Prevention of *Escherichia coli* K1 Penetration of the Blood-Brain Barrier by Counteracting the Host Cell Receptor and Signaling Molecule Involved in *E. coli* Invasion of Human Brain Microvascular Endothelial Cells[▽]

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Escherichia coli meningitis is an important cause of mortality and morbidity, and a key contributing factor is our incomplete understanding of the pathogenesis of *E. coli* meningitis. We have shown that *E. coli* penetration into the brain requires *E. coli* invasion of human brain microvascular endothelial cells (HBMEC), which constitute the blood-brain barrier. *E. coli* invasion of HBMEC involves its interaction with HBMEC receptors, such as *E. coli* cytotoxic necrotizing factor 1 (CNF1) interaction with its receptor, the 67-kDa laminin receptor (67LR), and host signaling molecules including cytosolic phospholipase A₂α (cPLA₂α). In the present study, we showed that treatment with etoposide resulted in decreased expression of 67LR on HBMEC and inhibited *E. coli* invasion of HBMEC. Pharmacological inhibition of cysteinyl leukotrienes, lipoxygenated products of arachidonic acid released by cPLA₂α, using montelukast (an antagonist of the type 1 cysteinyl leukotriene receptor) also inhibited *E. coli* invasion of HBMEC. *E. coli* penetration into the brain was significantly decreased by etoposide as well as by montelukast, and a combination of etoposide and montelukast was significantly more effective in inhibiting *E. coli* K1 invasion of HBMEC than single agents alone. These findings demonstrate for the first time that counteracting the HBMEC receptor and signaling molecule involved in *E. coli* invasion of HBMEC provides a novel approach for prevention of *E. coli* penetration into the brain, the essential step required for development of *E. coli* meningitis.

The mortality and morbidity associated with neonatal Gram-negative bacillary meningitis have remained significant despite advances in antimicrobial chemotherapy and supportive care. Inadequate knowledge of the pathogenesis has contributed to this mortality and morbidity (10–12). *Escherichia coli* is the most common Gram-negative organism that causes neonatal meningitis. Several lines of evidence from experimental animal models as well as human cases of *E. coli* meningitis indicate that *E. coli* penetrates into the brain initially in the cerebral vasculature (2, 13), but the underlying mechanisms contributing to *E. coli* penetration of the blood-brain barrier remain incompletely understood (10–12).

We have developed an *in vitro* blood-brain barrier model by isolation and cultivation of human brain microvascular endothelial cells (HBMEC) (14, 20–22). Upon cultivation on collagen-coated Transwell inserts, the HBMEC exhibit morphological and functional properties of tight junction formation and a polarized monolayer. These properties are shown by our demonstrations of tight junction proteins (such as ZO-1), adherens junction proteins (such as β-catenin), and their spatial separation, limited permeability to propidium iodide (molecular mass, 668 Da) and inulin (molecular mass, 4,000 Da), and development of high transendothelial electrical resistance (14,

20–22). We have also developed an infant rat model of experimental hematogenous meningitis (8, 13). This animal model has important similarities to *E. coli* meningitis in humans, such as hematogenous infection of the meninges. Using these *in vitro* and *in vivo* models, we have shown that *E. coli* binding to and invasion of HBMEC are prerequisites for penetration into the brain (10–12).

We have shown that *E. coli* K1 binding to and invasion of HBMEC require specific *E. coli* determinants (e.g., cytotoxic necrotizing factor 1 [CNF1] and OmpA), and these *E. coli* determinants contribute to HBMEC binding and invasion via interactions with their respective HBMEC receptors (10–12). For example, CNF1 contributes to *E. coli* K1 invasion of HBMEC via its interaction with 37-kDa laminin receptor precursor (37LRP)/67-kDa lamin receptor (67LR), while OmpA contributes to *E. coli* K1 binding to and invasion of HBMEC via its interaction with the HBMEC receptor, gp96 (3, 7, 9). We also showed that the *E. coli* determinants contributing to HBMEC binding and invasion exploit specific host signaling molecules for efficient invasion of HBMEC. For example, OmpA, NlpI, FliC, and IbeC (the *E. coli* structures contributing to HBMEC binding and invasion) are shown to exploit host cytosolic phospholipase A₂α (cPLA₂α) for *E. coli* invasion of HBMEC (4, 12, 25).

