Bordetella pertussis Filamentous Hemagglutinin Interacts with a Leukocyte Signal Transduction Complex and Stimulates Bacterial Adherence to Monocyte CR3 (CD11b/CD18)

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Summary

Bordetella pertussis, the causative agent of whooping cough, adheres to human monocytes/macrophages by means of a bacterial surface–associated protein, filamentous hemagglutinin (FHA) and the leukocyte integrin, complement receptor 3 (CR3, $\alpha_M\beta_2$, CD11b/CD18). We show that an FHA Arg-Gly-Asp site induces enhanced *B. pertussis* binding to monocytes, and that this enhancement is blocked by antibodies directed against CR3. Enhancement requires a monocyte signal transduction complex, composed of leukocyte response integrin ($\alpha_?\beta_3$) and integrin-associated protein (CD47). This complex is known to upregulate CR3 binding activity. Thus, a bacterial pathogen enhances its own attachment to host cells by coopting a host cell signaling pathway.

Attachment of the gram-negative bacterium *Bordetella pertussis* to host cells at or near the respiratory mucosal surface is a crucial feature of whooping cough pathogenesis in humans. Ciliated respiratory epithelial cells and leukocytes are the primary targets for adherence by this organism (1–3). This process leads to respiratory tract colonization, systemic intoxication, and altered host immune cell function. *B. pertussis* attachment involves a bacterial surface–associated and secreted protein, filamentous hemagglutinin (FHA)[1], and host galactose-containing glycoconjugates (4–7). In addition, FHA recognizes a leukocyte β_2-integrin, complement receptor type 3 (CR3, CD11b/CD18, $\alpha_M\beta_2$) (8). The biologic significance of FHA-CR3 recognition and *B. pertussis* binding to leukocytes in nature may reflect several possible outcomes, including competitive blockade of CR3 by secreted FHA, facilitated delivery of bacterial toxins to host leukocytes, and/or bacterial intracellular entry, survival, and persistence (9–13). A recent study suggests that cross-linking of the fibronectin receptor $\alpha_5\beta_1$ on human peripheral monocytes enhances CR3-mediated attachment of *B. pertussis* via FHA (14). Augmented β2-integrin–binding activity can be elicited by a number of other receptor–ligand binding interactions, including CD14 recognition of the LPS–LPS binding protein complex (15) and a β3-integrin–containing receptor signal transduction complex (description follows).

FHA and an intrinsic host ligand for CR3, complement fragment iC3b, contain Arg-Gly-Asp (RGD) cell recognition sites. These tripeptide motifs often denote binding domains that are recognized by integrins (16–18). It was initially assumed that the FHA and iC3b RGD sites were directly recognized by CR3. This assumption was based on the following observations: (*a*) an FHA RGD site-directed mutation in the *B. pertussis* chromosome significantly reduced binding of this bacterium to human macrophages (8); and (*b*) RGD-containing peptides inhibited both iC3b and *B. pertussis* binding to monocytes (8, 19). Although FHA and iC3b are ligands for CR3, and their RGD sites are involved in binding interactions between these ligands and monocytes/macrophages, binding studies with purified CR3 have demonstrated that the iC3b and FHA RGD sites are not recognized by CR3 (20, 21). These results shifted attention to a pair of surface-associated protein receptors found on monocytes/macrophages and neutrophils that do recognize RGD sequences, and that appear to regulate integrin activity.

Leukocyte response integrin (LRI) is a heterodimeric receptor ($\alpha_?\beta_3$) that is closely associated with a 50-kD protein known as integrin-associated protein (IAP) in phago-

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[1] *Abbreviations used in this paper:* FHA, filamentous hemagglutinin; IAP, integrin-associated protein; LRI, leukocyte response integrin; MDM, monocyte-derived macrophages; PT, pertussis toxin; RGD, Arg-Gly-Asp.

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cytes (22–24). IAP has recently been identified as CD47 (25). The LRI α chain remains poorly characterized; the β chain is antigenically closely related to the integrin β3 chain (CD61). LRI recognizes RGD and Lys-Gly-Ala-Gly-Asp-Val sequences in a number of basement membrane proteins, and together with IAP forms a signal transduction complex (26, 27). Ligation of either of these two proteins on a surface (cross-linking) induces enhanced neutrophil and monocyte chemotaxis, adherence, phagocytosis, and oxidative burst. Surface-bound ligation of LRI or IAP on neutrophils activates the binding activity of CR3 for iC3b, whereas soluble antibodies against LRI and IAP inhibit this interaction in a manner similar to that of RGD-containing peptides (21). LRI-mediated functions can be blocked by antibodies that bind to IAP; the individual functions of these two proteins have not been dissociated. The pathways that mediate LRI/IAP signaling remain to be characterized; however, LRI/IAP-initiated respiratory burst in neutrophils seems to be independent of CD18-dependent signaling (27). We sought to determine whether LRI and IAP might be involved in regulating *B. pertussis* adherence to human monocytes by an FHA-dependent mechanism.

