Differentiated Caco-2 cells as a model for enteric invasion by *Campylobacter jejuni* and *C. coli*

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Summary. A collection of 44 *Campylobacter* isolates (37 *C. jejuni* and seven *C. coli*) from children with colitis (21 strains) or watery diarrhoea (23 strains) was analysed for toxin production, association with HeLa cells, and invasion of differentiated Caco-2 cell cultures. There was no obvious association of clinical symptoms with species, biotype or enterotoxin production. All colitis strains and most of the isolates from watery diarrhoea were cytotoxic for Chinese hamster ovary cells. Measurements of bacterial association indices with HeLa cells varied with time, and were considered to be unreliable for discriminating between isolates from the two diagnostic groups. Statistically significant differences were observed between the two groups (all colitis strains and 65% of strains from non-inflammatory diarrhoea) with respect to invasion of both HeLa and Caco-2 cell monolayers. However, among the strains from non-inflammatory diarrhoea that did invade, numbers of internalised bacteria were similar to the range observed for colitis strains. Of the colitis strains, 86% were able to transcytose through polarised Caco-2 monolayers grown on filters, compared with 48% of isolates from non-inflammatory disease. We propose the use of Caco-2 cells as a model for studying invasion of intestinal epithelia by *C. jejuni* and *C. coli*.

Introduction

Campylobacter jejuni and *C. coli* are major causes of diarrhoea among children and adults.[1] Patients present with a range of symptoms of varying severity, from watery diarrhoea suggestive of an enterotoxin-induced process, to severe inflammatory diarrhoea indicating that tissue invasion may be important.[2,3] Bacteraemia is observed among a small proportion of patients with inflammatory disease.[3,4] Tissue invasion and bacteraemia by *C. jejuni* have been demonstrated in several animal models[5] but evidence for the presence of bacteria within cells is unconvincing,[6,7] and isolates give negative results in the Sérény test for conjunctival invasion.[8,9] Various in-vitro tests have been described for the detection of toxins,[10–13] and to demonstrate adherence and invasion properties of *Campylobacter* isolates.[14–17] While clinical correlation with toxigenicity is controversial, quantitative differences in the association of *C. jejuni* with HeLa cells were observed between strains causing diarrhoea with fever and those causing diarrhoea only.[14]

We report the detailed characterisation of *C. jejuni* and *C. coli* isolates from children with watery diarrhoea or colitis. In particular we have assessed the

limitations of the HeLa cell association model, and investigated the use of the differentiated cell line Caco-2[18,19] as a relevant model for the study of campylobacter pathogenicity.

Materials and methods

Bacteria

The 37 *C. jejuni* and seven *C. coli* strains used in this study were isolated from stool samples during routine bacteriological screening at the Department of Microbiology, St Pieters University Hospital, Brussels. Twenty-one strains were isolated from children with inflammatory diarrhoea. Colitis was confirmed by recto-colonoscopy and by light microscopic examination of biopsy specimens taken from the colon or the rectum or both. In all cases mucosal hyperaemia was observed on recto-colonoscopy, and moderate to severe polymorphonuclear leucocyte infiltration was detected in biopsy samples, with or without oedema and cryptitis. Twenty-three strains were isolated from children presenting with apparent non-inflammatory diarrhoea; absence of colitis was confirmed either by normal recto-colonoscopy, or by failure to detect leucocytes or erythrocytes in Giemsa-stained stool specimens. All faecal cultures were negative for *Salmonella* spp., *Shigella* spp., *Yersinia enterocolitica*, and

enterotoxigenic or enteropathogenic *Escherichia coli*. *Campylobacter* strains were biotyped according to the Lior scheme.[20] Isolates were stored both as freeze-dried samples and at $-70°C$ in glycerol 10 % broth; a new sample was used for each experimental procedure.