We also showed that blockade of the HBMEC receptors and/or host signaling molecules was effective in preventing *E. coli* K1 invasion of HBMEC. For example, anti-67LR and -gp96 antibodies inhibited *E. coli* K1 invasion of HBMEC in a ligand-dependent manner (7, 9), and pharmacological inhibi-

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tion of host cPLA₂ α exhibited a dose-dependent inhibition of *E. coli* invasion of HBMEC (4). These findings suggest that inhibition of the HBMEC receptors and host signaling molecules involved in *E. coli* K1 invasion of HBMEC is likely to affect the ability of *E. coli* to penetrate into the brain.

In screening drugs for their effects on the HBMEC receptors, we determined that etoposide (a topoisomerase inhibitor) decreased the expression of 67LR on HBMEC. cPLA₂ α mediates agonist-induced release of arachidonic acid (6). We showed that the contribution of host cPLA₂ α to *E. coli* invasion of HBMEC occurs via lipoxygenated products of arachidonic acid, cysteinyl leukotrienes (LTs), formed via LT biosynthetic pathways involving 5-lipoxygenase, and acting via the type 1 cysteinyl leukotriene receptor (CysLT1) (4, 12). More importantly, etoposide and montelukast (the CysLT1 antagonist) were additive in their prevention of *E. coli* K1 invasion of HBMEC and also efficient in preventing *E. coli* K1 penetration into the brain.

MATERIALS AND METHODS

Bacterial strains and culture condition. *E. coli* K1 strain RS218 (O18:K1:H7) is the cerebrospinal fluid (CSF) isolate from a neonate with meningitis. The mutants with deletions of *cnf1* and *ompA* from strain RS218 were described previously (7, 8, 26). *E. coli* strains were grown at 37°C overnight in brain heart infusion (BHI) broth containing appropriate antibiotics unless otherwise specified.

Reagents. Etoposide and montelukast were purchased from Sigma-Aldrich Corp. (St. Louis, MO) and Cayman Chemical Company (Ann Arbor, MI), respectively.

Isolation, characterization, and culture of human brain microvascular endothelial cells. HBMEC were isolated and characterized as described previously (22). Briefly, brain specimens were cut into small pieces and homogenized in Dulbecco's modified Eagle's medium (DMEM) containing 2% fetal bovine serum (FBS) (DMEM-S) using a Dounce homogenizer with a loose fitting. The homogenate was centrifuged in 15% dextran in DMEM-S for 10 min at 10,000 $\times$ g. The pellet containing crude microvessels was further digested in a solution containing 1 mg/ml collagenase-dispase in DMEM-S for 1 h at 37°C. Microvascular capillaries were isolated by adsorption to a column of glass beads (0.25 to 0.3 mm); the beads were washed off, and the microvascular capillaries were recovered in growth medium. HBMEC were plated on rat tail collagen-fibronectin-coated dishes or on glass coverslips and cultured in RPMI 1640-based medium with growth factors, 10% heat-inactivated FBS, 10% NuSerum, 5 U of heparin/ml, 2 mM L-glutamine, 1 mM sodium pyruvate, nonessential amino acids, vitamins, and 100 U of penicillin and streptomycin/ml. Viability of HBMEC was assessed by examining morphology and by trypan blue exclusion. HBMEC were positive for factor VIII-Rag, took up fluorescently labeled acetylated low-density lipoprotein, and expressed γ -glutamyl transpeptidase. HBMEC were maintained in RPMI-based medium, including 10% FBS and 10% NuSerum, at 37°C in a humid atmosphere of 5% CO₂ as described previously (20–22).

