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MINIREVIEW

Escherichia coli Translocation at the Blood-Brain Barrier

KWANG SIK KIM*

Division of Pediatric Infectious Diseases, Johns Hopkins University School of Medicine, Baltimore, Maryland 21287

The mortality and morbidity associated with bacterial meningitis have remained significant despite advances in antimicrobial chemotherapy and supportive care. A major contributing factor is the incomplete understanding of the pathogenesis of this disease. For example, most cases of bacterial meningitis develop as a result of hematogenous spread, but it is unclear how circulating bacteria cross the blood-brain barrier (12, 25, 33, 46). Recent studies have shown that *Escherichia coli* K1, group B *Streptococcus, Streptococcus pneumoniae*, and *Citrobacter* spp., the important pathogens that cause meningitis, translocate from blood to the central nervous system (CNS) without altering the integrity of the blood-brain barrier (2, 23, 27, 36, 42). These studies of bacterial translocation from blood to the CNS have become possible because of the availability of both in vitro and in vivo models of the blood-brain barrier (1, 3, 4, 7, 11, 13, 17–20, 22, 26, 28, 32, 41, 43, 47). The in vitro model of the blood-brain barrier is composed of brain microvascular endothelial cells (BMEC), which exhibit transendothelial resistance of 200 to 600 Ω/cm^2, a unique property of the brain microvascular endothelium monolayer compared to systemic vascular endothelium. The in vivo model of the blood-brain barrier utilizes experimental hematogenous meningitis in animals. In these experimental meningitis models, bacteria are injected via intravenous, intraperitoneal, subcutaneous, or intracardiac administration, resulting in bacteremia and subsequent entry into the CNS. At present, *E. coli*-BMEC interactions represent the most characterized system concerning how circulating bacteria cross the blood-brain barrier. This review summarizes our current understanding of the pathogenetic mechanisms involved in bacterial translocation of the blood-brain barrier, using *E. coli* as a paradigm.

A THRESHOLD LEVEL OF BACTEREMIA

Several studies of humans and experimental animals suggest a relationship between the magnitude of bacteremia and the development of meningitis due to *E. coli, Haemophilus influenzae* type b, and *S. pneumoniae* (Table 1), (5, 10, 13, 20, 22, 26, 44, 45). For example, Dietzman et al. (10) reported a significantly higher incidence of *E. coli* meningitis in neonates who had bacterial counts in blood $>10^3$ CFU/ml (6/11 [55%]) compared to those with bacterial counts in blood $<10^3$ CFU/ml (1/19 [5%]). Similarly, meningitis due to *S. pneumoniae* was observed more frequently in patients whose bacterial counts in blood were greater than 1×10^2 to 5×10^2 CFU/ml (6/7 [86%]) than those with bacterial counts in blood less than 1×10^2 to 5×10^2 CFU/ml (2/50 [4%]) (5, 45). For *H. influenzae* type b meningitis, the level of bacteremia required for the development of meningitis is around 10^2 CFU/ml of blood (5, 45). Consistent with these clinical findings, a high degree of bacteremia was shown to be a primary determinant for meningeal invasion by *E. coli* K1, group B *Streptococcus, H. influenzae* type b, and *S. pneumoniae* in an experimental hematogenous meningitis model (13, 20, 22, 26, 28). Thus, one of the reasons for the close association of *E. coli* K1, group B *Streptococcus, H. influenzae* type b, and *S. pneumoniae* with meningitis is their ability to escape from host defenses and then to achieve the threshold level of bacteremia necessary for invasion of the meninge. In contrast, it is unclear whether the development of meningococcal meningitis is associated with a threshold level of bacteremia as shown by inconsistent reports from clinical studies (Table 1) (44, 45). This issue is further hampered by the lack of a suitable animal model for studying the pathogenesis of meningococcal meningitis. *E. coli* is commonly associated with neonatal meningitis, and *E. coli* strains possessing the K1 capsular polysaccharide are predominant (approximately 80% among isolates from *E. coli* meningitis) (16, 24, 37). Of interest, rates of *E. coli* meningitis (defined as positive cerebrospinal fluid (CSF) cultures) were found to be similar between neonatal and adult animals developing a high degree of bacteremia (e.g., $>10^5$ CFU/ml of blood); however, an approximately 10^6-fold-greater inoculum of *E. coli* K1 was required to induce a similar high-level bacteremia in adult animals compared to neonatal animals (22). These findings indicate that the age dependency of *E. coli* meningitis is due to the relative resistance of adults to high-level bacteremia, which precedes the development of meningitis, and less likely due to greater invasion of neonatal BMEC compared to adults BMEC (43).

BACTERIAL STRUCTURES CONTRIBUTING TO INVASION OF BMEC

Recent studies indicate that a high degree of bacteremia is necessary but not sufficient for the development of meningitis from *E. coli* K1 and that invasion of BMEC is a prerequisite for *E. coli* K1 penetration of the blood-brain barrier in vivo (21). This was shown by the demonstration in infant rats with experimental hematogenous meningitis that several isogenic mutants of *E. coli* K1 strain RS 218 (018:K1:H7) were significantly

* Mailing address: Division of Pediatric Infectious Diseases, Johns Hopkins University School of Medicine, 600 N. Wolfe Street, Park 256, Baltimore, MD 21287. Phone: (410) 614-3917. Fax: (410) 614-1491. E-mail: kwangkim@jhmi.edu.

