Basolateral Invasion and Trafficking of *Campylobacter jejuni* in Polarized Epithelial Cells

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Abstract

Campylobacter jejuni is a major cause of bacterial diarrheal disease. Most enteropathogenic bacteria including *C. jejuni* can invade cultured eukaryotic cells via an actin- and/or microtubule-dependent and an energy-consuming uptake process. Recently, we identified a novel highly efficient *C. jejuni* invasion pathway that involves bacterial migration into the subcellular space of non-polarized epithelial cells (termed subvasion) followed by invasion from the cell basis. Here we report cellular requirements of this entry mechanism and the subsequent intracellular trafficking route of *C. jejuni* in polarized islands of Caco-2 intestinal epithelial cells. Advanced microscopy on infected cells revealed that *C. jejuni* invades the polarized intestinal cells via the subcellular invasion pathway. Remarkably, invasion was not blocked by the inhibitors of microtubule dynamics colchicine or paclitaxel, and was even enhanced after disruption of host cell actin filaments by cytochalasin D. Invasion also continued after dinitrophenol-induced cellular depletion of ATP, whereas this compound effectively inhibited the uptake of invasive *Escherichia coli*. Confocal microscopy demonstrated that intracellular *C. jejuni* resided in membrane-bound CD63-positive cellular compartments for up to 24 h. Establishment of a novel luciferase reporter-based bacterial viability assay, developed to overcome the limitations of the classical bacterial recovery assay, demonstrated that a subset of *C. jejuni* survived intracellularly for up to 48 h. Taken together, our results indicate that *C. jejuni* is able to actively invade polarized intestinal epithelial cells via a novel actin- and microtubule-independent mechanism and remains metabolically active in the intracellular niche for up to 48 hours.

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Introduction

Campylobacter is the most common cause of bacterial diarrheal disease worldwide [1]. It is estimated that each year up to 1% of the western population is infected with *Campylobacter* [2]. *Campylobacter jejuni* (*C. jejuni*) is the most prominent cause of human infections. Major infection sources are contaminated chicken and surface water. *C. jejuni* displays commensal behavior in chicken. The molecular basis of the difference in pathogenicity of *C. jejuni* in human and chicken still remains to be resolved. In the human intestine, *C. jejuni* penetrates the mucus and colonizes the intestinal crypts in a very efficient manner [3]. The crypts seem to be an optimal growth environment for *C. jejuni* [4]. Several studies suggest that after colonization, *C. jejuni* can cross the mucosal barrier and invade intestinal cells [5–8]. The exact mechanism(s) of invasion and the intracellular processing of the bacteria are not well understood.

Experimental studies using cell culture models indicate that *C. jejuni* can enter cells via different routes. Both actin-dependent and microtubule-dependent uptake into eukaryotic cells have been reported [7–11]. The uptake process may require cellular factors such as caveolin-1 and the small Rho GTPases Rac1 and Cdc42, but not dynamin [12–14]. The reports of different uptake requirements suggest that *C. jejuni* has evolved multiple mechanisms to gain access to eukaryotic cells, albeit with variably efficiency [8,15]. One of the most effective invasion pathways resulting in nearly 100% of bacterial uptake at low inocula, involves the subvasion entry pathway. This mechanism involves migration of *C. jejuni* underneath cultured cells, followed by bacterial invasion from the basal cell side instead of the apical side [17]. The sequence of events that drive this uptake process remains to be resolved.

Once inside the eukaryotic cells, *C. jejuni* is generally assumed to reside within a membrane-bound compartment. Both localization in endolysosomal compartments as well as in so-called *Campylobacter* containing vacuoles (CCV) have been reported [14]. CCV are supposed to be a special compartment specifically induced by *C. jejuni*, reminiscent of *Salmonella* that creates its own vacuole *Salmonella* containing vacuole SCV (for review: see [16]). Whether *C. jejuni* survives inside epithelial cells is still under investigation [14,17]. Intracellular survival may vary dependent on the nature of the *C. jejuni* containing compartment. Furthermore, the procedure to recover the intracellular *C. jejuni* may influence bacterial survival assay results [14,17,18].

The present study was designed to determine the unknown molecular events that are at the basis of the recently discovered *C. jejuni* subvasion entry route and to determine the trafficking and survival of *C. jejuni* after use of this infection pathway. Experiments were performed using polarized Caco-2 intestinal epithelial cells as a model system. A novel luciferase reporter system was developed to determine intracellular survival without the need of the debated