

DETERMINANTS THAT MAY BE INVOLVED IN VIRULENCE AND DISEASE IN *Campylobacter jejuni*

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Received for Publication September 30, 1995

Accepted for Publication January 6, 1996

006297

ABSTRACT

Campylobacter jejuni is the major cause of bacterial gastroenteritis in the United States and developed nations. In addition to gastroenteritis, *C. jejuni* can cause extraintestinal diseases such as the reactive arthritides and Guillain-Barré syndrome. Most of the illnesses induced by *C. jejuni* are not life-threatening but can be severe and long-lasting. A number of virulence determinants which are believed to be involved in the induction of gastroenteritis, reactive arthritides and Guillain-Barré syndrome have been demonstrated in *C. jejuni*. These determinants include attachment and invasion molecules, outer membrane proteins, heat shock proteins, flagella, iron acquiring mechanisms, cytotoxic and cytotoxic factors, arthritogenic antigens that may trigger reactive arthritides and bacterial factors that may induce the Guillain-Barré syndrome. The published literature concerning the pathogenic mechanisms of *C. jejuni* indicates that many of the virulence determinants of the organism are inadequately characterized and the role that they play in causing disease is not clear.

INTRODUCTION

Campylobacter jejuni/coli is the major cause of bacterial gastroenteritis in the United States (Anon 1994; Peterson 1994) and the United Kingdom (Cowden 1992; Skirrow 1991) with *C. jejuni* inducing the majority of cases (Tauxe *et al.* 1988). *C. jejuni* enteritis is mainly foodborne; food is four times more likely than water to be linked to outbreaks of campylobacteriosis (Tauxe *et al.* 1988). Therefore, *C. jejuni* is our most common foodborne bacterial pathogen.

¹ Mention of brand or firm names does not constitute an endorsement by the U.S. Department of Agriculture over others of a similar nature not mentioned.

Most of the infections are sporadic cases or small family outbreaks. Large outbreaks are rare and are generally due to drinking raw milk or untreated water (Skirrow 1991). Poultry is the predominant source of the organism in sporadic cases (Tauxe *et al.* 1988). Since *C. jejuni* is a commensal of the intestinal tract of poultry (Stern 1992) and since the consumption of poultry has doubled since 1970 (Putnam and Allshouse 1994; U.S. Bureau of the Census 1992), it is not surprising that poultry is a common source of the organism to humans.

The clinical manifestation generally seen in *C. jejuni* illness is enterocolitis. However, the effect of the organism on the gastrointestinal mucosa is poorly understood. Extra-intestinal clinical disease is seen in which various organs (heart, brain, liver, urinary tract, etc.) may be affected; bacteremia may occur, also. In addition, *C. jejuni* is associated with the reactive arthritides and the Guillain-Barré syndrome (Smith 1995). The various virulence determinants of *C. jejuni* which may play a role in the causation of gastroenteritis, reactive arthritides and the Guillain-Barré syndrome are discussed in this review.

DETERMINANTS THAT MAY BE INVOLVED IN GASTROENTERITIS

To induce gastroenteritis and extraintestinal disease, *Campylobacter* organisms must adhere to, invade and damage host cells by the production of adhesion and invasion factors, cytotoxic and/or cytotoxic toxins. A discussion of possible adhesive and invasive determinants as well as *Campylobacter* toxins is presented in the following sections.

Adhesion to and Invasion of Host Cells by *Campylobacter*

Uncharacterized Adhesion and Invasion Molecules. Carbohydrate moieties are important for adhesion since pretreatment of *C. jejuni* cells with L-fucose or D-mannose inhibits adherence to INT 407 epithelial cell monolayers (Cinco *et al.* 1984; McSweegan and Walker 1986) but heating of the bacteria (100C, 30 min) has no effect (Cinco *et al.* 1984). Surface proteins are involved in attachment, also, since treatment of bacterial cells with pepsin, trypsin, protease or glutaraldehyde interferes with attachment to the monolayers (McSweegan and Walker 1986). Treatment of both bacterial and monolayer cells with glutaraldehyde completely blocks adhesion of *C. jejuni* (McSweegan and Walker 1986). Association of *C. jejuni* with Caco-2 cells is reduced in a dose-dependent manner by D-galactose, D-mannose, D-glucose or D-maltose (Russell and Blake 1994). Invasion of the Caco-2 cell line is inhibited by D-glucose, D-mannose or D-maltose. Cell lysates of *C. jejuni* adsorbed onto HEP-2 cells prevents bacterial invasion but not attachment (Konkel and Joens 1989). Treatment of lysates with

sodium *meta*-periodate abolished lysate blockage of invasion indicating that invasion by *C. jejuni* depends on an intact carbohydrate moiety which is probably a glycoprotein (Konkel and Joens 1989; Konkel *et al.* 1990). These results indicate that *C. jejuni* has an adhesion molecule that initiates host-bacterial cell interaction and an invasion molecule that initiates invasion.

C. jejuni cultured in the presence of INT 407 epithelial cells synthesizes special proteins not found when the bacterium is grown in the absence of the epithelial cells (Konkel and Cieplak 1992). Chloramphenicol does not inhibit the adherence of *C. jejuni* to INT 407 cells, but significantly inhibits internalization of the bacteria. The antibiotic also inhibits the synthesis of the special proteins. The bacterial proteins induced by growth in epithelial cells probably play a role in the invasion of INT 407 cells by the bacteria (Konkel and Cieplak 1992). These bacterial proteins are not heat shock proteins. Oelschlaeger *et al.* (1993) found that invasion of INT 407 cells by *C. jejuni* depends on *de novo* protein synthesis. They proposed that *C. jejuni* synthesizes proteins with relatively short half-life which act as surface ligands triggering uptake of bacteria into epithelial cells.

C. jejuni is not invasive according to the Sereny test for keratoconjunctivitis in guinea pigs (Bukholm and Kapperud 1987; Manninen *et al.* 1982). However, *C. jejuni* does invade various tissue culture cells (Babakhani and Joens 1993; Konkel and Cieplak 1992; Konkel and Joens 1989; Russell and Blake 1994). Enteropathogenic *Escherichia coli* can invade epithelial cells but are negative by the Sereny test (Donnenberg and Kaper 1992; Donnenberg *et al.* 1989; Tesh and O'Brien 1992). Therefore, invasion of tissue culture cells indicates invasive capacity even though the Sereny test may be negative.

The ability of *C. jejuni* to invade and locate intracellularly in HEP-2 or A-549 epithelial cells is enhanced by enteroinvasive *Salmonella*, *Shigella* or *E. coli* as coinfectants (Bukholm and Kapperud 1987). The significance of enhanced invasion by *C. jejuni* in the presence of enteroinvasive bacteria is not clear at the present time.

Outer Membrane Proteins. A 27 kDa outer membrane protein (OMP) from strains of *C. jejuni* and *C. coli* preferentially adheres to HeLa cells (Fauchere *et al.* 1989, 1992). This OMP is similar to the *C. jejuni* protein, PEB1, which is involved both in binding to intestinal cells and in amino acid transport (Pei and Blaser 1993). De Melo and Pechere (1990) isolated OMPs of 28 and 32 kDa which bind to HEP-2 cell monolayers. Pretreatment of cell monolayers with neuraminidase or pre-incubation of OMP preparations with sialic acid, L-fucose, L-mannose or N-acetylgalactosamine prior to addition to HEP-2 cells does not affect binding of the *C. jejuni* OMPs to the cell monolayer (De Melo and Pechere 1990). A 69 kDa OMP from *C. jejuni* acts as an intestinal colonization factor for chickens (Meinersmann *et al.* 1990). Thus, a limited amount of data suggests that OMPs from *Campylobacter* may act as adherent and colonization factors.