TABLE 1. Association of the magnitude of bacteremia with meningitis in infants and children

Bacterium	No. of patients	CFU/ml of blood	No of patients with meningitis/total no. (percent)	Reference
E. coli	30	<1,000	1/19 (5)	10
		>1,000	6/11 (55)	
S. pneumoniae	24	≤100	1/20 (5)	5
		>100	3/4 (75)	
	33	<500	1/30 (3)	45
		≥500	3/3 (100)	
H. influenzae type b	34	<100	2/12 (17)	45
		≥100	11/22 (50)	
	26	≤100	7/11 (63)	5
		>100	11/15 (73)	
N. meningitidis	35	<500	7/22 (32)	44
		≥500	8/13 (62)	
	12	<1,000	5/8 (63)	45
		≥1,000	1/4 (25)	

less able to induce meningitis (defined as positive CSF cultures) than the parent strain despite similar levels of bacteremia, indicating that certain *E. coli* K1 structures are necessary for crossing the blood-brain barrier in vivo (Table 2). Similarly, many *E. coli* K1 structures which contribute to BMEC invasion in vitro have been identified (Table 3). The major findings are summarized below.

Outer membrane protein A (OmpA) was identified as a potential contributor to *E. coli* K1 invasion of BMEC on the basis of its homology with *Neisseria* Opa proteins, which have been shown to be involved in invasion of eukaryotic cells (32). OmpA is a major outer membrane protein in *E. coli*, and its N-terminal domain crosses the membrane eight times in antiparallel β-strands with four relatively large and hydrophilic surface-exposed loops. The N-terminal portion of OmpA, not the C-terminal portion, and surface-exposed loops have been shown to contribute to *E. coli* K1 invasion of BMEC (32). For example, the synthetic peptides representing a part of the first loop and the tip of the second loop of OmpA have been shown to inhibit *E. coli* K1 invasion of BMEC (32). In addition, anti-OmpA antibody inhibited *E. coli* K1 invasion of BMEC, indicating that OmpA is exposed to the surface of the K1 encapsulated *E. coli*. OmpA interacts with the GlcNAcβ1-4GlcNAc epitope of the BMEC receptor glycoprotein, and its first, and second loops are shown to be the sites for the interaction with the carbohydrate epitope of the BMEC glycoprotein (31). The receptor glycoprotein is found to be present on BMEC but is not detectable on systemic vascular endothelial cells (31). In addition, purified OmpA and chitooligomers prepared from the polymer of 1,4-linked GlcNAc inhibited *E. coli* K1 invasion of BMEC (31, 32). These findings indicate that OmpA contributes to BMEC invasion via a ligand-receptor interaction. *E. coli* K1 OmpA is highly homologous to *E. coli* K-12 OmpA. The nucleotide sequence of the K1 *ompA* gene (GenBank accession no. AF234269) differs from that of the *E. coli* K-12 *ompA* in 20 of 1,038 nucleotides, and only 3 of the 325 deduced amino acid residues differ between the K1 and K-12 OmpA proteins. The function of OmpA in *E. coli* invasion of BMEC was also found to be similar for *E. coli* K1 and K12 OmpA, as shown by successful complementation of the noninvasive *ompA* deletion mutant of *E. coli* K1 to invade BMEC with the *E. coli* K-12 *ompA* gene (32; Y. Wang and K. S. Kim, unpublished data).

Ibe (invasion of brain endothelial cells) proteins A, B, and C were identified by cloning and characterization the Tn*phoA* insertion sites from strains 10A-23, 7A-33, and 23A-20, respectively, which are the noninvasive mutants of *E. coli* K1 strain RS218 (018:K1:H7) (19, 20, 47). Sequence analysis indicates that the *E. coli* K1 *ibeA, ibeB*, and *ibeC* encode 50-, 50-, and 66-kDa membrane proteins, respectively. Both *ibeB* and *ibeC* were found to have K-12 homologues, *p77211* and *yijP*, respectively, while *ibeA* was unique to *E. coli* K1. The roles of IbeA, IbeB, and IbeC in *E. coli* K1 invasion of BMEC were verified by deletion and complementation experiments; i.e., isogenic deletion mutants were less invasive in BMEC in vitro and less able to cross the blood-brain barrier in vivo (Table 2), and their invasion abilities were restored by complementation in *trans* with individual genes. Of interest, recombinant Ibe proteins inhibited *E. coli* K1 invasion of BMEC, suggesting that Ibe proteins contribute to BMEC invasion by ligand-receptor interactions. This concept was supported by the demonstration of a 45-kDa BMEC surface protein interactive with IbeA and a polyclonal antibody raised against this receptor protein inhibited *E. coli* K1 invasion of BMEC (29). Partial characterization by N-terminal and internal amino acid sequencing of this receptor protein reveals that it represents a novel albumin-like protein present on BMEC (29). Studies are in progress to identify whether BMEC possess receptor proteins for IbeB and IbeC.