Growth media

Mueller-Hinton (MH) Broth and Agar (Oxoid) were used to prepare exponential cultures and for subculturing bacteria. They were incubated at 37°C in an atmosphere of H_2 6 %, CO_2 5 %, O_2 5 % and N_2 84 % in a variable atmosphere incubator cabinet, or at 42°C in an anaerobic jar with a nickel palladium catalyst and a Campylobacter micro-aerophilic generating envelope (Oxoid).

Cell culture

Chinese hamster ovary (CHO) cells were maintained in Eagle's minimal essential medium (MEM) with Earle's salts (Gibco) supplemented with fetal calf serum (FCS) 10 %. HeLa cells (Flow Laboratories) were maintained in MEM with Earle's salts containing non-essential amino acids 1 % and glutamine without antibiotics. The Caco-2 (human colon carcinoma) cell line[18,19] was maintained in MEM with FCS 10 % or 20 % without antibiotics. Cells were grown routinely in flasks at 37°C in CO_2 5 % and a humidified atmosphere. Confluent stock cultures were trypsinised and new stock cultures were seeded at c. 10^5 cells/ml.

Enterotoxin

Campylobacter strains were grown in Brucella Broth (Difco) supplemented as previously described.[21] Cell-free culture supernates (20 μl) were added to 400 μl of MEM with Earle's salts containing FCS 1 % and gentamicin 100 μg/ml in individual wells of a Lab Tek 8-chamber tissue-culture plate containing CHO monolayers. The cells were incubated for 16–18 h, fixed with methanol and stained with Giemsa solution (Merck) 1 %. Elongated CHO cells, indicative of enterotoxin activity, were those with a length:width ratio > 3:1. At least 200 cells were counted/well.

Cytotoxic activity

Strains were grown in a biphasic culture system[11] with MH agar (Difco) as the solid phase and Medium 199 (Gibco) with Hanks's salts and L-glutamine as the liquid phase. Incubation was at 42°C for 48 h in a micro-aerophilic atmosphere. Bacteria were pelleted by centrifugation and the supernates were sterilised by filtration. Pellets were resuspended in a one-tenth volume of medium 199, disrupted by sonication, centrifuged and filter sterilised. Dilution series of culture supernates and sonicates were added to CHO monolayers in MEM with Earle's salts and FCS 10 % in 96-well microtitration plates (Nunc), and incubated for 6 days. Morphological changes were observed by phase-contrast microscopy; cytotoxic activity (which was not neutralised by antiserum against purified

Shiga toxin) was indicated by rounding and death of CHO cells.

Adherence to HeLa cells

The association index (AI, adherent bacteria/cell) for each strain was determined according to the method of Fauchère *et al.*,[14] with the following modifications. Bacteria were harvested from exponential phase broth cultures and resuspended in Hanks's solution or MEM containing FCS to a density of c. 10^7 organisms/ml; exact titres were determined retrospectively by culture of dilutions of the cultures on MH agar plates. Bacterial suspensions (0·5 ml) were added to 75 % confluent HeLa cell monolayers on glass coverslips, three coverslips for each strain tested. Infections were incubated for 1 or 6 h, then the monolayers were washed with MEM containing FCS 1 % and incubated for a further 3 or 1 h, respectively. Monolayers were again washed before staining with carbol fuchsin or Giemsa.

Adherence to Caco-2 cells

Caco-2 cells, grown on membrane filters (0·4 or 3·0 μm pore size) in a Transwell unit (Costar), were used c. 10–12 days post-confluence, when the cells were fully differentiated. In this state they form a polarised monolayer with typical brush border microvilli on the apical surface separated from the basolateral domain by tight junctions.[18] Analysis of bacterial adherence to Caco-2 cells by light microscopy was difficult because of lack of contrast in stained preparations. Therefore, the method of Finlay *et al.*,[22] in which radiolabelled bacterial cultures were used to quantify cell adherence, was modified to screen the collection of *C. jejuni/coli* isolates. Bacteria in the mid-exponential growth phase were washed in phosphate-buffered saline (PBS), and suspended in Methionine Assay Medium (MAM, Difco). Bacterial suspensions were incubated micro-aerophilically at 37°C for 30–60 min, pelleted by centrifugation and suspended in MAM containing [^{35}S]methionine (Amersham, specific activity > 29·6 TBq/mmol) 1·85 mBq/ml. After incubation for a further 1 h, bacteria were again harvested, washed thoroughly, and suspended to a density of 10^7 organisms/ml in MAM.

