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Virulence Factors Determine Attachment and Ingestion of Nonopsonized and Opsonized *Bordetella pertussis* by Human Monocytes

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In the present study, the role of virulence factors in and the effect of opsonization on the interactions between *Bordetella pertussis* and human monocytes were investigated. The methods used facilitated the distinction between attachment and ingestion of bacteria by monocytes. Nonopsonized virulent *B. pertussis* cells attached to monocytes. Nonopsonized *B. pertussis* mutant strains deficient in filamentous hemagglutinin, fimbriae, or pertactin exhibited a reduced adherence to monocytes compared with that of their respective parental strains. Nonopsonized avirulent *B. pertussis* cells did not attach to monocytes. These results led to the conclusion that fimbriae and pertactin are involved in the adherence of nonopsonized virulent *B. pertussis* cells to monocytes and confirm the role of filamentous hemagglutinin in this process. In the absence of opsonins, about 40% of the monocyte-associated virulent *B. pertussis* cells were ingested. When *B. pertussis* cells were preopsonized with inactivated normal serum, about 50% of the monocyte-associated virulent *B. pertussis* cells were phagocytosed and about 80% of the monocyte-associated avirulent *B. pertussis* cells were ingested. These results indicate that virulence factors inhibit opsonin-mediated ingestion of *B. pertussis* by monocytes.

Bordetella pertussis is a gram-negative coccobacillus that causes whooping cough (pertussis), an infection of the respiratory tract. In nonimmune individuals not treated with antibiotics, such an infection can persist for prolonged periods. *B. pertussis* produces a number of virulence factors, the simultaneous expression of which is positively regulated by a locus designated *bvg* (*vir*) (41). These virulence factors are believed to play an important role in both colonization of the respiratory tract and resistance to host defense mechanisms. To date, three virulence factors, namely, filamentous hemagglutinin (FHA), pertussis toxin (PT), and a 69-kDa protein (pertactin), have been shown to mediate the adherence of *B. pertussis* to various mammalian cell lines and human ciliated epithelial cells (22, 23, 29, 31, 36, 37); adherence to human monocyte-derived macrophages can occur via FHA and PT (30, 31). In addition to the mediation of adherence, some virulence factors act as toxins. PT and adenylate cyclase (AC) can inhibit several phagocytic cell functions (5, 9, 11, 18, 26) and may thus contribute to the intracellular survival of the bacteria.

The main host defense mechanism against microorganisms is phagocytosis and subsequent intracellular killing. During an infection of the respiratory tract, alveolar macrophages and exudate macrophages, both of which are monocyte derived (1, 2), play an important role in the host defense. There is increasing evidence that *B. pertussis* can invade and survive in mouse macrophages (4), human monocyte-derived macrophages (12, 34), and human granulocytes (35). The mechanisms of the phagocytosis and killing of *B. pertussis* are not yet fully understood. Receptors on the surface of monocytes that are involved in adherence of nonopsonized *B. pertussis* include complement receptor type 3, which has been shown to bind FHA (30), and very late antigen 5, whose putative ligand on *B. pertussis* is unknown (17).

Preliminary studies demonstrated that during incubation of nonopsonized virulent *B. pertussis* cells with monocytes in suspension, the number of bacteria in the supernatant decreased; similar experiments with avirulent *B. pertussis* cells showed no decrease in the number of bacteria. These results indicate that the association of nonopsonized *B. pertussis* with monocytes requires virulence factors. During incubation of monocytes with avirulent *B. pertussis* cells that were preopsonized with inactivated normal human serum, the number of bacteria decreased significantly, presumably as a result of phagocytosis. However, the assay used has the limitation that it cannot discern between attachment of the bacteria to the cell surface and phagocytosis. Therefore, other methods were adapted for *B. pertussis* which allow differentiation between cell-adherent and ingested bacteria. The aim of the present study was to investigate the role of various virulence factors and the effect of opsonization on the attachment and ingestion of *B. pertussis* by human monocytes.