E. coli K1 *aslA* was identified by cloning and sequencing of the Tn*phoA* insertion site of the noninvasive mutant of *E. coli*

TABLE 2. Development of bacteremia and meningitis (defined as positive CSF cultures) in newborn rats receiving *E. coli* K1 strain E44 (RS 218 Rif^r, 018:K1:H7) or its isogenic mutants

E. coli strain	No. of animals	Bacteremia (log CFU/ml of blood)	No. (%) of animals with positive CSF	Reference(s)
E44	19	7.18 ± 0.63	12 (63)	48
Δ*ompA*	22	7.05 ± 0.49	6 (27)[a]	
E44	24	7.51 ± 1.25	16 (67)	20
Δ*ibeA*	25	6.97 ± 1.21	4 (16)[a]	
E44	27	7.01 ± 1.17	15 (56)	19
Δ*ibeB*	25	7.06 ± 1.29	4 (16)[a]	
E44	24	7.53 ± 0.40	18 (75)	47
Δ*ibeC*	24	7.80 ± 0.67	10 (42)[a]	
E44	17	7.50 ± 0.32	14 (82)	18
Δ*aslA*	22	7.60 ± 0.49	7 (32)[a]	
E44	51	7.22 ± 0.59	34 (6)	3, 4
Δ*traJ*	50	7.10 ± 0.44	23 (4)[a]	
E44	26	6.06 ± 1.49	10 (38)	4, Wang et al.[b]
Δ*cnf1*	28	6.07 ± 1.21	3 (11)[a]	

[a] Significantly less than result for E44.
[b] Wang et al., Abstr. 100th Gen. Meet. Am. Soc. Microbiol.

TABLE 3. *E. coli* K1 structures contributing to invasion of BMEC

K1 structure[a]	Orgin[b]	K12 homologue[c]	Mechanism	Reference(s)
ompA	Rs218/E44	*ompA* (98)	Ligand-receptor	31, 32; Wang and Kim, unpublished
ibeA	10A-23 (E44*ibeB*::Tn*phoA*)	None	Ligand-receptor	20
ibeB	7A-33 (E44*ibeB*::Tn*phoA*)	*p77211* (97)	Ligand-receptor interaction (?)	19
ibeC	23A-20 (E44*ibeC*::Tn*phoA*)	*yijP* (98)	Ligand-receptor interaction (?)	47
aslA	27A-6 (E44*aslA*::Tn*phoA*)	*aslA* (94)	ND	18
nilA	E44, DFI	None	ND	3
nilB	E44, DFI	None	ND	3
nilC	E44, DFI	None	ND	3
ygdP	E44, DFI	*ygdP* (98)	ND	3
traJ	E44, DFI, STM	None[d]	ND	3, 4
cnf_1	E44, STM	None	RhoA GTPase	4
cigA	E44, STM	None	ND	4
b2146 (o412)	E44, STM	*o412* (42–100)	ND	4
vibO (pmgl)	E44, STM	*pmgI* (60–74)	ND	4
b1983 (o347)	E44, STM	*o347* (49–100)	ND	4
yaiU	E44, STM	*yaiU* (44–100)	ND	4

[a] *ibe*, invasion brain endothelial cells; *nil*, newborn bovine serum-inducing loci; *cig*, CNS invasion gene.
[b] DFI, differential fluorescence induction assay; STM, signature-tagged mutagenesis.
[c] Numbers in parentheses indicate percentages of nucleotide identity of DNA sequenced.
[d] Homologous to F-like plasmid R1-19.

K1 strain RS218, 27A-6 (18). This mutant exhibited significantly decreased invasion of BMEC in vitro and an attenuated ability to cause meningitis compared to the parent strain in the newborn rat model of experimental hematogensis meningitis (18). The role of AslA in *E. coli* K1 invasion of BMEC was also verified by deletion and complementation experiments (18). The *E. coli* K1 *aslA* sequence is highly homologous to *E. coli* K-12 *aslA*, a putative arylsulfatase-like gene. This *E. coli* K-12 gene was named because the deduced protein sequence contains sulfatase consensus motifs I and II, which are homologous (55 and 70% identity, respectively) to those of *Klebsiella pneumoniae* AtsA, an arylsulfatase involved in sulfate metabolism. The *E. coli* K1 *aslA* encodes a 52-kDa protein with two transmembrane domains and an amino-terminal signal sequence (18). Its deduced protein sequence indicates that *E. coli* K1 AslA is also a member of the arylsulfatase family of enzymes that contain highly conserved sulfatase motifs. In bacteria, these genes are expressed under conditions of sulfur starvation. Of interest, unlike the *Klebsiella* protein, both *E. coli* K1 and K-12 AslA proteins failed to exhibit in vitro arylsulfatase activity (18). It remains unclear how AslA contributes to *E. coli* K1 invasion of BMEC in vitro and traversal of the blood-brain barrier in vivo.

