European Journal of Microbiology and Immunology 2 (2012) 1, pp. 32–40
DOI: 10.1556/EuJMI.2.2012.1.6

MOLECULAR MECHANISMS AND BIOLOGICAL ROLE OF *CAMPYLOBACTER JEJUNI* ATTACHMENT TO HOST CELLS

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Received: January 3, 2012; Accepted: January 4, 2012

Adhesion to host cells is an important step in pathogenesis of *Campylobacter jejuni,* which is the most prevalent bacterial cause of human gastroenteritis worldwide. In contrast to other bacteria such as *E. coli* and *Salmonella*, adherence of *C. jejuni* is not mediated by fimbria or pili. A number of *C. jejuni* adhesion-related factors have been described. However, the results obtained by different researchers in different laboratories are often contradictory and inconclusive, with only some of the factors described being confirmed as true adhesins. In this review, we present the current state of studies on the mechanisms of attachment of *C. jejuni* to host cells.

Keywords: *Campylobacter jejuni,* pathogenesis, host cell receptors, adhesion, adhesins, attachment, glycoproteins, evasion of immune response, colonisation

Introduction

Adhesion to host epithelial cells is an important step in the pathogenesis of many bacteria [1, 2]. Bacterial attachment may involve specific interaction between specialised proteins that can bind to receptors on host cell surfaces [3]. Bacterial adhesion depends on many factors including bacterial motility and adhesin production, and may lead to host cell invasion and subversion of both innate and adaptive immune response [4].

Campylobacter jejuni, which is the most prevalent bacterial cause of human gastroenteritis worldwide, is a Gram-negative, microaerophilic, curved or spiral bacillus with unipolar or bipolar flagella [5–7]. These bacteria colonise the gastrointestinal tract of birds, and infected poultry is considered to be the main source of human infection [8]. *Campylobacter* infection often occurs through the consumption of undercooked poultry, unpasteurised milk and untreated water [9]. The development of the disease involves adaptation of the bacteria to harsh gut environment, where they can survive and adhere to intestinal epithelial cells [10]. Adhesion is considered to play an important role in colonisation of chickens by *C. jejuni* and may be an important factor in human infection [11, 12]. It has been shown that severity of clinical symptoms in infected individuals depends on the level of *C. jejuni* adherence to HeLa cells [13]. Understanding molecular mechanisms involved in the interaction between host cell receptors and *C. jejuni* adhesins may allow the development of novel antibacterial agents based on the inhibition of bacterial attachment.

Factors and molecules involved in *Campylobacter* attachment

General features

Adherence of *Campylobacter* is not mediated by fimbria or pili, like in other Gram-negative bacteria such as *E. coli* and *Salmonella* [14, 15]. Various bacterial cell structures have been shown to contribute to interaction of *Campylobacter* with host cells. Some of these molecules were suggested to play a role of true adhesins directly interacting with host cell receptors, although in some cases, these data were either incomplete or contradictory. All currently known and putative adhesion-related factors of *C. jejuni* are summarised in *Tables 1* and *2*.

Confirmed protein adhesins

Host cell receptors have been identified only for outer membrane proteins CadF and FlpA specific to fibronectin (Fn) [16–19] and JlpA, specific to heat shock protein 90 [20].

Fn is a glycoprotein found in the extracellular matrix and has a molecular mass of 250 kDa [21]. Binding of bacteria to Fn is required for host cell invasion and colonisation [11, 16, 22]. For example, *cadF* mutants had a 50% reduced ability to adhere to human INT 407 cells compared to the wild type [16]. It was shown *cadF* mutation reduced the ability of *C. jejuni* strain F38011 to in-

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ISSN 2062-509X / $ 20.00 © 2012 Akadémiai Kiadó, Budapest

