Campylobacter jejuni: molecular biology and pathogenesis

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Abstract | *Campylobacter jejuni* is a foodborne bacterial pathogen that is common in the developed world. However, we know less about its biology and pathogenicity than we do about other less prevalent pathogens. Interest in *C. jejuni* has increased in recent years as a result of the growing appreciation of its importance as a pathogen and the availability of new model systems and genetic and genomic technologies. *C. jejuni* establishes persistent, benign infections in chickens and is rapidly cleared by many strains of laboratory mouse, but causes significant inflammation and enteritis in humans. Comparing the different host responses to *C. jejuni* colonization should increase our understanding of this organism.

Signature-tagged mutagenesis
A method for simultaneously screening pools of bacteria that have transposon-generated mutations that is used to identify genes that are required for survival under the conditions specified by the investigator. Widely used to identify genes in bacterial pathogens that are required for virulence or colonization.

As a human pathogen, *Campylobacter jejuni* is an accidental tourist that has reservoirs in water and various animals[1–3] (FIG. 1). In the developed world, where waterborne infection is less likely, animals are the primary source of human infection and disease (termed campylobacteriosis). Frequently, and perhaps most commonly, disease arises after the consumption of chicken products that have been contaminated during processing. *C. jejuni* is considered to be a commensal organism of chickens and other avian species. Although the experimental infection of chickens with *C. jejuni* can lead to diarrhoea[4,5], this is not typical, and it appears that the human response to *C. jejuni* infection is more symptomatic than that of the chicken. This situation is similar to that with the better-characterized pathogen enterohaemorrhagic *Escherichia coli*, which is a common colonizer of cattle and causes human disease through the ingestion of ground beef and other foods that have been contaminated by contact with cattle faeces. In both cases, control measures for human populations might be more successful if they were directed at reducing colonization of the natural host. Some potential control measures for *Campylobacter* infections are discussed in BOX 1.

C. jejuni belongs to the epsilon class of proteobacteria, in the order *Campylobacteriales*; this order includes two other genera, *Helicobacter* and *Wolinella*. Like *C. jejuni*, members of these genera have small genomes (1.6–2.0 megabases) and can establish long-term associations with their hosts, sometimes with pathogenic consequences. The genus *Helicobacter* includes the species *Helicobacter pylori*, which causes gastric ulcers and is clearly a pathogen, but which can be carried asymptomatically in humans for decades. The genus *Wolinella* contains a single species, *Wolinella succinogenes*, which colonizes cattle as a commensal organism. Thus, these related organisms appear to be host-adapted and can establish and maintain their niches without generating a response in the host that is sufficient for clearance.

The basis for the different outcomes of *C. jejuni* infection in humans versus chickens is not well understood. This is partly due to the lack of a good small-animal model that reproduces the human disease. Such a model would enable detailed investigations to be made of the basic mechanisms of *C. jejuni* pathogenesis. Ferrets colonized with pathogenic *C. jejuni* isolates can exhibit symptoms of disease that are seen in humans, including diarrhoea and inflammation[6], but the high cost and lack of suitable reagents and knockout technology to study the host factors that are involved in disease diminish the attractiveness of this model. Various murine models have been tried, but the results have been inconsistent; most do not replicate human disease by producing clinical symptoms, although inflammation and other pathological indicators have been observed[7–13]. Furthermore, these models often require high doses of infection, which limits the effectiveness of genetic approaches such as signature-tagged mutagenesis in identifying the important traits of *C. jejuni* infection. As a natural host and important food source for humans, the chicken is a good model for studying the basic aspects of host colonization and is potentially a good target for anti-*Campylobacter* strategies that could ultimately protect human populations. In addition to natural and experimental models of animal infection, human intestinal epithelial cell lines, such as the INT 407 cell line, have also been used in *C. jejuni* studies.

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The determination of the complete genome sequence of several *C. jejuni* strains and plasmids has heralded the beginning of a new era of *C. jejuni* research[14–18]. These projects have revealed the potential mechanisms by which *C. jejuni* associates with the host; for example, the complete sequencing of pVir, a plasmid that is found in some isolates of *C. jejuni*, has led to the identification of a type IV secretion system that has been demonstrated to have a role in cell invasion and pathogenicity in ferrets[16,19]. The publication of the genome sequence has also enabled the development of multiple genetic and genomic tools for use in *C. jejuni*, including microarrays, transposons for efficient random mutagenesis, signature-tagged mutagenesis, new reporter constructs and vectors for constructing in-frame deletion mutants and chromosomal point mutations[20–28].

The key findings in the molecular biology and pathogenicity of *C. jejuni* that have been uncovered and analysed using these models and techniques will be discussed in this Review, together with an analysis of the ways in which these factors contribute to colonization and disease in humans and chickens.

C. jejuni biology and pathogenicity

Genetic variation and natural transformation. *C. jejuni* displays extensive genetic variation, which has arisen from intragenomic mechanisms as well as genetic exchange between strains. Sequencing the genome of *C. jejuni* has revealed the presence of hypervariable sequences that consist of homopolymeric tracts[15]. Genome sequence data has also indicated that the frequency of variation within these sequences is high, which may be partly due to the lack of clear homologues of many *E. coli* DNA-repair genes[15]. Most of the hypervariable sequences that have been found are in regions that encode proteins that are involved in the biosynthesis or modification of surface-accessible carbohydrate structures, such as the capsule, lipooligosaccharide (LOS) and flagellum[15] (FIG. 2). Variation in these structures arises from mechanisms such as phase variation, gene duplication and deletion, frameshifts and point mutations[15,29–33].

C. jejuni is naturally competent, meaning that it can take up DNA from the environment. This leads to recombination between strains, which allows the generation of even more genetic diversity. The horizontal transfer of both plasmid and chromosomal DNA occurs both *in vitro* and during chick colonization, which indicates that natural transformation could have an important role in genome plasticity and in the spread of new factors such as antibiotic resistance, even in the absence of selective pressure[34–36]. *In vitro*, *C. jejuni* displays a marked preference for DNA from *C. jejuni* strains, as opposed to DNA from other species[35]. In addition, the frequency of natural transformation is affected by carbon dioxide and bacterial cell density, which indicates that horizontal exchange is probably environmentally regulated *in vivo*[35]. Transposon mutagenesis of *C. jejuni* has identified several genes that are required for natural transformation, including some components of the type II secretion system[37]. A candidate-gene approach that

Type IV secretion system
A bacterial secretion system that is related to bacterial conjugative pili and that consists of a secretion channel and often a pilus structure. It is involved in the secretion of proteins and/or DNA between two bacterial cells or between a bacterial cell and a eukaryotic cell.

Homopolymeric tract
A stretch of DNA that contains multiple repetitions of a single nucleotide. It can lead to slipped-strand mispairing, which can result in variation in the length of the homopolymeric tract and, potentially, phase variation.

Lipooligosaccharide
(LOS). Found in the outer leaflet of the outer membrane of some Gram-negative bacteria. LOS consists of lipid A linked to a polysaccharide, but lacks the *O*-specific polysaccharide of the LOS that is found in other Gram-negative bacteria.

Phase variation
A heritable but reversible 'on and off' switch that regulates the expression of a gene or operon.

Type II secretion system
A bacterial secretion system that transports proteins across the outer membrane after they have been transported across the inner membrane by the Sec or Tat machinery.

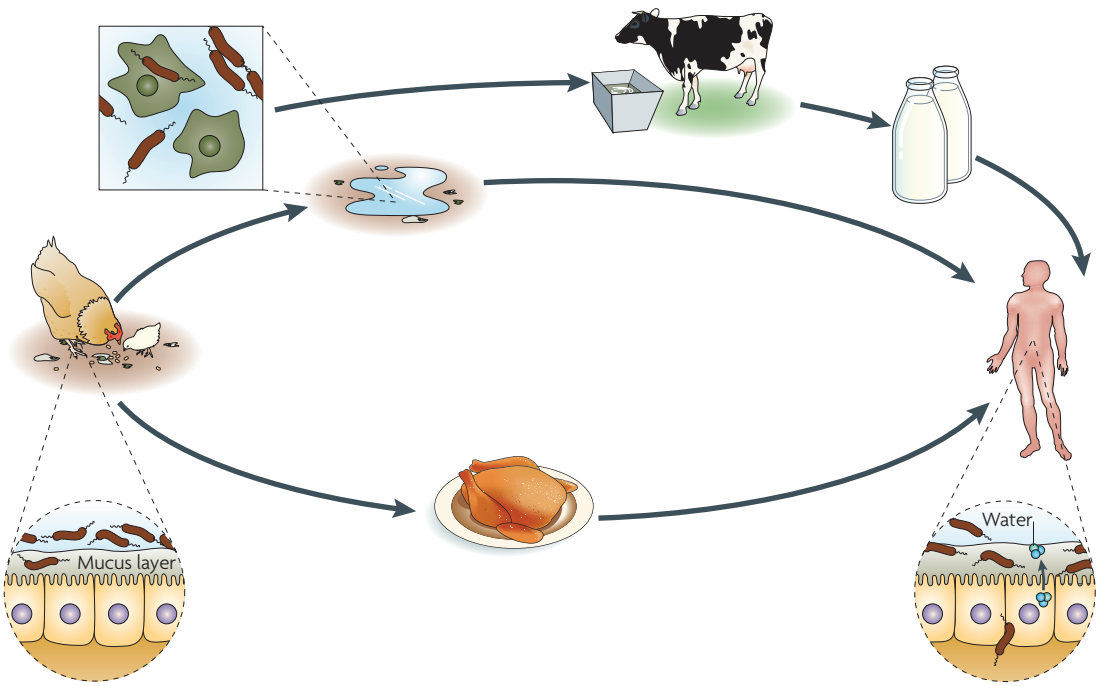

Figure 1 | **The sources and outcomes of *Campylobacter jejuni* infection.** Several environmental reservoirs can lead to human infection by *C. jejuni*. It colonizes the chicken gastrointestinal tract in high numbers, primarily in the mucosal layer, and is passed between chicks within a flock through the faecal–oral route. *C. jejuni* can enter the water supply, where it can associate with protozoans, such as freshwater amoebae, and possibly form biofilms. *C. jejuni* can infect humans directly through the drinking water or through the consumption of contaminated animal products, such as unpasteurized milk or meat, particularly poultry. In humans, *C. jejuni* can invade the intestinal epithelial layer, resulting in inflammation and diarrhoea.

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Box 1 | ***Campylobacter* control measures**

As a high percentage of retail chicken meat is contaminated with *Campylobacter jejuni*, chick colonization is a major factor in human exposure to this pathogen[183]. Although lowering the bacterial count in chickens would reduce the incidence of human infection, the best way to do this is not clear. Incorporating antibiotics into feed might help reduce the levels of *C. jejuni* colonization, but will almost certainly produce resistant strains, as usually occurs in response to wide-scale antibiotic use. One surprising finding that has raised further concerns about the use of antibiotics to control *C. jejuni* is that fluoroquinolone resistance, which arises from a mutation in the *gyrA* gene, leads to a concomitant increase in the fitness of *C. jejuni* for colonizing chickens, even in the absence of the antibiotic[184].

A good approach for identifying potential inhibitors is to use screens against specific virulence-related traits, as opposed to growth inhibitors *per se*. This general approach was validated in principle with the discovery of virstatin, a small-molecule inhibitor that blocks virulence-gene expression in *Vibrio cholerae*[185]. Virstatin inhibits a transcription activator, ToxT, that is required for the expression of crucial colonization determinants of *V. cholerae*, but it has no discernible effect on the growth of the bacterium in the laboratory. Thus, unlike antibiotics, the molecule blocks colonization without creating a strong selection for resistance. Screens for small molecules that inhibit the traits that are required for chick colonization by *C. jejuni* might lead to the development of new drugs that could be useful in reducing the levels of the bacterium in chickens and therefore lower the risk of human infection.

Vaccines are being investigated as a control measure[186], but there are some challenges to this approach. Vaccination could perhaps be accomplished by colonizing chickens with *Campylobacter* strains that, although immunogenic in chickens, are not pathogenic in humans. This requires knowledge about the different mechanisms by which the microorganism colonizes each host. In addition, the post-exposure disorders, such as Guillain–Barré syndrome, which might arise if the vaccine strain remains in the chicken after processing, are obvious concerns. Even if the serogroups that are prevalently associated with these post-infection sequelae are avoided in selecting strains to be used as vaccine platforms, it cannot be guaranteed that the strain would not eventually acquire the serogroup trait by natural transformation or some other horizontal exchange mechanism. Vaccines that use *Salmonella* to express *C. jejuni* antigens are also under investigation as a method of reducing chicken infection, but this approach requires an improved knowledge about what constitutes protection and why[187].

Competitive exclusion products that consist of microbiota from adult chickens have been successfully used to prevent *Salmonella* infection in chickens, but have had mixed success against *C. jejuni* (reviewed in REF. 188). These products consist of bacterial collections from the microbiota of adult chickens and are given to young chicks to prevent colonization by pathogenic species. For widespread acceptance by the food industry and regulatory agencies, the numbers and types of individual species that are present in these products must be determined. Although the technology for doing so is available (using methods adapted from microbial ecology), the complete identification of such a complex population remains a challenge.

identifies competence genes has implicated elements of a plasmid-encoded type IV secretion system as well as genes for *N*-linked glycosylation, LOS biosynthesis and a homologue of *H. pylori* DprA, a putative DNA-processing enzyme, as being necessary for wild-type levels of natural transformation[19,38–40]. However, other than the identification of these genes, no specific mechanism has yet been elucidated that explains how extracellular DNA is recognized and taken up by *C. jejuni*.

Lipooligosaccharide and capsule. Consistent with a role in immune avoidance, the LOS of *C. jejuni* is highly variable. Various *C. jejuni* LOS structures resemble human neuronal gangliosides. This molecular mimicry is thought to lead to autoimmune disorders, including Guillain–Barré syndrome (GBS), a paralytic neuropathy that occurs following approximately 1 in every 1,000 cases of campylobacterosis, and Miller–Fisher syndrome, a variant of GBS. Many advances have been made in the understanding of the mechanisms by which *C. jejuni* infections lead to such sequelae, and these have been extensively reviewed[41–44]. It is now known that mutations in the various genes that are involved in LOS biosynthesis affect serum resistance, as well as adherence to, and the invasion of, INT 407 cells[38].