Bacterial suspensions (5 μl, c. 5000 cpm) were added to polarised Caco-2 cell monolayers, either living—to permit quantitation of adherent and invasive bacteria —or fixed with glutaraldehyde 3 % in phosphate buffer so that bacterial invasion was inhibited, giving a measure only of adherence. After incubation for 6 h with bacteria, monolayers were washed thoroughly in cold PBS, and the radioactivity associated with each filter was determined in Optiphase (LKB) scintillant in a Packard Tri-Star liquid scintillation spectrometer.

Invasion of cultured cells

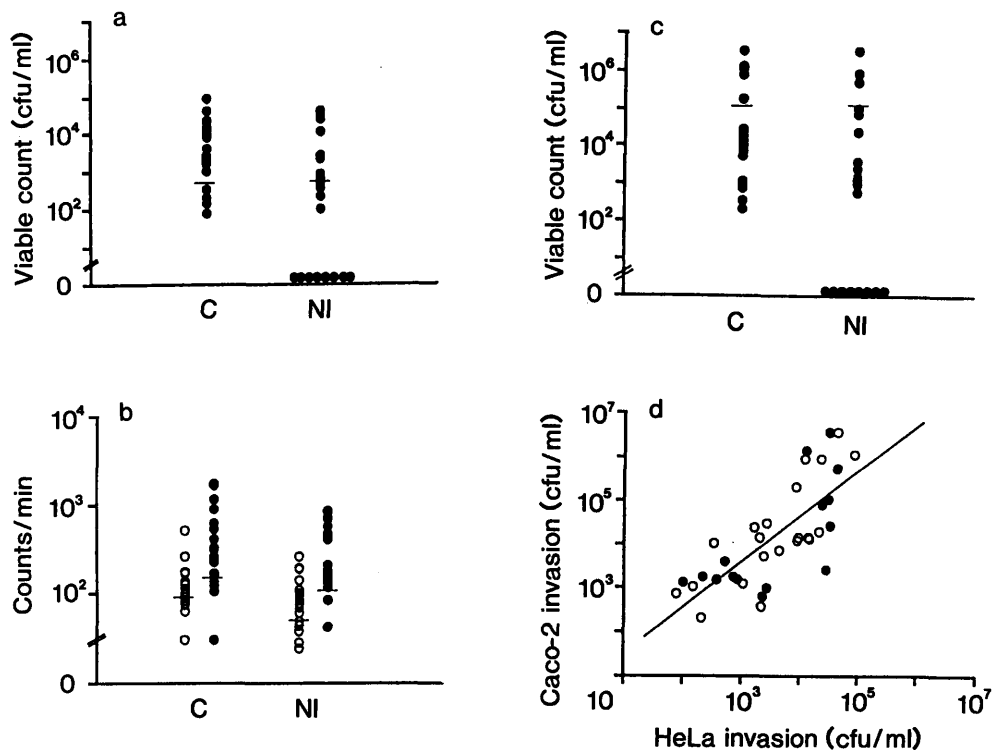Confluent monolayers of HeLa or fully differentiated Caco-2 cells in 24-well plastic tissue-culture plates

Fig. 1. Association of *Campylobacter* isolates with cultured human epithelial cell lines. Panels **a** and **c** show viable counts (cfu/ml) of intracellular bacteria recovered from gentamicin-treated HeLa and Caco-2 cells respectively. Horizontal bars indicate mean values, excluding strains unable to invade. Panel **b** shows the association of radioactively-labelled bacteria to live (○) or glutaraldehyde-fixed (●) Caco-2 cells. Horizontal bars indicate mean cpm for each group. C, colitis isolates; NI, isolates from non-inflammatory disease. Panel **d** is a direct comparison of invasion (determined as viable counts/ml of intracellular bacteria recovered from gentamicin—treated monolayers) of HeLa and Caco-2 cells by each *Campylobacter* isolate (○, colitis; ●, non-inflammatory).