Materials and Methods

Bacterial Strains and Strain Construction. *B. pertussis* BP536 (5) is a streptomycin-resistant derivative of BP338 (28), a virulent-phase member of the Tohama I lineage. All of the following *B. pertussis* strains used in this study are derivatives of BP536. BP101 contains a partial, in-frame deletion of the FHA structural gene, *fhaB*, resulting in truncation of the mature protein product and elimination of most FHA-associated adherence functions (5). BP-TOX6 contains a complete deletion of the pertussis toxin operon (5). BP1098 carries a site-directed mutation in *fhaB* that effects a substitution of Ala for Gly within the RGD site at amino acid positions 1097-1099 (8). The double mutant strain BP1098-TOX6 contains each of the last two described mutations.

BP200 contains a complete deletion of *fhaB* and was constructed as follows: a chromosomal PstI-EcoRI fragment of ∼700 bp, located 253-bp upstream of the *fhaB* open reading frame, was cloned from BP536. A chromosomal fragment of ∼550 bp, located 140-bp downstream of the *fhaB* open reading frame, was amplified from BP536 with the PCR using primers 11170E (5′GGA ATT CGT GAA ACT GAC CGA GTG T 3′) and 11721H (5′ GCG AAG CTT CCC GTC ACA AGC GTA TGT 3′). These two fragments were ligated in tandem within plasmid pSORTP1, a derivative of *B. pertussis* suicide vector pRTP1 (29) that encodes gentamicin resistance. This recombinant plasmid, pSΔFHABp1, was introduced into BP536 by conjugation. Merodiploid exconjugants with an integrated plasmid were selected with gentamicin. Streptomycin then allowed selection for loss of the suicide plasmid vector (resolved merodiploid exconjugants). These latter strains were examined for *fhaB* allelic exchange by Southern hybridization and loss of FHA production by Western blot analysis. BP200 was identified as one of these *fhaB* deletion strains (data not shown).

Monoclonal Antibodies and FHA Protein. The following monoclonal antibodies were used in this study (the cognate human receptor and source or reference are also indicated): mAb 73, directed against a 115-kD monocyte protein with no known function (D. Andrews, unpublished observations); KIM118, CD11b (M. Robinson, Celltech Ltd., Slough, UK); 6.5E, CD18 (M. Robinson, Celltech Ltd.); IB4, CD18 (S. D. Wright, The Rockefeller University, New York, NY; 30); 7G2, LRI β chain (CD61; F. Lindberg and E. Brown, Washington University School of Medicine, St. Louis, MO; 22); B6H12 and 2D3, IAP (F. Lindberg and E. Brown; 22, 23); mAb16, α5 integrin chain (S. K. Akiyama, National Institutes of Health, Bethesda, MD); mAb13, β1 integrin chain (S. K. Akiyama). 2D3 binds to an IAP epitope distinct from that recognized by B6H12 and with equal affinity, but causes none of the cellular activities that are associated with LRI/IAP signaling and are induced by surface-bound B6H12 (22–24).

Wild-type FHA (RGD) and mutant FHA (RAD) were isolated and purified from *B. pertussis* strains BP-TOX6 and BP1098-TOX6, respectively, by use of previously published techniques (31, 32) (some material was a gift from A. Kimura and J. Cowell, Lederle-Praxis Biologicals Division, American Cyanamid Co., West Henrietta, NY). These preparations were then further purified with concentrators (Centricon-3; Amicon Corp., Beverly, MA). Quantitative endotoxin determinations on the FHA preparations were performed with a limulus amebocyte ELISA assay (Microbiology Reference Laboratory, Cincinnati, OH).

Bacterial Binding to Monocytes. Monocytes were isolated from fresh human peripheral blood obtained from healthy donors by use of Ficoll-Hypaque (Pharmacia LKB Biotechnology, Inc., Piscataway, NJ) and standard procedures (33). Cells were resuspended in serum-free media consisting of PBS with 3 mM glucose, 150 nM $CaCl_2$, 500 nM $MgCl_2$, 0.3 U aprotinin/ml, and 0.05% human serum albumin. Adherence assays were performed as previously described (8), with some modifications. Terasaki tissue culture plate wells (Nunc, Inc., Naperville, IL) were precoated with 5 μl of mAb at 25 μg/ml, or BSA or FHA at variable concentrations overnight at 4°C (BSA = 500 μg/ml unless otherwise stated). After washing, well surfaces were blocked with serum-free media at room temperature for 2 h. After washing, 5 μl of mononuclear cell suspension (1.5×10^4 cells) was added to each well, and cells were allowed to spread at 37°C for 90 min. Nonadherent cells were removed by washing with serum-free media three times, and 5×10^5 bacteria were incubated in each well at 37°C in serum-free media for 30 min. Soluble mAbs were added at 25 μg/ml. After washing and staining with Giemsa, the number of bacteria adherent to 100 monocytes was determined by light microscopy (i.e., "attachment index"). Each well incubation was performed in triplicate, and each experiment was performed on at least three occasions.