Lipopolysaccharides. The lipopolysaccharide (LPS) of *C. jejuni* specifically binds to INT 407 cells and glutaraldehyde treatment of INT 407 cells prevents binding of LPS. Therefore, a protein on INT 407 cells must bind LPS (McSweegan and Walker 1986). Preincubation of INT 407 cells with L-fucose inhibits LPS binding to the cells and periodate oxidation of LPS reduces binding to INT 407 cells. Preincubation of INT 407 cells with LPS blocks adherence of *C. jejuni* cells (McSweegan and Walker 1986). LPS also binds to mucus gel from rabbit small intestine; however, more *C. jejuni* LPS binds to the epithelial cell surface than to mucus.

Stress Proteins. Stress proteins, ranging from 10 to 120 kDa, are induced and/or released by heat or alkaline pH treatment of *C. jejuni* (Wu *et al.* 1994). The 64 and 10 kDa proteins are homologous to *Escherichia coli* GroEL and GroES heat shock proteins (HSPs), respectively (Wu *et al.* 1994). Stress proteins play a role in the pathogenicity of microorganisms. For example, the GroEL protein of *Salmonella typhimurium* aids in binding of the bacteria to intestinal mucus (Ensgraber and Loos 1992). The agglutinin responsible for aggregation of *S. typhimurium* onto colonic mucus is a 15 kDa glycoprotein which is recognized and bound by the GroEL protein of the bacteria (Ensgraber and Loos 1992). During infection of macrophages, *S. typhimurium* synthesizes several stress proteins which enhance bacterial survival within macrophages (Buchmeier and Heffron 1990). The bacterial HSPs shield bacterial macromolecular complexes from the toxic metabolites and degradative enzymes produced by the macrophages. Stress proteins produced by *C. jejuni* may play a role in adhesion and invasion and also may protect the bacterium from host defenses during the disease process.

Flagella and Motility. *C. jejuni* has polar flagellum (at one or both ends of the cell) which is involved in virulence. The *Campylobacter* flagellar protein, flagellin, is highly immunogenic and appears to be the major protein antigen (Glenn-Calvo *et al.* 1994; Guerry *et al.* 1992; Khawaja *et al.* 1992; Nuijten *et al.* 1992). There are two flagellar genes in tandem chromosomal arrangement, *flaA* and *flaB*, separated by a short intervening sequence (Logan *et al.* 1989; Nuijten *et al.* 1990). The two genes are 95% homologous (Nuijten *et al.* 1991). Each gene, however, contains its own promoter (Guerry *et al.* 1992; Nuijten *et al.* 1992). The expression of *flaA* is predominant.

Campylobacter species undergo both phase and antigenic variations in flagella expression. Phase variation refers to the ability to express either flagellated (motile) or aflagellated (non-motile) phenotypes (Caldwell *et al.* 1985; Nuijten *et al.* 1989). Only the *flaA* gene is involved in phase variation (Nuijten *et al.* 1995). Antigenic variation is the ability of some strains to synthesize alternate flagellar subunits differing in molecular weight and antigenicity (Harris *et al.* 1987). Since flagella are a major target of the immune system, phase and antigenic variation enable *Campylobacter* to evade the immune response (Nuijten *et al.* 1989; 1995).

Ferrero and Lee (1988) demonstrated that *C. jejuni*, unlike *Vibrio cholerae*, *S. enteritidis* or *E. coli*, is actively motile in highly viscous solutions simulating intestinal mucus. The spiral morphology and rapid motility give *Campylobacter* a selective advantage in penetrating and colonizing the thick viscous mucus barrier of intestinal cells (Khawaja *et al.* 1992; Lee *et al.* 1986; Szymanski *et al.* 1995). However, the presence of anti-*Campylobacter* secretory antibody (sIgA) prevented bacterial adherence to INT 407 cells overlaid with rabbit small intestinal mucus (McSweegen *et al.* 1987).

Black *et al.* (1988) inoculated human volunteers with a strain of *C. jejuni* that contained a mixture of motile and nonmotile cells. The presence of only motile cells in feces suggest that motility is necessary for intestinal colonization. Animal experiments, utilizing Syrian hamsters and mice, have tended to confirm the importance of motility in infection and colonization of the intestinal tract (Aguero-Rosenfeld *et al.* 1990; Morooka *et al.* 1985; Newell *et al.* 1985). *C. jejuni* with intact flagella (fully motile) colonize the cecá of three-day-old chicks whereas mutants lacking flagella (nonmotile) or with altered flagella (partially motile) are unable to colonize (Nachamkin *et al.* 1993). However, flagella are not necessary for growth and survival of *C. jejuni* in the chick embryo (Field *et al.* 1993).

Nonflagellated *C. jejuni* do not adhere to INT 407 cells as well as flagellated bacteria (McSweegen and Walker 1986). Removal of flagella by shearing action decreased binding approximately 3-fold. When flagella activity was inhibited with KCN, bacteria bind to INT 407 cells more effectively than control cells. Isolated flagella also bind to culture cells (McSweegen and Walker 1986). These findings suggest that the flagellum of *C. jejuni* is an adhesion factor. *C. jejuni* with an active *flaA* gene penetrated INT 407 cells regardless of the status of the *flaB* gene (Wassenaar *et al.* 1991). Purified flagella from *C. jejuni* did not affect penetration of the bacteria into the culture cells. Wassenaar *et al.* (1991) state that flagella do not have specific adhesive properties which would block penetration of *C. jejuni* into INT 407 cells.

C. jejuni with *flaA*⁺*flaB*⁺ genotype is internalized 5-fold more efficiently than *flaA*⁻*flaB*⁺ cells, and 30 to 40-fold more efficiently than cells with *flaA*⁻*flaB*⁻ genotype (Grant *et al.* 1993). The invasive capacity of the *flaA*⁺ *flaB*⁺ strain for INT 407 cells was 98% as compared to 100% for the wild type *flaA*⁺*flaB*⁺ (Nuijten *et al.* 1992). Regardless of the status of *flaA* or *flaB* genes (active or inactive), the adherence of *C. jejuni* to INT 407 cells is approximately equal. These results suggest that flagella are not involved in cell adherence but are important in internalization of the bacterial cell into INT 407 cells (Grant *et al.* 1993). When polarized Caco-2 cell monolayers are used, only *flaA*⁺*flaB*⁺ bacteria translocate across the epithelial cell barrier. Thus, motility and/or the product of the *flaA* gene of *C. jejuni* is necessary for bacterial crossing of polarized epithelial cell monolayers with resultant cell invasion (Grant *et al.* 1993). Polarized Caco-2 cells, in contrast to nonpolarized cultured cells, have their apical and basolateral

surfaces separated by tight junctions and thereby have a permeability barrier. Caco-2 cells possess regular microvilli and brush borders and behave similarly to enterocytes. Thus, polarized culture cells are more suitable for the *in vitro* determination of bacterial invasion of the gastrointestinal tract than nonpolarized culture cells (Finlay and Falkow 1990; Konkel *et al.* 1992b).