MATERIALS AND METHODS

Bacteria. The *B. pertussis* strains used in this study were the parental virulent strains BP338, 18323, and Wellcome 28 (W28) and mutants derived from these strains, which are listed in Table 1. *B. pertussis* cells were cultured in Verwey medium (39) in gently shaken flasks at 37°C. The bacteria were harvested from overnight mid-log-phase cultures, washed twice with Hanks balanced salt solution containing 0.1% gelatin and 10 mM *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid (HEPES) (gelatin-HBSS), and suspended in gelatin-HBSS before use. Serial dilutions of the bacterial suspensions were applied to Bordet-Gengou agar plates (Difco Laboratories, Detroit, Mich.) supplemented with 15% sheep blood. Four days later, the number of CFU was determined. In one

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TABLE 1. *B. pertussis* strains used in this study

Strain	Relevant phenotype or genotype	Source or reference
Tohama derivatives		
BP338	Nalidixic acid-resistant derivative of Tohama I (wt)[a]	42
BP536	Streptomycin-resistant derivative of BP338 (wt)	29
BPTOX6	Contains a deletion in the pertussis toxin operon (PT^-)	29
BP349	*hly-2*::Tn*5*, deficient in secreted AC and hemolysin (AC/HLY^-)	14, 42
BP101	*fhaBU101*, defective in filamentous hemagglutinin (FHA^-)	29
B52	*fim2*::*SacI fim3*::*kan*, deficient in serotype 2 and 3 fimbriae ($Fim2^-$ $Fim3^-$); decreased FimD expression	25
BP347	*vir-1*::Tn*5* (Vir^-)	42
18323 and derivatives		
18323	Mouse challenge strain for vaccine potency test (wt)	ATCC 9797
SK8	*vag-8*::Tn*phoA*, deficient in 95-kDa protein ($95K^-$)	10, 19
SK34	*vag-34*::Tn*phoA*, deficient in 93-, 91-, 62-, and 30-kDa outer membrane proteins (OMP^-)	10, 19
W28 and derivatives		
W28	Wellcome 28 (wt)	
B176	*fimD*::Tn*phoA*, deficient in minor fimbrial subunit ($FimD^-$), has decreased Fim2/Fim3 and FHA expression	44
B172	*fimB*::*kan*, deficient in minor and major fimbrial subunits	44
BBC9	BBC8*prn*::Km, deficient in pertactin (Prn^-)	32

[a] wt, wild type.

experiment bacteria were killed by incubation of 10^8 *B. pertussis* BP338 per ml with 1% formaldehyde in gelatin-HBSS for 30 min at room temperature followed by two washes.

Opsonization. Human serum obtained from one healthy donor (normal serum) and a pool of sera obtained from pertussis patients (immune serum) were used. The levels of immunoglobulin G (IgG) antibodies against *B. pertussis* antigens in serum were determined by enzyme-linked immunosorbent assay (ELISA) as described previously (27). Briefly, microtiter plates were coated with 2×10^9 whole cells of *B. pertussis* per ml or with 2 μg of purified *B. pertussis* antigens (FHA, PT, serotype 2 or 3 fimbriae, or lipopolysaccharide [28]) per ml, washed, and incubated with 100-fold-diluted serum. Next, the plates were washed and incubated with 5,000-fold-diluted horseradish peroxidase-conjugated sheep anti-human IgG antibodies (National Institute of Health and Environmental Protection, Bilthoven, The Netherlands) and washed again. Tetramethylbenzidine (Sigma) was used as the substrate, and the optical density at 450 nm of each sample was determined. Since fresh human serum is bactericidal for both virulent and avirulent *B. pertussis* but heat-inactivated serum is not (data not shown), the latter was used for opsonization. Serum was heat inactivated by incubation at 56°C for 30 min. *B. pertussis* was preopsonized by incubating 10^7 bacteria per ml for 30 min at 37°C in gelatin-HBSS containing 10% heat-inactivated serum. After two washes with gelatin-HBSS, the bacteria were resuspended in gelatin-HBSS at a concentration of 10^7 bacteria/ml.