The percentage of monocytes among adherent cells in wells was determined by staining for α-naphthyl butyrate esterase by use of standard methods (34). Monocytes comprised 8.4 ± 1.0% (mean ± SD) of the total peripheral blood leukocytes from the donors used in this study. After Ficoll-Hypaque separation, the mononuclear cell fraction was 22.5 ± 0.18% (mean ± SD) monocytes. To assess possible differences in the percentage of monocytes bound by different substrates, adherent cells were stained in wells precoated with each of the proteins and antibodies described in this study. Determinations under each condition were performed in triplicate and expressed as the mean percentage of monocytes among total adherent cells. After a 90-min incubation with well surfaces and extensive washing, monocytes comprised >95% of the remaining adherent cells. The monocyte purity of the adherent cells did not vary in relation to any of the protein or antibody well coatings. Adherent bacteria were equally well distributed among the adherent monocytes in wells coated with different substrates.

In the experiments designed to identify upregulated *B. pertussis*-binding receptor(s) (Table 1), "pretreated" monocytes were incubated with mAbs, 25 μg/ml, for 15 min at 4°C before placement in precoated wells; they were then allowed to attach to the

Table 1. *Effect of Soluble Antibodies on B. pertussis Binding to Stimulated Monocytes*

Antibody	Anti-LRI–coated surfaces: Monocytes: Pretreated*	Anti-LRI–coated surfaces: Monocytes: Posttreated‡	FHA-coated surfaces (5 μg/ml): Monocytes: Pretreated*	FHA-coated surfaces (5 μg/ml): Monocytes: Posttreated‡
None (BSA)	158 ± 17	175 ± 4	172 ± 13	174 ± 16
73 (α-MO ag)	141 ± 23	180 ± 10	168 ± 28	188 ± 20
7G2 (α-LRI)	80 ± 18	175 ± 6	100 ± 29	172 ± 17
B6H12 (α-IAP)	64 ± 3	170 ± 8	99 ± 14	175 ± 27
2D3 (α-IAP)	179 ± 27	173 ± 7	165 ± 28	183 ± 12
KIM118 (α-CD11b)	53 ± 13	82 ± 4	70 ± 9	60 ± 8
IB4 (α-CD18)	72 ± 25	67 ± 13	66 ± 16	60 ± 6
6.5E (α-CD18)	56 ± 20	70 ± 4	71 ± 13	74 ± 5
mAb16 (α-alpha$_5$)	143 ± 13	188 ± 20	136 ± 14	171 ± 22
mAb13 (α-beta$_1$)	149 ± 31	178 ± 6	116 ± 6	168 ± 19

Data are expressed as mean percentages of bacterial binding to monocytes cultivated on uncoated surfaces in the absence of soluble antibody ± SE; "stimulated" refers to monocytes cultivated on surface-bound substrates (FHA and 7G2) that lead to enhanced bacterial binding.

* Monocytes treated with antibodies before cultivation in anti-LRI or FHA precoated wells.

‡ Monocytes treated with antibodies after cultivation in anti-LRI or FHA precoated wells.

well surface for 90 min at 37°C before bacterial infection. "Posttreated" monocytes were allowed to attach to the well surfaces for 90 min at 37°C, and were then incubated with mAbs, 25 μg/ml, for 15 min at room temperature, before bacterial infection.

Results

LRI/IAP Regulates B. pertussis Binding to Monocytes. Bacterial binding assays were performed on human peripheral monocytes in the presence of monoclonal antibodies directed against a variety of monocyte surface molecules. Monocytes were exposed to either antibody-coated surfaces or to soluble antibodies before incubation with bacteria. In the surface-coated format, mobile monocyte surface molecules are ligated by antibodies at the substrate-adherent domain of the cell and become cross-linked; this reduces their number on the cell apical surface (30, 35). The soluble format allows surface molecule blockade.

In assays of these types, the binding of a wild-type *B. pertussis* strain, BP536, was significantly reduced to 49–57% of control (using BSA) levels by three different surface-bound antibodies directed against either of the subunits of CR3 (CD11b/CD18) (Fig. 1 *A*). Binding of BP536 was also significantly reduced (41–63%) in the presence of the same antibodies in soluble form (Fig. 1 *B*). These results corroborated findings from an earlier study implicating CR3 in *B. pertussis* adherence to human peripheral monocyte–derived macrophages (MDM) (8). Interestingly, antibodies 7G2 and B6H12, directed against LRI and IAP, respectively, significantly enhanced BP536 binding to monocytes when used in surface-bound format (183 ± 17%, mean ± SE, and 189 ± 13%, respectively; Fig. 1 *A*). On the other hand, these two antibodies reduced bacterial binding (63 ± 4%, 52 ± 3%)

