Available online at www.sciencedirect.com
ScienceDirect
Biochemical and Biophysical Research Communications 365 (2008) 201–206
BBRC
www.elsevier.com/locate/ybbrc

Transcriptome of *Escherichia coli* K1 bound to human brain microvascular endothelial cells

Yi Xie [a], Geetha Parthasarathy [a], Francescopaolo Di Cello [a], Ching-Hao Teng [b,c], Maneesh Paul-Satyaseela [a], Kwang Sik Kim [a,*]

[a] *Division of Pediatric Infectious Diseases, Johns Hopkins University School of Medicine, 720 Rutland Avenue, Ross 1109, Baltimore, MD 21205, USA*
[b] *Division of Clinical Research, National Health Research Institutes, Tainan, Taiwan*
[c] *Institute of Molecular Medicine, National Cheng Kung University Medical College, Tainan, Taiwan*

Received 24 October 2007
Available online 5 November 2007

Abstract

Escherichia coli K1 is the most common Gram-negative organism causing neonatal meningitis. Binding to human brain microvascular endothelial cells (HBMEC) is an essential step for *E. coli* K1 traversal of the blood–brain barrier. In this study, we examined expression profiles of *E. coli* K1 strain RS218 during its binding to HBMEC. Comparison of HBMEC-bound *E. coli* K1 with collagen-bound *E. coli* revealed more than one hundred genes whose expression patterns were significantly changed in HBMEC-bound *E. coli* K1, but not in collagen-bound *E. coli* K1. These genes are involved mainly in cell surface decorations, cellular function, and nitrogen metabolism. The roles of several representative genes including *frdA*, *clpB*, *carA*, and *ompT* in HBMEC binding were verified with their isogenic mutants, which exhibited significantly less HBMEC binding capability compared to that of the parent strain. This transcriptome analysis provided us with the first genomic-level view of *E. coli* and HBMEC interactions.

Keywords: *E. coli* K1; Meningitis; Blood–brain barrier; Host–pathogen interaction; DNA microarray; Transcriptome

The blood–brain barrier is characteristic for its tight junctions and high transendothelial electrical resistance, and is highly efficient in blocking the entrance of bacteria into the central nervous system [1]. There are only a limited number of pathogens capable of crossing this barrier and causing meningitis. *Escherichia coli* K1 is the most common Gram-negative organism causing neonatal meningitis. *E. coli* meningitis usually occurs in newborns or infants under 3 months of age, and develops as the result of hematogenous spread after gastrointestinal colonization and bloodstream invasion of *E. coli* K1 [1]. During the last decade, several screening technologies have been applied to study the pathogenetic mechanisms of *E. coli* K1 meningitis in our laboratory [2–5]. These efforts indicate that a large number of bacterial virulence determinants are involved in the *E. coli* K1 traversal of the blood–brain barrier, such as K1 capsule, O-LPS, OmpA, Ibe proteins, AslA, TraJ, and CNF1 [1,6].

Escherichia coli K1 traversal of the blood–brain barrier is a dynamic process involving multiple interactions between the pathogen and host cells including binding, entry and transcytosis of human brain microvascular endothelial cells (HBMEC), which constitute of the blood–brain barrier. In order for *E. coli* to effectively bind and invade into HBMEC, expressions of virulence genes must be properly regulated in both temporal and spatial fashions. Up to date, there is only a limited knowledge on how those virulence factors are coordinated during *E. coli* K1 association with HBMEC.

As a leading technology in functional genomics studies, DNA microarray is able to profile thousands of mRNA transcripts in a single experiment and is a useful tool to

* Corresponding author. Fax: +1 410 614 1491.
E-mail address: kwangkim@jhmi.edu (K.S. Kim).

0006-291X/$ - see front matter © 2007 Elsevier Inc. All rights reserved.
doi:10.1016/j.bbrc.2007.10.174

interrogate the dynamic process such as the host–pathogen interaction. In this study, we examined the responses of *E. coli* K1 during its association with HBMEC using DNA microarray. The information derived from this study provided an overview of genetic regulation network involved in the pathogenesis of *E. coli* meningitis during bacterial binding to HBMEC.

