Journal of Microbiological Methods 95 (2013) 8–23

Contents lists available at ScienceDirect

Journal of Microbiological Methods

journal homepage: www.elsevier.com/locate/jmicmeth

Review

# Molecular methods to investigate adhesion, transmigration, invasion and intracellular survival of the foodborne pathogen *Campylobacter jejuni*

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ARTICLE INFO

*Article history:*
Received 26 February 2013
Received in revised form 18 June 2013
Accepted 28 June 2013
Available online 16 July 2013

*Keywords:*
Molecular pathogenesis
Cellular invasion
Signalling
Virulence

ABSTRACT

*Campylobacter jejuni* is a spiral-shaped Gram-negative pathogen and major agent of gastrointestinal foodborne illness in humans worldwide. This pathogen encodes numerous described pathogenicity-associated factors involved in important processes including bacterial adhesion to, transmigration across, invasion into and intracellular survival within intestinal epithelial cells. This review article highlights various molecular techniques applied in the studies of each of these individual steps of *C. jejuni* host cell interactions *in vitro* including gentamicin protection assay, chemotaxis and motility assays, transwell and intracellular survival assays, G-Lisa, siRNA knockdown, immunohistochemistry, immunofluorescence, electron microscopy and luciferase reporter assays. We discuss the strengths and limitations of the methods as well as the different cell model systems applied. Future work should employ new technologies including modern microscopic, proteomics-based and cell signaling approaches to identify and characterise novel virulence mechanisms, which are crucial to provide fresh insights into the diversity of strategies employed by this important pathogen to cause disease.



**Contents**



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http://dx.doi.org/10.1016/j.mimet.2013.06.031

## 1. Introduction

Zoonoses are a major health problem. An important example is the spiral-shaped flagellated bacterium *Campylobacter jejuni* which lives as a commensal in the gut of many birds and domestic animals, and thus can enter the food chain of humans in various ways. Infections with *C. jejuni* by fecal contaminated food products represent the major cause of bacterial gastroenteritis, and may be responsible for as many as 400–500 million cases in the human world population every year (Nachamkin et al., 2008; Dasti et al., 2010; Oyarzabal and Backert, 2011). Our knowledge about the interplay between *C. jejuni* and its hosts is still limited (Young et al., 2007), but several infection studies using different animal models elucidated the roles of motility and chemotaxis as crucial factors for a successful *C. jejuni* infection (Hendrixson and DiRita, 2004; Morooka et al., 1985; Nachamkin et al., 1993; Yao et al., 1997). The high motility of *C. jejuni* (Karim et al., 1998), which is even enhanced in medium with increased viscosity (Ferrero and Lee, 1988; Szymanski et al., 1995), allows this pathogen to efficiently reach its favoured colonisation site, the inner mucus layer of the intestine.

During the infection process *C. jejuni* interacts with non-phagocytic intestinal epithelial cells and encounters phagocytic immune cells (Babakhani et al., 1993; Black et al., 1988). Electron microscopic examinations of biopsies from patients with *Campylobacter* colitis showed *C. jejuni* closely associated with the colonic epithelial cells as well as inside intestinal cells (van Spreeuwel et al., 1985). Similar observations were made upon *C. jejuni* infections of infant *Macaca mulatta* monkeys (Russell et al., 1993) and newborn piglets (Babakhani et al., 1993). Interestingly, in other infection experiments *C. jejuni* was not seen attached to or inside intestinal epithelial cells of mice (Lee et al., 1986) and chicken (Beery et al., 1988), although *C. jejuni* was present in high numbers in the mucus within the intestinal crypts. These studies imply that adhesion to and invasion into intestinal epithelial cells are not necessarily essential steps for *C. jejuni* colonisation of the gut.

An essential objective of ongoing *C. jejuni* research is to clarify the precise role of bacterial adhesion to and invasion into enterocytes for the pathogenesis of these infections in different hosts. In general it is believed that the diverse clinical manifestations of campylobacteriosis in humans, ranging from a mild, watery to a severe inflammatory and bloody diarrhea, correlate with the variable virulence potential of different *C. jejuni* isolates and the immune predisposition of the patients (Allos, 2001; Janssen et al., 2008; van Putten et al., 2009; Dasti et al., 2010). Some studies suggest that *C. jejuni* strains isolated from patients with diarrhoea and fever adhere to and invade cultured cells *in vitro* more than *C. jejuni* isolates from patients with less obvious clinical manifestations or asymptomatic infections (Fauchere et al., 1986). In contrast, other studies have demonstrated that certain *C. jejuni* isolates from patients with non-inflammatory diarrhoea can exhibit similar invasion capacities *in vitro* as isolates causing severe colitis (Everest et al., 1992). These studies and other reports demonstrate convincingly that various *C. jejuni* isolates have significantly different capacities to interact with cultured epithelial cells *in vitro*.

The establishment of a functional interface with cultured epithelial cells by *C. jejuni* can be divided into three distinct processes: (i) adherence to the enterocytes, (ii) invasion into the intestinal epithelium cells and (iii) survival inside a defined intracellular compartment (called *Campylobacter*-containing vacuole; CCV) (Fig. 1). In addition, the translocation of *C. jejuni* across the intestinal epithelium into the subepithelial space of the lamina propria has been described during infection experiments (Humphrey et al., 1986) and *in vitro* experiments using polarised cultured epithelial cells were used with the aim to investigate if this process occurs via the transcellular or paracellular route (Fig. 1). During the last decades a main focus in the field of *C. jejuni* research has been to identify the bacterial factors mediating the efficient interaction with cultured epithelial cells. The methodology that has been used to characterise the interaction of *C. jejuni* with host cells *in vitro* will be presented and discussed in this review.

## 2. General aspects of experimental design

The efficiency by which *C. jejuni* interacts with cultured host cells depends on the specific properties of *C. jejuni* strains and the cultured epithelial cell lines. Consequently, the outcomes of *in vitro* assays being used to characterise the adherence, invasion, translocation or survival capacities of *C. jejuni* vary considerably in different studies. In addition, variable experimental settings as listed below make direct comparisons of published studies often difficult.

### 2.1. Selection of C. jejuni strains

Studies using *C. jejuni* isolates from various host origins for *in vitro* infection experiments with cultured epithelial cell lines suggest that no obvious host tropism occurs: *C. jejuni* isolates from humans, chicken or pigs are capable to adhere to and invade human, avian and porcine cell lines (Biswas et al., 2000; Gripp et al., 2011). However, *C. jejuni* isolates adhere to and invade cultured cell lines of certain host or tissue origins with different efficiencies (Larson et al., 2008; Poly et al., 2007; Wine et al., 2008), and the adherence and/or invasion capabilities between strains vary significantly (Biswas et al., 2000; Fauchere et al., 1986; Fearnley et al., 2008; Newell et al., 1985b; Zheng et al., 2008). While a positive correlation between adherence and invasion properties of *C. jejuni* isolates seems to exist (Biswas et al., 2000), it was shown that efficient adhesion is not sufficient for an effective invasion process (Song et al., 2004; Konkel et al., 1992a; Kim et al., 2008; Javed et al., 2012; Christensen et al., 2009). To perform *in vitro* infection experiments with *C. jejuni*, the strain of choice should exhibit efficient adherence and invasion characteristics but also robustly infect animal models (Ahmed et al., 2002; Hiett et al., 2008; Seal et al., 2007). Such strains allow the critical evaluation of *in vitro* characterised pathogen–host interactions in more complex infection models (Hermans et al., 2011; Bereswill et al.,

2011). Furthermore, the selected *C. jejuni* isolate should be suitable for genetic modification to enable the generation of isogenic mutants, considering that not all *C. jejuni* strains are equally manageable for genetic transformation (Gaasbeek et al., 2009, 2010; Javed et al., 2010).

The availability of complete genome sequences of *C. jejuni* strains including NCTC 11168 (Gundogdu et al., 2007; Parkhill et al., 2000), RM1221 (Fouts et al., 2005), 81-176 (Hofreuter et al., 2006) have facilitated targeted mutagenesis and enabled microarray based transcription analysis studies during *in vitro* experiments (Gaynor et al., 2005). In addition, comparative genome analysis of *C. jejuni* strains has helped to identify potential virulence traits and revealed that regions encoding for lipooligosaccharides (LOS), capsule and a flagellin modifying O-glycosylation system, are highly variable between *C. jejuni* isolates (Gilbert et al., 2002; Guerry et al., 2006; Karlyshev et al., 2005; Parkhill et al., 2000). It has been suggested that the variable LOS and capsule structures in different *C. jejuni* isolates influence the outcome of infections because these structures participate in the adherence and invasion processes of *C. jejuni* (Tables 1 and 3). Experiments with *C. jejuni* isolates expressing LOS structures that mimic human gangliosides require special safety rules as these strains are associated with the development of the Guillain–Barré syndrome (GBS) (Nachamkin et al., 1998).

The *C. jejuni* strains NCTC 11168, 81-176, 81116 and F38011 are the most commonly used isolates in laboratories for *in vitro* infection studies. In addition these isolates have been successfully used for *in vivo* infection experiments to characterise the pathogenicity potential of putative virulence factors identified previously by *in vitro* assays. Especially *C. jejuni* 81-176 has been widely used in the *Campylobacter* research community during recent years as it is highly invasive *in vitro* and can robustly colonise. Consequently, *C. jejuni* 81-176 is one of the few isolates that has been used for infection experiments in chicken (Hendrixson and DiRita, 2004), ferrets (Bacon et al., 2000; Yao et al., 1997), wild-type mice (Newell et al., 1985a; Pei et al., 1998; Naito et al., 2010), immune-deficient mice (Watson et al., 2007; Haag et al., 2012) and piglets (Naikare et al., 2006). In addition it is documented that *C. jejuni* 81-176 causes inflammatory colitis during challenge studies with human volunteers (Black et al., 1988; Prendergast et al., 2004; Tribble et al., 2010).

Interestingly, not every *C. jejuni* strain is suitable for *in vitro* infection experiments with any given cultured cell line, even if this strain is isolated from a patient with inflammatory diarrhoea. For example *C. jejuni* CG8486 is about 1000-fold less invasive in comparison to *C. jejuni* 81-176 in infection experiments using INT-407 cells, but both isolates show comparable invasion phenotypes with Caco-2 cells (Poly et al., 2007).

### 2.2. Cell line choice and cell confluency

The permissiveness by which *C. jejuni* interacts with a wide range of different eukaryotic cell types is striking. While *C. jejuni* adheres to different cell lines to similar extend (Konkel et al., 1992a), its internalisation efficiency varies dependent on the cell lines. It was suggested that *C. jejuni* invades epithelial cell lines of human origin more efficient than cell lines of non-human origin (Konkel et al., 1992a). Thus *C. jejuni* infection experiments have been most commonly studied with the human intestinal cell lines Caco-2, T84 and INT-407 (Tables 1–4). *In vitro* invasion of porcine IPEC-1 and IPEC-J2 small intestinal epithelial cells by *C. jejuni* has been described as well (Gripp et al., 2011; Naikare et al., 2006). Furthermore, *C. jejuni* is able to interact *in vitro* with cell lines of non-intestinal origin from various organisms like human HeLa cells, liver-derived HepG2 cells, LMH chicken hepatocellular carcinoma epithelial cells, and the African green monkey kidney derived COS-1 and Vero cell lines (Biswas et al., 2006; Flanagan et al., 2009; Konkel et al., 2007; Larson et al., 2008; Novik et al., 2010; Watson and Galan, 2008; Coote et al., 2007). Only few studies have worked with primary intestinal epithelial cell preparations from chicken (Byrne et al., 2007; Van Deun et al., 2008) or human biopsy material (Byrne et al., 2007).

The choice of cell line depends on the specific experimental question. Many studies have examined the adherence, invasion and survival capacity of *C. jejuni* during infection of non-differentiated INT-407, T84 or Caco-2 cells. The used cell confluency also influences the outcome of infection experiments. The term confluency refers to the coverage of cells on the given petri dish surface. For example, 100% confluency means the petri dish is completely covered by a monolayer of cells (confluent), whereas 50% confluency means that half of the dish is covered by cells (semi-confluent). Interestingly, *C. jejuni* invades cells of a semi-confluent cell monolayer more efficiently than cells of a confluent cell monolayer (Hu et al., 2008). Furthermore, *C. jejuni* infects Caco-2 less and T84 cells more efficiently than INT-407 cells (Poly et al., 2008; Wine et al., 2008). The use of INT-407 cells is preferred when investigating the detailed intracellular localisation and trafficking of *C. jejuni*: Co-localisation studies with transfected GFP-tagged phagolysosomal marker proteins have been used to investigate this

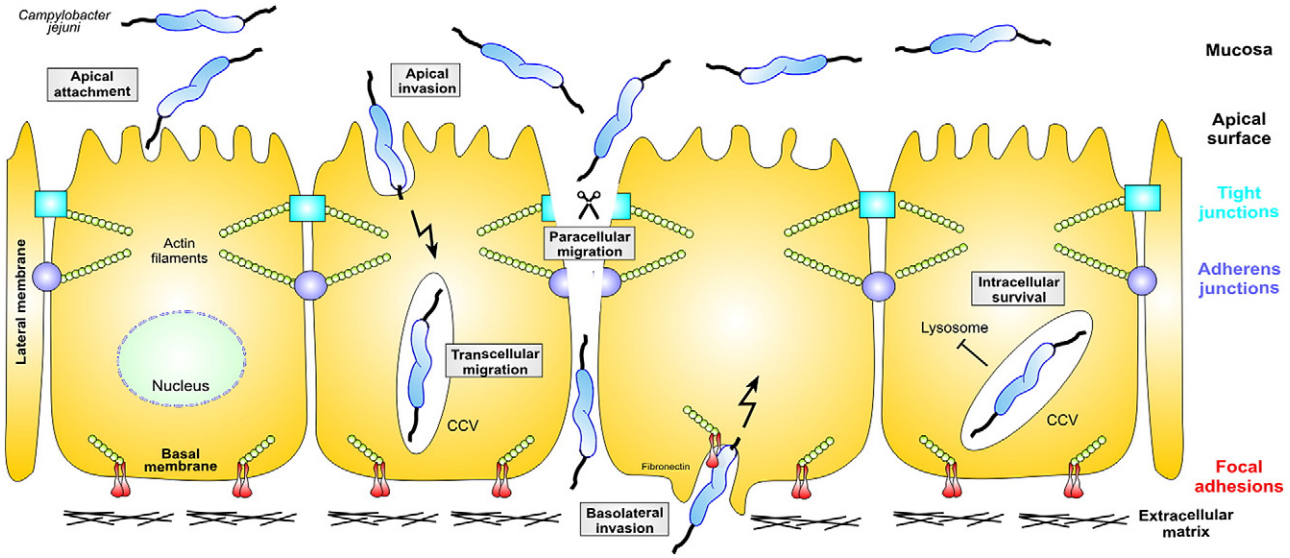


**Fig. 1. Hypothetical model for *C. jejuni* mechanisms of infection.** The pathogen can interact with, invade into, transmigrate across and survive within polarised intestinal epithelial cells as shown. For more details, see text.

