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REVIEW ARTICLE

Pathogenesis of enteric infection by *Campylobacter*

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Significance and historical context

Campylobacter jejuni and related species are causative agents of human enterocolitis. Being the most common bacterial cause of diarrhoea in many industrialized countries, *C. jejuni* infection is consequently responsible for a major public health and economic burden (Tauxe, 1992; ACMSF, 1993). Nevertheless, the level of public awareness remains limited. After nearly two decades of scientific investigation, much of the biology of campylobacters and the mechanisms by which they cause disease are still relatively poorly understood. More recently, scientific and governmental interest has been rekindled as the extent of the public health problem posed by *Campylobacter* has become clear (Tauxe, 1992; ACMSF, 1993).

The bacteria now recognized as members of the genus *Campylobacter* were first described at the beginning of this century (McFadyean & Stockman, 1913). It is probable that these were what are now known as *C. fetus*, which usually causes foetal and reproductive tract infection and abortion in animals (Mishu *et al.*, 1992). Although originally placed in the genus *Vibrio*, a new genus name of *Campylobacter* was proposed (Sebald & Véron, 1963) to reflect fundamental differences from the vibrios. It was not until the 1970s before they were isolated successfully from the stools of humans with acute enterocolitis (Dekeyser *et al.*, 1972; Butzler *et al.*, 1973; Skirrow, 1977). Their presence in the gut had been suspected before this time (Levy, 1946; King, 1957), but the techniques traditionally used in clinical laboratories were not suitable for the isolation of campylobacters. Although the species names of *C. jejuni* and *C. coli* were derived from an initial association with enteric disease in animals (Jones *et al.*, 1931; Doyle, 1948), they are the most important human pathogens in this genus, with the former usually responsible for the majority of enteric *Campylobacter* infections (80–90%). However, a variety of other species, including *C. upsaliensis*, *C. hyointestinalis* and *C. lari*, also infect humans (Mishu *et al.*, 1992); the true incidence of infection by these other species is still unclear (ACMSF, 1993). Research interest has been directed mainly towards *C. jejuni* and *C. coli* which have different clinical patterns of infection and virulence mechanisms to the other important pathogenic species, *C. fetus*. This review predominantly covers the major enteropathogenic species, *C. jejuni*. The term 'campylobacters' or the genus name refers to both *C. jejuni* and *C. coli*, unless otherwise specified.

Campylobacter biology

In morphological terms campylobacters are slim (1·5–6·0 μm long and 0·2–0·5 μm wide), Gram-negative rods which are spirally curved with tapering ends. The cell usually possesses a polar flagellum at one or both ends of the cell and this, presumably aided by its spiral morphology, imparts a high degree of motility to the cell. *C. jejuni* and *C. coli* are microaerophilic, requiring an O_2 concentration of 3–15% and a CO_2 concentration of 3–5%, and thermophilic, growing best at 42 °C; this latter characteristic may reflect an adaptation to the temperatures found in their normal habitat, the intestine of warm-blooded animals and birds.

Campylobacter spp. have a small genome of approximately 1·6–1·7 Mbp of AT-rich DNA; the GC ratio is approximately 30% (Owen & Leaper, 1981; Chang & Taylor, 1990; Nuijten *et al.*, 1990; Taylor *et al.*, 1992). An exception appears to be the *C. upsaliensis* genome which has a size of 2 Mbp. Its size difference may arise from a large duplication of chromosomal sequences (Bourke *et al.*, 1995). The small size of the genome in campylobacters is perhaps reflected in their requirement for complex media for growth and their inability to ferment carbohydrates and to degrade complex substances (Griffiths & Park, 1990). Extrachromosomal elements in the form of both conjugative plasmids and bacteriophages have been reported in *Campylobacter* spp. (Taylor, D. E., 1992).

The number of cloned genes from *Campylobacter* spp. is rather low in comparison to many other enteric

pathogens (Taylor, D. E., 1992). This is illustrated by the number of gene sequences deposited in GenBank (approximately 50 different entries, excluding rRNA and uncharacterized sequences), a perhaps surprising situation considering the importance of this pathogen. It is generally accepted that genes from *Campylobacter* spp. are often difficult to clone and subsequently analyse; this is thought to be due to several possible factors, including the high AT content resulting in promoter-like sequences, lack of expression and of required accessory factors or both in *Escherichia coli*, and different patterns of methylation or codon usage. A few genetic tools are now available (Taylor, D. E., 1992). A series of shuttle vectors have been constructed that contain both *E. coli* and *C. coli* origins of replication and *Campylobacter*-derived antibiotic resistance genes (Labigne-Roussel *et al.*, 1987; Wang & Taylor, 1990a, b; Purdy & Park, 1993; Yao *et al.*, 1993). The identification of genes and particularly of those unique to the campylobacters is even more difficult due to the fact that transposons of either Gram-negative or Gram-positive origin or 'recombinant' transposons (Ketley, 1995) have not been found to transpose in campylobacters. Genetic transformation, albeit at a low frequency, can be carried out by electroporation (Miller *et al.*, 1988). Broad-host-range plasmids, such as IncP-based systems, can be transferred by conjugation into the organism, but they are not maintained unless they contain an origin of replication from a *Campylobacter* spp. plasmid. Thus, without this origin a plasmid becomes a useful suicide vector. Campylobacters are naturally transformable (Wang & Taylor, 1990b; Taylor, D. E., 1992) and preliminary evidence suggests that this uptake requires a specific, but as yet unknown, sequence for uptake in a situation akin to that seen with naturally competent *Haemophilus* and *Neisseria* spp. It has been speculated that, like *N. gonorrhoeae*, the SOS response system of *C. jejuni* may have evolved to increase the chance of recombining exogenous DNA whilst avoiding the initiation of the SOS response (Ketley, 1995).

Clinical aspects

The clinical spectrum of enteric disease due to *C. jejuni* and *C. coli* ranges from a severe inflammatory diarrhoea to a generally mild, non-inflammatory, watery diarrhoea (reviewed by Butzler & Skirrow, 1979; Walker *et al.*, 1986). The former is the most common clinical presentation of patients from industrialized nations, whilst the latter is the usual pattern seen in developing nations.

C. jejuni and *C. coli* infection that results in inflammatory disease usually begins in about half the patients with a prodrome of characteristic acute abdominal pain, often with fever and general malaise. The symptoms then progress to include a profuse diarrhoea that becomes watery. The incubation period prior to the appearance of symptoms usually ranges from 1 to 7 d, although the source and exact timing of infection is often difficult to establish. The diarrhoeic stools often contain fresh blood, mucus and an inflammatory exudate with leucocytes. Bacteraemia, especially in the early stages of infection, is probably more common than suspected, but is rarely reported perhaps because of infrequent sampling and inappropriate culture conditions. The acute diarrhoea commonly lasts for 2–3 d and abdominal pain and discomfort persists during, and sometimes after, diarrhoea has stopped. Sigmoidoscopy usually reveals mucosal changes, ranging from oedema and hyperaemia with petechial haemorrhages to mucosal friability. Inflammation of some areas of the ileum and jejunum with mesenteric adenitis is usually evident. Sometimes relapses occur but they are usually less severe than the first attack. Campylobacters may be isolated from patients for as long as several weeks after the clinical symptoms have finished. Although infection can result in a severe illness lasting more than a week, it is usually self-limiting and complications are uncommon (Skirrow & Blaser, 1992). Perhaps the most notable complication is Guillain-Barré syndrome (Rhodes & Tattersfield, 1982; Kuroki *et al.*, 1991) which has a significant association with serological evidence of recent previous infection with *Campylobacter* spp. (see below) (Fujimoto *et al.*, 1992; Kuroki *et al.*, 1993; Mishu & Blaser, 1993).

