Subversion of integrins by enteropathogenic *Yersinia*

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Summary

Enteropathogenic *Yersinia* are gram-negative bacterial species that translocate from the lumen of the intestine and are able to grow within deep tissue sites. During the earliest stages of disease, the organism is able to bind integrin receptors that are presented on the apical surface of M cells in the intestine, which allows its internalization and subsequent translocation into regional lymph nodes. The primary integrin substrate is the outer-membrane protein invasin, which binds with extraordinarily high affinity to at least five different integrins that have the β_1 chain. Bacterial uptake into host cells is modulated by the affinity of receptor-substrate interaction, receptor concentration and the ability of the substrate to aggregate target receptors.

Key words: Yersinia, Invasin, Integrin, M-cell uptake

Introduction

The gram-negative enteropathogenic *Yersinia* are represented by two species of medical importance, *Y. enterocolitica* and *Y. pseudotuberculosis* (Bottone, 1997). Human disease caused by these organisms is the result of ingestion of contaminated foodstuffs followed by localized lymphadenopathy. The most important complication of such infections is reactive arthritis, particularly in HLA-B27 patients (Bottone, 1997). In mouse models, localized infection of the gut by *Yersinia* is rapidly followed by dissemination into other organ sites, and the ensuing growth of the organism in the liver and spleen leads to the eventual death of the animal (Autenrieth et al., 1996; Pepe et al., 1995). Systemic disease in the animal bears some resemblance to human typhoid fever, and the death of the infected animal requires most of the same bacterial proteins expressed by the closely related *Yersinia pestis*, the causative agent of bubonic plague (Cornelis et al., 1998).

During the course of disease, enteropathogenic *Yersinia* maintain an intimate relationship with host cells that requires adhesive contact by the microorganism. Shortly after entering the lumen of the intestine, the bacteria are internalized by M cells (Autenrieth and Firsching, 1996; Clark et al., 1998; Marra and Isberg, 1997), which lie within the lymphoid-follicle-associated epithelium (Fig. 1; FAE) (Neutra et al., 1996). The primary role of these cells is presumed to be presentation of antigens to immune cells found within the FAE, but M cells are also used by enteropathogens as portals for entry into host tissues (Neutra, 1999). There is no evidence for any further localization of the bacteria within host cells (Heesemann et al., 1993) after its translocation through these cells and subsequent entry into the lymphoid follicles (also called Peyer's patches, if located within the small intestine).

At the very earliest stages of disease, the organism establishes contact with host cell β_1 chain integrin receptors. The bacterial YadA protein binds to a variety of extracellular matrix proteins, such as collagen, laminin and fibronectin, which in turn are able to recognize integrin receptors (Flugel et al., 1994; Tahir et al., 2000). Another bacterial protein, invasin, binds to at least five different integrin receptors ($\alpha_3\beta_1$, $\alpha_4\beta_1$, $\alpha_5\beta_1$, $\alpha_6\beta_1$, and $\alpha_v\beta_1$; Isberg and Leong, 1990) and is responsible for the ability of the organism to enter M cells in the intestine (Clark et al., 1998; Marra and Isberg, 1997). Invasin mutants unable to bind to integrin receptors do not enter M cells or efficiently translocate into Peyer's patches (Marra and Isberg, 1997). In the small intestine, the only cells presenting β_1 chain integrins to the intestinal lumen are M cells, which thus explains the specificity of the microorganism for this cell type (Fig. 1; Clark et al., 1998).

Once across the intestinal lumen and localized in regional lymph nodes, the microorganism binds to and remains attached to the exterior of immune cells (Fig. 1), probably through invasin, YadA and the pH 6 antigen pilus, which binds to glycosphingolipids (Payne et al., 1998). Adhesion by at least one of these three proteins is a prerequisite for formation of a protein translocation channel between the bacterium and the host cell (Persson, 1995). This channel, which is a product of the plasmid-encoded *Yersinia* Type III secretion machinery (Cornelis et al., 1998), allows introduction of several bacterial proteins called Yops, at least four of which are cytoskeletal poisons, into the host cell cytosol (Bliska et al., 1991; Iriarte and Cornelis, 1998; Juris et al., 2000; Von Pawel-Rammingen et al., 2000). Two of these proteins (YopE and YopT) target mammalian RHO family members (Von Pawel-Rammingen et al., 2000; Zumbihl et al., 1999), whereas the third is a tyrosine phosphatase (YopH; Bliska et al., 1991). As a result, host cell phagocytosis, motility and cytoskeletal integrity are all severely compromised (Fallman et al., 1995; Rosqvist et al., 1991). Furthermore, T and B cell activation is inhibited by injection of YopH (Yao et al., 1999).

