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Original article

Expression of LAP, a SecA2-dependent secretory protein, is induced under anaerobic environment

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Abstract

Listeria adhesion protein (LAP), an alcohol acetaldehyde dehydrogenase homolog (*lmo1634*) in *Listeria monocytogenes*, promotes bacterial adhesion to intestinal epithelial cells *in vitro*. Investigation of the effect of anaerobiosis, an intrinsic gastrointestinal condition, on LAP expression and LAP-mediated infection should elucidate its significance during intestinal infection. The influence of anaerobiosis on LAP expression was determined by growing *L. monocytogenes* wild type (WT), and *lap*-deficient (KB208) and -complemented (CKB208) strains anaerobically and monitoring LAP in secreted, cell wall, and whole-cell protein fractions. The effect of anaerobiosis on LAP-mediated infection was evaluated in cell culture adhesion assays and mouse infection models. Additionally, the role of secretory system SecA2 in LAP secretion was investigated. Anaerobic growth induced significant increases in level of *lap* transcript and protein secretion, and secretion was SecA2-dependent. Anaerobiosis facilitated greater LAP-mediated adhesion of *L. monocytogenes* to cultured intestinal cells. Oral administration of WT, KB208 and CKB208 to mice confirmed that LAP is essential for full virulence, and anaerobically-grown WT exhibited greater translocation to liver and spleen relative to aerobically-grown organisms. LAP, a SecA2-dependent secreted virulence factor, plays an important role during intestinal infection, particularly when *L. monocytogenes* is subjected to an anaerobic environment.

Keywords: *Listeria monocytogenes*; LAP; Alcohol acetaldehyde dehydrogenase; Anaerobic environment; SecA2

1. Introduction

Listeria monocytogenes is an opportunistic, intracellular human pathogen that causes gastrointestinal listeriosis in healthy individuals and invasive, systemic listeriosis in immunosuppressed hosts. Listeriosis is contracted via consumption of contaminated foods or transplacental transmission to fetuses [1]. While cellular mechanisms of infection and host immune response are well documented [2], the gastrointestinal phase of infection remains largely unexplored.

A potential entry portal for foodborne pathogens, the host gastrointestinal tract (GIT) is designed to limit invasive infection. The stomach has low pH and the small intestine has high osmolarity, bile salts, reduced oxygen, mucus, and commensal microbiota (10^{4-8} CFU/g). The distal small intestine and colon contain high levels of natural microbiota (10^{12-13} CFU/g of content), large amounts of mucus, antimicrobial bacterial metabolic byproducts, and are anaerobic [3,4].

A successful enteric pathogen, *L. monocytogenes* survives GIT transit and regulates its virulence factor expression to enhance pathogenicity *in vivo* [3]. Expression of glutamate decarboxylase promotes acid resistance, and osmolyte uptake system, OpuC, confers osmotolerance during oral infection [3]. Resistance to bile salts involves multiple genes, including bile tolerance (*btlA*), a bile exclusion system (*bilE*) and bile salt hydrolase (*bsh*) [5,6]. Regulation of virulence genes in

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L. monocytogenes is a complex process, largely co-regulated by sigma B (σ^B or sigB) and PrfA, which promotes adaptation to extrinsic and intrinsic host factors [2,7].

In vivo, *L. monocytogenes* traverses the intestinal barrier by interacting with and invading phagocytic 'M' cells and non-phagocytic epithelial cells [1,8,9]. Our *in vitro* studies show that *Listeria* preferentially binds and invades epithelial cells originating from ileum and cecum [10]. Multiple bacterial factors are involved in epithelial adhesion and invasion [2]; fibronectin binding protein (FbpA) interacts with host fibronectin, internalin A with E-cadherin and internalin B with c-Met, gC1q-R, and proteoglycan receptors. Invasion protein Vip interacts with host Gp96 during intestinal infection [11], promoting internalin-independent *L. monocytogenes* invasion.

Listeria adhesion protein (LAP, *lmo1634*) [12] binds to host Hsp60 on the epithelial surface [13] during intestinal infection [10]. While Hsp60 is a mitochondrial chaperone, its plasma membrane expression has been observed in a variety of cell types, including intestinal epithelial cells [14].

LAP is primarily a cytosolic protein with a small percent of surface localization [10] during aerobic growth in laboratory media. Food-associated stressors including a nutrient-limiting environment, low glucose concentrations (<1.6 g/L) [15], and high temperature (42 °C) induce LAP expression [16]. On the basis of these observations, we hypothesized that LAP expression may be affected by conditions characteristic of the GIT, such as an anaerobic environment. Others demonstrated that anaerobiosis increased *L. monocytogenes* infectivity in cultured cells and in animals; however, involvement of specific virulence factors was not determined [17]. Here we investigated the effect of anaerobiosis on LAP expression, LAP-mediated adhesion, and pathogenesis in mice. A mechanism for LAP surface translocation and secretion was determined using an SecA2-deficient mutant strain.