A recent study has shown that certain environmental conditions positively and negatively affect *E. coli* K1 invasion of BMEC in vitro and traversal of the blood-brain barrier in vivo (1). For example, the following growth condition enhanced *E. coli* invasion of BMEC: media supplemented with 50% newborn bovine serum or iron. Growth conditions that significantly repressed invasion included iron chelation and high osmolarity (1). Using differential fluorescence induction and screening of *gfp* fusion library, TraJ was identified as a contributor to *E. coli* K1 invasion of BMEC (3). As expected, TraJ was found to be differentially expressed at the transcriptional level; e.g., transcript levels of *traJ* increased when *E. coli* K1 was grown in the presence of serum compared to that in medium alone. A *traJ* mutant was less invasive in BMEC in vitro and less able to cross the blood-brain barrier in vivo (4). *traJ* belongs to a cluster of genes within the F-like plasmid R1-19 transfer region called the *tra* operon. It is speculative whether *E. coli* K1 TraJ is an invasive factor itself or is needed for the expression of a gene(s) required for efficient penetration of BMEC and/or whether the F-like plasmid *tra* operon is required for *E. coli* K1 invasion of BMEC. Studies with signature tagged mutagenesis also identified TraJ as well as cytotoxic necrotizing factor 1 (CNF1) as contributors to *E. coli* K1 invasion of BMEC (4). An isogenic cnf_1-deletion mutant of *E. coli* K1 strain RS 218 is less invasive in BMEC in vitro and less able to penetrate the blood-brain barrier in vivo (Y. Wang, C. A. Wass, and K. S. Kim, Abstr. 100th Gen. Meet. Am. Soc. Microbiol. 2000, abstr. B-108, p. 65). CNF1 has been shown to activate Rho GTPases, resulting in polymerization of F-actin and increased formation of stress fibers (14, 39). Actin cytoskeletal rearrangements are required for *E. coli* K1 invasion of BMEC, as shown by invasive *E. coli* K1-associated F-actin condensation and blockade of invasion by the microfilament disrupting agents, cytochalasin D and latrunculin A (30). Taken together, these findings indicate that CNF1 contributes to *E. coli* K1 invasion of BMEC, most likely via Rho activation.

Recent studies have also indicated that other meningeal pathogens invade BMEC via ligand-receptor interations. For example, *S. pneumoniae* invades BMEC in part via interaction between cell wall phosphorylcholine and the BMEC platelet-activating factor receptor as shown by partial inhibition of pneumococcal invasion of BMEC by the platelet-activating factor receptor antagonists (36). *Listeria monocytogenes* invasion of BMEC has been shown to be mediated by internalin B (15). Two receptors for internalin B have been identified, gC1q-R (the receptor for the globular head of the complement component C1q) and Met tyrosine kinase (8, 40). However, it is unclear whether these receptors for internalin B are present on human BMEC. Group B *Streptococcus* and *Citrobacter* have also shown to invade BMEC (2, 27), but bacterial structures contributing to their invasion of BMEC have not been determined.

Comparative macrorestriction mapping and subtractive hy-

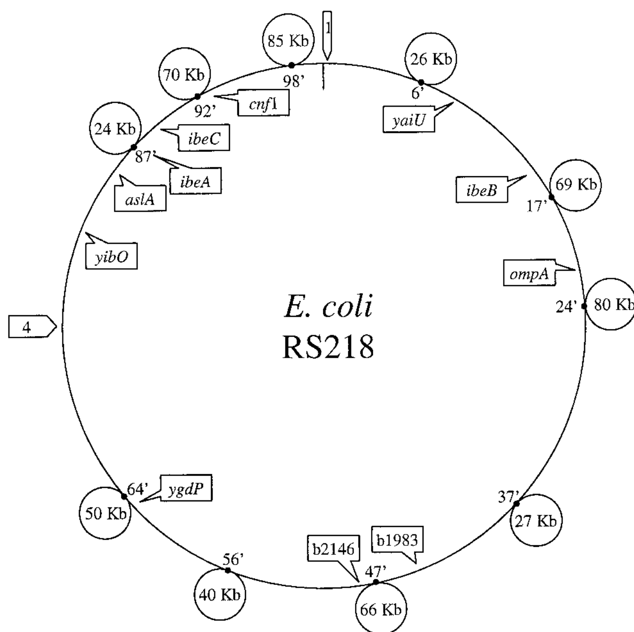

FIG. 1. Sizes and chromosomal locations of *E. coli* K1 strain RS 218 (018:K1:H7)-specific segments identified by comparative macrorestriction mapping (38) are shown by small circles. Numbers in the circles represent sizes of K1-specific DNA segments. The sizes of two additional segments shown by arrows labeled 1 and 4, identified by subtractive hybridization (6) are currently unknown. Also shown are 11 *E. coli* K1 genes contributing to invasion of BMEC, indicated by squares which include K1-specific genes (e.g., *ibeA*, and *cnf*1) as well as genes which have K-12 homologues (e.g., *ompA, ibeB, ibeC*, and *aslA*).

bridization of the chromosomes of meningitis-causing *E. coli* K1 (e.g., strains RS 218 and C5) compared to nonpathogenic *E. coli* have identified 500 kb spread over at least 12 chromosomal loci specific to *E. coli* K1 (6, 38) (Fig. 1). As shown in Table 3 and Fig. 1, several structures contributing to *E. coli* K1 invasion of BMEC are unique to *E. coli* K1, such as *ibeA, traJ*, and cnf_1, whereas other structures critical to *E. coli* K1 crossing of the blood-brain barrier are shown to have K-12 homologues such as *ompA, ibeB, ibeC*, and *aslA*. Thus, *E. coli* K1 determinants contributing to invasion of BMEC include K1 specific genes as well as K12 homologues. Mapping studies reveal that those *E. coli* loci involved in BMEC invasion are located at different regions of *E. coli* K1 chromosome (Fig. 1).