Until recently, many strains of *C. jejuni* were thought to produce both LOS and a high molecular weight lipopolysaccharide (HMW LPS). In fact, the HMW LPS is now known to be a highly variable capsular polysaccharide, rather than an LPS[45]. The structures of the capsules of several *C. jejuni* strains have been determined. The capsule structure of *C. jejuni* strain 11168 includes 6-methyl-D-glycero-α-L-glucoheptose, β-D-glucouronic acid modified with 2-amino-2-deoxyglycerol, β-D-Gal*f*NAc and β-D-ribose[46], and contains a novel modification on the Gal*f*NAc[47]. The capsule structure of *C. jejuni* strain RM1221 has also been determined and includes 6-deoxy-D-manno-heptose and D-xylose[48], which are two sugars that are not often detected in bacterial polysaccharides. Other strains possess teichoic acid-like or hyaluronic acid-like capsules[49,50]. The extensive variation in the capsule structure has been attributed to multiple mechanisms that include phase variation of structural genes and an *O*-methyl phosphoramidate modification[45–47,51]. The *C. jejuni* capsule is important for serum resistance, the adherence and invasion of epithelial cells, chick colonization and virulence in a ferret model[52–54]. A historical scheme for serotyping *C. jejuni* strains is now known to be based on differences in capsule structure[45], which indicates that the capsular polysaccharide is accessible to the immune system and that the extensive variation in its structure probably has a key role in the evasion of the host immune response. Additionally, the capsule might have a role beyond host colonization, such as protection against desiccation or phage infection, although these possibilities have not yet been explored.

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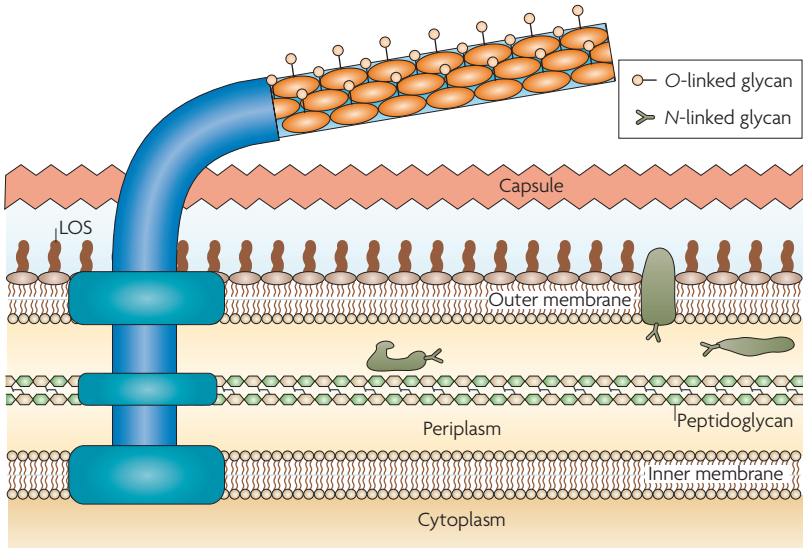

Figure 2 | **The *Campylobacter jejuni* glycome and surface structures.** The *C. jejuni* cell surface displays several structures, including many polysaccharides, that have vital roles in *C. jejuni* biology, particularly host–bacterium interactions. The capsule, a highly variable polysaccharide, is important for virulence, epithelial cell adherence and invasion. The lipooligosaccharide (LOS) is also highly variable and has a role in serum resistance, epithelial cell adherence and invasion. LOS structures of *C. jejuni* can display molecular mimicry of neuronal gangliosides, which is linked to Guillain–Barré syndrome and Miller–Fisher syndrome. The flagellum is required for colonization, virulence and epithelial cell invasion and also acts as a secretion apparatus for invasion antigens. The flagellin is modified by *O*-linked glycosylation. This modification is required for flagellar assembly and is, therefore, important for motility, virulence and epithelial cell adherence and invasion. The *N*-linked-glycosylation system modifies some periplasmic and outer-membrane proteins. The *N*-linked glycan is also important for colonization and epithelial cell adherence and invasion, but the role of this glycan in these processes is unclear.

Flagella. *C. jejuni* flagella and flagellar motility are vital to many aspects of *C. jejuni* biology, including host colonization, virulence in ferret models, secretion and host-cell invasion. Consequently, the regulation of flagella biogenesis and motility (that is, chemotaxis) is an active area of research. Various studies have elucidated a flagellar regulatory hierarchy that includes σ^{54} (encoded by *rpoN*) and σ^{28} (encoded by *fliA*) as the flagellar σ-factors and the two-component system FlgRS (which is phase variable)[22,23,26,55–57]. Homologues of the flagellar master regulators FlhC and FlhD, which are crucial for flagellar gene expression in other species, have not been identified in the *C. jejuni* genome[15]. Two proteins, FlgP and FlgQ, that are required for flagellar motility have recently been identified, but their roles are unclear; no homologues of these proteins are found in *E. coli* and only uncharacterized homologues have been identified in other species[58].

Chemotaxis probably has an important role in both the commensal and pathogenic lifestyles of *C. jejuni* (BOX 2). Genome sequence analysis has shown that the *C. jejuni* genome encodes most features of the *E. coli* chemotaxis system[15,59] (current knowledge of signal transduction and chemotaxis, derived primarily from studies on *E. coli*, has recently been reviewed[60,61]). *C. jejuni* displays chemotactic motility towards amino acids that are found in high levels in the chick gastrointestinal (GI) tract and towards components of mucus[62]. Mutants that lack either Cj0019c (DocB) or Cj0262c, which are both methyl-accepting chemotaxis receptors (there are ten in total), show decreased chick colonization, but the attractants to which these proteins respond are unknown[21]. Strains that lack or overexpress CheY, the response regulator that controls flagellar rotation, show decreased virulence in the ferret model[63]. In addition, *C. jejuni* lacks a homologue of the phosphatase CheZ, but does possess a homologue of the poorly understood protein CheV[15,59], which was first discovered in *Bacillus subtilis*[64] but is absent from *E. coli*. CheV has an amino-terminal CheW-like domain and a carboxyl-terminal CheY-like domain and might act as a phosphate sink for the chemotaxis signal-transduction machinery[59,65]. If CheV acts as a phosphate sink in *C. jejuni*, this might ameliorate the effect of the absence of a CheZ phosphatase on phosphate flow through this signal-transduction pathway[15,59]. Sequence and genetic analyses indicate that *C. jejuni* transduces an energy taxis (or aerotaxis) signal using two proteins, CetA and CetB, in place of the single protein (Aer) that is used by *E. coli* and other species[20]. It is apparent that *C. jejuni* combines elements of both the *E. coli* and *B. subtilis* chemotaxis signalling systems, as well as some proteins that are found in neither of these organisms. Clearly, understanding these model systems will aid our study of *C. jejuni* chemotaxis, but much remains to be understood in terms of the signal-transduction mechanisms that control *C. jejuni* motility.

Protein glycosylation. *C. jejuni* expresses two protein-glycosylation systems: one modifies serine or threonine residues on flagellin (*O*-linked glycosylation) and the other modifies asparagine residues on many proteins (*N*-linked glycosylation) (FIG. 3). Prior to the discovery of the *N*-linked-modification system in *C. jejuni*, *N*-linked glycosylation had been observed only in eukaryotes and archaea[66].

Proteins of the *O*-linked-glycosylation system, as well as many of their biochemical functions and a hypothetical biosynthetic pathway, have been elucidated by a combination of sequence analysis, targeted mutation and chemical analysis[67–70]. The flagellin in *C. jejuni* strain 81-176 is glycosylated with pseudaminic acid at up to 19 sites, which accounts for approximately 10% of its observed mass[67]. The flagellin of *Campylobacter coli* strain VC167 is modified with legionaminic acid, and the genes that encode the proteins that are involved in the biosynthesis of this glycan are shared by many strains of *C. jejuni* (not including strain 81-176)[71]. This indicates that this modification might also occur in these strains[71]. A specific recognition sequence for *O*-linked glycosylation has not been identified, and the addition of the glycan is thought to require surface exposure and hydrophobicity[67]. *O*-linked glycosylation of flagellin is necessary for the proper assembly of the flagellar filament[72], which has led to the hypothesis that the *O*-glycan might have a role in the interactions of flagellin subunits with one another or with other elements of the flagellar

Two-component system
Comprises two proteins, a sensor and a response regulator, that act together to regulate a cellular process (or processes). The sensor contains a histidine kinase domain that regulates the level of phosphorylation and, consequently, the activity of the response regulator (which is often, but not always, DNA binding and transcriptional regulation).

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Box 2 | **A brief overview of chemotaxis proteins**

The frequency of the directional changes that occur during bacterial swimming in response to extracellular signals is regulated by alternating between the clockwise and counter-clockwise rotation of the flagellum. The net result of this process, called chemotaxis, is that bacteria swim towards favourable environments and away from unfavourable environments. The extracellular signals, often sugars or amino acids, are sensed by chemoreceptors that are called methyl-accepting chemotaxis proteins (MCPs), which typically contain a periplasmic domain that binds to the signal. Another type of taxis, called energy taxis, is a response to an intracellular signal, such as the proton motive force or the redox state of the electron-transport system. Energy taxis is regulated by an MCP-like protein, which in *Escherichia coli* and some other species is called Aer (for aerotaxis or energy taxis).

The binding of the signal ligand is relayed by the MCP to CheA, a histidine kinase that forms a complex with the MCPs in conjunction with the adaptor protein CheW. CheA autophosphorylates and subsequently phosphorylates CheY, a response regulator. In *E. coli*, the phosphorylated CheY binds to the flagellar motor-switch protein to promote clockwise flagellar rotation. This results in an increase in tumbling frequency, which causes a change in direction. In *Bacillus subtilis*, however, phosphorylated CheY decreases the frequency of tumbling, thereby promoting smooth swimming. The *E. coli* phosphatase CheZ stimulates dephosphorylation of CheY, which leads to the rapid termination of the signal response. *B. subtilis* lacks a CheZ homologue. Instead, the CheV protein, which contains both CheW and CheY domains, might function as a phosphate sink in this system, so providing an alternative route to signal termination.

A type of memory known as adaptation is part of these systems and occurs by methylation of the MCPs, which is regulated by CheR, a methyltransferase, and CheB, a methylesterase. These proteins function to effectively reset the MCP during periods of constant stimulation. Recent detailed reviews of the current state of knowledge regarding MCPs and chemotaxis signalling are available[60,61].

apparatus. In keeping with the importance of flagella and motility to many aspects of *C. jejuni* biology, defects in *O*-linked glycosylation result in a loss of motility, a decrease in the adherence to and invasion of host cells and decreased virulence in ferrets[69]. It is unknown whether *O*-linked glycosylation has any role in immune avoidance or the host-cell interaction.

Unlike other surface carbohydrate structures of *C. jejuni* (such as LOS, the capsule and the *O*-linked glycan), the *N*-linked glycan is conserved in all *C. jejuni* strains that have been examined, as well as in *C. coli*[27,47,66]. The conservation of *N*-linked glycosylation, compared with the variability of other surface carbohydrate traits, suggests that *N*-linked glycosylation might have a more fundamental role in the biology of *C. jejuni*. The *N*-linked glycosylation system, which is encoded by the *pgl* genes[66], has been extensively studied since its discovery, both for a better understanding of its role in *C. jejuni* pathogenicity and for its potential importance in biotechnological applications[73–78]. The *N*-linked glycan that is assembled by the Pgl system consists of a heptasaccharide, unlike the tetradecasaccharide that is transferred by the eukaryotic *N*-linked-glycosylation machinery[79,80]. In contrast to the *O*-linked-glycosylation system, a consensus sequence element (sequon) for *N*-linked glycosylation — D/E-X_1-N-X_2-S/T (where X_1 and X_2 can be any amino acid except proline)[81,82] — has been identified. The glycosylation sequon is necessary, but not sufficient, for glycosylation, which indicates that other sequences or factors, such as tertiary or quarternary structure, also have a role[82].

Type III secretion system
A bacterial secretion system that consists of a needle-like apparatus that transports proteins from the bacterial cytoplasm directly into the cytoplasm of a eukaryotic cell.

The specific effect of a sequon mutation was tested with the periplasmic protein Cj1496c[83]. A strain that lacked *Cj1496c* was defective for both chick colonization and adherence to INT 407 human intestinal epithelial cells *in vitro*. However, a strain that expressed a Cj1496c sequon mutant, which expressed wild-type levels of protein that could not be glycosylated, colonized chicks in a similar way to the wild type and was not defective for INT 407 association[83]. By contrast, VirB10, a competence protein that is *N*-glycosylated by the Pgl system, might require glycosylation for its function. A mutant allele that lacked one of its two functional glycosylation sequons (*virB10N87A*) was unable to complement a *virB10* mutant to wild-type levels of competence[39].

The role of *N*-linked glycosylation in the biology of *C. jejuni* is not clear. Strains with *pgl* mutations exhibit reduced adherence and invasion in the INT 407 intestinal cell line as well as defects in natural competence[39] and colonization in mouse and chick models[21,83–86]. *N*-linked glycosylation changes the immunoreactivity of at least some glycosylated proteins[66], which suggests that *N*-linked glycosylation might be involved in the evasion of the immune system. However, most proteins that are modified by the Pgl system are predicted to be periplasmic, rather than surface exposed[80], and therefore it is unclear how modifying these proteins would enhance immune avoidance. Lectin-binding studies have identified numerous glycosylated proteins that are located mostly in the periplasm or membrane[80–82,87], but no obvious hypothesis for the role of *N*-linked glycosylation has arisen from the knowledge of which proteins are glycosylated.

