Invasion of brain microvascular endothelial cells (BMEC) is a prerequisite for successful crossing of the blood-brain barrier by Escherichia coli K1. We have previously demonstrated the requirement of cytoskeletal rearrangements and activation of focal adhesion kinase (FAK) in E. coli K1 invasion of human BMEC (HBMEC). The current study investigated the role of phosphatidylinositol 3-kinase (PI3K) activation and PI3K interaction with FAK in E. coli invasion of HBMEC. PI3K inhibitor LY294002 blocked E. coli K1 invasion of HBMEC in a dose-dependent manner, whereas an inactive analogue LY305511 had no such effect. In HBMEC, E. coli K1 increased phosphorylation of Akt, a downstream effector of PI3K, which was completely blocked by LY294002. In contrast, non-invasive E. coli failed to activate PI3K.

Overexpression of PI3K mutants Δp85 and catalytically inactive p110 in HBMEC significantly inhibited both PI3K/Akt activation and E. coli K1 invasion of HBMEC. Stimulation of HBMEC with E. coli K1 increased PI3K association with FAK. Furthermore, PI3K/Akt activation was blocked in HBMEC-overexpressing FAK dominant-negative mutants (FRNK and Phe397FAK). These results demonstrated the involvement of PI3K signaling in E. coli K1 invasion of HBMEC and identified a novel role for PI3K interaction with FAK in the pathogenesis of E. coli meningitis.

In neonates, Escherichia coli is the most common Gram-negative bacterium that causes meningitis, a serious disease affecting the central nervous system. The most distressing aspect of neonatal Gram-negative meningitis is high morbidity and mortality despite advances in antimicrobial chemotherapy and supportive care. Increased understanding of the pathogenesis and pathophysiology of this disease can lead to improved outcome. Intravascular survival and penetration of the blood-brain barrier by circulating bacteria represent the most critical events in the development of bacterial meningitis (1).

Invasion of brain microvascular endothelial cells (BMEC) is a requirement for E. coli crossing of the blood-brain barrier, and it involves attachment of E. coli to BMEC through interaction of bacterial ligands with corresponding receptors present on BMEC cell surface (2). Several E. coli determinants (OmpA, IbeA, IbeB, and YijP) involved in the invasion of BMEC have been identified (3–6). Receptors on the surface of BMEC for two of these E. coli determinants (OmpA and IbeA) have been characterized biochemically (7, 8). We have recently demonstrated that E. coli invasion of BMEC occurs via a zipper-like mechanism and requires cytoskeletal rearrangements in the host cell (9). We have further characterized in BMEC that E. coli K1 induces tyrosine phosphorylation of focal adhesion kinase (FAK) and paxillin, a cytoskeletal protein known to associate with FAK (10). Furthermore, using FAK dominant-negative mutants we have shown that FAK kinase activity and its autophosphorylation site tyrosine 397 (Tyr-397) are critical for E. coli K1 invasion of HBMEC (10). These results establish that FAK signaling is essential in E. coli K1 invasion of HBMEC.

FAK is a 125-kDa non-receptor tyrosine kinase and plays an important role in the assembly of signaling complexes that regulate the organization of the cytoskeleton and modulate the function of growth factors. An amino- and a carboxyl-terminal domain of about 400 amino acids each flank the kinase domain of FAK. The amino-terminal domain contains an autophosphorylation site (Tyr-397), and the carboxyl-terminal domain contains focal adhesion targeting sequences needed for targeting to focal adhesions and binding sites for intracellular signaling molecules and cytoskeletal proteins (11). However, the signaling molecules that interact with FAK in E. coli K1 invasion of BMEC have not been identified. The autophosphorylation site Tyr-397 of FAK has been shown to bind to Src kinases and phosphatidylinositol 3-kinase (PI3K), and binding to one or both is required for FAK-mediated functions (12–14). Interestingly, pretreatment of HBMEC with Src kinase-specific inhibitor, PP1, did not affect E. coli K1 invasion of HBMEC (10). Furthermore, overexpression of Src kinase dominant-negative mutants did not block E. coli K1 invasion of HBMEC, suggesting an insignificant role for Src kinases.