BACTERIAL TRAFFICKING OF BMEC

Transcytosis of BMEC by *E. coli* K1, group B *Streptococcus, S. pneumoniae*, and *Citrobacter* spp. occur without any change in the integrity of monolayers (2, 23, 27, 36, 42). Transmission electron microscopy revealed that *Citrobacter* has been shown to invade and replicate in BMEC (2). In contrast, *E. coli* K1 invades BMEC via a zipper-like mechanism and transmigrates through BMEC in an enclosed vacuole without intracellular multiplication (30). *E. coli* K1 invasion of BMEC requires actin cytoskeletal rearrangements and induces tyrosine phophorylation of focal adhesion kinase and paxillin, a cytoskeletal protein known to associate with focal adhesion kinase. Furthermore, using focal adhesion kinase-dominant-negative mutants, focal adhesion kinase activity and its autophosphorylation site tyrosine 397 are shown to be critical for *E. coli* K1 invasion of BMEC (35). The autophosphorylation site (Tyr 397) of focal adhesion kinase has been shown to bind Src kinases and phosphatidylinositol 3-kinase, and binding of one or both is required for focal adhesion kinase-mediated functions. Src kinases, however, were not critical in *E. coli* K1 invasion of BMEC. This was shown by the demonstration that pretreatment of BMEC with the Src kinase-specific inhibitor, PP1, did not affect *E. coli* K1 invasion of BMEC and also overexpression of a Src kinase-dominant-negative mutant did not block *E. coli* K1 invasion of BMEC (35).

In contrast, phosphatidylinositol 3-kinase activation and its association with focal adhesion kinase are required for *E. coli* K1 invasion of BMEC (34). This was shown by blockade of both phosphatidylinositol 3-kinase activation and *E. coli* K1 invasion of BMEC with specific phosphatidylinositol 3-kinase inhibitor (LY294002) as well as using dominant negative mutants of phosphatidylinositol 3-kinase and focal adhesion kinase. Phosphatidylinositol 3-kinase activation was abolished by focal adhesion kinase-dominant-negative mutants (34), indicating that focal adhesion kinase is upstream of phosphatidylinositol 3-kinase in *E. coli* K1 invasion of BMEC. Phosphatidylinositol 3-kinase has been shown to participate in actin reorganization, recruitment of early endosome proteins, and movement of the endosomes along the microtubules. It remains to be determined how focal adhesion kinase and phosphatidylinositol 3-kinase activation contribute to *E. coli* K1 invasion of BMEC.

Phospholipase A_2, particularly cytosolic phospholipase A_2, has been shown to contribute to *E. coli* K1 invasion of BMEC. This was shown by the demonstration that AACOCF3, a selective cytosolic phospholipase A_2 inhibitor, blocked *E. coli* K1 invasion of BMEC and *E. coli* K1 invasion was significantly decreased in BMEC derived from cytosolic phospholipase A_2 knockout mice compared to that in BMEC from control mice (9). Phospholipase A_2 hydrolyzes phospholipids at their sn-2 position, resulting in the release of fatty acids, e.g., arachidonic acid. Actin cytoskeletal rearrangements in mammalian cells have been linked to intracellular signaling via metabolites of arachidonic acid. These findings indicate that focal adhesion kinase, phosphatidylinositol 3-kinase, and cytostolic phospholipase A2 activation contribute to *E. coli* K1 invasion of BMEC, presumably via affecting the signaling mechanisms associated with BMEC actin cytoskeletal arrangements.

It should be noted that bacterial trafficking mechanisms in

TABLE 4. Comparison of host cell cytoskeleton and signaling mechanisms in bacterial invasion of BMEC

Bacterium	Mechanism					References
	Actin cytoskeletal rearrangements	Activation of[a]: FAK	Src	PI3K	cPLA2	
E. coli K1	+	+	−	+	+	9, 34, 35
Group B *Streptococcus*	+	ND	ND	−	−	9, 27, 34
L. monocytogenes	+	−	+	+	−	9, 15, 34, 35

[a] +, active participation in BMEC invasion; −, no role in BMEC invasion; ND, not examined.

BMEC are shown to differ between *E. coli* K1 and other meningitis-causing bacteria such as *L. monocytogenes* and group B *Streptococcus* (Table 4). For example, BMEC actin cytoskeletal rearrangements are shown to be a prerequisite for BMEC invasion by *E. coli* K1, *L. monocytogenes*, and group B *Streptococcus* (15, 27, 30). However, *L. monocytogenes* invasion of BMEC depends on Src kinases, not on focal adhesion kinase and cytosolic phospholipase A_2 (9, 35). In contrast, group B *Streptococcus* invasion of BMEC was independent of phosphatidylinositol 3-kinase and cytosolic phospholipase A_2 activation (9, 34). BMEC vacuoles containing *E. coli* K1 were found to have markers for early and late endosomes but devoid of lysosomal markers (K. J. Kim and K. S. Kim, unpublished data), suggesting that there is an escape from transport to lysosome and/or a blockade of fusion to lysosome. Additional studies are needed to understand the trafficking mechanisms involved in bacterial transcytosis of BMEC.