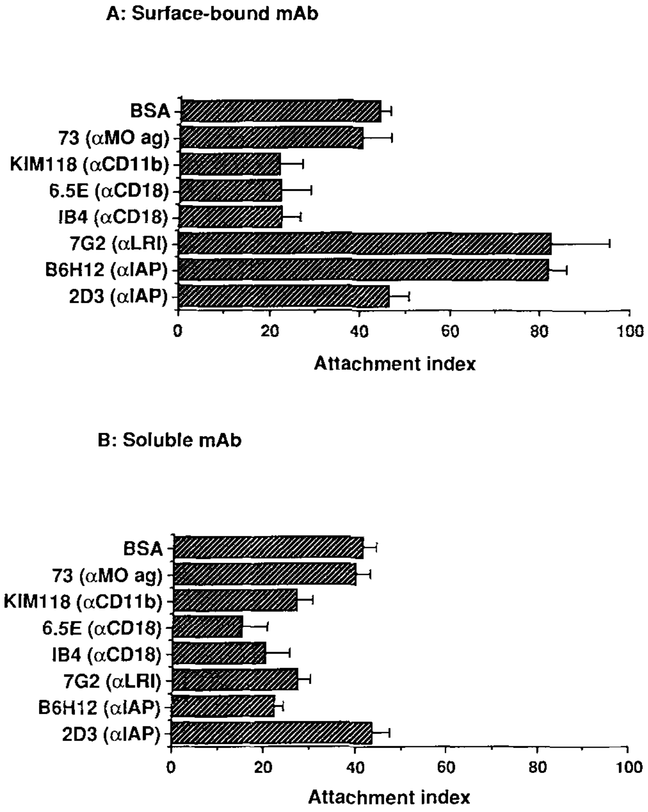


Figure 1. Effects of surface-bound (*A*) and soluble (*B*) mAbs on *B. pertussis* BP536 binding to human peripheral monocytes. Attachment index corresponds to the total number of bacteria adherent to 100 monocytes. Shown are mean values and standard errors, based on at least three experiments, each performed in triplicate.

when used in solution (Fig. 1 *B*). Neither enhancement nor inhibition was observed with control mAb 73 or IAP control (nonfunctional) antibody 2D3 (see Materials and Methods).

These results with 7G2 and B6H12 are reminiscent of, and consistent with, previous observations of upregulated CR3 binding activity, receptor-mediated phagocytosis, and activation of respiratory burst in neutrophils and monocytes under similar conditions in which LRI and IAP are cross-linked at a substrate–cell interface (21, 26, 27). The experiments with surface-bound and soluble 7G2, B6H12, 2D3, and control mAb 73 were repeated with fresh peripheral monocytes from two other independent human donors, each examined on three separate occasions. The results were essentially identical to those presented (data not shown).

FHA is Required for LRI/IAP-mediated B. pertussis Binding Enhancement. A number of *B. pertussis* proteins have been proposed as potential adherence factors. Previous studies have most strongly implicated FHA, and less so pertussis toxin (PT), in mediating binding to eukaryotic cells and tissues (4, 5), and, in particular, to human MDM (8). We studied the monocyte-binding activity of *B. pertussis* isogenic strains derived from BP536 that contain partial or total deletions of the FHA structural gene (BP101, BP200, respectively) or a total deletion of the PT operon (BP-TOX6) with antibodies in surface-bound and soluble formats. Using all of the antibodies previously mentioned in both formats with BP-TOX6, we found that the levels of binding enhancement and inhibition were indistinguishable from those observed with BP536 (Fig. 2, *A* and *B*). Conversely, monocyte binding of the FHA mutants BP101 and BP200 was significantly impaired (38 ± 5% mean ± SE, and 41 ± 9%, respectively, compared with BP536) and was not enhanced by surface-bound 7G2 and B6H12 nor inhibited by these antibodies in soluble form. mAbs other than 7G2 and B6H12 gave similar results with BP-TOX6 (data not shown) and BP536 (see Fig. 1); with the FHA mutant strains, there were no significant differences between results with any of the mAbs, including the negative control antibodies. These findings suggest that: (*a*) FHA is a more dominant *B. pertussis* adhesin for monocytes than is PT; (*b*) FHA is required for the LRI/IAP enhancement effect; and (*c*) FHA might directly interact with LRI/IAP.

Surface-bound FHA Stimulates LRI/IAP-mediated Binding Enhancement. To determine whether FHA could simulate the effects of anti-LRI and anti-IAP antibodies, purified FHA protein was used in surface-bound and soluble forms at varying densities and concentrations (Fig. 3). In surface-bound form, wild-type (RGD-containing) FHA protein produced two different effects: at lower densities, e.g., surface coating with a 5-μg/ml solution, *B. pertussis* monocyte adherence increased to 190 ± 44% of the levels achieved with BSA; with wells coated at higher densities, e.g., a 250-μg/ml solution, adherence was reduced to 45 ± 16% of BSA levels. The same protein used in soluble form caused no adherence enhancement, but did cause significant inhibition of adherence at higher concentrations. (If all FHA molecules in 5 μl of 5 μg/ml FHA were deposited on the well surface, ligand density would be ~5.7 × 10^3 molecules/μm^2.)