Determinants that lead to uptake

The most striking property of invasin is its ability to promote efficient uptake of bacteria into normally nonphagocytic cultured cell lines, which mimics entry of enteropathogenic *Yersinia* into host M cells. The *Y. pseudotuberculosis* invasin is a 986-residue member of the bacterial intimin/invasin family

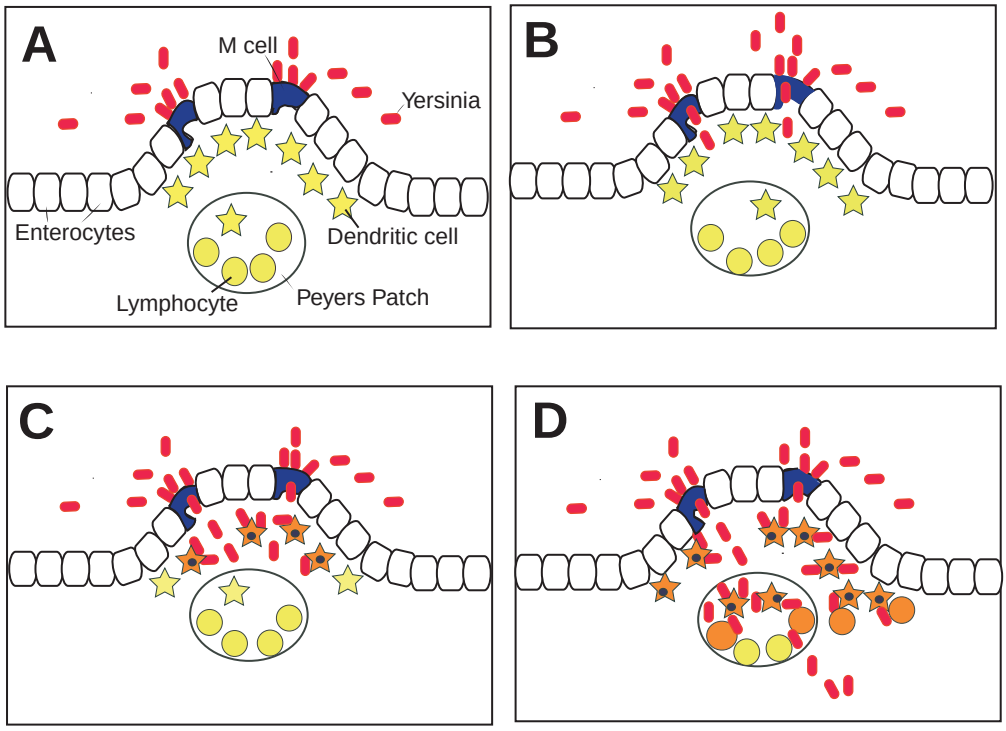

Fig. 1. Model for translocation of enteropathogenic Yersinia into the Peyer's patch of the small intestine. (A) Bacteria (red) encounter intestinal epithelium and target directly to M cells overlying the Peyer's patch. Organisms are internalized by these cells. (B) After translocation of bacteria across M cells, the bacteria encounter dendritic cells and other phagocytes in the region between the epithelium and the germinal center of the Peyer's patch. (C) Bacteria bound to phagocytes are found extracellularly localized, owing to deposition of Yops (yellow to orange color change). (D) Phagocytic cells bearing extracellular bacteria migrate to the germinal center, where replication of the bacteria occurs, and further dissemination follows.

(McGraw et al., 1999). The most highly conserved section is within the N-terminal 503 residues, and a portion of this region contains information necessary for localization in the outer member and presentation of the C-terminal cell adhesion module on the bacterial cell surface (Fig. 2A; Leong et al., 1990). The C-terminal region shows extensive sequence divergence among family members. On the basis of sequence analysis of homologs, as well as studies of the crystal structure and NMR analysis of the *Y. pseudotuberculosis* invasin and enteropathogenic *E. coli* intimin proteins (Batchelor et al., 2000; Hamburger et al., 1999; Luo et al., 2000), family members appear to consist of between 3 and 23 immunoglobulin (Ig)-like folds (Fig. 2A). These are arrayed in tandem on the bacterial cell surface and terminated by a C-type-lectin-like domain (CTLD) at the C-terminus (Weis et al., 1998). The CTLD (D5) and the upstream Ig domain (D4; Fig. 2A) form a large interface with each other, producing a superdomain that acts as a mammalian cell adhesion module (Fig. 3).

Three important factors enhance invasin-mediated uptake: (1) high-affinity binding of integrin receptors by the D4-D5 superdomain (Tran Van Nhieu and Isberg, 1993); (2) the ability of invasin monomers to undergo homotypic interactions (Dersch and Isberg, 1999); and (3) an increase in the concentration of integrin receptors available to bind invasin (Dersch and Isberg, 1999; Tran Van Nhieu and Isberg, 1993). Mutations that lower the affinity of the protein for receptors, deletion of a region of invasin necessary for homotypic interaction, and depletion of integrin receptors from the host cell all severely depress bacterial uptake, causing extracellular adhesion of the bacteria.