TRAVERSAL OF THE BLOOD-BRAIN BARRIER AS LIVE BACTERIA

Previous studies of *E. coli* K1 meningitis have shown that the K1 capsule is a critical determinant in the development of meningitis (22). This was shown by the demonstration of sterile CSF cultures from animals infected with $K1^-$ strains, which was interpreted to indicate that the K1 capsule was necessary for the bacterial crossing of the blood-brain barrier. A recent study, however, has shown that both *E. coli* $K1^+$ and $K1^-$ strains are able to traverse BMEC in vitro and enter the CNS in vivo, but infections caused by $K1^+$ strains resulted in positive CSF cultures (17). Thus, the K1 capsule has, in addition to its well-recognized serum resistance and antiphagocytic properties, a role in the traversal of *E. coli* K1 across the blood-brain barrier as live bacteria. The nature of this novel BMEC activity that is bactericidal to *E. coli* strains without a capsule is currently unknown. This has been shown not to be related to NO, peroxynitrites, superoxides, and other oxygen radicals (17). Similarly, most opaque variants of *S. pneumoniae* are shown to be killed in BMEC (36), but the basis of this BMEC killing activity is unclear.

CONCLUSION

A major limitation to advances in prevention and therapy of bacterial meningitis is our incomplete understanding of the pathogenesis of this disease, such as how circulating bacteria cross the blood-brain barrier. Successful isolation and cultivation of BMEC, which constitute the blood-brain barrier, and the development of an experimental hematogenous animal model that closely mimics the pathogenesis of human meningitis enabled dissection of the mechanisms of bacterial translocation across the blood-brain barrier. The studies, so far, have identified that crossing of the blood-brain barrier by *E. coli* K1, group B *Streptococcus, H. influenzae* type b, and *S. pneumoniae* require a high degree of bacteremia. However, a high degree of bacteremia alone is not sufficient for the development of meningitis. The microbial basis for successful traversal of the blood-brain barrier by circulating bacteria is incompletely understood. Recent studies with *E. coli* K1 have shown that several microbial determinants such as the K1 capsule, OmpA, Ibe proteins, AslA, TraJ, and CNF1 contribute to invasion of BMEC, which is required for successful penetration into the CNS in experimental hematogenous meningitis. In addition, bacterial trafficking of BMEC by *E. coli* K1 requires BMEC actin cytoskeletal reorganizations and activations of focal adhesion kinase, phosphatidylinositol 3-kinase, and cytosolic phospholipase A_2. Of interest, these *E. coli* trafficking mechanisms are shown to differ from those of other meningitis-causing bacteria, such as *L. monocytogenes* and group B *Streptococcus*. Structural genomic studies have identified DNA segments specific to the prototypes of meningitis-causing *E. coli* K1 (e.g., strains RS 218 and C5), and their sequencing is in progress. It is, however, unclear whether sequence information specific to *E. coli* K1 will identify all the microbial determinants relevant to the pathogensis of *E. coli* K1 meningitis. This was exemplified by the identification of *E. coli* K1 structures that contribute to crossing of the blood-brain barrier in vivo but having highly homologous structures in the *E. coli* K-12 genome (e.g., *ompA, ibeB, ibeC*, and *aslA*). Thus, it is likely that *E. coli* K1 determinants which contribute to crossing of the blood-brain barrier are not clustered within K1-specific segments and include K1-specific genes as well as K-12 homologues.

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REFERENCES

1. **Badger, J. L., and K. S. Kim.** 1998. Environmental growth conditions influence the ability of *Escherichia coli* K1 to invade brain microvascular endothelial cells and confer serum resistance. Infect. Immun. **66:**5692–5697.
2. **Badger, J. L., M. F. Stins, and K. S. Kim.** 1999. *Citrobacter freundii* invades and replicates in human brain microvascular endothelial cells. Infect. Immun. **67:**4208–4215.
3. **Badger, J. L., C. A. Wass, and K. S. Kim.** 2000. Identification of *E. coli* K1 genes contributing to human brain microvascular endothelia cell invasion by differential fluorescence induction. Mol. Microbiol. **36:**174–182.
4. **Badger, J. L., C. A. Wass, S. J. Weissman, and K. S. Kim.** 2000. Application of signature-tagged mutagenesis for the identification of *Escherichia coli* K1 genes that contribute to invasion of human brain microvascular endothelial cells Infect. Immun. **68:**5056–5061.
5. **Bell, L. M., G. Alpert, J. M. Campos, and S. A. Plotkin.** 1985. Routine quantitative blood cultures in children with *Haemophilus influenzae* or *Streptococcus pneumoniae* bacteremia. Pediatrics **76:**901–904.
6. **Bonacorsi, S. P. P., O. Clermont, C. Tinsley, I. Le Gall, J.-C. Beaudoin, J. Elion, X. Nassif, and E. Bingen.** 2000. Identification of regions of the *Escherichia coli* chromosome specific for neonatal meningitis-associated strains. Infect. Immun. **68:**2096–2101.
7. **Bowman, P. D., A. L. Betz, D. Ar, J. S. Wolinsky, J. B. Penney, R. R. Shivers, and G. W. Goldstein.** 1981. Primary culture of capillary endothelium from rat brain. In Vitro **17:**353–362.
8. **Braun, L., B. Ghebrehiwet, and P. Cossart.** 2000. gC1q-R/p32, a C1q-binding protein, is a receptor for the InlB invasion protein of *Listeria monocytogenes*. EMBO J. **19:**1458–1466.
9. **Das, A., L. Asatryan, M. A. Reddy, C. A. Wass, M. Stins, S. Joshi, J. V. Boventre, and K. S. Kim.** Differential role of cytosolic phospholipase A2 in the invasion of brain microvascular endothelial cells by *Escherichia coli* and *Listeria monocytogenes*. J. Infect. Dis., in press.
10. **Dietzman, D. E., G. W. Fischer, and F. D. Schoenknecht.** 1974. Neonatal *Escherichia coli* septicemia bacterial counts in blood. J. Pediatr. **85:**128–130.
11. **Dorovini-Zis, K., R. Prameya, and P. D. Bowman.** 1991. Culture and characterization of microvascular endothelial cells derived from human brain. Lab. Investig. **64:**425–436.
12. **Feigin, R. D., G. H. McCracken, Jr., and J. O. Klein.** 1992. Diagnosis and management of meningitis. Pediatr. Infect. Dis. J. **11:**785–814.
13. **Ferrieri, P., B. Burke, and J. Nelson.** 1980. Production of bacteremia and meningitis in infant rats with group B streptococcal serotypes. Infect Immun. **27:**1023–1032.