In the present study, we examined the role of PI3K in E. coli K1 invasion of HBMEC.
invasion of HBMEC. PI3K proteins are subdivided into three major groups, and group I PI3K has been identified as a downstream effector of both receptor and non-receptor tyrosine kinases (16). Group I PI3K is a heterodimer consisting of a regulatory subunit (p85) and a 110-kDa catalytic subunit (p110). Regulatory subunit p85 contains an SH3 domain and two SH2 domains that interact with various intracellular signaling molecules. Interaction of the SH2 domains of p85 subunit with phosphorytrosine residues on tyrosine kinases, including Src and FAK, results in PI3K activation. PI3K catalytic subunit p110 phosphorylates the β-3 position of the inositol ring of phosphatidylinositol, and its derivatives form the second messenger phosphatidylinositol 3,4,5-trisphosphate. Phosphatidylinositol 3,4,5-trisphosphate binding to pleckstrin homology (PH) domains of various cellular and cytoskeletal proteins mediates membrane recruitment of several kinases including, Akt (protein kinase B), PDK1, and PDK2. Akt is then activated by sequential phosphorylation on Thr-308 and Ser-473 by phosphoinositide-dependent protein kinases PDK1 and PDK2, respectively (17). PI3K signaling has been implicated in a variety of cellular processes, including survival, proliferation, migration, metabolic changes (18 and 19) and Listeria monocytogenes invasion of epithelial cells (20). PI3K has been shown to act downstream of FAK in cell migration and survival (21–23). In this paper, we report the activation of PI3K/Akt signaling in E. coli K1 invasion of HBMECs and demonstrate that PI3K interaction with FAK is required for the activation of PI3K/Akt signaling in this process.

EXPERIMENTAL PROCEDURES

Materials—Monoclonal antibodies to FAK and p85 were purchased from Transduction Laboratories (Lexington, KY). Polyclonal Akt antibody, which recognizes the activated form of Akt, was purchased from New England BioLabs (Beverly, MA). Protein A-agarose was from Roche Molecular Biochemicals (Beverly, MA). Protein A-agarose was from Roche Molecular Biochemicals. M2 monoclonal antibody that recognizes FLAG epitope was purchased from Sigma Chemical Co. (St. Louis, MO). PI3K inhibitor LY294002 was purchased from Calbiochem (San Diego, CA). Its inactive analogue LY303511 was a gift from Dr. Chris Vlahos (Eli Lilly & Co., Indianapolis, IN). Horseradish peroxidase-conjugated anti-goat antibody were from Santa Cruz Biotechnology (Santa Cruz, CA). Protein A-agarose was from Roche Molecular Biochemicals. M2 monoclonal antibody that recognizes FLAG epitope was purchased from Sigma Chemical Co. (St. Louis, MO). PI3K inhibitor LY294002 was purchased from Calbiochem (San Diego, CA). Its inactive analogue LY303511 was a gift from Dr. Chris Vlahos (Eli Lilly & Co., Indianapolis, IN). Horseradish peroxidase-conjugated anti-goat antibody were from Santa Cruz Biotechnology (Santa Cruz, CA). Protein A-agarose was from Roche Molecular Biochemicals.