(Nunc) were infected with *c.* 10⁷ bacteria. After incubation for 6 h to allow adherence and invasion, monolayers were washed with MEM, and incubated for 2 h in MEM containing gentamicin 200 μg/ml to kill extracellular bacteria. Monolayers were again washed thoroughly before lysis of cells with sodium deoxycholate (Difco) 0·5 % in PBS, and serial dilution for viable counts of bacteria on MH agar.

Caco-2 cell transcytosis

The ability of *C. jejuni/coli* to pass through confluent polarised Caco-2 cell monolayers grown on filters was determined as a measure of penetration of the host epithelial cell barrier.[22] Approximately 10⁷ exponential phase organisms in MEM were added to the top of the monolayers, incubated for 6–8 h, and the presence of bacteria in the wells beneath the filters was detected by culturing on MH agar. *E. coli* K-12 strain DH5α was used as a non-penetrating control organism.[22] Tight junctions in polarised monolayers were disrupted by incubation in calcium-free medium (Flow), after which DH5α cells could be detected in the medium beneath the filter.

Statistical analysis

Distribution of values within groups of strains was analysed by the χ^2 test; $p < 0.05$ was considered to be statistically significant.

Results

Detailed data for each *Campylobacter* strain are shown in table I; table II gives composite data for the two diagnostic groups.

Enterotoxin production and cytotoxic activity

Statistically significant differences between isolates from colitis and those from non-inflammatory disease were observed with respect to cytotoxin but not enterotoxin production (table II).

HeLa cell association and invasion

With AI values > 3·0 after 1-h infections as the criterion for HeLa cell association,[14] a statistically significant difference was observed between the two groups of strains (table II). However, preliminary time-course studies of infection indicated, in contrast with previously published data,[14] that adherence of the majority of strains tested increased to a maximum at about 6 h, at which time there was no significant difference between the two groups of strains (table II). Indeed, 12 of the 25 strains that were apparently non-adherent after 1 h (six *C. jejuni* strains from each diagnostic group), were observed to adhere after longer incubation. Conversely, five strains (P71, E116, V214, O69 and M233) that were positive for adherence at 1 h were negative at 6 h, perhaps because the ability

Table I. Characteristics of individual clinical *C. jejuni* and *C. coli* isolates.

