Proc. Natl. Acad. Sci. USA
Vol. 85, pp. 6682–6686, September 1988
Biochemistry

Cultured mammalian cells attach to the invasin protein of *Yersinia pseudotuberculosis*

(invasive pathogens)

RALPH R. ISBERG AND JOHN M. LEONG

Department of Molecular Biology and Microbiology, Tufts University School of Medicine, 136 Harrison Avenue, Boston, MA 02111

Communicated by Stanley Falkow, June 6, 1988

ABSTRACT **The expression of invasin, the product of the *Yersinia pseudotuberculosis inv* gene, allows enteric bacteria to enter cultured mammalian cells. The ability of invasin to bind animal cells and the potential significance of this interaction in the entry process were investigated. It was found that HEp-2 cells could attach to surfaces coated with bacterial membranes containing invasin. By fractionating bacterial membrane proteins on $NaDodSO_4$/polyacrylamide gels and transferring the protein to filters, we demonstrated that the cell-binding component of the membranes comigrated with invasin. Mutations that changed the electrophoretic mobility of the protein also caused a corresponding shift in the migration of the cell-binding activity, showing that the comigrating protein was indeed invasin. Monoclonal antibodies directed against invasin that blocked invasin–HEp-2 cell interaction also inhibited bacteria from penetrating HEp-2 cells, indicating that interaction of this protein with animal cells is critical for cellular penetration.**

Many intracellular bacterial pathogens are able to penetrate epithelial cells (1–3). For enteroinvasive microorganisms, such as *Salmonella* and *Yersinia*, cellular invasion is the initial step in an infectious process that ultimately results in systemic illnesses (4, 5). These intracellular pathogens may enter epithelial cells either to grow within this protective niche or to pass through the cell to spread into other tissues. The molecular bases of these important and common processes are relatively obscure, although many studies have been initiated that should allow dissection of intracellular penetration and growth (6–10).

Yersinia pseudotuberculosis is a bacterium that is able to enter cultured mammalian cells (5, 11). This *in vitro* property is used as a model for invasive diseases caused by the microorganism. To initiate analysis of cellular invasion, molecular clones have been isolated from this organism that allow the normally innocuous *Escherichia coli* K-12 strain to penetrate mammalian cells (10). Such clones encode a single *Yersinia* gene, called *inv*, which is sufficient to confer this phenotype (12). The product of this locus, termed invasin, is a 103-kDa protein exposed on the surface of the bacterium (12) that is synthesized at low levels in both *Y. pseudotuberculosis* and *E. coli* strains harboring the *inv* gene (13).

To date, the mechanism of action of invasin has not been elucidated. Bacteria encoding *inv* efficiently bind to the surface of mammalian cells prior to internalization, and inv^- mutants are defective for this binding activity (12). This indicates that expression of the *inv* gene must result in synthesis of a ligand, located on the surface of the bacterium, that is recognized and bound by the host cell. There are two simple models for how this cell-recognition structure could be produced. Invasin could enzymatically synthesize the ligand, which may be a small molecule such as a carbohydrate. Alternatively, invasin itself could be the ligand, since it is known that this protein is exposed on the surface of the bacterial cell (12). In this communication, we tested the latter model by investigating whether animal cells were able to bind invasin and by evaluating whether cellular attachment to invasin is important for the entry process.

MATERIALS AND METHODS

Bacterial Strains, Medium, and Growth Conditions. Bacterial strains, described in Table 1, and HEp-2 cells were grown and maintained as described (12, 14). The plasmid pRI253, which contains the *inv* gene under the control of the phage T7 RNA polymerase promoter ϕ10, was constructed by digesting pT7-4 (15) with *Hin*dIII and *Eco*RI and inserting the *Hin*dIII–*Eco*RI fragment that encompasses the *inv* gene from plasmid pRI220Δ42-14 (12).

pJL213 is a plasmid in which the *Cla* I site internal to invasin (Fig. 1) is fused to a translational start site, allowing production of a protein missing the 131 amino-terminal residues of invasin expressed under the control of the ϕ10 promoter from the plasmid pT7-7 (15). Overproduction of proteins encoded by pRI253 and its derivatives was accomplished by introducing the plasmids into a strain harboring pGP1, a plasmid that encodes the phage T7 RNA polymerase under thermoinducible control (15). Induction of expression of the ϕ10 promoter was performed in 2× YT medium (17) as described (15).