High affinity binding of integrin receptors

Competitive-inhibition studies, analysis of blocking monoclonal antibodies (mAbs), and studies using mutant integrin receptors indicate that invasin recognizes a site that is either identical to or overlaps that bound by natural substrates (Tran Van Nhieu and Isberg, 1991). Invasin is a competitive inhibitor of binding of fibronectin to the $\alpha_5\beta_1$ integrin, and the spectrum of mAbs able to block binding of this receptor is identical for both substrates. Furthermore, with the exception of one double mutant that appears to insert bulky interfering residues (see below, Fig. 2B; Krukonis and Isberg, 2000), mutations in the integrin β_1 chain that have severe defects in binding to invasin also have defective fibronectin binding (Krukonis et al., 1998; Krukonis and Isberg, 2000; Takada et al., 1992; Zhang et al., 1999).

That invasin and fibronectin appear to recognize similar residues on the integrin receptor is remarkable given that the solved crystal structures of the respective integrin-binding regions have very different contours (Hamburger et al., 1999; Leahy et al., 1996). In the case of fibronectin, maximal binding to the $\alpha_5\beta_1$ integrin requires the two IgSF repeats FnIII-9 and FnIII-10 (Aota et al., 1994; Fig. 3). Genetic studies indicate that residues in both domains are involved in contacting receptor, Asp1495 of the Arg-Gly-Asp (RGD) sequence in FnIII-10 being the most significant contributor to binding energy (Aota et al., 1994). Several other residues located on the same face of the molecule as the RGD sequence contribute to binding, including those within the so-called synergy region in FnIII-9 (Aota et al., 1994), as well as residues located between the synergy and RGD sites (Fig. 3; Redick et al., 2000). Unlike the D4-D5 adhesion module of invasin, the two IgSF domains in fibronectin have a small interface (342 $Å^2$), and there is a concave surface located between the residues involved in substrate recognition (Leahy et al., 1996). Furthermore, on the basis of NMR studies, this small interface appears to result in considerable interdomain flexibility (Copie et al., 1998; Spitzfaden et al., 1997).

The invasin D4-D5 cell adhesion module, by contrast, has a

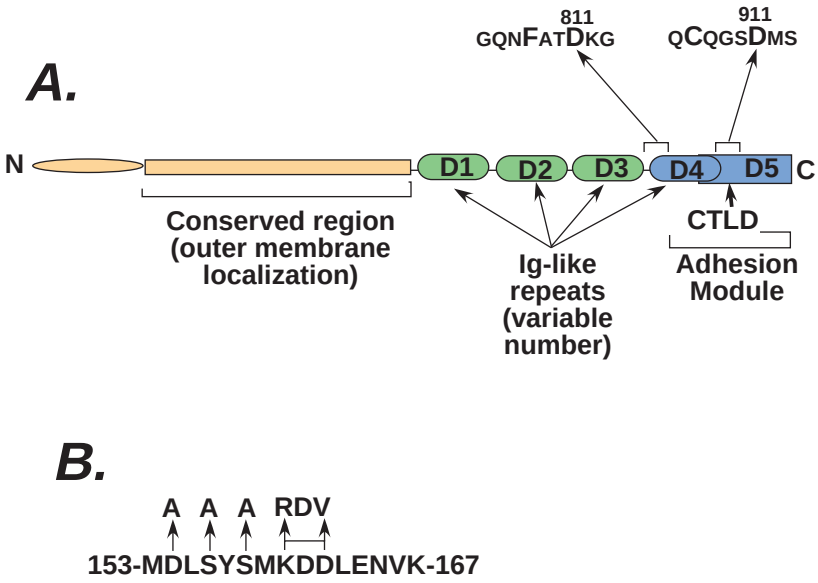

Fig. 2. Properties of receptor recognition by invasin. (A) Schematic of the significant features of the *Y. pseudotuberculosis* invasin. Displayed above schematic are residues believed to be important for receptor recognition. Enlarged single letter residue designations denote side chains that have been mutated and shown to be important for receptor recognition (Leong et al., 1993; Leong et al., 1995; Saltman et al., 1996). Conserved region: portion of protein that shows highest sequence identity to members of the intimin/invasin family (McGraw et al., 1999). Ig-like repeats: domains that have Ig-like folds showing loose sequence identity to each other as well as to other members of the invasin-intimin family (Batchelor et al., 2000; Hamburger et al., 1999; Luo et al., 2000). C-type-lectin-like domain (CTLD): the domain that has residues critical for receptor recognition (Batchelor et al., 2000; Hamburger et al., 1999; Luo et al., 2000). Adhesion module: the minimum region of the protein necessary for receptor recognition (Leong et al., 1990). D1-D5: individual domains, as determined by X-ray crystallography (Hamburger et al., 1999). (B) Integrin β_1 chain residues involved in recognition of invasin. Residue numbers refer to the chicken integrin β_1 chain (Krukonis and Isberg, 2000). Single alanine substitutions result in loss of recognition of both fibronectin and invasin by the integrin heterodimer. The double substitution KDD→RDV specifically destroys binding of integrin to invasin, and has small effects on fibronectin recognition.