Antibodies. Rabbit polyclonal antibody against factor VIII-Rag was purchased from Dako (Carpinteria, CA), mouse monoclonal VE-cadherin (F-8) was from Santa Cruz (Santa Cruz, CA), and R-phycoerythrin (PE) goat anti-rabbit IgG and Alexa Fluor 488 goat anti-mouse IgG were from Molecular Probes (Eugene, OR). Rabbit polyclonal anti-67LR antibody and -gp96 antibody were previously described (7, 9).

The effect of etoposide on expression of the HBMEC receptors by FACS. The expression of 67LR and gp96 on HBMEC was examined by fluorescence-activated cell sorting (FACS) analysis as described previously (23, 24). Briefly, HBMEC grown in six-well plates were treated with 0.5 mg/ml etoposide in serum-free medium (M199-Ham F12 medium at 1:1) for 2 h, lifted with 5 mM EDTA, and subsequently fixed with 2% paraformaldehyde in phosphate-buffered saline (PBS), quenched with 10 mM glycine-PBS, and blocked with 5% normal goat serum. Etoposide-treated and nontreated cells were then incubated for 30 min with primary antibodies (anti-67LR or anti-gp96), washed, and further incubated with either Alexa Fluor 488 or PE-conjugated secondary antibodies. Intracellular antigens (factor VIII-Rag and VE-cadherin) were demonstrated after permeabilization of the HBMEC with PBS containing 0.01% Triton X-100.

Nonstained samples were also prepared. Analysis was done with a BD FACScan flow cytometer and CellQuest software (Becton Dickinson, San Jose, CA).

***E. coli* binding and invasion assays in HBMEC.** The ability of *E. coli* K1 strains to bind to and invade HBMEC was examined as described previously (6–8, 26). Briefly, *E. coli* strains were grown overnight in BHI broth in the presence of streptomycin (50 μ g/ml). Bacteria were resuspended in experimental medium (M199-Ham F12 [1:1] medium containing 5% heat-inactivated fetal bovine serum, 2 mM glutamine, and 1 mM pyruvate) and added at a multiplicity of infection (MOI) of 100:1 to HBMEC grown in collagen-coated 24-well plates at 37°C in a 5% CO₂ incubator for 90 min for a binding assay. HBMEC were washed four times with PBS to remove unbound bacteria, lysed in 0.025% Triton X-100, and cultured for determinations of the numbers of CFU. The results were calculated as a percentage of the initial inoculum and expressed as percent relative binding compared to percent binding of strain RS218 in the presence of vehicle control (dimethyl sulfoxide [DMSO]). Each set was run in triplicate.

The HBMEC invasion assay with gentamicin treatment was performed to determine the number of viable intracellular bacteria recovered from the infected HBMEC. *E. coli* K1 strains were added to HBMEC as described above for the binding assay. HBMEC were subsequently washed with RPMI 1640 medium and incubated with experimental medium containing gentamicin (100 μ g/ml) for 1 h to kill extracellular bacteria. The cells were washed again with PBS and lysed in 0.025% Triton X-100. The released intracellular bacteria were enumerated by plating on sheep blood agar plates. The results were calculated as a percentage of the initial inoculum and expressed as percent relative invasion compared to percent invasion of strain RS218 in the presence of vehicle control (DMSO). Each set was run in triplicate.

To examine the effects of etoposide and montelukast, alone or in combination, on *E. coli* K1 binding to and invasion of HBMEC, HBMEC were pretreated with etoposide for 2 h, montelukast for 1 h, or DMSO (vehicle control, 0.1 to 0.5%) for 1 to 2 h before addition of *E. coli* and then processed for binding and invasion assays as described above.