FITC labeling of the bacteria. *B. pertussis* cells were labeled with fluorescein isothiocyanate (FITC; Sigma Chemical Co., St. Louis, Mo.) as described previously (45) with minor modifications by incubating 10^8 bacteria per ml in a solution of 1 mg of FITC per ml, 50 mM sodium carbonate, and 100 mM NaCl (pH 9.0) for 20 min at room temperature and then washing the bacteria three times with phosphate-buffered saline (PBS) containing 3 mM glucose, 0.3 U of aprotinin per ml, and 0.05% human serum albumin or gelatin-HBSS before use.

Cells. Mononuclear cells were isolated from buffy coats, obtained from healthy donors, by Ficoll-Hypaque differential centrifugation (3). Mononuclear cells of the interface layer, which contains about 30% monocytes and 70% lymphocytes, were harvested, washed four times with ice-cold PBS containing 0.5 U of heparin per ml, and suspended in ice-cold gelatin-HBSS. More than 95% of the cells remained viable after the isolation procedure, as determined by trypan blue exclusion.

Assay of the attachment to adherent monocytes. To determine the attachment of *B. pertussis* to the surface of adherent human monocytes, a previously described assay (30) was used with minor modifications. In each well of Terasaki plates (Greiner Labortechnik, Frickenhausen, Germany), 5×10^3 monocytes in PBS containing 3 mM glucose, 0.3 U of aprotinin per ml, and 0.05% human serum albumin were allowed to adhere for 2 h at 37°C, and then the nonadherent cells were removed by three washes with PBS. Next, 5×10^5 FITC-labeled bacteria in PBS containing 3 mM glucose, 0.3 U of aprotinin per ml, and 0.05% human serum albumin were added to each well and incubated for 30 min at 37°C. After five washes with PBS to remove nonattached bacteria, the plates were viewed under a fluorescence microscope (at a magnification of ×500) and the number of bacteria attached to 100 monocytes was determined. All of these experiments were performed in quadruplicate.

Localization of monocyte-associated fluorescent *B. pertussis* cells. Equal volumes containing 5×10^7 FITC-labeled *B. pertussis* cells per ml and 1×10^7 monocytes per ml of gelatin-HBSS were incubated for 90 min at 37°C under rotation (4 rpm). Preliminary studies have led to use of a bacterium-to-monocyte ratio of 5:1 because it gave the optimal readout. Bacteria not associated with cells were removed by differential centrifugation at $110 \times g$ for 4 min, and the cells were resuspended at a concentration of 10^7 monocytes per ml of gelatin-HBSS. Next, ethidium bromide (Calbiochem-Behring, La Jolla, Calif.) was added at a concentration of 50 μg/ml to quench the fluorescence of extracellular bacteria (7,

8, 20). The samples were viewed directly under a fluorescence microscope at a magnification of ×1,000. In the absence of ethidium bromide, FITC-labeled *B. pertussis* cells emit green fluorescence, which is visible with a barrier long-pass filter (365 nm). After addition of ethidium bromide, which does not enter live cells (7, 8), the green fluorescence of extracellular bacteria is quenched and they emit red fluorescence, which is visible with a 365-nm filter or with a band-pass filter (546 nm; width, 12 nm). In each sample, 50 positive monocytes, i.e., those which were associated with one or more bacteria, were examined. The mean numbers of green-fluorescing bacteria, i.e., those localized intracellularly, and quenched bacteria, i.e., those on the monocyte surface, per monocyte were determined. To inhibit ingestion of bacteria, mononuclear cells were pretreated for 5 min at 37°C with 10 μg of cytochalasin B per ml (Sigma) (6) dissolved in dimethyl sulfoxide at a final concentration of 0.5% (vol/vol); at this concentration cytochalasin B did not affect the viability of the cells as determined by trypan blue exclusion or the viability of the bacteria as determined by a microbiological assay.

