# *Campylobacter* Virulence Factors and Molecular Host–Pathogen Interactions

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## Contents

**Abstract** *Campylobacter jejuni* and *Campylobacter coli* can be frequently isolated from poultry and poultry-derived products, and in combination these two species

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cause a large portion of human bacterial gastroenteritis cases. While birds are typically colonized by these *Campylobacter* species without clinical symptoms, in humans they cause (foodborne) infections at high frequencies, estimated to cost billions of dollars worldwide every year. The clinical outcome of *Campylobacter* infections comprises malaise, diarrhea, abdominal pain and fever. Symptoms may continue for up to two weeks and are generally self-limiting, though occasionally the disease can be more severe or result in post-infection sequelae. The virulence properties of these pathogens have been best-characterized for *C. jejuni,* and their actions are reviewed here. Various virulence-associated bacterial determinants include the flagellum, numerous flagellar secreted factors, protein adhesins, cytolethal distending toxin (CDT), lipooligosaccharide (LOS), serine protease HtrA and others. These factors are involved in several pathogenicity-linked properties that can be divided into bacterial chemotaxis, motility, attachment, invasion, survival, cellular transmigration and spread to deeper tissue. All of these steps require intimate interactions between bacteria and host cells (including immune cells), enabled by the collection of bacterial and host factors that have already been identified. The assortment of pathogenicity-associated factors now recognized for *C. jejuni*, their function and the proposed host cell factors that are involved in crucial steps leading to disease are discussed in detail.

# 1   Introduction

Zoonotic infections by bacterial, viral and parasitic microbes represent a significant health burden to humans (Cunningham et al. 2017; Logue et al. 2017; Plowright et al. 2017). Among these, a number of important foodborne pathogens are responsible for high degrees of morbidity and mortality worldwide. Examples from the bacterial kingdom include *Campylobacter* species, which are often detected in the natural environment such as water surface habitats as well as in the gastrointestinal tract of certain birds and mammals, where they colonize asymptomatically as commensals (Young et al. 2007; Burnham and Hendrixson 2018). In addition, the presence of *Campylobacter* species, in particular *C. jejuni* and *C. coli*, in poultry flocks and other farm animals such as dairy cows provides a high zoonotic potential. By means of multilocus sequence typing (MLST), it was demonstrated that certain genetic *C. jejuni* variants can better survive in the environment than others and are frequently found in natural soil and water reservoirs, especially during the warm summer seasons (Epps et al. 2013; Bronowski et al. 2014). From such sources, the bacteria can transfer to new hosts, *e.g.,* to poultry flocks through rodents, flies or direct contact (Jorgensen et al. 2011; Bronowski et al. 2014). Even though *Campylobacter* spp. represent fastidious microaerophilic bacteria, they are well adapted to persistence in natural ecosystems, for instance through biofilm formation, aerotolerance mechanisms and starvation strategies (Gölz et al. 2012; Tram et al. 2020). Multiple surveys have demonstrated that the majority of commercial poultry flocks become colonized with *C. jejuni* or *C. coli* within about 2–4 weeks after hatching (Potturi-Venkata et al.

2007; van Gerwe et al. 2009). This colonization of chicks most frequently proceeds through horizontal transfer from the environment, rather than by vertical transmission from mother hens. Cross-transmission from other *Campylobacter*-positive flocks present on the same farm or from previous flocks can be prevented by strict hygiene procedures and fumigation (Herman et al. 2003; Wedderkopp et al. 2003; Bronowski et al. 2014). Taken together, although we now have a better understanding of the transmission routes by which *Campylobacter* enters the farm environment, it appears that more studies are required to find ways to effectively combat this pathogen on farms (see also Chaps. 4 and 5 of this book).

The major transmission route of *C. jejuni* (unless specifically stated otherwise, all reference to *C. jejuni* in this chapter also applies to *C. coli*) to humans mostly proceeds via the handling and consumption of contaminated poultry meat, raw milk, cross-contamination to other food products and, less frequently, by contact with freshwater or consumption of well water (Kaakoush et al. 2015). Occasionally, close contact to infected animals such as pets, particularly young dogs with diarrhea, can be sources of infection by *C. jejuni* (Campagnolo et al. 2018; Bronowski et al. 2014). Thus, the main infection route toward humans occurs by a fecal-to-oral pathway. Nevertheless, human-to-human spread is relatively uncommon. However, upon ingestion *Campylobacter* enters the gastrointestinal tract and colonizes the jejunal mucosa of the intestine by successfully competing with the intestinal microbiota (Masanta et al. 2013). The overall prevalence of *C. jejuni* infections is very high and represents a major fraction of all bacteria-caused gastroenteritis cases: It was estimated to account for about 400–500 million human incidences across the entire planet annually (Friedman et al. 2000). Infection by *C. jejuni* can lead to watery or bloody diarrheal disease, which can vary from non-inflammatory and self-limiting to a severe and inflammatory nature, resulting in significant medical and socioeconomic consequences (Nachamkin et al. 2008; Oyarzabal and Backert 2012). In a small subset of persons, the infection can be accompanied by more serious complications such as bacteraemia, or may result in the development of reactive arthritis and Reiter's syndrome or the neurological sequelae Guillain–Barré and Miller Fisher syndrome (Smith 2002; Yuki and Koga 2006). The rate of human infections by zoonotic foodborne *C. jejuni* has been progressively growing over the years, which creates a significant public health burden worldwide (Kaakoush et al. 2015).

Genetic typing tools and whole-genome sequencing have clearly demonstrated that *C. jejuni* isolates obtained from chicken meat can result in human campylobacteriosis (see Chap. 3 of this book). Nevertheless, the collective genetic diversity among *Campylobacter* isolates suggests that other transmission routes also exist, and that highly mutable sequences in multiple genetic loci across the chromosome appear to have an important function for bacterial adaptation in a new host (Sheppard and Maiden 2015). For example, comparative genomics and phenotypic analyses identified the emergence of specific *C. jejuni* lineages to become cattle specialists, which coincided with the enormous rise in the global cattle population in recent decades (Mourkas et al. 2020). Genome sequencing and other approaches have demonstrated the presence of various virulence factors, some of which have been well characterized in recent years. Once the bacteria have reached the human intestine, *C. jejuni*

has been shown to interact with the gut epithelium as well as with immune cells (as summarized in a model in Fig. 1). A major difference between the infection of chicken and human hosts is the markedly higher capability of *C. jejuni* to invade human vs. avian epithelial cells (Young et al. 2007; Ó Cróinín and Backert 2012; Burnham and Hendrixson 2018). These findings imply that *C. jejuni* adhesion to and invasion into the epithelium is most likely associated with disease outcome. Consequently, it can be assumed that pinpointing factors involved in bacteria-host interactions are crucial to understand *C. jejuni* pathogenesis and for the development of new antimicrobial therapies. This chapter reviews in detail the infection strategy by *C. jejuni* toward the human host and the interplay of bacterial factors with epithelial as well as immune cells, which is important for the development of gut disease.
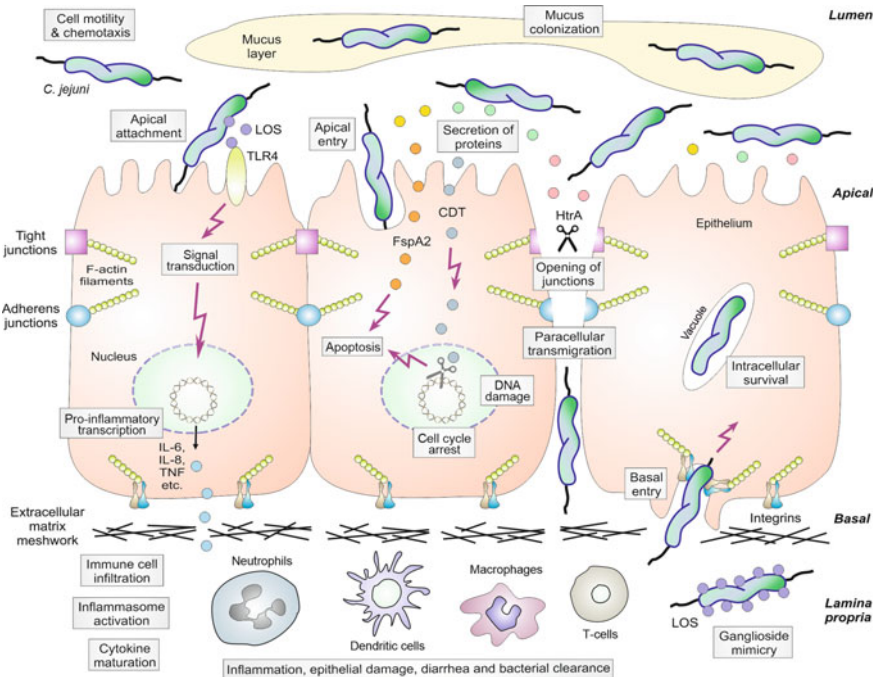