Mammalian expression vector pcDNA3-expressing kinase-negative p110 and Δp85 mutants with the FLAG epitope tag were kindly provided by L. C. Cantley (Harvard Medical School, Boston, MA). Expression vectors for FAK mutants lacking the autophosphorylation site (Phe397FAK) and amino-terminal domain (FRNK) were previously described (26, 27). Strain E44 is a rifampin-resistant mutant of E. coli K1 strain RS218 (serotype O157:H7) isolated from the cerebrospinal fluid of a neonate with meningitis and has been shown to invade brain microvascular endothelial cells (21). E. coli HB101 is a non-invasive laboratory strain (3). Endothelial Cell Culture and Transfections—Human brain microvascular endothelial cells (HBMEC) were isolated and cultured as described previously (28). HBMEC cultures were routinely grown on rat tail collagen-coated dishes in RPMI medium containing 10% heat-inactivated fetal bovine serum, 10% Nu-Serum, 2 mM glutamine, 1 mM pyruvate, penicillin (100 units/ml), streptomycin (100 µg/ml), essential amino acids, and vitamins. HBMECs were transfected with mammalian expression vectors coding for PI3K mutants using LipofectAMINE as described previously (10). Briefly, DNA-LipofectAMINE complex in RPMI was added to 50% confluent HBMEC monolayers. After 6 h of incubation at 37 °C, cells were washed with RPMI and complete medium was added. Three days after the transfection, HBMEC were transferred to medium containing an antibiotic G418 (400 µg/ml) for 2–3 weeks, and antibiotic-resistant colonies were pooled for further analysis. HBMEC-expressing FAK mutant Phe397FAK was maintained in medium containing G418 (400 µg/ml) and FRNK-expressing cells in the presence of hygromycin (100 µg/ml) as described previously (10).

Cell Lysates and Immunoprecipitation—Preparations of cell lysates and immunoprecipitation were performed as described previously (10). Confluent monolayers of HBMEC were stimulated with E. coli resuspended in experimental medium at a multiplicity of infection of 100 for the indicated periods of time, and the monolayers were washed with cold phosphate-buffered saline containing 1 mM sodium orthovanadate. Cells were lysed in modified radiolmune precipitation buffer (50 mM Tris, pH 7.4, 150 mM NaCl, 0.5% Triton X-100, 0.5% deoxycholate, 0.1% SDS, 1 mM sodium orthovanadate, 50 mM NaF, 10 mM sodium pyrophosphate, 25 mM β-glycerophosphate, 1 mM phenylmethylsulfonyl fluoride, and 10 µg/ml each of leupeptin and apro tin), at 4 °C for 20 min. The cell lysates were centrifuged at 16,000 × g for 20 min at 4 °C. The supernatant was collected, and protein content was determined using the bicinchoninic acid kit. For immunoprecipitation, 300–500 µg of protein was incubated with appropriate antibody overnight at 4 °C and incubated for 1 h with Protein A-agarose. The immune complexes were washed four times with radiolmune precipitation buffer without deoxycholate, and the proteins from immune complexes were eluted in SDS sample buffer for further analysis by Western blotting.

Western Blotting—Proteins in immune complexes or total cell lysates were electrophoresed on 10% SDS-polyacrylamide electrophoresis gels and transferred to polyvinylidene difluoride membranes. The blots were blocked with TBST (25 mM Tris, pH 7.4, 150 mM NaCl, and 0.1% Tween 20) containing 3% bovine serum albumin for 2 h at room temperature and incubated with appropriate primary antibody overnight at 4 °C or for 2 h at room temperature. They were washed with TBST and incubated with horseradish peroxidase-conjugated secondary antibody for 1 h at room temperature. Subsequently, the blots were washed with TBST and developed with Super Signal chemiluminescence reagent. The blots were exposed to x-ray film to visualize the proteins. The protein bands on autoradiograms were scanned by using a GS-700 imaging densitometer, and the intensity of bands was analyzed using Quantitation One software (Bio-Rad).

RESULTS

Inhibition of E. coli Invasion by PI3K Inhibitors—We have previously shown that the invasion of E. coli K1 induces activation of non-receptor tyrosine kinase FAK and its autophosphorylation site Tyr-397 is essential for E. coli invasion of HBMEC (10). Tyrosine 397 of FAK interacts with various intracellular signaling molecules, including Src kinases and PI3K, for FAK promoted biological effects. However, our earlier studies have demonstrated a minimal or no role for Src kinases in E. coli invasion of HBMEC. Hence, the involvement of PI3K in E. coli invasion of HBMEC was studied using a PI3K-specific inhibitor, LY294022. HBMEC were pre-treated with either experimental medium (Control) or experimental medium containing various concentrations of LY294002 (1–50 µM) for 30 min. Then, E. coli invasion assays were performed on these cells using invasive E. coli K1 strain E44, and the results were expressed as a percentage of the control. Fig. 1 shows that LY294002 inhibited E. coli K1 invasion of HBMEC in a dose-dependent manner with an IC50 of 5 µM (13,734 ± 1,000 cfu/well without inhibitor versus 834 ± 85 cfu/well with 50 µM LY294002, p < 0.001). Binding of E. coli to HBMEC was not
affected by LY294002 pretreatment, and LY294002 was not toxic to HBMEC even at 100 μM as determined by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium assay (data not shown). To confirm that the observed inhibition was specific to LY294002, E. coli invasion assays were also performed with LY303511, which is an inactive analogue of LY294002. The results (Fig. 1) showed that, even at 50 μM concentration, LY303511 had very little effect on E. coli K1 invasion of HBMEC. These results suggest the PI3K activity is required for E. coli K1 invasion of HBMEC.