Materials and methods

Bacteria strain and tissue culture. *E. coli* RS218^{str} is a spontaneous streptomycin-resistant mutant of RS218 (O18:K1:H7), isolated from the cerebrospinal fluid of a neonate with *E. coli* meningitis [7]. RS218^{str} was grown statically in brain heart infusion broth (BHI, BD Biosciences, Sparks, MD) with 100 μg/ml streptomycin overnight at 37 °C.

Human brain microvascular endothelial cells (HBMEC) were isolated and cultivated as previously described [8]. HBMEC were grown on collagen-coated tissue culture plates in RPMI medium containing 10% heat inactivated fetal bovine serum, 10% Nu-Serum, 2 mM glutamine, 1 mM pyruvate, 100 U/ml penicillin, 100 μg/ml streptomycin, 1% MEM non-essential amino acids, and vitamins at 37 °C in humid atmosphere of 5% CO_2 until confluence.

Bacteria-derived total RNA extraction and DNA microarray hybridization. *E. coli* K1 associated with and not-associated with HBMEC were recovered based on a differential lysis technique developed in our laboratory [9]. Confluent HBMEC were incubated with *E. coli* RS218^{str} at a multiplicity of infection of 100 for 90 min at 37 °C. The bacteria recovered from the supernatant after co-incubation were designated as non-HBMEC-associated *E. coli*. The HBMEC monolayers were washed twice with experimental media without serum. Subsequently, the HBMEC were lysed with guanidium thiocyanate-based RLT buffer (RNeasy Mini kit, Qiagen, CA) and the bacteria in the lysis suspension were recovered by centrifugation at 13,200*g* for 2 min. This batch of bacteria was designated as HBMEC-associated *E. coli*.

Bacterial total RNA was extracted with Ribopure Bacteria Kit (Ambion, Austin, TX). Cy3 and Cy5 labeled cDNA probes were generated with an indirect labeling procedure [9], which was subsequently combined and hybridized to *E. coli* K1 DNA microarray. This *E. coli* K1 DNA microarray is composed of total 8239 oligonucleotide probes (50-mer) [9,10], which were derived from a commercial *E. coli* K12 and O157 set (MWG Biotech, High Point, NC) supplemented with probes derived from extraintestinal pathogenic *E. coli*. Hybridized microarray slides were scanned with GenePix 4000B (Axon Instruments, Foster City, CA). The differential expression of genes was determined with limma package based on a generalized linear model [11]. The cutoff *p*-value of significance for differential expression was set as 0.005, i.e., less than one expected false positives in two hundred detected differentially expressed genes [11].

Construction of isogenic deletion mutants E. coli RS218. Isogenic deletion mutants were constructed with a "one-step PCR" gene inactivation method [12]. Briefly, each targeting ORF was replaced with a chloramphenicol cassette that was PCR-synthesized with two long primers using plasmid pKD3 as a template. Both primers had 50-nt extensions that were homologous to boundaries of the targeting ORF. After electroporation of those PCR products directly into *E. coli* RS218, the target ORF was replaced with the chloramphenicol cassette via homologous recombination through those two 50-bp homologous arms in the linear PCR product, which was facilitated by λ Red system [13]. Subsequently, chloramphenicol was used to identify mutants and correct deletions were confirmed PCR with screening primers and sequencing of PCR products.

Tissue cultures and in vitro infection experiments. Bacterial HBMEC association and invasion assays were performed as described previously [14]. For association assays, confluent HBMEC in 24-well tissue culture plates (Corning Costar, Corning, NY) were incubated with 10^7 CFU *E. coli* at a multiplicity of infection of 100 for 90 min at 37 °C. The monolayers were washed three times with PBS and lysed with sterile water for 30 min at room temperature. The released bacteria were enumerated by plating on sheep blood agar plates. Results were calculated as a percent of the initial inoculum and expressed as percent relative HBMEC association rates compared to HBMEC association of strain RS218^{str}.