**Table 1**
Methods applied to study *C. jejuni* adherence to cultured epithelial cells[a].

| Applied method | Cell model used | Bacterial factors involved | Host factors involved | *C.jejuni* strains used | References[b] |
|---|---|---|---|---|---|
| ALA | INT-407, Caco-2 | ND | ND | HC, 81-176 | McSweegan and Walker (1986); Russell and Blake (1994) |
| LM | HeLa | ND | ND | Human isolates | Fauchere et al. (1986) |
| MLA | Caco-2 | ND | ND | Human isolates | Everest et al. (1992) |
| CBA | INT-407 | FlaA | ND | 81116 | Grant et al. (1993) |
| CBA | INT-407 | PflA, FlaA, CheY | ND | 81-176 | Yao et al. (1994, 1997) |
| CBA | INT-407 | GalE | ND | 81116 | Fry et al. (2000) |
| CBA | INT-407 | KpsM | ND | 81-176 | Bacon et al. (2001) |
| CBA | Hep-2 | JlpA[c] | Hsp90alpha | TGH9011 | Jin et al. (2001, 2003) |
| CBA | INT-407 | PglB, PglE | ND | 81-176 | Szymanski et al. (2002) |
| CBA | T84, INT-407 | CadF | Fibronectin | F38011, 81-176 | Monteville and Konkel (2002); Monteville et al. (2003); Konkel et al. (2005) |
| CBA | INT-407 | PseA | ND | 81-176 | Guerry et al. (2006) |
| CBA | INT-407 | KpsE | ND | 81116 | Bachtiar et al. (2007) |
| IFM | hPIC, chPIC | ND | ND | 81-176, chicken and human isolates | Byrne et al. (2007) |
| CBA | Caco-2, LMH | CapA | ND | 11168, F38011 | Ashgar et al. (2007); Flanagan et al. (2009) |
| CBA | INT-407 | CsrA | ND | 81-176 | Fields and Thompson (2008) |
| CBA | INT-407 | Cj1461 | ND | 81-176 | Kim et al. (2008) |
| CBA | LMH, INT-407 | FlpA | Fibronectin | F38011 | Flanagan et al. (2009); Konkel et al. (2010) |
| CBA | INT-407 | Cj0497 | ND | 01/51 | Javed et al. (2010) |
| CBA, IOS | T84 | SodB | ND | 81-176 | Novik et al. (2010) |
| CBA | INT-407 | HtrA | ND | 11168, 81-176 | Brøndsted et al. (2005); Bæk et al. (2011); Hoy et al. (2012) |
| IFM | Caco-2 | Cst-II | ND | 81-176, GB11 | Louwen et al. (2012) |

[a] Abbreviations: ALA ($^3$H-acetic acid labelling assay), CBA (CFU-based adherence assay); IFM (immunofluorescence microscopy); IOS (inside/outside staining); LM (light microscopy); MLA ($^{35}$S-methionine labeling assay); hPIC (human primary intestinal cells), chPIC (chicken primary intestinal cells).
[b] Due to space limitations only a few key references are provided.
[c] Identified as an adhesin in *C. jejuni* strain TGH9011, but no effect observed with *jlpA* mutants in other isolates such as 11168 or 81-176 (van Alphen et al., 2008; Novik et al., 2010). Another report showed that JlpA is not required for adhesion of strain F38011 to chicken LMH cells (Flanagan et al., 2009).

process (Watson and Galan, 2008) and cultured INT-407 or COS-1 cell lines can be transfected more easily than T84 or Caco-2 cells.

Studies of the translocation properties of *C. jejuni* isolates across an intestinal epithelium layer *in vitro* require polarizing cell lines like T84 (Chen et al., 2006; Monteville and Konkel, 2002; Wine et al., 2008; Zheng et al., 2008), Caco-2 (Everest et al., 1992; Grant et al., 1993; Harvey et al., 1999; Hu et al., 2008; Konkel et al., 1992a), MDCK-I (Wine et al., 2008) or MKN-28 cells (Boehm et al., 2012), which express tight and adherens junctions thus properly connecting neighboring cells (Fig. 1). For this purpose, these cell lines are commonly grown as tight monolayers on transwell filter chambers before infection.

Mucus colonisation has been seen as a critical step in the infection process of *C. jejuni* (Beery et al., 1988; Lee et al., 1986). Until recently appropriate *in vitro* models have been missing to investigate the interaction of *Campylobacter* with mucus as most cultured cell lines generally do not synthesise and secrete mucin (Alemka et al., 2012). Lately it has been shown that the HT29-derivative E12 cell line produces a mucus layer when grown and differentiated on transwell filters increasing the binding efficiency of *C. jejuni* to E12 cells and subsequently its internalisation (Alemka et al., 2010; Behrens et al., 2001).

For immunological studies the significant variation in the dynamics of immune responses in cultured epithelial cells upon infection with *C. jejuni* should be considered. For example, it has been demonstrated that stimulation of INT-407 cells with *C. jejuni* leads to significant faster and more pronounced IL-8 secretion compared to stimulation of HeLa cells (MacCallum et al., 2006). The host cell type also seems to play an important role because the cytolethal distending toxin (CDT) has been reported to trigger IL-8 secretion in infected T84 (Zheng et al., 2008) and INT-407 (Hickey et al., 2000) but not in Colo-205 cells (Li et al., 2011).

### 2.3. Multiplicity of infection (MOI)

Differences in the ratio of bacteria to cultured epithelial cells, also referred to as multiplicity of infection (MOI), profoundly influence infection efficiency. Infection studies of INT-407 and Caco-2 cells using MOIs ranging from 0.02 to 20,000 demonstrated that the invasion efficiencies (the percentage of added bacteria that successfully invade cells) of *C. jejuni* 81-176 are highest at low MOIs of about 0.2 to 20 and decrease when higher MOIs are used (Hu and Kopecko, 1999). However, while the infection is less efficient, the use of MOIs of 100 and higher lead to the highest numbers of intracellular bacteria (Hu and Kopecko, 1999). It is possible that the reduced infection efficiencies with very high MOIs are the consequences of increased cytotoxic effects of *C. jejuni* on cultured epithelial cells (Kalischuk et al., 2007; Elmi et al., 2012). Therefore, MOIs between 10 and 500 are used for most *in vitro* infection studies, especially to compare the invasion capabilities of different *C. jejuni* strains or to investigate the intracellular fate of *C. jejuni* by microscopy. Using these MOIs up to 11 bacteria have been found inside infected cultured cells using fluorescence microscopy. COS-1 cells grown in 15 cm cell culture dishes were infected with *C. jejuni* in a ration 1:1000 to reach the critical number of intracellular bacteria for the intracellular proteome analysis of *C. jejuni* (Liu et al., 2012).

### 2.4. Motility and growth phase of bacteria

Many studies have undoubtedly proven that flagellum-driven motility of *C. jejuni* is a crucial requirement for the infection process *in vivo* and to make contact with host cells (Young et al., 2007; Guerry, 2007). Interestingly, recent studies have shown that motility is necessary but not sufficient for the efficient invasion process *in vitro* as the clinical isolates *C. jejuni* BH-01-0142, CG8421 and CG8486 are fully motile when compared to strain *C. jejuni* 81-176 but show significantly reduced invasion capability (Poly et al., 2007, 2008). Nevertheless, up to now many identified *C. jejuni* mutants severely (3 magnitudes or more) impaired in their capability to invade cultured epithelial cells, are negatively affected in their motility. Consequently, comparing the capability of different *C. jejuni* isolates to interact with host cells (Biswas et al., 2000; Ó Cróinín and Backert, 2012), or investigating specific mutants for invasion defects

**Table 2**
Methods applied to study *C. jejuni* invasion of human cells *in vitro*.[a]

| Applied methods | Cell models used | Bacterial factors involved | Host factors involved | *C. jejuni* strains used | References[b] |
|---|---|---|---|---|---|
| IHC, TEM | Human biopsies | ND | ND | 22 clinical isolates | Van Spreeuwel et al. (1985) |
| GPA, PIS | INT-407 | ND | Microtubules | 81-176 | Oelschlaeger et al. (1993) |
| GPA, ABB, PIS, IFM | INT-407 | CadF | FN, Paxillin, F-actin | F38011 | Monteville et al. (2003) |
| SEM, GAA, PIS | INT-407, mouse KO cells | CadF, FlpA, CiaC | Rac1 and Cdc42GTPases | 81-176, 11168, F38011 | Krause-Gruszczynska et al. (2007a, 2011); Boehm et al. (2011); Eucker and Konkel (2012) |
| GPA, ASPAB | INT-407, KO cell lines | CadF, FlpA | Paxillin, FAK,EGFR, PDGFR | 81-176, 11168, F38011 | Monteville et al. (2003); Krause-Gruszczynska et al. (2011); Boehm et al. (2011); Eucker and Konkel (2012) |
| IFM | INT-407, Caco-2, T84 | CadF, LOS | FN, ND | 81-176, 11168, F38011, clinical isolates | Krause-Gruszczynska et al. (2007a); Louwen et al. (2012) |
| T3SS assays, MLA | INT-407 | Flagellum, CiaB, CiaC | ND | 81-176, 11168 | Konkel et al. (1999); Christensen et al. (2009) |
| siRNA KD | INT-407 | CadF, Flagellum, CiaC | Rac1 and Cdc42GTPases, GEFs (Vav2,DOCK180, Tiam1) | 81-176, 11168, F38011 | Krause-Gruszczynska et al. (2011); Boehm et al. (2011); Eucker and Konkel (2012) |
| KO cell lines | Mouse fibroblasts | Flagellum, CadF | FN, integrin β1, FAK, Src kinases, Vav | 81-176, 11168, F38011 | Krause-Gruszczynska et al. (2011); Boehm et al. (2011) |
| DN/CA constructs | INT-407,T84, Cos-1 | CadF, ND | Rac1, RhoA, Cdc42, Vav2, EGFR, PDGFR, Caveolin | 81-176, 11168, F38011 | Krause-Gruszczynska et al. (2007); Boehm et al. (2011); Watson and Galan (2008) |
| Signaling constructs | INT-407, KO cell lines | Flagellum, CadF | Integrin β1, FAK, Vav2 | 81-176, 11168, F38011 | Krause-Gruszczynska et al. (2011); Boehm et al. (2011) |
| GPA, MA, CTA, AAA | Caco-2 | Cj0952c, Cj0951c, SOR | ND | B2, 81-176, 11168 | Tareen et al. (2010, 2011) |

[a] Abbreviations: AAA (autoagglutination assay); ABB (antibody blocking); ASPAB (activation specific phospho antibodies); CTA (chemotaxis assay); DN/CA (dominant-negative/constitutive active); TEM (transmission electron microscopy); FN (fibronectin); GAA (GTPase activation assays including CRIB pulldown and G-lisa); GEF (guanine exchange factor); GPA (gentamycin protection assay); IFM (immunofluorescence microscopy); IHC (immunhistochemistry); KD (knockdown); KO (knockout); LOS (Lipooligosaccharide); MA (motility assay); PIS (pharmacologoical inhibitor studies); MLA ($^{35}$S-methionine labelling assay); ND (not determined); SEM (scanning electron microscopy); siRNA (small interfering RNA); SOR (sulphite: cytochrome c oxidoreductase); T3SS assays (transolaction assay using the *Yersinia* flagellar type III secretion apparatus).
[b] Due to space limitations only a few key references are provided.

require the thorough pre-testing of their motility properties. Only if the tested strains exhibit a similarly high motility both on semi-solid motility agar plates and in liquid cultures, observed differences in adherence or invasion properties are potentially mediated by motility-independent virulence factors. Motility pre-tests with semi-solid motility agar plates are not always sufficient as *C. jejuni* mutants can lose motility upon cultivation in liquid medium and behave like the wild-type strain on semi-solid motility agar plates (Goon et al., 2006; Novik et al., 2010).

Motility is an important phase variable property of *C. jejuni* (Hendrixson, 2006, 2008; Karlyshev et al., 2002). *C. jejuni* motility is preferentially lost during *in vitro* cultivation (Gaynor et al., 2004), but can be regained throughout infection experiments (Jones et al., 2004; Caldwell et al., 1985). Especially during the process of generating isogenic *C. jejuni* mutants the resulting strains have to be screened for altered motility in comparison to the wild-type strain. To compensate the negative effect of slight motility alterations on the adherence efficiency of *C. jejuni* strains, many *in vitro* infection studies take advantage of an additional centrifugation step. For this purpose, *C. jejuni* can be enforced to interact with the epithelial cell monolayer using a centrifugation step for 5 min at 1000 ×*g*. This represents a suitable method to synchronise the infection process in order to overcome variations in the interaction of *C. jejuni* strains with cultured epithelial cells that are due to variable motility properties rather than altered adherence or invasion characteristics.

The motility of *C. jejuni* is not only phase variable as discussed above, but also growth-phase dependent. It peaks during the late logarithmic growth phase and diminishes during the stationary phase (Wösten et al., 2004). These findings imply that the growth phase of *C. jejuni* plays an additional role for the *in vitro* infection process (Konkel et al., 1992a) and it was shown that *C. jejuni* from mid-logarithmic growth cultures showed higher invasion efficiency than bacteria from early stationary phase (Hu and Kopecko, 1999). An important disadvantage of using agar plate grown *C. jejuni* for infection experiments is that the bacteria vary significantly in shape, flagellation, motility and viability (Ng et al., 1985a). Thus, before infection, the subcultivation of *C. jejuni* in liquid culture is highly recommended (Ng et al., 1985a). It has been shown that *C. jejuni* growth in liquid broth medium led to a higher percentage of highly motile *C. jejuni* cells with similar shape.

## 3. Host cell adhesion by *C. jejuni*

Though the precise molecular mechanisms involved in the attachment of *C. jejuni* to eukaryotic cells are still unknown, several studies have provided evidence in recent years that the adhesion process of *C. jejuni* is a multifactorial event (Young et al., 2007; Ó Cróinín and Backert, 2012). This is reflected by the circumstances that most of the described adherence-defective mutants exhibit only reduced rather than severely abolished interactions with the eukaryotic cells wherein the mutants reach adherence level of about 20% to 50% of the wild-type strain. These observations imply that redundancy may exist in the adhesion process and suggest that the co-operative action of several factors of *C. jejuni* is required to mediate optimal adherence to host cells.

### 3.1. Recovery of cell-associated bacteria

Traditionally, the adherence of *C. jejuni* to eukaryotic cells has been investigated by cell association experiments (Everest et al., 1992; Fauchere et al., 1986; Russell and Blake, 1994). These approaches enumerate all bacteria that adhere to and invade eukaryotic cells but fail to distinguish between these two populations and therefore provide no direct information about the number of bacteria solely attached to the surface of the host cell at a given time point of infection. Determination of colony forming units (CFU) has generally been used to characterise

**Table 3**
Methods applied to study *C. jejuni* migration across polarized human cells *in vitro*.[a]

| Applied methods | Cell models used | Bacterial factors involved | Host factors involved | *C. jejuni* strains used | References[b] |
|---|---|---|---|---|---|
| TWA, PIS, SEM, TEM | Caco-2 | ND | ND | 78-27, 81116, M129, F38011 | Konkel et al. (1992c) |
| CPT, TER | Caco-2 | ND | ND | 6 clinical isolates | Brás and Ketley (1999) |
| TWA | Caco-2, T84 | ND | ND | 81-176, F38011 | Monteville and Konkel (2002) |
| IFM, TER | Caco-2 | ND | Occludin | 11168 | MacCallum et al. (2005) |
| TWA, TEM | Caco-2, T84, HCA-7 | ND | ND | 81-176, 11168, 12189, 2801055 | Hu et al. (2008); Beltinger et al. (2008) |
| TWA, IHS, IFM | T84 | FlaA/B | Lipid rafts, caveolin | 81-176, CHR213 | Kalischuk et al. (2009) |
| ECA, TWA, TER, CA | MKN28 | HtrA | E-cadherin | 81-176, 11168 | Boehm et al. (2012); Hoy et al. (2012) |
| IFM, ISA | Caco-2, T84 | LOS | Late endosomal markers | 81-176, 11168, 34 clinical isolates | Louwen et al. (2012) |

[a] Abbreviations: CA (Casein assays); CPT (cell permeability test using $^{14}$C-Inulin labelling); ECA (E-cadherin cleavage assays), TEM (transmission electron microscopy); IFM (immunofluorescence microscopy); ISA (intracellular survival assay); LOS (Lipooligosaccharide); PIS (pharmacological inhibitor studies); ND (not determined); SEM (scanning electron microscopy); TER (transepithlial electrical resistance); TWA (transwell assay).
[b] Due to space limitations only a few key references are provided.

the attachment or association of *C. jejuni* to cultured eukaryotic cells and to identify bacterial factors involved in the adherence process (Table 1). The outcome of these assays depends on the plating efficiency of *C. jejuni*, which varies for different culture media (Ng et al., 1985b). Therefore, culture variations have also to be considered when comparing different studies.

For the standard CFU-based adherence (CBA) assay *C. jejuni* strains are added with a defined MOI to cultured epithelial cells in 24 well tissue plates. Non-adherent *C. jejuni* cells are removed by a repeated washing procedure before epithelial cells are lysed with 0.1% Triton X-100, 0.1% sodium deoxycholate (DOC) solutions or physical rupture. With this approach all cell-associated bacteria, either attached to the membranes or released from the intracellular compartments of infected cells, are spread in serial dilutions on appropriate agar plates to determine the bacterial counts. The cell association efficiency of *C. jejuni* is then calculated by dividing the number of bacteria determined by plate counting (CFU) after lysing the cultured epithelial cells by the total number of bacteria in the infection inoculum. This assay does not allow the direct quantification of extracellular attached bacteria as all cell-associated bacteria including intracellular bacteria are recovered after cell lysis. The accurate number of adherent bacteria can only be calculated when cell association experiments are performed in combination with a gentamicin protection assay (see below) that allows the determination of intracellular bacteria (see Section 6.1).