Activation of PI3K by Invasive E. coli K1 in HBMEC—Stimulation of PI3K results in activation of a Ser/Thr kinase Akt (protein kinase B). Products generated by PI3K recruit Akt to the membrane, where a Ser/Thr kinase PDK1 phosphorylates Thr-308 followed by Ser-473 by PDK2, resulting in the activation of Akt. Activation of Akt is sensitive to PI3K inhibitors, and the phosphorylated Akt can be detected using phosphospecific Akt antibodies. We, therefore, used Akt activation as a parameter for determining PI3K activation in HBMEC by E. coli. To test the activation of PI3K, HBMEC were stimulated with invasive E. coli K1 strain E44 and non-invasive E. coli strain HB101. As a positive control, HBMECs were stimulated with epidermal growth factor (EGF) in the same experiment. Then, the HBMEC monolayers were lysed and cell lysates were immunoprecipitated with anti-Akt antibody (Fig. 2, lane 3) or presence (+) of PI3K inhibitor LY294002 were immunoprecipitated with indicated antibodies. D, quantitation of phospho-Akt bands in C. Error bars indicate standard deviation.

Compared with control cells (Fig. 2C, lane 1), and it was abolished by 25 μM LY294002 (Fig. 2C, lane 4), a concentration at which E. coli K1 invasion was effectively inhibited. These results clearly demonstrate that Akt activation is specific to invasive E. coli K1 and is PI3K-dependent.

Dominant-Negative Mutants of PI3K Blocked the E. coli K1 Invasion of HBMEC—To further demonstrate the role of PI3K
in *E. coli* invasion, HBMEC were transfected with dominant-negative mutants of either p85 (Δp85) or p110 (p110ΔK) subunits of PI3K. The cDNAs of both mutants were cloned under the control of cytomegalovirus promoter with an amino-terminal FLAG epitope tag in the eucaryotic expression vector pcDNA3, which also confers G418 resistance. The Δp85 mutant contains a defective iSH2 region, fails to bind to PI3K catalytic subunit p110, and dominantly inhibits the activation of PI3K by titrating out the signaling molecules that interact with PI3K. Whereas, p110ΔK is a kinase inactive mutant, has a CAAX motif, and is constitutively translocated to the membrane. HBMEC transfected with the expression vector pcDNA3 was used as a control. HBMEC colonies resistant to G418 were pooled and used in further experiments. Lysates from transfected cells were immunoprecipitated with either p85 antibody or p110 antibody. Analysis of immunoprecipitates by immunoblotting with FLAG antibody (M2) showed that both p110 and p85 are expressed only in HBMEC transfected with appropriate vector but not in cells transfected with pcDNA3 (Fig. 3A). HBMEC transfected with pcDNA3 or PI3K mutants were used in *E. coli* invasion assays with E44, and the results of *E. coli* K1 invasion in HBMEC-expressing PI3K mutants were compared with those in cells expressing pcDNA3. As shown in Fig. 3B, *E. coli* K1 invasion of HBMEC was blocked by 85% and 50%, respectively, in cells expressing p110ΔK and Δp85 (12,400 ± 1,172 cfu/well with pcDNA3 versus 1,860 ± 168 cfu/well and 6,300 ± 651 cfu/well in HBMEC transfected with Δp110 and Δp85, respectively, *p* < 0.002). These results clearly support that PI3K is involved in *E. coli* K1 invasion of HBMEC.