FACS analysis of the association of *B. pertussis* with mononuclear cells. Nonopsonized virulent *B. pertussis* BP338 cells were labeled with 0.1 mg of FITC per ml as described above. Equal volumes containing 5×10^7 FITC-labeled *B. pertussis* cells per ml and 1×10^7 monocytes per ml of gelatin-HBSS were incubated for 90 min at 37°C under rotation (4 rpm). Non-cell-associated bacteria were removed by differential centrifugation for 4 min at $110 \times g$, and the cells were examined by fluorescence-activated cell sorter (FACS) analysis with a FACScan (Becton Dickinson, San Jose, Calif.) equipped with an argon-ion laser (excitation wavelength, 488 nm; laser power, 15 mW) and a band-pass filter of 530 nm (width, 30 nm). The fluorescence intensity of monocytes and lymphocytes was measured separately by gating these cells in the forward-sideward scatter.

Statistical analysis. Significant differences were assessed by means of the Mann-Whitney two-sample test, unless otherwise stated.

RESULTS

Role of virulence factors in the attachment of *B. pertussis* to adherent monocytes. To investigate the role of virulence factors in the interactions between *B. pertussis* and monocytes, the attachment of several mutant *B. pertussis* strains to adherent monocytes was analyzed. The results showed that virulent (strain BP338) but not avirulent (strain BP347) *B. pertussis* cells were able to attach to adherent monocytes (Fig. 1a). The attachment of strains deficient in PT (BPTOX6), extracellular AC and hemolysin (BP349), or the fimbrial major subunits (B52) (Fig. 1a) was similar to that of the parental virulent strain (BP338). Adhesion of a strain defective in FHA (BP101) was considerably reduced in comparison with that of the parental strain (BP338) (Fig. 1a). Strains lacking the 95-kDa protein (SK8) or outer membrane proteins (SK34) attached to the same extent as did their parental virulent strain (18323) to adherent monocytes (Fig. 1b). The attachment of a strain defective in pertactin (BBC9) or in the minor fimbrial subunit FimD (B176) to monocyte surfaces was reduced in comparison with that of their parental virulent strain (W28) (Fig. 1c). In separate experiments, binding of strain B172, which completely lacks minor and major fimbrial subunits, to monocytes was significantly ($P < 0.05$; $n = 4$) reduced (number of bacteria bound to 100 monocytes, 23.3 ± 8.0) compared with that of its parental strain, W28 (number of bound bacteria, 38.2 ± 12.9).