The CBA assay does not require expensive laboratory equipment, is easy to handle and enables the fast and economical test of *C. jejuni* mutants for their adherence properties. As such the assay has been successfully used to screen *C. jejuni* transposon mutant libraries for mutants with reduced capability to interact with cultured epithelial cells (Golden and Acheson, 2002; Javed et al., 2010; Novik et al., 2010). Since the experimental procedure usually involves the use of 0.1% DOC or 0.1% Triton X-100 to lyse the epithelial cell monolayer, potential adherence- or invasion-defective mutants have to be additionally tested for the susceptibility against these detergents. It is known that in particular inactivation of the *cme*-Gene cluster leads to hypersensitivity *C. jejuni* mutants against DOC (Lin et al., 2003; Raphael et al., 2005) and such mutants are overrepresented in CBA transposon mutant screens as false positive hits (Novik et al., 2010). Therefore it is recommended to retest potential mutants with a CBA assay that involves physical rupture of the cultured cells (Gaynor et al., 2005; Novik et al., 2010) instead of 0.1% DOC or 0.1% Triton X-100.

As an alternative to the CBA assay, experiments with radioactively-labelled *C. jejuni* have also been used. For this approach *C. jejuni* is radioactively marked prior to the infection of cultured epithelial cells by culturing the bacteria in medium with tritium-labeled acetic acid (McSweegan and Walker, 1986; Russell and Blake, 1994) or with [$^{35}$S]-methionine (Newell et al., 1985a). At the end of the infection experiment the cell monolayer is washed and lysed to release *C. jejuni*. The number of cell-associated *C. jejuni* is quantified by measuring the radioactivity with a liquid scintillation counter. Similar to the CBA assay described above, this approach does not allow the direct quantification of extracellular attached bacteria, which have to be determined in combination with a gentamicin protection assay.

To date, most *C. jejuni* mutants defective for efficient interaction (adhesion/invasion) with cultured epithelial cells have been characterised by the CBA/invasion assay (Table 1). This approach identified motility and chemotaxis (Yao et al., 1994, 1997; Tareen et al., 2010, 2011) as critical factors mediating the interaction of *C. jejuni* with host cells, but revealed also the important role of glycan surface structures for the adherence/invasion process of *C. jejuni*. In particular, inactivation of the capsule biosynthesis genes *kpsM* (Bacon et al., 2001) and *kpsE* (Bachtiar et al., 2007), the LOS biosynthesis gene *galE* (Fry et al., 2000) and the genes *pglB* and *pglE* (Szymanski et al., 2002) involved in the N-glycosylation of *C. jejuni* proteins resulted in a adherence defective phenotype. The *C. jejuni* 81-176 *pseA* mutant, unable to modify flagellin with the acetamidino form of pseudaminic acid, shows a decreased adherence to INT-407 cells (Guerry et al., 2006). Furthermore the involvement of the fibronectin-binding protein CadF (Monteville et al., 2003), the fibronectin-like protein A FlpA (Flanagan et al., 2009), the autotransporter protein CapA (Ashgar et al., 2007) and the HtrA protease (Brøndsted et al., 2005; Bæk et al., 2011; Hoy et al., 2012) in the adherence process have been identified by CBA assays. The surface-exposed lipoprotein JlpA has been described to facilitate the binding of *C. jejuni* TGH9011 to HEp-2 cells (Jin et al., 2001) but is surprisingly dispensable for the adherence of *C. jejuni* F38011 to chicken LMH hepatocellular carcinoma epithelial cells (Flanagan et al., 2009). Interestingly, although the *jlpA* mutant shows a reduced adherence phenotype by 20% of wild-type level (Jin et al., 2001), it is not defective for invasion (Flanagan et al., 2009; Novik et al., 2010; van Alphen et al., 2008). Another putative lipoprotein, the hypothetical protein Cj0497 of the hyper-invasive strain *C. jejuni* 01/51, has been identified as adherence factor by screening a transposon mutagenesis library for invasion defective mutants (Javed et al., 2010).

Though the antigenic protein Peb1A was originally identified as potential pathogenicity factor of *C. jejuni* 81-176 mediating adhesion to HeLa cells and invasion into INT-407 cells (Pei et al., 1998), it has been recently characterised as a periplasmic aspartate/glutamate-binding protein (Leon-Kempis Mdel et al., 2006; Muller et al., 2007) required for the utilisation of certain amino acids as growth substrates *in vitro* (Hofreuter et al., 2008; Leon-Kempis Mdel et al., 2006). These findings question the primary function of Peb1A as adhesin/invasin, especially since *peb1A* mutants exhibited no significant invasion defects in recent infection studies (Flanagan et al., 2009; Novik et al., 2010; van Alphen et al., 2008). It is feasible that the overnight-culture of the *C. jejuni* 81-176 *peb1A* mutant used for the *in vitro* infection experiment by Pei et al. (1998) lost the metabolic fitness required for the efficient invasion process as consequence for its inability to catabolise growth-supporting glucogenic amino acids. A similar phenotype has been described for the

**Table 4**
Methods applied to study *C. jejuni* intracellular survival in cultured epithelial cells[a].

| Applied method | Cell model used | Bacterial factors involved | Host factors involved[b] | *C.jejuni* strains used | References[c] |
|---|---|---|---|---|---|
| ISA | HEp-2 | ND | ND | Human isolates | De Melo et al. (1989) |
| ISA, IFM | INT-407, Caco-2 | ND | Microtubules, Dynein | 81-176 | Hu and Kopecko (1999) |
| ISA | INT-407 | SpoT | ND | 81-176 | Gaynor et al. (2005) |
| ISA | INT-407, IPEC-1 | FeoB | ND | 81-176 | Naikare et al. (2006) |
| ISA | INT-407 | Ppk1 | ND | 81-176 | Candon et al. (2007) |
| ISA, LDS, IFM | T84, Cos-1 | ND | EEA-1, PX-GFP, Rab4, Rab5, Lamp-1, GM130 | 81-176 | Watson and Galan (2008) |
| ISA | INT-407 | CprS | ND | 81-176 | Svensson et al. (2009) |
| ISA | T84, Cos-1 | VirK | ND | 81-176 | Novik et al. (2009) |
| ISA | Caco-2 | WaaF | ND | 81-176 | Naito et al. (2010) |
| ISA, LDS | T84 | SodB | ND | 81-176 | Novik et al. (2010) |
| ISA | INT-407 | Ppk2 | ND | 81-176 | Gangaiah et al. (2010) |
| ISA | INT-407, Hela | CiaI | Lamp-1 | F38011 | Buelow et al. (2011) |
| ISA | INT-407 | TatC | ND | 81-176 | Drozd et al. (2011) |
| ISA | T84 | AspA | ND | 81-176 | Liu et al. (2012) |
| ISA | T84 | FrdA | ND | 81-176 | Liu et al. (2012) |
| ISA | Caco-2 | Cst-II | EEA-1, Rab5, Lamp-1 | 81-176, GB11, GB19 | Louwen et al. (2012) |
| ISA, IFM, LRA | Caco-2 | ND | Lamp-1, CD63 | 81-176, 108 | Bouwman et al. (2013) |

[a] Abbreviations: IFM (immunofluorescence microscopy); ISA (intracellular survival assay); LDS (live/dead staining), LRA (luciferase reporter assay).
[b] Co-localisation of the indicated marker proteins occurs in a certain temporal fashion during the CCV maturation and represents merely an indirect indication for the host factors being involved in CCV development.
[c] Due to space limitations only a few key references are provided.

*aspA* and *aspB* mutants, defective in the catabolism of aspartate and glutamate, respectively (Guccione et al., 2008). Both mutants are recovered with reduced CFU in invasion assays, which can be compensated by fumarate supplementation in the culture medium (Novik et al., 2010). Though Peb1A plays probably no role in the direct interaction with host cells *in vitro*, it has a crucial role for the proliferation and persistence of *C. jejuni in vivo* as demonstrated in various animal infection studies (Flanagan et al., 2009; Hofreuter et al., 2012; Pei et al., 1998).

### 3.2. Immunofluorescence microscopy (IFM)

The above described CBA assay alone is not suitable to characterise the interaction of *C. jejuni* with individual cultured host cells. In order to gain more detailed information about the adherence process of *C. jejuni*, IFM studies have gained increased importance in recent years. The availability of *C. jejuni* strains expressing fluorescence proteins like GFP, its derivates or mCherry (Miller et al., 2000; Mixter et al., 2003; Krause-Gruszczynska et al., 2007a; van Alphen et al., 2008; Guerry et al., 2006) has facilitated studies imaging the interaction of *C. jejuni* with cultured host cells. Alternatively, immunostaining experiments with antibodies raised against *C. jejuni* (Apel et al., 2012; Byrne et al., 2007; Hu and Kopecko, 1999; Konkel et al., 2007; Louwen et al., 2012; Monteville et al., 2003; Novik et al., 2009, 2010; Watson and Galan, 2008) or direct labelling of *C. jejuni* cells by using a fluorescent dyes like FITC (Song et al., 2004), Texas-Red (Buelow et al., 2011) or TAMRA (Alemka et al., 2010; Byrne et al., 2007) have been successfully employed to investigate the infection process *in vitro*. Especially the inside outside staining approach overcomes the restriction of the above-described CFU-based assay and allows a precise quantification of the *C. jejuni* subpopulations which are extracellularly attached to the cultured cells or residing intracellularly (Apel et al., 2012; Byrne et al., 2007; Pryjma et al., 2012; Watson and Galan, 2008). IFM experiments have been also used to complement the results gained from CBA assay (Table 1). Recently, IFM-based infection experiments have demonstrated that efficient adherence of *C. jejuni* wild-type strains to Caco-2 cells occurs within 15 minutes and that ganglioside-like LOS enhances this interaction (Louwen et al., 2012). Consequently mutants with an inactivated sialyltransferase *cst-II* gene, which are unable to produce a ganglioside-like LOS, exhibit a significant adhesion defect (Louwen et al., 2012).

## 4. Cellular invasion of *C. jejuni*

### 4.1. Gentamicin protection assay (GPA) and microscopic methods

Early reports investigated intestinal biopsies from human patients using immunohistochemistry and transmission electron microscopy (TEM) and have shown that *C. jejuni* can be found in compartments within gut tissue cells *in vivo* (van Spreeuwel et al., 1985). Later, a multitude of studies have demonstrated that *C. jejuni* can invade and survive within a number of cultured cell lines *in vitro*, notably studies using non-polarised INT-407, Hep-2 or HeLa and polarised T84, MKN-28 or Caco-2 cells (Table 2). Mutagenesis of numerous *C. jejuni* genes involved in adhesion, glycosylation, capsular formation, bacterial motility and host adaptation or survival have been implicated to play a role during invasion (Grant et al., 1993; Szymanski et al., 2002; Yao et al., 1994; Pei et al., 1998; Bacon et al., 2001; Konkel et al., 2001; Hendrixson and DiRita, 2004; Kakuda and DiRita, 2006; Krause-Gruszczynska et al., 2007b; Watson and Galan, 2008; Novik et al., 2010). A widely-used but indirect laboratory method to monitor adherence and invasion is the gentamicin protection assay (GPA). This method uses the antibiotic substance gentamicin to kill all extracellular bacteria after infection, but is unable to penetrate host cells. Thus, intracellular bacteria stay alive and can be quantified as CFU on agar plates. However, this method is indirect and also bears some discussed limitations (Ó Cróinín and Backert, 2012). Direct visualisation of entering or intracellular *C. jejuni* in *in vitro* infected cells was performed by IFM, live cell imaging (LCI) and higher resolution approaches such as TEM or scanning electron microscopy (SEM), which were applied in some but not all studies (Table 2). For example, IFM was used to investigate the association of internalised *C. jejuni* with microtubules and the molecular motor protein dynein (Hu and Kopecko, 1999), filamentous actin (Konkel et al., 1992a; Rivera-Amill et al., 2001; Krause-Gruszczynska et al., 2007a), the Golgi protein GM130 (Watson and Galan, 2008) and the small Rho GTPases Rac1 and Cdc42 (Krause-Gruszczynska et al., 2007a; Eucker and Konkel, 2012).

In addition, Louwen et al. (2008, 2012) investigated whether sialylation of *C. jejuni* LOS structures, generating human nerve ganglioside mimetics, is important for intestinal epithelial invasion. They showed that *C. jejuni* isolates expressing ganglioside-like LOS bound to and invaded Caco-2 cells in larger numbers than Δ*cstII* mutants or natural isolates lacking such structures as determined by GPA and

quantitative IFM. Various TEM and SEM studies were then applied to visualise tightly bound *C. jejuni* to various host cell types or intracellular bacteria (Oelschlaeger et al., 1993; Hu et al., 2008; Watson and Galan, 2008; Eucker and Konkel, 2012). Investigation of infected INT-407 and other cells indicated that *C. jejuni* can induce membrane ruffling in a contact-dependent fashion (Krause-Gruszczynska et al., 2007a; Eucker and Konkel, 2012) and is associated with a specific host cell penetration mechanism, first entering with its flagellar tip followed by the opposite flagellar end (Krause-Gruszczynska et al., 2007a, 2011; Boehm et al., 2011).

### 4.2. Combination with inhibitor studies

To study the mode of *C. jejuni* host cell internalisation, commonly one (or two) of the above quantification and visualisation methods can be combined with certain functional assays. For example, GPA combined with pharmacological inhibitor studies indicated that mitogen-activated protein (MAP) kinases, protein kinase C (PKC) and phosphatidylinositol 3-kinase (PI3-kinase) are involved in signalling cascades permitting *C. jejuni* host cell entry (Wooldridge et al., 1996; Biswas et al., 2000; Hu et al., 2006). GPA in the presence of the lipid raft-disrupting substances methyl-beta cyclodextrin or filipin-III as well as specific protein toxins also reduced the numbers of intracellular CFU, suggesting that host caveolae structures and heterotrimeric G proteins may play a role in epithelial cell invasion by *C. jejuni* (Wooldridge et al., 1996; Hu et al., 2006; Watson and Galan, 2008). Further inhibitor-based studies have indicated that $Ca^{2+}$ release from intracellular stores (Hu et al., 2005), the activities of MAP kinase members ERK1/2 and p38 (Hu et al., 2006) as well as the activities of the tyrosine kinases EGF receptor, PDGF receptor, Src and focal adhesion kinase, FAK (Krause-Gruszczynska et al., 2011; Eucker and Konkel, 2012) are crucial for the recovery of internalised bacteria by GPA. In addition, host cell microfilaments (MFs, composed of actin subunits) and microtubules (MTs, composed of tubulin subunits) are known to play an important role in the invasion of certain bacterial and viral pathogens. To study their importance for *C. jejuni* invasion, GPA was done in the presence of inhibitors against MFs (e.g. cytochalasin B and latrunculin A) or MTs (e.g. nocodazole and colchicine), but the results of various groups provide a rather confusing picture. In numerous reports, *C. jejuni* uptake has been described to require MTs (Oelschlaeger et al., 1993; Hu and Kopecko, 2008), MFs (de Melo et al., 1989; Konkel and Joens, 1989), both MTs and MFs (Biswas et al., 2003; Monteville et al., 2003; Oelschlaeger et al., 1993), or neither MTs or MFs (Russell and Blake, 1994). The reason for these discrepancies is unknown. In addition, it should be noted that it is not yet clear whether the above signalling components are directly involved in the bacterial entry step or other events linked to intracellular survival of *C. jejuni*. Thus, inhibitor studies have clearly validity limitations. They can only serve as accompanying experiments and must be corroborated by other studies.

### 4.3. Use of activation-specific phospho antibodies to study invasion-related signal transduction

In agreement with some of the inhibitor studies described above, recent data using activation-specific phospho antibodies and Western blotting showed that infection with wild-type *C. jejuni* stimulated the phosphorylation of ERK1/2 and p38 MAP kinases during the first two hours (Hu et al., 2006; Watson and Galán, 2005). In addition, the autophosphorylation of EGF receptor at tyrosine residues Y-845 and Y-1068 (Krause-Gruszczynska et al., 2011; Eucker and Konkel, 2012), PDGF receptor at Y-754 and FAK at Y-397 (Krause-Gruszczynska et al., 2011) is also triggered by wild-type *C. jejuni* infection in a time-dependent fashion, and correlating with the time points of bacterial uptake. Moreover, FAK was shown to be phosphorylated at another tyrosine residue, Y-925, which has a proposed role in FAK downstream signalling to ERK1/2 and GTPases (Boehm et al., 2011). Parallel experiments using an isogenic Δ*cadF* deletion mutant in a time course showed that phosphorylation of FAK, EGF receptor and PDGF receptor was widely impaired (Krause-Gruszczynska et al., 2011; Boehm et al., 2011). Another signalling molecule, paxillin, also exhibited increased levels of tyrosine phosphorylation upon *C. jejuni* infection, which was not seen during infection with mutants of CadF (Monteville et al., 2003). These data support the view that the CadF → fibronectin interaction is upstream of various tyrosine kinases and other signalling molecules involved in *C. jejuni*-triggered host cell invasion.