Strain no.	Production of		HeLa cell association*		HeLa cell invasion†	Caco-2 cell association†		Caco-2 cell	
	enterotoxin	cytotoxin	1 h	6 h		live	fixed	invasion§	transcytosis||
(i) Colitis isolates									
C. jejuni biotype I									
A259	−	+	3·3	3·8	$2·8 \times 10^3$	126	265	$2·8 \times 10^4$	+
D246	+	+	1·6	3·1	$2·1 \times 10^3$	170	538	$1·4 \times 10^4$	+
H132	+	+	1·3	4·7	$9·0 \times 10^4$	513	331	$1·1 \times 10^6$	+
K85	+	+	0·1	3·9	$2·4 \times 10^3$	137	318	$5·3 \times 10^3$	+
L115	+	+	3·2	4·2	$2·3 \times 10^4$	116	232	$8·3 \times 10^5$	−
M288	−	+	1·4	4·5	$2·1 \times 10^2$	85	108	$2·1 \times 10^2$	+
P71	−	+	3·8	2·6	$1·7 \times 10^3$	77	227	$2·3 \times 10^4$	+
R143	+	+	0·6	3·8	$1·5 \times 10^2$	91	617	$1·0 \times 10^3$	+
T82	+	+	0·1	4·4	$4·4 \times 10^3$	136	1147	$7·3 \times 10^3$	+
C. jejuni biotype II									
A143	+	+	4·6	4·2	$7·8 \times 10^1$	90	122	$7·3 \times 10^2$	+
B404	+	+	2·3	1·8	$1·4 \times 10^4$	99	628	$1·4 \times 10^4$	+
C119	+	+	4·3	4·1	$9·0 \times 10^3$	266	168	$1·2 \times 10^4$	+
D217	+	+	4·3	3·0	$1·0 \times 10^4$	105	137	$1·4 \times 10^4$	+
E116	+	+	4·4	1·2	$1·3 \times 10^4$	102	234	$1·3 \times 10^6$	+
K131	+	+	0·1	1·2	$2·2 \times 10^3$	63	172	$3·6 \times 10^4$	+
O81	+	+	0·1	0·3	$1·1 \times 10^3$	30	420	$1·2 \times 10^3$	−
V214	+	+	4·1	0	$2·2 \times 10^4$	78	30	$1·9 \times 10^4$	−
V221	−	+	4·4	3·5	$1·5 \times 10^4$	100	249	$1·3 \times 10^4$	+
C. coli biotype I									
D272	−	+	3·0	3·8	$8·6 \times 10^3$	179	1768	$1·9 \times 10^5$	+
M175	+	+	ND	3·6	$4·5 \times 10^4$	134	913	$3·6 \times 10^6$	+
C. coli biotype II									
M254	−	+	3·0	3·2	$3·4 \times 10^2$	84	1716	$1·0 \times 10^4$	+
(ii) Isolates from non-inflammatory diarrhoea									
C. jejuni biotype I									
A365	+	−	1·5	3·4	$2·3 \times 10^3$	93	840	$6·0 \times 10^2$	−
B415	+	−	0·1	1·8	0	48	215	0	+
B378	+	+	0·1	0·2	0	75	171	0	−
E178	−	+	0·4	0·1	$1·2 \times 10^4$	ND	ND	$8·5 \times 10^5$	−
E206	+	+	3·1	3·4	0	96	859	0	−
E228	+	+	1·1	0·5	$2·5 \times 10^4$	79	135	$7·3 \times 10^4$	−
E230	+	+	0	0	0	28	42	0	+
J75	+	+	13·9	3·6	$2·2 \times 10^2$	91	160	$1·7 \times 10^3$	−
J78	+	−	0·1	0·4	$7·2 \times 10^2$	145	728	$1·7 \times 10^3$	−
H104	+	−	0	0·4	0	72	143	0	+
G136	+	+	0·9	3·1	$5·4 \times 10^2$	85	884	$4·0 \times 10^3$	−
K105	+	+	1·0	4·2	0	263	705	0	+
N82	+	−	0	1·5	$3·2 \times 10^4$	62	134	$1·0 \times 10^5$	+
R169	+	−	0·3	5·2	$3·2 \times 10^4$	28	163	$3·4 \times 10^6$	+
T117	+	+	1·2	3·7	$3·0 \times 10^4$	190	419	$2·5 \times 10^3$	−
V161	−	+	ND	2·5	$3·8 \times 10^2$	44	118	$1·5 \times 10^3$	−
C. jejuni biotype II									
A293	+	+	2·6	1·4	$8·7 \times 10^2$	95	210	$1·5 \times 10^3$	+
C139	+	+	0·6	4·0	$4·5 \times 10^4$	38	428	$5·2 \times 10^5$	−
O69	+	+	3·7	2·8	0	101	186	0	+
C. coli biotype I									
D265	−	+	3·5	6·2	0	99	488	0	+
M233	+	+	4·3	0	$2·7 \times 10^3$	24	575	$9·9 \times 10^2$	+
O73	−	+	0·5	0·1	$3·5 \times 10^4$	202	85	$2·4 \times 10^4$	−
C. coli biotype II									
A320	−	+	0·3	3·2	$1·0 \times 10^2$	112	579	$1·3 \times 10^3$	+

Data are averages of at least three independent experiments.