The effects of etoposide and montelukast on *E. coli* K1 penetration into the brain in the infant rat model of experimental hematogenous meningitis. Experimental hematogenous *E. coli* meningitis was induced in 5-day-old rats as described previously (8, 13). Briefly, outbred, specific-pathogen-free pregnant Sprague-Dawley rats with timed conception were purchased from Charles River Breeding laboratory (Wilmington, MA). The rats delivered in our vivarium 5 to 7 days after arrival. At 5 days of age, all members of each litter were randomly divided into three groups to receive intraperitoneally etoposide (50 μ g/10 g of weight in 50 μ l of PBS), montelukast (50 μ g/10 g of weight in 50 μ l of PBS), or vehicle control (3.3% DMSO in 50 μ l of PBS). One hour later, each animal received 1×10^7 CFU of *E. coli* K1 strain RS218 via intracardiac injection. At 1 h after bacterial injection, blood and CSF specimens were obtained as described previously (8, 13) for quantitative cultures. The development of *E. coli* meningitis (defined as positive CSF cultures) was compared between the recipients of etoposide or montelukast versus vehicle control.

RESULTS

The effect of etoposide on *E. coli* K1 binding to and invasion of HBMEC. We first examined the effect of etoposide on *E. coli* K1 binding to and invasion of HBMEC by pretreatment of HBMEC with etoposide for 2 h before *E. coli* binding and invasion assays. As shown in Fig. 1, etoposide treatment inhibited *E. coli* invasion of HBMEC in a dose-dependent manner while it did not affect *E. coli* binding to HBMEC. Etoposide did not affect the integrity of HBMEC, as assessed by live/dead staining (Molecular Probes), and also did not affect the growth of *E. coli* in experimental medium. These findings suggest that etoposide is most likely to affect the *E. coli*-HBMEC interactions involved in invasion but not in binding.

The effect of etoposide on expression of the HBMEC receptors by FACS. We have previously shown that *E. coli* binding to and invasion of HBMEC occur as the result of specific microbe-HBMEC interactions (10–12). For example, *E. coli* CNF1 contributes to invasion of HBMEC via its interaction with 67LR, while *E. coli* OmpA contributes to HBMEC binding and invasion via its interaction with gp96 on the HBMEC

FIG. 1. Etoposide inhibits *E. coli* K1 invasion of HBMEC in a dose-dependent manner. HBMEC were incubated with 0.1 mg/ml, 0.5 mg/ml, and 1 mg/ml of etoposide in serum-free experimental medium for 2 h, followed by bacterial binding and invasion assays. Results are expressed as percent relative binding and invasion (mean $\pm$ standard error of the mean) compared to percent binding and invasion of vehicle control-treated HBMEC. Each experiment was performed in triplicate. *, $P < 0.05$, by one-way analysis of variance followed by Dunnett's test, compared to vehicle control (0.5% DMSO).

surface (3, 7, 9). We next examined the effect of etoposide on the expression of the HBMEC receptors 67LR and gp96, which have been shown to interact with *E. coli* CNF1 and OmpA, respectively.

FACS analysis revealed that most cells express gp96 (approximately 95% of gated population), which did not change significantly by treatment with etoposide. In contrast, 67LR expression was found in approximately 28% of the gated population, which was decreased to 12% by treatment with etoposide (approximately 55% decline). Treatment of HBMEC with etoposide at 0.5 mg/ml for 2 h, thus, resulted in significantly decreased expression of 67LR while the expression of gp96 was not affected by treatment with etoposide (Fig. 2).

Etoposide decreases HBMEC invasion by the OmpA mutant but not by the CNF1 mutant compared to the parent strain RS218. Since treatment of HBMEC with etoposide decreased the expression of 67LR (the HBMEC receptor for CNF1), not

FIG. 3. Effect of etoposide on HBMEC invasion by *E. coli* K1 strain RS218, the CNF1 mutant, and the OmpA mutant. HBMEC were incubated with 0.5 mg/ml etoposide in serum-free experimental medium for 2 h, followed by bacterial invasion assays. Results are expressed as percent relative invasion (mean $\pm$ standard error of the mean) compared to percent invasion of vehicle control-treated HBMEC. Each experiment was performed in triplicate. **, $P < 0.01$, by a Student's *t* test between etoposide and vehicle control (0.5% DMSO); #, $P < 0.05$, by a Student's *t* test between the parent strain RS218 and the CNF1 mutant.