### 4.4. Application of GTPase activation assays

The GTPases Rac1, Cdc42 and RhoA are the best-characterised members of the small Rho family of GTP-binding proteins. They act as guanine nucleotide regulated switches, transmitting extracellular signals to regulate normal intracellular functions as well as invasion of various microbial pathogens (Boquet and Lemichez, 2003; Tegtmeyer et al., 2011). With the help of specific inhibitors (e.g. compactin) and GTPase-regulating toxins (e.g. CNFs, Toxin B) it was further shown by IFM, GPA and other methods that Rac1 and Cdc42 (but not RhoA) play a role in the cellular entry process of *C. jejuni* (Krause-Gruszczynska et al., 2007a). The uptake of *C. jejuni* into INT-407 cells is accompanied by a time-dependent accumulation of both increased Rac1-GTP and Cdc42-GTP levels as determined by classical CRIB-domain pulldown and G-LISA approaches (Krause-Gruszczynska et al., 2007a, 2011; Eucker and Konkel, 2012; Boehm et al., 2011). The induction of maximal GTPase activities by *C. jejuni* appears to require the fibronectin-binding proteins CadF and FlpA, the flagellum and the *Campylobacter* invasion antigen CiaC as well as the activities of FAK, Src, EGF receptor, PDGF receptor and PI3-kinase, as confirmed by inhibitor experiments (Krause-Gruszczynska et al., 2007a, 2011; Eucker and Konkel, 2012). IFM studies with wild-type and mutant *C. jejuni* also indicated that CiaC is required for re-localising Rac1 to the site of bacterial attachment (Eucker and Konkel, 2012).

### 4.5. Reporter assay to monitor injection of Cia proteins into host cells

One pressing question in the *C. jejuni* field is whether Cia and probably other pathogenicity-associated proteins can be delivered from the bacterium into the host cell cytoplasm in order to function as invasion-triggering molecules. To address this question, Neal-McKinney and Konkel (2012) utilised a reporter assay approach based on the adenylate cyclase domain of the *Bordetella pertussis* CyaA toxin expressed as a fusion construct with Cia proteins. The adenylate cyclase enzyme is inactive in the bacterium and activated in the presence of the eukaryotic factor calmodulin. Delivered adenylate cyclase then catalyses the production of cAMP from isotope-labelled ATP given to infected cells, which can be easily monitored in the assays. Expression of a CiaC-adenylate cyclase fusion protein exhibited significantly increased cAMP levels during infection with wild-type *C. jejuni* as compared to mutants of FlgE, FlgK and FlgL flagellar hook proteins (Neal-McKinney and Konkel, 2012). Similar results were obtained for another Cia protein, CiaI, suggesting that the flagellum is necessary for the delivery process (Neal-McKinney and Konkel, 2012). However, more functional studies are necessary to investigate this fascinating hypothesis in greater detail.

### 4.6. siRNA knockdown and useful mouse knockout cell lines

To investigate if a given host factor plays a role in *C. jejuni* invasion, the small interfering RNA (siRNA) approach can be employed to downregulate the expression of any gene of interest. This method was exemplarily used to confirm the importance of Cdc42 (Krause-Gruszczynska et al., 2011) and Rac1 (Boehm et al., 2011) for *C. jejuni* host cell entry. In addition, the siRNA method was applied to identify guanine exchange factors (GEFs), signalling compounds that can interact with GTPases and promote the active,

GTP-bound state by facilitating the exchange of GDP for GTP. In this way, two host cell GEFs (Tiam-1 and DOCK180) were downregulated and shown to mediate Rac1 activation downstream of FAK (Boehm et al., 2011; Eucker and Konkel, 2012). In addition, another GEF, Vav2, appears to act downstream of FAK, EGF receptor, PDGF receptor and PI3-kinase and stimulates the generation of elevated Cdc42-GTP levels (Krause-Gruszczynska et al., 2011). These studies were complemented with experiments using available fibroblast cell lines from knockout mice. Such knockout cell lines have the great advantage over siRNA studies that the respective gene of interest is completely deleted in the genome, and thus not even small traces of protein can be expressed. In fact, SEM and GPA studies showed that *C. jejuni* is severely impaired in its capability to enter cell lines derived from fibronectin$^{-/-}$, integrin-β1$^{-/-}$, focal adhesion kinase (FAK)$^{-/-}$, Vav1/2$^{-/-}$and Src$^{-/-}$/Yes$^{-/-}$/Fyn$^{-/-}$ (SYF) triple knockout mice, but can efficiently invade the corresponding wild-type control cells (Krause-Gruszczynska et al., 2011; Boehm et al., 2011). Finally, infection of integrin-β1$^{-/-}$ knockout cells stably expressing the integrin-β1 point mutant TT788/89AA exhibiting proper FAK signaling but defects in extracellular fibronectin fibril formation, and integrin-β1-Y783/795F cells, which have a pronounced defect in FAK autophosphorylation at Y-397, revealed large defects in *C. jejuni* invasion (Krause-Gruszczynska et al., 2011). Interestingly, the activation of Rac1 and Cdc42 by *C. jejuni* associated with the induction of membrane ruffling and filopodia formation was also significantly abrogated in infected fibronectin$^{-/-}$, integrin-β1$^{-/-}$, FAK$^{-/-}$ and SYF$^{-/-}$ knockout cells, although the bacteria expressed intact flagella and adhered to cells (Krause-Gruszczynska et al., 2011; Boehm et al., 2011). Collectively, these results strongly support the view that *C. jejuni* infection induces fibronectin-/integrin-dependent signal transduction to stimulate their own uptake by host target cells using signalling to activate the Rho GTPase members Rac1 and Cdc42.

### 4.7. Application of dominant-negative and constitutive active expression constructs

A common method in cell biology is the expression of dominant-negative (DN) and constitutive-active (CA) constructs in order to investigate the importance of a given factor of interest. A dominant-negative mutation adversely affects the normal, wild-type gene product within the same cell. This commonly occurs if the protein can still interact with the same elements as the wild-type protein, but inhibits its function. In contrast, a constitutively active protein is a mutated gene product whose activity is constant and active. Canonical point mutations that affect the GTPase cycle are well-described and lead to the generation of CA or DN Rho GTPase constructs (Heasman and Ridley, 2008). To confirm that Rac1 and Cdc42 activity was required for *C. jejuni* invasion, INT-407 cells were transfected with the DN alleles of Rac1 (Rac1-T17N) or Cdc42 (Cdc42-T17N). Transfection of INT-407 cells with both DN-Rac-1 and DN-Cdc42 resulted in a significantly reduced bacterial internalisation as determined by GPA and IFM; while expression of either DN-Rac1 or DN-Cdc42 alone was less effective (Krause-Gruszczynska et al., 2007a). When CA GTPase constructs were expressed the most pronounced effect was observed with CA-Rac1 (Rac-1-Q61L) whose expression stimulated *C. jejuni* invasion ~4.7-fold as compared to the empty vector control, while moderate effects (~1.7-fold increase) were seen with CA-Cdc42 (Cdc42-Q61L) (Krause-Gruszczynska et al., 2007a). Thus, activation of Rac1 and Cdc42 are involved in *C. jejuni* invasion. In addition, overexpression of DN constructs of caveolin-1 but not GTPase dynamin-II substantially inhibited *C. jejuni* uptake, suggesting that caveolin-1 in the classical caveolae structures of cell surfaces may also play a role in the uptake process (Watson and Galan, 2008). Finally, expression of DN forms PDGF and EGF receptors significantly reduced the amount of recovered intracellular *C. jejuni* by GPA, confirming the involvement of both receptor tyrosine kinases in the uptake of *C. jejuni* (Krause-Gruszczynska et al., 2011).

### 4.8. Application of other transient expression constructs

To further investigate the potential involvement of lipid rafts or caveolae in *C. jejuni* invasion, Watson and Galan (2008) examined the acquisition of caveolin-1 and flotillin-1, two markers associated with these membrane domains, to invading bacteria. Expression of the GFP (green fluorescence protein)-tagged proteins and LCI have shown that *C. jejuni* acquired caveolin-1-GFP and flotillin-1-GFP immediately after uptake (Watson and Galan, 2008). Quantification of this association determined that at early time points during infection, 60% of the *C. jejuni* containing vacuoles (CCVs) co-localised with both caveolin-1-GFP and flotillin-1-GFP. The association, however, was transient since at later points after infection, only ~10% of the CCVs were seen in association with these markers. In addition, the signalling of FAK and Vav2 was further investigated using transient transfection of point mutant constructs exhibiting specific signalling defects. First, FAK$^{-/-}$ knockout cells re-expressing wild-type FAK restored the capability of *C. jejuni* to invade these cells as determined by GPA, while expression of FAK mutants that were either not capable of autophosphorylation (FAKY397F), impaired kinase activity (FAK K454R), mutated in a binding site for the adapter protein Grb2 (FAKY925F), or mutated in several proline residues of two PxxP-motifs (FAKΔPR1/2) necessary for association with SH3-domain containing factors such as Graf and p130CAS, significantly reduced the invasion rates of *C. jejuni* by about 35–50% (Boehm et al., 2011). This indicates an important role of FAK signalling downstream of fibronectin and integrins in facilitating efficient uptake of *C. jejuni*. Second, INT-407 cells were transiently transfected with wild-type Vav2 and different Vav2 mutants that were either impaired in EGF receptor-dependent phosphorylation of Vav2 (Vav2 Y172/159F), lacking the primary phosphatidylinositol-3,4,5-triphosphate binding site (Vav2 R425C) or were unable in binding to activated EGF receptor (Vav2 W673R and Vav2 G693R) (Tamás et al., 2003). GPA revealed that overexpression of either Vav2 mutant construct significantly reduced the number of intracellular *C. jejuni*, further confirming the importance of Vav2 in bacterial uptake (Krause-Gruszczynska et al., 2011). These findings also support the view that Vav2, by binding to and signaling through *C. jejuni*-induced EGF/PDGF receptors and PI3-kinase activation pathways, may contribute Cdc42 activation during infection (Krause-Gruszczynska et al., 2011).

## 5. *C. jejuni* transmigration across polarised epithelial cells

Cell polarity of the epithelium is an important functional and protective feature in the gut system *in vivo*. While most of the above studies were done with non-polarised cells, polarised intestinal epithelial cells exhibit apical and basolateral surfaces that are separated by tight and adherens junctions (Fig. 1). They form microvilli structures with a well-defined brush border and express several defined marker proteins. Various important gut pathogens such as *Salmonella*, *Shigella* and *Listeria*, however, developed strategies to cross this epithelial barrier, gain access to submucosal tissues, trigger tissue damage and cause disease in humans. Generally, there are two different mechanisms, described as the paracellular and the transcellular transmigration routes (Kazmierczak et al., 2001; Tegtmeyer et al., 2011). Pathogens utilising the paracellular mechanism cross the epithelial barrier by passage between neighbouring epithelial cells and overcome the tight and adherens junction complexes. In contrast, pathogens specialised on the transcellular mechanism invade host cells at the apical membrane followed by intracellular trafficking and exit these cells at the basolateral membrane (Balkovetz and Katz, 2003).

### 5.1. Transwell assays and electron microscopy

Polarised cell models are particularly valuable for the study of microbial effects on cell barrier permeability, transepithelial electrical resistance (TER), mode of transmigration and cell invasion. To investigate whether

*C. jejuni* can cross the epithelial cell barrier reflective of a virulence mechanism, the transwell filter system has been applied (Table 3). For this purpose, various cell lines were seeded and differentiated for a few weeks in transwells. TER values were followed over time to ensure proper cell monolayers and junction formation, which have been confirmed by SEM illustrating the presence of normal microvilli and well-defined brush borders (Konkel et al., 1992c; Hu et al., 2008) as well as IFM staining for E-cadherin (adherens junction marker) and JAM (junction adhesion molecule, tight junction marker) as described (Boehm et al., 2012). SEM and TEM studies were used to compare the interaction of *C. jejuni* with non-polarised INT-407 cells with polarised Caco-2 cells. The efficiency of *C. jejuni* invasion of Caco-2 cells was two- to three fold less as compared to INT407 cells (Hu et al., 2008). *C. jejuni* bound to most INT407 cells and entered about two-thirds of the host cells over 2 h (two bacteria/cell). In contrast, only 11–17% of differentiated Caco-2 cells were observed to bind and internalise either *C. jejuni* strains 81-176 or NCTC11168, and a small percentage of infected Caco-2 cells contained 5–20 internalised bacteria per cell after 2 h (Hu et al., 2008). Further SEM demonstrated that *C. jejuni* were present intercellularly between two neighboring cells as well as intracellularly in CCV compartments (Konkel et al., 1992c; Hu et al., 2008). It was therefore suggested that *C. jejuni* can translocate across polarised Caco-2 cell monolayers both by passing through and between cells as summarised in Fig. 1.

### 5.2. Determination of CFU and functional studies

Migration of *C. jejuni* through polarised cells was confirmed by determination of CFU in the lower chamber of transwells, GPA and other functional assays. Chloramphenicol, an inhibitor of bacterial protein synthesis, reduced the translocation rates of *C. jejuni* (Konkel et al., 1992c). *C. jejuni* adherence, internalisation, and translocation were also inhibited at lower temperatures as examined at 20 °C and 4 °C compared to 37 °C (Konkel et al., 1992c). These data indicate that adherence, penetration, and translocation of *C. jejuni* require active bacterial and host cell processes. However, similar to the TEM studies described above some researchers supported the paracellular route as major transmigration pathway (Grant et al., 1993; Monteville and Konkel, 2002; Boehm et al., 2012), while other groups favoured the transcellular mode (Brás and Ketley, 1999; Kalischuk et al., 2009; Louwen et al., 2012) or proposed a mix of both (Konkel et al., 1992c; Hu et al., 2008). Because bacterial penetration through tight and adherens junctions might be expected to cause a loss in TER, one major argument for the transcellular mode is that TER values often did not change upon *C. jejuni* infection. In addition, the application of lipid raft inhibitors to the apical surface reduced the number of transmigrated CFU (Brás and Ketley, 1999; Kalischuk et al., 2009). Interestingly, *C. jejuni* induced not only its own internalisation and translocation but also that of commensal *Escherichia coli*, without affecting TER (Kalischuk et al., 2009).

Major arguments for the paracellular route came from competition experiments with soluble fibronectin and the observation that invasion-defective Δ*ciaB* or Δ*cadF* mutants transmigrated as effectively as wild-type *C. jejuni* (Monteville and Konkel, 2002). In addition, Δ*flaA*/*B* and motility mutants were unable to pass polarised Caco-2 or MKN-28 cells suggesting that certain flagellum-mediated activities play a role (Grant et al., 1993; Boehm et al., 2012).

Interestingly, two other types of mutants, LOS-deficient Δ*cstII* and Δ*htrA* serine protease which are highly motile, exhibited strong deficiencies in *C. jejuni* transmigration across polarised cells (Boehm et al., 2012; Louwen et al., 2012). This suggests that bacterial motility per se is not required for transmigration, but *cstII*- and *htrA*-mediated cell binding maybe involved. Another important observation is that HtrA was found to be secreted into the *C. jejuni* cell culture supernatant (Hoy et al., 2012; Boehm et al., 2012). Infection studies and *in vitro* cleavage assays showed that HtrA cleaves-off the extracellular NTF domain of E-cadherin on epithelial cells. Deletion of the *htrA* gene or expression of a protease-deficient S197A point mutant in *C. jejuni* led to severe defects in E-cadherin cleavage and bacterial transmigration across polarised MKN-28 cell monolayers (Boehm et al., 2012). Thus, cleavage of host junctional proteins like E-cadherin (and maybe others) by secreted HtrA may explain how *C. jejuni* can transmigrate paracellularly between neighboring cells. Interestingly, *C. jejuni* HtrA was unable to cleave the fibronectin receptor although this has been seen for its HtrA counterpart in *H. pylori* (Hoy et al., 2010; Boehm et al., 2012). This observation is in agreement with earlier findings that fibronectin is a major host cell factor necessary for *C. jejuni* binding and invasion of non-polarised cells. But can *C. jejuni* invade polarised cells from basolateral surfaces? The observation that Δ*cadF* mutants can transmigrate through but not invade into polarised cells would support this idea (Monteville and Konkel, 2002). Basolateral engulfment of *C. jejuni* in non-polarised Chang cells has been also shown by TEM and called subvasion, but studies on this phenotype indicated that the CadF, JlpA or PEB1 adhesins were not required (van Alphen et al., 2008). Thus, the processes of *C. jejuni* transmigration and basolateral invasion at the molecular level are still not clear. In future, more direct microscopic and genetic approaches are necessary to investigate in detail the involved bacterial and host factors.