bulging contour with a large interdomain interface. Even so, the critical invasin and fibronectin residues involved in receptor recognition appear to be similarly arrayed. As is true with fibronectin, a single aspartate residue in the more C-terminal domain (D5) appears to be the most important contributor to receptor binding (Leong et al., 1995). Furthermore, a region in invasin N-terminal to Asp911 appears to behave similarly to the synergy region in fibronectin. Mutations in this upstream region have considerably less drastic defects in binding than those observed for Asp911 mutants, which is reminiscent of the fibronectin synergy region (Saltman et al., 1996). In both molecules, the synergy region is located approximately 32 Å from the critical aspartate required for maximal binding (Hamburger et al., 1999; Leahy et al., 1996).

The most striking difference between invasin and fibronectin binding is the significantly higher affinity of invasin-receptor binding. This property is both critical for the protein to promote uptake as well as a central virulence determinant for the microorganism. Low-affinity integrin ligands, coated on either particles or bacteria, allow efficient adhesion to mammalian cells, but have a greatly reduced capacity to promote uptake relative to that seen with invasin (Tran Van Nhieu and Isberg, 1993). Furthermore, the mutation of Asp911 to glutamate in invasin allows *Y. pseudotuberculosis* to adhere to cells, but the lowered affinity caused by the lesion prevents bacteria from entering M cells and colonizing Peyer's patches in the mouse intestine (Marra and Isberg, 1997). There are a variety of explanations for why high-affinity receptor binding is critical for promoting uptake rather than simple adhesion, but most rely on the model that uptake requires circumferential binding of receptor molecules around the surface of the bacterium. Presumably, high-affinity binding allows invasin to compete efficiently with other ligands for integrin receptors and allows stable contact between the host and bacterial membranes. This then facilitates the sequestration of large numbers of integrin molecules at the surface of the bacterium. The basis for high affinity promoting uptake has been reviewed in detail elsewhere (Isberg et al., 2000).

Given the similar array of residues in fibronectin and invasin that are involved in binding to the receptor, the high affinity of invasin binding is probably a result of the very different contour of invasin relative to fibronectin. There are two possible advantages of the design of the invasin cell adhesion module. First, the large interdomain interface between D4 and D5 may lock the protein in a binding-competent conformation, allowing the residues involved in integrin recognition to be presented optimally to the receptor. Secondly, instead of the cleft found in fibronectin, the region between the critical Asp911 and the invasin synergy region contains a bulge with five aromatic residues that could contribute to binding receptor (Hamburger et al., 1999; Leahy et al., 1996). The fact that the only described integrin lesion that eliminates binding of invasin without significantly affecting binding to fibronectin involves the introduction of a double mutation in the β_1 chain (KDD160RDV) (Fig. 2B) is consistent with this model. The side chains altered in this mutant affect residues that can be changed to alanine without any noteworthy reduction in substrate adhesion; this indicates that these residues are presumably not directly involved in substrate recognition (Krukonis and Isberg, 2000). Instead, it would appear that the double mutant causes steric interference specifically with invasin.

The role of invasin homotypic interactions

Integrin receptors are able to transmit intracellular signals after engaging substrates (Cary et al., 1999). This signaling response is thought to require the engagement of several receptor molecules simultaneously, which allows the recruitment of cytoskeletal and other signaling proteins to the adhesion zone (Schlaepfer et al., 1997). Binding to simple monomeric substrates is much less efficient at promoting integrin-based signaling than binding to multimeric substrates (Stupack et al.,