Analysis of Membrane Proteins. $NaDodSO_4$/polyacrylamide gels were used to analyze protein preparations that had been solubilized in standard $NaDodSO_4$-containing loading buffer (18). Immunoprecipitations of [^{35}S]methionine-labeled samples were performed by the procedure of Kumamoto and Beckwith (16). Whole membranes were prepared by French pressure cell lysis (19) and pelleted at 300,000 × *g* in a Beckman TL100 ultracentrifuge. The insoluble material was washed twice in 10 mM Hepes (pH 8.0), and final membrane pellets were resuspended in 10 mM Tris·HCl (pH 8.0) at protein concentrations of 5–10 mg/ml. If outer membranes were to be isolated, freshly prepared washed insoluble material was loaded directly onto a discontinuous sucrose gradient and material of appropriate density was pooled and prepared as described (19).

Cell Binding to Invasin-Coated Surfaces. Dilutions of membrane preparations in isotonic phosphate-buffered saline (PBS) were crosslinked to 96-well microtiter dishes (Flow Laboratories) as described (20). To assay for cell attachment to invasin, HEp-2 cells were dispersed from confluent monolayers in trypsin, washed three times in soybean trypsin inhibitor (21, 22), and resuspended at 10^6 cells per ml in RPMI 1640 medium containing 20 mM Hepes (pH 7.0) and 0.4% bovine serum albumin. Aliquots (0.1 ml) of HEp-2 cells were allowed to attach to the bottoms of microtiter wells (20) and

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

Abbreviation: mAb, monoclonal antibody.

Table 1. Bacterial strains and plasmids

	Relevant properties	Ref. or source
Strains		
E. coli K12		
MC1000	F^- Δ(*ara-leu*) Δ(lac)X74 *rpsL galE galK*	16
MC4100	F^- *araD* Δ(*lac*) *rpsL*	16
SW5AA2Q	F^- *htpRam supFts lon*::miniTn*10*	34
Y. pseudotuberculosis		
YPIII(P^+)	Virulent, pIB1, inv^+	35
YP212	YPIII(P^+) *inv1*::*kan*	12
Plasmids		
pGP1	pACYC177 kan^R cI857 P_L-2	15
pT7-4	amp^R $P_{\phi 10}$	15
pT7-7	amp^R $P_{\phi 10}$	15
pRI253	pT7-4 inv^+	This study
pJL213	pT7-7 *inv Cla* I-*Eco*RI*	This study

*Deletion of first 450 base pairs of *inv* gene. This plasmid contains the coding sequence of invasin beginning at the *Cla* I site fused to an ATG start codon and ϕ10 promoter of plasmid pT7-7 (ref. 15 and Fig. 1).

the number of attached cells was quantitated by the hexosaminidase assay (23).

To visualize attachment of HEp-2 cells to filter replicas of polyacrylamide gels, proteins were fractionated electrophoretically on $NaDodSO_4$-containing gels (18) and electrotransferred in standard buffer (24) to either Immobilon (PVDF; Millipore) or nitrocellulose (HATF; Millipore) filters. The filters were incubated overnight in PBS containing 0.5% bovine serum albumin, washed twice in PBS, and then incubated with 1–2 × 10^7 live HEp-2 cells, freshly prepared as described above. Cells were allowed to attach to the protein on the filters, fixed in 3% (wt/vol) paraformaldehyde, and stained with amido black to visualize bands of cell attachment, as described (22, 25).