In summary, although our knowledge of these two post-translational modifications, *O*-linked and *N*-linked glycosylation, has greatly increased in recent years, there are still many gaps in our understanding. We have a good understanding of the mechanisms that are involved in *N*-linked glycosylation, but the biological function of this glycan is poorly understood. Conversely, although less is known about the mechanisms that are involved in *O*-linked glycosylation, its role in *Campylobacter* biology, particularly its importance in flagella assembly and, consequently, host-cell interactions, is better appreciated.

Secretion. The secretion mechanisms of *C. jejuni* are poorly characterized relative to those of other bacterial pathogens. *C. jejuni* secretes a protein, called CiaB, that is required for the invasion of cultured epithelial cells[88,89]. Mutants that lack *ciaB* exhibit reduced chick colonization levels[90], which implies that cell invasion might be an underappreciated factor in chick colonization.

The mechanism of CiaB secretion and its role in invasion has been likened to the model of type III secretion systems, in which effectors are injected directly into host cells[89,91]. However, *C. jejuni* does not encode a type III secretion system and evidence for the direct injection of CiaB is lacking. Rather, CiaB and other secreted Cia proteins (CiaA–H) require a functional flagellar export apparatus for their secretion[92], which is similar to the secretion of some proteins from

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Yersinia spp.[93] As well as the Cia proteins, the flagellar export apparatus of *C. jejuni* secretes FlaC, which is also required for invasion and shares limited homology with the major and minor flagellins (FlaA and FlaB)[94]. Thus, the flagellar export apparatus is an important secretion mechanism in *C. jejuni* and is required for host-cell invasion.

Cytolethal distending toxin. *C. jejuni* produces cytolethal distending toxin (CDT), which is also produced by a diverse group of other bacterial species, including *E. coli*, *Actinobacillus actinomycetemcomitans*, *Haemophilus ducreyi* and *Helicobacter hepaticus*. The toxin causes arrest at the G_1/S or G_2/M transition of the cell type[95–99]. The active holotoxin is a tripartite complex of CdtA, CdtB and CdtC[98] (FIG. 4), although one study has indicated that CdtB and CdtC combined have some cytotoxicity without CdtA[100].

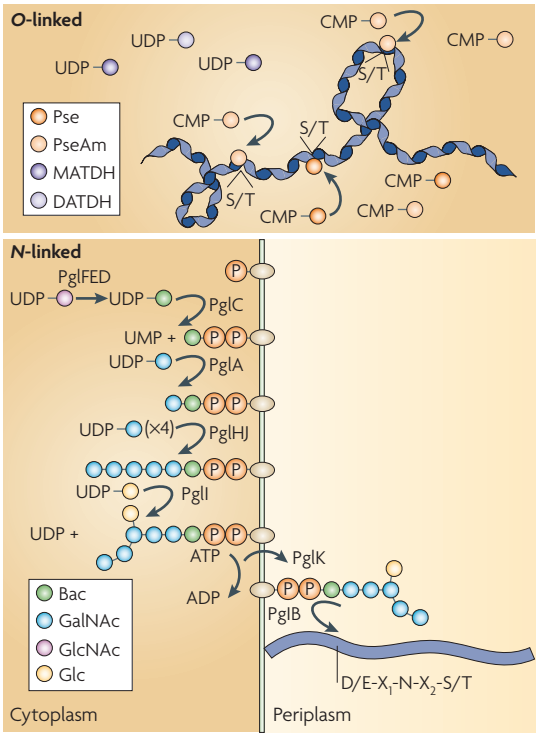

Figure 3 | ***O*- and *N*-linked glycosylation in *Campylobacter jejuni*.** The *O*-linked-glycosylation system in *C. jejuni* modifies flagellin, and the glycan is linked to flagellin through a serine or threonine residue. This modification is required for flagella assembly. The *N*-linked-glycosylation system of *C. jejuni* (Pgl) is a general glycosylation system that modifies asparagine residues on many proteins. Glycan assembly occurs in the cytoplasmic face of the inner membrane, where nucleotide-activated sugars are sequentially added to undecaprenylpyrophosphate. The *N*-glycan heptasaccharide is then flipped across the membrane and added as a block to target proteins in the periplasm. The consensus sequon for *N*-linked glycosylation is D/E-X_1-N-X_2-S/T (where X_1 and X_2 can be any amino acid except proline)[81,82]. This sequon is necessary, but not sufficient, for glycosylation. Bac, bacillosamine; CMP, cytosine monophosphate; DATDH, diacetamido-trideoxyhexose; GalNAc,*N*-acetylgalactosamine; Glc, glucose; GlcNAc, *N*-acetylglucosamine; HexNAc, *N*-acetylhexosamine; MATDH, monoacetamido-trideoxyhexose; Pse, pseudaminic acid; PseAm, 5-acetamidino analogue of Pse; UDP, uridine diphosphate; UMP, uridine monophosphate. This figure is modified with permission from REF. 189 © (2005) MacMillan Publishers Ltd.

The role of CDT in *C. jejuni* pathogenesis remains unclear, but its mechanism of action is becoming understood. CdtB is known to be the toxic component, as microinjection or transfection of this subunit alone into host cells leads to the effects that are observed with the holotoxin[96]. CdtB is thought to act as a DNase, as it shares similarity with a family of DNase I-like proteins. CdtB localizes to the nucleus of host cells, causes DNA damage and, ultimately, phosphorylation of the histone protein H2AX, thereby recruiting the DNA-repair protein Rad50 to double-strand breaks[99]. These activities require residues of CdtB that are shared with members of the DNase I family[96,101]. However, these residues are conserved within the larger phosphodiesterase family to which CdtB belongs and will therefore be required for catalytic activity even if CdtB is not a DNase[97]. CdtB has weak DNase activity *in vitro*[96], and studies that have attempted to determine whether DNA damage *in vivo* is a direct or indirect result of CdtB activity have had conflicting results[95,97,102–104].

CdtB nuclear localization has been evident for several years[96], and the mechanism behind this localization was recently established. CdtB sequences from several species contain putative bipartite nuclear-localization signals (NLSs), most of which are in the carboxy half of the protein[105]. At least one of the two putative NLSs in *E. coli,* CdtB-II, is definitely required for nuclear localization and cytotoxicity[105]. An amino-terminal region of *A. actinomycetemcomitans* CdtB is also required for nuclear localization, and this domain is necessary for cellular distension and cell-cycle arrest by *A. actinomycetemcomitans* CDT[106]. This region of *A. actinomycetemcomitans* CdtB contains one complete and one partial bipartite NLS, which potentially explains the nuclear localization[105]. Together, these studies emphasize the importance of the active transport of the toxin to the nucleus. A formal demonstration of the role of the NLS in *C. jejuni* CdtB has not been reported.

The functions of CdtA and CdtC in this family of toxins are unclear, but one or both might mediate binding to host cells. CdtA and CdtC have some similarity to the B chain of the ricin toxin, which is responsible for receptor-mediated endocytosis of ricin[98]. Additionally, CdtA and CdtC bind HeLa cells with specificity, probably using the same receptor[100]. As *H. ducreyi* CDT is taken up into cells by clathrin-coated pits, it seems likely that CdtA and CdtC mediate binding and subsequent internalization through this pathway[107] (FIG. 4).

The fact that a microorganism such as *C. jejuni*, which establishes long-term, asymptomatic associations with many hosts, has retained a toxin such as CDT is intriguing. CDT is responsible for some of the secretion of

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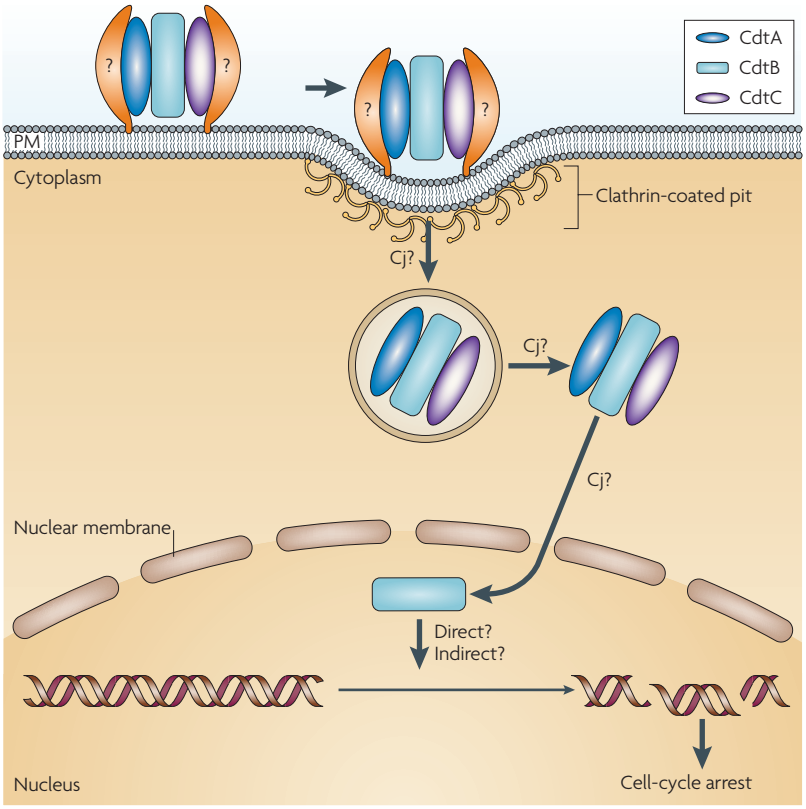

Figure 4 | **Uptake and activity of cytolethal distending toxin.** The cytolethal distending toxin (CDT) holotoxin consists of three subunits, CdtA, CdtB and CdtC. CdtA and CdtC are thought to bind to an unknown receptor on the host cell surface. CDT is taken up into host cells by way of clathrin-coated pits. Following internalization, nuclear localization signals on CdtB probably lead to its active transport into the nucleus through the classical nuclear-import cycle. Once in the nucleus, the toxin leads to double-strand DNA breaks and cell-cycle arrest. Whether or not CdtB acts as a DNase to cause the DNA damage directly, as opposed to this being an indirect effect of some other CdtB enzymatic activity, has yet to be definitely established. The arrows marked Cj? indicate the aspects of CDT uptake or activity that have only been studied using CDT from species other than *Campylobacter jejuni*.

interleukin (IL)-8 , a hallmark of *C. jejuni* pathogenesis, but there are also CDT-independent mechanisms of IL-8 stimulation[108]. CDT might have a role in asymptomatic, commensal infections, which would provide a way to either avoid host immune-response mechanisms or redirect them towards tolerance. *In vitro*, *C. jejuni* CDT induces apoptosis in monocytic cell lines[109]. Experiments that used a mouse model of *H. hepaticus* colonization suggest that CDT has a function in immune modulation and persistent colonization[110,111]. In addition, the persistent *C. jejuni* colonization of wild-type mice, but not mice that are deficient for nuclear factor (NF)-κB, requires CdtB, which indicates that CDT might allow *C. jejuni* to escape immune surveillance in an NF-κB-dependent manner[11]. In chickens, which are the more natural hosts for *C. jejuni*, CDT is expressed by bacteria in the caeca, a site of heavy colonization, although colonized chicks do not generate CDT-neutralizing antibodies[112]. Furthermore, mutants that lack CDT colonize chicks with wild-type efficiency[113].

ATP-binding cassette (ABC) transporter
A member of a large family of proteins that uses the energy provided by the hydrolysis of ATP to transport substrates across membranes.

Adherence mechanisms. To colonize hosts, microorganisms typically require adherence factors, which are often surface appendages such as the pili that are found on the surface of many Gram-negative and Gram-positive species. Genome annotations of several *C. jejuni* strains do not include obvious pilus or pilus-like open reading frames[14,15]. A multi-protein type II-like secretion system of a type that is associated with pilus assembly in *Vibrio cholerae* and *Neisseria gonorrhoeae* was identified as part of the competence machinery, but an actual pilus-like structure has not been identified[37].

Despite the lack of identifiable adherence organelles, several proteins contribute to *C. jejuni* adherence to eukaryotic cells. CadF binds specifically to fibronectin, which is located basolaterally on epithelial cells *in situ*[114–116]. The fibronectin-binding domain of CadF consists of amino acids 134–137 (FRLS), which represents a novel fibronectin-binding motif[117]. CadF is required for maximal binding and invasion by *C. jejuni in vitro*, and *cadF* mutants are greatly reduced in chick colonization compared with the wild type[116,118]. CadF is similar to *E. coli* OmpA and forms membrane channels, but the role of this activity, if any, has not been established[119].

Another characterized adhesin, JlpA, is a surface-exposed lipoprotein that is crucial for HEp-2 cell binding[120]. JlpA binds to Hsp90α, some of which is surface localized in these cells[121]. Binding to Hsp90α by JlpA activates NF-κB and p38 mitogen-activated protein (MAP) kinase, both of which contribute to proinflammatory responses[121]. This indicates that some of the inflammation that is observed during *C. jejuni* pathogenesis might be related to JlpA-dependent adherence. Another lipoprotein, CapA, was implicated as a possible adhesin[122]. CapA is an autotransporter that is homologous to an autotransporter adhesin, and CapA-deficient mutants have decreased adherence to Caco-2 cells and decreased colonization and persistence in a chick model[122].

Paradoxically, some putative adhesins of *C. jejuni* are located in the periplasm. The Peb1 adhesin, also known as CBF1, is one such adhesin. Although crucial for adherence to HeLa cells[123,124], Peb1 is periplasmic and shares homology to the periplasmic-binding proteins of amino acid ATP-binding cassette (ABC) transporters[125,126]. In fact, Peb1 binds to both aspartate and glutamate with high affinity, and *peb1*-deficient mutants cannot grow if these amino acids are the major carbon source[126]. Although Peb1 has not been localized to the inner or outer membrane, some has been observed in culture supernatants[126]. Furthermore, Peb1 contains a predicted signal peptidase II recognition site, a common motif in surface-localized lipoproteins, and so there is a possibility that some Peb1 is surface accessible, despite the failure of fractionation techniques to demonstrate this[125,126]. Mutants that lack *peb1* colonize mice poorly, but this could be attributed to the loss of either the adhesion or the amino-acid-transport functions, or both[124,126]. Another periplasmic protein, the glycoprotein Cj1496c, which has homology to a magnesium transporter, is

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also required for wild-type levels of adherence (see above)[83]. The mechanism by which these periplasmic proteins contribute to host-cell adherence by *C. jejuni* is unclear.