**FIG. 3.** Overexpression of PI3K mutants blocked *E. coli* K1 invasion of HBMEC. A, expression of p85 and p110ΔK mutants in HBMEC: Cell lysates from HBMEC, transfected with FLAG epitope-tagged PI3K mutants Δp85 or p110ΔK, were immunoprecipitated with p85 or p110 antibody, respectively. Immunoprecipitates were then immunoblotted with indicated antibodies. B, overexpression of PI3K mutants blocked *E. coli* K1 invasion of HBMEC: HBMEC, transfected with pcDNA3, or Δp85, or p110ΔK, were used in *E. coli* invasion assays. The results are expressed as percentage of HBMEC transfected with pcDNA3. The results represent mean of five independent experiments performed in triplicate. Error bars indicate standard deviation.

**PI3K Interaction with FAK in HBMEC Stimulated with *E. coli*—**The regulatory subunit p85 interacts with the autophosphorylation site (Tyr-397) of FAK. Because Tyr-397 is critical for FAK signaling in *E. coli* K1 invasion of HBMEC (10), we examined whether p85 interacts with FAK. The cell lysates from HBMEC stimulated with E44 were immunoprecipitated with FAK antibody, and immunoprecipitates were analyzed by immunoblotting with p85 antibody. Immunoprecipitates were also immunoblotted with FAK antibody to verify the presence of equal amounts of FAK protein levels in each immunoprecipitate. As shown in Fig. 5, p85 association with FAK was seen in control HBMEC (lane 1) and this association was increased in HBMEC stimulated with E44 for 5 min (lane 2), 10 min (lane 3), and 30 min (lane 4). Immunoblotting with FAK antibody showed that immunoprecipitates contained equal amounts of FAK in all the samples. These results demonstrated that PI3K association with FAK is increased in cells stimulated with *E. coli* K1, which may be required for HBMEC invasion.

**Inhibition of PI3K Activation by FAK Dominant-Negative Mutants—**In *E. coli* K1 invasion of HBMEC, FAK is involved in cells overexpressing FAK dominant-negative mutants FRNK and autophosphorylation mutant Phe397FAK (10). Having demonstrated the interaction of PI3K with FAK in HBMEC stimulated with *E. coli* K1, we next investigated if PI3K activation would be blocked by FAK dominant-negative mutants. HBMEC transfected with pcDNA3, FRNK, and Phe397FAK were either left alone (C) or stimulated with either E44 or epidermal growth factor (EGF) for 10 min, and the cell lysates were immunoprecipitated with anti-Akt antibody. Immunoprecipitates were immunoblotted with either anti-phospho-Akt antibody or anti-Akt antibody. The intensity of phospho-Akt bands was determined as described under “Experimental Procedures.” The phospho-Akt levels were expressed as compared with HBMEC transfectants treated with experimental medium (-fold increase of control). Data represent average of three independent experiments. Error bars indicate standard deviation.

**FIG. 4.** Inhibition of Akt activation in HBMEC-overexpressing PI3K mutants. A, HBMEC transfected with pcDNA3 or Δp85 or p110ΔK were either left alone (C) or stimulated with either E44 or epidermal growth factor (EGF) for 10 min, and the cell lysates were immunoprecipitated with anti-Akt antibody. Immunoprecipitates were immunoblotted with either anti-phospho-Akt antibody or anti-Akt antibody. B, the phospho-Akt bands was determined as described under “Experimental Procedures.” The phospho-Akt levels were expressed as compared with HBMEC transfectants treated with experimental medium (-fold increase of control). Data represent average of three independent experiments. Error bars indicate standard deviation.
tion was completely abolished in HBMEC transfected with Phe397FAK and partially abolished in cells expressing FRNK (Fig. 6). Thus, PI3K activation is inhibited in cells where FAK activation is blocked by dominant-negative mutants.