At the other end of the spectrum, non-inflammatory, watery diarrhoea is a presentation occasionally seen in industrialized nations but seemingly the most common clinical pattern of disease seen in developing countries. Sigmoidoscopy does not reveal any significant mucosal changes and the watery diarrhoea does not contain any blood, mucus or leucocytes.

Transmission and epidemiology

Campylobacter enteritis is considered to be a food-borne disease rather than food poisoning, with infection often being derived from a range of foods and also water-based environmental sources (reviewed in Griffiths & Park, 1990; ACMSF, 1993). Campylobacters are part of the natural intestinal flora of a wide range of domestic and wild birds and animals. Although the identification of the origin of a particular infection is rarely made, transmission is probably most commonly via the surface of meat as a result of faecal contamination during slaughtering. Other sources include untreated water, untreated milk and sewage contamination. Pet contact, particularly with puppies with diarrhoea, is also a probable source. Robinson's own experience (Robinson, 1981) and volunteer studies carried out at the Center for Vaccine Development, Baltimore, MD, USA (Black *et al.*, 1988) have shown that the infective dose that results in symptoms can be as low as 5–800 organisms with the attack rate correlating with increasing dose.

Under certain conditions, for example in the stationary phase or on exposure to atmospheric oxygen, campylobacters become spherical or coccoid in shape. This shape change has been associated with a transition from a viable culturable form to a viable non-culturable

(VNC) state (Rollins & Colwell, 1986). It has been suggested that this 'dormant'-like state is an adaptation to survival in adverse environments. Consequently, VNC campylobacters and their role in transmission has stimulated a great deal of interest. There is evidence that VNC campylobacters are infectious in neonatal mice (Jones *et al.*, 1991), but in a perhaps more relevant chicken model, the evidence is more contradictory (Medema *et al.*, 1992; Stern *et al.*, 1994). Such investigations are very difficult to perform and interpret, for example, not all coccoid cells may progress to a VNC state or VNC 'development' may advance through several stages in a coccoid cell, and a 'coccoid culture' may contain a small number of fully viable spiral forms.

It is clear that *C. jejuni* and *C. coli* enteritis is a major public health problem in industrialized countries (Tauxe, 1992; ACMSF, 1993) with the number of reported intestinal infections being significantly greater than those due to any other enteric pathogen, including the more newsworthy *Salmonella* (ACMSF, 1993). The incidence has also clearly risen dramatically and this probably reflects a change in our eating patterns, for example, an increase in the popularity of chicken. The peak incidence rate is in young adults and young children (Tauxe, 1992). However, when considered as a proportion of faecal samples within each age group, the number of stools containing campylobacters is higher in young adults (Butzler & Skirrow, 1979; Tauxe, 1992). Below the age of 45 there is an as yet unexplained, but consistently higher incidence of infection in males compared to females. Most cases appear to be sporadic and show a consistent seasonality (Tauxe, 1992; ACMSF, 1993). The incidence of infection as derived from laboratory isolations is almost certainly a significant underestimate of the true rate of the disease in the general population (Tauxe, 1992). A limited study in a general practice population (Kendall & Tanner, 1982) calculated an annual incidence of 1.1% and is close to an estimate of 1.0% made for the US population (Tauxe, 1992).

Campylobacter infection in developing countries appears to have different clinical and epidemiological characteristics to that described above for industrialized nations (Taylor, D. N., 1992). Disease is usually restricted to children with no apparent peak in adults, no strong pattern of seasonality and a higher incidence of infection complicated by a higher rate of asymptomatic carriage. Many of these differences are probably due to higher rates of exposure and infection early in life, resulting in a different pattern of immunity. Although strain differences have been correlated with clinical symptoms (Ruiz-Palacios *et al.*, 1983, 1985, 1992; Everest *et al.*, 1992), there is little evidence for the presence of different types of strains in developing countries to those found in industrialized countries (Taylor, D. N., 1992). Indeed, the importance of *C. jejuni* as a cause of travellers' diarrhoea (Taylor, D. N., 1992) has led to the observation that the spectrum of illness in travellers is similar to that described in their country of origin.

Pathogenesis

Since the association of *Campylobacter* spp. with human enteric disease, a reasonable understanding of the general clinical, microbiological and epidemiological aspects of infection has been achieved. However, the molecular mechanisms involved in pathogenesis are still rather poorly understood. The factors or virulence determinants required to establish an infection and to generate pathological changes by pathogens are multifactorial in nature and certainly campylobacters are no exception to this. Few of the determinants involved in *Campylobacter* pathogenesis are known or have a proven role. These virulence determinants are generally not well characterized and some are rather controversial.

C. jejuni and *C. coli* are food-borne pathogens, and therefore factors involved in survival and resistance to physiological stresses encountered in food and water are important for successful transmission and infection. Thus, the possibility that campylobacters can enter a VNC state may be of great significance (Rollins & Colwell, 1986; Jones *et al.*, 1991). In association with food or water, campylobacters enter the host intestine via the stomach acid barrier and colonize the distal ileum and colon. Following colonization of the mucus and adhesion to intestinal cell surfaces, campylobacters perturb the normal absorptive capacity of the intestine by damaging epithelial cell function either directly, by cell invasion or the production of toxin(s), or indirectly, following the initiation of an inflammatory response. As these possible mechanisms are not mutually exclusive, any combination may have a role depending on the host status and attributes of the infecting strain.

Chemotaxis and motility

Effective colonization requires chemotaxis. Thus, campylobacters have mechanisms to detect chemical gradients and linked motility functions that enable the cell to move up or down the gradient. The importance of chemotaxis has been demonstrated by testing chemically mutagenized, non-chemotactic mutants in an animal model (Takata *et al.*, 1992); such mutants failed to colonize the suckling mouse intestine. *In vitro* studies (Hugdahl *et al.*, 1988) have revealed various chemoattractants, including mucin, L-serine and L-fucose, and several bile acids have chemorepellant effects. Little is known about the molecular basis for campylobacter chemotaxis. However, this situation is likely to change as one of the regulatory components, *cheY*, has been identified (J. E. Marchant, J. Henderson, B. Wren & J. M. Ketley, unpublished data). Mutation of this gene does not affect motility or invasion but does result in a loss of chemotaxis *in vitro*. The description of the effect of this mutation on colonization is awaited.

Motility of *Campylobacter* spp. necessitates the production of the flagellum, the best characterized virulence determinant of campylobacters. A combination of the flagellum and cell shape are believed to give campylobacters an unusually high level of motility in viscous

environments. Observations suggest that, at high viscosity, cell shape and flagellar conformation or both may change and this perhaps results in campylobacters remaining motile with longer path lengths (Ferrero & Lee, 1988). This behaviour has relevance to the penetration of the mucus that overlays the intestinal epithelium. It has been speculated (Lee *et al.*, 1986) that adhesion to host cells is not actually necessary as the bacterium is able to remain in the intestine by successfully colonizing the mucus.