Infection in humans

Campylobacter infection commonly presents as an acute gastroenteritis that is characterized by inflammation, abdominal pain, fever and diarrhoea, with the infectious dose as low as 500–800 bacteria[127,128]. The incubation period that precedes the development of acute diarrhoea is 2–5 days and, although the disease is typically resolved in one week, symptoms can last for up to 2 weeks. Epidemiological studies of *Campylobacter* infection indicate that there are two disease manifestations, which are dependent on socio-economic status[129] (reviewed by Blaser[130]). In the developed world, campylobacteriosis manifests as bloody diarrhoea with mucus, and is usually self-limiting. In the developing world, watery diarrhoea predominates, and infection is more frequent among children, which might naturally vaccinate them against becoming infected as adults. The reason for this disparity of outcomes is not clear but could reflect the different levels (and the T-helper (T_H)-1 versus T_H-2 bias) of pre-existing immunity that arises from differing natural immune stimulants in these environments.

To establish an infection, *C. jejuni* must bypass the mechanical and immunological barriers of the GI tract. The mucus layer of the GI epithelium serves as the first line of defence, but several traits contribute to the ability of *C. jejuni* to penetrate this barrier. These include the motility and corkscrew morphology of *C. jejuni* and the relatively short *O*-sidechain of its LOS, which is proposed to reduce nonspecific binding to the mucin glycoproteins[131]. Once *C. jejuni* passes through the mucus layer, it can interact with the underlying epithelial cells by using the various mechanisms already discussed.

Cia protein synthesis is stimulated by the bile component deoxycholate, but Cia secretion is not[91]. This observation has led to the suggestion that Cia production might be stimulated early in colonization, in the small intestine, but that secretion occurs only after adherence at the site of long-term colonization[91].

Campylobacter apparently invades intestinal epithelial cells, as intracellular bacteria have been observed in patients[132] and invasion can be reproduced in cell lines *in vitro*. The mechanism that controls this invasion is being dissected experimentally, but complete understanding is complicated by differences between strains. It is clear that all strains require microtubule polymerization for maximal invasion, although some also require microfilament polymerization[116,133–136]. This is different from the microfilament-dependent mechanism of entry that is used by many other invasive bacteria, in which the disruption and subversion of actin-based processes has been well described (and reviewed elsewhere[137,138]). Scanning electron microscopy has captured epithelial cell membrane pseudopods extending towards and enveloping *C. jejuni*[135], and immunofluorescence experiments have indicated that these pseudopods contain microtubules[134]. Once internalized, *C. jejuni*-containing vacuoles appear to move along microtubules to the perinuclear region of the cell by interactions with dynein[134]. The fate of these internalized bacteria and their role in pathogenesis, possibly by immune evasion or establishment of a protected reservoir, has not yet been determined.

The responses of intestinal epithelial cells to *C. jejuni* are generally characterized by induction of cytokines, such as IL-8, which is a proinflammatory cytokine that is a hallmark of campylobacteriosis, although this response is not universal for all *C. jejuni* strains[108]. *C. jejuni* infection of two polarized intestinal epithelial lines, Caco-2 and T84, as well as of human intestinal tissue explants results in the activation of MAP kinase family proteins ERK and p38 (REFS 139,140); for T84 cells, ERK activation is essential for the stimulation of IL-8 (REF. 139). CDT contributes to IL-8 secretion in the INT 407 intestinal cell line[108]. Thus, although *C. jejuni* has invasion mechanisms to breach the physical barrier that is presented by the intestinal epithelium, these cells can in turn signal for the recruitment of inflammatory cells to the site of infection (FIG. 5).

Human immune responses to* C. jejuni *infection. As with many other pathogens, Toll-like receptors (TLRs) presumably represent the first immunological challenge that *C. jejuni* must overcome during infection. *Campylobacter* evades the flagellin-mediated stimulation of TLR-5 owing to key alterations in the flagellin primary structure relative to that of TLR-5-stimulatory flagellins[139,141,142]. *C. jejuni* does not stimulate TLR-9, which recognizes unmethylated CpG dinucleotides, owing to the AT-rich nature of the genome[143]. However, the intracellular pathogen-recognition receptor NOD1 does have a crucial role in immune stimulation by *C. jejuni*[144].

The primary response that is needed to clear *C. jejuni* is polarized towards cell-mediated (or T_H1) immunity and presumably involves dendritic cells (DCs) and macrophages, as opposed to antibody-mediated immunity[11,145,190]. Fox and colleagues demonstrated *in vivo* that the clearance of a *C. jejuni cdtB* mutant was mediated by a T_H1-dependent IgG2a response[11]. Although several *in vitro* studies have contributed to the understanding of the interaction of *Campylobacter* with these immune cells, their specific contributions to *C. jejuni* clearance *in vivo* have not yet been determined. The exposure of the T84 epithelial cell line to *C. jejuni* results in the increased expression of CXCL20, a cytokine that has been implicated in the recruitment of DCs[142]. On the basis of *in vitro* studies, it is likely that DCs encounter and rapidly internalize *C. jejuni*[146]. This results in NF-κB activation and the secretion of several cytokines and tumour necrosis factor-α, which are two traits associated with maturing DCs[146]. Much of the DC-maturation response to *C. jejuni* is attributed to bacterial LOS[146].

T helper (T_H)-1 versus T_H-2
A T helper (T_H) cell is derived from one of two subsets that regulate the immune response through the secretion of cytokines. T_H-1 mediates an inflammatory, cell-mediated response, whereas T_H-2 cell activity enhances the humoral response and suppresses cell-mediated responses.

Toll-like receptor
(TLR). A key recognition molecule in the host innate immune response. A membrane-spanning protein that recognizes conserved ligands on pathogens, such as flagellin, lipopolysaccharide or DNA. Such ligands are widely found in pathogens and are known as pathogen-associated molecular patterns.

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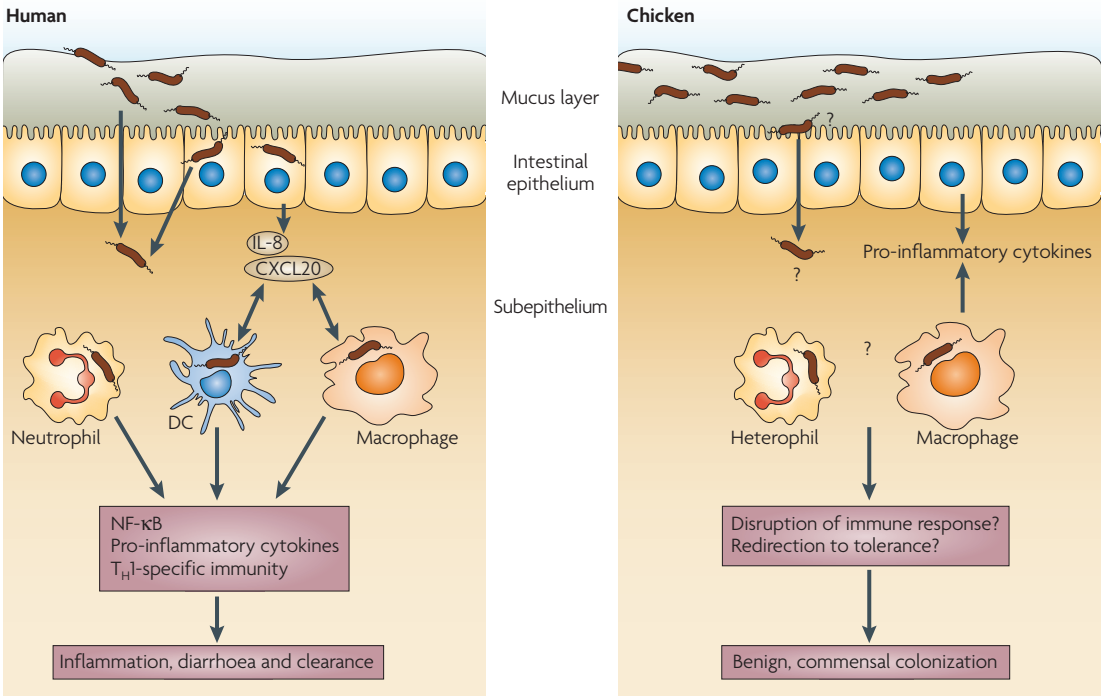

Figure 5 | **Molecular and cellular features of the innate immune response to *Campylobacter jejuni* in humans and chickens.** *C. jejuni* circumvents the mucus layer in humans and interacts with the intestinal epithelial cells causing interleukin (IL)-8 production. *C. jejuni* binds to, and is internalized by, epithelial cells. The induction of IL-8 causes the recruitment of dendritic cells (DC), macrophages and neutrophils, which interact with *C. jejuni*. These interactions result in a massive pro-inflammatory response and increases in the corresponding cytokines. By contrast, *C. jejuni* resides primarily in the mucosal layer in chicken intestines. *In vitro* evidence shows that *C. jejuni* can stimulate the production of IL-1β, IL-6 and intracellular nitric oxide synthase from epithelial cells and macrophages, but the ensuing host response does not typically lead to inflammatory diarrhoea in chickens. Unknown factors either dampen the immune response or redirect it towards tolerance. Heterophils and macrophages might also have a role in the establishment of *C. jejuni* colonization in chickens, but epithelial cell invasion is not typically reported. Question marks indicate areas that lack clarity in our current knowledge of *Campylobacter*–chicken interactions. NF-κB, nuclear factor-κB.

The role of monocytes and macrophages in *C. jejuni* infection is unclear because results vary with different cell lines or primary cells. Both NF-κB and the pro-inflammatory cytokine IL-1β are induced in the monocyte cell line THP-1 stimulated with *C. jejuni*, which has provided evidence that monocytes also have a role in inflammation during *C. jejuni* infection[147,148]. However, a significant proportion of monocytic cells infected with *C. jejuni* also undergo apoptosis[109,148]. Confounding the issue, one report showed that *C. jejuni* is killed by macrophages derived from human monocytes[149], whereas other groups found that clinical isolates of *C. jejuni* survived for several days in murine peritoneal macrophages and the J774A.1 macrophage cell line[109,150,151]. The differences in these observations might be due to strain variation or, perhaps more likely, to the use of different macrophage or macrophage-like cell lines.

Although the multiple levels of variation that exist in *C. jejuni* surface structures could help it to evade antibody responses, an adaptive immune response has been demonstrated during *C. jejuni* infection. Antibodies to several bacterial components have been observed in human sera, including antibodies to flagella, major outer-membrane protein (MOMP), outer membrane proteins and LOS[152,153]. The unusual structure of the LOS layer of *C. jejuni* has focused its investigation to its potential role in virulence. The absence of *N*-acetylneuraminic acid (NeuNAc) from the LOS core decreases its immunogenicity[154]. CDT has been implicated as a major antigen for antibody production, and neutralizing antibodies that are directed against CDT are elicited during human infection; additionally, pooled anti-sera from infected patients neutralized the toxin[112].

Infections in children younger than 6 months of age resulted in low levels of specific IgA, IgG and IgM, possibly owing to the presence of maternal antibodies (reviewed by Coker[155]). However, 80–90% of patients that were infected with culturable *C. jejuni* produced specific serum immunoglobulins against *C. jejuni*[156]. Elevated levels of IgG persisted after clearance, but elevated IgA was detected only from the onset of symptoms until the clearance of *C. jejuni*[156].

Infection in chickens

A better understanding of the responses of chickens to *C. jejuni* infection, which typically does not lead to the same symptoms and pathological inflammatory response that are seen in humans, might reveal ways to

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target the natural source of human infections. *C. jejuni* can colonize chickens in extremely high numbers, up to 10^{10} colony-forming units per gram of infected intestine, and the primary site of colonization is the deep crypts of the caecum, where *C. jejuni* is found in the mucus layer close to the epithelial cells (reviewed by Lee[157]). Avian caeca are large closed pouches, found off the colon and located just past the ileal junction. Dietary cellulose is broken down in the caeca and fermentation products such as lactic acid and short-chain fatty acids are abundant, probably because of the metabolic action of the microbiota[158]. This environment is, therefore, probably similar to that of the human colon. A slight inhibition of human epithelial cell invasion by *C. jejuni* in the presence of chicken intestinal mucus has been observed, which prompted the suggestion that the mucus might contribute to the asymptomatic nature of chick infection[159].

C. jejuni *factors required for chick colonization.* Studies using genetic screens and/or targeted mutagenesis of candidate genes have led to a growing understanding of which *C. jejuni* traits are important in chicken colonization. A common emerging theme from this work is the importance of flagella and flagellar motility. Signature-tagged mutagenesis of *C. jejuni* in a chick model of infection resulted in the identification of two methyl-accepting chemotaxis receptors and other elements of the flagellar and chemotactic machinery as being important for wild-type chick colonization[21]. Additionally, mutants in the genes that encode the flagellins and flagellar biosynthesis regulators FlgR, σ^{54} and σ^{28} all display defects in chick colonization[21,56,57,160,161]. Other regulators that are not associated with flagellar motility are also important for efficient chick colonization. These include CbrR, which regulates deoxycholate resistance and contains two response-regulator domains and a GGDEF domain, thereby implicating this protein in cyclic-di-GMP regulation[162].