ND, not done.

* Association indices (bacteria/cell) determined at 1 or 6 h.

† Viable bacteria/ml recovered after gentamicin treatment of infected HeLa cells.

‡ Radioactivity (cpm) associated with monolayers infected with radiolabelled (^{35}S) bacteria.

§ Viable bacteria/ml recovered after gentamicin treatment of infected Caco-2 cells.

\|\| Recovery of bacteria beneath polarised Caco-2 monolayers.

to adhere is a reversible process, or because rapid adherence and subsequent invasion prevent further adherence by down-regulation of receptor proteins.

A statistically significant difference was observed between colitis strains and strains from non-inflammatory diarrhoea with regard to invasion of HeLa cell monolayers, as determined by recovery of gentamicin-protected (internalised) bacteria after incubation for 6 h (table II). However, among the strains from non-inflammatory disease that did invade, viable counts of recovered bacteria were similar to the range observed for colitis strains (fig. 1a). In the majority of

Table II. Comparative analysis of *C. jejuni* and *C. coli* isolates from colitis and non-inflammatory diarrhoea

Property	Number (%) of isolates from		Statistical significance
	colitis (21)	non-inflammatory diarrhoea (23)	
Enterotoxin	15 (71%)	18 (78%)	$\chi^2 = 0.6$, p = 0.4
Cytotoxin	21 (100%)	17 (74%)	$\chi^2 = 4.2$, p = 0.03
HeLa cell association			
1 h, AI > 3.0	11 (55%)*	5 (23%)*	$\chi^2 = 4.6$, p = 0.03
6 h, AI > 3.0	15 (71%)	10 (43%)	$\chi^2 = 2.0$, p = 0.15
HeLa cell invasion	21 (100%)	15 (65%)	$\chi^2 = 7.4$, p = 0.005
Caco-2 cell invasion	21 (100%)	15 (65%)	$\chi^2 = 7.4$, p = 0.005
Caco-2 cell transcytosis	18 (86%)	11 (48%)	$\chi^2 = 7.0$, p = 0.008

* One strain in each group was not tested.

cases of invasion, < 1 % of the original inoculum was internalised (table I).

Caco-2 cell association

The human colonic carcinoma line Caco-2, that differentiates to give microvillated monolayers,[18] was investigated as an alternative model for the study of campylobacter adherence and invasion. Radioactively labelled bacteria were used for this test, primarily because adherent bacteria are difficult to visualise directly in stained preparations, but also because the method permits independent determination of adherence and invasion with gluteraldehyde-fixed and living monolayers respectively. All *C. jejuni/coli* strains in our collection adhered to Caco-2 cells (table I). Colitis isolates generally gave higher counts than strains from non-inflammatory disease but levels of adherence varied considerably between strains, and there was no significant difference in the mean levels of adherence between the two diagnostic groups with either gluteraldehyde-fixed or living cells (fig. 1b). With fixed cells, in which association represents surface attachment of bacteria only, the average cell-associated radioactivity was 474 cpm for colitis strains compared with 375 cpm for isolates from non-inflammatory diarrhoea. With living Caco-2 cells, associated counts were generally lower than with fixed cells (154 and 100 cpm for strains from colitis and non-inflammatory disease, respectively). This may reflect changes in cells due to the process of fixation, or the dynamic process of transcytosis (see below) permitting release of labelled bacteria from the monolayers. Kinetic studies revealed that adherence to Caco-2 cells occurred within 4 h of infection, depending on the strains used (data not shown).