**DISCUSSION**

Bacterial entry of mammalian cells involves a complex interplay of bacterial determinants with host cell receptors and is associated with activation of specific signaling pathways (2). Our previous studies have demonstrated that FAK plays a central role in *E. coli* K1 invasion of HBMEC (10). However, the mechanisms of FAK activation and signaling molecules that interact with FAK in this process have not been identified. In this paper, we report that PI3K activation and its interaction with FAK are required for *E. coli* K1 invasion of HBMEC. This was shown by blockade of both PI3K activation and *E. coli* invasion of HBMEC with specific PI3K inhibitor (LY294002) as well as using dominant-negative mutants of PI3K (A85 and p110A) and FAK (FRNK and FAKY397F).

The regulatory subunit p85 of PI3K contains an amino-terminal SH3 domain, a breakpoint cluster homology domain and two SH2 domains. These domains allow p85 to simultaneously interact with multiple intracellular signaling molecules (16). Thus, PI3K can recruit a variety of signaling molecules to the site of bacterial entry. There are several phosphoinositide binding proteins, which can be potential downstream effectors of PI3K, that can participate at several steps in *E. coli* invasion of HBMEC, including cytoskeletal rearrangements or the endosome formation. One of them is the Rho family of GTP binding proteins that regulates actin rearrangement. Rho family members cycle between the inactive GDP binding form and the active GTP binding form. Guanine nucleotide exchange factors (GEF) and GTPases regulate the cycling of Rho-like GTPases. GEFs mediate conversion of inactive GDP-bound form to active GTP-bound form. PI3K products can bind to pleckstrin homology domains in GEF and target them to plasma membrane to activate Rho family of GTPases (29). We showed that the *E. coli* K1 mutant lacking cytotoxic necrotizing factor-1 failed to invade HBMEC (30), suggesting the involvement of Rho GTPases. Cytotoxic necrotizing factor-1 is known to activate Rho GTPases in mammalian cells (31, 32). PI3K may be required for the regulation of Rho-like GTPases in HBMEC. Many cytoskeletal proteins involved in the regulation of actin polymerization bind to phosphoinositides through their pleckstrin homology (PH) domains, e.g., profilin and capping proteins bind to monomeric actin and prevent its polymerization. Binding of phosphoinositides to the PH domains of these proteins releases actin and promotes polymerization (33). Phosphoinositide-induced changes in the conformation of another cytoskeletal protein vinculin may also play a role in focal adhesion assembly by promoting cross-linking of talin to actin. Another mechanism for the PI3K involvement could be the activation of Akt, a Ser/Thr kinase, which is recruited to plasma membrane by binding to phosphoinositides through its PH domain. Evidence exists for the involvement of Akt in actin reorganization and migration of microvascular endothelial cells (34). Upon entry into HBMEC, *E. coli* have been shown to be located in vacuoles and cross the HBMEC by transcytosis (9). This is another level where PI3K may be involved, because PI3K has been shown to participate in the recruitment of early endosomes as well as movement of the endosomes along the microtubules (35, 36). Studies are in progress to examine the role of PI3K in cytoskeleton rearrangements and trafficking of *E. coli*-containing vacuoles in HBMEC.

Our recent results have demonstrated the cytoskeletal changes in HBMEC at the site of bacterial entry (9). These sites were similar to focal adhesions that are intracellularly associated with protein complexes consisting of cytoskeletal proteins and tyrosine kinase FAK (37). The role of FAK has been studied in many cellular responses associated with cytoskeleton rearrangement, including adhesion and migration (38). However, the mechanism of FAK activation and the proteins recruited by FAK in *E. coli* invasion of HBMEC have not been identified. The present study identified that PI3K interacts with FAK in HBMEC and its interaction is enhanced in HBMEC stimulated with *E. coli* K1, suggesting that FAK recruits PI3K to the site of bacterial entry. PI3K activation was abolished by FAK dominant-negative mutants FRNK and FAKY397F, indicating that FAK may be upstream of PI3K in *E. coli* K1 invasion of HBMEC. In contrast, tyrosine phosphorylation of FAK was not affected by PI3K inhibitors (data not shown), providing further support for this mechanism.