Early studies with genetically undefined mutants indicated that the flagellum was needed for adhesion and for colonization in a range of animals (Caldwell *et al.*, 1985; Morooka *et al.*, 1985; Newell *et al.*, 1985; McSweegan & Walker, 1986; Aguero-Rosenfeld *et al.*, 1990). The unsheathed flagellum exhibits phase (Caldwell *et al.*, 1985) and antigenic (Harris *et al.*, 1987) variation. The flagellin gene has been cloned and extensively characterized mainly by two groups, one in North America (*C. coli*) and one in Utrecht (*C. jejuni*) (reviewed by Guerry *et al.*, 1992 and Nuijten *et al.*, 1992). The locus has been mapped to the chromosome and, in the majority of isolates, contains two adjacent genes, *flaA* and *flaB*. The two genes are of equal size (approximately 1.7 kbp) and in *C. jejuni* encode proteins with predicted molecular masses of 59538 and 59909, respectively. The *flaA* and *flaB* genes share a high level of base sequence identity (> 93%) with most heterogeneity being found in the 5′ and 3′ regions and in a small central region of the genes. A comparison of the *C. coli* and *C. jejuni flaA* gene products reveals an overall amino acid sequence similarity of 87%, with most variability being found in the central regions. In addition, *Campylobacter* FlaA shows a high degree of amino acid sequence conservation with other bacterial flagellins including those from *Salmonella typhimurium* and *Bacillus subtilis*. The *flaA* and *flaB* genes are independently transcribed and are regulated by different types of promoter (see below). Under those conditions tested so far, the *flaA* is expressed at higher levels than *flaB*. Defined mutagenesis of each gene has shown that in the absence of FlaA, the *flaB* gene encodes a flagellin protein that forms a short, truncated non-functional flagellum. However, a role in normal flagallar function is supported by the observation that FlaB is incorporated into the whole filament (albeit in small amounts) and that although a *flaA*$^+$ *flaB* mutant produces a normal length flagellum, it demonstrates slightly decreased motility in comparison to the wild-type strain. A molecular genetic approach has also led to the first genetic characterization of a role in virulence for a particular *Campylobacter* gene. Defined mutants have shown that *flaA* and hence the flagellum is essential for colonization (Nachamkin *et al.*, 1993; Wassenaar *et al.*, 1993).

Genetic analysis of the *fla* genes from some antigenic variants indicated that the amino acid sequence differences in the flagellins were not able to account for the variation observed. Peptide sequencing studies indicated that the flagellin is post-translationally modified and it was postulated that this is due to phosphorylation of serine residues (Logan *et al.*, 1989). Antigenic variation arising from post-translational modification of the flagellin protein by glycosylation has also been described (Constantinidou *et al.*, 1996; Doig *et al.*, 1996a). Although modification of flagellins is not unusual, sialylation has only been described in campylobacters (Guerry *et al.*, 1996). A search for the *fla*-linked loci involved in the modification identified two genes, *ptmA*, which has similarity to genes encoding alcohol dehydrogenases and *ptmB*, with similarity to cytidine-5′-monophospho-*N*-acetylneuraminic acid (CMP-NANA) synthase genes (Guerry *et al.*, 1996). Mutations in either of these genes affected glycosyl modification of the flagellin but the nature of the changes at the molecular level have not yet been determined (Doig *et al.*, 1996a). In a rabbit model (Guerry *et al.*, 1996), a mutation in *ptmA* did not affect motility or intestinal colonization but did reduce the ability to elicit protection against subsequent challenge with heterologous strains of the same Lior serotype compared to the parental wild-type strain. Thus, surface-exposed post-translational modifications of flagellin may play a role in the protective immune response.

Adhesion

Although over the years several candidates have emerged as putative *Campylobacter* adhesins, the role of these determinants is still not clear. *C. jejuni* and *C. coli* are certainly able to adhere to tissue culture cells without subsequent invasion (Everest *et al.*, 1992). One would assume that adhesion to the epithelial cell surface is necessary for subsequent invasion of the cell (see below), although adhesion and invasion may not be needed for intestinal colonization *per se* (Lee *et al.*, 1986). A variety of outer-membrane proteins (DeMelo & Pechere, 1990; Fauchere *et al.*, 1992; Kervella *et al.*, 1993) and LPS (McSweegan & Walker, 1986) have been described that bind to eukaryotic cells. One protein, PEB1, binds to cells and the gene coding for this potential adhesin has been cloned (Pei & Blaser, 1993). The predicted PEB1 protein has amino acid sequence similarity to amino acid transporter systems of other bacteria. Colonization studies have indicated that the binding of PEB1 to cells may be an artifact as the protein is not required for the short-term colonization of chicks (Meinersmann *et al.*, 1996). Similarly, the gene encoding another outer-membrane protein, PEB4, is now known to have DNA sequence similarity to a gene encoding a part of a protein export system (Burucoa *et al.*, 1995) and therefore is perhaps also unlikely to be an adhesin.

Until recently the production of fimbriae by *Campylobacter* had not been observed, However, Doig *et al.* (1996b) described some exciting work demonstrating the production of fimbriae. Production of fimbriae, which is enhanced by the presence of bile salts in the media, was shown by electron microscopy and conferred an aggregative phenotype in bile-salt-containing media. The fimbriae were observed to have a

width of 4–7 nm. Although the gene encoding the fimbrial subunit has not been described, the role of the fimbriae was investigated using a mutant constructed in a prepilin peptidase gene (*pspA*) which resulted in the loss of fimbrial production. The non-fimbriated mutant was still able to adhere to and invade INT407 cells and colonize ferrets, but with ameliorated disease symptoms. This provides strong evidence of some role in virulence for this structure.

There has also been some investigation of a role for the flagellum in adhesion. However, there is little direct evidence which is able to separate the involvement of flagellar components and motility. Initial studies showed that the presence of exogenous purified flagella did not block adhesion (McSweegan & Walker, 1986; Wassenaar *et al.*, 1991). In addition, strains with genetically defined mutations in either one or both of the *flaA* and *flaB* genes appear to still adhere to host cells after centrifugation (Grant *et al.*, 1993). Yao *et al.* (1994) showed (1) that a *flaA flaB*$^+$ defined mutant showed a 50-fold reduction in adhesion and (2) that a mutation in a gene called *pflA* (paralysed flagella) results in immobilized full-length flagella, the mutant exhibiting a twofold reduced adherence and an inability to invade enterocyte-like cells. Therefore, FlaA (or a component of the flagellum that requires the presence of FlaA) can adhere to host cells but, significantly, this work suggests that FlaA-mediated adhesion may be different to the adhesive process that results in (induces?) invasion (see below).

Iron acquisition

In order to colonize the intestine, campylobacters must be able to compete with the resident flora and to avoid non-specific host defences. Iron is an essential element for all living organisms and pathogenic bacteria obtain iron throughout the infection process. Campylobacters have not been shown to produce siderophores, but they are able to use exogenous siderophores (Field *et al.*, 1986). They possess a transport system, encoded by the *ceu* operon (*ceuBCDE*; campylobacter enterochelin uptake) that might scavenge siderophores in the intestinal tract (Richardson & Park, 1995). However, chick colonization studies with a *ceuE* mutant showed that this system is not necessary for chick colonization (Crawthraw *et al.*, 1996). This observation suggests that campylobacters possess an additional iron uptake system(s) that complements for the loss of the Ceu uptake system. Alternatively, as the chick model involves colonization but not tissue invasion, this particular uptake system may only play a role during tissue invasion.

Iron storage systems are also used by micro-organisms to allow growth in low-iron environments. In addition, such storage systems help to protect the bacterium against iron overload which may result in iron-catalysed oxidative damage to cellular components. The iron storage protein ferritin is produced by *C. jejuni* (Wai *et al.*, 1995) and a *C. jejuni* mutant in the gene encoding ferritin, *cft* (Wai *et al.*, 1996), was found to grow poorly in iron-deficient media and was sensitive to oxidative stress. Thus, production of ferritin may facilitate the colonization of the host by *C. jejuni* and may also help protect the bacterium in conditions of high O_2 levels.

Invasion

There is evidence supporting a role for host cell invasion in campylobacter-mediated disease. Inflammation and bacteraemia strongly suggest that cellular invasion is an important pathogenic mechanism. Although evidence of epithelial cell invasion *in vivo* is sparse, host cell invasion has been observed in both experimentally infected infant macaque monkeys (Russell *et al.*, 1993) and in the colon of patients (van Spreeuwel *et al.*, 1985); unfortunately, the latter work presents no direct evidence. In addition, *C. jejuni* readily invades primary swine intestinal cells (Babakhani & Joens, 1993) and the ability to invade tissue culture cells is well-established (Fauchere *et al.*, 1986; DeMelo *et al.*, 1989; Konkel & Joens, 1989; DeMelo & Pechere, 1990; Everest *et al.*, 1992).