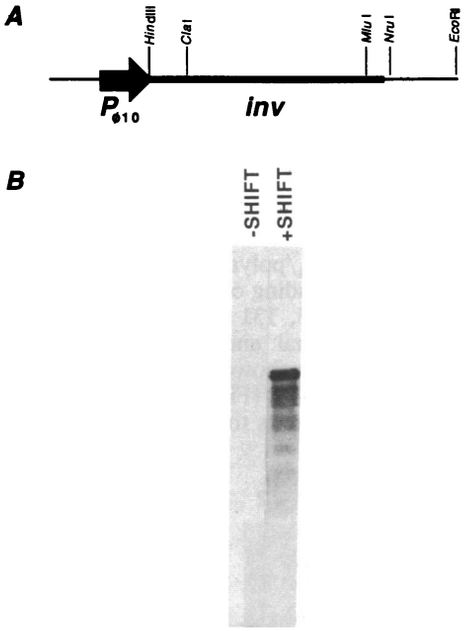

FIG. 1. Overproduction of invasin under the control of phage T7 RNA polymerase promoter. (*A*) Structure of pRI253, a plasmid that places the *inv* gene under the control of the phage T7 ϕ10 promoter (15). Denoted are restriction sites that were used for construction of invasin derivatives. (*B*) SW5AA2Q(pGP1/pRI253) was aerated at 30°C in M9-glucose medium (14) containing complete amino acids except methionine and cysteine. At a Klett reading of 40 (using a green filter), the culture was either labeled with [^{35}S]methionine (10 μCi/ml; 1 Ci = 37 GBq) (lane − SHIFT), or shifted to 42°C for 30 min and further aerated at 30°C for 30 min prior to labeling with [^{35}S]methionine (10 μCi/ml) (lane + SHIFT). Labeling periods were terminated by removing 1 ml of labeled cultures and adding it to 0.5 ml of 20% (wt/vol) ice-cold trichloroacetic acid. The protein precipitates were pelleted in a microcentrifuge and the trichloroacetic acid-insoluble material was washed twice with ice-cold acetone before solubilizing in $NaDodSO_4$ and processing for immunoprecipitation with polyclonal anti-invasin antibody, as described (16).

Preparation of Antisera and Monoclonal Antibodies. Monoclonal antibodies (mAbs) were raised by immunizing RBF/DnJ mice with Triton/EDTA-insoluble debris from MC1000 pGP1/pRI253 after overproduction of invasin, as above. Spleen cells from immune mice were fused to the FOX-NY myeloma cell line (26), and hybridoma supernatants were screened for anti-invasin antibody by the ELISA (27) and immunoblotting techniques (24). Selected hybridomas were cloned twice in soft agar (28).

One antibody, mAb 1B10 (IgG2a), was purified by affinity methods on a 3-ml protein A-Sepharose 4B column (Pharmacia), as described (29). The purified antibody retained full activity, and only heavy and light chains were apparent when analyzed by gel electrophoresis. Concentrations of antibodies were determined by protein assay (30) or indirect ELISA assay (31).

Electron Microscopy of HEp-2 Cells Bound to Invasin. Invasin, fractionated on an $NaDodSO_4$/polyacrylamide gel, was electrotransferred to Immobilon filters and probed with HEp-2 cells, as above. The filters were fixed in a solution of 50 mM Pipes (pH 6.8), 1% glutaraldehyde, 150 mM KCl, and 2 mM $MgCl_2$ for 30 min and washed three times in the identical buffer without glutaraldehyde. The region of the filter corresponding to invasin was cut out with a scalpel and prepared for scanning or transmission electron microscopy as described (32).

RESULTS

Construction and Analysis of an Invasin Overproducer. The *inv* gene was fused to the phage T7 ϕ10 promoter and the plasmid so constructed (Fig. 1*A*, pRI253) was then introduced into a strain encoding the phage T7 RNA polymerase under thermoinducible control (15). Biosynthetically labeled proteins produced by this strain were analyzed by immunoprecipitation with polyclonal anti-invasin antibody to identify the invasin-related polypeptide species that were produced after induction. As can be seen in Fig. 1*B*, bacteria grown at 30°C expressed little [^{35}S]methionine-labeled invasin (Fig. 1*B*, lane − SHIFT). In contrast, thermal induction of T7 RNA polymerase resulted in a large increase in synthesis of invasin-related protein products immunoprecipitable by anti-invasin antibody (Fig. 1*B*, lane + SHIFT).

A family of invasin-related peptides was thermally induced in the strains analyzed here (Fig. 1*B*). This phenomenon does not appear to be unique to strains that overproduce this protein, inasmuch as multiple species of invasin also have been seen with strains that produce low levels of invasin (12), with *in vitro* translation reactions driven by clones containing *inv* (12), and with the wild-type *Y. pseudotuberculosis* strain (see Fig. 4; R.R.I., unpublished data). This raises the possibility that if invasin is able to attach to animal cells, then many of these species may participate in the binding reaction. The following experiments illustrate this point.

Animal Cells Attach to Invasin. To determine if mammalian cells could attach to a membrane component from the invasin-overproducing strain, dispersed HEp-2 cells were incubated in 96-well polystyrene dishes that had been coated with membrane extracts from bacterial strains (20). The mammalian cells were allowed to attach to the well bottoms, and the number of bound cells was quantitated by assaying for lysosomal hexosaminidase (Fig. 2). It is clear from Fig. 2 that the HEp-2 cells were able to attach to membranes prepared from a strain that overproduced invasin, with >50% of the added cells binding to the bottom of the wells. Membranes prepared from an isogenic *E. coli* strain that does not contain the *inv* locus were unable to promote attachment of the animal cells to the polystyrene wells; <0.5% of the input cells attached to these wells over a wide range of membrane concentrations (Fig. 2).

