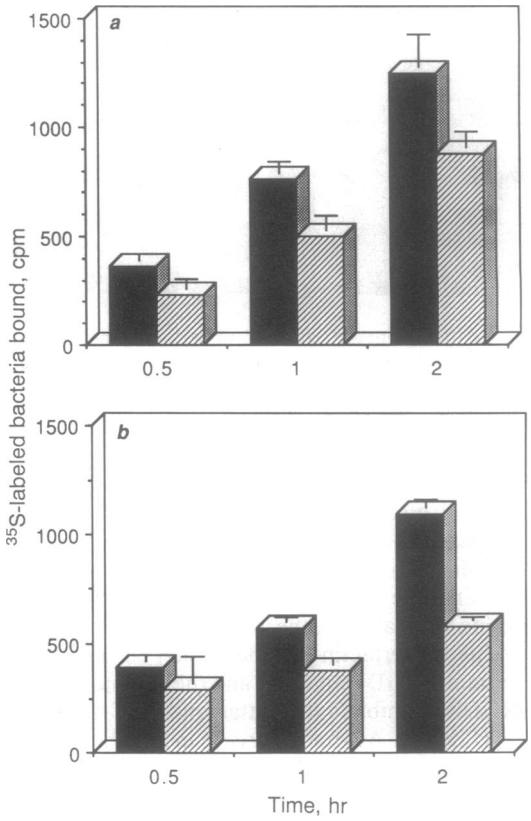

FIG. 6. Adherence of *B. pertussis* to mammalian cells. ^{35}S-labeled *B. pertussis* BBC8 (solid bars) and the BBC9 pertactin-minus mutant (hatched bars) were incubated with monolayer cultures of CHO (*a*) or HeLa (*b*) cells. Adhered bacteria were measured at 0.5, 1, and 2 hr. When bacteria were added to plastic wells alone, 82 cpm at 0.5 hr, 129 cpm at 1 hr, and 252 cpm at 2 hr were obtained and have been subtracted from the values shown. The determinations represent the mean of triplicate wells and standard deviation bars are shown. The decrease in attachment of BBC9 compared to BBC8 was statistically significant at all time points with CHO cells ($P < 0.02$) and at 1 and 2 hr with HeLa cells ($P < 0.001$) using Student's *t* test.

BBC9 suggests, however, that *B. pertussis* also attaches to these cells by other mechanisms. Our unpublished observations indicate that most of the remaining attachment of the BBC9 to mammalian cells occurs through FHA, a *B. pertussis* protein shown (2) to function as a bacterial adhesin. DNA sequence analysis has predicted (3) the presence of RGD sites in FHA and it has been reported (3) that a *B. pertussis* mutant with a 2.4-kilobase deletion in the FHA gene lacks one of these RGD sites and fails to adhere to mammalian cells. Many proteins that contain the RGD sequence in their cell binding domains recognize a family of specific cellular receptors called integrins (23). Recent results have implicated an RGD sequence in the attachment of FHA to integrins present on macrophages (26). We are experimentally testing the hypothesis that the receptor on eukaryotic cells for pertactin may belong to the integrin family.

At present it is not known whether pertactin plays a role in the attachment of *B. pertussis* to ciliated human cells as has been suggested for both FHA and pertussis toxin (1). Pertactin could potentially mediate the interaction of the bacteria with integrins that are present on macrophages and leukocytes (28, 29) or play a role in the recently described process of invasion of mammalian cells by *Bordetella* spp. (30, 31). An outer membrane protein similar to pertactin is present on both *Bordetella bronchiseptica* and *Bordetella parapertussis* (7, 32). These proteins exhibit similar molecular masses on SDS/PAGE (≈69 kDa) and are antigenically cross reactive (7); therefore, they may also promote adherence of the related *Bordetella* spp. to host tissues.

In this report we have provided evidence that the 69-kDa outer membrane protein of *B. pertussis* promotes mammalian cell attachment and may play a role in the interaction of the bacteria with host cells. We propose to name this molecule pertactin based on its function as an adhesin of *B. pertussis*. Our results, in combination with previous reports that have shown that this protein can elicit a substantial immune response in humans (11) and mice (10) and can function as a protective antigen in animal models (33), suggest that this molecule should be seriously considered for inclusion in an acellular pertussis vaccine.

We thank Dr. Kenneth Seamon and Ms. Judy Regan (Laboratory of Molecular Pharmacology, Center for Biologics Evaluation and Research, Food and Drug Administration, Bethesda, MD) for the synthesis of the five peptides used in this study.