gp96 (the HBMEC receptor for OmpA), we next examined whether pretreatment of HBMEC with etoposide affects *E. coli* invasion of HBMEC by comparing the percent HBMEC invasion of the CNF1 mutant and the OmpA mutant with that of the parent strain. As expected from our previous findings (7–9), both the OmpA mutant and the CNF1 mutant were significantly defective in invasion of HBMEC compared to invasion by the parent strain, with a mean HBMEC invasion of 0.02% and 0.1%, respectively, versus 0.2% for the parent strain. As shown in Fig. 3, the percent HBMEC invasion was significantly decreased by pretreatment of HBMEC with etoposide at 0.5 mg/ml compared to vehicle control treatment for the parent *E. coli* K1 strain and its CNF1 and OmpA mutants. Of interest, the magnitudes of inhibition that occurred with etoposide treatment compared to that of the vehicle control were similar for the parent strain and the OmpA mutant. In contrast, the magnitude of inhibition by etoposide compared to that of the vehicle control was significantly less with the CNF1 mutant than with the parent strain. These findings support the concept that etoposide-induced inhibition of *E. coli* invasion was partly due to its decreased expression of 67LR, thus mitigating the contribution of the CNF1-67LR interaction to HBMEC invasion by the CNF1 mutant.

Montelukast affects *E. coli* K1 invasion of HBMEC. We have shown that cPLA₂α contributes to *E. coli* K1 invasion of HBMEC through lipxygenated products of arachidonic acid, cysteinyl LTs, acting via the CysLT1 (4, 12). We also showed that several *E. coli* determinants exploit cPLA₂α for invasion of HBMEC (4, 12, 25). As shown in Fig. 4, montelukast (the CysLT1 antagonist) caused a dose-dependent inhibition of HBMEC invasion by the parent *E. coli* K1 strain and its OmpA and CNF1 mutants while it did not affect their binding to HBMEC. These findings support the idea that several *E. coli* determinants with and without CNF1 or OmpA use cPLA₂α for their contributions to HBMEC invasion.

Combined effect of etoposide and montelukast on *E. coli* K1 invasion of HBMEC. Since etoposide and montelukast inhibited *E. coli* K1 invasion of HBMEC using different mecha-

Flow cytometry: 67LR and gp96 surface expression on etoposide treated and non-treated HBMEC

FIG. 2. Relative expression of 67LR and gp96 on etoposide-treated and nontreated HBMEC, as examined by FACS. The expression levels of 67LR and gp96 on nontreated HBMEC were set at 100%, and their expression levels on etoposide-treated HBMEC were expressed as a percentage of the expression on nontreated HBMEC.

FIG. 4. Montelukast inhibits HBMEC invasion by *E. coli* K1 strain RS218, the CNF1 mutant, and the OmpA mutant but not their binding to HBMEC. HBMEC were incubated with 10 and 50 μ M montelukast for 1 h, followed by bacterial binding and invasion assays. Results are expressed as percent relative binding and invasion (mean $\pm$ standard error of the mean) compared to percent binding and invasion of vehicle control-treated HBMEC. Each experiment was performed in triplicate. *, $P < 0.05$; **, $P < 0.01$ (Student's t test between 50 μ M montelukast and vehicle control [0.1% DMSO]).

nisms, we next examined the effects of etoposide and montelukast, alone or in combination, on *E. coli* K1 invasion of HBMEC. As shown in Fig. 1 and 4, etoposide at 0.1 mg/ml and montelukast at 10 μ M did not significantly affect *E. coli* K1 invasion of HBMEC. The combination of both, however, exhibited a significant inhibition compared to that of the vehicle control (Fig. 5).

The effects of etoposide and montelukast on *E. coli* K1 penetration into the brain in the infant rat model of experimental hematogenous meningitis. We have previously shown that *E. coli* penetration into the brain involves *E. coli* binding to and invasion of HBMEC (3, 7–9). Using our well-characterized infant rat model of experimental hematogenous meningitis, we next examined whether etoposide and montelukast affect *E. coli* K1 penetration into the brain by virtue of their affecting the HBMEC receptor and signaling molecule.