Localization of *B. pertussis* associated with monocytes in

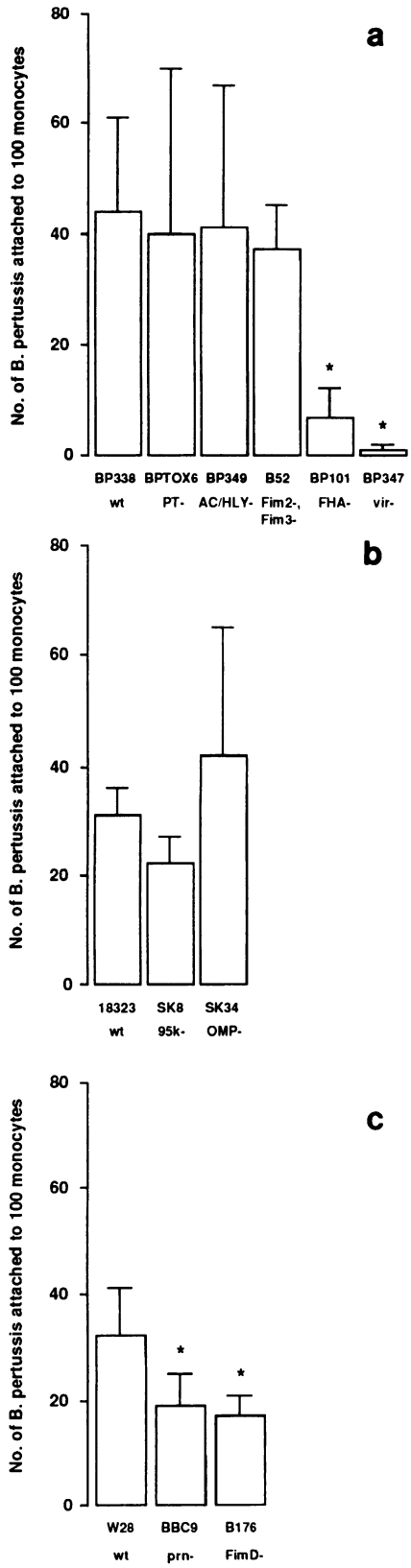

FIG. 1. Role of virulence factors in the attachment of *B. pertussis* to adherent monocytes. Nonopsonized *B. pertussis* mutant strains deficient in various virulence factors and derived from parental strain BP338 (a), 18323 (b), or W28 (c) were allowed to attach to adherent monocytes. Values are the means ± standard deviations of at least four experiments; an asterisk indicates a significant difference ($P < 0.05$) from the respective parental strain. wt, wild type.

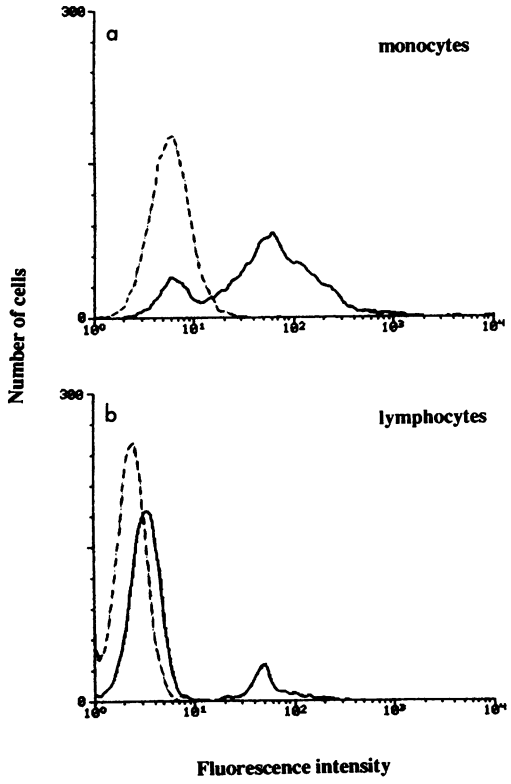

FIG. 2. FACS analysis of the association of *B. pertussis* with monocytes (a) and lymphocytes (b). A suspension of mononuclear cells was incubated with FITC-labeled nonopsonized virulent *B. pertussis* BP338 (solid line) or, as a control, with unlabeled nonopsonized virulent *B. pertussis* (dotted line) for 90 min at 37°C. The results represent the fluorescence intensity of 5×10^3 cells in one representative experiment.

suspension. To study the localization of *B. pertussis* associated with monocytes, FITC-labeled bacteria were incubated with mononuclear cells in suspension. First, the association of *B. pertussis* with monocytes and lymphocytes was quantitated by incubation of mononuclear cell suspensions for 90 min with FITC-labeled nonopsonized virulent *B. pertussis* BP338 and subsequent analysis by FACS. The results revealed that within a mononuclear cell suspension, most (92.4% ± 11.6%; $n = 7$) of the monocytes but only a few (8.3% ± 5.1%; $n = 7$) of the lymphocytes expressed increased fluorescence, compared with the results for control cells incubated with unlabeled bacteria (Fig. 2).