## 6. Intracellular survival of *C. jejuni*

While the entry process into eukaryotic cells by *C. jejuni* has been the focus of many studies in the past (Ó Cróinín and Backert, 2012), only a few recent reports have examined the survival of *C. jejuni* within eukaryotic cells. Consequently, by comparison to other invasive enteropathogenic bacteria, the intracellular fate of *C. jejuni* within eukaryotic cells is still poorly understood. It has been generally suggested that *C. jejuni* is able to persist within cultured epithelial cells *in vitro* for 1–3 days in a membrane-surrounded compartment termed the CCV (Buelow et al., 2011; Day et al., 2000; Gaynor et al., 2005; Naikare et al., 2006; Watson and Galan, 2008; Bouwman et al., 2013). Methods that have been applied to characterise the intracellular fate of *C. jejuni* are summarised in Table 4 and described below.

### 6.1. Intracellular survival assay (ISA)

Most studies investigating the intracellular survival capability of *C. jejuni* have used a CFU-based intracellular survival assay (ISA) demonstrating that *C. jejuni* is able to survive within various types of non-phagocytic cells for 24 h (Gaynor et al., 2005; Watson and Galan, 2008) or even up to 72 h (Konkel et al., 1992b; Naikare et al., 2006). The experimental settings of the ISA approach are similar to the GPA assay described in Section 4.1. After an initial infection time, cultured cells are treated with a high dose of gentamicin to kill extracellular bacteria. The follow-up cell lysis performed for invasion assays to release the intracellular bacteria is skipped; instead the cultured epithelial cells are further cultivated in cell culture medium for various time periods to investigate the survival capacity of *C. jejuni*. During this extended incubation time the experimental procedure varies significantly between studies as gentamicin is either omitted (Konkel et al., 1992b; Naikare et al., 2006) or added in concentrations of 10 μg/ml (Candon et al., 2007; Louwen et al., 2012; Watson and Galan, 2008), 20 μg/ml (Novik et al., 2010) or 50 μg/ml (Konkel et al., 1992b; Van Deun et al., 2008; Bouwman et al., 2013) to the cell culture medium. After this incubation and subsequent washing steps, *C. jejuni* is finally released from the cells by treatment with detergents or by physical sheering, and intracellular bacteria CFUs are quantified by plating of serial dilutions. The survival is often presented as absolute number of intracellular bacteria recovered over time (Buelow et al., 2011; Candon et al., 2007; Gaynor et al., 2005; Watson and Galan, 2008). Alternatively, the survival rate is calculated either by dividing the numbers of recovered bacteria in relation to the inoculum or by dividing the number of bacteria recovered at the end of an infection experiment in relation to the number of invaded bacteria recovered about four hours after infection.

Until now, only few *C. jejuni* mutants with intracellular persistence defects have been identified using the ISA test (Table 4). Inactivation of the stringent response regulator SpoT results, among other defects, in a reduced intracellular survival capability (Gaynor et al., 2005). Polyphosphate kinase 1 and 2 (Ppk 1 & Ppk 2) mediating polyphosphate metabolism also support the viability of *C. jejuni* in the CCVs (Candon et al., 2007; Gangaiah et al., 2010). Furthermore, CiaI, a newly identified member of the *Campylobacter* invasion antigen family, is suggested to be involved in efficient intracellular survival (Buelow et al., 2011). An active superoxide dismutase SodB is not only required for efficient adherence and invasion (Novik et al., 2010), but also for intracellular persistence (Novik et al., 2010; Pesci et al., 1994). It is surprising in this context that the catalase KatA, involved in the detoxification of reactive oxygen species, is apparently not required by *C. jejuni* to survive within Hep-2 cells (Day et al., 2000). In addition, *aspA* (aspartate ammonia-lyase) and *frdA* (fumarate reductase A) mutants, potentially defective in fumarate respiration, also showed a reduced survival capability (Liu et al., 2012). Only one mutation has so far been identified that leads to slightly increased intracellular survival: Inactivation of CprS, the sensor kinase of a two-component regulatory system involved in *Campylobacter* planktonic growth regulation, improves intracellular fitness (Svensson et al., 2009).

The ISA technique requires attentiveness to avoid misleading results because extracellular growing bacteria that have survived the high dose gentamicin treatment can be mistaken for intracellular bacteria recovered from the CCVs. It was also suggested that intracellular *C. jejuni*, which are protected from gentamicin treatment, are able to escape their host cells and re-infect other cells (Van Deun et al., 2008). In addition, a so-called subvasion mechanism, the basolateral invasion of epithelial cells accompanied by preceding migration into a subcellular, gentamicin-protected space, of certain *C. jejuni* has been suggested (van Alphen et al., 2008; Bouwman et al., 2013). Few extracellular bacteria that survive gentamicin treatment can skew survival experiments as *in vitro* studies have demonstrated that various cell culture media with or without serum supplementation can promote the growth of certain *C. jejuni* isolates. In particular, the high glutamine concentration (4 mM) present in certain cell culture media provides a suitable growth substrate for *C. jejuni* isolates expressing a secreted, active γ-glutamyltranspeptidase (Hofreuter et al., 2008). So the continuous presence of low gentamicin concentrations up to 20 μg/ml for the extended incubation period during survival experiments is highly recommended to prevent the ongoing proliferation of extracellular *C. jejuni* that have potentially survived the initial gentamicin treatment. Higher gentamicin concentrations over a long time period are problematic and can lead to the killing of intracellular bacteria in the CCVs since gentamicin is not completely excluded from eukaryotic cells (Elsinghorst, 1994).

Numerous survival studies using ISA have clearly demonstrated that the number of intracellular, viable bacteria declines substantially during the first 24 h. Recent reports showed that the significant decrease in the number of intracellular surviving bacteria after 24 h reflects a less efficient recovery with the standard cultivation methods used rather than a reduced viability of intracellular *C. jejuni*. This phenomenon is due to pronounced physiological adaptation of *C. jejuni* to the specific environment present in the CCVs (Liu et al., 2012; Watson and Galan, 2008). The metabolic alterations of *C. jejuni* are most prominently reflected by a general metabolic down shift and changes in the respiratory activity (Liu et al., 2012) that allows better adaptation to oxygen-independent respiration. It was further shown that the physiological adaptions of *C. jejuni* to the CCV negatively affect its efficient recovery from this intracellular compartment unless specific cultivation methods are used. Consequently, *C. jejuni* isolated from eukaryotic cells 24 h after infection can be recovered with higher CFUs when cultivated under very low oxygen/anerobic conditions than under microaerobic or 10% $CO_2$ cultivation conditions (Liu et al., 2012; Watson and Galan, 2008). After 48 h, the transfer of the recovered *C. jejuni* colonies to a microaerobic or 10% $CO_2$ atmosphere is required for maximal growth. This is in agreement with previous observations demonstrating that the *in vitro* growth of *C. jejuni* is restricted in anerobic atmosphere (Sellars et al., 2002). Moreover, it was recently suggested that *C. jejuni* undergoes an intracellular metabolic reprogramming that reduces its recovery on standard Mueller–Hinton cultivation plates after survival or invasion experiments (Pryjma et al., 2012). Addition of 20 mM sodium sulfite, a suitable respiratory electron donor substrate for *C. jejuni* (Myers and Kelly, 2005), enhanced the recovery of *C. jejuni* wild-type and especially of its formate dehydrogenase mutants from infected host cells (Pryjma et al., 2012).

Although the intracellular survival of *C. jejuni* in epithelial cells is well documented, there are conflicting data about the survival capability of *C. jejuni* in phagocytic cells. Some studies have described that *C. jejuni* can persist for several days in isolated BALB/c macrophages, in the murine J774G8 macrophage cell line (Kiehlbauch et al., 1985), in human monocytes and in the human monocyte cell line 28SC (Hickey et al., 2005) as well as in murine peritoneal macrophages (Day et al., 2000). In contrast, other reports have shown that *C. jejuni* is efficiently killed by chicken peripheral macrophages (Myszewski and Stern, 1991), macrophages derived from human peripheral monocytes (Wassenaar et al., 1997), and murine bone marrow-derived macrophages (Watson and Galan, 2008). Experimental variances like the use of different phagocytic immune cells, different *C. jejuni* isolates, variable MOIs and infection times and the usage or non-usage of gentamicin to prevent the extracellular growth of *C. jejuni* as discussed previously (Wassenaar et al., 1997) could be reasons for the conflicting observations. Furthermore, the altered induction of immune cell differentiation and activation could influence *C. jejuni* survival in these experiments. Further studies are required to clarify the capability of *C. jejuni* to survive within macrophages of certain hosts and to identify bacterial factors that are involved in this potential process.

As the ISA is based on the recovery of intracellular bacteria by standard cultivation methods, potential survival defects of mutants have to be characterised in more detail. Follow up experiments like the live/dead straining procedure described below can provide additional information to exclude the possibility that the observed phenotype is a defect in culturability of investigated *C. jejuni* strains upon release from the CCV rather than a loss of their intracellular viability.

### 6.2. Live/Dead Staining (LDS)

Several reports have investigated the *in vitro* viability of *C. jejuni* upon various environmental stresses using DNA-specific dyes that allow the discrimination of live from dead bacteria according to their membrane integrity (Cameron et al., 2012; Ica et al., 2012; Asakura et al., 2007). This live/dead staining (LDS) technique has been successfully applied as an alternative to the ISA to investigate the survival of *C. jejuni* within the human monocytic 28 SC cells (Hickey et al., 2005), the intestinal T84 epithelial cells (Novik et al., 2010; Watson and Galan, 2008) and the waterborne protozoa *Tetrahymena pyriformis* and *Acanthamoeba castellanii* (Snelling et al., 2005). The LDS method does not depend on bacterial growth and overcomes therewith the problem of enumerating viable but non-culturable *C. jejuni* that can be missed by ISA experiments. Instead viable intracellular *C. jejuni* cells are identified by staining with a cell viability kit and can be counted visually by IFM or enumerated by flow cytometry.

LDS-based survival experiments are set up as described in Section 6.1 with the following modifications: At the end of the experiment host cells are lysed and the released *C. jejuni* cells are not tested for viability by direct plating and enumerating but separated from the cell debris by a low speed spin. Subsequently, the bacteria in the supernatant are stained with a mixture of two solutions: one dye is membrane permeable and stains all cells (e.g. thiazole orange) whereas the second dye is membrane impermeable (e.g. propidium iodide) and

only enters dead bacterial cells with disrupted membranes. Using IFM *C. jejuni* cells that remain viable inside the CCV can be seen as green, whereas dead *C. jejuni* cells are stained red (Watson and Galan, 2008). Direct counting by microscopic examination is possible but time consuming. In addition, remaining cell debris particles of lysed host cells could interfere with the precise counting by fluorescence microscopy. The use of flow cytometry can overcome these problems and makes it possible to quantify the stained viable and dead *C. jejuni* cells of a mixed population (Novik et al., 2010; Watson and Galan, 2008). The FACS (fluorescence activated cell sorting) analysis method has to be carefully adapted and optimised for each *C. jejuni* isolate, as this pathogen has rather small dimensions (Walker et al., 1986) in comparison to other bacteria and different strains vary in their tendencies to self-aggregate (Golden and Acheson, 2002; Misawa and Blaser, 2000).

### 6.3. Microscopic methods

TEM studies have revealed that *C. jejuni* remains tightly surrounded by a host-derived membrane after invasion into epithelial cells *in vitro* and in *vivo* (Babakhani et al., 1993; De Melo et al., 1989; Hu et al., 2008; Konkel et al., 1992b; van Spreeuwel et al., 1985; Watson and Galan, 2008). Recent infection studies with COS-1 cells suggest that *C. jejuni* 81-176, once internalised, actively subverts the classical endocytic pathway to establish its intracellular compartment: Shortly after invasion, *C. jejuni* resides in an early-endosomal-like compartment as demonstrated by IFM experiments in which *C. jejuni* co-localises with the antibody-stained endocytic marker EEA-1, with the transfected GFP-fusion proteins of the GTPases Rab4 and Rab5 as well as with a PX-domain-containing probe (Ellson et al., 2002) for phosphoinisitide 3 phosphate presence (Watson and Galan, 2008). Subsequently, the CCV co-localises transiently with a transfected GFP-tagged late endosomal marker protein Rab7. One hour after infection the CCV can be efficiently stained by IFM using an antibody against the late endosomal/lysosomal protein Lamp1. At this time point only very limited co-localisation with the previously detected EEA-1, Rab4, Rab5 and Rab7 occurs (Watson and Galan, 2008). A similar maturation of the CCV during the first 2 h after invasion was recently described upon *C. jejuni* infection of Caco-2 cells (Louwen et al., 2012). Though marked with Lamp1 the CCV does not acquire lysosomal properties supported by the observation that cathepsin B is not detected by IFM staining in significant amounts on the vesicle where *C. jejuni* resides (Watson and Galan, 2008). It was shown recently that *C. jejuni* CiaI may contribute to the exclusion of cathepsin D from the CCV (Buelow et al., 2011). About 5 h after invasion *C. jejuni* resides in a survival-permissive vacuolar compartment that is localised in the perinuclear region of the host cells as demonstrated by TEM (Hu and Kopecko, 1999; Watson and Galan, 2008) and can be immuno-stained with the Golgi marker protein GM130 (Watson and Galan, 2008). The translocation of the CCV from the cell periphery to the perinuclear space could be mediated by the vesicle transport machinery involving microtubules and dynein motor protein (Hu and Kopecko, 1999; Watson and Galan, 2008). Infection experiments with polarised Caco-2 islands revealed that *C. jejuni* resides in CCVs that can be immuno-stained with antibodies against Lamp1 and CD63 but not with GM130 (Bouwman et al., 2013). Further studies have to clarify if the perinuclear localisation of the CCV varies with the usage of non-polarised or polarised cultured epithelial cells.

*C. jejuni* resides in the non-spacious CCV during its entire intracellular persistence. This vacuole usually contains a single *C. jejuni* bacterium (Hu et al., 2008; Watson and Galan, 2008) suggesting that no obvious intracellular replication of *C. jejuni* occurs *in vitro*. Only occasionally multiple bacteria have been observed in one CCV but it is unclear if this event is the consequence of intracellular *C. jejuni* replication or the fusion of several phagosomes containing individual *C. jejuni* cells. The latter seems more feasible: While inside/outside IFM showed up to 11 bacteria inside infected cultured epithelial cells (Novik et al., 2009), that *C. jejuni* does not replicate intracellularly is supported by experiments based on the previous described survival assays (see Sections 6.1 and 6.2) where after 24 h no more bacteria can be recovered from infected cells than after 4 h.

## 7. Conclusions and perspectives

*C. jejuni* is a predominant foodborne pathogen causing enterocolitis in humans worldwide. This zoonotic bacterium represents part of the commensal flora in many wild and domestic animals. Its importance for humans attracted many researchers worldwide to investigate the molecular mechanisms of associated infections. However, despite the high prevalence of *C. jejuni* induced disease and research progress made in recent years, our knowledge is still relatively limited as compared to other invasive pathogens such as *Salmonella*, *Listeria*, *Shigella* or *Legionella*. Efforts with rodent and chicken model systems have been made to study *C. jejuni* infection *in vivo*. Currently, disease manifestations such as gastroenteritis can be achieved in gnotobiotic, immunodeficient and CH3 mice with a defined gut flora, but each model system has limitations (Bereswill et al., 2011; Haag et al., 2012). No model alone seems ideal to investigate pathogenicity and virulence mechanisms of *C. jejuni. In vivo* and *in vitro* research studies performed in the last two decades revealed that *C. jejuni* exhibits remarkable features during infection. Several key processes such as glycosylation, molecular mimicry, tissue entry and intracellular survival have been described. Many genes have been discussed to be involved in the above processes including the capsule, flagellum, LOS, CadF, HtrA, respiratory factors and others. Future studies should combine *in vivo* and *in vitro* studies and should take advantage of powerful modern technologies including high-resolution IFM and LCI as well as proteomics-based and cellular signal transduction approaches. It will be particularly important to investigate in future, the detailed mechanisms by which *C. jejuni* enters polarised epithelial cells from apical and/or basolateral surfaces as well as how it survives and spreads intracellularly.

## Acknowledgements

We thank Dr. Nicole Tegtmeyer (University Erlangen) for critical reading of this manuscript and advice. The work of SB is supported through a DFG grant (project B10 of CRC-796). The work of DH is supported by the German Research Foundation through the DFG priority program SPP1316 (HO 4553/1-2) and the German Ministry for Education and Research (BMBF; Consortium FBI-Zoo).

## References

Ahmed, I.H., Manning, G., Wassenaar, T.M., Cawthraw, S., Newell, D.G., 2002. Identification of genetic differences between two *Campylobacter jejuni* strains with different colonization potentials. Microbiology 148, 1203–1212.

Alemka, A., Clyne, M., Shanahan, F., Tompkins, T., Corcionivoschi, N., Bourke, B., 2010. Probiotic colonization of the adherent mucus layer of HT29MTXE12 cells attenuates *Campylobacter jejuni* virulence properties. Infect. Immun. 78, 2812–2822.