As shown in Table 1, bacterial counts in the blood did not differ between the three groups of animals receiving etoposide,

FIG. 5. Combined effect of montelukast and etoposide on *E. coli* K1 invasion of HBMEC. HBMEC were incubated with 10 μ M montelukast for 1 h, with 0.1 mg/ml etoposide for 2 h, or with a combination of montelukast and etoposide, followed by invasion assays with strain RS218. Results are expressed as percent relative invasion (mean $\pm$ standard error of the mean) compared to percent invasion of vehicle control-treated HBMEC. Each experiment was performed in triplicate. *, $P < 0.05$, by a Student's t test between the combined treatment and vehicle control (0.2% DMSO).

TABLE 1. Comparison of bacterial counts in the blood and development of meningitis between three groups of 5-day-old rats receiving etoposide, montelukast, or vehicle control

Inhibitor (n) ^a	Bacteremia (log CFU/ml of blood [mean $\pm$ SD])	No. (%) of animals with meningitis ^c
DMSO ^b (9)	6.97 $\pm$ 0.57	9 (100)
Etoposide (12)	6.81 $\pm$ 0.90	1 (8)*
Montelukast (9)	7.07 $\pm$ 0.26	2 (22)*

^a n, number of animals.

^b Vehicle control, 3.3% DMSO.

^c Meningitis was defined as positive CSF cultures. *, $P < 0.01$ compared to vehicle control, by Fisher's exact test.

montelukast, or vehicle control. However, the development of *E. coli* meningitis (defined as positive CSF cultures) was significantly less in the recipients of etoposide or montelukast than in mice that received the vehicle control (Table 1). These findings support the idea that 67LR and cysteinyl LTs are likely to contribute to *E. coli* K1 penetration into the brain.

DISCUSSION

We and others have previously shown that *E. coli* K1 penetration into the brain requires a high degree of bacteremia as well as *E. coli* binding to and invasion of HBMEC, involving specific *E. coli* interactions with HBMEC (so-called ligand-receptor interactions) and host signaling molecules (5, 10–12), but the contributions of microbe-HBMEC interactions to *E. coli* penetration into the brain remain incompletely understood.

In this report, we showed for the first time that pharmacologically induced decreased expression of the HBMEC receptor that interacts with the *E. coli* K1 structure contributing to HBMEC invasion (i.e., CNF1) and the antagonist of the host signaling molecule that contributes to HBMEC invasion (e.g., CysLT1) provide a novel approach for preventing *E. coli* K1 invasion of HBMEC and penetration into the brain. This novel concept was shown by our demonstrations, as follows: (i) etoposide treatment decreased the expression of 67LR (the receptor for CNF1) on the surface of HBMEC but did not affect the expression of gp96 (the HBMEC receptor for OmpA, the structure involved in HBMEC binding and invasion); (ii) etoposide significantly inhibited *E. coli* invasion of HBMEC for the parent K1 strain and its CNF1 and OmpA mutants, but its inhibition was significantly less for the CNF1 mutant than for the parent strain; (iii) montelukast (the CysLT1 antagonist) inhibited HBMEC invasion for the parent *E. coli* strain as well as its CNF1 and OmpA mutants; and (iv) administration of etoposide or montelukast was efficient in significantly inhibiting *E. coli* K1 penetration into the brain. Of interest, the combination of etoposide and montelukast exhibited significantly greater inhibition of *E. coli* K1 invasion of HBMEC than the individual agents alone.

We have previously shown that a high degree of bacteremia is a primary determinant for *E. coli* K1 penetration into the brain (8, 13). The magnitudes of bacteremia between the three groups of animals receiving etoposide, montelukast, or vehicle control, however, were similar, indicating that decreased penetration of *E. coli* K1 into the brain of the recipients of eto-

poside or montelukast was not an artifact of not having sufficient numbers of circulating bacteria in the bloodstream. These findings demonstrate that etoposide and montelukast are likely to prevent *E. coli* K1 penetration into the brain by virtue of affecting the host cell receptor and signaling molecule that contribute to *E. coli* invasion of HBMEC.