Table 1. *Campylobacter* protein adhesins with identifed host cell receptors*

No	Gene number in NCTC 11168 genome	Product name	Summary of experimental data	Host cell receptors	Conclusion
1	*cj1478*	CadF	Adhesion of F38011/*cadF* mutant INT407 cells was reduced [16] Purified CadF from strain F38011 adhered to INT407 cell-line [16]	Fn	Both mutagenesis and adhesion studies confirm specific interaction of CadF with host cell receptors
2	*cj1279*	FlpA	Reduced adhesion of F38011/*flpA* mutant to LMH cell-line [17] Reduced adhesion of F38011/*flpA* mutant to INT407 cell-line [17] Phenotypic changes were confirmed by complementation studies [18]	Fn	Specific interaction with host cell receptors present on cells of human and chicken origin
3	*cj0983*	JlpA	Reduced adhesion of TGH9011/*jlpA* mutant in strain to HEp-2 cell-line [23] Adherence of TGH9011 to HEp-2 cell-line was reduced in the presence of purified JlpA in a dose dependent manner [23] No affect of *jlpA* mutation on adhesion of strain F38011 to chicken LMH cell-line [17] No difference in adhesion in strain 81-176 *jlpA* mutant to human T48 cell-line [24]	HSP90	Confirmed adhesin with TGH9011 cells but not with other cells

*This table lists the genes and products involved in direct interaction with host cells receptors.

hibit binding of a clinical isolate 81-176 to the INT 407 cell line [16].

Fn-binding protein FlpA contains Fn type III domains [17, 18]. Disruption of the *flpA* gene impairs bacterial ability to adhere to chicken LMH hepatocellular carcinoma epithelial cells and to human INT 407 cells, and reduces bacterial ability to colonise chickens [17]. In addition, bacterial binding to host cells was inhibited by anti-FlpA specific antiserum in a dose-dependent way [18]. These findings, which were supported by complementation studies, confirmed that FlpA is an Fn-specific adhesin.

JlpA is a lipoprotein involved in adhesion [23]. This protein, which is loosely associated with the outer membrane, is able to bind heat shock protein 90 on the surface of HEp-2 epithelial host cells, leading to the activation of NF-κB and p38 MAP kinase [20, 23]. Mutations in *jlpA* gene resulted in reduced adherence of JlpA to HEp-2 epithelial cells [23]. Purified JlpA inhibited adherence of *C. jejuni* to HEp-2 cells confirming the role of the former as an adhesin [23]. However, inactivation of *jlpA* gene did not affect the ability of *C. jejuni* to bind to chicken LMH cells or to colonise broiler chickens [17], and also did not reduce attachment to human T84 human colonic adenocarcinoma cell [24] suggesting that only certain host cells are able to produce receptors for this adhesin.

Unconfirmed and putative adhesion-related proteins

A number of proteins of *C. jejuni* have been suggested to play a role in adhesion. However, insufficient and/or contradictory results, as well as the lack of data on a possible nature of putative host cell receptors, do not allow to provide conclusive evidence on their role as adhesins. These factors are therefore referred to as 'putative' and are listed in *Table 2*.

Cj1349 is annotated as a putative Fn/fibrinogen-binding protein. *Cj1349* mutant also had reduced (by 14%) ability to adhere to chicken LMH cells, but *cj1349c* mutation had no effect on colonisation of chicks [19]. As no complementation studies have been conducted to confirm these data, and no detailed characterisation of the protein was performed, this protein is regarded as a putative adhesin.

CapA is an autotransporter lipoprotein reported to be involved in *C. jejuni* adherence to host cells [25]. *CapA* mutant showed decreased adherence to human Caco-2 cells and also low colonisation efficiency in chicks [25]. In another study, mutation of *capA* also showed decreased adherence to chicken epithelial cells *in vitro*, although it did not result in a reduced ability of mutants to colonise chicks (47%) [17].

Cj0091 is another lipoprotein, which has been reported to be involved in *Campylobacter* adhesion. It was shown