**Fig. 1   Model for crucial steps and *C. jejuni* mechanisms during infection in the human intestine**. The intestinal epithelium functions as a tight physical barrier and serves as sensor of microbial infections such as *C. jejuni*. Various indicated surface-exposed and secreted bacterial factors are proposed to enable colonization of the mucus layer, adhere to epithelial cells, open the tight and adherens junctions, allow cell invasion as well as trafficking and survival in intracellular vacuoles. In addition, factors produced by *C. jejuni* can trigger nuclear responses such as cell cycle arrest, DNA damage, apoptosis and pro-inflammatory cytokine production. The latter leads to the infiltration of various immune cell types to the sites of infection. Production of reactive oxygen species and other anti-bacterial responses enhance cell damage, leading to campylobacteriosis and eventually to bacterial clearance. Furthermore, modified lipooligosaccharide (LOS) structures of *C. jejuni* mimic human gangliosides, which can lead to auto-antibody production and neural disorders in some patients

## 2 Bacterial Virulence Factors and Epithelial Cell Responses

### 2.1 *Specialized Metabolism and Enteric Life Style*

It is well established that *C. jejuni* does not utilize sugar metabolites as a carbon source, which was shown to be due to absence of the glycolytic enzyme phosphofructokinase (Parkhill et al. 2000; Velayudhan and Kelly 2002). Instead, *C. jejuni* growth depends on the presence of single amino acids or keto acids, either supplied by the host or by the residual gut microbiota (Lee and Newell 2006; Hofreuter 2014). The amino acids present in the chicken gut were quantified, and the most abundant ones were those that *C. jejuni* depends on for its metabolism, demonstrating its adaptation to this host (Parsons et al. 1983). Early *C. jejuni* growth experiments in vitro have shown that aspartate, serine, proline and glutamate are favorably utilized as nutritive substances (Leach et al. 1997; Elharrif and Mégraud 1986; Leon-Kempis et al. 2006; Velayudhan et al. 2004; Guccione et al. 2008). The bacteria contain dedicated membrane transporters for certain amino acids that are essential for serine metabolism. By means of mutagenesis, it was demonstrated that these transporter systems are required for colonization in the intestine of chickens (Hendrixson and DiRita 2004; Velayudhan et al. 2004; Ribardo and Hendrixson 2011). Furthermore, it appears that natural *C. jejuni* strains display a high genetic diversity and sometimes utilize specific pathways to metabolize a given amino acid (Hofreuter 2014; Gao et al. 2017). In addition, specific genetic polymorphisms have been associated with varying capabilities to acquire nutrients and establish colonization in mice. For example, *C. jejuni* strains expressing a certain γ-glutamyltranspeptidase (GGT) enzyme were also able to metabolize glutathione and glutamine, leading to elevated colonization rates in the murine gut (Hofreuter et al. 2008; Floch et al. 2014). Similarly, when an asparaginase enzyme was present with a sec-mediated secretion motif, *C. jejuni* could acquire asparagine from the environment, which not only improved colonization of the intestinal tract, but also of the liver of mice (Hofreuter et al. 2008). Finally, a recent comprehensive in vitro analysis of a *C. jejuni* transposon mutant library was performed for bacterial multiplication in distinct broth media and subsequently for the capability to colonize mice, and this was merged with isotopolog profiling experiments and metabolic flow studies (Gao et al. 2017). This work identified that *C. jejuni* can consume various metabolic end products from the gut microbiome, including acetate and carbon dioxide in the form of hydrogen carbonate, and particularly single amino acids as mentioned above, as well as oligo-peptides made available from food and degraded host proteins. Furthermore, it was observed that single amino acids and di-peptides are present in the intestinal mucus layer in considerable quantities. However, certain required amino acids may not be available in abundances high enough to support *C. jejuni* growth, as demonstrated by auxotrophic mutations that prevented the production of serine or aromatic and branched amino acids and led to the inability to colonize mice (Gao et al. 2017). It seems that *C. jejuni* surmounts these metabolic substrate constraints by utilizing the tricarboxylic acid cycle, the

non-oxidative pentose phosphate pathway and gluconeogenesis, which can collectively promote growth in vitro and in vivo (Gao et al. 2017). Together, this detailed study has pinpointed multiple routes of a highly specialized metabolism and life style of *C. jejuni* to achieve bacterial fitness in the gut.

## 2.2   Campylobacter *Motility and Chemotaxis*

Motility is a characteristic property of *C. jejuni* and is essential for effective colonization in the avian, murine or human host (Wassenaar et al. 1993; Guerry 2007; Chang and Miller 2006; Artymovich et al. 2013; Black et al. 1988; Schmidt et al. 2019). The bacteria are motile by means of two flagella, one on each end of the spirally-shaped bacterial cell body. The flagellum functions as a propeller driven by a rotating motor, which enables the bacterium to swim by a corkscrew-like mechanism (Purcell 1997; Karim et al. 1998; Shigematsu et al. 1998; Cohen et al. 2020). The *C. jejuni* flagellar structure resembles that of *Salmonella* or *E. coli,* but *C. jejuni* has three additional disk structures making up the motor, called the basal disk (mainly consisting of FlgP), the medial disk (composed of PflA) and the proximal disk (formed by PflB and MotAB stator units) (Chen et al. 2011; Beeby et al. 2016). These disk structures are located in the periplasm, where they surround the flagellar rod (Beeby et al. 2016). In addition, the *C. jejuni* flagellum has an MS ring and a C ring of higher complexity that contributes to enhanced activity of the flagellar type III secretion system (Henderson et al. 2020), which will be further described in Sect. 2.6 below. Connected to this structure is the so-called surface hook (FlgE) to which the flexible extracellular flagellar filament (composed of FlaA and FlaB) is attached (Guerry et al. 1991; Hendrixson and DiRita 2003; Chen et al. 2011; Beeby et al. 2016). In addition to a function in bacterial motility, the flagellum may also be used for the secretion of proteins into the extracellular space (Konkel et al. 1999; Poly et al. 2007; Christensen et al. 2009; Barrero-Tobon and Hendrixson 2012; Faber et al. 2015). It was also reported that the flagellum can play a role in the adhesion of *C. jejuni* to certain host cells (Yao et al. 1994). In line with that observation, it was described that the secreted flagellin-like protein FlaC binds to the host cell, may contribute to *C. jejuni* invasion and as such may play a role by modulation of the host immune response (Song et al. 2004). The flagellar filament undergoes O-linked glycosylation, which enables it to colonize chickens (Howard et al. 2009). Protein glycosylation is also important for the correct assembly of the filament's building blocks, flagellin (FlaA and FlaB). To this end, it appears that glycosylation allows the flagellin subunits to interact with each other (Goon et al. 2003; Guerry et al. 2006; Kreutzberger et al. 2020). A two-component signal transduction system (FlgS and FlgR) regulates transcription of many flagellar genes essential for flagellar biosynthesis, some of which are produced with Sigma 54 (Hendrixson and DiRita 2003; Wösten et al. 2004). They are controlled by supercoiling of chromosomal DNA (Shortt et al. 2016) as well as by phase variation and phosphorylation, which is unique in this bacterium (Hendrixson 2006, 2008).