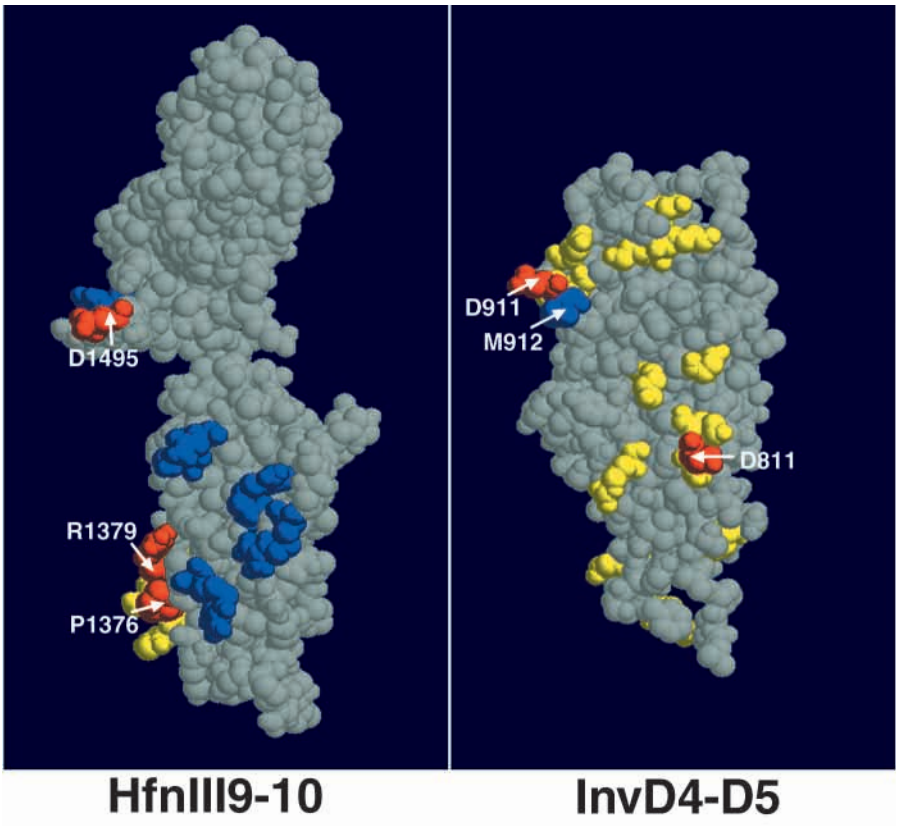

Fig. 3. Comparison of the integrin-binding domains of invasin and human fibronectin. Shown are space-filling models of human fibronectin (Hfn) Type III repeats 9 and 10 (HfnIII9-10) and domains 4 and 5 of the *Yersinia pseudotuberculosis* invasin protein (InvD4-D4) (Hamburger et al., 1999; Leahy et al., 1996). Displayed in yellow, red and blue are amino acid residues that have been changed to alanine, which are color coded to indicate the effects of mutations on recognition of substrate (Aota et al., 1994; Leong et al., 1995; Redick et al., 2000; Saltman et al., 1996). Red represents side-chain alterations that have strong effects on binding, residue changes represented by blue have mild effects on binding, and yellow residues can be changed to alanine without causing any drastic defects on substrate adhesion. Amino acid numbers refer to residues discussed in the text. D1495 in Hfn and D911 in invasin are hypothesized to play similar roles in substrate recognition, whereas the region defined by R1379 in Hfn and D811 in invasin are presumed to be functionally similar (Aota et al., 1994; Leong et al., 1995; Saltman et al., 1996).

1999; Fig. 4). Similarly, the monomeric cell adhesion domain of invasin is strikingly inefficient at promoting uptake. Latex beads coated with the Fab-immobilized monomeric D4-D5 superdomain are efficiently bound, but not internalized, by target cells (Dersch and Isberg, 1999). In contrast, dimerization of the superdomain by antibody allows the beads to be internalized.

Deletion analysis of invasin indicates that the IgSF domain D2 promotes the homotypic interaction necessary for efficient uptake (Fig. 2A). Bacterial mutants lacking D2 are inefficiently internalized by host cells (Dersch and Isberg, 2000), and the isolated D2 domain is able to promote dimerization in the lambda repressor one-hybrid assay (Dersch and Isberg, 1999). Furthermore, the *Y. enterocolica* invasin protein, which lacks D2, is much less efficient at promoting uptake than the *Y. pseudotuberculosis* protein (Dersch and Isberg, 2000). Crosslinking studies further support the model that D2 is important in presenting a multimeric form of invasin to the mammalian cell. The rate of recruitment of tyrosine-phosphorylated proteins to the phagocytic cup is considerably enhanced by dimerization of invasin, which may be a reason for the importance of multimerization (P. Dersch, personal communication).

The importance of substrate and receptor density

By increasing the concentration of both invasin and integrin receptors, one can partially bypass the requirements for high receptor-substrate affinity and multimerization (Fig. 4). Cell lines transfected with the integrin α_5 chain have a greatly enhanced ability to internalize particles coated by low-affinity substrates such as fibronectin (Tran Van Nhieu and Isberg, 1993). Furthermore, bacteria expressing high levels of invasin derivatives lacking D2 can be internalized at efficiencies approaching that of bacteria expressing similar levels of wild-type protein (Dersch and Isberg, 2000). Presumably, placing receptor-substrate contacts in sufficiently close proximity allows internalization to take place in the absence of multimerization (Fig. 4B). Finally, downmodulation of receptor availability, by plating cells on high-affinity antibodies directed against integrin receptors, interferes with the ability of substrate-coated particles to be internalized (Fig. 4A; Tran Van Nhieu and Isberg, 1993).