For invasion assays, HBMEC monolayers were incubated with 10^7 CFU *E. coli* for 90 min as described above. The monolayers were washed once and then incubated with experimental medium containing gentamicin (100 μg/ml) for 1 h to kill extracellular bacteria. The monolayers were washed with PBS for three times, lysed with sterile water, and released internalized bacteria were enumerated as described above. Results were expressed as percent relative HBMEC invasion rates compared to HBMEC invasion of strain RS218^{str}.

Results

Overviews of transcriptome of HBMEC-associated E. coli K1

We detected a total of 227 genes that were differentially expressed in *E. coli* K1 associated with HBMEC compared to the non-associated bacteria in the supernatant (see Supplementary Table S1). We also profiled the expression changes of *E. coli* K1 strain RS218 bound to collagen-coated tissue culture plate as a control, where the expression patterns of 239 genes were significantly changed compared to the unbound bacteria (see Supplementary Table S2). Nearly half of differentially expressed genes are common irrespective that the binding surface is live human cells or collagen (Fig. 1), suggesting that physical binding process plays an important role in *E. coli* interactions with HBMEC and collagen.

These gene changes common to surface-bound *E. coli* could be categorized into several groups according to their functional annotations, i.e., respiration genes (aerobic and anaerobic respiration), iron utilization genes, carbohydrate catabolic genes, and transcriptional regulator genes (Supplementary Fig. S1). About half of these transcriptional changes were associated with surface-bound *E. coli* K1 switching from aerobic to anaerobic respiration. It appears that HBMEC- and collagen-bound *E. coli* RS218 switches from aerobic to anaerobic respiration with fumurate as

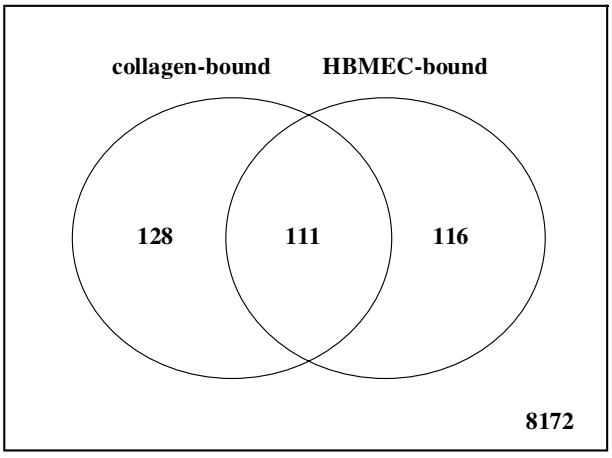

Fig. 1. Vann diagram of differentially expressed genes in HBMEC-associated as well as collagen-bound *E. coli* RS218.

electron acceptors and hydrogen as electron donors. The genes involved in this anaerobic respiration pathway (fumarate reductase genes *frdABCD* and hydrogenase 2 genes *hybOABCDEFG*) were significantly induced, while the aerobic respiration-related genes such as pyruvate dehydrogenase (*aceEF-lpdA*) and cytochrome *o* ubiquinol oxidase (*cyoABCDE*) were significantly repressed. Induction of *fdhGH* genes suggests that formate may also serve as electron donors for the fumurate respiration.

Expression changes that are specific to HBMEC-associated E. coli K1

There were a total of 116 genes whose expression changes were unique to HBMEC-associated *E. coli* RS218. These genes could be roughly categorized into five groups based on their functional annotations, i.e., surface remodeling genes, amino acid metabolism genes, nucleotide metabolism genes, stress related genes, and ribosomal genes (Fig. 2).

The most interesting transcriptional changes in HBMEC-associated *E. coli* RS218 were associated with genes involved in bacterial surface decorations. Type 1 fimbriae (*fimC* and *fimI*) were significantly increased about twofold in HBMEC-associated *E. coli* RS218 (Fig. 2). Type 1 fimbriae is a known virulence factor that is involved in *E. coli* K1 association with HBMEC, and type 1 fimbrial expression is related to phase-variation [15]. In addition, the flagella-specific ATP synthase gene (*fliI*) induced nearly 6 folds (Fig. 2). FliI ATPase serves as the energy supplier for flagella exportation apparatus. Interestingly, the structural gene of flagellar *fliC* was significantly repressed in both HBMEC-associated and collagen-bound *E. coli* (Fig. 2). Flagellar is another virulence factor known to contribute to *E. coli* K1 binding to HBMEC [16].