Infection of INT 407 culture cells with nonmotile, short flagellated *flaAflaB*⁺ *C. jejuni* cells yields a motile variant with full length flagella containing type B flagellin. The increased motility of the variant *C. jejuni* does not lead to improved invasive capacity (Wassenaar *et al.* 1994). Nonmotile *flaA*⁺*flaB*⁺ mutants with full-length flagella show only about 1% of the capacity to invade INT 407 cells of the wild-type motile strain but do have about 40% of the adhesive ability of the wild-type (Yao *et al.* 1994).

Wild-type motile *C. jejuni* but not an aflagellate variant colonize the gastrointestinal tract of infant mice. However, a flagellated non-motile variant colonize infant mice as well as wild-type (Newell *et al.* 1985). Everest *et al.* (1993b) found that a mutant *C. jejuni* with reduced motility did not colonize the intestine of infant mice and did not induce tissue damage or fluid secretion in rabbit ileal loops. Chick cecal colonization is decreased 100-fold in *flaAflaB* (nonmotile) mutants of *C. jejuni* as compared to wildtype motile (*flaA*⁺*flaB*⁺) strains (Wassenaar *et al.* 1993). There is more than a 1000-fold decrease in colonization of chicks with *flaAflaB*⁺ (partially motile) mutants. Interestingly, *flaA*⁺*flaB*⁻ (motile) strains colonize the chick cecum 1000-fold that of the wildtype. Thus, inactivation of the *flaB* gene enhances chicken colonization. Wassenaar *et al.* (1993) suggested that the presence of flagellin A, rather than motility, is essential for bacterial colonization of chicken ceca.

Flagella and its associated motility appear to be pathogenic determinants for *C. jejuni*. Motility is necessary for *C. jejuni* to penetrate the thick gastric mucosa. Flagella, in particular flagella with type A flagellin, do appear to be necessary for invasion and internalization of *C. jejuni* but flagella may or may not act as adhesion factors. The conflicting data concerning the role of flagella in adhesion may reflect differences in strains or procedures used or may be due to variations within the species itself. Penn and Luke (1992) have reviewed the role of flagella in the pathogenesis of gram-negative bacteria.

M Cells and Entry into Colonic Mucosa. Walker *et al.* (1988) postulated that *Campylobacter* species utilize M cells to pass from the intestinal lumen into the intestinal mucosa. M cells are closely associated with the Peyer's patches found in the lamina propria of the colonic submucosa. Peyer's patches consist of lymphoid aggregates containing B-cells, T-cells and antigen-presenting macrophages. Peyer's patches are covered by M cells; thus, the cells are part of the mucosal membrane. Luminal bacteria and other antigens are ingested by the M cells and are then transported, in an unaltered state, to the underlying macrophages (Salyers and Whitt 1994; Sneller and Strober 1986; Stites and Terr 1991).

Thus, M cells serve as channels for the entry of antigens or bacteria from the intestinal lumen to Peyer's patches. The role of M cells is protective since they alert the gastrointestinal-associated lymphoid tissue (GALT) to the presence of disruptive bacteria on the intestinal mucosa. Normally, bacteria ingested by M cells and transported to the underlying tissue are killed by the macrophages (which are antigen presenting cells) of the GALT. The antigen presenting cells stimulate the T- and B-cells which leads to sIgA antibody production (Owen *et al.* 1986; Salyers and Whitt 1994; Sneller and Strober 1986).

However, some microorganisms use M cells as a conduit for entering the body and causing disease. These include reoviruses (Rubin *et al.* 1985; Wolf *et al.* 1981, 1983), *S. typhi* (Kohbata *et al.* 1986), *Yersinia enterocolitica* (Grutzkau *et al.* 1990; Hanski *et al.* 1989; Pepe and Miller 1993), and *S. flexneri* (Wassef *et al.* 1989). Walker *et al.* (1988) demonstrated that *C. jejuni* adheres to M cells and are transported intact to macrophages of Peyer's patches. This phenomenon may explain the movement of the organism through the intestinal mucosa, proliferation in the lamina propria and mesenteric lymph nodes and transport of the bacteria to the circulatory system with resultant bacteremia. M cells may be important in the pathogenesis of *C. jejuni* by acting as channels for infection as well as leading to the formation of protective sIgA antibodies on the intestinal mucosal surface (Sneller and Strober 1986; Walker and Owen 1990; Walker *et al.* 1988).

Role of Iron. Iron is required by pathogens for growth but is extremely limited in the mammalian host. Pathogens can not establish an infection unless they can utilize the host's iron-binding compounds directly or have mechanisms to release iron from host sources (Litwin and Calderwood 1993). Iron overload in the animal host leads to increased susceptibility to microbial infections (Barclay 1985). When embryonated chicken eggs were inoculated with *C. jejuni* suspended in a solution of iron dextran, the LD₅₀ of the pathogen was decreased several-fold (Field *et al.* 1986). Using *C. jejuni* serially passaged through weanling mice, Kazmi *et al.* (1984) found that the intragastric LD₅₀ of the pathogen for neonatal mice is decreased from 10¹¹ to 10⁵ colony forming units (CFU) when iron dextran is included with the bacterial inoculum.

Iron is necessary for *C. jejuni in-vitro* growth (Baig *et al.* 1986; Field *et al.* 1986; Stern *et al.* 1988). In the absence of iron, cells elongate, lack septa and become filamentous; iron is necessary for normal cell division (Field *et al.* 1986). The addition of iron along with sodium bisulfite and sodium pyruvate to growth medium enhances the aerotolerance of the organism (George *et al.* 1978; Hoffman *et al.* 1979; Stern *et al.* 1988). Iron also enhances the production of *Campylobacter* cytotoxic toxin by *C. jejuni* (McCardell *et al.* 1986).

Bacterial hemolysins may promote infectivity by releasing iron-containing heme and hemoglobin from erythrocytes. Most strains of *C. jejuni* or *C. coli* produce a cell-associated hemolysin (Arimi *et al.* 1990; Tay *et al.* 1995).

Hemolytic activity of *Campylobacter* is not regulated by iron (Pickett *et al.* 1992). Hemin, hemoglobin, hemin-hemopexin and hemoglobin-haptoglobin stimulates the growth of *C. jejuni* strains in low-iron medium but transferrin, lactoferrin and ferritin do not (Pickett *et al.* 1992). Haptoglobin specifically and irreversibly binds hemoglobin and its derivatives; free heme is insoluble under physiological conditions and its solubility is maintained in the body by binding to hemopexin or albumin (Mietzner and Morse 1994). *C. jejuni* must have some mechanism to transport hemolysis products into the cell and to release iron from complexes. However, the role of *C. jejuni* hemolysins in virulence is not known.

Siderophores are iron chelators secreted by microorganisms in response to low environmental iron levels. They are low-molecular weight nonporphyrin and nonprotein compounds which solubilize iron and can effectively compete with host iron-binding compounds to mobilize iron for bacterial use. Specific cell-surface receptors are required for the siderophore-iron complex (Litwin and Calderwood 1993; Mietzner and Morse 1994). Field *et al.* (1986) reported that 9/26 strains of *C. jejuni* produced siderophores in low iron media. However, these siderophores were not chemically identified. *C. jejuni* or *C. coli* do not produce siderophores capable of removing iron from host transferrin, but they were able to obtain iron by utilizing exogenous siderophores produced by other microorganisms including enterobactin and ferrichrome. However, rhodotorulic acid, aerobactin or desferroxamine B are not utilized (Baig *et al.* 1986; Field *et al.* 1986). Even though *Campylobacter* do not appear to produce siderophores (Baig *et al.* 1986), the proper receptors for exogenous siderophores must be present. Field *et al.* (1986) demonstrated that under conditions of iron-deprivation, new OMPs are produced by *C. jejuni*. While they did not characterize these OMPs, they may be receptors for the exogenous siderophores. *C. jejuni* must have mechanisms by which iron is released from the siderophore-iron complex. These may include reduction of the ferric ion in the complex and/or production of enzymes which decrease the affinity of the siderophore for the attached iron (Barclay 1985; Guerinot 1994).