Signaling pathways required for uptake

Clustering of integrins by extracellular substrates generates a variety of intracellular signals, including tyrosine phosphorylation of cytoskeleton-associated factors and activation of MAP kinase cascades (Fincham et al., 2000). Invasin-mediated adhesion of enteropathogenic *Yersinia* to target cells induces similar responses. A series of experiments using chemical inhibitors and toxins have demonstrated that the activity of tyrosine kinases (Rosenshine et al., 1992), phosphoinositide 3-kinase (Mecsas et al., 1998) and RHO family members are involved in uptake (Black and Bliska, 2000; Von Pawel-Rammingen et al., 2000). For the most part, it is not clear which specific proteins affected by these inhibitors are critical for uptake or why there is a requirement for 3-phosphoinositides.

The deposition of the tyrosine phosphatase YopH into target mammalian cells significantly reduces invasin-mediated uptake (Persson et al., 1997). Analysis of the substrate specificity of YopH gives some insight into which tyrosine phosphorylated proteins are involved in internalization. The cytoskeletal proteins focal adhesion kinase (FAK), Cas, paxillin and Fyn-

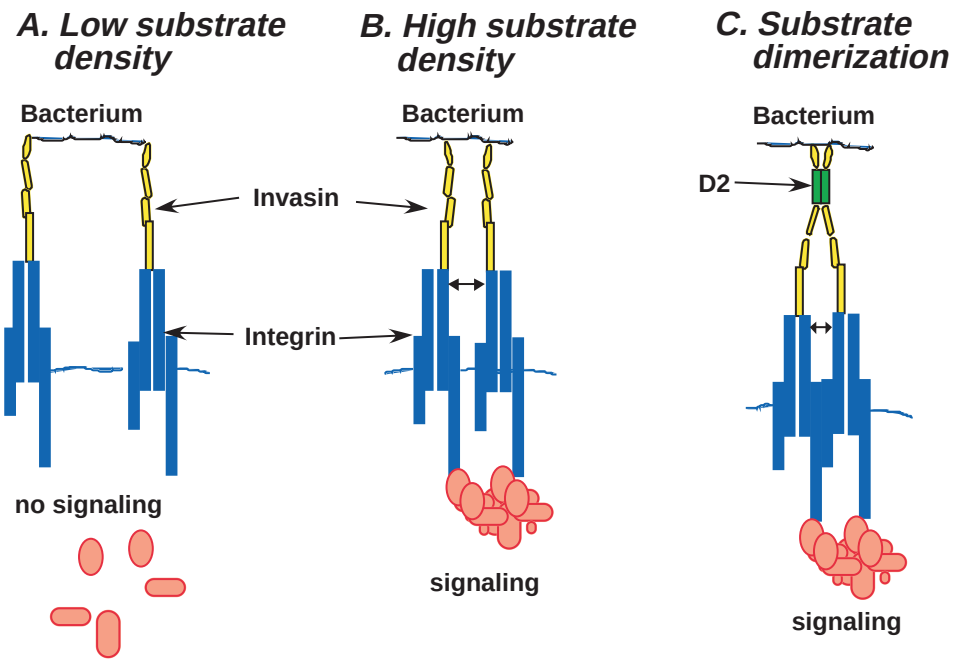

Fig. 4. Conditions that lead to integrin signaling and microbial uptake (Dersch and Isberg, 1999). (A) Bacteria having low concentrations of integrin substrate can engage receptor and allow adhesion, but no uptake signal is conveyed, and bacteria are immobilized on host cell surface. (B) Bacteria having high concentrations of integrin substrate can aggregate receptor efficiently and convey the uptake signal. (C) Bacteria having dimerized integrin substrate (due to domain D2 of *Y. pseudotuberculosis* invasin in this depiction) can cluster receptor and promote uptake, even if the substrate density is relatively low on the bacterial cell surface.

binding protein (Fyb) are all targets of YopH (Black and Bliska, 1997; Black et al., 1998; Hamid et al., 1999; Persson et al., 1997). The rate of dephosphorylation of these proteins varies significantly, however, the kinetics of phosphate loss from Cas and paxillin being quite rapid. Of this group, paxillin appears to be the most rapidly phosphorylated protein in response to bacterial adhesion to J774 cells (Andersson et al., 1996); this strongly suggests that it plays a key role in promoting invasin-mediated uptake. In addition, YopH alters an early response of cells to invasin-mediate adhesion (Andersson et al., 1999). Within seconds after contact of neutrophils by bacteria, invasin-mediated adhesion results in a transient increase in intracellular Ca^{2+}. The presence of YopH inhibits this event, although it is not clear whether inhibition of this Ca^{2+} spike also inhibits bacterial uptake. In fact, Pace et al. have argued that the intracellular concentration of Ca^{2+} plays little role in invasin-mediated uptake by a cultured epithelial cell line (Pace et al., 1993).