Another interesting change in HBMEC-bound *E. coli* RS218 is related to K1 capsule. In HBMEC-bound *E. coli*, there was a significant induction of *kps* genes as well as *siaL* gene (Fig. 2). The *kps* genes were responsible for the K1 capsular polysaccharide biosynthesis [17], while *siaL* gene encoded an endo-sialidase (endo-*N*) that digested polysialic acid [18]. In contrast to these K1 capsule related changes in HBMEC-bound *E. coli*, *neuAB* genes were significantly repressed in collagen-bound *E. coli.* The expression patterns of outer membrane proteases were also uniquely changed during *E. coli* K1 interaction with HBMEC. For example, outer membrane protease gene *ompT* was significantly induced in HBMEC-bound *E. coli*, while another outer membrane protease *ompX* was significantly repressed in collagen-bound *E. coli*.

Other than bacterial surface remodeling, genes involved in amino acids and nucleotide metabolisms were significantly induced in HBMEC-associated *E. coli*. For example, in HBMEC-associated *E. coli* K1, several genes involved in acidic amino acids (aspartate, asparagine, and glutamate), threonine, and branch-chain amino acids metabolisms were induced around 2 folds (Fig. 2). Associated with these inductions, both glutamine transporter (*glnHPQ*) and arginine transporter (*artIQ*) were induced 2–4 folds (Fig. 2). Furthermore, the oligopeptide transport genes (*oppA*, *oppC*, and *oppF*) were significantly induced 2 folds (Fig. 2). Besides amino acid metabolic genes, there was a significant induction of carbamoyl phosphate biosynthesis operon *carAB*, which encoded a key enzyme shared