2. Materials and methods

2.1. Bacterial strains, growth conditions and antibodies

L. monocytogenes F4244 (WT, serovar 4b), isogenic *lap*-deficient mutant KB208 (*lap*$^-$), *lap*-complemented CKB208 (*lap*$^+$) [18], *secA2* deletion mutant AKB-103 (Δ*secA2*) and AKB-104, a complementation of Δ*secA2* (*secA2*$^+$) were used for anaerobic LAP expression studies. All *L. monocytogenes* strains were grown in brain heart infusion broth (BHI; Difco Laboratories) at 37 °C with antibiotics, unless indicated otherwise. *lap*$^-$ was grown with erythromycin (5 μg/ml) at 42 °C, *lap*$^+$ with erythromycin (5 μg/ml) and chloramphenicol (5 μg/ml) at 37 °C and *secA2*$^+$ with erythromycin (10 μg/ml). Growth curves were determined for all strains under aerobic and anaerobic conditions at 37 °C (Fig. 1). Antibodies to LAP (EM-H7) and NamA, a 66-kDa *N*-acetylmuramidase (MAb-C11E9; EM-7G1) from our laboratory were used for immunoblots and immunoabsorption experiments.

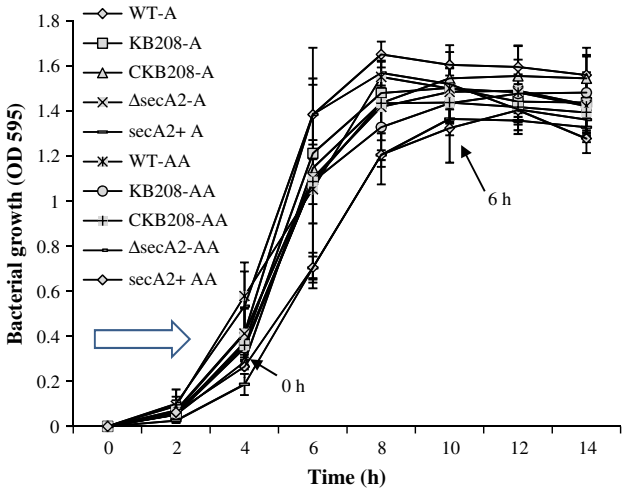

Fig. 1. Growth curves of *L. monocytogenes* WT, KB208 (*lap*$^-$), CKB208 (*lap*$^+$), AKB-103 (Δ*secA2*) and AKB-104 (*secA2*$^+$) in aerobic (solid symbols) and anaerobic (open symbols) environments at 37 °C. Open arrow indicates when the culture was transferred to anaerobic conditions.

2.2. Construction of in-frame Δ secA2 L. monocytogenes mutant using splice-by-overextension (SOE) PCR, and complementation of Δ secA2 mutant

A modification of the SOE method [19] was used to generate a 5′–3′ in-frame deletion of *secA2* ORF from codons 32 to 774. Oligonucleotide primers SecA2USF and SecA2USR generated a 403 bp product at the 5′ end of the *secA2* locus. A 400 bp 3′ product was amplified using primers SecA2DSF and SecA2DSR. The 5′ and 3′ products were combined in equimolar concentrations in a ligation reaction to yield the 803 bp 5′–3′ SOE fragment. This product was digested with BamH1 and EcoR1, cloned into pGMTeasy (Promega, Madison, WI), and subcloned into temperature-sensitive pKSV7 shuttle vector (Provided by D. Portnoy). Electrocompetent *L. monocytogenes* F4244 WT were transformed with the construct and subjected to temperature-dependent allelic exchange. In-frame deletion of *secA2* was confirmed by PCR using primers SecA2USF and SecA2DSR.

To complement the Δ*secA2* mutant with the *secA2* gene, oligonucleotide primers SecA2FSma and SecA2RPst (Table 1) were used to amplify the entire *secA2* ORF from *L. monocytogenes* F4244 genomic DNA. Amplified *secA2* was cloned via SmaI and PstI sites into pGMTeasy, and subcloned into pNF8 [20]. Electrocompetent *L. monocytogenes* Δ*secA2* were transformed with pNF8-*secA2* and positive transformants were selected by plating on erythromycin.

2.3. Exposure to anaerobic growth conditions

Fresh cultures were inoculated (1% inoculum) and incubated at 37 °C in a shaking (150 rpm) incubator (New Brunswick, Edison, NJ) until reaching $OD_{595} = 0.4$ (~4 h). Cultures were transferred to anaerobic jars with a hydrogen–carbon dioxide atmosphere produced by a gas-generating system (Becton Dickinson, Cockeysville, MD). Aerobic