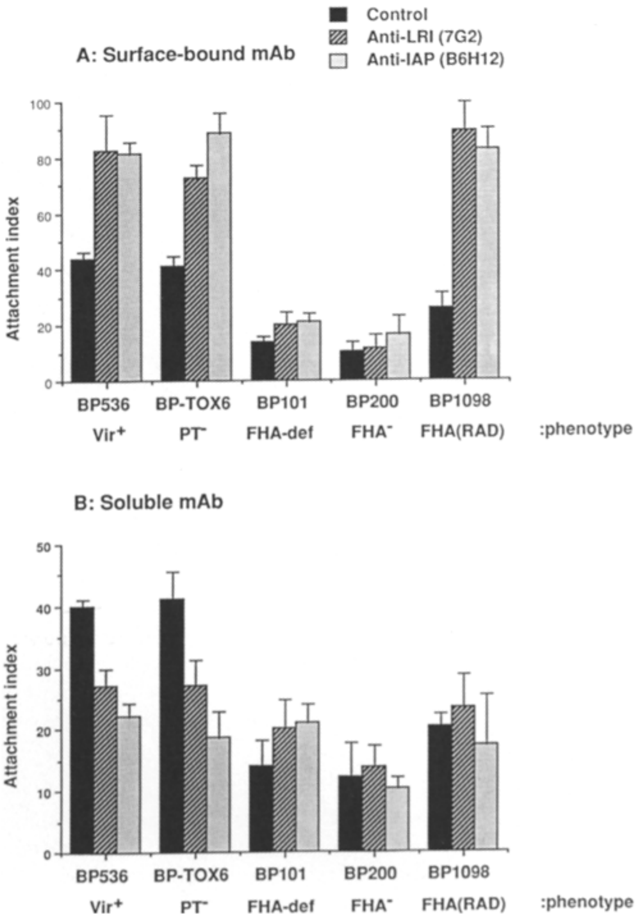
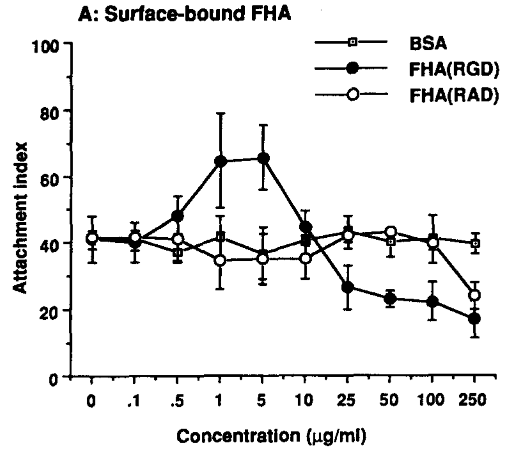


Figure 2. Effects of surface-bound (*A*) and soluble (*B*) LRI and IAP mAbs on the binding of *B. pertussis* wild-type, PT-mutant, and FHA-mutant strains to human peripheral monocytes. Phenotypes: *Vir*⁺, virulent phase; *PT*⁻, *FHA*⁻, absence of PT and FHA expression, respectively; *FHA-def*, expresses truncated FHA protein; FHA (RAD), expresses FHA protein with G→A substitution at RGD site. Shown are mean values and standard errors, based on at least three experiments, each performed in triplicate.

Because FHA-mediated enhancement resembled the LRI/IAP effects previously associated with some RGD-containing ligands, we examined the activity of a mutant FHA protein secreted by BP1098, an isogenic derivative of BP536 that contains a site-directed chromosomal mutation resulting in a Gly → Ala substitution at the RGD site (8). This FHA(RAD) in surface-bound form did not enhance *B. pertussis* monocyte binding at any tested density; however, this protein significantly diminished BP536 adherence when used at 250 μg/ml in surface-bound and soluble forms. Although endotoxin may contribute to monocyte activation, the endotoxin concentrations in the FHA(RGD) and FHA(RAD) preparations were similar: 1.7 and 2.0 ng/ml, respectively. These experiments with BSA, FHA(RGD) (at 5 μg/ml) and FHA(RAD) (at 5 μg/ml) were repeated with fresh peripheral monocytes from two other independent donors, each examined on three separate occasions. The results were essentially identical to those presented (data not shown).