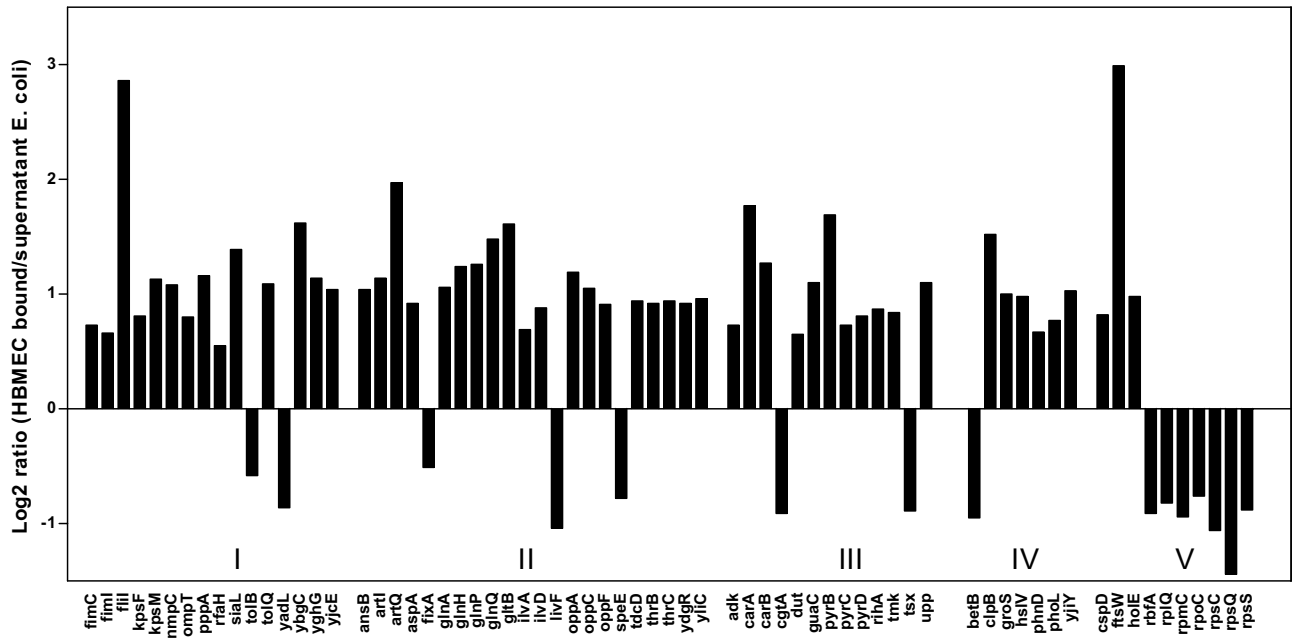

Fig. 2. Representative gene expression profiles that are specific to HBMEC-associated *E. coli* RS218. Group I are bacterial surface structures; Group II are peptide and amino acid metabolism genes; Group III are nucleotide metabolism genes; Group IV are stress-related genes; Group V are cellular function and ribosomal genes (adjusted p-value <0.005).

between the arginine and pyrimidine biosynthesis pathways. Other genes that encoded enzymes downstream carbamoyl phosphate synthetase in de novo pyrimidine ribonucleotide and deoxyribonucleotide biosynthesis (i.e., *pyrB*, *pyrC*, *pyrD*, *dut*, and *tmk*) were also induced around 2 folds (Fig. 2).

One of the most induced genes in HBMEC-associated *E. coli* K1 is cell division gene *ftsW*, which was induced 8 folds compared to the unbound *E. coli* (Fig. 2). At the same time, DNA polymerase III gene (*holE*) was induced 2 folds in the HBMEC-associated bacteria. The induction of both cell division and DNA synthesis genes implies that HBMEC-surface associated bacteria are likely to undergo the active growth. Interestingly, protein synthesis machinery including ribosomal genes (*rplQ*, *rpmC*, *rpoC*, *rpsC*, *rpsQ*, and *rpsS*) was repressed nearly 2 fold in HBMEC-associated bacteria. Interestingly, several stress genes, such as *clpB*, *groS*, *hslV*, and *phoL* were also significantly induced in HBMEC-associated *E. coli* K1.

Roles of HBMEC association-induced genes in E. coli K1 interaction with HBMEC

Expression profiling of HBMEC-associated *E. coli* RS218 revealed that bacteria experienced a drastic change when they were in close contact with host cells. However, differential expression of genes did not provide direct evidences demonstrating their contributions to *E. coli* binding to HBMEC. Previously, we have shown that the Δ*fimH* or Δ*fliI* mutants of *E. coli* RS218 were significantly defective in HBMEC binding and invasion compared to the parent strain [15,16], confirming that both type 1 fimbriae and flagella play the important roles in *E. coli* K1 binding to and invasion of HBMEC. In this study, we constructed deletion mutants for additional genes from *E. coli* RS218 using the "one-step PCR" method [12]. These genes included *frdA*, *narQ*, *clpB*, *artQ*, *carA*, *oppA*, *ompT*, *kps*, and *siaL* that were shown to be involved in anaerobic respiration, stress, peptide and amino acid nutrition, nucleotide nutrition, and cell surface remodeling. Since a quite few of unknown or hypothetical ORFs were also significantly induced in *E. coli* bound to HBMEC (Supplementary Table S1), we selected an ORF that encoded an unknown autotransporter protein (orthologue of *E. coli* CFT073 c0426) and deleted it from *E. coli* RS218 genome. Most of those genes (excepting *frdA*) were significantly induced in HBMEC-associated *E. coli*, but remained relatively unchanged in collagen-bound *E. coli*.