By means of chemotactic sensors, *C. jejuni* is able to senses metabolic concentration gradients, such as those represented by certain components surrounding the mucosa of the gut (Korolik 2019). For the human intestine, these are mainly aspartate, asparagine and lactate, while in the chicken intestine L-fucose is present (Vegge et al. 2009; Hartley-Tassell et al. 2010; Rahman et al. 2014; Dwivedi et al. 2016). Thus, chemotaxis plays an important role both in commensal and pathogenic microbe–host interactions. Through a broad genome sequence analysis among multiple *C. jejuni* strains, a number of orthologous chemotaxis genes including *cheA, cheW, cheV, cheY, cheR* and *cheB* were identified (Marchant et al. 2002). It was shown that CheY acts as a response regulator and interacts with the flagellar motor to affect the rotation direction to turn either clockwise or counterclockwise. Therefore, it is a particularly important factor for flagellar function (Yao et al. 1997). A recent publication demonstrated that CheY has no effect on the speed of rotation, because speed was not affected by deletion of *cheY* (Cohen et al. 2020). Deletion of one of the above mentioned chemotaxis components leads to a colonization defect as shown in ferret and mouse models (Yao et al. 1997; Chang and Miller 2006). As a result, *C. jejuni* is no longer able to induce the associated disease during infection. Together, it can be concluded that motility in combination with the chemotaxis cascade is important for colonization and proper interaction of *C. jejuni* with its host, either asymptomatic as in chickens or symptomatic as in humans (Korolik 2019).

## 2.3 CDT Toxin Production

The cytolethal distending toxin (CDT) is the only known toxin found in most, though not all *C. jejuni* strains (Mortensen et al. 2011). In contrast to other diarrheal pathogens, *C. jejuni* does not encode other toxins, which makes the CDT unique for this bacterium (Lai et al. 2016). When cultured host cells are exposed to CDT, it leads to a characteristically enlarged cell surface and to cell death, which gave the toxin its name. This was originally shown for several sensitive cell lines upon infection with *C. jejuni* (Johnson and Lior 1988). CDT is a holotoxin comprising of three subunits, CdtA, CdtB and CdtC. Of these, CdtB exhibits enzymatic Dnase activity that ultimately results in cell-cycle arrest and cell death, while CdtA and CdtC are responsible for the translocation of CdtB across the target cell membrane (Lara-Tejero and Galán 2001). Subunits CdtA, CdtB and CdtC are located on the bacterial cell surface, where they assist in binding to the host cell (Guerra et al. 2011); however, the exact mechanism of CdtA/CdtC-assisted CdtB translocation remains controversial. Similarities to the B chain of ricin toxin, which is important for the receptor-induced endocytosis of ricin, have been demonstrated (Lara-Tejero and Galán 2001). It has also been shown that the CDT holotoxin is either directly secreted into the extracellular space or packaged into outer membrane vesicles (OMVs) that are continuously being shed from the bacteria (Lindmark et al. 2009). OMVs are commonly formed by Gram-negative bacteria and fulfil a number of tasks such as the delivery of toxins (Wai et al. 2003). So far it remains unclear, however, how the OMVs carrying CDT

bind to target cells, or whether the OMVs enter the cell. It appears that the holotoxin, when packed in OMVs, can enter the host cell by membrane fusion (DiRienzo 2014). Thus, it remains to be determined how exactly CdtB is translocated into the host cell.

Once CdtB has reached the cytoplasm of the target cell, it is transported to the endoplasmic reticulum with the help of the Golgi apparatus to get into the cell nucleus (Heywood et al. 2005). Both actin and the microtubulin systems assist in this transport of CdtB into the nucleus (Méndez-Olvera et al. 2016). Inside the cell nucleus, CdtB induces DNA double-strand breaks, which lead to arrest of the cell cycle in the G2/M phase (Whitehouse et al. 1998). This mechanism results in the activation of DNA repair mechanisms and blocking of the nuclear CDC2 kinase via phosphorylation, which is responsible for entering mitosis. This ultimately leads to apoptosis of the cell (Pickett and Whitehouse 1999; Guerra et al. 2005). In some human cell lines (Hela, Caco-2), cell enlargement is indeed observed during infection with *C. jejuni*, leading to cell death (Johnson and Lior 1988; Elmi et al. 2016). Further studies showed that CDT is able to trigger the secretion of IL-8 as an immune response, which leads to the recruitment of macrophages and neutrophils to the infected site (Hickey et al. 2000; Purdy et al. 2000; Zheng et al. 2008). This leads to a massive infiltration of immune cells into the infected tissue, which is a typical histopathological hallmark of campylobacteriosis. In support of this, a Δ*cdt* knockout mutant was still able to colonize immuno-suppressed mice but was no longer able to induce symptoms or systemic infection (Purdy et al. 2000; Fox et al. 2004). Thus, these results clearly show that CDT plays a role in the pathogenicity of *C. jejuni* and can therefore be counted as virulence factor. Nevertheless, human symptomatic infections are sometimes caused by strains defective in CDT production (Mortensen et al. 2011).

## 2.4 Serine Protease HtrA and Epithelial Barrier Disruption

The trypsin-like serine protease HtrA (high-temperature requirement A) is a highly conserved enzyme found in both prokaryotes and eukaryotes and was first described in *Escherichia coli* (Lipinska et al. 1989). That species typically contains three *htrA* orthologous genes, called *degQ, degP* and *degS*, while other bacteria such as *C. jejuni* encode only one *htrA* gene copy, whose product is most similar to DegQ. HtrA has a domain-like structure and consists of a signal peptide required for secretion, a protease domain and two PDZ domains. The bi-functional protein acts as a chaperone and also has protease activity. The protease domain contains a catalytic triad which is composed of the amino acids histidine (His), aspartate (Asp) and serine (Ser). Following removal of the signal peptide, the protein reaches the periplasm, where it forms proteolytically active multimers that carry out protein quality control functions (Clausen et al. 2002; Kim and Kim 2005; Krojer et al. 2010). Cryo-electron microscopy of *C. jejuni* HtrA revealed that it forms a dodecamer, built of four trimers (Zarzecka et al. 2020). Biochemical studies disclosed proteolytically active hexamers, dodecamers and even larger oligomers with a remarkable stability compared to previously investigated HtrA orthologs in other bacteria (Zarzecka et al.

2020). It was further shown that HtrA protects the *C. jejuni* bacteria against the enrichment of denatured or non-properly folded proteins in the periplasm under stress conditions (Brøndsted et al. 2005; Bæk et al. 2011a). Indeed, a *C. jejuni* Δ*htr*A deletion mutant exhibited reduced growth compared to the wild-type bacteria. This was attributed to the chaperone activity of the protein, since a protease-inactive S197A mutant showed no impairment in terms of growth (that single amino acid mutation does not affect the chaperonin activity of HtrA). Similar behavior was also observed in response to oxidative stress (Brøndsted et al. 2005; Bæk et al. 2011a). Further studies demonstrated that the Δ*htr*A deletion mutant had defects to adhere and invade into host cells. The defective phenotype was restored after genetic complementation with the wild-type *htrA* gene (Bæk et al. 2011a; Boehm et al. 2012, 2013, 2015). However, the ability to adhere to the host cell did not require protease activity of HtrA (Bæk et al. 2011b). Altogether, these findings suggest that the chaperone part of HtrA has an important role in the adherence and invasion process during *C. jejuni* infection (Konkel et al. 2001).

It has been demonstrated that HtrA of *C. jejuni* is also secreted into the extracellular space, which suggests stress response and survival is not its only function (Boehm et al. 2012, 2018; Backert et al. 2018). A plausible hypothesis is that the HtrA protein is released as a soluble enzyme and/or packaged as cargo into OMVs (Boehm et al. 2012; Elmi et al. 2012, 2016; Yoon 2016). The number of HtrA molecules secreted per *C. jejuni* cell has been quantified and is considerably high: On average, about 4000–5000 HtrA molecules can be secreted by a single bacterium during 2 h of culture in liquid broth (Neddermann and Backert 2019). When the protein is secreted into the extracellular environment during infection, HtrA comes in direct contact with host cell surface proteins, where it may exhibit protease activity, as has been experimentally demonstrated. The first described HtrA target was the adherens junction and tumor suppressor protein E-cadherin, which HtrA cleaves into various fragments (Boehm et al. 2012). Another recently discovered target protein which is cleaved by *C. jejuni* HtrA is the tight junction protein occludin (Harrer et al. 2019). These cleavage events lead to the temporary opening of cell-to-cell junctions in the epithelium, allowing *C. jejuni* to transmigrate between two neighboring cells (Fig. 1). Interestingly, it was observed that such a temporary opening of the junctions has no major impact on the transepithelial electrical resistance (TER), which demonstrates the overall tightness of an epithelial layer (Boehm et al. 2012; Harrer et al. 2019). It was also shown that both the Δ*htrA* and an S197A mutants were unable to transmigrate across a cell monolayer, despite being fully motile, which clearly demonstrated the importance of the HtrA protease of *C. jejuni* for crossing the epithelial cell monolayer. Thus, our hypothesis is that HtrA has a dual function for the pathogen: (i) intracellular protein quality control and (ii) extracellular cleavage of host cell junctional proteins to establish a proper infection (Boehm et al. 2012, 2013; Backert et al. 2018).