Superoxide dismutases (SODs) catalyze the degradation of superoxide molecules to H_2O_2 and O_2 . SOD is a defense against oxidative stress and protects cytoplasmic enzymes, DNA and various membrane factors against damage by oxygen radicals (Pesci *et al.* 1994). Since macrophages can kill ingested bacterial pathogens by oxidative mechanisms, the production of SOD contributes to virulence by protecting bacteria from macrophage attack (Purdy and Park 1994). The gene coding for the production of an iron-containing SOD (FeSOD or SodB) in *C. jejuni* and *C. coli* has been cloned and sequenced (Pesci *et al.* 1994; Purdy and Park 1994). Pesci *et al.* (1994) demonstrated that FeSOD promotes the entry and survival of *C. jejuni* in INT 407 culture cells. It is probable that the iron-containing enzyme protects the organism from oxygen-mediated intracellular death during invasion.

Fur, the protein product of the *fur* gene, acts as a corepressor with ferrous ion to negatively regulate certain genes in *E. coli* including those involved in siderophore biosynthesis and transport (Litwin and Calderwood 1993), synthesis of outer membrane siderophore receptors (Litwin and Calderwood 1993), synthesis of Shiga-like toxin (Calderwood and Mekalonos 1987), and expression of SODs (Niederhoffer *et al.* 1990). Wooldridge *et al.* (1994) demonstrated that *C. jejuni* has a *fur* gene which allows the expression of a Fur protein with 35% identity with the Fur protein in *E. coli*. The *fur* gene of *C. jejuni* has been cloned and characterized (Chan *et al.* 1995). Since Fur repressors regulate virulence genes in other bacteria (Litwin and Calderwood 1993), the Fur-like repressor protein in *C. jejuni* probably regulates genes involved in pathogenesis. These could include genes involved in the synthesis of OMPs acting as receptors for exogenous siderophores, toxins and SOD as well as the synthesis of other factors. Determination of the genes repressed by the Fur protein by the use of *fur* mutants should provide information concerning putative virulence factors in *C. jejuni*.

Since iron is an absolute requirement for most pathogenic bacteria, mechanisms for acquiring iron from the host are an important aspect of virulence. *C. jejuni* can utilize hemin and hemoglobin released from erythrocytes by hemolysin action and they can utilize siderophores produced by other microorganisms. It is not completely clear if *C. jejuni* produces its own siderophores; Field *et al.* (1986) reported the synthesis of siderophores but this does not appear to have been confirmed. The campylobacter FeSOD may protect the organism *in-vivo* against the macrophagic oxidative burst. The Fur protein repressor, important in other pathogens, may also play a role in virulence of *C. jejuni*.

Cytotoxic and Cytotonic Toxins Produced by *Campylobacter*

Cytotoxins are toxins that kill mammalian cells. In *Campylobacter*, there appear to be several types of cytotoxins: a number (at least two and possibly more) of poorly characterized cytotoxins, Shiga-like cytotoxin (SLT) and a cytotoxin termed cytolethal distending toxin (CLDT). *Campylobacter jejuni* toxin (CJT), similar to *Vibrio cholerae* cholera toxin (CT) and *E. coli* heat-labile toxin (LT), is a cytotonic toxin. Cytotonic exotoxins exert their effects on enterocytes by perturbing the physiological regulation of ion and water transport mediated by cyclic nucleotides (Polotsky *et al.* 1994). The result is efflux of fluid and electrolytes into the gut. There is no direct damage to enterocytes by cytotonic toxins but the excessive transport of ions and water from cells eventually lead to cell damage.

Poorly-Characterized Cytotoxins. It is difficult to determine if there is one cytotoxin or several in *Campylobacter* species. Part of the problem is the lack of consistency among the various investigators concerning tissue culture cell lines used and in the way the assays are conducted. Only one group appears to have

purified a cytotoxin (Mahajan and Rodgers 1990). Most of the cytotoxins listed in Table 1 are poorly characterized.

The cytopathic effects observed for *Campylobacter* cytotoxins (Table 1) include cell rounding with nuclear pyknosis (condensation and reduction in size of the nucleus), loss of cell monolayer adhesiveness and cell death within 24-48 h. The cytotoxins are proteins with 50,000-70,000 molecular weight, inactivated by trypsin, do not lead to accumulation of fluid in ligated ileal loop or suckling mouse assays, and are not related to LT, CT, SLT or *E. coli* heat-stable toxin (ST). The heat stability data are inconsistent because of great variability in the temperatures and times used. The behavior of *Campylobacter* cytotoxins on cultured cells (Table 1) would suggest that there is a toxin that is cytopathic on Vero cells and another that is not; consistent use of Vero, HeLa, and CHO cells by investigators would clarify this issue.

Misawa *et al.* (1994) demonstrated that the expression of cytotoxicity by *C. jejuni* depends on the presence of serum in the cell culture medium. Cytotoxic activity is seen in culture filtrates from *C. jejuni* using CHO, C-6, HeLa, Vero, INT 407 and HEP-2 cells when newborn calf serum was added; in the presence of fetal calf serum, activity is seen only with CHO and C-6 cells. In the absence of serum, toxic effects were seen with CHO, C-6 and HeLa cells (Misawa *et al.* 1994). Misawa *et al.* (1994) suggested that these results indicate the presence of several different cytotoxins.

Shiga-Like Cytotoxin. The cytotoxicity of some *Campylobacter* cell lysates toward HeLa cells is neutralized by Shiga toxin antibody or by a monoclonal antibody against the B subunit of *E. coli* SLT-1 (Moore *et al.* 1988). The level of SLT produced by *Campylobacter* is < 10,000-fold that produced by *Shigella dysenteriae* 1 or *E. coli* O157:H7. There is no homology between the phage-encoded SLT-1 gene and the putative *Campylobacter* SLT gene (Moore *et al.* 1988). The inability to show a genetic basis for SLT production in *Campylobacter* casts a large measure of doubt concerning the production of the toxin by the organism.

Cytolethal Distending Toxin. CLDT is a toxin found in culture filtrates of *Campylobacter* species (Johnson and Lior 1988a), *E. coli* strains associated with diarrhea (Johnson and Lior 1988b), enteropathogenic *E. coli* (Bouzari and Varghese 1990, 1992; Guth *et al.* 1994) and *Shigella* spp. (Johnson and Lior 1987a). The CLDT genes of *E. coli* have been cloned and sequenced (Pickett *et al.* 1994).

CLDT of *Campylobacter* is cytolethal to CHO, Vero, HeLa and HEP-2 cell lines but is negative on Y-1 cells (Johnson and Lior 1988a). CHO cells exposed to CLDT are characterized by extensive distension of the cells with 90% of the cells killed within 96 h (Johnson and Lior 1988a). Johnson and Lior (1987b; 1988a) caution that unless the assay for CLDT is carefully done, it will be labeled as enterotoxin rather than CLDT. At 96 h, CLDT causes distension and cytolethal

TABLE 1.
SOME PROPERTIES OF CYTOTOXINS FOUND IN *CAMPYLOBACTER JEJUNI*.