Alemka, A., Corcionivoschi, N., Bourke, B., 2012. Defense and adaptation: the complex inter-relationship between *Campylobacter jejuni* and mucus. Front. Cell. Infect. Microbiol. 2, 15.

Allos, B.M., 2001. *Campylobacter jejuni* Infections: update on emerging issues and trends. Clin. Infect. Dis. 32, 1201–1206.

Apel, D., Ellermeier, J., Pryjma, M., Dirita, V.J., Gaynor, E.C., 2012. Characterization of *Campylobacter jejuni* RacRS reveals roles in the heat shock response, motility, and maintenance of cell length homogeneity. J. Bacteriol. 194, 2342–2354.

Asakura, H., Yamasaki, M., Yamamoto, S., Igimi, S., 2007. Deletion of peb4 gene impairs cell adhesion and biofilm formation in *Campylobacter jejuni*. FEMS Microbiol. Lett. 275, 278–285.

Ashgar, S.S., Oldfield, N.J., Woolridge, K.G., Jones, M.A., Irving, G.J., Turner, D.P., Ala'Aldeen, D.A., 2007. CapA, an autotransporter protein of *Campylobacter jejuni* mediates association with human epithelial cells and colonization of the chicken gut. J. Bacteriol. 189, 1856–1865.

Babakhani, F.K., Bradley, G.A., Joens, L.A., 1993. Newborn piglet model for campylobacteriosis. Infect. Immun. 61, 3466–3475.

Bachtiar, B.M., Coloe, P.J., Fry, B.N., 2007. Knockout mutagenesis of the KpsE gene of *Campylobacter jejuni* 81116 and its involvement in bacterium host interactions. FEMS Immunol. Med. Microbiol. 49, 149–154.

Bacon, D.J., Alm, R.A., Burr, D.H., Hu, L., Kopecko, D.J., Ewing, C.P., Trust, T.J., Guerry, P., 2000. Involvement of a plasmid in virulence of *Campylobacter jejuni* 81-176. Infect. Immun. 68, 4384–4390.

Bacon, D.J., Szymanski, C.M., Burr, D.H., Silver, R.P., Alm, R.A., Guerry, P., 2001. A phase-variable capsule is involved in virulence of *Campylobacter jejuni* 81-176. Mol. Microbiol. 40, 769–777.

Bæk, K.T., Vegge, C.S., Brøndsted, L., 2011. HtrA chaperone activity contributes to host cell binding in *Campylobacter jejuni*. Gut Pathog. 3, 13.

Balkovetz, D.F., Katz, J., 2003. Bacterial invasion by a paracellular route: divide and conquer. Microbes Infect. 5, 613–619.

Beery, J.T., Hugdahl, M.B., Doyle, M.P., 1988. Colonization of gastrointestinal tracts of chicks by *Campylobacter jejuni*. Appl. Environ. Microbiol. 54, 2365–2370.

Behrens, I., Stenberg, P., Artursson, P., Kissel, T., 2001. Transport of lipophilic drug molecules in a new mucus-secreting cell culture model based on HT29-MTX cells. Pharm. Res. 18, 1138–1145.

Beltinger, J., del Buono, J., Skelly, M.M., Thornley, J., Spiller, R.C., Stack, W.A., Hawkey, C.J., 2008. Disruption of colonic barrier function and induction of mediator release by strains of *Campylobacter jejuni* that invade epithelial cells. World J. Gastroenterol. 14, 7345–7352.

Bereswill, S., Fischer, A., Plickert, R., Haag, L.M., Otto, B., Kühl, A.A., Dasti, J.I., Zautner, A.E., Muñoz, M., Loddenkemper, C., Gross, U., Göbel, U.B., Heimesaat, M.M., 2011. Novel murine infection models provide deep insights into the "menage a trois" of *Campylobacter jejuni*, microbiota and host innate immunity. PLoS One 6, e20953.

Biswas, D., Itoh, K., Sasakawa, C., 2000. Uptake pathways of clinical and healthy animal isolates of *Campylobacter jejuni* into INT-407 cells. FEMS Immunol. Med. Microbiol. 29, 203–211.

Biswas, D., Itoh, K., Sasakawa, C., 2003. Role of microfilaments and microtubules in the invasion of INT-407 cells by *Campylobacter jejuni*. Microbiol. Immunol. 47, 469–473.

Biswas, D., Fernando, U., Reimau, C., Willson, P., Potter, A., Allau, B., 2006. Effect of cytolethal distending toxin of *Campylobacter jejuni* on adhesion and internalization in cultured cells and in colonization of the chicken gut. Avian Dis. 50, 586–593.

Black, R.E., Levine, M.M., Clements, M.L., Hughes, T.P., Blaser, M.J., 1988. Experimental *Campylobacter jejuni* infection in humans. J. Infect. Dis. 157, 472–479.

Boehm, M., Krause-Gruszczynska, M., Rohde, M., Tegtmeyer, N., Takahashi, S., Oyarzabal, O.A., Backert, S., 2011. Major host factors involved in epithelial cell invasion of *Campylobacter jejuni*: role of fibronectin, integrin beta1, FAK, Tiam-1, DOCK180 in activating Rho GTPase Rac1. Front. Cell. Infect. Microbiol. 1, 17.

Boehm, M., Hoy, B., Rohde, M., Tegtmeyer, N., Baek, K.T., Oyarzabal, O.A., Brondsted, L., Wessler, S., Backert, S., 2012. Rapid paracellular transmigration of *Campylobacter jejuni* across polarized epithelial cells without affecting TER: role of proteolytic-active HtrA cleaving E-cadherin but not fibronectin. Gut Pathog. 4, 3.

Boquet, P., Lemichez, E., 2003. Bacterial virulence factors targeting Rho GTPases: parasitism or symbiosis? Trends Cell Biol. 13, 238–246.

Bouwman, L.I., Niewold, P., van Putten, J.P., 2013. Basolateral invasion and trafficking of *Campylobacter jejuni* in polarized epithelial cells. PLoS One 8, e54759.

Brás, A.M., Ketley, J.M., 1999. Transcellular translocation of *Campylobacter jejuni* across human polarised epithelial monolayers. FEMS Microbiol. Lett. 179, 209–215.

Brøndsted, L., Andersen, M.T., Parker, M., Jørgensen, K., Ingmer, H., 2005. The HtrA protease of *Campylobacter jejuni* is required for heat and oxygen tolerance and for optimal interaction with human epithelial cells. Appl. Environ. Microbiol. 71, 3205–3212.

Buelow, D.R., Christentsen, J.E., Neal-McKinney, J.M., Konkel, M.E., 2011. *Campylobacter jejuni* survival within human epithelial cells is enhanced by the secreted protein CiaI. Mol. Microbiol. 80, 1296–1312.

Byrne, C.M., Clyne, M., Bourke, B., 2007. *Campylobacter jejuni* adhere to and invade chicken intestinal epithelial cells *in vitro*. Microbiology 153, 561–569.

Caldwell, M.B., Guerry, P., Lee, E.C., Burans, J.P., Walker, R.I., 1985. Reversible expression of flagella in *Campylobacter jejuni*. Infect. Immun. 50, 941–943.

Cameron, A., Frirdich, E., Huynh, S., Parker, C.T., Gaynor, E.C., 2012. Hyperosmotic stress response of *Campylobacter jejuni*. J. Bacteriol. 194, 6116–6130.

Candon, H.L., Allan, B.J., Fraley, C.D., Gaynor, E.C., 2007. Polyphosphate kinase 1 is a pathogenesis determinant in *Campylobacter jejuni*. J. Bacteriol. 189, 8099–8108.

Chen, M.L., Ge, Z., Fox, J.G., Schauer, D.B., 2006. Disruption of tight junctions and induction of proinflammatory cytokine responses in colonic epithelial cells by *Campylobacter jejuni*. Infect. Immun. 74, 6581–6589.

Christensen, J.E., Pacheco, S.A., Konkel, M.E., 2009. Identification of a *Campylobacter jejuni*-secreted protein required for maximal invasion of host cells. Mol. Microbiol. 73, 650–662.

Coote, J.G., Stewart-Tull, D.E., Owen, R.J., Bolton, F.J., Siemer, B.L., Candlish, D., Thompson, D.H., Wardlaw, A.C., On, S.L., Candlish, A., Billcliffe, B., Jordan, P.J., Kristiansen, K., Borman, P., 2007. Comparison of virulence-associated in vitro properties of typed strains of *Campylobacter jejuni* from different sources. J. Med. Microbiol. 56, 722–732.

Dasti, J.I., Tareen, A.M., Lugert, R., Zautner, A.E., Gross, U., 2010. *Campylobacter jejuni*: a brief overview on pathogenicity-associated factors and disease-mediating mechanisms. Int. J. Med. Microbiol. 300, 205–211.

Day Jr., W.A., Sajecki, J.L., Pitts, T.M., Joens, L.A., 2000. Role of catalase in *Campylobacter jejuni* intracellular survival. Infect. Immun. 68, 6337–6345.

De Melo, M.A., Gabbiani, G., Pechere, J.C., 1989. Cellular events and intracellular survival of *Campylobacter jejuni* during infection of Hep-2 cells. Infect. Immun. 57, 2214–2222.

Drozd, M., Gangaiah, D., Liu, Z., Rajashekara, G., 2011. Contribution of TAT system translocated PhoX to *Campylobacter jejuni* phosphate metabolism and resilience to environmental stresses. PLoS One 6, e26336.

Ellson, C.D., Andrews, S., Stephens, L.R., Hawkins, P.T., 2002. The PX domain: a new phosphoinositide-binding module. J. Cell Sci. 115, 1099–1105.

Elmi, A., Watson, E., Sandu, P., Gundogdu, O., Mills, D.C., Inglis, N.F., Manson, E., Imrie, L., Bajaj-Elliott, M., Wren, B.W., Smith, D.G., Dorrell, N., 2012. *Campylobacter jejuni* outer membrane vesicles play an important role in bacterial interactions with human intestinal epithelial cells. Infect. Immun. 80, 4089–4098.

Elsinghorst, E.A., 1994. Measurement of invasion by gentamicin resistance. Methods Enzymol. 236, 405–420.

Eucker, T.P., Konkel, M.E., 2012. The cooperative action of bacterial fibronectin-binding proteins and secreted proteins promote maximal *Campylobacter jejuni* invasion of host cells by stimulating membrane ruffling. Cell. Microbiol. 14, 226–238.

Everest, P.H., Goossens, H., Butzler, J.P., Lloyd, D., Knutton, S., Ketley, J.M., Williams, P.H., 1992. Differentiated Caco-2 cells as a model for enteric invasion by *Campylobacter jejuni* and *C. coli*. J. Med. Microbiol. 37, 319–325.

Fauchere, J.L., Rosenau, A., Veron, M., Moyen, E.N., Richard, S., Pfister, A., 1986. Association with HeLa cells of *Campylobacter jejuni* and *Campylobacter coli* isolated from human feces. Infect. Immun. 54, 283–287.

Fearnley, C., Manning, G., Bagnall, M., Javed, M.A., Wassenaar, T.M., Newell, D.G., 2008. Identification of hyperinvasive *Campylobacter jejuni* strains isolated from poultry and human clinical sources. J. Med. Microbiol. 57, 570–580.

Ferrero, R.L., Lee, A., 1988. Motility of *Campylobacter jejuni* in a viscous environment: comparison with conventional rod-shaped bacteria. J. Gen. Microbiol. 134, 53–59.

Fields, J.A., Thompson, S.A., 2008. *Campylobacter jejuni* CsrA mediates oxidative stress responses, biofilm formation, and host cell invasion. J. Bacteriol. 190, 3411–3416.

Flanagan, R.C., Neal-McKinney, J.M., Dhillon, A.S., Miller, W.G., Konkel, M.E., 2009. Examination of *Campylobacter jejuni* putative adhesins leads to the identification of a new protein, designated FlpA, required for chicken colonization. Infect. Immun. 77, 2399–23407.

Fouts, D.E., Mongodin, E.F., Mandrell, R.E., Miller, W.G., Rasko, D.A., Ravel, J., Brinkac, L.M., DeBoy, R.T., Parker, C.T., Daugherty, S.C., Dodson, R.J., Durkin, A.S., Madupu, R., Sullivan, S.A., Shetty, J.U., Ayodeji, M.A., Shvartsbeyn, A., Schatz, M.C., Badger, J.H., Fraser, C.M., Nelson, K.E., 2005. Major structural differences and novel potential virulence mechanisms from the genomes of multiple campylobacter species. PLoS Biol. 3, e15.

Fry, B.N., Feng, S., Chen, Y.Y., Newell, D.G., Coloe, P.J., Korolik, V., 2000. The galE gene of *Campylobacter jejuni* is involved in lipopolysaccharide synthesis and virulence. Infect. Immun. 68, 2594–2601.

Gaasbeek, E.J., Wagenaar, J.A., Guilhabert, M.R., Wosten, M.M., van Putten, J.P., van der Graaf-van Bloois, L., Parker, C.T., van der Wal, F.J., 2009. A DNase encoded by integrated element CJIE1 inhibits natural transformation of *Campylobacter jejuni*. J. Bacteriol. 191, 2296–2306.

Gaasbeek, E.J., Wagenaar, J.A., Guilhabert, M.R., van Putten, J.P., Parker, C.T., van der Wal, F.J., 2010. Nucleases encoded by the integrated elements CJIE2 and CJIE4 inhibit natural transformation of *Campylobacter jejuni*. J. Bacteriol. 192, 936–941.

Gangaiah, D., Liu, Z., Arcos, J., Kassem, I.I., Sanad, Y., Torrelles, J.B., Rajashekara, G., 2010. Polyphosphate kinase 2: a novel determinant of stress responses and pathogenesis in *Campylobacter jejuni*. PLoS One 5, e12142.

Gaynor, E.C., Cawthraw, S., Manning, G., MacKichan, J.K., Falkow, S., Newell, D.G., 2004. The genome-sequenced variant of *Campylobacter jejuni* NCTC 11168 and the original clonal clinical isolate differ markedly in colonization, gene expression, and virulence-associated phenotypes. J. Bacteriol. 186, 503–517.

Gaynor, E.C., Wells, D.H., MacKichan, J.K., Falkow, S., 2005. The *Campylobacter jejuni* stringent response controls specific stress survival and virulence-associated phenotypes. Mol. Microbiol. 56, 8–27.

Gilbert, M., Karwaski, M.F., Bernatchez, S., Young, N.M., Taboada, E., Michniewicz, J., Cunningham, A.M., Wakarchuk, W.W., 2002. The genetic bases for the variation in the lipo-oligosaccharide of the mucosal pathogen, *Campylobacter jejuni*. J. Biol. Chem. 277, 327–337.

Golden, N.J., Acheson, D.W., 2002. Identification of motility and autoagglutination *Campylobacter jejuni* mutants by random transposon mutagenesis. Infect. Immun. 70, 1761–1771.

Goon, S., Ewing, C.P., Lorenzo, M., Pattarini, D., Majam, G., Guerry, P.A., 2006. sigma28-regulated nonflagella gene contributes to virulence of *Campylobacter jejuni* 81-176. Infect. Immun. 74, 769–772.

Grant, C.C.R., Konkel, M.E., Cieplak Jr., W., Tompkins, L.S., 1993. Role of flagella in adherence, internalization, and translocation of *Campylobacter jejuni* in nonpolarized and polarized epithelial cell cultures. Infect. Immun. 61, 1764–1771.

Gripp, E., Hlahla, D., Didelot, X., Kops, F., Maurischat, S., Tedin, K., Alter, T., Ellerbroek, L., Schreiber, K., Schomburg, D., Janssen, T., Bartholomaus, P., Hofreuter, D., Woltemate, S., Uhr, M., Brenneke, B., Gruning, P., Gerlach, G., Wieler, L., Suerbaum, S., Josenhans, C., 2011. Closely related *Campylobacter jejuni* strains from different sources reveal a generalist rather than a specialist lifestyle. BMC Genomics 12, 584.

Guccione, E., Leon-Kempis Mdel, R., Pearson, B.M., Hitchin, E., Mulholland, F., van Diemen, P.M., Stevens, M.P., Kelly, D.J., 2008. Amino acid-dependent growth of *Campylobacter jejuni*: key roles for aspartase (AspA) under microaerobic and oxygen-limited conditions and identification of AspB (Cj0762), essential for growth on glutamate. Mol. Microbiol. 69, 77–93.

Guerry, P., 2007. *Campylobacter* flagella: not just for motility. Trends Microbiol. 15, 456–461.