In two different mouse models (one using IL-10$^{-/-}$ knockout mice and the other using infant wild-type mice), the impact of *C. jejuni* HtrA on the infection process has been monitored (Boehm et al. 2018). When IL-10$^{-/-}$ knockout mice were infected with wild-type *C. jejuni*, strong changes in the crypt architecture were observed,

accompanied by a strong infiltration of immune cells into the tissue. These features were diminished in mice infected with the Δ*htrA* mutant (Heimesaat et al. 2014a). Infection of infant mice with wild-type and Δ*htrA* mutant *C. jejuni* resulted in similar observations, and in this model presence or absence of HtrA did not affect the colonization rates of *C. jejuni* in the intestine (Heimesaat et al. 2014b). Taken together, these observations clearly identified HtrA as a virulence factor of *C. jejuni*. We therefore consider HtrA an ideal candidate for future development of new antimicrobial drugs. The development of new drugs against this foodborne pathogen is very important, as *Campylobacter*-mediated enteritis is highly frequent in a number of countries (Gölz et al. 2014). Efforts to develop an inhibitor against HtrA have been conducted in vitro, mostly targeting the proteins of *E. coli* or *Helicobacter pylori* HtrA (Hauske et al. 2009; Perna et al. 2014, 2015; Schmidt et al. 2016; Tegtmeyer et al. 2016). These attempts had shown some success; however, the identified inhibitors are still somewhat unspecific and can also inhibit HtrAs of other bacteria, including commensals. Thus, further research must be carried out in this area to narrow down the specificity of HtrA inhibitors toward the proteins of pathogenic bacteria only.

## 2.5   Outer Membrane Adhesins and Host Cell Binding

*C. jejuni* invasion into host epithelial cells is first initiated through cell adherence provided by various adhesion proteins (called adhesins), which recognize and bind to specific host cell receptors resulting in stable attachment. The binding activity of adhesins is widely accepted to be fundamental for the effective interaction of a given bacterium with host cells and is a necessary prerequisite for subsequent invasion (Hermans et al. 2011; Backert et al. 2013). Several major *C. jejuni* adhesins have been shown to result in attachment to the host cell (Fig. 2), with CadF (*Campylobacter* adhesin to fibronectin) believed to play a key role in this process. Other major adhesins include fibronectin like protein A (FlpA), *jejuni* lipoprotein A (JlpA), major outer membrane protein (MOMP), *Campylobacter* autotransporter protein A (CapA), a 95 kDa outer membrane protein (p95) and periplasmic binding protein 1 (PEB1) (Ó Cróinín and Backert 2012). CadF is a 37-kDa outer membrane protein that mediates bacterial attachment to the host cells through the extracellular matrix protein fibronectin (Konkel et al. 1997; Schmidt et al. 2019; Krause-Gruszczynska et al. 2007a). The CadF/fibronectin interaction during *C. jejuni* colonization was mostly studied using INT-407 cells as a model for infection. Originally thought to be derived from normal embryonic intestinal tissue, INT-407 was subsequently found to have been established via HeLa cell contamination, which should be taken into account when working with these cells (Nelson-Rees and Flandermeyer 1976; Neimark 2015). Disruption of the *cadF* gene resulted in reduction of bacterial adherence to the INT-407 cells (Monteville and Konkel 2002; Monteville et al. 2003; Krause-Gruszczynska et al. 2007b). CadF deficiency further rendered the bacteria less capable to colonize chickens in comparison with wild-type *C. jejuni* (Ziprin et al. 1999). It was proposed that CadF is responsible for the uptake of *C. jejuni* at

**Fig. 2 Major surface-exposed adhesins and involved host cell receptors described for *C. jejuni*.** At least eight membrane-anchored bacterial proteins have been described to act as binding factors to host target cells. For three of these factors, the corresponding host cell interaction partner has been identified. For the others, the host receptor is still unknown and these are labeled with question marks. FlaC* is secreted via the flagellar T3SS and PEB1** has been described as an aspartate/glutamate-binding protein of an ABC transporter. For more details on these adhesins, see text
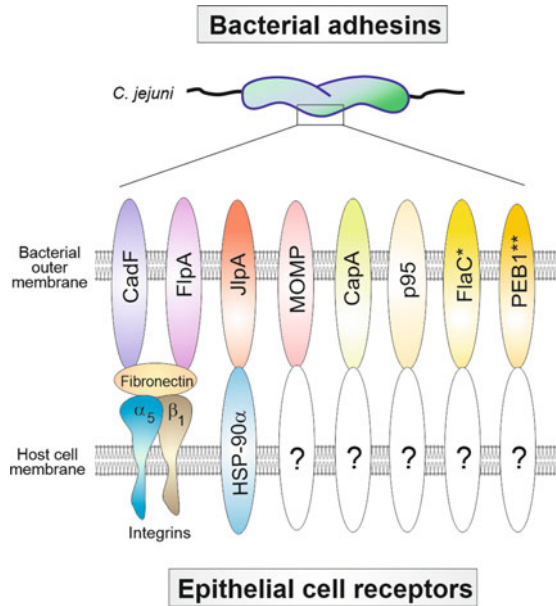


the basolateral area of host cells, where fibronectin is linked to the integrin-based receptor complex (Monteville and Konkel 2002). However, in the IL-10$^{-/-}$ mouse model observations revealed that *C. jejuni* flagellin A and B, but not cell adhesion mediated by CadF, are essential for inducing murine campylobacteriosis (Schmidt et al. 2019).

Another binding factor of *C. jejuni* acting through interaction with fibronectin is the 46-kDa protein FlpA. Deletion of *flpA* resulted in a significant reduction of bacterial attachment to INT407 cells, while in wild-type *C. jejuni* the FlpA protein interacted with cellular fibronectin in a dose-dependent manner (Konkel et al. 2010; Eucker and Konkel 2012). This interaction was studied in more detail, revealing that the FlpA/fibronectin interaction is mediated by the fibronectin-binding linear motif in domain-2 of FlpA with the gelatin-binding domain of fibronectin (Larson et al. 2013). Altogether, it seems that both CadF and FlpA proteins enable attachment of *C. jejuni* to host cells via fibronectin in a cooperative manner.

A third adhesin is the constitutively expressed JlpA, a 43-kDa surface lipoprotein that was shown to interact with intestinal heat shock protein 90α. Mutation in the *jlpA* gene led to reduced adherence of *C. jejuni* to cultured HEp-2 cells by 18–19.4% (Jin et al. 2001, 2003). In addition, pretreatment of HEp-2 cells with purified JlpA decreased *C. jejuni* adhesion in a dose-dependent manner (Jin et al. 2001), suggesting saturation of a receptor. In an interesting study, recombinant JlpA was expressed in *Lactococcus lactis* and when these bacteria were fed to chickens, IgA antibodies were raised against the protein that was present in chicken feces. When these antibodies were used for pretreatment of either human INT407 or primary chicken embryo

intestinal cells, it resulted in a significant reduction in bacterial adherence and invasion of *C. jejuni* (Gorain et al. 2020), again suggesting the interaction between JlpA and its targets can be blocked, this time with JlpA-directed antibodies.

Similarly, inactivation of the *peb1A* gene, which encodes a 28-kDa protein PEB1, decreased adherence of *C. jejuni* to cultured HeLa cells, and prevented colonization of mice (Pei et al. 1998); however, the exact adhesion mechanism of this protein remains unclear. Later studies showed that PEB1 had less influence on adherence of *C. jejuni* to T84 cells or chicken epithelial cells (Novik et al. 2010; Flanagan et al. 2009). PEB1 actually functions as a periplasmic binding protein as part of an aspartate/glutamate ABC transporter system, which is required for optimal microaerobic growth on dicarboxylic amino acids (Leon-Kempis et al. 2006). This could explain why PEB1-deficient bacteria are less able to colonize a given host.