Under nonstimulated monocyte conditions, the FHA RAD



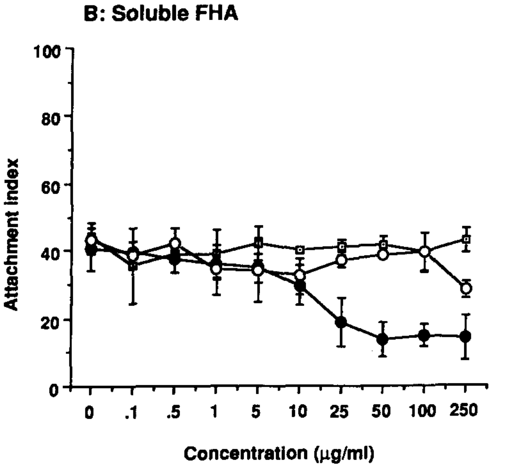


Figure 3. Effects of surface-bound (*A*) and soluble (*B*) FHA on BP536 binding to human peripheral monocytes. Wild-type RGD-containing FHA and mutant RAD-containing FHA are compared with BSA. The concentration of protein solutions used to precoat wells is indicated on the abscissa. Shown are mean values and standard errors, based on at least three experiments, each performed in triplicate.

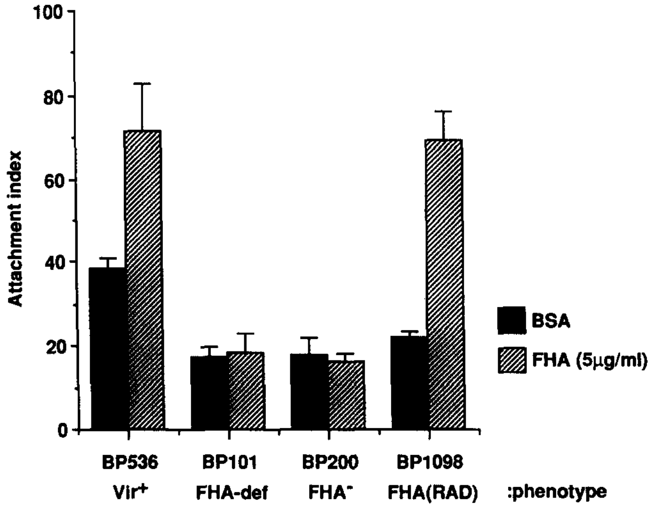


Figure 4. Enhancement of wild-type *B. pertussis* and FHA(RAD) mutant strain binding to monocytes by surface-bound low-density FHA(RGD). For strain phenotypes, see Fig. 2 legend. Shown are mean values and standard errors, based on at least three experiments, each performed in triplicate.

mutant strain, BP1098, was partially deficient in monocyte binding (62 ± 10% of BP536 levels; Figs. 2 and 4). Low density FHA(RGD) as a surface-bound monocyte ligand was able to restore fully the binding of this strain, as did surface-bound 7G2 and B6H12. In contrast, these three stimuli of enhanced *B. pertussis* adherence did not increase the binding of the partial or total FHA deletion mutants (Figs. 2 and 4). As a soluble ligand at 50 µg/ml, FHA(RGD) blocked surface-bound 7G2-stimulated BP536 binding to the same degree as soluble 7G2 or B6H12 (59 ± 14% of control antibody, 56 ± 5%, and 47 ± 7%, respectively). In addition, soluble 7G2 and soluble B6H12 blocked equally well the enhanced bacterial binding stimulated by surface-bound FHA and surface-bound 7G2 when monocytes were pretreated with these antibodies (Table 1). Taken together, these findings are consistent with the hypothesis that the FHA RGD site interacts directly with LRI/IAP. We propose that FHA, as a surface-bound ligand, cross-links the LRI/IAP complex by means of the RGD site and initiates LRI/IAP-mediated intracellular signaling in a manner similar to entactin (26). A separate FHA domain may mediate binding with the receptor(s) that is upregulated by this signaling event, as evidenced by the fully restored adherence of BP1098 to monocytes cultivated on low density FHA(RGD)-coated surfaces. We speculate that high density surface-bound FHA reduces, rather than enhances, *B. pertussis* binding by capturing the binding receptor at the cell–substrate interface. Since we have not formally demonstrated direct binding of FHA to LRI/IAP, we cannot rule out at the same time the possibility of an indirect mechanism for FHA-induced, LRI/IAP-mediated binding enhancement.

Enhanced B pertussis Monocyte Binding Is Due to Upregulated CR3 Activity. To identify the upregulated binding receptors on the monocyte apical surface, soluble antibodies were used to block monocyte receptors either before or after monocyte incubation in 7G2- and low density FHA(RGD)-coated wells (Table 1). 7G2, B6H12, and the three antibodies directed against CR3 all significantly reduced the LRI and FHA enhancement effects when used to block monocytes before well incubation. However, only the antibodies directed against CR3 caused significant inhibition of the enhancement effects when used to treat monocytes after their attachment to coated wells. In separate experiments, the three anti-CR3 antibodies in soluble form reduced BP1098 monocyte binding to 42–59% of the control antibody levels in uncoated wells. We interpret these data to indicate that CR3 is the monocyte receptor that is upregulated during LRI/IAP signaling and binds directly to *B. pertussis* FHA at a domain other than RGD. This leads to enhanced bacterial attachment to monocytes.