Interactions with eukaryotic cells. The ability to invade and the degree to which campylobacters invade appears to be strain-dependent (Konkel & Joens, 1989; Everest *et al.*, 1992; Konkel *et al.*, 1992a; Oelschlaeger *et al.*, 1993). Clinical isolates tend to be more invasive (Konkel & Joens, 1989) and extensive *in vitro* passage reduces the invasiveness of isolates (Konkel *et al.*, 1990; Babakhani & Joens, 1993). As motility is important for invasion (Wassenaar *et al.*, 1991; Grant *et al.*, 1993), clearly the appearance of aflagellate, non-motile variants *in vitro* could contribute to this effect. Everest *et al.* (1992) found a significant, but not complete, correlation between the ability to invade Caco-2 cells and the presence of symptoms of colitis in patients from which the isolates were obtained. Notably, some strains classified as 'non-inflammatory' were able to invade cells. In contrast, using Hep-2 cells no correlation was found between invasiveness and the type of symptoms observed (Tay *et al.*, 1996). Thus, although patient symptoms may be a reflection of strain differences in the ability to invade, host factors such as immune status are also important.

Like several other invasive pathogens, campylobacters are not positive in the Séreny test (Manninen *et al.*, 1982). They show variability in the ability to invade a range of tissue culture cell lines (Oelschlaeger *et al.*, 1993; Konkel *et al.*, 1992d) and invasion of cultured epithelial cells by clinical isolates appears to be more efficient when cells of human origin are used, whereas isolates adhere efficiently to cells of both non-human and human origin (Konkel *et al.*, 1992d). When binding to host cells campylobacters were observed to associate preferentially with intercellular junctions (Konkel *et al.*, 1992c; Oelschlaeger *et al.*, 1993), which may be significant given the ability of campylobacters to transcytose polarized monolayers (see below). Cell attachment appears to be promoted by centrifugation and involves close binding with the host cell membrane

(DeMelo *et al.*, 1989; Konkel *et al.*, 1992a) without any evidence of fimbriae. At least in HEp-2 cells, attachment may be patchy (Konkel *et al.*, 1992d) and thus, perhaps, similar to localized adherence in enteropathogenic *E. coli* (EPEC) (Knutton *et al.*, 1989). The accumulation of a dense fibrillar material in the host cell in close apposition to the attached bacterial cell has been noted *in vivo* (Russell *et al.*, 1993) and in tissue culture cells (DeMelo *et al.*, 1989; Konkel *et al.*, 1992a). Labelling with fluorescein-conjugated phalloidin indicated that condensed actin co-localizes with attached (and therefore invading?) campylobacters (Konkel *et al.*, 1992a). However, other work directly contradicts this observation (Konkel *et al.*, 1992d; P. H. Everest, unpublished data). New proteins are induced in campylobacters on contact with both viable and non-viable host cells with a subset of these proteins being induced by released host cell components (Konkel & Cieplak, 1992). Non-viable campylobacters are still able to attach to host cells indicating that *de novo* protein synthesis is not required for the bacterium to bind to the eukaryotic cell (Konkel & Cieplak, 1992). In contrast, invasion does require bacterial protein synthesis, whereas host protein synthesis is not essential (Konkel & Cieplak, 1992; Oelschlaeger *et al.*, 1993). As a small proportion of campylobacters still attach and invade despite inhibition of bacterial protein synthesis, there may be a subpopulation that constitutively expresses the necessary factors; this is supported by the fact that campylobacters start to invade host cells within a very short time period. Interestingly, unidentified proteins of the same size (43 and 45 kDa) as some of those observed during association with tissue culture cells are specifically induced in the rabbit ileum (Panigrahi *et al.*, 1992)

Bacterial factors involved in invasion. It is reasonable to conclude that, although there is as yet no clear idea as to which host cell mechanism(s) is involved when campylobacters invade the cell, there is good evidence that they are probably capable of invasion *in vivo*. So what bacterial determinants are important in stimulating endocytic entry into the host cell? Until recently there were data which pointed towards an involvement of the flagellum but the exact nature of this involvement was unclear. More recent work with the *pflA* mutation goes some way towards answering the question. Mutants with a defined insertion in the *flaA* gene were found to demonstrate significantly reduced levels of invasion of tissue culture cells which could only be slightly improved by centrifugation of the mutant onto the cell monolayers (Wassenaar *et al.*, 1991). Thus, it seemed that, although FlaA may not be involved in adhesion, the subunit itself, or a functional flagellum was needed for invasion. The demonstration that the *pflA* mutant with a paralysed but full-length flagellum (containing FlaA) shows slightly reduced adhesion and greatly reduced invasion of enterocyte-like cells (Yao *et al.*, 1994) would indicate that campylobacters invade by a process requiring active motility and an unidentified adhesin whose interaction with the host ligand results in uptake. Furthermore, the invasion-related aspect of motility cannot be effectively reproduced by centrifugation and interestingly the FlaA-receptor interaction does not result in the initiation of endocytic uptake. It is notable that, in at least one *C. jejuni* strain, increased viscosity leading to a greater proportion of cells with non-tumbling motility increases the level of invasion (Szymanski *et al.*, 1995).

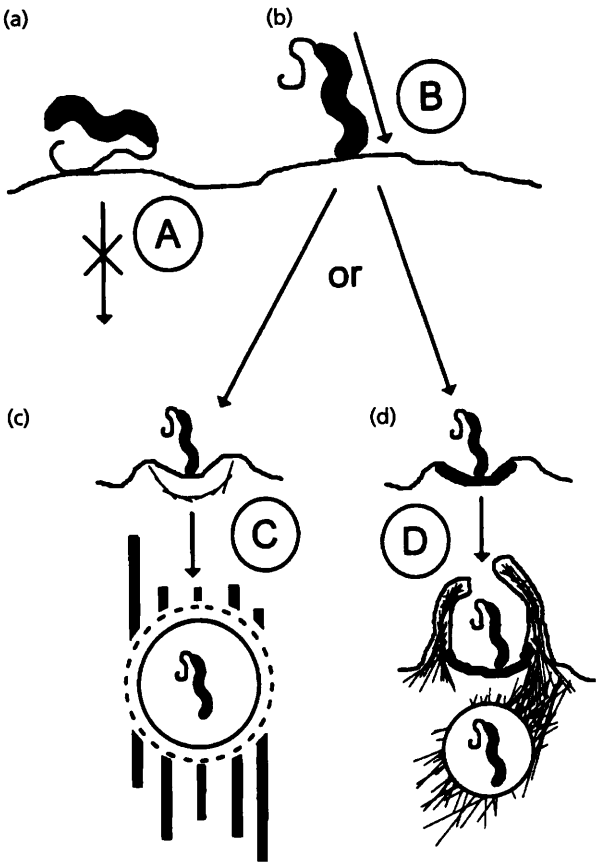

Fig. 1. Diagrammatic overview of proposed mechanisms of host cell invasion by campylobacters (see text for details). (a) Adhesion by secondary adhesin on flagellum does not stimulate uptake. (b) Motility and binding via primary adhesin leads to invasion by pathway C, pathway D or both. (c) Endocytic uptake via coated pits and association of vacuole with microtubules. (d) Interaction with molecules associated with caveolae leads to transduction of signal resulting in endocytosis involving actin filaments.