In contrast to the Ca^{2+} spike, phosphorylation of the tyrosine kinase FAK in response to bacterial binding and dephosphorylation of FAK by YopH have rather slow kinetics. In spite of these observations, FAK is an attractive candidate for the agent that transmits a signal from the clustered integrin to the cytoskeleton, because the cytoplasmic domain of the integrin β_1 chain binds to FAK (Schaller et al., 1995). Dominant inhibitory mutations in FAK interfere strongly with invasin-mediated uptake (Alrutz and Isberg, 1998). Of particular note is the dominant inhibitory mutant FAK-Y397F, which prevents phosphorylation of Tyr397, a site required for binding of SRC family members to FAK. Given that a kinase-defective SRC also inhibits uptake, a FAK-SRC complex might play a regulatory role in uptake. Also consistent with a role for FAK in uptake is the observation that a knockout cell line that fails to express FAK exhibits highly defective invasin-mediated internalization (Alrutz and Isberg, 1998).

FAK could have more than one role in promoting bacterial internalization. The simplest possibility is that, after bacteria engage the integrin receptor and induce clustering, FAK is recruited to the phagocytic cup, localization of SRC at this site occurring shortly afterward. SRC, in turn, phosphorylates downstream effector molecules involved in promoting cytoskeletal rearrangements. The alternative model is that FAK is involved in release of integrin molecules from focal adhesions. Results consistent with this latter model include the observation that the FAK-knockout cell line has greatly lowered migration rates relative to wild-type cell lines, and a similar reduction in migration results from overexpression of interfering forms of FAK. Therefore the ability of FAK to release receptors immobilized in focal adhesions results in receptors that are highly mobile within the plane of the membrane and can be more easily recruited to the front of moving cells or to the phagocytic cup than can receptors immobilized in focal adhesions. Kucik et al. have demonstrated the importance of receptor mobility in promoting phagocytosis previously, using $\alpha_{mac}\beta_2$ integrin as the phagocytic receptor (Kucik et al., 1996).

Analysis of another Yop gives insight into a second family of signaling proteins involved in invasin-dependent uptake. The YopE protein is the most potent cytotoxin deposited by *Yersinia* species into host cells and significantly contributes to inhibition of *Yersinia* uptake (Mecsas et al., 1998). The protein is highly homologous to portions of the SptP protein of *Salmonella* and the ExoS cytotoxin encoded by *Pseudomonas* species, both of which are RHO-family-GTPase-activating proteins (GAPs) (Fu and Galan, 1999; Goehring et al., 1999). YopE similarly shows GAP activity, and a mutation in a region highly conserved in all RHOGAPs, called the arginine finger, abolishes both phagocytosis inhibition and GAP activity. Therefore, YopE appears to inhibit invasin-mediated uptake by depleting activated RHO family members of GTP, blocking their interaction with downstream effectors (Black and Bliska, 2000).

The RHO family member most likely to be involved in invasin-promoted uptake is RAC1. Overexpression of a

constitutively active RAC1, but not activated mutants of other RHO family members, reverses inhibition of phagocytosis by YopE (Black and Bliska, 2000). Furthermore, a RAC1 mutant locked in the GDP-bound state (RACN17) has a strong dominant interfering effect on uptake, whereas a mutant locked in the GTP-bound state (RACV12) stimulates uptake (Alrutz et al., 2000). This latter result is consistent with observations that GTP-γ-S stimulates uptake in semipermeabilized cells. RAC1 appears to be involved in uptake throughout the early stages of the process, because nascent phagosomes showing partially engulfed bacteria have large amounts of RAC1 localized about their surfaces. In contrast to RAC1, RHOABC proteins appear to have a negative regulatory role on uptake. Inactivation of these proteins by Clostridial C3 toxin stimulates uptake (Alrutz et al., 2000), whereas a constitutively active form of the protein inhibits uptake (Black and Bliska, 2000).

Phagocytosis of IgG and C3b-coated particles, as well as *Salmonella* and *Shigella* uptake, requires RHO family members, but in no case has such a distinct requirement for RAC1 been observed. The unique requirement for RAC1 in invasin-mediated uptake can be explained if one examines the behavior of other RHO family members in response to inactivation or integrin clustering. Inactivation of RHOABC causes loss of actin stress fibers, breakdown of focal adhesions and induction of cell rounding (Tapon and Hall, 1997). The loss of stress fibers presumably destabilizes focal contacts promoted by integrin receptors. The consequence of breakdown of a supramolecular array containing integrins is that the receptor becomes something of a free agent, which can be recruited to the phagocytic cup. This then facilitates sequestration of the receptor around the phagosome and consequent stimulation of uptake. Activation of RHOABC should have the opposite effect, rigidifying the cytoskeleton and making complex integrin-cytoskeleton contacts that prevent migration of the receptor to the site of contact between the bacterium and the mammalian cell. Why interfering forms of CDC42 have little effect on invasin-mediated uptake is less clear, but this may simply be the result of a lack of efficient recruitment of the protein to the site of bacterial binding. Only a small percentage of phagosomes bearing *Y. pseudotuberculosis* are associated with Cdc42 (Alrutz et al., 2000); presumably, therefore, the kinetics of Cdc42 recruitment to the site of β_1 chain integrin clustering are slow relative to those of RAC1 recruitment. In the absence of significant localization of Cdc42, invasin-mediated uptake might be dependent on RAC1.