TABLE 3. Relative levels of IgG antibodies against *B. pertussis* in serum

Antigen coating	OD_{450}[a] for:	
	Normal serum	Immune serum
Virulent *B. pertussis*[b]	0.754	1.761
Avirulent *B. pertussis*[b]	0.575	1.353
FHA	0.280	1.619
PT	0.306	2.135
Fimbriae, serotype 2	1.121	2.424
Fimbriae, serotype 3	0.460	1.970
LPS[c]	1.928	2.290

[a] Data are expressed as optical densities at 450 nm (OD_{450}) determined by ELISA. In the absence of serum, when only the conjugated antibody was added, optical densities ranged between 0.09 and 0.12 for all antigens used.
[b] Whole cells of virulent *B. pertussis* BP536 or avirulent *B. pertussis* BP347 were used.
[c] LPS, lipopolysaccharide.

After quenching with ethidium bromide, intracellular *B. pertussis* organisms, which remained clearly fluorescent green, could be distinguished from extracellularly attached bacteria, which were red, by fluorescence microscopy. A major proportion of the nonopsonized virulent *B. pertussis* BP338 cells were attached to the surface of the monocytes; 38% of these bacteria were ingested (Table 2). After opsonization of virulent *B. pertussis* cells with normal serum, 47% of the monocyte-associated bacteria were intracellular (Table 2). When virulent *B. pertussis* cells were opsonized with immune serum, which contained higher levels of IgG antibodies against *B. pertussis*, various purified virulence factors, and lipopolysaccharide than normal serum did (Table 3), the number of bacteria per monocyte amounted to 5.2 ± 0.2 ($n = 5$), of which 61% were localized intracellularly, which was significantly higher ($P < 0.05$) than after opsonization with normal serum.

Treatment of the monocytes with cytochalasin B partially inhibited the numbers of both intracellularly localized nonopsonized and normal serum-opsonized virulent *B. pertussis* cells and had no effect on the numbers of extracellularly attached bacteria (Table 2). A single experiment with nonopsonized, killed, virulent *B. pertussis* organisms revealed that 65% of these bacteria were localized intracellularly and 36% of bac-

TABLE 2. Localization of monocyte-associated *B. pertussis* cells[a]

Strain	Cytochalasin B	No. of expt	No. (%) of *B. pertussis* cells/monocyte		
			Total[b]	Fluorescent (intracellular)[c]	Quenched (extracellular)[d]
Nonopsonized BP338 (wt)	−	15	2.5 ± 1.4	1.0 ± 0.5 (38)	1.6 ± 1.1 (62)
Nonopsonized BP338 (wt)	+	9	2.1 ± 1.0	0.3 ± 0.2[e] (15)	1.8 ± 1.0 (85)
Opsonized BP338 (wt)	−	13	4.1 ± 2.2[e]	1.9 ± 1.3[e] (47)	2.2 ± 1.1[e] (53)
Opsonized BP338 (wt)	+	9	3.0 ± 0.8	0.8 ± 0.4[f] (27)	2.2 ± 0.6 (73)
Opsonized BP347 (Vir^-)	−	5	4.2 ± 1.5[e]	3.5 ± 1.4[e,f] (83)	0.7 ± 0.2[e,f] (17)

[a] FITC-labeled virulent wild-type (wt) (BP338) or avirulent (BP347) *B. pertussis* cells were incubated for 90 min at 37°C with monocytes in suspension in the presence or absence of cytochalasin B. The monocytes were treated with ethidium bromide and examined under a fluorescence microscope. Values are means ± standard deviations.
[b] Number of bacteria per positive monocyte.
[c] Number of bacteria that remained fluorescent, i.e., localized intracellularly, per positive monocyte.
[d] Number of quenched bacteria, i.e., localized on the monocyte surface, per positive monocyte.
[e] Significantly different ($P < 0.05$) from value for nonopsonized BP338 without cytochalasin B by nonpaired two-tailed t test.
[f] Significantly different ($P < 0.05$) from value of opsonized BP338 without cytochalasin B by nonpaired two-tailed t test.

teria not treated with formaldehyde were intracellular, while the number of bacteria per positive monocyte was similar.