The MOMP protein, which is also known as PorA, has also been suggested to contribute to the adherence of *C. jejuni* to host cells, although its major role seems to be the transport of nutrients and other small molecules. Nevertheless, a recent study showed that the transcription terminator of the *porA* gene enhances the expression level of MOMP by stabilizing its mRNA and therefore influences the virulence of *C. jejuni* (Dai et al. 2019). Some of the proposed *C. jejuni* adhesins are controversial in the literature, and their contribution to the bacterial adhesive properties might be indirect, as may apply to, among others, PEB1, MOMP, CapA and p95 (Ó Cróinín and Backert 2012). Interestingly, novel genes regulating adhesion factors remain to be discovered, as illustrated by the recent report of a new two-component signal transduction system which is involved in regulating adhesion (Xi et al. 2020). These genes (*cj1492c* and *cj1507c*) encode a histidine kinase and a transcriptional regulator, respectively, and when inactivated, this impaired motility, adherence and invasion, and fewer bacteria survived intracellularly. The gene pair has been renamed BumR and BumS and has also been found to be involved in directing a response to butyrate (Luethy et al. 2015; Goodman et al., 2020). Together, these findings illustrate that adhesion is a key prerequisite for the successful colonization of host by *C. jejuni*.

## 2.6   The Flagellum as a Specialized Type III Secretion System

*C. jejuni* does not possess classical type III or type IV secretion systems (T3SS or T4SS) to inject effector molecules into host cells; however, T3SS functions were found to be provided by the flagellum, which has been demonstrated to export effector proteins that can control bacteria–host interactions as discussed below (Barrero-Tobon and Hendrixson 2012; Christensen et al. 2009; Young et al. 1999; Ziprin et al. 1999). The flagellar filament of *C. jejuni* consists of two glycosylated structural flagellins, FlaA and FlaB, as described above. One of the proteins secreted through the flagellar filament is the non-structural protein FlaC, which is implemented in host cell invasion. The flagellar proteins FliS and FliW in *C. jejuni* assist in the secretion of FlaC protein (Radomska et al. 2017). The FliS protein (a flagellar chaperone) preferentially binds to the glycosylated flagellins and is essential for flagellar assembly;

it also directs FlaC toward the flagella for its secretion, while FliW mainly acts as sensor of intracellular FlaA/FlaB flagellin levels. The FlaC protein is thus secreted from the flagellar apparatus of *C. jejuni* cells and plays an important role in entry to epithelial cells (Song et al. 2004). Moreover, FlaC has been shown to directly interact with toll-like receptor 5 (TLR5), resulting in p38 activation (Faber et al. 2015). Preincubation with FlaC modulated the immune responses of chicken and human macrophage-like cells toward the bacterial TLR4 agonist lipopolysaccharide (LPS) by promoting cross-tolerance with subsequent reduction of interleukin-1β (IL-1β) expression (Faber et al. 2015). Consequently, the flagellum is a complex machinery that not only renders *C. jejuni* motile, but also enables protein secretion and administration into the host cell, providing a crucial step in the process leading to host invasion (Burnham and Hendrixson 2018). In this context, it is noteworthy that the flagellum is evolutionarily related to T3SSs used by, for instance, pathogenic *Salmonella* or *Yersinia* species, and was therefore named flagellar T3SS (fT3SS).

FlaC is not the only protein secreted by the *C. jejuni* fT3SS. There are two other distinct groups of proteins secreted this way, described as (i) flagellar co-expressed determinants (FedA-D) and (ii) *Campylobacter* invasion antigens (CiaA-I) (Konkel et al. 1999; Eucker and Konkel 2012; Burnham and Hendrixson 2018). The Fed proteins were found to be important in commensal colonization of chickens, while FedA is also involved in invasion of human intestinal cells (Barrero-Tobon and Hendrixson 2012). However, the individual functions of Fed proteins remain largely unknown (Burnham and Hendrixson 2018). The Cia proteins have been reported to influence *C. jejuni* interaction with human intestinal cells; however, their mechanisms of action during adhesion and invasion are also relatively unclear. One of the best-characterized Cia members, the 73-kDa protein CiaB, appears to be necessary for the secretion process itself, and is required for maximal invasion of *C. jejuni* into host target cells (Konkel et al. 1999). *C. jejuni* strain F38011 with a deleted ΔciaB gene exhibited significantly lower invasion capacity into human cells, along with reduced colonization in chickens (Ziprin et al. 1999). However, inactivation of *ciaB* in strain 81–176 did not influence invasion capacity toward cultured intestinal epithelial cells (Novik et al. 2010). These conflicting outcomes may be related to strain differences or differences in experimental procedures. A gene screening of *C. jejuni* strain NCTC 11168 revealed at least 42 proteins with putative fT3SS amino-terminal sequences directing their export through the flagellum (Christensen et al. 2009). CiaC is an example and was reported to be essential for maximal invasion of epithelial cells, which took place through the recruitment and activation of small Rho GTPase member Rac1, while *ciaC*-deficient *C. jejuni* resulted in significant decrease of Rac1 activation (Eucker and Konkel 2012). Another Cia member, CiaI, may have a function in intracellular survival in human cells (Buelow et al. 2011) and/or colonization in chickens (Barrero-Tobon and Hendrixson 2012) and is discussed below. Taken together, it appears that the flagellar export machinery fT3SS represents a crucial secretory apparatus, which enables *C. jejuni* to invade and manipulate host cells.

## 2.7 Bacterial Factors and Signaling Involved in Host Cell Invasion

A major disease-associated feature of *C. jejuni* is its capability to invade host tissues, which is believed to represent a primary mechanism of pathogenesis associated with host tissue damage. A number of molecular players of the bacterium and host cell involved in invasion have been discovered (Fig. 3). High-resolution electron microscopy of infected epithelial cells revealed that *C. jejuni* can induce membrane rearrangements upon contact with a host membrane, which eventually leads to cell invasion (Boehm et al. 2011; Krause-Gruszczynska et al. 2011). According to multiple studies, there are two major strategies utilized by *C. jejuni* to enter cultured cells, either microtubule-dependent or actin-filament-dependent mechanisms, which
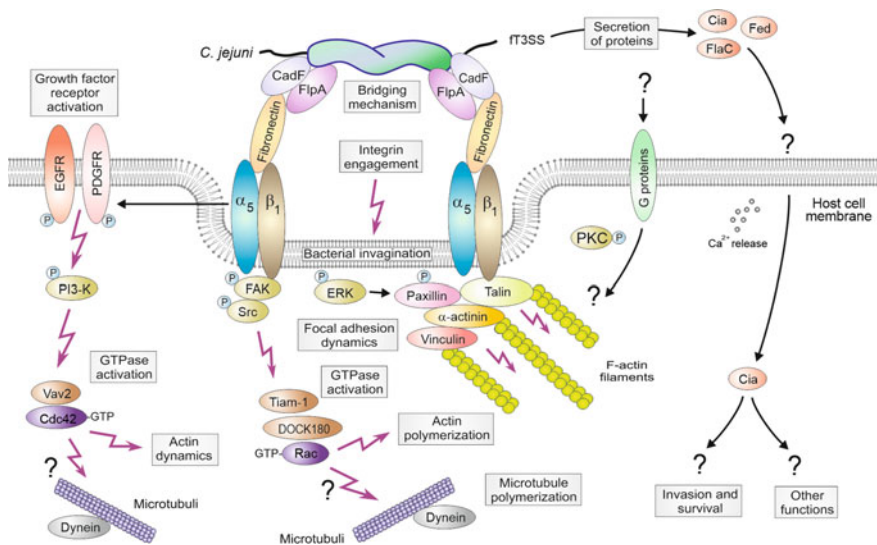