In all of our experiments, 2D3 was noninhibitory and nonfunctional in soluble and surface-bound forms, as previously shown by others (22–24), despite IAP-binding affinity equivalent to that of B6H12. Since surface-bound 2D3 should capture LRI/IAP complexes, the fact that *B. pertussis* bound

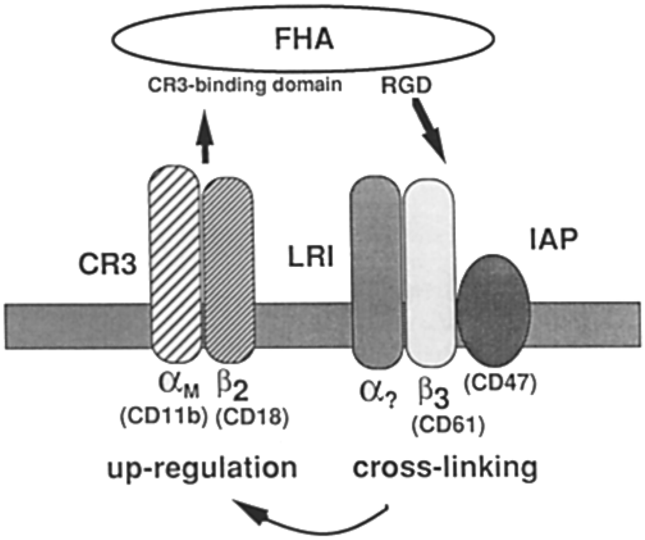


Figure 5. Proposed model indicating recognition of monocyte LRI/IAP by the *B. pertussis* FHA RGD site, leading to LRI/IAP cross-linking, intracellular signaling with upregulation of CR3 activity, and enhanced CR3 recognition of a separate *B. pertussis* FHA domain. In this manner, *B. pertussis* FHA upregulates its own binding to monocyte CR3. Nonetheless, there is substantial CR3–*B pertussis*–binding activity in the absence of surface-bound FHA stimulation. The CR3-binding domain of FHA is currently undefined.

equally well to the apical surfaces of monocytes adherent to 2D3- and control mAb-coated surface suggests that if bacteria do bind directly to LRI/IAP, only a small proportion of the total number of monocyte-adherent bacteria are bound to this complex.

Discussion

A number of microbial pathogens bind to integrin receptors on eukaryotic cell surfaces, either directly or through soluble host integrin ligands adsorbed to the microbial surface (36, 37). The resulting intracellular signaling events are incompletely understood, but may be manifested by tyrosine phosphorylation, cytoskeletal rearrangements, and altered cellular morphology. The choice of ligand and receptor combinations dictate the subsequent fate of the microorganism. Binding affinity may also play a role in these events. For example, high-affinity binding of the *Yersinia* invasin protein for the $\beta 1$ chain integrins is crucial for the internalization of this organism by nonprofessional phagocytes (36, 38). A 100-fold higher dissociation constant of fibronectin for the $\alpha_5\beta_1$ integrin, compared to invasin, may explain why the latter and not the former promotes microbial internalization. The leukocyte β_2 chain integrin, CR3, also serves as a receptor for a variety of microbial pathogens, including *Legionella pneumophila*, *Rhodococcus equi*, *Histoplasma capsulatum*, and *Leishmania* (39–42). The attachment of some of these microorganisms to leukocyte CR3 is mediated by deposition of iC3b on the microbial surface. In addition to the concurrent role of other leukocyte receptors, CR3 receptor activity may be crucial in determining the likelihood of phagocytosis, the generation of an oxidative burst, and other cellular responses to the adherent microorganism.

CR3 and other leukocyte receptors exhibit variable states of activation. Stimuli such as phorbol esters, divalent cations such as Mn^{2+}, integrin modulating factor, and contact with surface-bound RGD-containing extracellular matrix ligands dramatically enhance CR3 activity (21, 43–45). Enhanced activity probably reflects various combinations of increased surface receptor number, receptor binding affinity, and receptor signaling capabilities. Manganese ion enhances *B. pertussis* binding to monocytes (our unpublished data). Non-$\beta 2$ chain integrins and associated membrane proteins, such as $\alpha_5\beta_1$ and LRI/IAP, may play important roles in regulating CR3 activity. Presumably, these forms of receptor activity regulation are crucial for professional phagocytes as they move between bloodstream and tissue sites (27). Microbial pathogens may benefit from their own manipulations of leukocyte receptor activity modulation. *B. pertussis* FHA may mimic RGD-containing extracellular matrix proteins by ligating the LRI/IAP complex and stimulating CR3 binding activity.