Nonopsonized avirulent *B. pertussis* BP347 cells did not associate with monocytes (data not shown). After opsonization with normal human serum, most (83%) of the avirulent *B. pertussis* cells were localized intracellularly (Table 2).

DISCUSSION

The major conclusions to be drawn from this study are that nonopsonized virulent *B. pertussis* cells adhere to human monocytes and that this process is mediated by the virulence factors FHA, fimbriae, and pertactin. A minor proportion of the nonopsonized virulent *B. pertussis* cells become ingested, and opsonization with normal serum only slightly enhances the ingestion of the virulent strain. However, opsonization results in ingestion of the great majority of avirulent *B. pertussis* cells. This indicates that virulence factors inhibit the opsonin-mediated ingestion of virulent *B. pertussis* cells.

These conclusions are supported by the following observations. The attachment of nonopsonized *B. pertussis* mutant strains deficient in FHA, fimbriae, or pertactin to adherent monocytes was reduced compared with that of their respective parental strains, while avirulent *B. pertussis* cells did not attach to monocytes. The finding that the adherence of the FHA mutant strain BP101 to monocytes was reduced more than that of strains defective in fimbrial subunits (strains B176 and B172) or in pertactin (strain BBC9) may reflect a stronger adhesive capacity of FHA than of fimbriae and pertactin for their respective counterstructures on the monocyte. Strains deficient in various other virulence factors, such as PT, extracellular AC and hemolysin, the major fimbrial subunits, the 95-kDa protein, or outer membrane protein, exhibited adherence to monocytes similar to that of their parental strains.

It has been shown previously that FHA mediates adhesion of *B. pertussis* to human ciliated epithelial cells (36), mammalian tissue culture cell lines (29, 37), or human monocyte-derived macrophages (30, 31). FHA contains two distinct binding sites for human monocyte-derived macrophages, one which has binding affinities for carbohydrate structures and one containing an Arg-Gly-Asp (RGD) sequence which is involved in the binding of FHA to complement receptor type 3 (30). Pertactin, which also contains an RGD sequence, has been demonstrated to play a role in adherence of *B. pertussis* to mammalian cell lines (22, 23, 32). Recently it has been reported that fimbriae are involved in the adherence of *B. pertussis* to baboon tracheal rings (13). However, the role of fimbriae and pertactin in adherence of *B. pertussis* to monocytes had not been documented earlier.

B. pertussis fimbriae are extracellular filamentous heteropolymers composed of major subunits encoded by *fim2* and *fim3* genes and at least one minor subunit encoded by *fimD* (44). Mutations in the major subunit genes (*B. pertussis* B52) had no effect on the adherence to monocytes, which is consistent with the observation by others (30) that the attachment of this mutant to monocyte-derived macrophages was not reduced. A mutation in *fimD* (strain B176) did affect the adherence of *B. pertussis* to monocytes, which suggests that the minor fimbrial subunit, FimD, is involved in this binding. However, it cannot be excluded that indirect effects of the mutation in *fimD* of strain B176 on the expression of *fha* genes (43) also affected the adherence. The role of fimbriae in the adherence of *B. pertussis* to monocytes was confirmed by the observation that the adherence of another strain (B172), which completely lacks minor and major fimbrial subunits and which is not affected in the expression of FHA (43), to monocytes was reduced compared with that of its parental strain. The role of fimbriae was further confirmed by our finding that purified *B. pertussis* fimbriae bind to monocytes, as determined by FACS analysis (16).

PT has previously been shown to mediate the adherence of *B. pertussis* to human monocyte-derived macrophages (30, 31). We found no evidence that PT is involved in the attachment of *B. pertussis* to monocytes. Possibly PT recognizes a macrophage-specific structure which is not present on freshly isolated monocytes.