To determine if the mammalian cells had directly attached to invasin in the above experiment, sucrose-gradient-isolated outer membrane material was fractionated on a polyacrylamide gel (18) and transferred to filters. The filters were then incubated with live, dispersed HEp-2 cells. If the mammalian cells were able to attach to invasin, then bands of cells, revealed by histochemical staining, should form where the protein has been transferred (20, 22). Such an experiment, which is based on techniques devised by Pierschbacher, Hayman, and coworkers (22), is displayed in Fig. 3. No animal cells attached to lanes loaded with membranes from an *E. coli* K12 strain (Fig. 3*B*, lanes pT7-4). In contrast, if membrane extracts from the isogenic invasin-overproducing strain were analyzed, animal cells formed four or five bands that attached to components comigrating with the invasin species (Fig. 3*B*, lane pR1253 at 25°C). Extracts solubilized at 95°C, rather than at room temperature, were unable to promote cell binding to the filter replicas (Fig. 3*B*). We conclude, therefore, that animal cells were able to attach to several of the invasin species and that heat denaturation destroyed the binding activity.

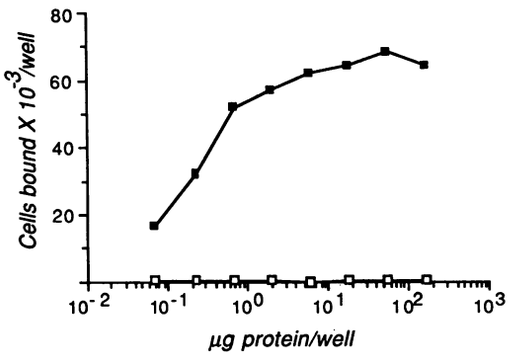

FIG. 2. HEp-2 cells attach to surfaces coated with membrane preparations containing invasin. Membranes were prepared from either MC1000pGP1/pRI253 (■) or MC1000pGP1/pT7-4 (□), and dilutions were used to coat 96-well microtiter dishes. Approximately 10^5 HEp-2 cells were allowed to incubate in the wells for 60 min at 37°C, and unattached cells were removed. The number of cells bound was determined by assaying for lysosomal hexosaminidase. Displayed is the number of cells bound as a function of amount total membrane proteins used to coat microtiter wells.

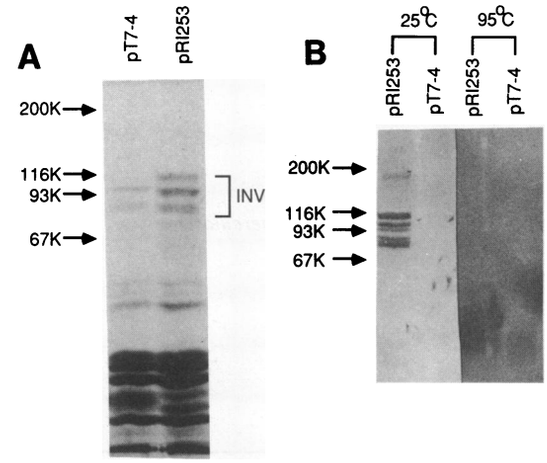

FIG. 3. HEp-2 cells bind to invasin. (*A*) $NaDodSO_4$/7.5% polyacrylamide gel electrophoresis profile (18) of sucrose gradient-purified outer membranes (19), stained with Coomassie blue. Lanes: pT7-4, material from MC1000 pGP1/pT7-4; pRI253, material from MC1000 pGP1/pRI253. INV, protein bands corresponding to invasin that are specific for strains containing the pRI253 plasmid. (*B*) Attachment of HEp-2 cells to proteins transferred to filters. Proteins fractionated on $NaDodSO_4$/7.5% polyacrylamide gel, as above, were transferred to a nitrocellulose filter and then probed with live HEp-2 cells to detect attachment of cells to protein bands. Lanes: pRI253, MC1000 pGP1/pRI253 membranes solubilized in $NaDodSO_4$ loading buffer (18) at 25°C or 95°C before loading on gel; pT7-4, MC1000 pGP1/pT7-4 solubilized in $NaDodSO_4$ at 25°C or 95°C before loading on gel.

To eliminate the possibility that the mammalian cells do not attach to invasin but merely bind to comigrating proteins, deletion derivatives of the *inv* gene were isolated that generated proteins migrating faster than invasin on $NaDodSO_4$/polyacrylamide gels. If invasin is able to bind mammalian cells, then filter replicas of such proteins fractionated on $NaDodSO_4$/polyacrylamide gels should reveal faster migrating cell-binding components. In one such deletion derivative, pJL213, 131 amino acids were eliminated from the amino-terminal end of invasin (Fig. 4*A*). The cell-attachment activity showed a corresponding change in electrophoretic migration (Fig. 4*B*). Therefore, the cell-binding component appears to be bona fide invasin.