Reference	Properties
Wong <i>et al.</i> 1983; Pang <i>et al.</i> 1987	Cytopathic on HeLa, MRC-5 and HEp-2 cells (negative on Vero and other monkey cells); inactivated at 100 C/30 min but not at 56 C/30 min; trypsin-sensitive; molecular weight > 30,000; no fluid accumulation in suckling mouse assay
Klipstein <i>et al.</i> 1985	Cytopathic on HeLa and Vero cells (negative on CHO cells); no fluid with rat ligated ileal loop assay
Johnson and Lior 1986	Cytopathic on HeLa and Vero cells (negative on CHO and Y-1 cells ?); inactivated at 70 C/30 min; no fluid accumulation in suckling mouse assay; activity not neutralized by antibody against <i>E. coli</i> SLT
Johnson and Lior 1986	Cytopathic on HeLa and CHO cells (negative on Vero cells)
Guerrant <i>et al.</i> 1987, 1992	Cytopathic on HeLa and CHO cells; inactivated ~10% at 50 C/30 min and ~70% at 60 C/30 min; trypsin-sensitive; molecular weight 50,000-70,000; no fluid accumulation in rabbit ligated ileal loop or suckling mouse assays; activity not neutralized by antibody against <i>E. coli</i> SLT I or II
Akhtar and Huq 1989	Cytopathic on HeLa and Vero cells (negative on CHO cells); inactivated at 60 C/30 min or 56 C/60 min
Mahajan and Rodgers 1990	68,000 molecular weight protein isolated by gel electrophoresis; cytopathic on CHO and INT 407 cells; inactivated at 60 C/15 min; trypsin-sensitive; inactivated at pH 3 or pH 9; activity not neutralized by antibodies against CT or LT
Florin and Antillon 1992	Cytopathic on MRC-3, Vero and HeLa cells; inactivated at 100 C/30 min
Mizuno <i>et al.</i> 1994	Cytopathic on CHO and HeLa cells; inactivated ~50% at 70 C/30 min and ~90% at 80 C/30 min; trypsin-sensitive

effects on CHO cells whereas CJT produces a rounded effect on CHO cells and >85% of CHO cells are still viable (Johnson and Lior 1988a). CLDT was present in 237/583 (40.7%) of *C. jejuni* and 46/109 (42.2%) of *C. coli* strains. Of 715 *Campylobacter* strains (*C. jejuni*, *C. coli*, *C. laridis* and *C. fetus*) tested, 41.0% were CLDT⁺ (Johnson and Lior 1988a).

Campylobacter CLDT is heat-labile (70C for 30 min), trypsin-sensitive, nondialyzable, >30,000 molecular weight, and is neutralized only by homologous rabbit antitoxin. CLDT is negative in the adult rabbit ligated ileal loop, suckling mouse and rabbit skin permeability assays indicating that the toxin is not similar to LT or ST (Johnson and Lior 1988a). CLDT-positive *Campylobacter* produced hemorrhagic fluid and inflammation when inoculated into rat ligated ileal loops. No cyclic AMP accumulates; therefore, CLDT does not appear to activate adenylate cyclase to induce fluid accumulation (Johnson and Lior 1988a). At present, nothing is known concerning the toxic mechanism of CLDT on cultured cell lines and the role of CLDT in pathogenesis of *Campylobacter* is not clear.

The mechanism of CLDT action appears to be at the level of cell division. Inhibition of cell division without a corresponding inhibition of cell replication may lead to accumulation of nuclear and cytoplasmic material. The resultant accumulation of cellular material leads to bulging and distension with ultimate bursting of the cell (Bouzari and Varghese 1990).

***Campylobacter jejuni* Toxin.** Some properties of CJT are given in Table 2. Only those reports in which CJT was detected and/or quantified by the use of LT or CT antibodies or in which the activity of CJT was neutralized by LT or CT antibodies are cited in Table 2. LT, CT and CJT bind strongly to the ganglioside, GM1, on target cells. LT and CT also bind to GD1b gangliosides whereas CJT binds poorly to GD1b (Fukuta *et al.* 1988; Suzuki *et al.* 1994). CJT can be detected and quantitated by use of GM1 ganglioside and CT, CJT or LT antibodies (Table 2); indicating a close relationship between the toxins.

CJT is cytopathic toward CHO or Y-1 cells and the effects are neutralized by CT or LT antibodies. The effect of heat on CJT (Table 2) is unclear. Fluid and cyclic AMP accumulation is induced by CJT in ligated ileal loops. Thus, the mode of action of CJT is similar to CT or LT but is unlike ST since the suckling mouse assay was negative (Smith 1988). McCardell *et al.* (1984) and Collins *et al.* (1992) suggest that CJT lacks the A and B subunit structure found in CT or LT. However, the data of Daikoku *et al.* (1990) and Klipstein and Engert (1985) suggest that CJT has a subunit structure. Obviously, more work is needed to determine the actual structure of CJT.

Konkel *et al.* (1992a) and Perez-Perez *et al.* (1989, 1992) were unable to detect CT-like activity in *C. jejuni* and could not show genetic homology between *Campylobacter* DNA and DNA probes for CT or LT genes. Using strains obtained from Ruiz-Palacios (Table 2), Olsvik *et al.* (1984) were unable to show

homology between *C. jejuni* DNA and DNA probes for CT or LT subunits A and B. Wadstrom *et al.* (1983) could not confirm the presence of cytotoxic nor cytotoxic enterotoxins in their *C. jejuni* strains. However, Calva *et al.* (1989) found nucleotide sequences on the *C. jejuni* chromosome which were similar to the coding region for the GM1 binding site of LT or CT. Interestingly, chromosomal DNA from both enterotoxin and non-enterotoxin producing strains hybridized with the DNA probe for the GM1-binding site. Therefore, it is possible that all *C. jejuni* strains have the enterotoxin gene but it is not always expressed (Calva *et al.* 1989).

Everest *et al.* (1993b) demonstrated low levels of CJT *in vitro* in *C. jejuni* strains isolated from stools of children with inflammatory diarrhea (Table 2). Whole cells inoculated into rabbit ligated ileal loops led to fluid accumulation but CJT could not be detected in the fluids. Therefore, Everest *et al.* (1993b) suggest that CJT is not responsible for fluid secretion in the gut but the effect is due to host-derived mediators of secretion which are associated with tissue inflammation. Indeed, Everest *et al.* (1993a) demonstrated an increase in cyclic AMP, prostaglandin E₂ (PGE₂), leukotriene B₄ (LTB₄) and leukocytes in rabbit ileal loop fluid from loops inoculated with inflammatory *C. jejuni* strains producing little or no CJT. Leukotriene and prostaglandin are released from leukocytes during inflammation (Donowitz 1985). LTB₄ attracts neutrophils which infiltrate the areas of inflammation. PGE₂ enhances the activity of LTB₄ and activates adenylate cyclase causing fluid secretion in the gut (Everest *et al.* 1993a). Thus, CJT does not appear to have a role in the pathogenic process; however, Everest *et al.* (1993a) did not search for cytotoxins or CLDT in their *C. jejuni* strains. CLDT does not appear to activate adenylate cyclase but does induce bloody fluid in rat ligated ileal loops (Johnson and Lior 1988a). Reports concerning the effect of cytotoxins on adenylate cyclase were not found but it is possible that the results obtained by Everest *et al.* (1993a) were due to cytotoxins since Klipstein *et al.* (1985) found that viable cells of cytotoxin-positive *C. jejuni* induced fluid accumulation in rat ligated ileal loops. On the other hand, Ruiz-Palacios *et al.* (1992) stated that there was a strong correlation between CJT production and diarrhea. There was no correlation between cytotoxin production and diarrhea.