Our data suggest that *B. pertussis* FHA may interact with the monocyte LRI/IAP complex through the FHA RGD site, and that FHA-induced LRI/IAP cross-linking leads to upregulated CR3-mediated binding of *B. pertussis* to monocytes. Several types of evidence favor the direct interaction of FHA with LRI/IAP: (*a*) soluble antibodies against LRI/IAP block wild-type *B. pertussis* binding to monocytes, but do not further affect the binding of FHA-deficient *B. pertussis* mutants; (*b*) pretreatment of monocytes with soluble antibodies against LRI/IAP block surface-bound FHA-mediated enhancement of *B. pertussis* binding to the same degree that they block surface-bound 7G2-mediated enhancement; and (*c*) soluble FHA inhibits surface-bound anti-LRI antibody from stimulating enhanced *B. pertussis* binding. At the same time, we have not ruled out the possibility that the interaction between FHA and LRI/IAP is indirect. Because LRI has not been cloned, nor purified in large amounts, it is technically difficult to prove direct binding of FHA with LRI. Cross-linking reagents may provide one approach to this problem.

As with other microbial pathogens, *B. pertussis* adherence to mammalian cells is certain to be a complex process. Bacterial adhesin density at the contact points with the eukaryotic surface, multiple binding domains within a single adhesin, and cooperation among different adhesins are all relevant. The density of FHA at the bacteria–monocyte interface is unknown; it is also unclear whether bacterial surface ligands cross-link monocyte receptors in a manner similar to ligand-coated plastic surfaces. Secreted FHA may coat other bacteria (46), host epithelial and basal cells, and exposed basement membrane. FHA is also thought to contain a carbohydrate recognition domain, as well as other binding domains for eukaryotic proteins, besides the RGD site (47–49). In addition, a separate *B. pertussis* adhesin, PT, appears to contain lectin-like binding domains within subunits S2 and S3 that may mediate and possibly regulate *B. pertussis* attachment to leukocytes (50–52). However, our data demonstrate that a PT-deficient strain adheres to human peripheral monocytes and responds to LRI/IAP cross-linking as well as the wild-type organism.

Hazenbos et al. (14) provide evidence that *B. pertussis* may interact with the fibronectin receptor $\alpha_5\beta_1$ on the monocyte surface and that cross-linking of this receptor causes enhanced CR3-mediated, FHA-dependent binding of the bacterium to these cells. In our study, when soluble antibodies directed against α_5 chain or β_1 chain were incubated with monocytes already attached to anti-LRI antibody– or FHA-coated surfaces, there was no inhibition of enhanced *B. pertussis* binding (Table 1). This finding suggests that activated CR3 may be dominant to $\alpha_5\beta_1$ as an FHA-binding receptor. However, α_5 chain and β_1 chain antibodies did appear to inhibit partially the FHA-enhanced bacterial binding when they were used to pretreat monocytes. It is possible that FHA interacts with $\alpha_5\beta_1$, as well as with CR3 and LRI/IAP, and may also cause some degree of $\alpha_5\beta_1$ cross-linking on monocyte surfaces.

We suggest that a bacterial adherence protein may regulate the binding activity of its own eukaryotic receptor by coopting a host signal transduction complex. This concept is illustrated by a model in which *B. pertussis* FHA may enhance its own binding interactions with human monocytes (Fig. 5). If one were to postulate a role for the FHA carbohydrate recognition domain in the initial recognition of monocyte glycoconjugates, then this model would become strikingly similar to a multistep model of leukocyte–endothelial cell recognition (53). The latter process consists of primary lectin-mediated transient adhesion, followed by leukocyte activation, and then activation (CR3)-dependent binding.

What might be the consequences and relevance in vivo of LRI/IAP recognition of FHA and subsequent enhanced binding of *B. pertussis* to monocyte CR3? Some data suggest that *B. pertussis* enters and survives within host phagocytes to a limited degree (9–13). Prolonged intracellular survival within alveolar macrophages or within cells of the respiratory-associated lymphoid tissue might suggest mechanisms for (*a*) *B. pertussis* persistence within the human host; (*b*) prolonged or delayed clinical manifestations and immune responses; and (*c*) establishment of a *B. pertussis* human reservoir, the existence of which remains speculative (54, 55). LRI/IAP-enhanced CR3 binding activity may facilitate *B. pertussis* intracellular entry and survival by means of either increased CR3 binding avidity or receptor number. In support of this notion, we have preliminary evidence that FHA/(RGD) cross-linking of LRI/IAP increases the number of *B. pertussis* that enter human peripheral monocytes (our unpublished data). LRI/IAP-enhanced CR3 binding of *B. pertussis* may also lead to enhanced delivery of *B. pertussis* toxins. Thus, the ultimate outcome of the *B. pertussis*–monocyte encounter probably reflects PT and adenylate cyclase toxin inhibition of various phagocyte intracellular signalling pathways (56, 57). From a general perspective, *B. pertussis* and FHA may serve as important tools for characterizing the functions of LRI/IAP and other phagocyte receptors.

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