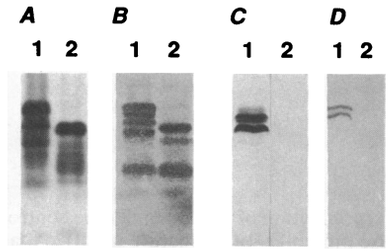

FIG. 4. Animal cells bind to derivatives of invasin. Membrane proteins were fractionated on $NaDodSO_4$/polyacrylamide gels, transferred to PVDF filters, and probed with either anti-invasin mAbs or live HEp-2 cells, as described in Fig. 3. (*A*) Immunoblot of 10 μg of membrane proteins prepared from SW5AA2Q pGP1/pRI253 (lane 1) or SW5AA2Q pGP1/pJL213 (lane 2) and probed with a mixture of anti-invasin mAbs. (*B*) Attachment of HEp-2 cells to membranes (100 μg of crude protein) prepared from SW5AA2Q/pRI253 (lane 1) or SW5AA2Q/pJL213 (lane 2). (*C*) Immunoblot of 100 μg of membrane proteins prepared from *Y. pseudotuberculosis* strains YPIII(P^+) (lane 1) and YP212, a *Y. pseudotuberculosis* inv^- strain (lane 2) probed with mAb 1B10. (*D*) Attachment of HEp-2 cells to membranes (100 μg of crude protein) prepared from YPIII(P^+) (lane 1) or YP212 (lane 2).

We analyzed the attachment activity of invasin expressed in small amounts by the wild-type bacteria because it is possible that overproduction of invasin may generate species that have activities not normally exhibited by this protein. To this end, membrane extracts were prepared from two *Y. pseudotuberculosis* strains and subjected to the filter binding assay, as above. HEp-2 cells were still able to attach to proteins that comigrated with the invasin species produced from the wild-type strain (Fig. 4 *C* and *D*). A mutant *Y. pseudotuberculosis* strain harboring an insertion mutation in the *inv* locus (ref. 12 and Fig. 4*C*), in contrast, showed no such cell-binding components (Fig. 4*D*). Therefore, invasin appears to possess a cell-attachment activity when expressed in the parental pathogenic microorganism and binding is not dependent on overproduction of the protein.

To establish that the bands observed on filters represented mammalian cells, regions of the filter corresponding to the amido black-staining bands were cut out and prepared for electron microscopy. Fig. 5 *A–C* shows that HEp-2 cells were tightly bound to this region of the filter and appeared to spread out on this surface. In contrast, if a region of the filter showing no histochemical staining was analyzed, then bound cells were not found (Fig. 5*D*). We conclude that the bands seen on the filter replicas were indeed due to staining of HEp-2 cells.

Invasin-Mediated Penetration of Animal Cells Requires Cell Binding by Invasin. To test the model that the invasin–animal cell interaction is necessary for cellular penetration, we wanted to determine whether disruption of this binding eliminated bacterial entry into HEp-2 cells. To this end, a battery of hybridoma cell lines were derived that produced mAbs directed against invasin. Two of the hybridoma cell lines, called 1B10 and 2G2, produced mAbs that recognized invasin and prevented the binding of HEp-2 cells to invasin-coated polystyrene dishes as well (data not shown). Inasmuch as these antibodies disrupted invasin–HEp-2 cell interaction, we tested whether they were also able to block inv^+ bacteria from penetrating the mammalian cell line.

Dilutions of hybridoma supernatants were incubated with an *E. coli* K12 inv^+ strain [MC4100(pRI203)], and the treated bacteria were assayed for the ability to enter HEp-2 cells. The addition of a saturating amount of mAb 2G2 to the bacteria caused a 1000-times decrease in the number of bacteria that were able to penetrate the HEp-2 cells, with one-half maximal inhibition resulting from 20 ng of antibody added to 10^7 bacteria (Fig. 6*A*). In contrast, mAb 2B10, which is able to recognize invasin but not disrupt binding of the HEp-2 cells to invasin-coated polystyrene wells, had no effect on the ability of bacteria to penetrate HEp-2 cells. As a further control, bacteria were treated with an mAb isolated in our screen that recognized an unidentified *E. coli* membrane component. This mAb, 3C12, also had no effect on cellular penetration (Fig. 6*A*). In fact, among 11 anti-invasin mAbs that we have analyzed in this fashion, only antibodies that disrupted invasin–host cell interaction in polystyrene dishes could prevent entry of inv^+ bacteria into animal cells (unpublished results).

