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Original article

Mitogen-activated protein kinases are required for effective infection of human choroid plexus epithelial cells by *Listeria monocytogenes*

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Abstract

Listeria monocytogenes, a Gram-positive bacterium, can cause meningitis after invading the human central nervous system. The blood-cerebrospinal fluid barrier (BCSFB), located at the epithelium of the choroid plexus, is a possible entry site for *L. monocytogenes* into the brain, and *in vitro L. monocytogenes* invades human choroid plexus epithelial papilloma (HIBCPP) cells. Although host cell signal transduction subsequent to infection by *L. monocytogenes* has been investigated, the role of mitogen-activated protein kinases (MAPK) is not clarified yet. We show that infection with *L. monocytogenes* causes activation of the MAPKs Erk1/2 and p38 preferentially when bacteria are added to the physiologically more relevant basolateral side of HIBCPP cells. Deletion of the listerial virulence factors Internalin (InlA) and InlB reduces MAPK activation. Whereas inhibition of either Erk1/2 or p38 signaling significantly attenuates infection of HIBCPP cells with *L. monocytogenes*, simultaneous inhibition of both MAPK pathways shows an additive effect, and Erk1/2 and p38 are involved in regulation of cytokine and chemokine expression following infection. Blocking of endocytosis with the synthetic dynamin inhibitor dynasore strongly abrogates infection of HIBCPP cells with *L. monocytogenes*. Concurrent inhibition of MAPK signaling further reduces infection, suggesting MAPKs mediate infection with *L. monocytogenes* during inhibition of dynamin-mediated endocytosis.

Keywords: Human; Choroid plexus; Blood-cerebrospinal fluid barrier; *Listeria monocytogenes*; Mitogen-activated protein kinase; Dynamin

1. Introduction

The Gram-positive bacterium *Listeria monocytogenes* (*L. monocytogenes*) is a facultative intracellular pathogen that can invade into and multiply inside of host cells. Infection with *L. monocytogenes* in humans can cause listeriosis, a disease that can lead to central nervous system (CNS) infections in neonates, elderly and immunocompromised individuals. Following entry into the host across the gastrointestinal epithelium, the bacteria disseminate via the blood and the lymph stream and establish themselves in the spleen and the liver. From here the bacteria can be re-released into the blood stream and subsequently cross the fetoplacental barrier in pregnant women, as well as the blood–brain barriers for entry into the CNS, where they can cause meningitis and meningoencephalitis [1,2].

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Invasion of *L. monocytogenes* into non-phagocytic cells is helped by an arsenal of virulence factors, including the bacterial surface proteins Internalin (InlA) and InlB. InlA and InlB interact with specific receptors on the host cell surfaces, which are the adherens junction protein E-cadherin (Ecad) for InlA and the receptor tyrosine kinase Met for InlB, respectively. Following binding to these receptors, the bacteria hijack host cell endocytosis mechanisms to enter target cells and can subsequently spread from cell to cell [3].

Interaction of Ecad and Met with InlA and InlB, respectively, leads to ubiquitination of their receptors and subsequent recruitment of the clathrin-mediated endocytosis machinery, which then causes polymerization of the actin cytoskeleton. A critical role during this process is particularly played by the GTPase dynamin that can recruit further factors initiating two waves of actin rearrangements, which mediate entry of the bacteria inside of vacuoles [3–5]. Once in the cell, *L. monocytogenes* can escape the vacuole by lysis, a process which involves the bacterial virulence factor listeriolysin O (LLO), a cholesterol-dependent cytolysin, and bacterial phospholipases. Subsequently, *L. monocytogenes* multiplies in the cytoplasm. Finally, with the help of actin comet tails the pathogens move through the cell and enter neighboring cells via membrane protrusions [3,6,7].