Host factors involved in entry. Although several groups have investigated the mechanisms by which *Campylobacter* invade host cells, no consensus has yet been established (Fig. 1). Like other well characterized invasive pathogens, including *Salmonella*, *Shigella* and *Listeria*, *C. jejuni* may enter host cells via a microfilament-dependent process (DeMelo *et al.*, 1989; Konkel & Joens, 1989; Konkel *et al.*, 1992a) with no evidence of microtubule involvement (Konkel *et al.*, 1992a). Such an entry mechanism might correlate with microfilament accumulation (DeMelo *et al.*, 1989; Konkel *et al.*, 1992a; Russell *et al.*, 1993). In contrast, other studies (Oelschlaeger *et al.*, 1993; Russell & Blake, 1994) have found that microfilament inhibitors did not

affect invasion, thereby supporting the involvement of a microtubule-dependent pathway (Oelschlaeger *et al.*, 1993). Oelschlaeger *et al.* (1993) also noted that invasion was inhibited by antagonists of coated pit formation and uptake and suggested that *C. jejuni* may enter host cells via coated-pit-associated receptors with the resultant endosome interacting with microtubules; such a pathway would be unusual for an invasive bacterium. Wooldridge *et al.* (1996) have described the involvement of a novel host signal transduction pathway in the entry of campylobacters into differentiated enterocyte-like cells. Disruption of the receptor-mediated endocytosis pathway involving caveolae (Schnitzer *et al.*, 1994) with filipin III abolishes endocytosis of campylobacters into Caco-2 cells but not adhesion. Inhibition of protein phosphorylation, tyrosine protein kinases and $G\alpha_s$ heterotrimeric G-proteins also inhibited entry but not binding. These results support a hypothesis (Wooldridge *et al.*, 1996) that campylobacters stimulate a receptor that co-localizes to caveolae, resulting in a signal transduction event across the membrane via a G-protein or tyrosine phosphorylation which in turn leads to the activation of PI_3 kinase substrates that stimulate Rac-mediated membrane ruffling and subsequent phagocytosis.

Epithelial translocation. Campylobacters have been observed to translocate across an epithelial cell barrier (Everest *et al.*, 1992; Konkel *et al.*, 1992c; Grant *et al.*, 1993). Translocation was first observed in *Salmonella* (Finlay *et al.*, 1988). Clearly, this behaviour is of potential pathophysiological significance. Translocated bacteria can be observed below the cell monolayer less than 1 h after inoculation above the cells (Konkel *et al.*, 1992c) and continue to translocate for at least 6 h, although experiments with pulse-labelled bacteria suggest that the majority of campylobacters that are destined to transcytose leave the apical compartment by 40 min post-inoculation (A. Brás-Goldberg & J. M. Ketley, unpublished observations).

While the ability to cross the cell monolayer may be due to transcytosis (i.e. translocation via a cytoplasmic pathway) with the exit to the basolateral surface following initial cell invasion, there is evidence that campylobacters may also cross the monolayer via a paracellular route. Electron microscopic observations (Konkel *et al.*, 1992c; Oelschlaeger *et al.*, 1993) indicate that campylobacters pass between cells and some isolates appear to transcytose without invasion (Everest *et al.*, 1992). These strains would be predicted to take a paracellular route between tight junctions. Paracellular translocation does not appear to result in a large scale loss of tight junction integrity as, in contrast to *Salmonella* (Finlay *et al.*, 1988), transmonolayer electrical resistance does not significantly change during translocation (A. Brás-Goldberg & J. M. Ketley unpublished data; Konkel *et al.*, 1992c).

Campylobacters may, therefore, cross the intestinal epithelium by translocation or epithelial cell invasion followed by cell lysis. An additional epithelial translocation pathway would be via M cells. Such a route has been proposed for several enteric pathogens including *Shigella* (Sansonetti *et al.*, 1991). Interaction with and translocation via M cells by campylobacters has been observed in rabbits (Walker *et al.*, 1988, 1992), but not in macaque monkeys (Russell *et al.*, 1993).

Intracellular survival. Several studies have addressed the fate of campylobacters after entering the host cell. In Hep-2 cells a strong lysosomal response was observed following invasion by campylobacters (DeMelo *et al.*, 1989). Endosome–lysosome fusion was observed with acid phosphatase activity evident at the surface of the internalized bacteria, most of which then showed signs of degradation by changing into a coccoid form. The gentamicin protection assay indicated that bacterial viability declined after 6 h, with few remaining viable after 36 h. Campylobacters were found in INT407 cells up to 96 h post-inoculation if gentamicin treatment was reduced in steps; electron microscopy confirmed the presence of viable intracellular campylobacters (Konkel *et al.*, 1992a). Removal of the antibiotic resulted in a cytopathic effect after 48 h which was coincident with the increase in extracellular bacteria. The bacterial and host factors important in determining the fate of intracellular campylobacters are not understood. Endosome acidification may not play a role as inhibition of acidification with monensin did not affect intracellular survival (Oelschlaeger *et al.*, 1993). Reduction of short-term intracellular survival in INT407 cells (Pesci *et al.*, 1994) of a *C. jejuni* strain mutated in the superoxide dismutase gene, *sodB* (Pesci *et al.*, 1994; Purdy & Park, 1994), when compared to the isogenic parent, indicates that reactive oxygen species influence intracellular survival. The identification of the gene encoding catalase, *katA* (Grant & Park, 1995), indicates that *C. jejuni* may have other determinants that form part of a defence system against oxidative stress. Interestingly, recent data has indicated that oxidative stress can increase the invasive potential of *C. jejuni* (Harvey *et al.*, 1996). Campylobacters may not necessarily remain within membrane-bound vacuoles in the cytoplasm of tissue culture cells (Konkel *et al.*, 1992a), although some reports did not observe cytoplasmic bacteria (Konkel *et al.*, 1992c; Oelschlaeger *et al.*, 1993). *In vivo*, free *C. jejuni* within the cytoplasm were observed and were associated with a cytopathic effect (Russell *et al.*, 1993).

Interactions with leucocytes

Intestinal infection with campylobacters is often associated with an inflammatory response where polymorphonuclear leucocytes (PMNLs) and monocytes infiltrate the intestinal epithelium (Duffy *et al.*, 1980; Ruiz-Palacios *et al.*, 1981; Black *et al.*, 1988; Russell *et al.*, 1989). Given this inflammatory response, the interaction between campylobacters and professional phagocytes is of potential importance. In addition, in order for translocation via M cells to be of pathological significance, campylobacters must resist killing by

monocytes following entry into underlying lymphoid tissue. Antibody and complement-opsonized *C. jejuni* are readily phagocytosed by PMNLs, induce an oxidative burst and are efficiently killed. Without opsonization, phagocytosis and killing is less efficient and strain-dependent (Pennie *et al.*, 1986; Walan *et al.*, 1992; Autenrieth *et al.*, 1995). In contrast to phagocytosis by PMNLs, opsonization by antibody or complement is not required for efficient uptake by macrophages (Field *et al.*, 1991). *C. jejuni* can survive within macrophages for up to 6–7 d (Kiehlbauch *et al.*, 1985) despite a complete conversion of intracellular bacteria to coccoid forms. Strain and species (*C. jejuni* vs *C. coli*) differences have been observed with macrophage killing of campylobacters (Kiehlbauch *et al.*, 1985; Banfi *et al.*, 1986) but too few have been tested to allow general conclusions to be drawn. The depletion of macrophages, but not complement, increased mouse mortality following injection with a clinical isolate of *C. jejuni*, suggesting a role for macrophages in defence against *C. jejuni* infection.