To ensure that the actual blocking activity was due to the mAb present in the hybridoma supernatants, and not some serum component, another mAb that blocks cellular attachment to invasin was affinity purified with protein A-Sepharose 4B column chromatography. Addition of homogeneous preparations of this antibody, 1B10, also prevented cellular penetration by *E. coli* K12 inv^+ bacteria (Fig. 6*B*), with no effect on viability of the bacteria. Saturating amounts of the mAb depressed entry into HEp-2 cells to a level similar to the isogenic inv^- strain, with one-half maximal inhibition at 9 ng of antibody per 10^7 bacteria (≈3700 antibody molecules per bacterial cell; Fig. 6*B*). These antibody studies indicate, therefore, that the binding of invasin to HEp-2 cells is required for invasin-mediated entry into this cultured cell line.

DISCUSSION

We have demonstrated that invasin immobilized on solid matrices was able to recognize and bind HEp-2 cells. By using our assay conditions, the attachment of cells to this protein resulted in the formation of monolayers, in which individual cells spread across invasin-coated surfaces a few minutes after attachment (Fig. 5). This allowed us to utilize assays identical to those employed in the analysis of mammalian cell adhesion proteins, such as fibronectin and vitronectin (33), which interact with cultured cells in a similar fashion (20, 22). In spite of this similarity, it should be emphasized that the protein sequence of invasin (12) does not

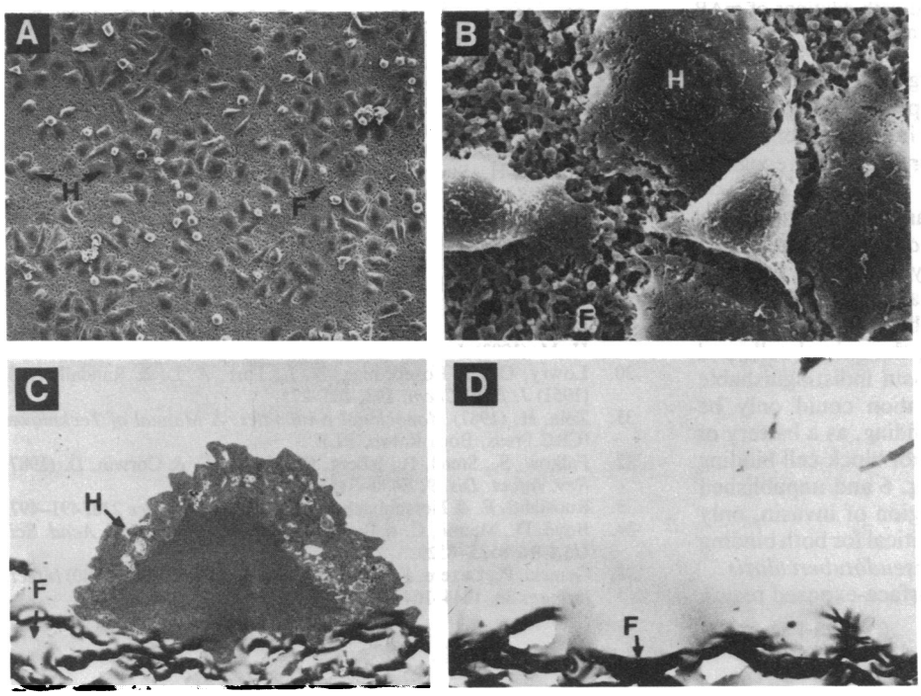

FIG. 5. Analysis of mammalian cell attachment to invasin-coated filters. Membrane proteins from the invasin-overproducing strain MC1000 pGP1/pRI253 were fractionated on two lanes of a polyacrylamide gel (18) and transferred to Immobilon filters. The filters were probed with freshly prepared HEp-2 cells, washed three times in PBS, and fixed in glutaraldehyde. One-half of the filter, corresponding to one of the lanes transferred from the gel, was stained with amido black to localize the region of cell binding on the filter. By using the stained filter as a guide, the region of the unstained filter corresponding to the cell bands was cut out and processed for electron microscopy. (*A* and *B*) Scanning electron microscopy of region of filter corresponding to amido black-staining bands. (*C*) Transmission electron microscopy of a thin section corresponding to amido black-staining band. (*D*) Thin section of region where no staining was evident. F, Filter; H, HEp-2 cell.