The role of integrin binding in disease

Enteropathogenic *Yersinia* species simultaneously encode proteins that antagonize and promote uptake via integrin receptors; it is therefore reasonable to ask why these presumably contradictory activities exist. One possibility is that invasin does not promote uptake during animal infections. Binding of invasin to host cells induces the production of several cytokines (Kampik et al., 2000), activates B cells (Lundgren et al., 1996) and participates with YopE in inducing a cytotoxic T cell response (Falgarone et al., 1999). Although these are primarily anti-microbial responses, they may be important for the pathogenesis of *Yersinia* disease and induction of an inflammatory response. Alternatively, because disease within deep organ sites primarily involves replication of extracellular bacteria, binding of bacteria to target integrin receptors may function to facilitate deposition of the Yop proteins within host cells. Several properties of the infection argue against this latter model being the primary role of invasin. First, mutations in invasin fail to prevent systemic disease, indicating that the protein is dispensable for deposition of Yops (Pepe and Miller, 1993; Rosqvist et al., 1988). Secondly, only *Yersinia* species that initiate disease in the gut encode invasin, whereas the closely related *Y. pestis* species, which does not express invasin, still efficiently spreads from a flea bite to cause a lethal disease (Rosqvist et al., 1988). Thirdly, *Y. pseudotuberculosis* invasin mutants that can bind integrin receptors, but cannot promote bacterial uptake into cultured cells, are unable to enter M cells and colonize the Peyer's patch (Marra and Isberg, 1997). This indicates that internalization by M cells is required for colonization of the Peyer's patch, and argues that the most important role for binding of integrin receptors is to facilitate infection of intestinal lymph nodes.

Given that Yops interfere with uptake, it would appear that there should be no opportunity for invasin to promote internalization during infection, and yet bacteria are clearly seen within M cells after 60 minutes of oral inoculation (Marra and Isberg, 1997). There are two explanations for this phenomenon. Firstly, the most severe oral disease proceeds after the bacteria have been grown at ambient temperature. Growth at this temperature results in high levels of invasin expression, but very little expression of Yops. Therefore, shortly after entry into the small intestine, the bacteria may not have undergone sufficient biosynthesis at 37ºC to allow for maximal Yop expression, and consequently the bacteria remain uptake competent during the encounter with the M cell. Secondly, experiments using an in vitro culture system in which epithelial cells have been differentiated into cells that resemble M cells indicate that there may be inefficient targeting of Yops into M cells (Schulte et al., 2000). The cells that mimic M cells show little Yop-dependent cytotoxicity and little translocation of Yops into the cytosol.

Perspectives

Numerous microbial pathogens, in addition to enteropathogenic *Yersinia*, encode substrates that either attach to integrin receptors on host cells or bind to host proteins that adhere to integrins (Krukonis and Isberg, 1997). These include entry proteins, such as the adenovirus penton base protein (Mathias et al., 1998), *Leishmania* gp63 (Talamas-Rohana et al., 1990; Van Strijp et al., 1993), as well as proteins involved in extracellular adhesion, such as the *Bordetella pertussis* filamentous hemmaglutanin (Ishibashi et al., 1994). The widespread use of integrins as substrates presumably allows the pathogen to take advantage of the signaling properties of these receptors. Integrins are highly regulated proteins, and regulating the localization of a pathogen on either the extracellular surface of the host cell or within a phagosome is dependent on both the activation state of the host cell and the number of receptor available to contact microorganisms. Furthermore, engagement of integrin receptors by pathogens allows stimulation of a variety of signaling pathways within host cells. These include cytoskeleton-associated functions that allow stabilization of the pathogen–host-cell interaction, as well as induction of specific host cell transcripts. Given that

binding of pathogens through integrins can induce expression of cytokines (Kampik et al., 2000), proteases (Huhtala et al., 1995), and a variety of proteins regulated by MAP kinase pathways within host cells (Fincham et al., 2000), attachment has consequences for the infection process that go far beyond localized effects on the actual adhesion site.

The analysis of *Yersinia* entry into mammalian cells has allowed a detailed analysis of the elements involved in a phagocytic process as well as facilitated investigation of the determinants required for recognition of substrates by integrins. Further investigation of this process should reveal important signaling pathways that transmit information from engaged integrin receptors to processes involved in cytoskeletal activity and membrane trafficking.

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