A mutation in the *htrA* stress response gene of *S. typhimurium* confers sensitivity to oxidative stress and thus oxidative killing within macrophages (Johnson *et al.*, 1991). Indeed this *htrA* mutant is attenuated *in vivo* (Chatfield *et al.*, 1992). The *C. jejuni htrA* gene has been identified and the chromosomal locus mutated (J. Henderson, A. Wood, B. Wren & J. M. Ketley, unpublished data). The mutant is not sensitive to oxidative stress and invades and survives intracellularly in Caco-2 cells as well as the wild-type parent.

Toxins

Although tissue invasion could be solely responsible for the clinical picture resulting from infection, toxins may also contribute to the disease process. The production of toxins is another unclear area. Campylobacters reportedly produce a variety of toxic activities including a cholera-like toxin (CLT) and several cytotoxins. The work concerning these toxins has been reviewed recently elsewhere (Wassenaar, 1997). Enterotoxin production by *C. jejuni* was first described in 1983 (Ruiz-Palacios *et al.*, 1983). The evidence for a role and even the production of the enterotoxin is not convincing. In support (reviewed by Wassenaar, 1997), there is a cell product that, like cholera toxin, elongates CHO cells, is detected with a GM1-based ELISA and produces fluid accumulation in intestinal loops. In addition, CLT crossreacts immunologically with *E. coli* labile toxin (LT) and *Vibrio cholerae* cholera toxin (CT), has been partially purified and specific antisera raised to it. CLT production was also reported to correlate with the watery diarrhoea observed in patients in developing countries (reviewed by Guerrant *et al.*, 1992), but CLT-positive strains have been isolated from non-symptomatic carriers (Mathan *et al.*, 1984; Belbouri & Mégraud, 1988). In the rat ileum enterotoxigenic *C. jejuni* has been observed to stimulate a Ca^{2+}-dependant secretion that involved the activation of protein kinase C in the absence of invasion or mucosal damage (Kanwar *et al.*, 1995). In contrast, even using similar strains, production of CLT and an antibody response could not be demonstrated by other workers (Mathan *et al.*, 1984; Perez-Perez *et al.*, 1989, 1992; Konkel *et al.*, 1992b; McFarland & Neill, 1992; Ruiz-Palacios *et al.*, 1992). When found, CLT is produced more frequently by *C. jejuni* than *C. coli*. It is not yet clear if CLT is an artifact arising from a non-toxic protein containing similar epitopes to cholera toxin or if the strains in which it is expressed and the conditions of expression are very restricted.

A comprehensive and conclusive description of the cytotoxin(s) has yet to emerge, but several different activities have been observed, including cytotoxins with different patterns of cell specificity, a cytolethal distending toxin (CLDT), a shiga-like toxin, and a haemolysin(s) (Wassenaar, 1997). CLDT has been perhaps the most widely reported cytotoxin and recently genes with similarity to those encoding *E. coli* CLDT have been isolated from *C. jejuni* (Pickett *et al.*, 1996). It is not yet clear how many distinct cytotoxic moieties are produced and certainly the gene(s) that might encode the other cytotoxin(s) has not yet been identified. Nevertheless, the clinical presentation, which often involves intestinal tissue damage and an associated inflammatory response, is not inconsistent with the action of cytotoxin(s). As with other bacterial pathogens, it is possible that the nature of toxin production by *Campylobacter* spp. is complex and involves a range of different toxins expressed under a variety of, as yet, unknown conditions.

LPS

LPS is a virulence determinant in many species of Gram-negative bacteria. It contributes to several aspects of the pathogenic process, including serum resistance, resistance to phagocytic killing and cell toxicity. Sialylation of LPS plays a role in virulence in some pathogens (for example in *Neisseria* sp. and *Haemophilus* sp., Demarco de Hormaeche *et al.*, 1991; Moxon & Maskell, 1992) by enhancing serum resistance. Much progress has been made on the biochemical characterization of *C. jejuni* LPS (Conrad & Galanos, 1990; Moran *et al.*, 1991; Aspinall *et al.*, 1992a, b, 1993a, b, c; Mills *et al.*, 1992). It either consists of a low-molecular-mass fraction similar to *Neisseria* and *Haemophilus* LPS or, in addition, it can also contain a high-molecular-mass fraction (Mills *et al.*, 1992). Until recently, the genetic basis for the production and variation in *C. jejuni* LPS was completely unknown. However, a region of the chromosome containing a range of genes likely to have a role in LPS biosynthesis has been isolated (B. Fry & B. A. M. van der Zeijst, unpublished data; A. Wood & J. M. Ketley, unpublished data) and inter-strain comparisons have revealed RFLPs and differing gene content. The role of LPS in the virulence of other Gram-negative pathogens provides a basis for the search for such role in *C. jejuni*. A single report (McSweegan &