Treatment of HBMEC with etoposide resulted in significantly decreased invasion for the parent strain RS218 as well as for the OmpA and the CNF1 mutants. Etoposide (the topoisomerase inhibitor) has been shown to induce apoptosis in vascular endothelial cells (17), but, as described before, treatment of HBMEC with etoposide at 0.5 mg/ml for 2 h did not affect the viability of HBMEC and also did not affect the growth of *E. coli* strains in experimental medium. The reasons for the etoposide-induced decrease in HBMEC invasion by the *E. coli* K1 strain and its OmpA and CNF1 mutants remain unclear and may involve other microbe-host interactions contributing to *E. coli* K1 invasion of HBMEC (12). The etoposide-induced inhibition of HBMEC invasion, however, was significantly greater with the parent strain and the OmpA mutant than with the CNF1 mutant. It is, therefore, likely that decreased 67LR expression by etoposide treatment mitigates the contribution of 67LR and its interaction with CNF1 to HBMEC invasion by the CNF1 mutant. The mechanisms for decreased expression of 67LR, but not gp96, in response to etoposide treatment in HBMEC are not clear. Topoisomerase enzymes are essential in higher-order eukaryotes as they are likely to be involved in DNA supercoiling generated by transcription, replication, and chromatin remodeling (1). Our transcriptional analysis of 37LRP (laminin precursor protein) with quantitative reverse transcription-PCR (RT-PCR) did not reveal any change in the expression of 37LRP in HBMEC before or after treatment with etoposide (data not shown). At present, the nature of the mechanism by which 37LRP gives rise to its mature form, 67LR, is unclear. Recent data suggest that a posttranslational modification of 37LRP involving acylation might lead to dimerization of 37LRP (15, 18), and etoposide may affect the steps involved in dimerization, resulting in decreased expression of 67LR on the HBMEC surface. Additional studies are needed to clarify this issue.

In contrast, montelukast was equally effective in inhibiting HBMEC invasion by the parent *E. coli* K1 strain as well as its OmpA and CNF1 mutants. These findings are consistent with our preliminary demonstration that several *E. coli* determinants use cysteinyl LTs for invasion of HBMEC (e.g., OmpA, NlpI, FliC, and IbeC) (4, 12, 25). It remains to be determined how cysteinyl LT interaction with the CysLT1 contributes to *E. coli* K1 invasion of HBMEC and penetration into the brain. More importantly, the combination of etoposide and montelukast was significantly more effective in inhibiting *E. coli* K1 invasion of HBMEC, as shown by the demonstration that *E. coli* invasion of HBMEC was significantly less with the combination of ineffective doses of etoposide and montelukast, suggesting that counteracting both the HBMEC receptor(s) and signaling molecule(s) is likely to be more efficient in inhibiting *E. coli* invasion of HBMEC and penetration into the brain than targeting single molecules.

Our findings demonstrate that pharmacological inhibition and/or blockade of the HBMEC receptor(s) that interacts with *E. coli* structure(s) and host signaling molecule(s) that contrib-

utes to HBMEC invasion is likely to prevent *E. coli* penetration into the brain, and studies are in progress to identify additional pharmacological agents for their ability to inhibit the HBMEC receptors and signaling molecules involved in *E. coli* invasion of HBMEC.

Increasing resistance to antimicrobial agents, as shown, for example, by extended-spectrum β -lactamase-producing *E. coli*, is an important factor contributing to mortality and morbidity associated with *E. coli* sepsis and meningitis (16, 19, 27). Our findings illustrate that targeting host cell receptors and signaling molecules involved in microbial invasion of the blood-brain barrier, as shown here with down-modulation of 67LR by etoposide and blockade of the CysLT1 by montelukast, may limit the exposure to emerging antimicrobial-resistant bacteria and is likely to provide a novel approach for prevention of *E. coli* meningitis.

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The animal experiments were approved by the Animal Care and Use Committee of the Johns Hopkins University.

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