**Fig. 3 Molecular signal transduction model for *C. jejuni*-induced events leading to bacterial invasion of the human intestinal epithelium**. *C. jejuni* express two fibronectin-binding proteins, CadF and FlpA, which mediate attachment of the bacteria to integrin-based focal adhesion structures. In this way, integrin receptors are activated leading to various indicated signaling events. For example, the growth factor receptors EGFR and PDGFR are activated by phosphorylation, which leads to stimulation of PI3-kinase (PI3-K) and the guanine exchange factor Vav-2, activating the small Rho GTPase Cdc42. In addition, integrin engagement leads to autophosphorylation of the cytoplasmic kinases FAK and Src as well as paxillin, which in turn activate the guanine exchange factors Tiam-1 and Dock180, stimulating small Rho GTPase Rac1. Both Cdc42 and Rac1 can then trigger microtubule and F-actin polymerization/reorganization events leading to the membrane engulfment and subsequent uptake of *C. jejuni* into the host cells. Activation of additional host receptors such as G proteins by yet unknown bacterial factor(s) also appears to contribute to bacterial host cell entry. Finally, various secreted effector proteins of the *C. jejuni* flagellum (fT3SS) are proposed to function in bacterial attachment and invasion, but their exact mechanisms are yet unclear. For more details, see text

seem to vary between *C. jejuni* strains (Ó Cróinín and Backert 2012). While the detailed signaling mechanism of the microtubule-dependent invasion pathway is unclear, members of the small Rho GTPase proteins are known as key regulators, which trigger actin polymerization, membrane ruffling and bacterial internalization (Stradal and Schelhaas 2018). Various experiments reported that entry of *C. jejuni* into intestinal epithelial cells depends on the activation of the small Rho GTPase members Cdc42 and Rac1 (Krause-Gruszczynska et al. 2007b; Krause-Gruszczynska et al. 2011; Boehm et al. 2011; Eucker and Konkel 2012). *C. jejuni* binds to fibronectin by means of its CadF and FlpA adhesins, which may act together, and in turn drive the phosphorylation of EGF receptor by activation of the integrin receptor (Eucker and Konkel 2012). Upon attachment to the host cell surface, CadF invokes two signaling cascades that lead to either Cdc42 or Rac1 activation, both resulting in F-actin-dependent engulfment and uptake of *C. jejuni*. This process involves a wide range of intermediate factors initiated by binding of CadF to fibronectin, followed by signal cascades involving PDGF and EGF receptors, integrins, cytosolic kinases and guanine exchange factors. The activation of Cdc42 and Rac1, respectively, takes place by the following proposed pathways. The difference of these two routes is the step following integrin/focal adhesion kinase (FAK) interaction: CadF → fibronectin → $\beta$1-integrin → FAK/Src → PDGFR/EGFR → PI3-kinase → Vav2 → Cdc42 (Krause-Gruszczynska et al 2011) (left side of Fig. 3); or CadF → fibronectin → $\beta$1-integrin → FAK → Tiam-1/DOCK180 → Rac1 (Krause-Gruszczynska et al. 2011; Boehm et al. 2011) (middle part of Fig. 3). The importance of all these host factors for efficient bacterial uptake was demonstrated by *C. jejuni* infection of fibroblasts derived from fibronectin$^{-/-}$, integrin-$\beta_1$$^{-/-}$, FAK$^{-/-}$ and Src$^{-/-}$/Yes$^{-/-}$/Fyn$^{-/-}$ (SYF) triple knockout mice, each of which resulted in invasion failure (Krause-Gruszczynska et al. 2011; Boehm et al. 2011). Whether the activation of Cdc42 and Rac1 are involved in microtubule-dependent uptake of *C. jejuni* has not yet been investigated. In addition, various studies using inhibitors and gentamycin protection assays suggested that heterotrimeric G proteins, the mitogen-activated kinases ERK and p38, protein kinase C (PKC), phosphatidylinositol-3-kinase (PI3K) and Ca$^{2+}$ release from host cytoplasmic compartments all play a role in *C. jejuni* host cell entry (Wooldridge et al. 1996; Biswas et al. 2000; Hu et al. 2005; 2006), but the involved bacterial factors are not yet clear and need further investigation.

It should be mentioned that many of the above in vitro studies were performed with non-polarized host cells that lack proper cell-to-cell junctions, so that the basolateral fibronectin/integrin complex is easily accessible, a situation that vastly differs from an intact intestinal epithelium encountered in vivo. By means of polarized intestinal Caco-2 cells, it was demonstrated that the serine protease HtrA enables disruption of the cellular tight and adherens junctions as discussed above, which facilitates bacterial invasion of epithelial cells at the basolateral site (Harrer et al. 2019). Taken together, invasion of the epithelium by *C. jejuni* is a complex process involving dozens of bacterial effector molecules as well as a range of host receptors and proteins. While fibronectin-mediated effects of the proteins CadF and FlpA are well described, the molecular mechanisms of action of other proteins, such as adhesins PEB1, MOMP, p95 or CapA, remain to be elucidated.

## 2.8   Intracellular Survival and Trafficking of Campylobacter

Invasion of *C. jejuni* into the gut epithelium has been examined in detail; however, there are only a handful of studies investigating the fate and persistence of the bacteria once they are inside the host cells (Pesci et al. 1994; Gaynor et al. 2005; Naikare et al. 2006; Buelow et al. 2011; Bouwman et al. 2013). Gentamicin protection assays and electron microscopy studies have shown that *C. jejuni* can survive for up to 1–3 days in intestinal epithelial cell lines in vitro. In particular, intracellular *C. jejuni* were observed in a membrane-enclosed compartment in the cellular cytoplasm that was named CCV (short for *Campylobacter*-containing vacuole) (Watson and Galán 2008). These findings initiated investigations toward the *C. jejuni* factors that are involved in intracellular survival and trafficking. The first *C. jejuni* gene reported to contribute to survival within epithelial cells was *sodB*, encoding a superoxide dismutase catalyzing the breakdown of superoxide radicals, which represents an important defense mechanism against oxidative damage (Pesci et al. 1994). Another intracellular bacterial survival factor is *spoT*, a gene encoding a bifunctional ppGpp synthetase/ pyrophosphohydrolase (Gaynor et al. 2005). Microarray expression studies showed that SpoT regulates the so-called stringent stress response by *C. jejuni*. This response appears to be important for bacterial survival in the stationary phase as well as persistence during changing $O_2$ or $CO_2$ concentrations. In addition, the stringent response was necessary for various pathogenicity-related phenotypes including *C. jejuni* viability inside the cultured intestinal epithelial cells (Gaynor et al. 2005). Other genes essential to intracellular survival include *aspA* (aspartate ammonia-lyase) and *aspB* (aspartate aminotransferase) as demonstrated by their inactivation, which decreased intracellular survival, probably due to decreased viability and/or unidentified consequences of the bacterial physiology (Novik et al. 2010). However, in such studies it is difficult to differentiate factors specifically needed from intracellular survival, as mutation of many housekeeping enzymes would result in impaired intracellular survival as well. In another study, the gene of the fT3SS-delivered protein CiaI was inactivated, which compromised invasion and reduced intracellular survival levels (Buelow et al. 2011). However, the latter function is not yet fully clear because other studies supported the view that CiaI may exhibit a different function in establishing commensalism during colonization of chicken (Barrero-Tobon and Hendrixson 2011). Thus, more work is required to characterize the *C. jejuni* factors facilitating intracellular survival functions.

It appears that LOS enhances *C. jejuni* attachment and endocytosis into intestinal epithelial cells (Louwen et al. 2008, 2012). Survival of *C. jejuni* inside CCVs, a compartment in which other pathogens can be killed, may be due to a mechanism evading their maturation to a typical lysosome (Watson and Galán 2008). It has been reported that the CCV diverges from the conventional "canonical" endocytic route (Fig. 4). Interestingly, experimental recovery of intracellular *C. jejuni* from CCVs was only possible by culturing the bacteria under oxygen-limiting conditions (Watson and Galán 2008). This implies that the bacteria undergo crucial physiological adaptations inside the CCVs that may be irreversible. Furthermore, the CCVs were shown
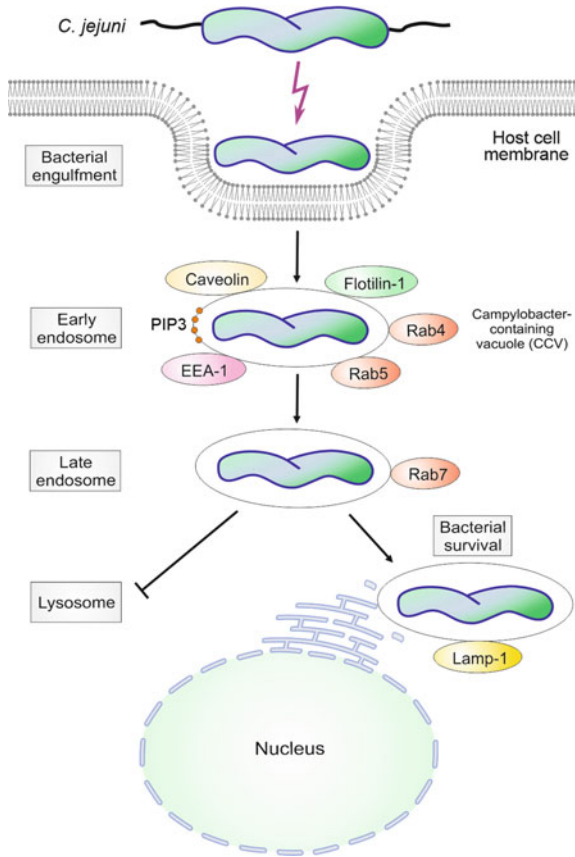
**Fig. 4 Model for the establishing and maturation of *C. jejuni*-containing vacuoles (CCVs) in the cytoplasm of infected epithelial cells**. *C. jejuni* enters the intestinal epithelium via mechanisms described in Fig. 3. After internalization, a so-called CCV is formed by membrane engulfment of invading *C. jejuni*. This CCV transiently recruits various marker molecules of the endocytic cascade including flotilin-1, Rab4, Rab5, Rab7, Caveolin and phosphatidylinositol 3,4,5 trisphosphate (PIP3). This leads to trafficking and intracellular survival of the bacteria in this compartment, inhibiting the canonical endocytic route toward lysosome development. In particular, intracellular *C. jejuni* inhibit the fusion of the late endosome to form lysosomes, and thus avoid bacterial killing. The CCVs also contain the marker protein Lamp-1 and localize in close proximity to the host Golgi apparatus near the nucleus. For more details, see text