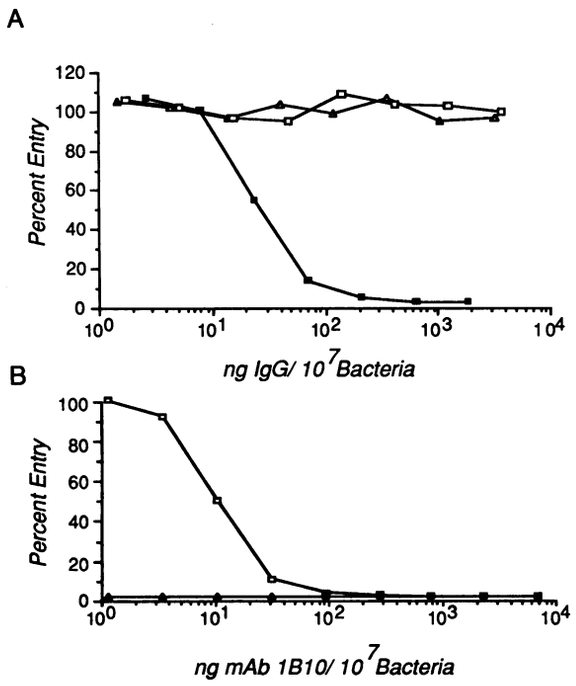

FIG. 6. Anti-invasin antibody blocks invasin-mediated cellular penetration. (*A*) Approximately 10^7 MC1000(pRI203 *inv*$^+$) were incubated for 30 min at 37°C in RPMI 1640 medium with various dilutions of mAbs from hybridoma cell line culture supernatants. The adsorbed bacteria were allowed to bind to HEp-2 cell monolayers for 90 min on ice and then assayed for the ability to penetrate HEp-2 cells during a 1-hr incubation at 37°C (10). The quantity of bacteria that entered the HEp-2 cells was determined by using resistance to gentamicin killing, as described (10), and is plotted as a function of the amount of added mAb. The 100% entry was determined to be 8.1 $\times 10^5$ bacteria surviving gentamicin treatment. □, Bacteria incubated with mAb 2B10, a mAb that recognizes invasin but does not block cellular attachment; △, bacteria treated with 3C12, a mAb that recognizes an unidentified *E. coli* membrane protein; ■, bacteria treated with 2G2, a mAb that recognizes invasin and blocks attachment of HEp-2 cells to invasin-coated surfaces. (*B*) MC4100 (pRI203*inv*$^+$) or MC4100(*inv*$^-$) were incubated, as above, with affinity-purified mAb 1B10 and assayed for the ability to penetrate HEp-2 cells. □, MC4100 (pRI203) incubated with dilutions of mAB 1B10; △, MC4100 treated with dilutions of mAb 1B10.

contain the cell-binding tripeptide Arg-Gly-Asp present in most of the characterized cell adhesion proteins (33). It seems likely that the unidentified receptor for invasin either has not been implicated in cellular adhesion or is able to recognize more than one protein sequence.

The attachment of invasin to the animal cell surface is essential for cellular penetration by *E. coli* strains producing this protein. This was demonstrated by incubating bacterial strains encoding invasin with mAbs that recognized invasin as well as disrupted invasin–HEp-2 cell binding. This treatment prevented bacteria from entering the animal cells and rendered *E. coli* strains encoding invasin indistinguishable from *inv*$^-$ bacteria. Bacterial penetration could only be blocked by mAbs that disrupted cell binding, as a battery of mAbs that recognized invasin but did not block cell binding had no effect on the entry process (Fig. 6 and unpublished data). This indicates that a specific region of invasin, only recognized by some of these mAbs, is critical for both binding and penetration of animal cells by *Y. pseudotuberculosis*.

Our results indicate that a specific surface-exposed region of invasin is recognized and bound by animal cells that subsequently internalize the bacterial cell. It is clear that the binding event promoted by this domain is critical for penetration by the bacterium, although the actual endocytic process may require other bacterial proteins, or parts of invasin other than the cell-binding domain. The elucidation of the nature of this cell-binding domain, and the receptor that binds this structure, should allow the further dissection of this process and identification of the ligand mimicked by invasin.

We thank John Forger of the Harvard University Monoclonal Antibody Facility for performing hybridoma fusions, Judith Reichler for electron microscopy, Michael Pierschbacher for experimental advice, and Carol Kumamoto for review of the text. This work was supported by Grant 1 RO1-AI23538 from the National Institutes of Health and a Presidential Young Investigator Award from the National Science Foundation to R.R.I. J.M.L. is a Helen Hay Whitney Fellow and R.R.I. is a Searle–Chicago Community Trust Scholar.