Compared to the HBMEC association rates of wild type *E. coli* RS218, we found that four out of ten deletion mutants, Δ*carA*, Δ*clpB*, Δ*frdA*, and Δ*ompT*, exhibited significantly decreased abilities to bind to HBMEC (Table 1). Furthermore, three of these four mutants (i.e., Δ*carA*, Δ*clpB*, and Δ*ompT*) also exhibited significantly decreased HBMEC invasion rates compared with the parental strain *E. coli* RS218, suggesting the reduced HBMEC invasion rates of these mutants most likely stem from their

Table 1
Relative HBMEC association and invasion rates of *E. coli* RS218 isogenic mutants

Gene	Functional annotation	Relative HBMEC association rate % (mean ± SD)[a]	Relative HBMEC invasion rate % (mean ± SD)[b]
frdA	Fumarate reductase, anaerobic, catalytic, and NAD/flavoprotein subunit	63 ± 10*	79 ± 17
narQ	Sensory histidine kinase in two-component regulatory system with NarP (NarL), regulates anaerobic respiratory gene expression, senses nitrate/nitrite	139 ± 36	129 ± 13
clpB	ATP-dependent protease, Hsp 100, part of multi-chaperone system with DnaK, DnaJ, and GrpE	58 ± 18*	44 ± 1*
artQ	Arginine transport protein (ABC superfamily, membrane)	123 ± 8	93 ± 15
carA	Carbamoyl phosphate synthetase, glutamine amidotransferase small subunit	18 ± 6*	28 ± 3*
oppA	Oligopeptide transport protein (ABC superfamily, peri_bind)	84 ± 28	97 ± 28
ompT	Protease VII, outer membrane protein 3b (a), putative porin	71 ± 12*	63 ± 5*
kps	K1 capsular polysaccharide biosynthesis	148 ± 29	250 ± 9
siaL	Endo-*N*-acylneuraminidase, endo-*N*	109 ± 34	109 ± 55
c0426	Unknown autotransporter protein	121 ± 3	81 ± 13

[a] HBMEC-association rate of *E. coli* RS218 was treated as 100%. *E. coli* HB101 was used as a negative control.
[b] HBMEC-invasion rate of *E. coli* RS218 wild type was treated as 100%. *E. coli* HB101 was used as a negative control.
* The isogenic mutants exhibited significantly decreased HBMEC association or invasion rates compared to the parental strain *E. coli* RS218 (p-value <0.10).

decreased binding. The HBMEC association and invasion rates of Δ*narQ*, Δ*artQ*, Δ*oppA*, Δ*kps*, Δ*siaL*, and Δ*c0426* mutants were not significantly lower from those of the wild type strain *E. coli* RS218.

Discussion

In this report, we conducted a detailed comparison of expression profiles of *E. coli* K1 strain RS218 associated or not-associated with HBMEC after 90 min co-incubation. *E. coli* K1 associated with HBMEC after 90 min incubation include bacteria both bound to HBMEC and internalized into HBMEC. However, our previous studies have shown that the majority of HBMEC-associated *E. coli* K1 belongs to those that bound to the surface of HBMEC, and HBMEC-internalized bacteria represent approximately 1% of the total HBMEC-associated bacteria [15]. Therefore, the expression profiles obtained from HBMEC-associated bacteria are most likely to represent surface-bound bacteria.

Previous studies demonstrated that *E. coli* grown under microaerobic condition were more invasive into HBMEC than those grown in aerobic condition [19]. Consistent with this result, we demonstrated that the *frdA* deletion mutant exhibited decreased HBMEC association and invasion rates. It remains, however, unclear whether the contribution of anaerobic respiration to *E. coli* K1 association with HBMEC is biologically relevant because this anaerobic lifestyle was also demonstrated with collagen-bound *E. coli*.

Based on the transcriptome data, the metabolic status of HBMEC-bound *E. coli* was drastically different from that of the bacteria that are not associated with HBMEC. HBMEC-associated *E. coli* uniquely enhanced the expression of genes involved in peptide and amino acids metabolisms. However, deletion of *oppA* and *artQ* did not affect HBMEC association and invasion rates, suggesting that these nitrogen nutrients may not be critical for *E. coli* K1 interaction with HBMEC. In contrast, deletion of *carA* significantly reduced both HBMEC association and invasion rates. The importance of nucleotide metabolic genes in *E. coli* K1 invasion of HBMEC has been demonstrated previously. For example, *purA* (encoding adenylosuccinate synthetase) was shown to contribute to *E. coli* K1 invasion of HBMEC [20].