The literature devoted to CJT presents a very confusing picture of the toxin. In particular, studies on the pathogenesis, chemical structure and the genetics of CJT give conflicting information. A concerted and sustained research effort is needed in order to bring order and consistency to the literature.

On the basis of clinical symptoms, there are three mechanisms by which *Campylobacter* can cause disease. The bacterium attaches to intestinal epithelial cells and produces a cytotoxic enterotoxin (CJT) which leads to a secretory diarrhea. The clinical presentation consists of episodes of loose watery stools. A second pathogenic mechanism involves the invasion and proliferation of the organism within the intestinal epithelium with production of cytotoxins which

TABLE 2.
SOME PROPERTIES OF CAMPYLOBACTER JEJUNI TOXIN (CJT)

Reference	Properties
Ruiz-Palacios <i>et al.</i> 1983	Elongation induced in CHO cells; inactivated at 56 C/60 min and at 96 C/10 min; inactivated at pH 2.0 and 8.0; fluid accumulation in rat ligated loop assay (negative with rabbit ileal loop or suckling mouse assays); increase in cyclic AMP in CHO cells; fluid accumulation in ileal loops and cyclic AMP production neutralized by antibody against CT
Mathan <i>et al.</i> 1984	Cytopathic on CHO cells; detected and quantitated by ELISA with GM ₁ ganglioside and LT antibody; ELISA for ST was negative
McCardell <i>et al.</i> 1984	Cell rounding induced in Y-1 cells and cell elongation in CHO cells; not inactivated at 100 C/10 min; trypsin-sensitive; fluid accumulation in rabbit ligated ileal loop assay (negative in suckling mouse assay); positive in rabbit skin permeability assay; cytopathic effect in Y-1 cells neutralized with antibody against CT
Klipstein and Engert 1984a, 1984b; Klipstein <i>et al.</i> 1985, 1986	Purified CJT had molecular weight of 70,000; no subunit structure; CJT and CT demonstrated partial immunological identity by immunodiffusion; did not bind to GM ₁ ganglioside
	Elongation induced in CHO cells; inactivated at 70 C/120 min and at 96 C/10 min; detected and quantitated by ELISA with GM ₁ ganglioside and antibody against LT; fluid accumulation in rabbit ligated ileal loop assay; cytopathic effect on CHO cells neutralized with antibodies against LT and CT; rats immunized with LT or B subunit of LT produced significantly less fluid in ligated ileal loops when challenged with CJT
Goossens <i>et al.</i> 1985	Partially purified CJT gave lines of identity with LT and partial lines of identity with CT in immunodiffusion tests
	Elongation induced in CHO cells; cytopathic effects on CHO cells neutralized by antibodies against LT and CT
Saha <i>et al.</i> 1988; Saha and Sanyal 1990	Fluid accumulation in rat ligated ileal loop assay (negative with rabbit ligated ileal loops); fluid accumulation neutralized by antibody against CT
	Partially purified CJT inactivated at 56 C/30 min and 60 C/10 min; inactivated at pH 3.0 and pH 9.0; optimum pH for fluid accumulation was 6.0; not inactivated by trypsin but inactivated by pronase and papain; not hemolytic
Belbouri and Megraud 1988	Elongation induced in CHO cells; not inactivated at 100 C/10 min; cytopathic effect partially neutralized by antibody against LT

TABLE 2. (Cont.)

Lindblom <i>et al.</i> 1989	Elongation induced in CHO cells; detected and quantitated by ELISA with GM ₁ and antibody against LT
Daikoku <i>et al.</i> 1990	Partially purified CJT detected and quantitated by ELISA with ganglioside and antibody against LT; adenylate cyclase activity stimulated in HeLa cells with accumulation of cyclic AMP
Bok <i>et al.</i> 1991	Rounding induced in Y-1 cells; cytopathic effect neutralized by antibody against CT
Florin and Antillon 1992	Rounding induced in Y-1 cells; cytopathic effect neutralized by antibody against LT
Collins <i>et al.</i> 1992	Elongation induced in CHO cells; cross-reaction with LT and CT; negative in rat ligated ileal loop assay Partially purified CJT had molecular weight ~50,000 with no subunit structure; detected on Western blots by ELISA with GM ₁ and antibody against CT
Everest <i>et al.</i> 1993b	Elongation induced in CHO cells; detected by ELISA with GM ₁ ganglioside and antibody against B subunit of CT

induce cell damage. Clinically, there is an inflammatory diarrhea with bloody stools and fecal leukocytes. A third mechanism leading to extraintestinal infections involves penetration of intestinal mucosa (probably through the M-cells) and proliferation of bacteria in the lamina propria and mesenteric lymph nodes with eventual entry of the organisms into the blood system (Calva *et al.* 1989; Klipstein *et al.* 1985; Ruiz-Palacios 1992).

Studies by Klipstein *et al.* (1985, 1986) and Florin and Antillon (1992) indicate that the toxin pattern of *C. jejuni* strains isolated from patients do not correlate well with symptoms seen in gastroenteritis. For example, strains negative for both CJT and cytotoxins were isolated from asymptomatic individuals in one study by Klipstein *et al.* (1985); yet such strains were isolated from symptomatic patients (some of whom had bloody stools) in a different study by the same group (Klipstein *et al.* 1986). Florin and Antillon (1992) found patients with either watery diarrhea or bloody diarrhea even though the *C. jejuni* strains were negative for both CJT and cytotoxin. CJT positive-cytotoxin negative strains were found in patients with watery diarrhea by Klipstein *et al.* (1985, 1986) but in a different study, patients infected with CJT positive-cytotoxin negative *C. jejuni* strains had either watery or bloody diarrhea (Florin and Antillon 1992). It is apparent that disease symptoms can not necessarily be predicted on the basis of toxin type of the infecting *C. jejuni*.

Recently, Ketley (1995) reviewed various aspects of the virulence of *Campylobacter* with emphasis on molecular genetics.

DETERMINANTS THAT MAY BE INVOLVED IN THE INDUCTION OF THE REACTIVE ARTHRITIDES BY *Campylobacter*

The reactive arthritides (reactive arthritis, Reiter's syndrome) are arthritic syndromes characterized by sterile inflammation of joints from infections originating at nonarticular sites. The arthritides may be "triggered" by gastrointestinal infections involving *Campylobacter*, *Salmonella*, *Shigella* or *Yersinia* and in urethral infections by *Chlamydia* or *Ureaplasma* (Smith *et al.* 1993). An important feature of reactive arthritides is that viable microorganisms are not present in the joints; however, bacterial antigens are found. Lipopolysaccharides (LPS) from arthritis-causing *S. enteritidis*, *S. typhimurium*, *Shigella flexneri*, and *Yersinia enterocolitica* are present in synovial cells (polymorphonuclear leukocytes and mononuclear phagocytes) of arthritic joints (Granfors *et al.* 1989, 1990, 1992). After infection with the triggering organisms, bacterial LPS and other antigens are probably transferred to the joints within phagocytic cells (Gaston 1994).