One trait of chickens that is different to humans and other mammals that could contribute to the different outcomes of infection with *C. jejuni* is body temperature. Chickens have a body temperature that ranges from 41 to 45°C, as opposed to the 37°C that is normal in humans, which makes temperature a potential signal for host-specific infection. Transcription profiles of *C. jejuni* cultures that were shifted from 37°C to 42°C showed evidence of potential alterations in membrane structure by the upregulation of genes for transport and binding proteins, as well as cell wall and envelope constituents[163]. A regulatory system that might contribute to survival at the higher temperature is discussed below. RacRS is a two-component system that is required for wild-type chick colonization, and mutants that lack it have a growth defect at 42°C[164]. The RacRS system can act as both an activator and repressor to regulate gene expression, sometimes in a temperature-dependent manner[164]. Mutants in the DccRS two-component system, for which an activating signal is unknown, are poor colonizers of chicks compared with the wild type[165]. The DccRS-regulated genes that have been identified have no known or predicted functions, but one appears to be essential for growth and mutants in two others lead to chick colonization defects[165].

Several genes that control mechanisms other than motility and gene regulation are also required for chick colonization. These include genes that encode the enzymes that are responsible for the *N*-linked glycosylation of several proteins[21,83,85] as well as various adherence and invasion factors, such as *cadF* and *ciaB*[90,118]. Finally, antimicrobial resistance mechanisms and elements of metabolism that are related to low iron, low oxygen (but not anaerobic) and high serine or other amino-acid environments might have significant effects on chick colonization[166–171].

Chick immune responses to* C. jejuni *infection. The chicken innate immune response to *C. jejuni* infection has been investigated using both epithelial and macrophage cell lines of *C. jejuni*[172], although the epithelial line used was a chicken kidney cell line as opposed to an intestinal line. An elevated production of IL-1β, IL-6 and inducible nitric oxide synthase was observed from both cell types, which indicates that *C. jejuni* can stimulate innate responses by the chick immune system (FIG. 5). The microbial ligands and host receptors that control this mechanism are unknown. For instance, chickens have orthologues of mammalian TLR molecules[173–175], but the stimulation of TLR signalling by *C. jejuni* ligands has not been described. It is unknown whether flagellin is unrecognized by the relevant chicken TLR as it is by human TLR-5; if so, then other as-yet unidentified molecules must be implicated in innate immune signalling in chickens.

Young chicks are exposed to *C. jejuni* during the period when their innate intestinal immune system is developing. Within the first two weeks of life, exposure to feed and low doses of bacteria can cause the recruitment of heterophils and lymphocytes, as well as the release of the inflammatory cytokines IL-1β, IL-8 and K203 (REF. 175). Maternal antibodies that recognize *C. jejuni* can also be present in newborn chicks for the first 2 weeks, so providing possible protection from *C. jejuni* colonization[176]. Such antibodies, which recognize surface components including LOS, MOMP and flagellin[177], lead to the complement-mediated killing of *C. jejuni* in a strain-specific manner[178]. Therefore, for successful early colonization, *C. jejuni* must bypass the young innate immune response and the presence of maternal antibodies.

By 2 weeks of age, maternal antibodies are no longer present, and by 3 weeks of age the chick produces its own antibodies in response to *C. jejuni*, which tend to react primarily to flagellin[177,179]. Although CDT is expressed by *C. jejuni* in chicks, neutralizing antibodies against CDT are not produced, unlike in humans[112]. This might point to a mechanistic difference in the way *C. jejuni* antigens are recognized by the two hosts. Although the capsule that is expressed in many pathogens is strongly antigenic, the chicken humoral response towards polysaccharides is weak[179]. This is probably due to an incomplete response by chickens to T-cell independent type 2 (TI-2) antigens (usually polysaccharides) in general[179]. TI-2 antigens activate B cells

Cyclic-di-GMP regulation
Regulation through the second-messenger cyclic-di-GMP. This molecule is generated by diguanylate cyclases, which often carry the conserved residues GGDEF, and is hydrolysed by phosphodiesterase A, which often carries the conserved residues EAL. These regulatory domains are found in a wide range of proteins, thereby allowing various input signals to influence the production or hydrolysis of the second messenger.

Heterophil
A granular leukocyte that is defined by its variable size and staining characteristics; the human version is the neutrophil.

Complement-mediated killing
The binding of an antibody to an antigen often triggers the complement system, which comprises approximately 30 proteins. A proteolytic cascade sequentially activates the complement proteins, which results in the formation of a complex and either opsonization or lysis of the foreign material.

Humoral response
The humoral immune response refers to the production of antibodies for pathogen clearance. The term encompasses complement activation, opsonization, T_H2 activation and cytokine production.

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Box 3 | **Open questions in *Campylobacter jejuni* pathogenesis**

Genetic variation and natural transformation
- What is the mechanism of DNA uptake?
- What is the role of each of the genes that have been identified as being required for wild-type natural transformation?
- Are there receptors for DNA?
- What is the mechanism of specificity for *C. jejuni* DNA?

Flagella
- What is the role of FlgR phase variation, and thus flagella, in colonization or transmission?
- What signals do each of the methyl-accepting chemotaxis proteins (MCPs) sense?
- Do these MCPs have a subtle role in colonization or virulence that is not detected in single-mutant studies?
- How is phosphate flow through the chemotaxis machinery regulated without the phosphatase CheZ? Is this the role of the CheV protein ?
- What are the roles of the novel proteins FlgP and FlgQ in flagellar motility?

Protein glycosylation
- What are the specific requirements for *O*- and *N*-linked glycosylation (other than the *N*-linked glycosylation consensus site)?
- What function does *O*-linked glycosylation serve in flagellar assembly?
- Is there a role for *O*-linked glycosylation other than flagellar assembly? (Such as immune avoidance?)
- What is the role of *N*-linked glycosylation in the biology of *C. jejuni*?
- Does any protein require *N*-linked glycosylation for its function?

Secretion
- What are the roles or functions of the Cia proteins and the flagellin FlaC once they are secreted?
- Does secretion occur in chickens and humans *in vivo*? When does it occur during colonization and pathogenesis?
- Are there other secreted proteins?

***Campylobacter* physiology**
- How does the stationary phase of *C. jejuni* contribute to its persistence *in vivo*?
- What are the growth substrates that encourage long-term colonization in chicken caeca?
- What signals stimulate the two-component-like regulatory systems that are associated with colonization (for example, CbrR, RacRS, DccRS or FlgRS)?

Cytolethal distending toxin
- What is the role of cytolethal distending toxin (CDT) in colonization and immune evasion?
- What is the role of CDT in pathogenesis?
- What is the host-cell receptor for the proteins CdtA and CdtC?
- How is CDT secreted from *C. jejuni*?

Adherence mechanisms
- What is the relative importance (and contribution) of each of the known adhesins for colonization and pathogenesis?
- What other adhesins, if any, does *C. jejuni* possess?
- What are the receptors for the adhesins Peb1, CapA and Cj1496c?
- Do Peb1 and Cj1496c ever localize to the outer membrane or cell surface? If not, how do they contribute to adherence?

Human infection
- What factors lead to the different disease outcomes in the developed versus the developing world?
- What is the role of invasion in pathogenesis?
- What is the mechanism of microtubule-mediated invasion?
- Does microtubule and microfilament mediated invasion occur *in vivo*?
- What is the nature of *C. jejuni*–monocyte interactions *in vivo*?

Chicken infection
- Which of the genes that are regulated by CbrR, RacRS and DccRS contribute to the effect of these regulators on colonization?
- Why is *N*-linked glycosylation required, given that there is no variation in this glycan and most glycosylated proteins are periplasmic?
- What is the timing of expression of the various required genes? In other words, which are required for early colonization, which are required for persistence and which are required for transmission to new hosts?
- Is there a role for invasion in commensalism?
- What other factors are required for chicken colonization by *C. jejuni*?

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independently of T cells, presumably because of their ability to crosslink cell-surface immunoglobins. This ineffective TI-2 response might contribute to *C. jejuni* colonization in chicks.

Conclusions

Despite the progress that has been made in recent years, there are still gaps in our knowledge of some of the basic aspects of the molecular biology and pathogenicity of *C. jejuni* (BOX 3). For example, *C. jejuni* has three σ-factors that are encoded in its genome. Two of these, σ^{28} (encoded by *fliA*) and σ^{54} (encoded by *rpoN*), are involved in biogenesis of the flagellum, and the third is the housekeeping σ-factor, σ^{70} (encoded by *rpoD*). How does a species that spends so much of its time persistently infecting hosts regulate its stationary phase? Some clues have come from work that has shown that the stringent response, which is regulated by the SpoT protein, is an important component of *C. jejuni* stationary-phase survival and contributes to virulence-associated phenotypes and potentially to phenotypes that are related to transmission[28]. Future studies should provide more clues about the basic physiology of *C. jejuni* and how it relates to its pathogenicity.

The lack of a good small-animal model that mimics disease caused by *C. jejuni* in humans has clearly limited our understanding of *C. jejuni* pathogenicity and the host response to infection. A new murine model shows promise for closing this gap. This model is based on the hypothesis that competitive exclusion by the normal complex intestinal microbiota can limit *C. jejuni* colonization[180]. C3H mice with limited gut flora were colonized to several orders of magnitude higher than mice with normal flora. The animals remained colonized for up to 3 weeks before *C. jejuni* titres began to diminish considerably. Dosing experiments demonstrated that the limited-flora animals could be infected by as few as 200 bacteria, which is similar to results that were obtained using day-of-hatch chicks[21,181,182]. However, the drawbacks of using immunocompetent limited-flora mice as a disease model for colonization include minimal inflammation and a lack of observable clinical symptoms. Nonetheless, when combined with a severe combined immunodeficiency mutation that eliminates B and T cells, *C. jejuni* colonization persisted for several weeks and was accompanied by marked inflammation, particularly in the lower intestine[180]. Furthermore, flagellar motility and chemotaxis, which are two traits that are required for *C. jejuni* colonization in chicks and ferrets, were required for detectable colonization in the limited-flora murine model. Thus, this model holds promise for using both bacterial and host genetics to uncover and study the pathogenic mechanisms of *C. jejuni*.

With the widespread appreciation that *C. jejuni* is an important human pathogen, the efforts of many investigators have focused on this microorganism. However, our understanding of its pathogenic processes still lags considerably behind that of pathogenic genera such as *Salmonella*, *Shigella*, *Vibrio* and *Listeria*. *Campylobacter* is only distantly related to other enteropathogens, and so has probably evolved distinct infection and virulence mechanisms that have not been observed before. Developing new animal models and other experimental tools as well as acquiring more genome information are the necessary first steps on a challenging journey of discovery.

1. Szewzyk, U., Szewzyk, R., Manz, W. & Schleifer, K. H. Microbiological safety of drinking water. *Annu. Rev. Microbiol.* **54**, 81–127 (2000).
2. Axelsson-Olsson, D., Waldenstrom, J., Broman, T., Olsen, B. & Holmberg, M. Protozoan *Acanthamoeba polyphaga* as a potential reservoir for *Campylobacter jejuni*. *Appl. Environ. Microbiol.* **71**, 987–992 (2005).
3. Blaser, M. J., LaForce, F. M., Wilson, N. A. & Wang, W. L. Reservoirs for human campylobacteriosis. *J. Infect. Dis.* **141**, 665–669 (1980).
4. Ruiz-Palacios, G. M., Escamilla, E. & Torres, N. Experimental *Campylobacter* diarrhea in chickens. *Infect. Immun.* **34**, 250–255 (1981).
5. Sanyal, S. C. *et al.* *Campylobacter jejuni* diarrhea model in infant chickens. *Infect. Immun.* **43**, 931–936 (1984).
6. Fox, J. G., Ackerman, J. I., Taylor, N., Claps, M. & Murphy, J. C. *Campylobacter jejuni* infection in the ferret: an animal model of human campylobacteriosis. *Am. J. Vet. Res.* **48**, 85–90 (1987).
7. Yrios, J. W. & Balish, E. Colonization and pathogenesis of *Campylobacter* spp. in athymic and euthymic germfree mice. *Prog. Clin. Biol. Res.* **181**, 199–202 (1985).
8. Yrios, J. W. & Balish, E. Immune response of athymic and euthymic germfree mice to *Campylobacter* spp. *Infect. Immun.* **54**, 339–346 (1986).
9. Yrios, J. W. & Balish, E. Colonization and infection of athymic and euthymic germfree mice by *Campylobacter jejuni* and *Campylobacter fetus* subsp. *fetus*. *Infect. Immun.* **53**, 378–383 (1986).
10. Yrios, J. W. & Balish, E. Pathogenesis of *Campylobacter* spp. in athymic and euthymic germfree mice. *Infect. Immun.* **53**, 384–392 (1986).
11. Fox, J. G. *et al.* Gastroenteritis in NF-κB-deficient mice is produced with wild-type *Campylobacter jejuni* but not with *C. jejuni* lacking cytolethal distending toxin despite persistent colonization with both strains. *Infect. Immun.* **72**, 1116–1125 (2004).
12. Mansfield, L. S. *et al.* C57BL/6 and congenic interleukin-10-deficient mice can serve as models of *Campylobacter jejuni* colonization and enteritis. *Infect. Immun.* **75**, 1099–1115 (2007).
13. Watson, R. O., Novik, V., Hofreuter, D., Lara-Tejero, M. & Galan, J. E. A MyD88-deficient mouse model reveals a role for Nramp1 in *Campylobacter jejuni* infection. *Infect. Immun.* **75**, 1994–2003 (2007).
14. Fouts, D. E. *et al.* Major structural differences and novel potential virulence mechanisms from the genomes of multiple *Campylobacter* species. *PLoS Biol.* **3**, e15 (2005).
15. Parkhill, J. *et al.* The genome sequence of the food-borne pathogen *Campylobacter jejuni* reveals hypervariable sequences. *Nature* **403**, 665–668 (2000).
This paper presented the first *C. jejuni* genome sequence by using strain 11168 as the source for DNA-sequence analysis.
16. Bacon, D. J. *et al.* DNA sequence and mutational analyses of the pVir plasmid of *Campylobacter jejuni* 81-176. *Infect. Immun.* **70**, 6242–6250 (2002).
17. Hofreuter, D. *et al.* Unique features of a highly pathogenic *Campylobacter jejuni* strain. *Infect. Immun.* **74**, 4694–4707 (2006).
18. Gundogdu, O. *et al.* Re-annotation and re-analysis of the *Campylobacter jejuni* NCTC11168 genome sequence. *BMC Genomics* **8**, 162 (2007).
Reannotated the 11168 genome of the *C. jejuni* genome sequence (presented in reference 15).
19. Bacon, D. J. *et al.* Involvement of a plasmid in virulence of *Campylobacter jejuni* 81-176. *Infect. Immun.* **68**, 4384–4390 (2000).
20. Hendrixson, D. R., Akerley, B. J. & DiRita, V. J. Transposon mutagenesis of *Campylobacter jejuni* identifies a bipartite energy taxis system required for motility. *Mol. Microbiol.* **40**, 214–224 (2001).
21. Hendrixson, D. R. & DiRita, V. J. Identification of *Campylobacter jejuni* genes involved in commensal colonization of the chick gastrointestinal tract. *Mol. Microbiol.* **52**, 471–484 (2004).
Work reported in this paper included a signature-tagged mutagenesis screen to identify genes that have key roles in *C. jejuni* colonization.
22. Hendrixson, D. R. & DiRita, V. J. Transcription of σ–54-dependent but not σ–28-dependent flagellar genes in *Campylobacter jejuni* is associated with formation of the flagellar secretory apparatus. *Mol. Microbiol.* **50**, 687–702 (2003).
23. Colegio, O. R., Griffin, T. J., Grindley, N. D. & Galan, J. E. *In vitro* transposition system for efficient generation of random mutants of *Campylobacter jejuni*. *J. Bacteriol.* **183**, 2384–2388 (2001).
24. Golden, N. J., Camilli, A. & Acheson, D. W. Random transposon mutagenesis of *Campylobacter jejuni*. *Infect. Immun.* **68**, 5450–5453 (2000).
25. Grant, A. J. *et al.* Signature-tagged transposon mutagenesis studies demonstrate the dynamic nature of cecal colonization of 2-week-old chickens by *Campylobacter jejuni*. *Appl. Environ. Microbiol.* **71**, 8031–8041 (2005).
26. Carrillo, C. D. *et al.* Genome-wide expression analyses of *Campylobacter jejuni* NCTC11168 reveals coordinate regulation of motility and virulence by *flhA*. *J. Biol. Chem.* **279**, 20327–20338 (2004).
27. Dorrell, N. *et al.* Whole genome comparison of *Campylobacter jejuni* human isolates using a low-cost microarray reveals extensive genetic diversity. *Genome Res.* **11**, 1706–1715 (2001).