Several *E. coli* K1 determinants involved in the HBMEC invasion have been identified, and some of these bacterial proteins induced cytoskeleton rearrangements in HBMEC (2). However, at present, the identity of *E. coli* K1 structures responsible for the activation of FAK and/or PI3K and the mechanism of activation in HBMEC are not yet defined. Of interest, uropathogenic *E. coli* invasion of bladder epithelial cells was found to require host actin reorganization, FAK phosphorylation at Tyr-397 and PI3K activation, but not activation of Src-family tyrosine kinases (39). These events were mediated by FimH and correlated with the formation of complexes between FAK and PI3K. Thus, our findings of PI3K activation and PI3K/FAK interactions reported here with *E. coli* K1 invasion of HBMEC are similar to those observed with type 1 pilus *E. coli* invasion of uroepithelial cells.

We have previously demonstrated that other meningitis-causing bacteria, e.g., group B streptococcus (40), *Citrobacter freundii* (41), and *L. monocytogenes* (42), are able to invade HBMEC. PI3K involvement has been demonstrated in *L. monocytogenes* invasion of epithelial cells. However, PI3K involvement is independent of FAK in *L. monocytogenes* invasion of epithelial cells (15). In contrast, our findings indicate the requirement of PI3K interaction with FAK in *E. coli* K1 invasion of HBMEC. Thus, the mechanism of *E. coli* K1 invasion of HBMEC is strikingly different from that of *L. monocytogenes*. This may reflect the differences in cell types, bacterial ligands, and mechanisms of diseases. It would be interesting to see whether PI3K/FAK interactions are also involved in the invasion of HBMEC by other meningitis-causing bacteria.
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REFERENCES