ClpB is an ATP-dependent protease belonging to the family of heat shock proteins. Mutation of *clpB* gene in *Salmonella typhimurium* significantly decreased its ability to colonize in the alimentary tract of 3-week-old chickens [21]. The main function of ClpB is to protect bacteria from environmental stresses, suggesting that some forms of stresses might have occurred during *E. coli* interaction with HBMEC. The outer-membrane protease OmpT is an outer membrane serine protease belonging to the family of the omptins. OmpT is a virulence factor in uropathogenic *E. coli* and may participate in the degradation of antimicrobial peptides secreted by uroepithelial cells [22]. HBMEC was known to secrete antimicrobial compounds such as indoleamine 2,3-dioxygenase and cathelicidins [23–25]. It is possible that OmpT might execute similar roles in degradation of host-derived antimicrobial peptides or proteins during *E. coli* interaction with HBMEC.

Transcriptional profiling of *E. coli* K1 during its interaction with HBMEC, the constituent of the blood–brain barrier, provided us with a large amount of information to depict the global picture of *E. coli* K1 during its interaction with the relevant target tissue. Complete elucidation of these transcriptional changes will provide the additional information on the *E. coli*-HBMEC interactions, which is essential to our understanding of the pathogenesis of *E. coli* meningitis.

Acknowledgments

This work was supported by American Heart Association BGIA-0665442U (to Y.X.) and National Institutes of Health Grants RO1-NS 26310 and AI 71894 (to K.S.K.).

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bbrc.2007.10.174.

References

[1] K.S. Kim, Pathogenesis of bacterial meningitis: from bacteraemia to neuronal injury, Nat. Rev. Neurosci. 4 (2003) 376–385.
[2] J.L. Badger, C.A. Wass, K.S. Kim, Identification of *Escherichia coli* K1 genes contributing to human brain microvascular endothelial cell invasion by differential fluorescence induction, Mol. Microbiol. 36 (2000) 174–182.
[3] J.L. Badger, C.A. Wass, S.J. Weissman, K.S. Kim, Application of signature-tagged mutagenesis for identification of *Escherichia coli* K1 genes that contribute to invasion of human brain microvascular endothelial cells, Infect. Immun. 68 (2000) 5056–5061.
[4] C.A. Bloch, S.H. Huang, C.K. Rode, K.S. Kim, Mapping of noninvasion TnphoA mutations on the *Escherichia coli* O18:K1:H7 chromosome, FEMS Microbiol. Lett. 144 (1996) 171–176.
[5] K.S. Kim, H. Itabashi, P. Gemski, J. Sadoff, R.L. Warren, A.S. Cross, The K1 capsule is the critical determinant in the development of *Escherichia coli* meningitis in the rat, J. Clin. Invest. 90 (1992) 897–905.
[6] Y. Xie, K.J. Kim, K.S. Kim, Current concepts on *Escherichia coli* K1 translocation of the blood–brain barrier, FEMS Immunol. Med. Microbiol. 42 (2004) 271–279.
[7] R.P. Silver, W. Aaronson, A. Sutton, R. Schneerson, Comparative analysis of plasmids and some metabolic characteristics of *Escherichia coli* K1 from diseased and healthy individuals, Infect. Immun. 29 (1980) 200–206.
[8] M. Stins, N. Prasadarao, L. Ibric, C. Wass, P. Luckett, K. Kim, Binding characteristics of S fimbriated *Escherichia coli* to isolated brain microvascular endothelial cells, Am. J. Pathol. 145 (1994) 1228–1236.
[9] F. Di Cello, Y. Xie, M. Paul-Satyaseela, K.S. Kim, Approaches to bacterial RNA isolation and purification for microarray analysis of *Escherichia coli* K1 interaction with human brain microvascular endothelial cells, J. Clin. Microbiol. 43 (2005) 4197–4199.