Guerry, P., Ewing, C.P., Schirm, M., Lorenzo, M., Kelly, J., Pattarini, D., Majam, G., Thibault, P., Logan, S., 2006. Changes in flagellin glycosylation affect *Campylobacter* autoagglutination and virulence. Mol. Microbiol. 60, 299–311.

Gundogdu, O., Bentley, S.D., Holden, M.T., Parkhill, J., Dorrell, N., Wren, B.W., 2007. Re-annotation and re-analysis of the *Campylobacter jejuni* NCTC11168 genome sequence. BMC Genomics 8, 162.

Haag, L.M., Fischer, A., Otto, B., Plickert, R., Kuhl, A.A., et al., 2012. *Campylobacter jejuni* induces acute enterocolitis in gnotobiotic IL-10$^{-/-}$ mice via Toll-like-receptor-2 and -4 signaling. PLoS One 7, e40761.

Harvey, P., Battle, T., Leach, S., 1999. Different invasion phenotypes of *Campylobacter* isolates in Caco-2 cell monolayers. J. Med. Microbiol. 48, 461–469.

Heasman, S.J., Ridley, A.J., 2008. Mammalian Rho GTPases: new insights into their functions from in vivo studies. Nat. Rev. Mol. Cell Biol. 9, 690–701.

Hendrixson, D.R., 2006. A phase-variable mechanism controlling the *Campylobacter jejuni* FlgR response regulator influences commensalism. Mol. Microbiol. 61, 1646–1659.
Hendrixson, D.R., 2008. Restoration of flagellar biosynthesis by varied mutational events in *Campylobacter jejuni*. Mol. Microbiol. 70, 519–536.
Hendrixson, D.R., DiRita, V.J., 2004. Identification of *Campylobacter jejuni* genes involved in commensal colonization of the chick gastrointestinal tract. Mol. Microbiol. 52, 471–484.
Hermans, D., Van Deun, K., Martel, A., Van Immerseel, F., Messens, W., Heyndrickx, M., Haesebrouck, F., Pasmans, F., 2011. Colonization factors of *Campylobacter jejuni* in the chicken gut. Vet. Res. 42, 82.
Hickey, T.E., McVeigh, A.L., Scott, D.A., Michielutti, R.E., Bixby, A., Carroll, S.A., Bourgeois, A.L., Guerry, P., 2000. *Campylobacter jejuni* cytolethal distending toxin mediates release of interleukin-8 from intestinal epithelial cells. Infect. Immun. 68, 6535–6541.
Hickey, T.E., Majam, G., Guerry, P., 2005. Intracellular survival of *Campylobacter jejuni* in human monocytic cells and induction of apoptotic death by cytholethal distending toxin. Infect. Immun. 73, 5194–5197.
Hiett, K.L., Stintzi, A., Andacht, T.M., Kuntz, R.L., Seal, B.S., 2008. Genomic differences between *Campylobacter jejuni* isolates identify surface membrane and flagellar function gene products potentially important for colonizing the chicken intestine. Funct. Integr. Genomics 8, 407–420.
Hofreuter, D., Tsai, J., Watson, R.O., Novik, V., Altman, B., Benitez, M., Clark, C., Perbost, C., Jarvie, T., Du, L., Galan, J.E., 2006. Unique features of a highly pathogenic *Campylobacter jejuni* strain. Infect. Immun. 74, 4694–4707.
Hofreuter, D., Novik, V., Galan, J.E., 2008. Metabolic diversity in *Campylobacter jejuni* enhances specific tissue colonization. Cell Host Microbe 4, 425–433.
Hofreuter, D., Mohr, J., Wensel, O., Rademacher, S., Schreiber, K., Schomburg, D., Gao, B., Galan, J.E., 2012. Contribution of amino acid catabolism to the tissue specific persistence of *Campylobacter jejuni* in a murine colonization model. PLoS One 7, e50699.
Hoy, B., Löwer, M., Weydig, C., Carra, G., Tegtmeyer, N., Geppert, T., Schröder, P., Sewald, N., Backert, S., Schneider, G., Wessler, S., 2010. *Helicobacter pylori* HtrA is a new secreted virulence factor that cleaves E-cadherin to disrupt intercellular adhesion. EMBO Rep. 11, 798–804.
Hoy, B., Geppert, T., Boehm, M., Reisen, F., Plattner, P., Gadermaier, G., Sewald, N., Ferreira, F., Briza, P., Schneider, G., Backert, S., Wessler, S., 2012. Distinct roles of secreted HtrA proteases from Gram-negative pathogens in cleaving the junctional protein and tumor suppressor E-cadherin. J. Biol. Chem. 287, 10115–10120.
Hu, L., Kopecko, D.J., 1999. *Campylobacter jejuni* 81-176 associates with microtubules and dynein during invasion of human intestinal cells. Infect. Immun. 67, 4171–4182.
Hu, L., Kopecko, D.J., 2008. Cell biology of human host cell entry by *Campylobacter jejuni*. In: Nachamkin, I., Szymanski, Blaser, M.J. (Eds.), Campylobacter. ASM Press, Washington, DC, pp. 297–313.
Hu, L., Raybourne, R.B., Kopecko, D.J., 2005. Ca2 + release from host intracellular stores and related signal transduction during *Campylobacter jejuni* 81-176 internalization into human intestinal cells. Microbiology 151, 3097–3105.
Hu, L., McDaniel, J.P., Kopecko, D.J., 2006. Signal transduction events involved in human epithelial cell invasion by *Campylobacter jejuni* 81-176. Microb. Pathog. 40, 91–100.
Hu, L., Tall, B.D., Curtis, S.K., Kopecko, D.J., 2008. Enhanced microscopic definition of *Campylobacter jejuni* 81-176 adherence to, invasion of, translocation across, and exocytosis from polarized human intestinal Caco-2 cells. Infect. Immun. 76, 5294–5304.
Humphrey, C.D., Montag, D.M., Pittman, F.E., 1986. Morphologic observations of experimental *Campylobacter jejuni* infection in the hamster intestinal tract. Am. J. Pathol. 122, 152–159.
Ica, T., Caner, V., Istanbullu, O., Nguyen, H.D., Ahmed, B., Call, D.R., Beyenal, H., 2012. Characterization of mono- and mixed-culture *Campylobacter jejuni* biofilms. Appl. Environ. Microbiol. 78, 1033–1038.
Janssen, R., Krogfelt, K.A., Cawthraw, S.A., van Pelt, W., Wagenaar, J.A., Owen, R.J., 2008. Host–pathogen interactions in *Campylobacter* infections: the host perspective. Clin. Microbiol. Rev. 21, 505–518.
Javed, M.A., Grant, A.J., Bagnall, M.C., Maskell, D.J., Newell, D.G., Manning, G., 2010. Transposon mutagenesis in a hyper-invasive clinical isolate of *Campylobacter jejuni* reveals a number of genes with potential roles in invasion. Microbiology 156, 1134–1143.
Javed, M.A., Cawthraw, S.A., Baig, A., Li, J., McNally, A., Oldfield, N.J., Newell, D.G., Manning, G., 2012. Cj1136 is required for lipooligosaccharide biosynthesis, hyperinvasion, and chick colonization by *Campylobacter jejuni*. Infect. Immun. 80, 2361–2370.
Jin, S., Joe, A., Lynett, J., Hani, E.K., Sherman, P., Chan, V.L., 2001. JlpA, a novel surface-exposed lipoprotein specific to *Campylobacter jejuni*, mediates adherence to host epithelial cells. Mol. Microbiol. 39, 1225–1236.
Jin, S., Song, Y.C., Emili, A., Sherman, P.M., Chan, V.L., 2003. JlpA of *Campylobacter jejuni* interacts with surface-exposed heat shock protein 90alpha and triggers signalling pathways leading to the activation of NF-kappaB and p38 MAP kinase in epithelial cells. Cell. Microbiol. 5, 165–174.
Jones, M.A., Marston, K.L., Woodall, C.A., Maskell, D.J., Linton, D., Karlyshev, A.V., Dorrell, N., Wren, B.W., Barrow, P.A., 2004. Adaptation of *Campylobacter jejuni* NCTC11168 to high-level colonization of the avian gastrointestinal tract. Infect. Immun. 72, 3769–3776.
Kakuda, T., DiRita, V.J., 2006. Cj1496c encodes a *Campylobacter jejuni* glycoprotein that influences invasion of human epithelial cells and colonization of the chick gastrointestinal tract. Infect. Immun. 74, 4715–4723.
Kalischuk, L.D., Inglis, G.D., Buret, A.G., 2007. Strain-dependent induction of epithelial cell oncosis by *Campylobacter jejuni* is correlated with invasion ability and is independent of cytolethal distending toxin. Microbiology 153, 2952–2963.
Kalischuk, L.D., Inglis, G.D., Buret, A.G., 2009. *Campylobacter jejuni* induces transcellular translocation of commensal bacteria via lipid rafts. Gut Pathog. 1, 2.
Karim, Q.N., Logan, R.P., Puels, J., Karnholz, A., Worku, M.L., 1998. Measurement of motility of *Helicobacter pylori*, *Campylobacter jejuni*, and *Escherichia coli* by real time computer tracking using the Hobson BacTracker. J. Clin. Pathol. 51, 623–628.
Karlyshev, A.V., Linton, D., Gregson, N.A., Wren, B.W., 2002. A novel paralogous gene family involved in phase-variable flagella-mediated motility in *Campylobacter jejuni*. Microbiology 148, 473–480.
Karlyshev, A.V., Champion, O.L., Churcher, C., Brisson, J.R., Jarrell, H.C., Gilbert, M., Brochu, D., St Michael, F., Li, J., Wakarchuk, W.W., Goodhead, I., Sanders, M., Stevens, K., White, B., Parkhill, J., Wren, B.W., Szymanski, C.M., 2005. Analysis of *Campylobacter jejuni* capsular loci reveals multiple mechanisms for the generation of structural diversity and the ability to form complex heptoses. Mol. Microbiol. 55, 90–103.
Kazmierczak, B.I., Mostov, K., Engel, J.N., 2001. Interaction of bacterial pathogens with polarized epithelium. Annu. Rev. Microbiol. 55, 407–435.
Kiehlbauch, J.A., Albach, R.A., Baum, L.L., Chang, K.P., 1985. Phagocytosis of *Campylobacter jejuni* and its intracellular survival in mononuclear phagocytes. Infect. Immun. 48, 446–451.
Kim, J.S., Li, J., Barnes, I.H., Baltzegar, D.A., Pajaniappan, M., Cullen, T.W., Trent, M.S., Burns, C.M., Thompson, S.A., 2008. Role of the *Campylobacter jejuni* Cj1461 DNA methyltransferase in regulating virulence characteristics. J. Bacteriol. 190, 6524–6529.
Konkel, M.E., Joens, L.A., 1989. Adhesion to and invasion of Hep-2 cells by *Campylobacter* spp. Infect. Immun. 57, 2984–2990.
Konkel, M.E., Corwin, M.D., Joens, L.A., Cieplak, W., 1992a. Factors that influence the interaction of *Campylobacter jejuni* with cultured mammalian cells. J. Med. Microbiol. 37, 30–37.
Konkel, M.E., Hayes, S.F., Joens, L.A., Cieplak Jr., W., 1992b. Characteristics of the internalization and intracellular survival of *Campylobacter jejuni* in human epithelial cell cultures. Microb. Pathog. 13, 357–370.
Konkel, M.E., Mead, D.J., Hayes, S.F., Cieplak Jr., W., 1992c. Translocation of *Campylobacter jejuni* across human polarized epithelial cell monolayer cultures. J. Infect. Dis. 166, 308–315.
Konkel, M.E., Kim, B.J., Rivera-Amill, V., Garvis, S.G., 1999. Identification of proteins required for the internalization of *Campylobacter jejuni* into cultured mammalian cells. Adv. Exp. Med. Biol. 473, 215–224.
Konkel, M.E., Monteville, M.R., Rivera-Amill, V., Joens, L.A., 2001. The pathogenesis of *Campylobacter jejuni*-mediated enteritis. Curr. Issues Intest. Microbiol. 2, 55–71.
Konkel, M.E., Christensen, J.E., Keech, A.M., Monteville, M.R., Klena, J.D., Garvis, S.G., 2005. Identification of a fibronectin-binding domain within the *Campylobacter jejuni* CadF protein. Mol. Microbiol. 57, 1022–1035.
Konkel, M.E., Christensen, J.E., Dhillon, A.S., Lane, A.B., Hare-Sanford, R., Schaberg, D.M., Larson, C.L., 2007. *Campylobacter jejuni* strains compete for colonization in broiler chicks. Appl. Environ. Microbiol. 73, 2297–2305.
Konkel, M.E., Larson, C.L., Flanagan, R.C., 2010. *Campylobacter jejuni* FlpA binds fibronectin and is required for maximal host cell adherence. J. Bacteriol. 192, 68–76.
Krause-Gruszczynska, M., Rohde, M., Hartig, R., Genth, H., Schmidt, G., Keo, T., Koenig, W., Miller, W.G., Konkel, M.E., Backert, S., 2007a. Role of the small Rho GTPases Rac1 and Cdc42 in host cell invasion of *Campylobacter jejuni*. Cell. Microbiol. 9, 2431–2444.
Krause-Gruszczynska, M., van Alphen, L.B., Oyarzabal, O.A., Alter, T., Hänel, I., Schliephake, A., König, W., van Putten, J.P., Konkel, M.E., Backert, S., 2007b. Expression patterns and role of the CadF protein in *Campylobacter jejuni* and *Campylobacter coli*. FEMS Microbiol. Lett. 274, 9–16.
Krause-Gruszczynska, M., Boehm, M., Rohde, M., Tegtmeyer, N., Takahashi, S., Buday, L., Oyarzabal, O.A., Backert, S., 2011. The signaling pathway of *Campylobacter jejuni*-induced Cdc42 activation: role of fibronectin, integrin beta1, tyrosine kinases and guanine exchange factor Vav2. Cell Commun. Signal. 9, 32.
Larson, C.L., Shah, D.H., Dhillon, A.S., Call, D.R., Ahn, S., Haldorson, G.J., Davitt, C., Konkel, M.E., 2008. *Campylobacter jejuni* invade chicken LMH cells inefficiently and stimulate differential expression of the chicken CXCLi1 and CXCLi2 cytokines. Microbiology 154, 3835–3847.
Lee, A., O'Rourke, J.L., Barrington, P.J., Trust, T.J., 1986. Mucus colonization as a determinant of pathogenicity in intestinal infection by *Campylobacter jejuni*: a mouse cecal model. Infect. Immun. 51, 536–546.
Leon-Kempis Mdel, R., Guccione, E., Mulholland, F., Williamson, M.P., Kelly, D.J., 2006. The *Campylobacter jejuni* PEB1a adhesin is an aspartate/glutamate-binding protein of an ABC transporter essential for microaerobic growth on dicarboxylic amino acids. Mol. Microbiol. 60, 1262–1275.
Li, Y.P., Vegge, C.S., Brøndsted, L., Madsen, M., Ingmer, H., Bang, D.D., 2011. *Campylobacter jejuni* induces an anti-inflammatory response in human intestinal epithelial cells through activation of phosphatidylinositol 3-kinase/Akt pathway. Vet. Microbiol. 148, 75–83.
Lin, J., Sahin, O., Michel, L.O., Zhang, Q., 2003. Critical role of multidrug efflux pump CmeABC in bile resistance and in vivo colonization of *Campylobacter* jejuni. Infect. Immun. 71, 4250–4259.
Liu, X., Gao, B., Novik, V., Galan, J.E., 2012. Quantitative proteomics of intracellular *Campylobacter jejuni* reveals metabolic reprogramming. PLoS Pathog. 8, e1002562.
Louwen, R., Heikema, A., van Belkum, A., Ott, A., Gilbert, M., Ang, W., Endtz, H.P., Bergman, M.P., Nieuwenhuis, E.E., 2008. The sialylated lipooligosaccharide outer core in *Campylobacter jejuni* is an important determinant for epithelial cell invasion. Infect. Immun. 76, 4431–4438.
Louwen, R., Nieuwenhuis, E.E., van Marrewijk, L., Horst-Kreft, D., de Ruiter, L., Heikema, A.P., van Wamel, W.J., Wagenaar, J.A., Endtz, H.P., Samsom, J., van Baarlen, P., Akhmanova, A., van Belkum, A., 2012. *Campylobacter jejuni* translocation across intestinal epithelial cells is facilitated by ganglioside-like lipooligosaccharide structures. Infect. Immun. 80, 3307–3318.
MacCallum, A., Hardy, S.P., Everest, P.H., 2005. *Campylobacter jejuni* inhibits the absorptive transport functions of Caco-2 cells and disrupts cellular tight junctions. Microbiology 151, 2451–2458.
MacCallum, A.J., Harris, D., Haddock, G., Everest, P.H., 2006. *Campylobacter jejuni*-infected human epithelial cell lines vary in their ability to secrete interleukin-8