to interact with endosomal compartments, and since they can be stained for the early endosomal marker EEA-1 (Early Endosome Antigen 1) as well as for two trafficking GTPases (called Rab4 and Rab5) (Watson and Galán 2008; Louwen et al. 2012); the presence of these markers on the outside of CCVs can be assumed. Nevertheless, this early event obviously appears only temporary and does not continue along the canonical cascade of endocytosis and is evidently different from conventional lysosome formation. However, the CCVs stained positive for the well-known late

endosomal marker protein Lamp-1 (Fig. 4). In contrast, the CCVs did not contain signals for cathepsin B, a well-known lysosomal marker protease, and it is also not stainable for specific other endocytic tracer proteins (Watson and Galán 2008). Altogether, the recruitment of Lamp-1, which happens at a very early stage of CCV development appears to progress by a unique *C. jejuni*-triggered signaling cascade and does not lead to fusion with lysosomes. And this pathway does not demand the functional expression of the GTPases Rab5 and Rab7, despite their presence in the CCVs. Thus, further analyses are necessary to clarify in better detail how *C. jejuni* hijacks endocytic compartments for trafficking and to cause disease in humans.

## 3 Bacterial Virulence Factors and Immune Cell Responses

Innate immunity identifies multiple microbes through the action of various immune receptors. The recognition of *C. jejuni* by such receptors has raised much consideration because their activities could explain the immune pathology of this pathogen (Phongsisay 2016). Below we discuss important receptors binding to *C. jejuni* factors and examine their downstream signaling cascades and resulting immune responses. Numerous pro- and anti-inflammatory pathways that are triggered by *C. jejuni* and control the infection, respectively, are highlighted in Figs. 5 and 6. In vitro and in vivo studies have demonstrated that cytokines including TNF-α, IL-1β, IL-6, IL-8, IFN-β, IFN-γ and others are induced by *C. jejuni*, which not only leads to a pronounced inflammatory response (Phongsisay 2016), but also disturbs the intestinal epithelial barrier function in various ways (Bücker et al. 2018; for details see Chapter 8 in this book).

### 3.1 Interaction with Toll-Like Receptors

Toll-like receptors (TLRs) are key players in the activation of innate immunity. These transmembrane proteins are typically expressed on the surface of macrophages and dendritic cells but can also be found on intestinal epithelial cells. TLRs comprise specific patter-recognition receptors that recognize structurally conserved components of pathogenic microbes, including fungi, bacteria and viruses (Trinchieri and Sher 2007). For example, TLR2 recognizes peptidoglycan and lipoteichoic acid from Gram-positive bacteria (Schwandner et al. 1999), TLR4 recognizes LPS of Gram-negative bacteria (Poltorak et al. 1998), while TLR5 commonly recognizes bacterial flagellin (Andersen-Nissen et al. 2005). In vitro studies using mouse dendritic cells revealed that contact with *C. jejuni* activates their signal transduction protein MyD88 via the receptors TLR2 and TLR4 (Rathinam et al. 2009). Specifically, it is the contact of TLR4 with specific bacterial glycoconjugates that stimulates this signal cascade. At least five different *C. jejuni* glycoconjugates were found to trigger this response, three of which may be components of low-molecular-weight LOS. A
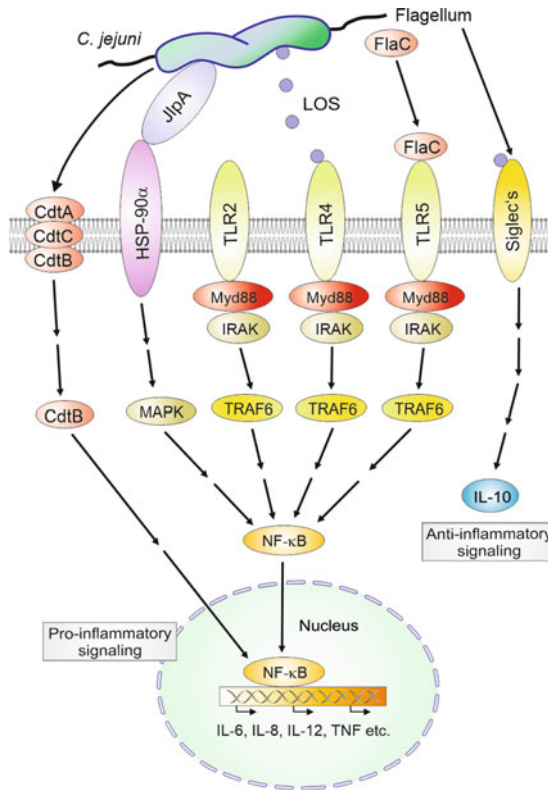
**Fig. 5** *C. jejuni* **targets various pro-inflammatory and anti-inflammatory immune signaling pathways by interaction of bacterial factors with indicated receptor molecules**. *C. jejuni* passes the mucus layer in the human gut and interacts with the intestinal epithelial cells, triggering nuclear responses such as cytokine expression including IL-6, IL-8, IL-12 or TNF. *C. jejuni*-induced cytokine secretion can be stimulated by CDT toxin, adhesin JlpA, LOS and functional flagella. The pro-inflammatory signal transduction proceeds via the activation of host cell receptors HSP-90α, toll-like receptors (TLR2, TLR4 and TLR5), intracellular signaling proteins and transcription factor NF-κB; while engagement of Siglec receptors by *C. jejuni* stimulate the production of anti-inflammatory cytokine IL-10

fourth glycoconjugate appears as a ladder-like band with a molecular size between 30 and 50 kDa on SDS–polyacrylamide gels, while the fifth is a large protein of approximately 150 kDa (Phongsisay et al. 2015). However, the exact *C. jejuni* factor(s) that trigger TLR2 receptor activation remain to be identified. In addition, *C. jejuni* flagellin FlaA is a very poor stimulator of TLR5 (Watson and Galán 2005). Instead, cellular contact to the *C. jejuni* flagellar factor FlaC activates TLR5 (Faber et al. 2015). Upon stimulation, TRL2, TLR4 and TLR5 activate MyD88, which in turn mediates an inflammatory response via the MyD88-NF-κB signaling pathway that involves IRAK, TRAF6 and possibly other mediators, and results in the release of
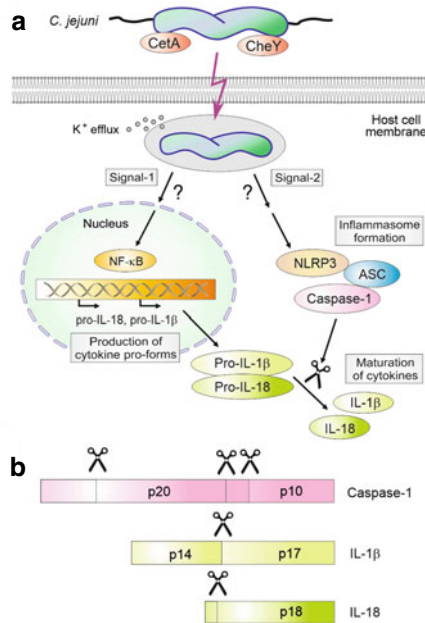
◄**Fig. 6 Signal transduction events leading to inflammasome activation by *C. jejuni*. a** *C. jejuni* stimulates NLRP3 inflammasome formation, typically assembling in infected human dendritic cells, macrophages or monocytes, which finally leads to bacterial clearance. The canonical pathway of inflammasome activation comprises two signals coming from the bacterium, signal-1 and signal-2. Signal-1 leads to activation of transcription factor NF-κB and mRNA production of NLRP3 and the pro-forms of IL-1β and IL-18. Engagement of the NLRP3 protein through signal-2 and adaptor protein ASC induces the recruitment and cleavage of autoproteolytic pro-caspase-1. Activated caspase-1 then cleaves interleukin pro-forms leading to the production of mature IL-1β and IL-18 cytokines. It was described that energy taxis protein CetA and CheY-controlled host cell invasion by *C. jejuni* play an important role, but the actual bacterial factors representing signal-1 and signal-2, respectively, are yet unknown and labeled with question marks. **b** Autoproteolytic processing events in pro-caspase-1 leading to the activation of caspase-1 and maturation steps of IL-1β and IL-18 pro-forms through caspase-1 are shown. For more details, please see text