Caco-2 cell invasion

A statistically significant difference was observed between strains from colitis and non-inflammatory disease with regard to invasion of Caco-2 cell monolayers, as determined by recovery of gentamicin-
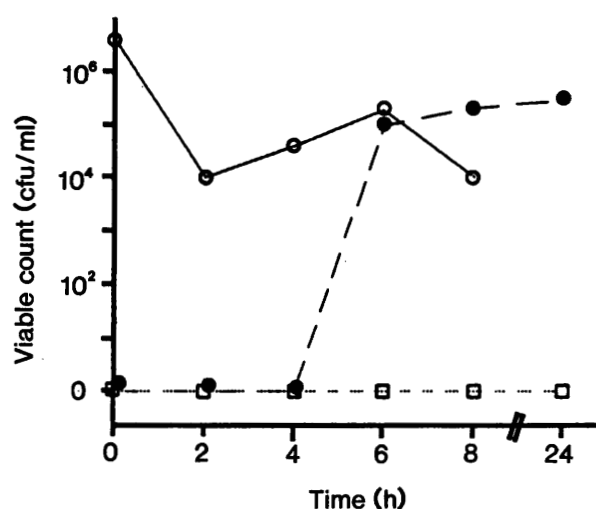
Fig. 2. Time course of transcytosis of strain E116 through polarised Caco-2 cell monolayers: ○, viable counts (cfu/ml) recovered from monolayers by deoxycholate lysis (adherent and internalised bacteria); ●, viable counts (cfu/ml) recovered from the medium below the support filter. Strain DH5α (□) was used to confirm integrity of monolayers.

protected bacteria after incubation for 6 h (table II). However, among the strains from non-inflammatory disease that did invade, viable counts of recovered bacteria were similar to the range observed for colitis strains (fig. 1c). The proportion of the original inoculum internalised was somewhat greater than with HeLa cells, but there was a strong correlation between the extent of invasion of the two cell types (fig. 1d). Kinetic studies with representative strains indicated that invasion of Caco-2 cells occurred between 2 and 4 h (data not shown).

Transcytosis through Caco-2 monolayers

There was a statistically significant difference between the ability of isolates from colitis and non-inflammatory disease to pass through intact Caco-2 cell monolayers from the apical to the basolateral surface (tables I and II). Bacteria were recovered in the medium below the filter in numbers between 10^2 and > 10^4/ml 4–6 h after infection (fig. 2). However, since

culture conditions in this test are suboptimal for growth and survival of *Campylobacter* strains, exact figures were not determined for each strain. Instead, simply the presence of bacteria in the lower compartment was taken as a qualitative indicator of transcytosis. It should be noted that six strains (B415, E230, H104, K105, O69 and D265) capable of penetrating the polarised Caco-2 cell monolayer were found by other criteria to be non-invasive. Further experiments are required to establish whether these strains pass directly through cell junctions rather than by an intracellular pathway.

Discussion

In this paper we present a detailed study of 44 *Campylobacter* isolates from children with colitis (21 strains) or watery diarrhoea (23 strains). There was no obvious association of species or biotype with clinical symptoms. In common with previous reports, we found that enterotoxin production was more prevalent among *C. jejuni* isolates (31 of 37, 84%) than *C. coli* (2 of 7, 29%), although numbers are too small for statistical analysis. There was no statistically significant difference between isolates from patients with watery diarrhoea and those from children with inflammatory disease with respect to enterotoxin production. There have been several previous attempts to correlate the toxigenic profile of *C. jejuni* isolates with associated clinical illness.[10,23-26] In separate studies in India[25] and Algeria,[10] for example, the proportion of enterotoxin-producing strains did not differ significantly between symptomatic and asymptomatic children. On the other hand, enterotoxin-producing strains were isolated more frequently from symptomatic patients than from asymptomatic carriers in Mexico.[26] Thus the role of enterotoxin in disease remains uncertain. Several workers have reported *C. jejuni* cytotoxins active against various cell lines.[23,27] All the colitis strains in our collection, and 18 of 23 isolates from non-inflammatory diarrhoea, produced activity that was lethal to cultured CHO cells. This is a statistically significant difference, and may suggest a role in disease. However, the absence of neutralising antibodies in convalescent sera in one study[28] raises doubts about the significance of such cytotoxins in patients.