Table 2. *Campylobacter* adhesion-related proteins*

No	Gene number in NCTC 11168 genome	Product name	Summary of experimental data	Amino acid sequence similarity to known adhesins in other bacteria: best hits (E-value less than 1.0e–7, BlastP, SwissProt database)	Pfam domains	Conclusion
1	*cj1349*	Cj1349	Reduced adhesion of F38011/*cj1349* mutant to LMH cell-line [17] Contains Fn-binding domain [17]	None Note: annotated by Sanger Institute as fibronectin/fibrinogen-binding protein similar to *Streptococcus pyogenes* fibronectin/fibrinogen-binding protein FBP54 (23.2% identity in overlap of 466 amino acid sequence, E value: 1.7e–05)	Fn-binding domain (FbpA)	Studies with purified protein not performed Fibronectin predicted as a receptor (by similarity, not confirmed)
2	*cj0628/ cj0629*	CapA	Reduced adhesion of NCTC11168H/*capA* mutant to Caco-2 cell-line [25] Reduced adhesion of F38011/*capA* mutant to LMH cell-line [17]	None	None	Complementation studies to confirm the results not conducted Studies with purified protein not performed
3	*cj0091*	Cj0091	Reduced adhesion of NCTC 11168/*cj0091* mutant to INT 407 cell-line [26]	None	None	Studies with purified protein not performed
4	*cj1339*	FlaA	Purified flagella from strain 81116 adhered to INT407 cell-line [34] Purified flagella did not prevent binding of strain 81116 to INT407 cell-line [36] No difference in adhesion of 81116/*flaA* mutant to INT407 cell-line [35] Reduced adhesion of mutant in strain 81-176/*flaA* mutant to INT407 cell-line [30]	None	Flagellin	Contradictory data Possible reason: difference in strains and/or assay conditions
5	*cj0588*	TlyA	Reduced adhesion of *cj0588* mutants in strains 81-176 and 81116 to Caco-2 cell-line [38] Purified TlyA protein from 81-176 binds Caco-2 cell-line [38]	*Treponema hyodysenteriae (Serpulina hyodysenteriae)* haemolysin A SP: HLYA_TREHY Identities = 90/263 (34%) Positives = 137/263 (52%)	FtsJ-like methyltransferase	No complementation studies to confirm the results
6	*cj0921*	PEB1	Purified PEB1 from strain 85H binds to HeLa cells [41] Reduced adhesion of 81-176/*peb1A* mutant to HeLa cell-line [43] No difference in adhesion of F38011/*peb1A* mutant to LMH cell-line [17] No difference in adhesion in strain 81-176/*peb1A* mutant to T84 cell-line [24]	None	Bacterial extracellular solute-binding proteins, family 3	Contradictory data

Table 2. *Continued*

No	Gene number in NCTC 11168 genome	Product name	Summary of experimental data	Amino acid sequence similarity to known adhesins in other bacteria: best hits (E-value less than 1.0e–7, BlastP, SwissProt database)	Pfam domains	Conclusion
7	*cj0289*	PEB3	Major antigenic protein [40] Transporter protein [50] Cell surface located glycoprotein [46]	None	Bacterial extracellular solute-binding protein	Original data on its role as an adhesin not confirmed
8	*cj0596*	PEB4	Purified PEB4 from strain 85H did not bind to HeLa cell-line [41] Reduced adhesion of NCTC11168/*peb4* mutant of strain to INT407 cell-line [51] Pleiotropic effect of mutation (severe changes in outer membrane profile) [52]	None	Peptidyl prolyl isomerase	Contradictory data/ Complementation studies did not confirm changes in phenotype Possible function as a periplasmic chaperone
9	*cj1677/ cj1678*	CapB	Expression not detected [25]	None	None	No experimental data available Putative adhesin (high level of similarity to CapA)
10	*cj0737*	P95	Identified as a putative adhesin using comparative genomics [53]	*H. influenzae* HxuA haem:haemopexin-binding protein SP:P44602 Identities = 69/214 (32%) Positives = 97/214 (45%)	Haemagglutination activity domain	No mutagenesis, or adhesion studies performed to confirm a proposed function of P95 as an adhesin

*This table lists proteins either involved in interaction with host cell receptors indirectly or those whose function as adhesins is not conclusive (e.g. insufficient experimental evidence or contradictory data). Host cell receptors for these proteins are not known/not confirmed.

that Cj0091 mediates binding of *C. jejuni* to INT 407 cells and is necessary for colonisation of the gastrointestinal tract of chickens [26]. Since *cj0091* mutation affected colonisation at the early stages of the infectious process, these data suggest that Cj0091 is required only for initial adherence.

Major outer membrane protein (MOMP) is a pore-forming protein implicated in the adherence of *C. jejuni* to INT 407 cells [27]. MOMP consists of 16–18 membrane strands connected by short periplasmic turns and several external loops, which are antigenically variable [28]. In addition to a possible role in bacterial attachment, MOMP is also involved in transport of ions across the bacterial cell wall [29]. Due to essentiality of this function and inability to generate a mutant in the respective gene, it is not currently possible to make the final conclusion on whether this protein directly interacts with host cells and plays a role of an adhesin [17].