14. **Flatau, G., E. Lemichez, M. Gauthier, P. Chardin, C. Fiorentini, and P. Boquet.** 1997. Toxin-induced activation of the G protein p21 Rho by deamidation of glutamine. Nature **387:**729–733.
15. **Greiffenberg, L., W. Goebel, K. S. Kim, I. Weiglein, A. Bubert, F. Engelbrecht, M. Stins, and M. Kuhn.** 1998. Interaction of *Listeria monocytogenes* with human brain microvascular endothelial cells: InlB-dependent invasion, long-term intracellular growth, and spread from macrophages to endothelial cells. Infect Immun. **66:**5260–5267.
16. **Gross, R. J., L. R. Ward, E. J. Threlfall, T. Cheasty, and B. Rowe.** 1982. Drug resistance among *Escherichia coli* strains isolated from cerebrospinal fluid. J. Hyg. **90:**195–198.
17. **Hoffman, J. A., C. Wass, M. F. Stins, and K. S. Kim.** 1999. The capsule supports survival but not traversal of *Escherichia coli* K1 across the blood-brain barrier. Infect. Immun. **67:**3566–3570.
18. **Hoffman, J. A., J. L. Badger, Y. Zhang, S.-H. Huang, and K. S. Kim.** 2000. *Escherichia coli* K1 *aslA* contributes to invasion of brain microvascular endothelial cells in vitro and in vivo. Infect. Immun. **68:**5062–5067.
19. **Huang, S.-H., Y.-H. Chen, Q. Fu, M. Stins, Y. Wang, C. Wass, and K. S. Kim.** 1999. Identification and characterization of an *Escherichia coli* invasion gene locus, *ibeB*, required for penetration of brain microvascular endothelial cells. Infect. Immun. **67:**2103–2109.
20. **Huang, S.-H., C. A. Wass, Q. Fu, P. N. V. Prasadarao, M. Stins, and K. S. Kim.** 1995. *Escherichia coli* invasion of brain microvascular endothelial cells in vitro and in vivo: molecular cloning and characterization of invasion gene *ibe10*. Infect. Immun. **63:**4470–4475.
21. **Kim, K. S.** 2000. *Escherichia coli* invasion of brain microvascular endothelial cells as a pathogenetic basis of meningitis. Subcell Biochem. **33:**47–59.
22. **Kim, K. S., H. Itabashi, P. Gemski, J. Sadoff, R. L. Warren, and A. S. Cross.** 1992. The K1 capsule is the critical determinant in the development of *Escherichia coli* meningitis in the rat. J. Clin. Investig. **90:**897–905.
23. **Kim, K. S., C. A. Wass, and A. S. Cross.** 1997. Blood brain barrier permeability during the development of experimental bacterial meningitis in the rat. Exp. Neurol. **45:**253–257.
24. **Korhonen, T. K., M. V. Valtonen, J. Parkkinen, V. Vaisanen-Rhen, J. Finne, F. Ørskov, I. Ørskov, S. B. Svenson, and P. H. Mäkelä.** 1985. Serotypes, hemolysin production, and receptor recognition of *Escherichia coli* strains associated with neonatal sepsis and meningitis. Infect. Immun. **48:**486–491.
25. **Leib, S. L., and M. G. Tauber.** 1999. Pathogenesis of bacterial meningitis. Infect. Dis. Clin. N. Am. **13:**527–548.
26. **Moxon, E. R., and P. T. Ostrow.** 1977. *Haemophilus influenzae* meningitis in infant rats: role of bacteremia in pathogenesis of age-dependent inflammatory responses in cerebrospinal fluid. J. Infect. Dis. **135:**303–307.
27. **Nizet, V., K. S. Kim, M. Stins, M. Jonas, E. Y. Chi, D. Nguyen, and C. E. Rubens.** 1997. Invasion of brain microvascular endothelial cells by group B streptococci. Infect. Immun. **65:**5074–5081.
28. **Petersdorf, R. G., D. R. Swarner, and M. Garcia.** 1962. Studies on the pathogenesis of meningitis. II. Development of meningitis during pneumococcal bacteremia. J. Clin. Investig. **41:**320–327.
29. **Prasadarao, N. V., C. A. Wass, S.-H. Huang, and K. S. Kim.** 1999. Identification and characterization of a novel Ibe10 binding protein that contributes to *Escherichia coli* invasion of brain microvascular endothelial cells. Infect Immun. **67:**1131–1138.