1. Tuomanen, E. & Weiss, A. (1985) *J. Infect. Dis.* **152,** 118–125.
2. Urisu, A., Cowell, J. L. & Manclark, C. R. (1986) *Infect. Immun.* **52,** 695–701.
3. Relman, D. A., Domenighini, M., Tuomanen, E., Rappuoli, R. & Falkow, S. (1989) *Proc. Natl. Acad. Sci. USA* **86,** 2637–2641.
4. Brennan, M. J., David, J. L., Kenimer, J. G. & Manclark, C. R. (1988) *J. Biol. Chem.* **263,** 4895–4899.
5. Tuomanen, E., Towbin, H., Rosenfelder, G., Braun, D., Larson, G., Hansson, G. C. & Hill, R. (1988) *J. Exp. Med.* **168,** 267–277.
6. Beachey, E. H. (1981) *J. Infect. Dis.* **143,** 325–345.
7. Brennan, M. J., Li, Z. M., Cowell, J. L., Bisher, M. E., Steven, A. C., Novotny, P. & Manclark, C. R. (1988) *Infect. Immun.* **56,** 3189–3195.
8. Stibitz, S., Aaronson, W., Monack, D. & Falkow, S. (1989) *Nature (London)* **338,** 266–268.
9. Weiss, A. A. & Hewlett, E. L. (1986) *Annu. Rev. Microbiol.* **40,** 661–686.
10. Novotny, P., Chubb, A. P., Cownley, K., Montaraz, J. A. & Beesley, J. E. (1985) *Dev. Biol. Stand.* **61,** 27–41.
11. De Magistris, M. T., Romano, M., Nuti, S., Rappuoli, R. & Tagliabue, A. (1988) *J. Exp. Med.* **168,** 1351–1362.
12. Novotny, P., Chubb, A. P., Cownley, K. & Montaraz, J. A. (1985) *Infect. Immun.* **50,** 199–206.
13. Charles, I. G., Dougan, G., Pickard, D., Chatfield, S., Smith, M., Novotny, P., Morrissey, P. & Fairweather, N. F. (1989) *Proc. Natl. Acad. Sci. USA* **86,** 3554–3558.
14. Pierschbacher, M. D. & Ruoslahti, E. (1984) *Nature (London)* **309,** 30–33.
15. Pytela, R., Pierschbacher, M. D. & Ruoslahti, E. (1985) *Proc. Natl. Acad. Sci. USA* **82,** 5766–5770.
16. Plow, E. F., Pierschbacher, M. D., Ruoslahti, E., Marguerie, G. A. & Ginsberg, M. H. (1985) *Proc. Natl. Acad. Sci. USA* **82,** 8057–8061.
17. Li, Z. M., Cowell, J. L., Brennan, M. J., Burns, D. L. & Manclark, C. R. (1988) *Infect. Immun.* **56,** 699–702.
18. Li, Z. M., Brennan, M. J., David, J. L., Carter, P. H., Cowell, J. L. & Manclark, C. R. (1988) *Infect. Immun.* **56,** 3184–3188.
19. Ruoslahti, E., Hayman, E. G., Pierschbacher, M. D. & Engvall, E. (1982) *Methods Enzymol.* **82,** 803–831.
20. Stibitz, S., Black, W. & Falkow, S. (1986) *Gene* **50,** 133–140.
21. Towbin, H., Staehelin, T. & Gordon, J. (1979) *Proc. Natl. Acad. Sci. USA* **76,** 4350–4354.
22. Manclark, C. R., Meade, B. D. & Burstyn, D. G. (1986) in *Manual of Clinical Laboratory Immunology*, eds. Rose, N. R., Friedman, H. & Fahey, J. L. (Am. Soc. Microbiol., Washington, DC), 3rd Ed., pp. 388–394.
23. Ruoslahti, E. & Pierschbacher, M. D. (1987) *Science* **238,** 491–497.
24. Ouaissi, M. A. (1988) *Parasitol. Today* **4,** 169–173.
25. Fox, G., Parry, N. R., Barnett, P. V., McGinn, B., Rowlands, D. J. & Brown, F. (1989) *J. Gen. Virol.* **70,** 625–637.
26. Relman, D., Tuomanen, E., Falkow, S., Golenbock, D. T., Saukkonen, K. & Wright, S. D. (1990) *Cell* **61,** 1375–1382.
27. Ruoslahti, E. & Pierschbacher, M. D. (1986) *Cell* **44,** 517–518.
28. Corbi, A. L., Kisttimoto, T. K., Miller, L. J. & Springer, T. A. (1988) *J. Biol. Chem.* **263,** 12403–12411.
29. Hemler, M. E. (1988) *Immunol. Today* **9,** 109–113.
30. Ewanowich, C. A., Sherburne, R. K., Man, S. F. P. & Peppler, M. S. (1989) *Infect. Immun.* **57,** 1240–1247.
31. Evanowich, C. A., Melton, A. R., Weiss, A. A., Sherburne, R. K. & Peppler, M. S. (1989) *Infect. Immun.* **57,** 2698–2704.
32. Montaraz, J. A., Novotny, P. & Ivanyi, J. (1985) *Infect. Immun.* **47,** 744–751.
33. Shahin, R. D., Brennan, M. J., Li, Z. M., Meade, B. D. & Manclark, C. R. (1990) *J. Exp. Med.* **171,** 63–73.