A number of studies have suggested a role of flagella as an adhesin [30–33]. In particular, McSweegan and Walker demonstrated that purified flagella specifically adhered to INT407 cells [34]. Motility appears to interfere with adhesion of *C. jejuni* 81116 to INT 407 cell line since immobilization of flagella increased adhesion [34]. These findings suggest that flagella may work as an adhesin. However, this could not be confirmed in later studies demonstrating that both flagellated and aflagellated *C. jejuni* 81116 adhered to INT 407 cells [35]. Wassenaar et al. [36] investigated the adhesive properties of flagella by competition experiments, where purified flagella were added to INT407 cells prior to infection with *C. jejuni*. As incubation with flagella showed no effect on penetration compared to control, authors concluded that flagella do not have specific adhesive properties. These data contradicted subsequent studies demonstrating that inactivation

of a flagellin-encoding gene *flaA* resulted in reduction of *C. jejuni* adhesion to the INT 407 cells [30, 37]. A non-flagellated, non-motile mutant was induced by ultraviolet irradiation of wild-type strain of *C. jejuni* CF84-340. Cellular adherence and invasiveness were compared with fluorescent antibody staining. Only 6.1% of flagella-defective mutants invaded the INT 407 cells, while 21.4% of the organisms of the wild-type strain were able to invade the cultured cells [37]. Mutation of *flaA* gene generated a non-adherent non-invasive mutant that had reduced ability to adhere to INT 407 cells [30]. Overall, it seems that flagella may play a role in initial attachment to epithelial cells although further studies are required to confirm it.

TlyA, which is homologous to bacterial haemolysins found in other bacteria, was suggested to play a role in adhesion of *C. jejuni* [38]. Mutation in *tlyA* resulted in 56% reduction of the ability of *C. jeuni* 81-176 to adhere to cultured Caco-2 cells. Moreover, purified TlyA protein was found to interact with Caco-2 cells, indicating the presence of specific host cell receptors. It is possible that TlyA is a bifunctional protein as *tlyA* mutant in a closely related bacterium *H. pylori* had decreased haemolytic activity and decreased ability to adhere to gastric epithelial cells [39]. These finding supports the concept that TlyA of *Campylobacter* may also be involved in interaction with host cells.

In 1991, Pei et al. [40] purified four proteins called PEB1 to PEB4. Later, PEB1 was identified as a major antigenic protein of *C. jejuni* that was able to bind to HeLa cells [41]. Convalescent sera from infected patients commonly recognise PEB1, which is encoded by *peb1A* gene [42]. PEB1, which is also known as PEB1a, plays an important role in adherence and host colonisation [43]. PEB1 is a surface exposed and/or a periplasmic component of an aspartate/glutamate ABC transporter [44]. Despite having no leader peptide, the protein was also found to be secreted after cloning into *E. coli* by as yet unknown mechanism [45]. Mutation of *peb1A* resulted in 100-fold reduction of *C. jejuni* attachment to HeLa cells and also impaired bacterial ability to colonise intestinal cells of rats [43]. These results contradicted with another study reporting no effect of peb1A mutation on the ability of *C. jejuni* to attach to cultured epithelial cells [24]. Flanagan et al. [17] also demonstrated that *peb1A* mutant did not show a reduced ability to bind to chicken LMH cells, even though it was unable to colonise chicks. A BLAST search did not reveal similarity with any known experimentally confirmed adhesins found in other bacteria. It was suggested that PEB1 does play an important role in aspartate and glutamate transport [17]. Overall, these findings suggest that either PEB1 is not involved in adhesion directly or it is a bifunctional protein.