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28. Gaynor, E. C., Wells, D. H., MacKichan, J. K. & Falkow, S. The *Campylobacter jejuni* stringent response controls specific stress survival and virulence-associated phenotypes. *Mol. Microbiol.* **56**, 8–27 (2005). **Determined the necessity of the stringent response for pathogenic and, potentially, transmission traits for *C. jejuni*.**
29. Linton, D. *et al.* Phase variation of a β-1,3 galactosyltransferase involved in generation of the ganglioside GM1-like lipo-oligosaccharide of *Campylobacter jejuni*. *Mol. Microbiol.* **37**, 501–514 (2000).
30. Gilbert, M. *et al.* The genetic bases for the variation in the lipo-oligosaccharide of the mucosal pathogen, *Campylobacter jejuni*. Biosynthesis of sialylated ganglioside mimics in the core oligosaccharide. *J. Biol. Chem.* **277**, 327–337 (2002). **Described the spectrum of genetic mechanisms that lead to the high level of variation in LOS structure seen in *C. jejuni*.**
31. Guerry, P. *et al.* Phase variation of *Campylobacter jejuni* 81-176 lipooligosaccharide affects ganglioside mimicry and invasiveness *in vitro*. *Infect. Immun.* **70**, 787–793 (2002).
32. Karlyshev, A. V., Linton, D., Gregson, N. A. & Wren, B. W. A novel paralogous gene family involved in phase-variable flagella-mediated motility in *Campylobacter jejuni*. *Microbiology* **148**, 473–480 (2002).
33. Karlyshev, A. V., Ketley, J. M. & Wren, B. W. The *Campylobacter jejuni* glycome. *FEMS Microbiol. Rev.* **29**, 377–390 (2005).
34. de Boer, P. *et al.* Generation of *Campylobacter jejuni* genetic diversity *in vivo*. *Mol. Microbiol.* **44**, 351–359 (2002).
35. Wilson, D. L. *et al.* Variation of the natural transformation frequency of *Campylobacter jejuni* in liquid shake culture. *Microbiology* **149**, 3603–3615 (2003).
36. Avrain, L., Vernozy-Rozand, C. & Kempf, I. Evidence for natural horizontal transfer of *tetO* gene between *Campylobacter jejuni* strains in chickens. *J. Appl. Microbiol.* **97**, 134–140 (2004).
37. Wiesner, R. S., Hendrixson, D. R. & DiRita, V. J. Natural transformation of *Campylobacter jejuni* requires components of a type II secretion system. *J. Bacteriol.* **185**, 5408–5418 (2003).
38. Fry, B. N. *et al.* The *galE* gene of *Campylobacter jejuni* is involved in lipopolysaccharide synthesis and virulence. *Infect. Immun.* **68**, 2594–2601 (2000).
39. Larsen, J. C., Szymanski, C. & Guerry, P. *N*-linked protein glycosylation is required for full competence in *Campylobacter jejuni* 81-176. *J. Bacteriol.* **186**, 6508–6514 (2004).
40. Takata, T., Ando, T., Israel, D. A., Wassenaar, T. M. & Blaser, M. J. Role of *dprA* in transformation of *Campylobacter jejuni*. *FEMS Microbiol. Lett.* **252**, 161–168 (2005).
41. Nachamkin, I. Chronic effects of *Campylobacter* infection. *Microbes Infect.* **4**, 399–403 (2002).
42. Hughes, R. *Campylobacter jejuni* in Guillain–Barre syndrome. *Lancet Neurol.* **3**, 644 (2004).
43. Komagamine, T. & Yuki, N. Ganglioside mimicry as a cause of Guillain–Barre syndrome. *CNS Neurol. Disord. Drug Targets* **5**, 391–400 (2006).
44. Yu, R. K., Usuki, S. & Ariga, T. Ganglioside molecular mimicry and its pathological roles in Guillain–Barre syndrome and related diseases. *Infect. Immun.* **74**, 6517–6527 (2006).
45. Karlyshev, A. V., Linton, D., Gregson, N. A., Lastovica, A. J. & Wren, B. W. Genetic and biochemical evidence of a *Campylobacter jejuni* capsular polysaccharide that accounts for Penner serotype specificity. *Mol. Microbiol.* **35**, 529–541 (2000).
46. St Michael, F. *et al.* The structures of the lipooligosaccharide and capsule polysaccharide of *Campylobacter jejuni* genome sequenced strain NCTC 11168. *Eur. J. Biochem.* **269**, 5119–5136 (2002).
47. Szymanski, C. M. *et al.* Detection of conserved *N*-linked glycans and phase-variable lipooligosaccharides and capsules from *Campylobacter* cells by mass spectrometry and high resolution magic angle spinning NMR spectroscopy. *J. Biol. Chem.* **278**, 24509–24520 (2003).
48. Gilbert, M., Mandrell, R. E., Parker, C. T., Li, J. & Vinogradov, E. Structural analysis of the capsular polysaccharide from *Campylobacter jejuni* RM1221. *Chembiochem.* **8**, 625–631 (2007).
49. McNally, D. J. *et al.* The HS:19 serostrain of *Campylobacter jejuni* has a hyaluronic acid-type capsular polysaccharide with a nonstoichiometric sorbose branch and *O*-methyl phosphoramidate group. *FEBS J.* **273**, 3975–3989 (2006).
50. McNally, D. J. *et al.* The HS:1 serostrain of *Campylobacter jejuni* has a complex teichoic acid-like capsular polysaccharide with nonstoichiometric fructofuranose branches and *O*-methyl phosphoramidate groups. *FEBS J.* **272**, 4407–4422 (2005).
51. Karlyshev, A. V. *et al.* 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 (2005).
52. Bacon, D. J. *et al.* A phase-variable capsule is involved in virulence of *Campylobacter jejuni* 81-176. *Mol. Microbiol.* **40**, 769–777 (2001).
53. Bachtiar, B. M., Coloe, P. J. & Fry, B. N. Knockout mutagenesis of the *kpsE* gene of *Campylobacter jejuni* 81116 and its involvement in bacterium–host interactions. *FEMS Immunol. Med. Microbiol.* **49**, 149–154 (2007).
54. Jones, M. A. *et al.* Adaptation of *Campylobacter jejuni* NCTC11168 to high-level colonization of the avian gastrointestinal tract. *Infect. Immun.* **72**, 3769–3776 (2004).
55. Jagannathan, A., Constantinidou, C. & Penn, C. W. Roles of *rpoN*, *fliA*, and *flgR* in expression of flagella in *Campylobacter jejuni*. *J. Bacteriol.* **183**, 2937–2942 (2001).
56. Wosten, M. M., Wagenaar, J. A. & van Putten, J. P. The FlgS/FlgR two-component signal transduction system regulates the *fla* regulon in *Campylobacter jejuni*. *J. Biol. Chem.* **279**, 16214–16222 (2004).
57. Hendrixson, D. R. A phase-variable mechanism controlling the *Campylobacter jejuni* FlgR response regulator influences commensalism. *Mol. Microbiol.* **61**, 1646–1659 (2006).
58. Sommerlad, S. M. & Hendrixson, D. R. Analysis of the roles of FlgP and FlgQ in flagellar motility of *Campylobacter jejuni*. *J. Bacteriol.* **189**, 179–186 (2007).
59. Marchant, J., Wren, B. & Ketley, J. Exploiting genome sequence: predictions for mechanisms of *Campylobacter* chemotaxis. *Trends Microbiol.* **10**, 155–159 (2002).
60. Baker, M. D., Wolanin, P. M. & Stock, J. B. Signal transduction in bacterial chemotaxis. *Bioessays* **28**, 9–22 (2006).
61. Wadhams, G. H. & Armitage, J. P. Making sense of it all: bacterial chemotaxis. *Nature Rev. Mol. Cell Biol.* **5**, 1024–1037 (2004).
62. Hugdahl, M. B., Beery, J. T. & Doyle, M. P. Chemotactic behavior of *Campylobacter jejuni*. *Infect. Immun.* **56**, 1560–1566 (1988).
63. Yao, R., Burr, D. H. & Guerry, P. CheY-mediated modulation of *Campylobacter jejuni* virulence. *Mol. Microbiol.* **23**, 1021–1031 (1997).
64. Fredrick, K. L. & Helmann, J. D. Dual chemotaxis signaling pathways in *Bacillus subtilis*: a σ-D-dependent gene encodes a novel protein with both CheW and CheY homologous domains. *J. Bacteriol.* **176**, 2727–2735 (1994).
65. Pittman, M. S., Goodwin, M. & Kelly, D. J. Chemotaxis in the human gastric pathogen *Helicobacter pylori*: different roles for CheW and the three CheV paralogues, and evidence for CheV2 phosphorylation. *Microbiology* **147**, 2493–2504 (2001).
66. Szymanski, C. M., Yao, R., Ewing, C. P., Trust, T. J. & Guerry, P. Evidence for a system of general protein glycosylation in *Campylobacter jejuni*. *Mol. Microbiol.* **32**, 1022–1030 (1999). **Identified the genes that encode the *O*-linked glycosylation system of *C. jejuni*, which is responsible for glycosylation of flagellin.**
67. Thibault, P. *et al.* Identification of the carbohydrate moieties and glycosylation motifs in *Campylobacter jejuni* flagellin. *J. Biol. Chem.* **276**, 34862–34870 (2001).
68. Chou, W. K., Dick, S., Wakarchuk, W. W. & Tanner, M. E. Identification and characterization of NeuB3 from *Campylobacter jejuni* as a pseudaminic acid synthase. *J. Biol. Chem.* **280**, 35922–35928 (2005).
69. Guerry, P. *et al.* Changes in flagellin glycosylation affect *Campylobacter* autoagglutination and virulence. *Mol. Microbiol.* **60**, 299–311 (2006).
70. McNally, D. J. *et al.* Functional characterization of the flagellar glycosylation locus in *Campylobacter jejuni* 81-176 using a focused metabolomics approach. *J. Biol. Chem.* **281**, 18489–18498 (2006).
71. McNally, D. J. *et al.* Targeted metabolomics analysis of *Campylobacter coli* VC167 reveals legionaminic acid derivatives as novel flagellar glycans. *J. Biol. Chem.* **282**, 14463–14475 (2007).
72. Goon, S., Kelly, J. F., Logan, S. M., Ewing, C. P. & Guerry, P. Pseudaminic acid, the major modification on *Campylobacter* flagellin, is synthesized via the *Cj1293* gene. *Mol. Microbiol.* **50**, 659–671 (2003).
73. Glover, K. J., Weerapana, E. & Imperiali, B. *In vitro* assembly of the undecaprenylpyrophosphate-linked heptasaccharide for prokaryotic *N*-linked glycosylation. *Proc. Natl Acad. Sci. USA* **102**, 14255–14259 (2005).
74. Glover, K. J., Weerapana, E., Numao, S. & Imperiali, B. Chemoenzymatic synthesis of glycopeptides with PglB, a bacterial oligosaccharyl transferase from *Campylobacter jejuni*. *Chem. Biol.* **12**, 1311–1315 (2005).
75. Weerapana, E., Glover, K. J., Chen, M. M. & Imperiali, B. Investigating bacterial *N*-linked glycosylation: synthesis and glycosyl acceptor activity of the undecaprenyl pyrophosphate-linked bacillosamine. *J. Am. Chem. Soc.* **127**, 13766–13767 (2005).
76. Glover, K. J., Weerapana, E., Chen, M. M. & Imperiali, B. Direct biochemical evidence for the utilization of UDP-bacillosamine by PglC, an essential glycosyl-1-phosphate transferase in the *Campylobacter jejuni N*-linked glycosylation pathway. *Biochemistry* **45**, 5343–5350 (2006).
77. Kowarik, M. *et al. N*-linked glycosylation of folded proteins by the bacterial oligosaccharyltransferase. *Science* **314**, 1148–1150 (2006).
78. Olivier, N. B., Chen, M. M., Behr, J. R. & Imperiali, B. *In vitro* biosynthesis of UDP-N, *N*-diacetylbacillosamine by enzymes of the *Campylobacter jejuni* general protein glycosylation system. *Biochemistry* **45**, 13659–13669 (2006).
79. Wacker, M. *et al. N*-linked glycosylation in *Campylobacter jejuni* and its functional transfer into *E. coli*. *Science* **298**, 1790–1793 (2002). **Determined that the *pgl* locus of *C. jejuni* encodes an *N*-linked protein glycosylation system, the first described in bacteria, and moved the *pgl* locus into *E. coli*, allowing for the *N*-linked glycosylation of recombinant proteins.**
80. Young, N. M. *et al.* Structure of the *N*-linked glycan present on multiple glycoproteins in the Gram-negative bacterium, *Campylobacter jejuni*. *J. Biol. Chem.* **277**, 42530–42539 (2002).
81. Nita-Lazar, M., Wacker, M., Schegg, B., Amber, S. & Aebi, M. The N-X-S/T consensus sequence is required but not sufficient for bacterial *N*-linked protein glycosylation. *Glycobiology* **15**, 361–367 (2005).
82. Kowarik, M. *et al.* Definition of the bacterial *N*-glycosylation site consensus sequence. *EMBO J.* **25**, 1957–1966 (2006).
83. Kakuda, T. & DiRita, V. J. *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 (2006).
84. Szymanski, C. M., Burr, D. H. & Guerry, P. *Campylobacter* protein glycosylation affects host cell interactions. *Infect. Immun.* **70**, 2242–2244 (2002).
85. Karlyshev, A. V. *et al.* The *Campylobacter jejuni* general glycosylation system is important for attachment to human epithelial cells and in the colonization of chicks. *Microbiology* **150**, 1957–1964 (2004).
86. Kelly, J. *et al.* Biosynthesis of the *N*-linked glycan in *Campylobacter jejuni* and addition onto protein through block transfer. *J. Bacteriol.* **188**, 2427–2434 (2006).
87. Linton, D., Allan, E., Karlyshev, A. V., Cronshaw, A. D. & Wren, B. W. Identification of *N*-acetylgalactosamine-containing glycoproteins PEB3 and CgpA in *Campylobacter jejuni*. *Mol. Microbiol.* **43**, 497–508 (2002).
88. Konkel, M. E., Kim, B. J., Rivera-Amill, V. & Garvis, S. G. Identification of proteins required for the internalization of *Campylobacter jejuni* into cultured mammalian cells. *Adv. Exp. Med. Biol.* **473**, 215–224 (1999).
89. Konkel, M. E., Kim, B. J., Rivera-Amill, V. & Garvis, S. G. Bacterial secreted proteins are required for the internalization of *Campylobacter jejuni* into cultured mammalian cells. *Mol. Microbiol.* **32**, 691–701 (1999).
90. Ziprin, R. L. *et al.* Role of *Campylobacter jejuni* potential virulence genes in cecal colonization. *Avian Dis.* **45**, 549–557 (2001).
91. Rivera-Amill, V., Kim, B. J., Seshu, J. & Konkel, M. E. Secretion of the virulence-associated *Campylobacter* invasion antigens from *Campylobacter jejuni* requires a stimulatory signal. *J. Infect. Dis.* **183**, 1607–1616 (2001).
92. Konkel, M. E. *et al.* Secretion of virulence proteins from *Campylobacter jejuni* is dependent on a functional flagellar export apparatus. *J. Bacteriol.* **186**, 3296–3303 (2004).