The assays used in the present study allowed us to distinguish between extracellularly attached bacteria and those ingested by monocytes. The results revealed that in the absence of opsonins, a minor proportion of the monocyte-associated virulent *B. pertussis* cells were localized intracellularly. Opsonin-independent ingestion was shown to be microfilament dependent, since it could be inhibited partially by pretreatment of the monocytes with cytochalasin B. This finding is consistent with the observation that cytochalasin D inhibited the ingestion of nonopsonized virulent *B. pertussis* cells by human monocytes cultured overnight (12).

Opsonization with normal serum resulted in a slightly enhanced ingestion of monocyte-associated virulent *B. pertussis* cells, although about 50% of the opsonized virulent *B. pertussis* cells remained extracellular, i.e., attached to the monocyte surface. On the other hand, when avirulent *B. pertussis* cells were opsonized with normal serum, they were found to be almost exclusively intracellular. These differences in localization of virulent and avirulent *B. pertussis* cells are probably not due to a difference in opsonization, because in normal serum the level of IgG antibodies against virulent *B. pertussis* was even slightly higher than the level of antibodies against avirulent *B. pertussis*. Thus, the presence of virulence factors appears to preclude opsonin-mediated ingestion partially. When virulent *B. pertussis* cells were opsonized with immune serum, which contains higher levels of anti-*B. pertussis* antibodies than normal serum does, about 60% of the bacteria were localized intracellularly, indicating that the antiphagocytic activity of virulence factors can be counteracted by antibodies.

It is not known which virulence factors are responsible for this antiphagocytic effect. Treatment of virulent *B. pertussis* with formaldehyde enhanced the ingestion of these bacteria, indicating that the inhibition of phagocytosis is mediated by factors produced and/or secreted by viable bacteria during their interaction with monocytes. Purified PT and AC can inhibit various functions of monocytes and granulocytes, including the phagocytosis of bacteria (5, 9, 11, 18, 26). We observed that *B. pertussis* BP349, defective in extracellular AC and hemolysin, became ingested better by monocytes, while the total number of bacteria per positive monocyte was similar to that of the parental wild-type strain (15). Others reported a reduced uptake of another AC-deficient *B. pertussis* strain (BC67) by monocytes cultured overnight compared with uptake of the parental strain (12). However, a decreased intracellular survival rather than a decreased uptake could explain the latter results, since serum which was added to remove extracellular bacteria also activates the intracellular killing of bacteria by monocytes (21). Support for this interpretation is provided by the observation that AC purified from *B. pertussis* can inhibit the killing of *Staphylococcus aureus* by human granulocytes (5). The relationship between antiphagocytic activity of bacteria and their virulence is not specific for *B. pertussis*, since similar differences were found for the phagocytosis of virulent and avirulent *Yersinia enterocolitica* strains by

granulocytes (24, 40) and *Yersinia pseudotuberculosis* strains by mouse peritoneal macrophages (33).

Virulence factor-mediated antiphagocytic activity may be relevant during an infection with *B. pertussis*. When no opsonizing antibodies are present, e.g., in a nonimmune host, the bacteria can attach to the monocyte surface via virulence factors. These bacteria may become ingested via the RGD-dependent FHA-complement receptor type 3 interaction, possibly not resulting in the activation of a respiratory burst (30, 34), thus facilitating intracellular survival of the bacteria. In the presence of antibodies, e.g., in an immune host, opsonized virulent *B. pertussis* cells can also be ingested via Fc receptors, which can activate antimicrobial mechanisms (38), and this may be partially impeded by the presence of virulence factors. The contribution of Fc receptors to the ingestion of *B. pertussis* probably depends on the amount and specificity of antibodies. This is supported by the observation that after opsonization with immune serum, a greater proportion of virulent *B. pertussis* cells were localized intracellularly than after opsonization with normal serum. Therefore, it may be relevant that vaccines will be developed which induce the formation of antibodies that neutralize virulence factors which inhibit ingestion of *B. pertussis*.

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