PEB3 is a highly immunogenic protein reactive with convalescent sera from patients with Campylobacteriosis [40]. It was shown that PEB3 is a surface glycoprotein interacting with soybean agglutinin (SBA) *in vitro,* due to the presence of α-linked GalNAc residues [46]. PEB3 has 56% sequence identity with *E. coli* Paa protein [47] and 54% identity with *Vibrio cholerae* AcfC, an accessory colonisation factor [48]. Despite a possible role in colonisation, the exact function of Acf protein is unknown. Based on its location on the cell surface and the presence of a sulfite-binding domain, it was suggested that Paa protein may play a role of an adhesin with specificity to highly sulfated heparin receptors present on the surface of host cell [47]. However, no experimental evidence has been presented to support this hypothesis. It is equally possible that this protein performs a transport or other function indirectly affecting expression of adhesin(s). According to its annotation Paa has a "substrate binding domain of LysR-type transcriptional regulators" suggesting a possible regulatory function of this protein. Similarly, despite a suggestion by Rangarajan et al. [49] that PEB3 may functions as an adhesin, there are no published data confirming it. A role of PEB3 in transport of 3-phosphoglycerate was reported by Min et al. [50] suggesting a possible dual function of this protein.

Inactivation of *peb4* gene led to a reduction in ability of bacteria to adhere to INT407 cells, to form biofilms and colonise mice [51]. Subsequent studies demonstrated induction of *peb4* gene expression at 37 °C. Combined with the findings that *peb4* mutation reduced bacterial motility and ability to invade host cells, the results suggest a possible role of this protein in human infection [52]. However, phenotypic changes in the mutant were not supported by complementation and did not allow researchers to arrive at any firm conclusion about the function(s) of this protein.

The presence of *capB* gene coding for a putative autotransporter was identified in *Campylobacter* genome. According to Sanger Institute annotation *capB* gene was assigned two pairs of ORFs, in the same way as *capA*. In contrast to CapA, playing a role in adhesion to cultured cells, no expression of CapB was detected [25]. Due to high level of amino acid sequence similarity between the two proteins, CapB might also be considered a putative adhesin. There is a possibility of recombinant events occurring between these two genes, which might lead to production of new antigenic variants.

A putative adhesin P95 was detected by genomics analysis of two strains of *Campylobacter* (one isolated from a healthy patient and another from a pathogenic isolate) different in their ability to adhere to two Caco-2 cells [53]. The study identified a sequence of an ORF encoding a protein of 869 amino acids or 95 kDa. This gene product showed significant sequence similarity to adhesins found in other Gram-negative bacteria, such as *Haemophilus* and *Bordetella*, supporting a role of P95 as adhesin. However, the function prediction and preliminary results were not supported by construction and analysis of a respective mutant, or by investigation of a purified protein, and there are no data on a possible nature of a putative cognate host cell receptor.

Campylobacter *adhesion involving lectin-glycan interaction*

Host cell interaction in such bacteria such as *H. pylori* and *E. coli* may involve interaction of adhesins with oligosac-

charides found on the surface of host cells [1, 54, 55]. A similar lectin–glycan interaction appears to take place in case of *Campylobacter*. It was found that in the presence of certain lectins *C. jejuni* adherence to Caco-2 cells was reduced by more than 85% and that such reduction was due to inhibition of bacterial interaction with oligosaccharides present on the surface of Caco-2 cells [56]. On the other hand, *Campylobacter* is also known to produce glycans [lipo-oligosaccharide (LOS) and glycoproteins] that can potentially be involved in interaction with lectin-like host cell receptors. There are two types of glycoproteins produced by *C. jejuni:* O-linked and N-linked. In contrast to structures of oligosaccharides decorating O-linked glycoproteins the structure of the glycan present in N-linked glycoproteins is highly conserved consisting of GalNac-α1,4-GalNAc-α1,4-(Glcβ1,3)-GalNAc-α1,4-GalNAc-α1,4-GalNAc-α1,3-Bac [57]. Inactivation of N-linked glycosylation machinery leads to decreased colonisation ability of bacteria, as well as to a reduction of adherence to and invasion of human epithelial cells suggesting that some of these glycoproteins may play a role of adhesins [57–60]. Although it remains unknown whether *Campylobacter* N-glycosylation is required for adhesion there is some evidence that it might influence host immune response [61, 62].