30. **Prasadarao, N. V., C. A. Wass, M. F. Stins, H. Shimada, and K. S. Kim.** 1999. Outer membrane protein A-promoted actin condensation of brain microvascular endothelial cells is required for *Escherichia coli* invasion. Infect. Immun. **67:**5775–5783.
31. **Prasadarao, N. V., C. A. Wass, and K. S. Kim.** 1996. Endothelial cell GlcNAcβ1-4GlcNAc epitopes for outer membrane protein A enhance traversal of *Escherichia coli* across the blood-brain barrier. Infect. Immun. **64:** 154–160.
32. **Prasadarao, N. V., C. A. Wass, J. N. Weiser, M. F. Stins, S.-H. Huang, and K. S. Kim.** 1996. Outer membrane protein A of *Escherichia coli* contributes to invasion of brain microvascular endothelial cells. Infect. Immun. **64:**146–153.
33. **Quagliarello, V. Q., and W. M. Scheld.** 1992. Bacterial meningitis: pathogenesis, pathophysiology and progress. N. Engl. J. Med. **327:**864–871.
34. **Reddy, M. A., N. V. Prasadarao, C. A. Wass, and K. S. Kim.** 2000. Phosphatidylinositol 3-kinase activation and interaction with focal adhesion kinase in *Escherichia coli* K1 invasion of human brain microvascular endothelial cells. J. Biol. Chem. **275:**36769–36774.
35. **Reddy, M. A., C. A. Wass, K. S. Kim, D. D. Schlaepfer, and N. V. Prasadarao.** 2000. Involvement of focal adhesion kinases in *Escherichia coli* invasion of human brain microvascular endothelial cells. Infect. Immun. **68:**6425–6430.
36. **Ring, A., J. N. Weiser, and E. I. Tuomanen.** 1998. Pneumococcal trafficking across the blood brain barrier. Molecular analysis of a novel bi-directional pathway. J. Clin. Investig. **102:**347–360.
37. **Robbins, J. B., G. H. McCracken, Jr., E. C. Gotschlich, F. Orskov, I. Orskov, and L. A. Hanson.** 1974. *Escherichia coli* K1 capsular polysaccharide associated with neonatal meningitis. N. Engl. J. Med. **290:**1216–1220.
38. **Rode, C. K., L. J. Melkerson-Watson, A. T. Johnson, and C. A. Bloch.** 1999. Type-specific contributions to chromosome size differences in *Escherichia coli*. Infect. Immun. **19:**230–236.
39. **Schmidt, G., P. Sehr, M. Wilm, J. Selzer, M. Mann, and K. Aktories.** 1997. Gln 63 of Rho is deamidated by *Escherichia coli* cytotoxic necrotizing factor 1. Nature **387:**725–729.
40. **Shen, Y., M. Naujokas, M. Park, and K. Ireton.** 2000. InIB-dependent internalization of *Listeria* is mediated by the Met receptor tyrosine kinase. Cell **103:**501–510.
41. **Smith, A. L., D. H. Smith, D. R. Averill, J. Marino, and E. R. Moxon.** 1973. Production of *Haemophilus influenzae* b meningitis in infant rats by intraperitoneal inoculation. Infect. Immun. **8:**278–290.
42. **Stins, M. F., J. L. Badger, and K. S. Kim.** 2001. Bacterial invasion and transcytosis in transfected human brain microvascular endothelial cells. Microb. Pathog. **30:**19–28.
43. **Stins, M. F., P. V. Nemani, C. Wass, and K. S. Kim.** 1999. *Escherichia coli* binding to and invasion of brain microvascular endothelial cells derived from humans and rats of different ages. Infect. Immun. **67:**5522–5525.
44. **Sullivan, T. D., and L. J. LaScolea, Jr.** 1987. *Neisseria meningitidis* bacteremia in children: quantitation of bacteremia and spontaneous clinical recovery without antibiotic therapy. Pediatrics **80:**63–67.
45. **Sullivan, T. D., L. J. LaScolea, and E. Neter.** 1982. Relationship between the magnitude of bacteremia in children and the clinical disease. Pediatrics **69:**699–702.
46. **Tuomanen, E.** 1996. Entry of pathogens into the central nervous system. FEMS Microbiol. Rev. **18:**289–299.
47. **Wang, Y., S.-H. Huang, C. A. Wass, M. F. Stins, and K. S. Kim.** 1999. The gene locus *yijP* contributes to *Escherichia coli* K1 invasion of brain microvascular endothelial cells. Infect. Immun. **67:**4751–4756.

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