One of several intracellular host cell signaling cascades that can be manipulated by bacterial pathogens to their advantage is the mitogen-activated protein kinase (MAPK) pathway [8]. The best described members of the MAPK family are the extracellular signal-regulated kinases (ERK) 1 and 2, as well as members of the p38 and the JUN N-terminal kinases (JNK), which are all activated by phosphorylation [9]. Activation of MAPKs has been described to be involved during infection of host cells by *L. monocytogenes*. Experiments with epithelial cells and murine macrophage-like cells indicated a role of MAPKs during uptake of *L. monocytogenes* [10–12], and inhibition of MAPKs attenuated listerial invasion into mouse trophoblast giant cells [13]. Activation of MAPKs is at least partly mediated by LLO [14,15]. Noteworthy, binding of InlB to Met leads to phosphorylation of Erk1/2 [16,17]. Furthermore, activation of MAPKs during infection by *L. monocytogenes* induces production of cytokines and chemokines in endothelial cells [18,19].

For brain entry, *L. monocytogenes* needs to cross either the blood–brain barrier (BBB) or the blood-cerebrospinal fluid (CSF) barrier (BCSFB), which is located at the choroid plexus (CP). At the CP the epithelial cells are interconnected by tight junctions, display polarity, and are the morphological correlate of the BCSFB [20]. *In vivo* studies have suggested that *L. monocytogenes* invades the CP, and the bacteria are detected in the CSF during infection [21–23]. We have developed an *in vitro* model of the BCSFB based on human CP papilloma (HIBCPP) cells [24,25]. In this model, InlA and InlB are interdependently required to mediate invasion of *L. monocytogenes* specifically from the basolateral cell side in a polar fashion. The bacteria are detected in vacuoles, as well as free in the cytoplasm with attached actin comet tails, indicating that they follow their regular life cycle in HIBCPP cells [26].

The role of MAPKs during infection and invasion of *L. monocytogenes* at the BCSFB is not clarified yet. Here, we use HIBCPP cells as model of the CP epithelium to demonstrate that *L. monocytogenes* activates Erk1/2 and p38 *in vitro* preferentially when added to the basolateral side. Both MAPKs are required for maximum infection of HIBCPP cells by the bacteria, and simultaneous inhibition of both MAPK pathways leads to additive repression of infection. Activation of Erk1/2 and p38 is furthermore involved in regulation of cytokine and chemokine expression following bacterial challenge. Infection with *L. monocytogenes* is strongly dependent on the activity of dynamin and activated MAPK signaling might mediate infection during blocking of dynamin-dependent endocytosis.

2. Material and methods

2.1. Bacterial strains and growth conditions

L. monocytogenes EGDe was stored in stock cultures at −80 °C in brain-heart infusion (BHI) containing 10% glycerol. Deletion of InlA and/or InlB has been described [27,28]. For invasion experiments, 100 µl of the stock cultures were inoculated in 10 ml BHI and grown at 37 °C in a water bath for 6 h under moderate agitation to mid-logarithmic phase. Bacteria were then washed with phenol-red free DMEM/F12 and adjusted to an optical density at 600 nm (OD_{600}) of 0.65. This solution was further diluted in DMEM/F12 supplemented with 1% fetal calf serum (FCS) and 5 µg/ml insulin to a 1:3 ratio in case for the wild type, ΔInlA and ΔInlAB mutant strains as well as a 1:6 ratio for bacteria lacking InlB. These suspensions contained approximately 1×10^8 colony forming units (CFU) per ml. To exclude an influence of the inhibitors on bacterial growth, the latter was monitored in presence and absence of inhibitors, showing no effect.

2.2. Standard and inverted filter insert culture of HIBCPP cells and TEER measurements

HIBCPP cells were cultured in DMEM/F12 supplemented with 10% fetal calf serum (FCS), 5 µg/ml insulin, 100 U/ml penicillin and 100 µg/ml streptomycin. For experiments, cells grown in the standard and the inverted cell culture insert system were used as previously described [24]. To determine barrier function and confluency of the cell layer on the filter inserts, the transepithelial electrical resistance (TEER) was measured with an epithelial voltohmmeter, model Millicell-ERS STX-2 electrode system (Millipore, Schwalbach, Germany).

2.3. Determination of FITC-inulin flux

For determination of the paracellular permeability of HIBCPP cells grown on filter supports, a FITC-inulin tracer