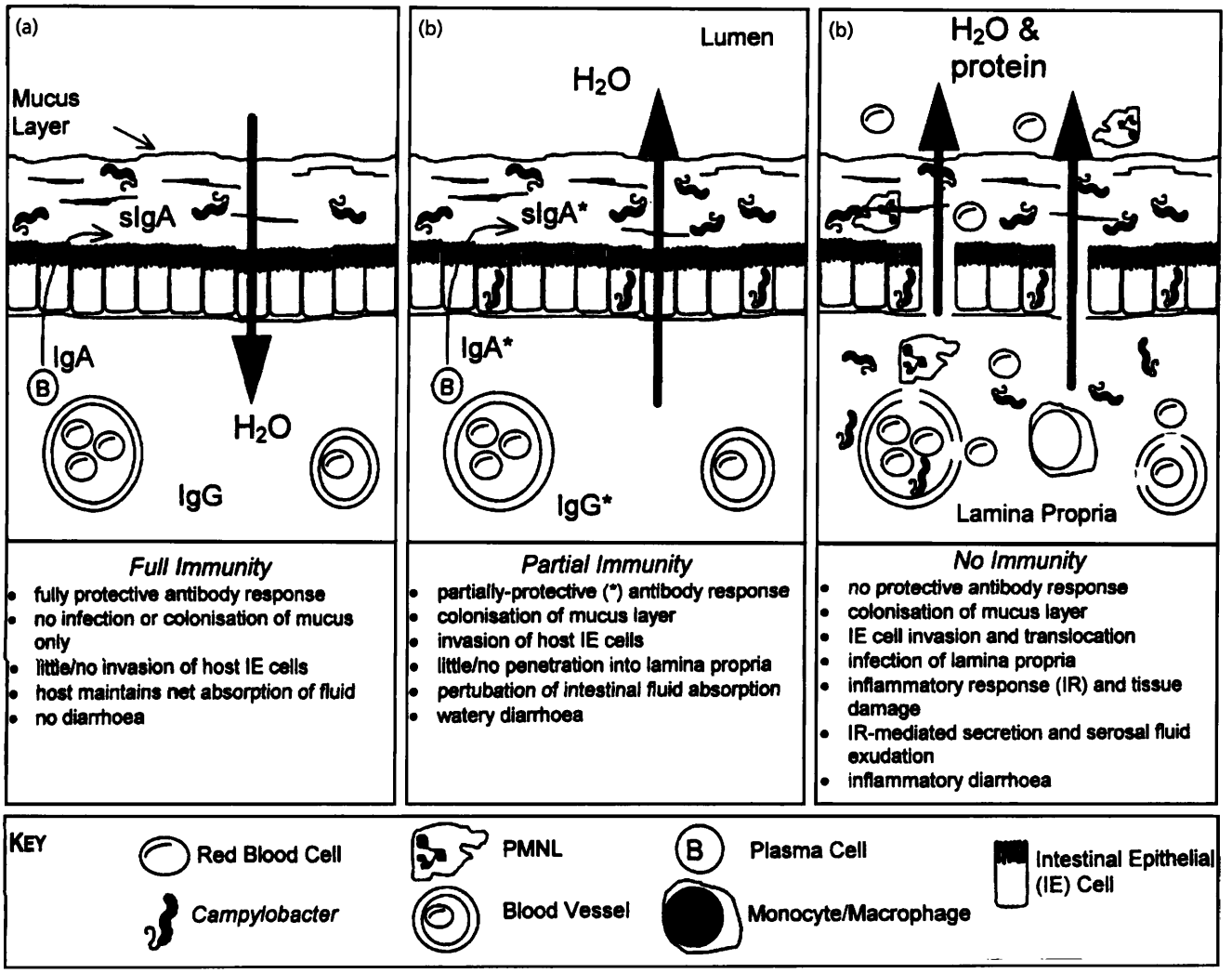

Fig. 2. Hypothetical model to explain the different clinical outcomes of infection by *C. jejuni*. Panels (a), (b) and (c) illustrate how the contribution of host response may result in inflammatory diarrhoea, watery diarrhoea or asymptomatic infection. (a) In a host with protective immunity, *C. jejuni* may colonize the mucus but the activity of toxin(s) and invasion is limited to a sub-pathologic level by the action of specific antibodies (and an effective cellular response?). (b) In a host previously infected by a heterologous strain with some shared epitopes, *C. jejuni* colonizes the mucus and elaborates toxin(s). Tissue invasion is restricted to the epithelial cell layer by the presence of partially-protective (*) cross-reacting antibodies [secretory IgA* (sIgA*), IgA* and IgG*]. Limited epithelial damage and toxin activity results in a loss of net fluid absorption and therefore milder, watery diarrhoea. (c) In an host with no previous history of *Campylobacter* infection, *C. jejuni* colonizes the mucus blanket, elaborates toxin(s), invades epithelial cells and translocates across the epithelium. The presence of bacteria and damaged host cells in the epithelium and lamina propria stimulates an inflammatory response. Tissue damage and inflammatory mediators, or the action of toxin(s) or both, result in net fluid secretion. As a specific immune response develops, tissue invasion by *C. jejuni* is restricted and the diarrhoea becomes less severe and watery.

Walker, 1986), suggests a role of LPS as an adhesin. It should soon be possible to determine whether or not LPS plays any role in colonization, invasion or inflammation using genetically defined mutants.

Inflammation and clinical disease

Infection by *C. jejuni* and *C. coli* leads to enterocolitis involving intestinal tissue damage. Thus, host cell invasion and perhaps cytotoxin production with subsequent tissue destruction are likely to be key elements in pathogenesis. One could postulate that enterocyte damage (responsible for a loss of net fluid absorption) and eventual perturbation of epithelial integrity (with a resultant leakage of serosal fluid) would lead to diarrhoea. Epithelial disruption, however, may not be the only mechanism that results in net fluid loss. One of the notable pathological changes associated with clinical disease is intestinal inflammation. This has also been observed in many animal models (Fox, 1992; Russell, 1992; Walker *et al.*, 1992; Everest *et al.*, 1993a; Russell *et al.*, 1993). Everest *et al.* (1993b) observed the elevation of cAMP, prostaglandin E_2 (PGE_2) and leukotriene B_4 (LTB_4) levels in infected intestinal tissues and lumenal

fluids. The lumenal fluids were found to elevate cellular cAMP levels in Caco-2 cell monolayers, an effect that could be inhibited by anti-PGE_2 but not by anti-cholera toxin. These results suggest an element of active intestinal fluid secretion in diarrhoea arising from acute intestinal inflammation following tissue damage.

Host status and clinical presentation

It is not clear what mechanism underlies the different clinical presentations of infection in patients from developed or developing countries. The simple answer would be that infection by non-invasive, non-cytotoxic, enterotoxigenic strains results in a non-inflammatory clinical presentation. Some studies have described strain differences that correlate with clinical symptoms (Everest *et al.*, 1992; Fauchere *et al.*, 1992; Ruiz-Palacios *et al.*, 1992); however, others (Perez-Perez *et al.*, 1992; Tay *et al.*, 1996) have not found such an association. With respect to invasion, strains isolated from patients with non-inflammatory, watery diarrhoea are capable of invading cultured host cells (Everest *et al.*, 1992; Tay *et al.*, 1996). In addition, the observation that travellers infected with *Campylobacter* have the pattern of clinical symptoms associated with their country of origin (Taylor, D. N., 1992) is evidence for a different explanation.

One current opinion, which remains untested, is that host status modulates disease presentation. In a speculative model (Fig. 2), the interaction of host immune status and a particular strain might result in inflammatory diarrhoea (no immunity), mild diarrhoea (partial immunity due to cross-reaction) or asymptomatic colonization (fully protective immunity). Alternatively, in some populations concurrent infection by other pathogens could affect the expression of the host's immune response, for example, altering the balance of T_{H1} (cellular response)- and T_{H2} (antibody response)-mediated pathways. Clearly, these two hypotheses are not the only possibilities and are not mutually exclusive. Unfortunately, our knowledge of the immune response during infection is limited (Newell & Nachamkin, 1992). Infection of adult volunteers with *C. jejuni* produced an inflammatory illness and a serum antibody response. When subsequently re-challenged with the same strain, these volunteers were protected from illness but not colonization (Black *et al.*, 1988). It would seem that a different pattern of immunity is seen in patients in developed and developing countries. Thus, in developing nations recurrent infection with different *Campylobacter* strains leads to the progressive development of fully protective immunity which, in turn, results in successively milder symptoms and an age-related decrease in the number of episodes of *Campylobacter*-mediated illness.

The regulation of *Campylobacter* virulence

Bacterial pathogens are highly adapted micro-organisms which express a virulence phenotype that is complex and multifactorial. Given the number of genes probably involved in virulence and the possibility that their pattern of expression may need to be modulated during infection, pathogens require effective signal transduction systems with mechanisms to coordinately regulate virulence determinant expression. These systems respond to differing host-specific conditions encountered throughout infection; such signals include temperature, pH, osmolarity and iron (Calderwood *et al.*, 1988). Regulatory systems may be part of the existence of a global network of interacting regulatory cascades used by pathogens to further direct and fine-tune the expression of genes associated with virulence. It is highly likely that *Campylobacter* species also utilize similar, perhaps interacting, regulatory systems to adapt to and survive within the host. Such systems would be essential for successful transfer from a low nutrient environment to a host intestinal tract. They are also likely to play a role in any adaptive response necessary for survival during cell and tissue invasion in humans following commensalism in an animal or avian intestine. Consequently, a range of strategies have been used to identify potentially important regulatory systems in *Campylobacter* sp.

Regulation by Fur

The restriction of free iron by mammalian hosts is a non-specific defence mechanism and consequently, bacterial pathogens have evolved systems to obtain iron during infection. These systems are maximally expressed under iron-restricted conditions with negative regulation being controlled by the Fur protein (ferric uptake regulator; Bagg & Neilands, 1987) which utilizes ferrous iron as co-repressor and binds to specific operators (Hantke, 1981; Calderwood & Mekalanos, 1988). In addition, genes encoding virulence determinants not directly involved in iron scavenging may also be regulated by iron (for example, Pappenheimer, 1955; Calderwood & Mekalanos, 1987). Highly conserved homologues of *fur* have been cloned from several pathogenic species (for example, Berish *et al.*, 1993). Moreover, iron-regulated genes in some species are preceded by promoters containing sequences with similarity to Fur-responsive operators in *E. coli* (Chen *et al.*, 1993; Thompson *et al.*, 1993). In common with other bacteria, *C. jejuni* synthesize new envelope-associated proteins in response to iron stress (Field *et al.*, 1986) and one such protein is probably a component of a high-affinity uptake pathway for haemin and haemoglobin (Pickett *et al.*, 1992). Thus, an iron-responsive regulatory circuit similar to the Fur system probably regulates a subset of virulence-associated genes in *C. jejuni*.

