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International Journal of Medical Microbiology 295 (2005) 463–470
IJMM
www.elsevier.de/ijmm

REVIEW

Invasion processes of pathogenic *Escherichia coli*

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Abstract

Pathogenic *Escherichia coli* causes extraintestinal infections such as urinary tract infection and meningitis, which are prevalent and associated with considerable morbidity. Previous investigations have identified common strategies evolved by pathogenic *E. coli* to exploit host cell function and cause extraintestinal infections, which include the invasion into non-phagocytic eukaryotic cells such as epithelial and endothelial cells and associated host cell actin cytoskeletal rearrangements. However, the mechanisms involved in pathogenic *E. coli* invasion of eukaryotic cells are shown to differ depending upon types of host tissues and microbial determinants. In this mini-review, invasion processes of pathogenic *E. coli* are discussed using *E. coli* K1 invasion of human brain microvascular endothelial cells (HBMEC) as a paradigm. *E. coli* K1 is the most common Gram-negative organism causing neonatal meningitis, and *E. coli* invasion of HBMEC is shown to be a prerequisite for *E. coli* traversal of the blood-brain barrier in vivo. Previous studies have demonstrated that *E. coli* translocation of the blood-brain barrier is the result of specific *E. coli*–host interactions including specific signal transduction pathways and modulation of endocytic pathways. Recent studies using functional genomics have identified additional microbial determinants contributing to *E. coli* K1 invasion of HBMEC. Complete understanding of microbial-host interactions that are involved in *E. coli* K1 invasion of HBMEC should help in the development of new strategies to prevent *E. coli* meningitis.

Keywords: *E. coli*; Binding; Invasion; Human brain microvascular endothelial cells; Blood-brain barrier; Meningitis

Contents

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doi:10.1016/j.ijmm.2005.07.004

Introduction

The most distressing aspect of neonatal Gram-negative bacillary meningitis is limited improvement in the mortality and morbidity attributable to advances in antimicrobial chemotherapy and supportive care (Gladstone et al., 1990; Unhanand et al., 1993; Anonymous, 1996, 1998; Klinger et al., 2000; Stevens et al., 2003). Inadequate knowledge of the pathogenesis has contributed to this high mortality and morbidity. The most common Gram-negative organism causing meningitis during the neonatal period is *Escherichia coli*. Most cases of *E. coli* meningitis develop as a result of hematogenous spread (Dietzman et al., 1974; Kim, 2003), but it is not completely understood how circulating *E. coli* traverses the blood-brain barrier.

We have established both in vitro and in vivo models of the blood-brain barrier using human brain microvascular endothelial cells (HBMEC) and experimental hematogenous meningitis in neonatal rats, respectively. Using these in vitro and in vivo models of the blood-brain barrier, we have shown that *E. coli* invasion of HBMEC is a prerequisite for its traversal of the blood-brain barrier in vivo (Kim, 2000, 2001, 2002, 2003; Huang et al., 1995, 1999, 2001; Wang et al., 1999; Hoffman et al., 2000; Khan et al., 2002; Wang and Kim, 2002).

Pathogenic bacteria have exploited varied strategies to penetrate their host cells such as non-professional phagocytes. Microbial internalization into non-professional phagocytic cells such as epithelial and endothelial cells are shown to occur mainly via two different mechanisms involving the host cell actin cytoskeletal rearrangements, such as a zipper mechanism involving the formation of cell protrusions in contact with the pathogens and a trigger mechanism involving the formation of membrane ruffling around the pathogens (Cossart and Sansonetti, 2004). Our studies revealed that *E. coli* K1 internalizes into HBMEC via a zipper-like mechanism (Kim, 2003; Nemani et al., 1999). *E. coli* invasion of HBMEC requires rearrangements of the host cell actin cytoskeleton and specific signal transduction pathways (Kim, 2003; Nemani et al., 1999; Chung et al., 2003; Reddy et al., 2000a, b; Das et al., 2001; Khan et al., 2003).

Of interest, HBMEC actin cytoskeleton rearrangements are shown to be a prerequisite for HBMEC invasion by meningitis-causing bacteria such as *E. coli*, group B streptococci and *Listeria monocytogenes*, but the signaling mechanisms that are involved in HBMEC invasion are shown to differ between *E. coli* K1 and other bacteria such as group B streptococci and *L. monocytogenes* (Kim, 2001, 2002, 2003). These findings suggest that the mechanisms involved in the same phenotype may differ depending upon types of organisms. This concept is also relevant to pathogenic *E. coli* including the source of isolates such as urinary tract and meningitis isolates and types of host tissues such as uroepithelial cells and endothelial cells.

Once *E. coli* K1 is internalized into HBMEC, *E. coli* K1 resides inside the membrane-bound vacuoles and modulates intracellular trafficking to avoid lysosomal fusion (Kim et al., 2003). Blockade of lysosomal fusion has been evolved to avoid degeneration by lysosomal enzymes, which is an important determinant of pathogenesis for a variety of organisms (Kim et al., 2003; Roy et al., 2004) including *E. coli* K1 and meningitis. This mini-review summarizes our current understanding of invasion processes by pathogenic *E. coli* using *E. coli* K1 invasion of HBMEC as a paradigm.

E. coli determinants contributing to invasion of HBMEC

Our previous studies using Tn*phoA* mutagenesis, signature-tagged mutagenesis and differential fluorescence induction with screening of *gfp* fusion library identified several *E. coli* K1 determinants contributing to invasion of HBMEC, which include the Ibe proteins, AslA, TraJ and cytotoxic necrotizing factor 1 (CNF1) (Huang et al., 1995, 1999, 2001; Wang et al., 1999; Hoffman et al., 2000; Badger et al., 2000a, b). The roles of these *E. coli* K1 determinants in HBMEC invasion were verified by deletion and complementation experiments. For example, isogenic deletion mutants were less invasive in HBMEC in vitro and less able to cross the blood-brain barrier in vivo, and their invasive abilities were restored by complementation in trans with individual genes. Of interest, recombinant Ibe proteins inhibited *E. coli* K1 invasion of HBMEC (Huang et al., 1999, 2001; Wang et al., 1999), suggesting that Ibe proteins contribute to HBMEC invasion by ligand–receptor interactions. This concept was supported by our demonstration of a 45-kDa HBMEC surface protein interactive with IbeA, and a polyclonal antibody raised against this receptor protein inhibited *E. coli* K1 invasion of HBMEC (Prasadarao et al., 1999).

CNF1 is a bacterial virulence factor associated with pathogenic *E. coli* strains causing urinary tract infection and meningitis (Khan et al., 2002; Boquet, 2001). CNF1 is an AB-type toxin, composed of the N-terminal cell binding domain and the C-terminal catalytic domain possessing a deaminase activity through the site-specific deamination of a Gln residue to Glu. CNF1 has been shown to activate Rho GTPases such as Rho, Rac and Cdc 42. CNF1 has been suggested to be internalized via receptor-mediated endocytosis upon binding to a cell surface receptor by a clathrin-independent, caveolin-independent mechanism (Contamin et al., 2000). Once endocytosed, CNF1-containing vesicles are delivered to