Information concerning mechanisms by which *Campylobacter* may induce the reactive arthritides is not available. However, a discussion of how other organisms, particularly *Yersinia enterocolitica*, are believed to induce arthritis may provide some clues as what may be happening when *Campylobacter* gastroenteritis proceeds to arthritis.

The reactive arthritides are T-cell mediated diseases (Toivanen *et al.* 1994). In *in vitro* experiments utilizing ³H-thymidine incorporation, T-cells isolated from the joints of reactive arthritis patients incorporated the greatest amount of isotope (this assay is termed T-cell proliferation) when they were exposed to the specific organism such as *Chlamydia*, *Campylobacter*, *Salmonella* or *Yersinia* which triggered the arthritis (Gaston *et al.* 1989). A cationic 19-kDa antigen which is a beta-subunit of *Yersinia* urease is a target antigen for synovial T-cells isolated from patients with *Yersinia*-induced reactive arthritis (Probst *et al.* 1993a; Skurnik *et al.* 1993). The 19-kDa antigen is arthritogenic in rats. The interaction of the cationic antigen with negatively charged cartilage proteoglycan may allow the antigen to be retained in the joint as a continual immune stimulus (Gaston 1994; Probst *et al.* 1993a). Other antigenic targets of synovial T-cell response include the cationic 18-kDa chlamydial histone-like protein (Gaston 1994) and the 14 kDa 50s subunit of the ribosomal R2 protein from *Yersinia* (Toivanen *et al.* 1994).

Some investigators have postulated that HSPs are arthritogenic proteins targeted by synovial T cells (Gaston *et al.* 1994; Schultz and Arnold 1993). Exposure of prokaryotic and eukaryotic cells to a variety of stresses induces an

immediate reprogramming of protein synthesis with production of unique proteins, the HSPs, which play a protective role against stressful conditions. The proteins are highly conserved with a great deal of amino acid homology in HSPs from different species (Polla 1988; Schultz and Arnold 1993). Pathogens produce HSPs during invasion of a mammalian host and these proteins serve to protect bacteria from host defense mechanisms. Similarly, host cells synthesize HSPs (homologous to the bacterial HSPs) in response to the stress of bacterial invasion. If bacterial HSPs become targets for T-cells, the host homologues of the bacterial HSPs may be cross-reactive and result in autoimmunity (Polla 1988; Schultz and Arnold 1993).

Hermann *et al.* (1991) isolated a *Yersinia*-reactive T-cell clone from synovial fluid of a patient with Reiter's syndrome which proliferated in the presence of *Yersinia* antigens and human 65-kDa HSP. The homologous HSP from the bacterium was not available for testing. Probst *et al.* (1993b) could not isolate T-cell clones from reactive arthritis patients which reacted with purified *Yersinia* 61-kDa HSP, the homologue of the human 65-kDa HSP used by Hermann *et al.* (1991). Since the T-cells used by Hermann *et al.* (1991) and Probst *et al.* (1993b) were from different patients, it may not be possible to compare their results. Not enough data are available at the present time to conclude that cross-reaction between homologous HSPs from humans and bacteria plays no role in the reactive arthritides.

Synovial T-cells from patients with *Yersinia*-, *Salmonella*- or *Chlamydia*-induced arthritis show a limited spectrum of cytokine production: interferon- γ (IFN- γ) and interleukin-2 (IL-2). IL-4, -5 and -10 are not found (Hermann 1993; Lahesmaa *et al.* 1992; Schlaak *et al.* 1992). T cells that produce IL-2 and IFN- γ are labeled "helper T cells type 1" (Th1) and are involved in cell-mediated responses such as delayed-type hypersensitivity. IFN- γ and IL-2 stimulate the activity of cytotoxic T-cells, macrophages, and natural killer cells. Th2 cells produce IL-4, -5, and -10 cytokines. The Th2 cell cytokines are involved in humoral immunity and assist in antibody production by inducing B-cell differentiation and expansion and promote allergic type responses (Clerici and Shearer 1994; O'Garra and Murphy 1994; Sartor 1994; Scott 1993). Cross-regulation of the subsets occurs since IFN- γ can inhibit cytokine production by Th2 cells. IL-4 and IL-10, produced by Th2 cells, can suppress (down-regulate) Th1 responses (O'Garra and Murphy 1994).

Lahesmaa *et al.* (1992) suggested that Th1 cytokines are involved in the generation of and propagation of joint inflammation. However, Schlaak *et al.* (1992) demonstrated that the Th1 cells control the intracellular replication of *Yersinia*. IFN- γ promotes intracellular killing of *Yersinia*, whereas IL-4 (produced by Th2 cells) antagonizes IFN- γ killing of the bacteria. In addition, the Th2 cytokine, IL-10, inhibits *in vitro* proliferation of *Yersinia*-specific T-cells and represses (down-regulates) production of IFN- γ and IL-2 by Th1 cells (Schlaak

et al. 1992). Hermann (1993) interpreted the data of Schlaak *et al.* (1992) as indicating that Th1 cells, by production of *Yersinia*-killing IFN- γ , may promote eradication of bacterial antigens from inflamed tissue. Therefore, the pathology of the reactive arthritides is probably due to an imbalance of Th1 and Th2 cell activities rather than due to excessive Th1 cell expansion.

Arthritogenic pathogens may produce proteins which behave as superantigens. Superantigens are those antigens which, at levels lower than that required for conventional antigens, can stimulate proliferation of a large number of T-cells bearing a particular variable part of the β chain (V β sequence) of the T-cell receptor. Each superantigen recognizes one to five different V β sequences. As many as 5-25% of T-cells having the proper V β sequences may be activated by a superantigen whereas a conventional antigen may engage only about 0.01% of the T-cell pool. The result of such massive T-cell stimulation is substantial cytokine release including IL-2, -4, and -6, tumor necrosis factor and IFN- γ (Imberti *et al.* 1992; Schlievet 1993; Sissons 1993; Taub and Blank 1993; Zumla 1992).

Stuart and Woodward (1992) demonstrated an antigen in *Y. enterocolitica* which behaves as a T-cell superantigen and a superantigen from *Y. pseudotuberculosis* has been purified and partially sequenced (Abe *et al.* 1993; Uchiyama *et al.* 1993; Yoshino *et al.* 1994). Unfortunately, no information is available concerning potential superantigen activity in other microorganisms that induce reactive arthritides. Lahesmaa *et al.* (1994) envisions the role of bacterial superantigens in the reactive arthritides as follows: inflammation of the joint may result from massive cytokine production due to superantigen stimulation of large numbers of T-cells. Alternatively, the superantigen could contribute to inflammatory synovitis by expanding a population of autoreactive T cells that recognize a particular synovial antigen.

Lipopolysaccharides are present in synovial cell fluid cells (Granfors *et al.* 1989, 1990, 1992) but are not considered as arthritogenic antigens since lipopolysaccharides do not activate T-cells (Abbas *et al.* 1991; Hermann 1993). The OMPs, YadA and YopH, of *Y. enterocolitica* may be arthritogenic since mutant strains not producing these OMPs demonstrate decreased arthritis-inducing ability in rats (Gaede and Heesemann 1995; Gripenberg-Lerche *et al.* 1994). YadA binds to extracellular matrix proteins such as collagen, laminin and fibronectin which are present in joints (Flugel *et al.* 1994; Schultz-Koops *et al.* 1992, 1993; Tamm *et al.* 1993; Terti *et al.* 1992). A *Y. enterocolitica* strain lacking the chromosomal gene for yersiniabactin, an iron-chelator, does not induce arthritis in rats (Gaede and Heesemann 1995). It is not known if the *Y. enterocolitica* OMPs or yersiniabactin play a role in the induction of the reactive arthritides in humans.