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93. Young, G. M., Schmiel, D. H. & Miller, V. L. A new pathway for the secretion of virulence factors by bacteria: the flagellar export apparatus functions as a protein-secretion system. *Proc. Natl Acad. Sci. USA* **96**, 6456–6461 (1999).
94. Song, Y. C. *et al.* 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 (2004).
References 89, 92 and 94 identified secreted proteins of *C. jejuni* as important for host cell invasion, and reported that the flagella is a major export apparatus of these proteins.
95. Whitehouse, C. A. *et al. Campylobacter jejuni* cytolethal distending toxin causes a G2-phase cell cycle block. *Infect. Immun.* **66**, 1934–1940 (1998).
96. Lara-Tejero, M. & Galan, J. E. A bacterial toxin that controls cell cycle progression as a deoxyribonuclease I-like protein. *Science* **290**, 354–357 (2000).
97. Hassane, D. C., Lee, R. B., Mendenhall, M. D. & Pickett, C. L. Cytolethal distending toxin demonstrates genotoxic activity in a yeast model. *Infect. Immun.* **69**, 5752–5759 (2001).
98. Lara-Tejero, M. & Galan, J. E. CdtA, CdtB, and CdtC form a tripartite complex that is required for cytolethal distending toxin activity. *Infect. Immun.* **69**, 4358–4365 (2001).
99. Hassane, D. C., Lee, R. B. & Pickett, C. L. *Campylobacter jejuni* cytolethal distending toxin promotes DNA repair responses in normal human cells. *Infect. Immun.* **71**, 541–545 (2003).
100. Lee, R. B., Hassane, D. C., Cottle, D. L. & Pickett, C. L. Interactions of *Campylobacter jejuni* cytolethal distending toxin subunits CdtA and CdtC with HeLa cells. *Infect. Immun.* **71**, 4883–4890 (2003).
101. Elwell, C. A. & Dreyfus, L. A. DNase I homologous residues in CdtB are critical for cytolethal distending toxin-mediated cell cycle arrest. *Mol. Microbiol.* **37**, 952–963 (2000).
References 96 and 101 demonstrated that the mechanism of action of the cytolethal distending toxin may involve DNase I activity.
102. Sert, V. *et al.* The bacterial cytolethal distending toxin (CDT) triggers a G2 cell cycle checkpoint in mammalian cells without preliminary induction of DNA strand breaks. *Oncogene* **18**, 6296–6304 (1999).
103. Li, L. *et al.* The *Haemophilus ducreyi* cytolethal distending toxin activates sensors of DNA damage and repair complexes in proliferating and non-proliferating cells. *Cell. Microbiol.* **4**, 87–99 (2002).
104. Mao, X. & DiRienzo, J. M. Functional studies of the recombinant subunits of a cytolethal distending holotoxin. *Cell. Microbiol.* **4**, 245–255 (2002).
105. McSweeney, L. A. & Dreyfus, L. A. Nuclear localization of the *Escherichia coli* cytolethal distending toxin CdtB subunit. *Cell. Microbiol.* **6**, 447–458 (2004).
106. Nishikubo, S. *et al.* An N-terminal segment of the active component of the bacterial genotoxin cytolethal distending toxin B (CDTB) directs CDTB into the nucleus. *J. Biol. Chem.* **278**, 50671–50681 (2003).
107. Cortes-Bratti, X., Chaves-Olarte, E., Lagergard, T. & Thelestam, M. Cellular internalization of cytolethal distending toxin from *Haemophilus ducreyi*. *Infect. Immun.* **68**, 6903–6911 (2000).
108. Hickey, T. E. *et al. Campylobacter jejuni* cytolethal distending toxin mediates release of interleukin-8 from intestinal epithelial cells. *Infect. Immun.* **68**, 6535–6541 (2000).
Demonstrated a role for CDT in the intracellular survival of *C. jejuni* in a human monocytic cell line.
109. Hickey, T. E., Majam, G. & Guerry, P. Intracellular survival of *Campylobacter jejuni* in human monocytic cells and induction of apoptotic death by cytholethal distending toxin. *Infect. Immun.* **73**, 5194–5197 (2005).
110. Ge, Z. *et al.* Cytolethal distending toxin is essential for *Helicobacter hepaticus* colonization in outbred Swiss Webster mice. *Infect. Immun.* **73**, 3559–3567 (2005).
111. Pratt, J. S., Sachen, K. L., Wood, H. D., Eaton, K. A. & Young, V. B. Modulation of host immune responses by the cytolethal distending toxin of *Helicobacter hepaticus*. *Infect. Immun.* **74**, 4496–4504 (2006).
112. AbuOun, M. *et al.* Cytolethal distending toxin (CDT)-negative *Campylobacter jejuni* strains and anti-CDT neutralizing antibodies are induced during human infection but not during colonization in chickens. *Infect. Immun.* **73**, 3053–3062 (2005).
113. Biswas, D. *et al.* 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 (2006).
114. Konkel, M. E., Garvis, S. G., Tipton, S. L., Anderson, D. E. Jr & Cieplak, W. Jr. Identification and molecular cloning of a gene encoding a fibronectin-binding protein (CadF) from *Campylobacter jejuni*. *Mol. Microbiol.* **24**, 953–963 (1997).
115. Monteville, M. R. & Konkel, M. E. Fibronectin-facilitated invasion of T84 eukaryotic cells by *Campylobacter jejuni* occurs preferentially at the basolateral cell surface. *Infect. Immun.* **70**, 6665–6671 (2002).
116. Monteville, M. R., Yoon, J. E. & Konkel, M. E. Maximal adherence and invasion of INT 407 cells by *Campylobacter jejuni* requires the CadF outer-membrane protein and microfilament reorganization. *Microbiology* **149**, 153–165 (2003).
117. Konkel, M. E. *et al.* Identification of a fibronectin-binding domain within the *Campylobacter jejuni* CadF protein. *Mol. Microbiol.* **57**, 1022–1035 (2005).
118. Ziprin, R. L., Young, C. R., Stanker, L. H., Hume, M. E. & Konkel, M. E. The absence of cecal colonization of chicks by a mutant of *Campylobacter jejuni* not expressing bacterial fibronectin-binding protein. *Avian Dis.* **43**, 586–589 (1999).
119. Mamelli, L., Pages, J. M., Konkel, M. E. & Bolla, J. M. Expression and purification of native and truncated forms of CadF, an outer membrane protein of *Campylobacter*. *Int. J. Biol. Macromol.* **39**, 135–140 (2006).
120. Jin, S. *et al.* JlpA, a novel surface-exposed lipoprotein specific to *Campylobacter jejuni*, mediates adherence to host epithelial cells. *Mol. Microbiol.* **39**, 1225–1236 (2001).
121. Jin, S., Song, Y. C., Emili, A., Sherman, P. M. & Chan, V. L. JlpA of *Campylobacter jejuni* interacts with surface-exposed heat shock protein 90α and triggers signalling pathways leading to the activation of NF-κB and p38 MAP kinase in epithelial cells. *Cell. Microbiol.* **5**, 165–174 (2003).
122. Ashgar, S. S. *et al.* CapA, an autotransporter protein of *Campylobacter jejuni*, mediates association with human epithelial cells and colonization of the chicken gut. *J. Bacteriol.* **189**, 1856–1865 (2007).
123. Kervella, M. *et al.* Isolation and characterization of two *Campylobacter* glycine-extracted proteins that bind to HeLa cell membranes. *Infect. Immun.* **61**, 3440–3448 (1993).
124. Pei, Z. *et al.* Mutation in the *peb1A* locus of *Campylobacter jejuni* reduces interactions with epithelial cells and intestinal colonization of mice. *Infect. Immun.* **66**, 938–943 (1998).
125. Pei, Z. & Blaser, M. J. PEB1, the major cell-binding factor of *Campylobacter jejuni*, is a homolog of the binding component in gram-negative nutrient transport systems. *J. Biol. Chem.* **268**, 18717–18725 (1993).
126. Leon-Kempis Mdel, R., Guccione, E., Mulholland, F., Williamson, M. P. & Kelly, D. J. 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 (2006).
127. Robinson, D. A. Infective dose of *Campylobacter jejuni* in milk. *Br. Med. J. (Clin. Res. Ed.)* **282**, 1584 (1981).
128. Black, R. E., Levine, M. M., Clements, M. L., Hughes, T. P. & Blaser, M. J. Experimental *Campylobacter jejuni* infection in humans. *J. Infect. Dis.* **157**, 472–479 (1988).
129. Blaser, M. J. *et al.* Isolation of *Campylobacter fetus* subsp. *jejuni* from Bangladeshi children. *J. Clin. Microbiol.* **12**, 744–747 (1980).
130. Blaser, M. J., Taylor, D. N. & Feldman, R. A. Epidemiology of *Campylobacter jejuni* infections. *Epidemiol. Rev.* **5**, 157–176 (1983).
131. McSweegan, E. & Walker, R. I. Identification and characterization of two *Campylobacter jejuni* adhesins for cellular and mucous substrates. *Infect. Immun.* **53**, 141–148 (1986).
132. van Spreeuwel, J. P. *et al. Campylobacter colitis*: histological immunohistochemical and ultrastructural findings. *Gut* **26**, 945–951 (1985).
First description of the novel microtubule-dependent invasion mechanism of *C. jejuni*.
133. Oelschlaeger, T. A., Guerry, P. & Kopecko, D. J. Unusual microtubule-dependent endocytosis mechanisms triggered by *Campylobacter jejuni* and *Citrobacter freundii*. *Proc. Natl Acad. Sci. USA* **90**, 6884–6888 (1993).
134. Hu, L. & Kopecko, D. J. *Campylobacter jejuni* 81-176 associates with microtubules and dynein during invasion of human intestinal cells. *Infect. Immun.* **67**, 4171–4182 (1999).
135. Biswas, D., Itoh, K. & Sasakawa, C. Uptake pathways of clinical and healthy animal isolates of *Campylobacter jejuni* into INT-407 cells. *FEMS Immunol. Med. Microbiol.* **29**, 203–211 (2000).
136. Biswas, D., Itoh, K. & Sasakawa, C. Role of microfilaments and microtubules in the invasion of INT-407 cells by *Campylobacter jejuni*. *Microbiol. Immunol.* **47**, 469–473 (2003).
137. Finlay, B. B. Bacterial virulence strategies that utilize Rho GTPases. *Curr. Top. Microbiol. Immunol.* **291**, 1–10 (2005).
138. Selbach, M. & Backert, S. Cortactin: an Achilles' heel of the actin cytoskeleton targeted by pathogens. *Trends Microbiol.* **13**, 181–189 (2005).
139. Watson, R. O. & Galan, J. E. Signal transduction in *Campylobacter jejuni*-induced cytokine production. *Cell. Microbiol.* **7**, 655–665 (2005).
Mapped the signal-transduction pathway during *C. jejuni* infection of intestinal epithelial cells, showing stimulation of ERK, p38 and MAP kinase activity. Also demonstrated that *C. jejuni* flagellin stimulation of TLR5 is minimal.
140. MacCallum, A., Haddock, G. & Everest, P. H. *Campylobacter jejuni* activates mitogen-activated protein kinases in Caco-2 cell monolayers and *in vitro* infected primary human colonic tissue. *Microbiology* **151**, 2765–2772 (2005).
141. Andersen-Nissen, E. *et al.* Evasion of Toll-like receptor 5 by flagellated bacteria. *Proc. Natl Acad. Sci. USA* **102**, 9247–9252 (2005).
142. Johanesen, P. A. & Dwinell, M. B. Flagellin-independent regulation of chemokine host defense in *Campylobacter jejuni*-infected intestinal epithelium. *Infect. Immun.* **74**, 3437–3447 (2006).
143. Dalpke, A., Frank, J., Peter, M. & Heeg, K. Activation of toll-like receptor 9 by DNA from different bacterial species. *Infect. Immun.* **74**, 940–946 (2006).
144. Zilbauer, M. *et al.* A major role for intestinal epithelial nucleotide oligomerization domain 1 (NOD1) in eliciting host bactericidal immune responses to *Campylobacter jejuni*. *Cell. Microbiol.* (2007).
145. Hu, L., Bray, M. D., Osorio, M. & Kopecko, D. J. *Campylobacter jejuni* induces maturation and cytokine production in human dendritic cells. *Infect. Immun.* **74**, 2697–2705 (2006).
Described the maturation of dendritic cells and their activation in response to *C. jejuni* infection.
146. Jones, M. A., Totemeyer, S., Maskell, D. J., Bryant, C. E. & Barrow, P. A. Induction of proinflammatory responses in the human monocytic cell line THP-1 by *Campylobacter jejuni*. *Infect. Immun.* **71**, 2626–2633 (2003).
147. Siegesmund, A. M., Konkel, M. E., Klena, J. D. & Mixter, P. F. *Campylobacter jejuni* infection of differentiated THP-1 macrophages results in interleukin 1-β release and caspase-1-independent apoptosis. *Microbiology* **150**, 561–569 (2004).
148. Wassenaar, T. M., Engelskirchen, M., Park, S. & Lastovica, A. Differential uptake and killing potential of *Campylobacter jejuni* by human peripheral monocytes/macrophages. *Med. Microbiol. Immunol.* **186**, 139–144 (1997).
149. Kiehlbauch, J. A., Albach, R. A., Baum, L. L. & Chang, K. P. Phagocytosis of *Campylobacter jejuni* and its intracellular survival in mononuclear phagocytes. *Infect. Immun.* **48**, 446–451 (1985).
150. Day, W. A. Jr, Sajecki, J. L., Pitts, T. M. & Joens, L. A. Role of catalase in *Campylobacter jejuni* intracellular survival. *Infect. Immun.* **68**, 6337–6345 (2000).
151. Nachamkin, I. & Yang, X. H. Human antibody response to *Campylobacter jejuni* flagellin protein and a synthetic N-terminal flagellin peptide. *J. Clin. Microbiol.* **27**, 2195–2198 (1989).
152. Panigrahi, P., Losonsky, G., DeTolla, L. J. & Morris, J. G. Jr. Human immune response to *Campylobacter jejuni* proteins expressed *in vivo*. *Infect. Immun.* **60**, 4938–4944 (1992).
153. Guerry, P., Ewing, C. P., Hickey, T. E., Prendergast, M. M. & Moran, A. P. Sialylation of lipooligosaccharide cores affects immunogenicity and serum resistance of *Campylobacter jejuni*. *Infect. Immun.* **68**, 6656–6662 (2000).
154. Coker, A. O., Isokpehi, R. D., Thomas, B. N., Amisu, K. O. & Obi, C. L. Human campylobacteriosis in developing countries. *Emerg. Infect. Dis.* **8**, 237–244 (2002).