compared to in vitro-infected primary human intestinal tissue. Microbiology 152, 3661–3665.
McSweegan, E., Walker, R.I., 1986. Identification and characterization of two *Campylobacter jejuni* adhesins for cellular and mucous substrates. Infect. Immun. 53, 141–148.
Miller, W.G., Bates, A.H., Horn, S.T., Brandl, M.T., Wachtel, M.R., Mandrell, R.E., 2000. Detection on surfaces and in Caco-2 cells of *Campylobacter jejuni* cells transformed with new gfp, yfp, and cfp marker plasmids. Appl. Environ. Microbiol. 66, 5426–5436.
Misawa, N., Blaser, M.J., 2000. Detection and characterization of autoagglutination activity by *Campylobacter jejuni*. Infect. Immun. 68, 6168–6175.
Mixter, P.F., Klena, J.D., Flom, G.A., Siegesmund, A.M., Konkel, M.E., 2003. *In vivo* tracking of *Campylobacter* jejuni by using a novel recombinant expressing green fluorescent protein. Appl. Environ. Microbiol. 69, 2864–2874.
Monteville, M.R., Konkel, M.E., 2002. Fibronectin-facilitated invasion of T84 eukaryotic cells by *Campylobacter jejuni* occurs preferentially at the basolateral cell surface. Infect. Immun. 70, 6665–6671.
Monteville, M.R., Yoon, J.E., Konkel, M.E., 2003. Maximal adherence and invasion of INT 407 cells by *Campylobacter jejuni* requires the CadF outer-membrane protein and microfilament reorganization. Microbiology 149, 153–165.
Morooka, T., Umeda, A., Amako, K., 1985. Motility as an intestinal colonization factor for *Campylobacter jejuni*. J. Gen. Microbiol. 131, 1973–1980.
Muller, A., Leon-Kempis Mdel, R., Dodson, E., Wilson, K.S., Wilkinson, A.J., Kelly, D.J., 2007. A bacterial virulence factor with a dual role as an adhesin and a solute-binding protein: the crystal structure at 1.5 A resolution of the PEB1a protein from the food-borne human pathogen *Campylobacter jejuni*. J. Mol. Biol. 372, 160–171.
Myers, J.D., Kelly, D.J., 2005. A sulphite respiration system in the chemoheterotrophic human pathogen *Campylobacter jejuni*. Microbiology 151, 233–242.
Myszewski, M.A., Stern, N.J., 1991. Phagocytosis and intracellular killing of *Campylobacter jejuni* by elicited chicken peritoneal macrophages. Avian Dis. 35, 750–755.
Nachamkin, I., Yang, X.H., Stern, N.J., 1993. Role of *Campylobacter jejuni* flagella as colonization factors for three-day-old chicks: analysis with flagellar mutants. Appl. Environ. Microbiol. 59, 1269–1273.
Nachamkin, I., Allos, B.M., Ho, T., 1998. *Campylobacter* species and Guillain–Barre syndrome. Clin. Microbiol. Rev. 11, 555–567.
Nachamkin, I., Szymanski, C.M., Blaser, M.J., 2008. Campylobacter. ASM Press, Washington, DC.
Naikare, H., Palyada, K., Panciera, R., Marlow, D., Stintzi, A., 2006. Major role for FeoB in *Campylobacter jejuni* ferrous iron acquisition, gut colonization, and intracellular survival. Infect. Immun. 74, 5433–5444.
Naito, M., Frirdich, E., Fields, J.A., Pryjma, M., Li, J., Cameron, A., Gilbert, M., Thompson, S.A., Gaynor, E.C., 2010. Effects of sequential *Campylobacter jejuni* 81-176 lipooligosaccharide core truncations on biofilm formation, stress survival, and pathogenesis. J. Bacteriol. 192, 2182–2192.
Neal-McKinney, J.M., Konkel, M.E., 2012. The *Campylobacter jejuni* CiaC virulence protein is secreted from the flagellum and delivered to the cytosol of host cells. Front. Cell. Infect. Microbiol. 2, 31.
Newell, D.G., McBride, H., Dolby, J.M., 1985a. Investigations on the role of flagella in the colonization of infant mice with *Campylobacter jejuni* and attachment of *Campylobacter jejuni* to human epithelial cell lines. J. Hyg. 95, 217–227.
Newell, D.G., McBride, H., Saunders, F., Dehele, Y., Pearson, A.D., 1985b. The virulence of clinical and environmental isolates of *Campylobacter jejuni*. J. Hyg. 94, 45–54.
Ng, L.K., Sherburne, R., Taylor, D.E., Stiles, M.E., 1985a. Morphological forms and viability of *Campylobacter* species studied by electron microscopy. J. Bacteriol. 164, 338–343.
Ng, L.K., Stiles, M.E., Taylor, D.E., 1985b. Comparison of basal media for culturing *Campylobacter jejuni* and *Campylobacter coli*. J. Clin. Microbiol. 21, 226–230.
Novik, V., Hofreuter, D., Galan, J.E., 2009. Characterization of a *Campylobacter jejuni* VirK protein homolog as a novel virulence determinant. Infect. Immun. 77, 5428–5436.
Novik, V., Hofreuter, D., Galan, J.E., 2010. Identification of *Campylobacter jejuni* genes involved in its interaction with epithelial cells. Infect. Immun. 78, 3540–3553.
Ó Cróinín, T., Backert, S., 2012. Host epithelial cell invasion by *Campylobacter jejuni*: trigger or zipper mechanism? Front. Cell. Infect. Microbiol. 2, 25.
Oelschlaeger, T.A., Guerry, P., Kopecko, D.J., 1993. Unusual microtubule-dependent endocytosis mechanisms triggered by *Campylobacter jejuni* and *Citrobacter freundii*. Proc. Natl. Acad. Sci. U. S. A. 90, 6884–6888.
Oyarzabal, O.A., Backert, S., 2011. Microbial Food Safety. Springer, New York978-1-4614-1176-5.
Parkhill, J., Wren, B.W., Mungall, K., Ketley, J.M., Churcher, C., Basham, D., Chillingworth, T., Davies, R.M., Feltwell, T., Holroyd, S., Jagels, K., Karlyshev, A.V., Moule, S., Pallen, M.J., Penn, C.W., Quail, M.A., Rajandream, M.A., Rutherford, K.M., van Vliet, A.H., Whitehead, S., Barrell, B.G., 2000. The genome sequence of the food-borne pathogen *Campylobacter jejuni* reveals hypervariable sequences. Nature 403, 665–668.
Pei, Z., Burucoa, C., Grignon, B., Baqar, S., Huang, X.Z., Kopecko, D.J., Bourgeois, A.L., Fauchere, J.L., Blaser, M.J., 1998. Mutation in the peb1A locus of *Campylobacter jejuni* reduces interactions with epithelial cells and intestinal colonization of mice. Infect. Immun. 66, 938–943.
Pesci, E.C., Cottle, D.L., Pickett, C.L., 1994. Genetic, enzymatic, and pathogenic studies of the iron superoxide dismutase of *Campylobacter jejuni*. Infect. Immun. 62, 2687–2694.
Poly, F., Read, T., Tribble, D.R., Baqar, S., Lorenzo, M., Guerry, P., 2007. Genome sequence of a clinical isolate of *Campylobacter jejuni* from Thailand. Infect. Immun. 75, 3425–3433.
Poly, F., Read, T.D., Chen, Y.H., Monteiro, M.A., Serichantalergs, O., Pootong, P., Bodhidatta, L., Mason, C.J., Rockabrand, D., Baqar, S., Porter, C.K., Tribble, D., Darsley, M., Guerry, P., 2008. Characterization of two *Campylobacter jejuni* strains for use in volunteer experimental-infection studies. Infect. Immun. 76, 5655–5667.
Prendergast, M.M., Tribble, D.R., Baqar, S., Scott, D.A., Ferris, J.A., Walker, R.I., Moran, A.P., 2004. *In vivo* phase variation and serologic response to lipooligosaccharide of *Campylobacter jejuni* in experimental human infection. Infect. Immun. 72, 916–922.
Pryjma, M., Apel, D., Huynh, S., Parker, C.T., Gaynor, E.C., 2012. FdhTU-modulated formate dehydrogenase expression and electron donor availability enhance recovery of *Campylobacter jejuni* following host cell infection. J. Bacteriol. 194, 3803–3813.
Raphael, B.H., Pereira, S., Flom, G.A., Zhang, Q., Ketley, J.M., Konkel, M.E., 2005. The *Campylobacter jejuni* response regulator, CbrR, modulates sodium deoxycholate resistance and chicken colonization. J. Bacteriol. 187, 3662–3670.
Rivera-Amill, V., Kim, B.J., Seshu, J., Konkel, M.E., 2001. Secretion of the virulence-associated *Campylobacter* invasion antigens from *Campylobacter jejuni* requires a stimulatory signal. J. Infect. Dis. 183, 1607–1616.
Russell, R.G., Blake Jr., D.C., 1994. Cell association and invasion of Caco-2 cells by *Campylobacter jejuni*. Infect. Immun. 62, 3773–3779.
Russell, R.G., O'Donnoghue, M., Blake Jr., D.C., Zulty, J., DeTolla, L.J., 1993. Early colonic damage and invasion of *Campylobacter jejuni* in experimentally challenged infant Macaca mulatta. J. Infect. Dis. 168, 210–215.
Seal, B.S., Hiett, K.L., Kuntz, R.L., Woolsey, R., Schegg, K.M., Ard, M., Stintzi, A., 2007. Proteomic analyses of a robust versus a poor chicken gastrointestinal colonizing isolate of *Campylobacter jejuni*. J. Proteome Res. 6, 4582–4591.
Sellars, M.J., Hall, S.J., Kelly, D.J., 2002. Growth of *Campylobacter jejuni* supported by respiration of fumarate, nitrate, nitrite, trimethylamine-N-oxide, or dimethyl sulfoxide requires oxygen. J. Bacteriol. 184, 4187–4196.
Snelling, W.J., McKenna, J.P., Lecky, D.M., Dooley, J.S., 2005. Survival of *Campylobacter jejuni* in waterborne protozoa. Appl. Environ. Microbiol. 71, 5560–5571.
Song, Y.C., Jin, S., Louie, H., Ng, D., Lau, R., Zhang, Y., Weerasekera, R., Al Rashid, S., Ward, L.A., Der, S.D., Chan, V.L., 2004. FlaC, a protein of *Campylobacter jejuni* TGH9011 (ATCC43431) secreted through the flagellar apparatus, binds epithelial cells and influences cell invasion. Mol. Microbiol. 53, 541–553.
Svensson, S.L., Davis, L.M., MacKichan, J.K., Allan, B.J., Pajaniappan, M., Thompson, S.A., Gaynor, E.C., 2009. The CprS sensor kinase of the zoonotic pathogen *Campylobacter jejuni* influences biofilm formation and is required for optimal chick colonization. Mol. Microbiol. 71, 253–272.
Szymanski, C.M., King, M., Haardt, M., Armstrong, G.D., 1995. *Campylobacter jejuni* motility and invasion of Caco-2 cells. Infect. Immun. 63, 4295–4300.
Szymanski, C.M., Burr, D.H., Guerry, P., 2002. *Campylobacter* protein glycosylation affects host cell interactions. Infect. Immun. 70, 2242–2244.
Tamás, P., Solti, Z., Bauer, P., Illés, A., Sipeki, S., Bauer, A., Faragó, A., Downward, J., Buday, L., 2003. Mechanism of epidermal growth factor regulation of Vav2, a guanine nucleotide exchange factor for Rac. J. Biol. Chem. 278, 5163–5171.
Tareen, A.M., Dasti, J.I., Zautner, A.E., Gross, U., Lugert, R., 2010. *Campylobacter jejuni* proteins Cj0952c and Cj0951c affect chemotactic behaviour towards formic acid and are important for invasion of host cells. Microbiology 156, 3123–3135.
Tareen, A.M., Dasti, J.I., Zautner, A.E., Gross, U., Lugert, R., 2011. Sulphite: cytochrome c oxidoreductase deficiency in *Campylobacter jejuni* reduces motility, host cell adherence and invasion. Microbiology 157, 1776–1785.
Tegtmeyer, N., Rohde, M., Backert, S., 2011. Clinical presentations and pathogenicity mechanisms of foodborne infections. In: Oyarzabal, O.A., Backert, S. (Eds.), Microbial Food Safety. Springer, New York, NY, pp. 13–31.
Tribble, D.R., Baqar, S., Scott, D.A., Oplinger, M.L., Trespalacios, F., Rollins, D., Walker, R.I., Clements, J.D., Walz, S., Gibbs, P., Burg III, E.F., Moran, A.P., Applebee, L., Bourgeois, A.L., 2010. Assessment of the duration of protection in *Campylobacter jejuni* experimental infection in humans. Infect. Immun. 78, 1750–1759.
van Alphen, L.B., Bleumink-Pluym, N.M., Rochat, K.D., van Balkom, B.W., Wösten, M.M., van Putten, J.P., 2008. Active migration into the subcellular space precedes *Campylobacter jejuni* invasion of epithelial cells. Cell. Microbiol. 10, 53–66.
Van Deun, K., Pasmans, F., Ducatelle, R., Flahou, B., Vissenberg, K., Martel, A., Van den Broeck, W., Van Immerseel, F., Haesebrouck, F., 2008. Colonization strategy of *Campylobacter jejuni* results in persistent infection of the chicken gut. Vet. Microbiol. 130, 285–297.
van Putten, J.P., van Alphen, L.B., Wosten, M.M., de Zoete, M.R., 2009. Molecular mechanisms of *campylobacter* infection. Curr. Top. Microbiol. Immunol. 337, 197–229.
van Spreeuwel, J.P., Duursma, G.C., Meijer, C.J., Bax, R., Rosekrans, P.C., Lindeman, J., 1985. *Campylobacter colitis*: histological immunohistochemical and ultrastructural findings. Gut 26, 945–951.
Walker, R.I., Caldwell, M.B., Lee, E.C., Guerry, P., Trust, T.J., Ruiz-Palacios, G.M., 1986. Pathophysiology of *Campylobacter enteritis*. Microbiol. Rev. 50, 81–94.
Wassenaar, T.M., Engelskirchen, M., Park, S., Lastovica, A., 1997. Differential uptake and killing potential of *Campylobacter jejuni* by human peripheral monocytes/macrophages. Med. Microbiol. Immunol. 186, 139–144.
Watson, R.O., Galán, J.E., 2005. Signal transduction in *Campylobacter jejuni*-induced cytokine production. Cell. Microbiol. 7, 655–665.
Watson, R.O., Galan, J.E., 2008. *Campylobacter jejuni* survives within epithelial cells by avoiding delivery to lysosomes. PLoS Pathog. 4, e14.
Watson, R.O., Novik, V., Hofreuter, D., Lara-Tejero, M., Galan, J.E., 2007. A MyD88-deficient mouse model reveals a role for Nramp1 in *Campylobacter jejuni* infection. Infect. Immun. 75, 1994–2003.
Wine, E., Chan, V.L., Sherman, P.M., 2008. *Campylobacter jejuni* mediated disruption of polarized epithelial monolayers is cell-type specific, time dependent, and correlates with bacterial invasion. Pediatr. Res. 64, 599–604.
Wooldridge, K.G., Williams, P.H., Ketley, J.M., 1996. Host signal transduction and endocytosis of *Campylobacter jejuni*. Microb. Pathog. 21, 299–305.
Wösten, M.M., Wagenaar, J.A., van Putten, J.P., 2004. The FlgS/FlgR two-component signal transduction system regulates the fla regulon in *Campylobacter jejuni*. J. Biol. Chem. 279, 16214–16222.

Yao, R., Burr, D.H., Doig, P., Trust, T.J., Niu, H., Guerry, P., 1994. Isolation of motile and non-motile insertional mutants of *Campylobacter jejuni*: the role of motility in adherence and invasion of eukaryotic cells. Mol. Microbiol. 14, 883–893.

Yao, R., Burr, D.H., Guerry, P., 1997. CheY-mediated modulation of *Campylobacter jejuni* virulence. Mol. Microbiol. 23, 1021–1031.

Young, K.T., Davis, L.M., Dirita, V.J., 2007. *Campylobacter jejuni*: molecular biology and pathogenesis. Nat. Rev. Microbiol. 5, 665–679.

Zheng, J., Meng, J., Zhao, S., Singh, R., Song, W., 2008. *Campylobacter*-induced interleukin-8 secretion in polarized human intestinal epithelial cells requires *Campylobacter*-secreted cytolethal distending toxin- and Toll-like receptor-mediated activation of NF-kappaB. Infect. Immun. 76, 4498–4508.