Wooldridge *et al.* (1994) and now others (Chan *et al.*, 1995) have cloned the *fur* gene from *C. jejuni*. Analysis of the *C. jejuni fur* gene has identified sequences with similarity to the *E. coli* Fur-binding consensus sequence (Calderwood & Mekalanos, 1988) with overlapping putative -10 and -35 promoter sequences. This

suggests that, like its counterparts in *E. coli* (de Lorenzo *et al.*, 1988) and *N. gonorrhoeae* (Berish *et al.*, 1993), the *fur* gene of *C. jejuni* is autoregulated. This observation is supported by experiments on the expression of a *fur* promoter fusion to a promoterless *cat* gene which showed a degree of repression by iron (K. G. Wooldridge & J. M. Ketley, unpublished data). The Fur homologues of other Gram-negative bacteria are highly conserved, but, in comparison, the Fur-like protein of *C. jejuni* is highly diverged (Wooldridge *et al.*, 1994). The degree of dissimilarity of the *C. jejuni* Fur protein with respect to its *E. coli* counterpart is also apparent antigenically and functionally (Wooldridge *et al.*, 1994). Thus, if the *C. jejuni* Fur-like protein is truly the major iron-dependent regulator in this organism, its recognition sequence, as well as the repressor itself, may have significantly diverged between the two species. Since Fur and Fur-like repressors are known to regulate some virulence determinant genes in other bacteria, it is likely that the Fur-like repressor protein also regulates a subset of genes with a role in pathogenesis. To date, two genes, in addition to *fur* itself, show Fur operator-like sequences in the promoter. These are the *sod* (Pesci *et al.*, 1994; Purdy & Park, 1994) and *katA* (Grant & Park, 1995) genes. These sequences, along with the altered pattern of protein expression with iron limitation, supports the presence of a Fur regulon in campylobacters. A *C. jejuni fur* mutant has now been constructed (A. H. M. van Vliet & J. M. Ketley, unpublished data) which will facilitate the characterization of the Fur regulon and the role of this system in *C. jejuni* pathogenesis.

Two-component regulatory systems

Many of the regulatory systems that have been identified can be grouped into families of bacterial transcriptional regulators. The superfamilies include the two-component regulatory systems, the LysR group of regulatory elements and the AraC group (Deretic *et al.*, 1989). Amino acid sequence analysis of the members of such families has revealed a large degree of sequence conservation within the same organism and between bacteria from different species. One group of regulatory signal transduction systems which are often involved in pathogenicity belong to a family of two-component transcriptional regulators that direct responses to external environmental stimuli (Parkinson, 1993). The two components often consist of histidine protein kinase (HPK) sensor proteins and response regulator (RR) proteins that interact to coordinately regulate the transcription of a number of genes. Four members of this regulatory family have been identified in campylobacters. The chemotaxis system involves a signal transduction pathway which includes the RR CheY. The *cheY* gene of *C. jejuni* has been cloned and characterized. The predicted amino acid sequence of the gene is characteristic of known RR proteins from other bacterial species and of the CheY sub-family in particular. A genetically defined *C. jejuni cheY* mutant is nonchemotactic on motility agar and in a chemotaxis assay (J. E. Marchant, J. Henderson, B. Wren & J. M. Ketley, unpublished data). With another RR gene, *regX1*, mutation results in an altered pattern of protein expression and a change in the ability of the mutant to invade Caco-2 cells (A. Brás-Goldberg & J. M. Ketley, unpublished data) and to colonize chick intestine (A. Brás-Goldberg, S. Crawthraw, D. G. Newell & J. M. Ketley, unpublished data). These results would indicate that it is highly likely that campylobacters utilize HPK/RR systems to allow adaptation to and to survive within the intestinal tract and that these two-component regulatory systems are also likely to be important for survival in the environment.

fla gene regulation

There is now some insight into the regulation of flagella production. The *flaA* and *flaB* genes are independently transcribed, with the *flaA* gene regulated by a σ^{28} promoter and the *flaB* gene by a σ^{54} promoter. Under the conditions assessed so far, the *flaA* gene is expressed at much higher levels than the *flaB* gene. Transcription from the σ^{54} promoter has been found to be environmentally modulated by conditions such as temperature, pH, and inorganic salt and divalent cation concentrations (Alm *et al.*, 1993). Miller *et al.* (1993) have identified a gene (*flbA*) that may play a role in the regulation of flagellin expression. Interestingly, the *C. jejuni* FlbA protein might be a member of a group of proteins involved in the secretion or regulation of virulence-related proteins. The group includes the *Yersinia pestis* LcrD protein and *S. typhimurium* InvA protein. A *C. jejuni flbA* mutant did not produce functional flagella and flagellin was not present in the cytosol; these data suggest that FlbA (or the product of a co-transcribed gene) is regulating the expression of the *fla* gene. Flagellar synthesis has been observed to undergo both phase and antigenic variation (Caldwell *et al.*, 1985; Harris *et al.*, 1987). The molecular mechanisms responsible for such variation are not clear but the former does not involve DNA sequence changes and can be induced (Nuijten *et al.*, 1995).

Future perspectives

Further progress is urgently required in both our understanding of the molecular basis of the virulence of the campylobacters and the nature of the bacterial interactions with the host during the progress of infection. One clear objective is to establish exactly which toxins campylobacters express and to determine the role of these toxins in disease. Another important aim of future work is to develop a detailed understanding of the mechanisms of host cell invasion and transcytosis by campylobacters and to, again, confirm their role in the pathophysiology of disease. The range of apparently conflicting data is certainly frustrating as it is difficult to draw any definitive conclusions as to the exact mechanisms and pathways involved in invasion. The observed differences may stem from the range of different host cell types, strains and experimental conditions that have been applied. The challenge is now

to identify the factor(s) that stimulates uptake of campylobacters, to compare the ligand with that associated with FlaA binding and determine exactly how motility contributes to the invasive process. Once a genetic understanding of toxin expression and invasion has been established then a more defined molecular investigation of strain differences can be undertaken.

As the work with the flagellin genes has so elegantly demonstrated, a molecular genetic approach is a powerful and rapid strategy to achieve this objective. However, the molecular genetic methodologies currently available for the analysis of campylobacters are not as well-developed as in other common bacterial pathogens. Although limited success has been achieved with some current commonly used molecular genetic approaches, the identification of genetic determinants that mediate complex virulence phenotypes (for example invasion) would be greatly facilitated by a detailed knowledge of the *Campylobacter* genome. The limitations encountered with many molecular genetic methodologies and the small size of the *C. jejuni* chromosome argue that the most efficient and powerful future course would be the direct sequence analysis of the entire genome. This would revolutionize our understanding of the biology and, particularly, the genetics of this important food-borne pathogen. Given the huge cost of food-borne disease to society, such an effort is justified both scientifically and economically. The completion of such a project would not, of course, be the end point for research on campylobacters, but it would provide the genetic information to enable the rapid analysis of gene function and regulation. With respect to virulence, although various cell assays are now well established, an improvement of the available *in vivo* models will also be necessary to precisely characterize any putative pathophysiological mechanisms that are inferred from sequence analysis.

During the past decade it has become clear that *Campylobacter* species are a significant cause of debilitating enteric disease in developed countries. After two decades of investigation many strong leads but few conclusive answers exist as to how campylobacters cause disease in humans. There is good evidence that motility and invasion play a role in pathogenesis but, although several toxins may be produced, their roles are far from clear. Advances in the understanding of the physiology and pathogenesis of campylobacters will without doubt lead to new strategies for detecting, controlling and even eliminating campylobacters from food.

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