It appears that the response of synovial T-cells to antigens of the arthritis triggering microorganism plays an important part in the pathogenesis of the

reactive arthritides. However, it is not clear which bacterial antigen or antigens are arthritogenic and activate T-cells.

Determinants That May Be Involved in *Campylobacter* Induced Guillain-Barré Syndrome

Guillain-Barré syndrome (GBS) is an acute, progressive inflammatory demyelinating polyneuropathy characterized by paralysis, pain, muscular weakness and mild distal sensory loss. In severe cases, respiration, eye movements, swallowing and autonomic functions may be affected. Patients are usually bed-ridden due to paralysis, pulmonary support may be required and often the patients may require nasogastric feeding. Generally, the symptoms are preceded by viral or bacterial infections, vaccinations or administration of certain drugs (Ropper 1992; Smith 1995). *C. jejuni* is the most common bacterial pathogen associated with GBS (Rees *et al.* 1993).

An infection by *C. jejuni* may induce GBS by an immune cross-reaction between bacterial and peripheral nerve antigens. Antibodies to neural gangliosides are found in sera from 231/847 (27.3%) GBS patients. The most common antibody is directed against the ganglioside, GM1 (Willison and Kennedy 1993). GBS patients with antibodies that react with GM1 or GD1b had a higher incidence of prior *Campylobacter* infections than other GBS patients (Walsh *et al.* 1991; von Wulffen *et al.* 1994). It is interesting that patients without neurologic disease who were seropositive for *Campylobacter* demonstrated antibodies against GM1 and GD1b, also (von Wulffen *et al.* 1994). Lipopolysaccharides isolated from *C. jejuni* strains associated with GBS have regions homologous to the human gangliosides, GM1 and GD1b (Aspinall 1994a, 1994b; Yuki *et al.* 1994a). Thus, the molecular mimicry between homologous regions of the bacterial lipopolysaccharides and the peripheral nerve gangliosides may lead to autoimmunity and GBS.

The Miller-Fisher syndrome (MFS), a variant of GBS, is an acute neuropathic disorder characterized by paralysis of the eye muscle (ophthalmoplegia), muscle incoordination, absence of reflexes, and facial weakness. Except for ophthalmoplegia, the clinical features of MFS overlap those of GBS (Smith 1995). Patients with MFS show high levels of antibody against the ganglioside, GQ1b (Chiba *et al.* 1992; Willison *et al.* 1993; Yuki *et al.* 1993). Yuki *et al.* (1994b) demonstrated that the GQ1b epitope was present on lipopolysaccharides from *C. jejuni* strains isolated from patients with MFS. Molecular mimicry between the GQ1b epitope on lipopolysaccharides of *C. jejuni* strains which induce MFS and human ganglioside may function in the pathology of the syndrome.

The toxin, CJT, binds to GM1. The toxin-GM1 complex may alter recognition of self antigens and triggers the production of antibodies directed

against neural antigens (Rees *et al.* 1993). LT and CT bind to GM1, also, but toxigenic *E. coli* or *V. cholerae* do not induce GBS. There may be epitopes on the CJT molecule differing from those on LT or CT which may allow the CJT-GM1 complex to produce antibodies which act against neural tissue. However, there is no indication that CJT-GM1 plays a role in the induction of GBS.

A preliminary report by Fujimoto and Amako (1990) suggests another potential mechanism for *Campylobacter* induction of GBS: anti-bacterial antibodies may cross-react with myelin proteins. They found that antisera from patients with *C. jejuni*-induced GBS reacted with the myelin proteins, P0 and P2. An antibody against a bacterial protein similar to myelin protein may lead to an autoimmune reaction with resultant GBS.

Rees *et al.* (1993) suggest that *C. jejuni* may induce GBS via superantigens. The superantigen could activate large numbers of anti-myelin or anti-neural T-cells with resultant cytokine production. Substantial cytokine production would damage nerve tissue and induce GBS. However, there is no evidence that *Campylobacter* species synthesize superantigens.

What little is known about *C. jejuni* induction of GBS suggest that molecular mimicry between bacterial and neural antigens may be the mechanism for disease. Due to immune cross-reactions, antibodies against *C. jejuni* may cause GBS (and Miller-Fisher syndrome) by reacting with and damaging the host's antigenically related neural tissue.

PERSPECTIVES

There is no doubt that *C. jejuni* is a major pathogen responsible for gastroenteritis, arthritis, Guillain-Barré syndrome and a host of other diseases affecting many parts of the body. Yet it is not clear how the organism exerts its pathogenic effects. A number of putative virulence determinants have been found in *C. jejuni* but, in spite of much effort, there is confusion and inconsistency concerning these virulence determinants and the role that they play in disease. Part of the problem is due to the disparate assays and types of tissue culture cells used to determine the presence of virulence factors and disagreement about how the virulence factors exert their toxic effects.

Another deterrent to good information concerning the virulence and disease determinants in *Campylobacter* is the lack of appropriate animal models. Small animals have been used in attempts to gain some understanding of campylobacter enteritis (reviewed by Fox 1992; Stern and Kazmi 1989; Walker *et al.* 1986). Unfortunately, an appropriate small animal model that mimics human enteric campylobacteriosis is not available. Recently, Babakhani *et al.* (1993) have indicated that the colostrum-deprived newborn piglet, when infected with *C. jejuni*, developed clinical symptoms and histopathological lesions similar to those in infected humans. Further studies using the newborn piglet should indicate if the

model can give much-needed information concerning gastroenteritis. Suitable animal models to study the *Campylobacter*-induced Guillain-Barré syndrome are not yet available. Utilization of certain strains of rats can give some information concerning the pathogenesis of reactive arthritis (Smith *et al.* 1993); however, the arthritis is really not typical of that seen in humans.

Hopefully, this review will focus the attention of research investigators on the problems of *Campylobacter* virulence and pathogenicity. The control and treatment of *C. jejuni*-induced diseases depend on increased and concerted research efforts on the chemical nature and mode of action of the *C. jejuni* virulence factors.

ABBREVIATIONS

AMP = adenosine monophosphate; CFU = colony forming unit; CJT = *Campylobacter jejuni* heat-labile toxin; CLDT = cytolethal distending toxin; CT = *Vibrio cholerae* heat-labile toxin; GBS = Guillain-Barré syndrome; HSP = heat shock protein; GALT = gastrointestinal-associated lymphoid tissue; IFN- γ = gamma interferon; IL = interleukin; LPS = lipopolysaccharide; LT = *Escherichia coli* heat-labile toxin; LTB₄ = leukotriene B₄; OMP = outer membrane protein; PGE₂ = prostaglandin E₂; SI_gA = secretory I_gA; SLT = *E. coli* Shiga-like toxin; SOD = superoxide dismutase; ST = *E. coli* heat-stable toxin; Th1 = T helper cell, type 1; Th2 = T helper cell, type 2.

CELL LINES

A-549 = human lung carcinoma; C-6 = rat glial tumor; Caco-2 = human colon adenocarcinoma; CHO = Chinese hamster ovary; Hela = human cervix epithelioid carcinoma; HEp-2 = human larynx epidermoid carcinoma; INT 407 = human embryonic intestine; MRL-5 = human diploid lung; Vero = African green monkey kidney; Y-1 = mouse adrenal cortex tumor.

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