1. Formal, S. B., Hale, T. L. & Sansonetti, P. J. (1983) *Rev. Infect. Dis.* **5**, S702–S707.
2. Devenish, J. A. & Shiemann, D. A. (1981) *Infect. Immun.* **36**, 48–55.
3. Sansonetti, P. J., Ryter, A., Clerc, P., Maurelli, A. T. & Mounier, J. (1986) *Infect. Immun.* **51**, 461–469.
4. Takeuchi, A. & Sprinz, H. (1967) *Am. J. Pathol.* **51**, 137–161.
5. Carter, P. B. (1975) *Infect. Immun.* **11**, 164–170.
6. Maurelli, A. T., Baudry, B., d'Hauteville, H. & Sansonetti, P. J. (1985) *Infect. Immun.* **49**, 164–171.
7. Sasakawa, C., Kamata, K., Sakai, T., Murayama, S. Y., Makino, S. & Yoshikawa, M. (1986) *Infect. Immun.* **51**, 470–475.
8. Fields, P. I., Swanson, R. V., Haidiris, C. G. & Heffron, F. (1986) *Proc. Natl. Acad. Sci. USA* **83**, 5189–5193.
9. Small, P. L. C., Isberg, R. R. & Falkow, S. (1987) *Infect. Immun.* **55**, 1674–1679.
10. Isberg, R. R. & Falkow, S. (1985) *Nature (London)* **317**, 262–264.
11. Bovallius, A. & Nillson, G. (1975) *Can. J. Microbiol.* **21**, 1997–2007.
12. Isberg, R. R., Voorhis, D. L. & Falkow, S. (1987) *Cell* **50**, 769–778.
13. Isberg, R. R., Swain, A. & Falkow, S. (1988) *Infect. Immun.* **56**, 2133–2138. .
14. Miller, J. H. (1972) *Experiments in Molecular Genetics* (Cold Spring Harbor Lab., Cold Spring Harbor, NY).
15. Tabor, S. & Richardson, C. C. (1985) *Proc. Natl. Acad. Sci. USA* **82**, 1074–1078.
16. Kumamoto, C. A. & Beckwith, J. (1983) *J. Bacteriol.* **154**, 253–260.
17. Messing, J. (1983) *Methods Enzymol.* **101**, 20–78.
18. Laemmli, U. K. (1970) *Nature (London)* **227**, 680–685.
19. Smit, J., Kamio, Y. & Nikaido, H. (1975) *J. Bacteriol.* **124**, 942–958.
20. Pierschbacher, M., Hayman, E. G. & Ruoslahti, E. (1983) *Proc. Natl. Acad. Sci. USA* **80**, 1224–1227.
21. Ruoslahti, E., Hayman, E. G., Pierschbacher, M. & Engval, E. (1981) *Methods Enzymol.* **82**, 803–831.
22. Hayman, E. G., Engval, E., A'Hearn, E., Barnes, D., Pierschbacher, M. & Ruoslahti, E. (1982) *J. Cell Biol.* **95**, 20–23.
23. Landegren, U. (1984) *J. Immunol. Methods* **67**, 379–388.
24. Towbin, H., Staehelin, T. & Gordon, J. (1979) *Proc. Natl. Acad. Sci. USA* **76**, 4350–4354.
25. Bell, M. L. & Engval, E. (1982) *Anal. Biochem.* **123**, 329–335.
26. Taggart, R. T. & Samloff, I. M. (1983) *Science* **219**, 1228–1230.
27. Engval, E. & Perlmann, P. (1971) *Immunocytochemistry* **8**, 871–874.
28. Civin, C. I. & Banquerigo, M. L. (1986) *Methods Enzymol.* **121**, 322–327.
29. Underwood, P. A., Kelly, J. F., Harmon, D. F. & Macmillan, H. M. (1983) *J. Immunol. Methods* **60**, 33–45.
30. Lowry, O. H., Rosebrough, N. J., Farr, A. L. & Randall, R. J. (1951) *J. Biol. Chem.* **193**, 265–275.
31. Zola, H. (1987) *Monoclonal Antibodies: A Manual of Techniques* (CRC Press, Boca Raton, FL).
32. Falkow, S., Small, P., Isberg, R., Hayes, F. & Corwin, D. (1987) *Rev. Infect. Dis.* **9**, S450–S455.
33. Ruoslahti, E. & Pierschbacher, M. D. (1987) *Science* **238**, 491–497.
34. Boyd, D., Manoil, C. & Beckwith, J. (1987) *Proc. Natl. Acad. Sci. USA* **84**, 8525–8529.
35. Gemski, P., Lazere, J. R., Casey, T. & Wohlhieter, P. (1980) *Infect. Immun.* **28**, 1044–1047.