pro-inflammatory cytokines such as IL-6, IL-8 and IL-12. In addition to MyD88-NF-κB, TRL4 also induces TIR-domain-containing adapter-inducing interferon-β (TRIF)-mediated phosphorylation of Interleukin release factor 3 (IRF3), which stimulates IFN-β secretion from the challenged cells (Rathinam et al. 2009). All these responses are part of immune stimulation that eventually recruits immune cells. Taken together, recognition of *C. jejuni* biomolecules by a variety of TLRs activates pro-inflammatory responses (Fig. 5) and finally results in clearance of the pathogen.

## *3.2 Role of Siglec Receptors*

Sialic acid-binding immunoglobulin-like lectins (Siglecs) are transmembrane proteins comprising 15 members in humans and that are expressed by various immune cell types. The N-terminus is exposed to the extracellular space and binds to ligands containing sialic acid components (von Gunten and Bochner 2008). Due to their specificity, several of the known Siglecs can bind to a variety of pathogens, including Group B *Streptococcus* and *Trypanosoma cruzi*, resulting in the production of inter-leukins (Crocker et al. 2007). Systematic analyses of the binding capacity of 10 different Siglecs revealed that *C. jejuni* LOS interacted with Siglec7 (Avril et al. 2006). As expected, this binding was only observed for sialylated, but not with unsia-lylated LOS components. In addition, sialylated LOS, particularly α2,3-sialylated LOS, was shown to interact with the soluble Siglec-1/Sn (Heikema et al. 2010), while Siglec7 preferably binds to α2,8-sialylated LOS (Bax et al. 2011). Further-more, modified LOS structures of *C. jejuni* mimic human gangliosides, which can lead to auto-antibody production and neural disorders in some patients (Yuki et al. 2004). However, in addition to Siglec1/Sn and Siglec7, *C. jejuni* is also recognized by Siglec10, which was reported to bind both live *C. jejuni* bacteria and purified flagella, suggesting that activation of Siglec10 might be mediated by the flagella. In contrast to TLRs that activate a pro-inflammatory response, flagellin-Siglec10 contact increased the expression of IL-10 (Stephenson et al. 2014) and thus anti-inflammatory signaling (Fig. 5). Further studies are required to confirm these findings in in vivo models of infection.

## *3.3 Activation of the NLRP3 Inflammasome*

Inflammasomes represent cytosolic multiprotein complexes of the host innate immune system. A wide variety of pathogens, including yeasts, bacteria and viruses, induce inflammasome-dependent production of IL-1β (Schroder and Tschopp 2010). Upregulation of pro-IL-1β transcription and subsequent secretion of IL-1β by mouse macrophages was also observed following infection with *C. jejuni*, which suggested processing of the pro-cytokine by the inflammasome (Bouwman et al. 2014). There are several inflammasome types, e.g., NLRP1, NLRP3 and NLRC4 (Schroder and Tschopp 2010), of which *C. jejuni* activates NLRP3 (Bouwman et al. 2014). The NLRP3 inflammasome is triggered by two bacterial signals (signal-1 and signal-2, respectively) and assembles with pro-caspase-1 and adaptor protein ASC, which stimulates maturation and formation of active caspase-1 (Fig. 6a). Active caspase-1 then conventionally processes pro-IL-1β and pro-IL-18 into the respective mature cytokines (Fig. 6b). An analysis of a series of *C. jejuni* mutants defective for a variety of known virulence-associated factors, including LOS, flagella and adhesins, attempted to identify *C. jejuni* components involved in triggering this inflamma-some response (Bouwman et al. 2014). Only two of the tested mutants, resulted in

decreased IL-1β production, namely the deletion of the chemotaxis protein CheY and of the energy taxis protein CetA. This suggests that there is a direct correlation between the number of intracellular *C. jejuni* bacteria (which is regulated by at least CheY and CetA) and NLRP3 inflammasome activation. Yet, all engineered deletion mutants still induced mature IL-1β secretion, albeit at slightly lower levels, suggesting that multiple components may activate this response. It is suggested that contact of macrophages with *C. jejuni* induces a transmembrane ion flux (e.g., K⁺-efflux), as seen for other pathogens (Muñoz-Planillo et al. 2013), but the exact signals that trigger the increased transcription of pro-IL-1β and possibly pro-IL-18, as well as the formation of the NLRP3 inflammasome, remain to be elucidated.

## 4  Concluding Remarks

*C. jejuni* is a fascinating microorganism which colonizes the chicken intestinal tract as a commensal but is associated with various diseases when infecting humans. Interestingly, by comparison to other enteric pathogens, *C. jejuni* comprises only a relatively small array of known virulence factors. While other pathogens like *Salmonella, Yersinia* or *Listeria* species exhibit a broad collection of "weapons" including various toxins, multiple secretion systems and effector molecules, *C. jejuni* has a relatively limited number of identified disease-associated factors, most notably CDT, LOS, HtrA, CadF and fT3SS. As reviewed here, some of these are involved in adhesion to and invasion into host epithelial cells, where the bacteria can survive and even spread into neighboring tissue. Although all details are still not fully clear, most data support an invasion mechanism that depends on the fibronectin-integrin-$\beta_1$ receptor and the small Rho GTPases Rac1 and Cdc42, which appear to dominate *C. jejuni* host cell entry (Fig. 3). However, the process of cell invasion would involve molecular dissection of the mechanic forces activated by host cells, which then trigger bacterial engulfment, allow entry and result in membrane closing behind the invading *C. jejuni*. Future studies should examine how this works in detail. It is also important to investigate in more detail the mechanisms by which *C. jejuni* survives and spreads intracellularly, or how the bacteria cause infection of other organs in the human body that can include the spleen, mesenteric lymph nodes and the liver (Burnham and Hendrixson 2018; Backert et al. 2013). It also appears that *C. jejuni* survives inside the macrophages using its catalase enzyme KatA (Day et al. 2000), a phenomenon which deserves further investigation. Furthermore, for many of the proposed *C. jejuni* virulence proteins, a general knowledge of how they function mechanistically in vivo is still missing. Also, the actual advantage of some bacterial factors such as the CDT toxin for the bacterium is still unclear. It might be that the DNA damage triggered by its DNase activity not only initiates apoptosis but also modulates the host immune response, operating as an immunoregulatory factor, which helps the bacteria producing CDT to establish a suitable niche in its host. In this context, the process of apoptotic cell death induced by *C. jejuni* is more complicated than originally envisaged. Besides CDT described above (Pickett and Whitehouse 1999; Guerra et al.

2005), the serine protease HtrA (Heimesaat et al. 2014b) and the fT3SS substrate FspA2 (Poly et al. 2007) were also reported to stimulate apoptosis in epithelial cells, which can be counteracted by the *C. jejuni*-induced ubiquitin-editing enzyme A20 that negatively regulates apoptotic cell death (Lim et al. 2017). Thus, the interplay of apoptotic and anti-apoptotic signal pathways modulated by *C. jejuni* are still not fully understood, deserving further investigation. Lastly, it must be mentioned that there are controversies and contradictions in various reports describing the functional mechanistics and importance of particular *C. jejuni* virulence factors, such as the Cia proteins or some proposed adhesins. Strain-dependent differences, variation in experimental procedures, and a disconnect between in vitro work, in vivo (mouse) models, and the human situation (where individual immunological variation may be of crucial importance to clinical outcome) further murken the waters. These important issues also need to be addressed and clarified in future studies. Thus, it occurs that *C. jejuni* will continue to be an attractive and gratifying research subject with high importance to public health.

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