Indeed, human immune cells including macrophages do express various classes of lectins that recognise specific glycan structures presented on the surface of pathogens [63]. Of particular interest are *Campylobacter* cell surface-located glycoproteins PEB3 and JlpA, which may interact with lectin-like host cell receptors such as a subset C-type lectins specific to GalNac residues. C-type lectins are calcium dependent carbohydrate-binding proteins that have been shown to function as receptors recognised by various pathogens. A role of C-type lectins in evasion of innate host immune response has been demonstrated in such bacteria. For example, interaction of *M. tuberculosis* cell-surface glycans with C-type lectins present on dendritic cells stimulated production of IL-10, a known anti-inflammatory molecule [64]. Excessive production of IL-10 also inhibited maturation of dendritic cells, thus, further assisting bacteria to evade host immune response [64]. In the study by van Sorge et al. [61] it was shown that N-linked glycoproteins of *C. jejuni* interact with C-type lectins of macrophage galactose-type lectins (MGL). These findings suggest a possible role of *Campylobacter* N-lined glycosylation system for modulation of host immune responses.

In addition to galactose-specific C-type lectins described above, other C-type lectins as well as sialic acid-binding immunoglobulin-like lectins (siglecs) may be involved in host-pathogen interaction [61, 62]. Siglecs are a family of type I membrane proteins widely expressed on immune cells that have specificity for sialic acid-containing glycans present in some types of *Campylobacter* lipo-oligosaccharides (LOSs). Therefore, siglec-LOS interaction may play a role in pathogen recognition [63, 65]. Sialic acid containing LOS of *Campylobacter* has been shown to interact specifically with Siglec-7 on monocytes and natural killer cells [66]. It is possible that *Campylobacter* benefits from expression of sialylated LOS as it might modulate the host innate immune response.

Characterization of the glycan receptors essential for *C. jejuni* adhesion may allow the design of new intervention strategies based on inhibition of bacterial interaction with host cells.

A role of capsule in Campylobacter *adhesion*

Another cell surface structure of *Campylobacter* that may also influence bacterial interaction with host cells is a capsular polysaccharide (CPS) [67–69]. CPSs may be involved in attachment, as capsule deficient mutants (*kpsM* mutants) of 81–176 strain were twofold less adhesive to INT407 cells compared to the wild type strain [70]. A similar reduction in attachment to INT407 cells was found with acapsulate mutant 81116/*kpsE* [71]. Although this data might suggest requirement of *Campylobacter* capsule for attachment, the results were not supported by complementation. These results were in disagreement with a finding that *kpsM* mutant of strain 11168H showed higher level of adhesion to Caco-2 cells [72]. It was suggested that reduced ability of capsulated strains to adhere is due to the masking effect of the capsule on cells surface adhesins. The exact role of the capsule in adhesion is still unclear. Karlyshev et al. [72] suggested that the production of capsule and adhesins may be differentially regulated and can be expressed at different stages of infection. Production of CPS might be necessary in the beginning of bacterial interaction with the mucus layer. This may be followed by down regulation of CPS production leading to exposure of bacterial adhesins.

Summary

Campylobacter interaction with host cells is a complicated process involving a variety of bacterial cell surface structures interacting with particular host cell receptors. Attachment to these receptors may be required for colonisation, invasion of host cell tissues and/or evasion of host immune response. The findings that attachment may involve glycan–lectin interaction may serve a basis for the development of novel intervention strategies using analogues of such glycans as inhibitors of bacterial adhesion. For example, targeting adhesins specific to chicken epithelial cells may allow elimination of the pathogen from poultry, whilst targeting adhesins specific to human tissues may assist in the development of novel anti-*Campylobacter* drugs. Remarkably, preliminary experiments did show inhibitory effect of fucosyl-oligosaccharides on *Campylobacter* attachment and colonisation using a mice model of infection [73]. The synthesis and usage of specific oligosaccharides as inhibitors of bacterial adhesion is a highly promising research direction [74]. How-

ever, for most putative adhesins the receptors still remain unknown. The fact that there are at least two Fn-specific adhesins (FlpA and CadF) may indicate their cooperation during bacterial binding to host cells. Alternatively, the respective genes may be differentially expressed and induced under certain stages of infection. Indeed, a Fn binding protein of *Staphylococcus epidermidis* was found to be expressed only *in vivo* during infection [75]. Further studies on attachment to host cell receptors should lead to better understanding of the lifestyle and the mechanisms of pathogenicity of *C. jejuni*.

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