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155. Kaldor, J., Pritchard, H., Serpell, A. & Metcalf, W. Serum antibodies in *Campylobacter enteritis*. *J. Clin. Microbiol.* **18**, 1–4 (1983).
156. Lee, M. D. & Newell, D. G. *Campylobacter* in poultry: filling an ecological niche. *Avian Dis.* **50**, 1–9 (2006).
157. Clench, M. H. & Mathias, J. R. The avian cecum: a review. *The Wilson Bulletin* **107**, 93–121 (1995).
158. Byrne, C. M., Clyne, M. & Bourke, B. *Campylobacter jejuni* adhere to and invade chicken intestinal epithelial cells *in vitro*. *Microbiology* **153**, 561–569 (2007).
159. Nachamkin, I., Yang, X. H. & Stern, N. J. Role of *Campylobacter jejuni* flagella as colonization factors for three-day-old chicks: analysis with flagellar mutants. *Appl. Environ. Microbiol.* **59**, 1269–1273 (1993).
160. Wassenaar, T. M., van der Zeijst, B. A., Ayling, R. & Newell, D. G. Colonization of chicks by motility mutants of *Campylobacter jejuni* demonstrates the importance of flagellin A expression. *J. Gen. Microbiol.* **139**, 1171–1175 (1993).
161. Raphael, B. H. *et al.* The *Campylobacter jejuni* response regulator, CbrR, modulates sodium deoxycholate resistance and chicken colonization. *J. Bacteriol.* **187**, 3662–3670 (2005).
162. Stintzi, A. Gene expression profile of *Campylobacter jejuni* in response to growth temperature variation. *J. Bacteriol.* **185**, 2009–2016 (2003).
163. Bras, A. M., Chatterjee, S., Wren, B. W., Newell, D. G. & Ketley, J. M. A novel *Campylobacter jejuni* two-component regulatory system important for temperature-dependent growth and colonization. *J. Bacteriol.* **181**, 3298–3302 (1999).
164. MacKichan, J. K. *et al.* The *Campylobacter jejuni dccRS* two-component system is required for optimal *in vivo* colonization but is dispensable for *in vitro* growth. *Mol. Microbiol.* **54**, 1269–1286 (2004).
165. Purdy, D., Cawthraw, S., Dickinson, J. H., Newell, D. G. & Park, S. F. Generation of a superoxide dismutase (SOD)-deficient mutant of *Campylobacter coli*: evidence for the significance of SOD in *Campylobacter* survival and colonization. *Appl. Environ. Microbiol.* **65**, 2540–2546 (1999).
166. Lin, J., Sahin, O., Michel, L. O. & Zhang, Q. Critical role of multidrug efflux pump CmeABC in bile resistance and *in vivo* colonization of *Campylobacter jejuni*. *Infect. Immun.* **71**, 4250–4259 (2003).
167. Luo, N., Sahin, O., Lin, J., Michel, L. O. & Zhang, Q. *In vivo* selection of *Campylobacter* isolates with high levels of fluoroquinolone resistance associated with *gyrA* mutations and the function of the CmeABC efflux pump. *Antimicrob. Agents Chemother.* **47**, 390–394 (2003).
168. Palyada, K., Threadgill, D. & Stintzi, A. Iron acquisition and regulation in *Campylobacter jejuni*. *J. Bacteriol.* **186**, 4714–4729 (2004).
169. Velayudhan, J., Jones, M. A., Barrow, P. A. & Kelly, D. J. L-serine catabolism via an oxygen-labile L-serine dehydratase is essential for colonization of the avian gut by *Campylobacter jejuni*. *Infect. Immun.* **72**, 260–268 (2004).
170. Woodall, C. A. *et al.* *Campylobacter jejuni* gene expression in the chick cecum: evidence for adaptation to a low-oxygen environment. *Infect. Immun.* **73**, 5278–5285 (2005).
Described the *C. jejuni* gene-expression profile during chick colonization, so providing clues as to the adaptation of *C. jejuni* to its commensal colonization niche.
171. Smith, C. K. *et al.* *Campylobacter jejuni*-induced cytokine responses in avian cells. *Infect. Immun.* **73**, 2094–2100 (2005).
Showed that *Campylobacter* can stimulate a proinflammatory response in avian cells, so suggesting that colonization does not occur because of the inability to stimulate the chick immune system.
172. Kogut, M. H. *et al.* Expression and function of Toll-like receptors in chicken heterophils. *Dev. Comp. Immunol.* **29**, 791–807 (2005).
173. Kogut, M. H., Swaggerty, C., He, H., Pevzner, I. & Kaiser, P. Toll-like receptor agonists stimulate differential functional activation and cytokine and chemokine gene expression in heterophils isolated from chickens with differential innate responses. *Microbes Infect.* **8**, 1866–1874 (2006).
174. Fukui, A. *et al.* Molecular cloning and functional characterization of chicken toll-like receptors. A single chicken toll covers multiple molecular patterns. *J. Biol. Chem.* **276**, 47143–47149 (2001).
175. Bar-Shira, E. & Friedman, A. Development and adaptations of innate immunity in the gastrointestinal tract of the newly hatched chick. *Dev. Comp. Immunol.* **30**, 930–941 (2006).
176. Sahin, O. *et al.* Prevalence, antigenic specificity, and bactericidal activity of poultry anti-*Campylobacter* maternal antibodies. *Appl. Environ. Microbiol.* **67**, 3951–3957 (2001).
177. Cawthraw, S., Ayling, R., Nuijten, P., Wassenaar, T. & Newell, D. G. Isotype, specificity, and kinetics of systemic and mucosal antibodies to *Campylobacter jejuni* antigens, including flagellin, during experimental oral infections of chickens. *Avian Dis.* **38**, 341–349 (1994).
178. Sahin, O., Luo, N., Huang, S. & Zhang, Q. Effect of *Campylobacter*-specific maternal antibodies on *Campylobacter jejuni* colonization in young chickens. *Appl. Environ. Microbiol.* **69**, 5372–5379 (2003).
179. Jeurissen, S. H., Janse, E. M., van Rooijen, N. & Claassen, E. Inadequate anti-polysaccharide antibody responses in the chicken. *Immunobiology* **198**, 385–395 (1998).
180. Chang, C. & Miller, J. F. *Campylobacter jejuni* colonization of mice with limited enteric flora. *Infect. Immun.* **74**, 5261–5271 (2006).
Reported a promising new mouse model of *C. jejuni* infection that enabled low-dose infection and analysis of immune-clearance mechanisms.
181. Young, C. R., Ziprin, R. L., Hume, M. E. & Stanker, L. H. Dose response and organ invasion of day-of-hatch Leghorn chicks by different isolates of *Campylobacter jejuni*. *Avian Dis.* **43**, 763–767 (1999).
182. Wong, T. L. *et al.* Prevalence, numbers, and subtypes of *Campylobacter jejuni* and *Campylobacter coli* in uncooked retail meat samples. *J. Food Prot.* **70**, 566–573 (2007).
183. Stern, N. J., Bailey, J. S., Blankenship, L. C., Cox, N. A. & McHan, F. Colonization characteristics of *Campylobacter jejuni* in chick ceca. *Avian Dis.* **32**, 330–334 (1988).
184. Luo, N. *et al.* Enhanced *in vivo* fitness of fluoroquinolone-resistant *Campylobacter jejuni* in the absence of antibiotic selection pressure. *Proc. Natl Acad. Sci. USA* **102**, 541–546 (2005).
185. Hung, D. T., Shakhnovich, E. A., Pierson, E. & Mekalanos, J. J. Small-molecule inhibitor of *Vibrio cholerae* virulence and intestinal colonization. *Science* **310**, 670–674 (2005).
186. de Zoete, M. R., van Putten, J. P. & Wagenaar, J. A. Vaccination of chickens against *Campylobacter*. *Vaccine* **25**, 5548–5557 (2006).
187. Sizemore, D. R., Warner, B., Lawrence, J., Jones, A. & Killeen, K. P. Live, attenuated *Salmonella typhimurium* vectoring *Campylobacter* antigens. *Vaccine* **24**, 3793–3803 (2006).
188. Wagner, R. D. Efficacy and food safety considerations of poultry competitive exclusion products. *Mol. Nutr. Food Res.* **50**, 1061–1071 (2006).
189. Szymanski, C. M. & Wren, B. W. Protein glycosylation in bacterial mucosal pathogens. *Nature Rev. Microbiol.* **3**, 225–237 (2005).
190. Al-Salloom, F. S. *et al.* *Campylobacter*-stimulated INT407 cells produce dissociated cytokine profiles. *J. Infect.* **47**, 217–224 (2003).

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Competing interests statement
The authors declare no competing financial interests.

DATABASES
Entrez Gene: http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=gene
Cj1496c
Entrez Genome: http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=genome
pVir | RM1221 | 11168 | 81-176
Entrez Genome Project: http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=genomeprj
Actinobacillus actinomycetemcomitans | *Bacillus subtilis* | *Campylobacter coli* | *Campylobacter jejuni* | Escherichia coli | *Haemophilus ducreyi* | *Helicobacter hepaticus* | *Helicobacter pylori* | *Neisseria gonorrhoeae* | *Vibrio cholerae* | *Wolinella succinogenes*
Entrez Protein: http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=protein
CadF | CetA| CetB | CbrR | CdtA | CdtB | CdtC | CDT | CheV | CheY | CiaB | Cj1496c | FlaC | IlpA | NOD1 | Peb1 | TLR-5 | TLR-9 | σ^{28} | σ^{54}

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