Fauchère *et al.*[14] suggested an AI of 3·0 after 1-h infections of HeLa cells as the arbitrary criterion for distinguishing between adherent and non-adherent organisms. Moreover, they observed a statistically significant correlation between in-vitro adherence and clinically apparent diarrhoea with fever (presumably indicative of invasion). In our study on a much larger collection of clinically documented strains, the same parameter also yielded a statistically significant discrimination between isolates from colitis and those from non-inflammatory disease. We feel, however, that this is of little predictive value, since the majority of isolates apparently did not adhere to HeLa cells in

1 h (i.e., AI < 3·0). Even taking AI > 1·0 as the criterion for adherence, on the assumption that intracellular proliferation may result from invasion by a single bacterium, 25% of colitis strains and 45% of strains from non-inflammatory disease would still be considered non-adherent. Moreover, in this case there was no significant difference between the two groups of isolates ($\chi^2 = 3·7$, p = 0·05).

The clinicopathological characteristics of *Campylobacter* enteritis suggest that mucosal invasion is an important component of pathogenicity. Penetration of cultured HeLa cells by *Campylobacter* strains has been demonstrated by several groups,[14-17] and suggested as a convenient model system for discriminating between isolates from environmental sources and different types of disease. The data we present here, however, indicate that for systematic analysis of pathogenicity the HeLa cell model suffers from variability and unreliability inherent in the fact that a time-dependent process is observed at a single arbitrarily chosen experimental time point. We observed a statistically significant difference between isolates from colitis and non-inflammatory disease with respect to invasion of HeLa cells. However, strains from non-inflammatory disease that were able to invade HeLa cells were indistinguishable from colitis isolates in terms of numbers of bacteria recovered. Moreover, the fact that several strains that were invasive as defined by gentamicin protection were non-adherent in terms of AI (< 3 after 6 h) is a cause for concern.

We propose the use of Caco-2 cells, that differentiate to give a microvillous brush border characteristic of intestinal enterocytes,[18,19,29,30] as an alternative model for the study of epithelial-cell invasion by clinical isolates of *Campylobacter* spp. Apart from the obvious benefit of using cells similar to those for which campylobacters may show natural tropism *in vivo*, the major advantage of this model is that it makes it possible to assess the ability of *Campylobacter* isolates to penetrate and pass through an epithelial barrier, a process that is likely to be important in the intestinal damage and occasional bacteraemia observed in enterocolitis. Transcytosis[31] of *Salmonella choleraesuis* through the canine kidney cell line MDCK has been described previously.[22] *C. jejuni*, on the other hand, was reported to be unable to penetrate MDCK cells,[32] possibly because the necessary receptors were not present. However, transcytosis through polarised Caco-2 monolayers was a feature of 18 of the 21 colitis strains in our collection, compared with only 11 of the 23 isolates from non-inflammatory diarrhoea. Interestingly, six strains of this latter type were found to be non-invasive; in such cases transcytosis may be due to disruption of tight junctions and paracellular passage across the monolayer.

It is important to note that strains classified as "non-inflammatory" that were nevertheless able to invade cultured cells or pass through polarised monolayers or both, may have been isolated from cases of colitis in which inflammatory cells were missed, or in

which characteristic symptoms of the disease were absent at the time of diagnosis. Host factors may also critically influence the progress of an infection and contribute to difficulties in defining isolates purely on the basis of symptoms. Nevertheless, the data presented in this paper clearly indicate that the range of symptoms observed in campylobacter infections is, at least in part, a reflection of strain differences between causative organisms. Future work in our laboratories will seek to exploit the Caco-2 cell system in the study of host-pathogen interactions in campylobacter virulence.

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