[10] Y. Yao, Y. Xie, K.S. Kim, Genomic comparison of *Escherichia coli* K1 strains isolated from the cerebrospinal fluid of patients with meningitis, Infect. Immun. 74 (2006) 2196–2206.
[11] G.K. Smyth, Limma: linear models for microarray data, in: W. Huber (Ed.), Computational Biology Solutions using R and Bioconductor, Springer, New York, 2005, pp. 397–420.
[12] K.A. Datsenko, B.L. Wanner, One-step inactivation of chromosomal genes in *Escherichia coli* K-12 using PCR products, Proc. Natl. Acad. Sci. USA 97 (2000) 6640–6645.
[13] K.C. Murphy, Use of bacteriophage lambda recombination functions to promote gene replacement in *Escherichia coli*, J. Bacteriol. 180 (1998) 2063–2071.
[14] N. Prasadarao, C. Wass, J. Weiser, M. Stins, S. Huang, K. Kim, Outer membrane protein A of *Escherichia coli* contributes to invasion of brain microvascular endothelial cells, Infect. Immun. 64 (1996) 146–153.
[15] C.H. Teng, M. Cai, S. Shin, Y. Xie, K.J. Kim, N.A. Khan, F. Di Cello, K.S. Kim, *Escherichia coli* K1 RS218 interacts with human brain microvascular endothelial cells via type 1 fimbria bacteria in the fimbriated state, Infect. Immun. 73 (2005) 2923–2931.
[16] G. Parthasarathy, Y. Yao, K.S. Kim, Flagella promote *Escherichia coli* K1 association with and invasion of human brain microvascular endothelial cells, Infect. Immun. 75 (2007) 2937–2945.
[17] E. Vimr, S. Steenbergen, M. Cieslewicz, Biosynthesis of the polysialic acid capsule in *Escherichia coli* K1, J. Ind. Microbiol. 15 (1995) 352–360.
[18] E.L. Deszo, S.M. Steenbergen, D.I. Freedberg, E.R. Vimr, *Escherichia coli* K1 polysialic acid *O*-acetyltransferase gene, neuO, and the mechanism of capsule form variation involving a mobile contingency locus, Proc. Natl. Acad. Sci. USA 102 (2005) 5564–5569.
[19] J.L. Badger, K.S. Kim, Environmental growth conditions influence the ability of *Escherichia coli* K1 to invade brain microvascular endothelial cells and confer serum resistance, Infect. Immun. 66 (1998) 5692–5697.
[20] J.A. Hoffman, J.L. Badger, Y. Zhang, K.S. Kim, *Escherichia coli* K1 purA and sorC are preferentially expressed upon association with human brain microvascular endothelial cells, Microb. Pathog. 31 (2001) 69–79.
[21] A.K. Turner, M.A. Lovell, S.D. Hulme, L. Zhang-Barber, P.A. Barrow, Identification of Salmonella typhimurium genes required for colonization of the chicken alimentary tract and for virulence in newly hatched chicks, Infect. Immun. 66 (1998) 2099–2106.
[22] S. Stumpe, R. Schmid, D.L. Stephens, G. Georgiou, E.P. Bakker, Identification of OmpT as the protease that hydrolyzes the antimicrobial peptide protamine before it enters growing cells of *Escherichia coli*, J. Bacteriol. 180 (1998) 4002–4006.
[23] R. Adam, D. Russing, O. Adams, A. Ailyati, K.S. Kim, H. Schroten, W. Daubener, Role of human brain microvascular endothelial cells during central nervous system infection. Significance of indoleamine 2,3-dioxygenase in antimicrobial defence and immunoregulation, Thromb. Haemost. 94 (2005) 341–346.
[24] H. Schroten, B. Spors, C. Hucke, M. Stins, K.S. Kim, R. Adam, W. Daubener, Potential role of human brain microvascular endothelial cells in the pathogenesis of brain abscess: inhibition of *Staphylococcus aureus* by activation of indoleamine 2,3-dioxygenase, Neuropediatrics 32 (2001) 206–210.
[25] P. Bergman, L. Johansson, H. Wan, A. Jones, R.L. Gallo, G.H. Gudmundsson, T. Hokfelt, A.B. Jonsson, B. Agerberth, Induction of the antimicrobial peptide CRAMP in the blood–brain barrier and meninges after meningococcal infection, Infect. Immun. 74 (2006) 6982–6991.