1. Leib, L. S., and Tauber, M. G. (1999) Infect. Dis. Clin. North Am. 13, 527–548
2. Kim, K. S. (2000) Subcell. Biochem. 33, 47–59
3. Prasad J.R., N.V., Wuss, C. A., Weiser, J. N., Stins, M. F., Huang, S. H., and Kim, K. S. (1996) Infect. Immun. 64, 146–153
4. Huang, S. H., Wuss, C. A., Fu, Q., Prasad J.R., N.V., Stins, M. F., and Kim, K. S. (1995) Infect. Immun. 63, 4470–4475
5. Huang, S. H., Chen, Y. H., Fu, Q., Stins, M., Wang, Y., Wuss, C., and Kim, K. S. (1999) Infect. Immun. 67, 2103–2109
6. Wang, Y., Huang, S. H., Wuss, C. A., Stins, M. F., and Kim, K. S. (1999) Infect. Immun. 67, 4781–4786
7. Prasad J.R., N.V., Wuss, C. A., and Kim, K. S. (1996) Infect. Immun. 64, 154–160
8. Prasad J.R., N.V., Wuss, C. A., Huang, S. H., and Kim, K. S. (1999) Infect. Immun. 67, 1131–1138
9. Prasad J.R., N.V., Wuss, C. A., Stins, M. F., Shimada, H., and Kim, K. S. (1999) Infect. Immun. 67, 5775–5783
10. Reddy, M. A., Wuss, C. A., Kim, K. S., Schlaepfer, D. D., and Prasad J.R., N.V. (2000) Infect. Immun. 68, 6423–6430
11. Schlaepfer, D. D., Hauck, C. R., and Sieg, D. J. (1999) Prog. Biophys. Mol. Biol. 71, 435–478
12. Xing, Z., Chen, H.-C., Nowlen, J. K., Taylor, S., Shalloway, D., and Guan, J.-L. (1994) Mol. Biol. Cell 5, 413–442
13. Cobb, B. S., Schaller, M. D., Leu, T.-H., and Parsons, J. T. (1994) Mol. Cell. Biol. 14, 147–155
14. Chen, H.-C., and Guan, J.-L. (1994) Proc. Natl. Acad. Sci. U. S. A. 91, 10148–10152
15. Ireton, K., Payrastre, B., and Cossart, P. (1999) J. Biol. Chem. 274, 17025–17032
16. Wymann, M. P., and Pirola, L. (1998) Biochim. Biophys. Acta 1436, 127–150
17. Downward, J. (1998) Science 279, 673–674
18. Martin, J. F. (1998) Annu. Rev. Cell Dev. Biol. 14, 231–264
19. Ramesh, L. E., and Canley, L. C. (1999) J. Biol. Chem. 274, 8347–8350
20. Ireton, K., Payrastre, B., Chap, H., Ogawa, W., Sakaue, H., Kasuga, M., and Cossart, P. (1997) Science 274, 780–782
21. Reiske, H. R., Kao, S.-C., Cary, L. A., Guan, J.-L., Lai, J.-F., and Chen, H.-C. (1999) J. Biol. Chem. 274, 12361–12366
22. Sonoda, Y., Watanabe, S., Matsumoto, Y., Aizu-Yokota, E., and Kasahara, T. (1999) J. Biol. Chem. 274, 15066–15070
23. Chan, P.-C., Lai, J.-F., Cheng, C.-H., Tang, M.-J., Cjiu, C.-C., and Chen, H.-C. (1999) J. Biol. Chem. 274, 26601–26606
24. Kara, H., Yonezawa, K., Sakaue, H., Ando, A., Kotani, K., Kitamura, T., Kitamura, Y., Ueda, H., Stephens, L., Jackson, T. R., Hawkins, P. T., Dhand, R., Clark, A. E., Holman, G. D., Waterfield, M. D., and Kasuga, M. (1994) Proc. Natl. Acad. Sci. U. S. A. 91, 7415–7419
25. Rodriguez-Viciana, P., Warne, P. H., Vanhaesebroeck, B., Waterfield, M. D., and Downward, J. (1996) EMBO J. 15, 2442–2451
26. Schlaepfer, D. D., and Hunter, T. (1997) J. Biol. Chem. 272, 13189–13195
27. Sieg, D. J., Hauck, C. R., and Schlaepfer, D. D. (1999) J. Cell Sci. 112, 2677–2691
28. Stins, M. F., Gilles, F., and Kim, K. S. (1997) J. Neuroimmunol. 76, 81–90
29. Carpenter, C. L., Tobias, K. F., Courvillon, A. C., and Hartwig, J. H. (1997) Adv. Enz. Regul. 37, 377–390
30. Wang, Y., Wuss, C., and Kim, K. S. (2000) in Abstracts of the 100th American Society for Microbiology General Meeting, Los Angeles, May 21–25; Abstr. B108, p. 65
31. Schmidt, G., Sehr, P., Wilm, M., Selzer, J., Mann, M., and Aktories, K. (1997) Nature 387, 725–729
32. Flatau, G., Lemichez, E., Gauthier, M., Chardin, P., Fiorentini, C., and Boquet, P. (1997) Nature 387, 729–733
33. Schmidt, A., and Hall, M. N. (1998) Ann. Rev. Cell Dev. Biol. 14, 305–338
34. Morales-Ruiz, M., Fulton, D., Sowa, G., Languino, L. R., Fujiw, Y., and Wash, K. (2000) Circ. Res. 86, 892–896
35. Nielsen, E., Severin, F., Backer, J. M., Hyman, A. A., and Zerial, M. (1999) Nat. Cell Biol. 1, 376–382
36. Gauthier, J. M., Simonson, A., D’Arrigo, A., Bremnes, B., Sternmark, H., and Ausland, R. (1998) Nature 394, 432–433
37. Brigitte, M. J., Bubeck, P., Giegl, K., Kroemker, M., Moschner, J., Rothkegel, M., Rudiger, M., Schluter, K., Stanke, G., and Winkler, J. (1995) Ann. Rev. Cell Dev. Biol. 11, 379–416
38. Guan, J.-L., and Chen, H.-C. (1996) Int. Rev. Cytol. 168, 81–108
39. Martine, J. J., Mulvey, M. A., Schilling, J. D., Pinkner, J. S., and Hultgren, S. J. (2000) J. Biol. Chem. 274, 12361–12366
22. Sonoda, Y., Watanabe, S., Matsumoto, Y., Aizu-Yokota, E., and Kasahara, T. (1999) J. Biol. Chem. 274, 12361–12366