Campylobacter jejuni activates mitogen-activated protein kinases in Caco-2 cell monolayers and in vitro infected primary human colonic tissue

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The mitogen-activated protein kinases (MAPKs) play a central role in many host signalling pathways. These signalling proteins are known to be involved in host responses against invasive bacteria including generation of chemotactic and inflammatory cytokines. It was hypothesized that Campylobacter jejuni may activate MAPKs, as intestinal infection may induce a clinical and pathological picture of acute colonic inflammation. Infection of Caco-2 cell monolayers (human colonic epithelial cell line) and human colonic tissue with C. jejuni in vitro demonstrated increased MAPK activity for ERK 1/2 (p44/p42 MAPK), JNK and p38 MAPKs. Kinase activity and phosphorylated forms were increased in infected Caco-2 cells and human colonic explants, suggesting that these pathways are important in inflammatory responses induced by C. jejuni in man.

INTRODUCTION

Campylobacter jejuni is the most common cause of bacterial enteritis in man, causing both watery and bloody diarrhoea with symptoms of acute inflammation in colonic mucosa (Black et al., 1988; Wassenaar & Blaser, 1999; Skirrow & Blaser, 2000; Coker et al., 2002). The mechanisms by which C. jejuni causes diarrhoeal disease and inflammation in the infected human host are not fully understood. Like other human enteric bacterial pathogens, C. jejuni infects humans by colonizing the mucus layer of the intestine followed by adherence and invasion of epithelial cells (Skirrow, 1986; Everest et al., 1992; Konkel et al., 1992, 2001; Ketley, 1997; Harvey et al., 1999; Kopecko et al., 2001). Bacteria transcytose through the host cell (Everest et al., 1992; Konkel et al., 1992; Harvey et al., 1999), to emerge on the basolateral surface, allowing access to the lamina propria. Recruitment of neutrophils occurs at the site of bacterial invasion (basolateral surface) via interleukin-8 (IL8) (Hickey et al., 1999, 2000) and release from infected enterocytes by activation of NF-κB (Mellits et al., 2002). This innate immune response involves secretion of cytokines and other mediators leading to an inflammatory response.

Mitogen-activated protein kinases (MAPKs) are a group of serine/threonine-specific protein kinases, which regulate the transcription of inflammatory cytokines in response to various extracellular and stress stimuli (Garrington & Johnson, 1999). Upon activation by upstream kinases, MAPKs phosphorylate downstream kinases and transcription factors, and transmit signals from the cell surface to the nucleus via distinct but related pathways. MAPK activation is achieved through kinase cascades, which include a MAPK kinase (MAPKK or MEK) and a MAPKK/MEK kinase (MAPKKK/MEKK). Several distinct MAPK subgroups have been identified, the best characterized of which are: extracellular signal regulated kinase (ERK) 1/2 (also known as p44/p42 MAPK), stress-activated protein kinase (SAPK)/c-jun N-terminal kinase (JNK) and p38 MAPK. The MAPK subgroups are activated by different extracellular stimuli and the signal transduced through kinase cascades, which only partially overlap. In general, the ERKs are largely involved in pathways leading to cell proliferation as a consequence of growth factor stimulation, whereas the JNK and p38 MAPKs are activated in response to a variety of cytokines and stress conditions, which include bacterial infection (Kyriakis & Avruch, 1996).

A variety of bacterial factors can stimulate or inhibit MAPKs. The best studied is LPS, which activates various MAPK pathways, in particular the p38 MAPKs (Cario et al., 2000). Jin et al. (2003) recently demonstrated that a C. jejuni cell-surface protein JlpA interacts with a host heat-shock protein Hsp 90, and activates NF-κB and p38 MAPK in Hep-2 epithelial cells. p38 MAPK plays an important role in regulation of proinflammatory responses to bacterial infections.
Host cell responses to bacterial components, and especially, host cell signal transduction, are considered to be important in the pathogenesis of disease caused by *C. jejuni* (Wooldridge et al., 1996; Wooldridge & Ketley, 1997; Hu & Kopecko, 1999; Konkel et al., 1999; Kopecko et al., 2001) and the resulting inflammation and fluid-loss from the gastro-intestinal mucosa. However, the actual host cell signalling pathways activated upon infection are not yet fully established. The aim of this study was to determine if MAPK activation could have a role in the signalling events associated with host cell responses to *C. jejuni*.

**METHODS**

**Bacterial isolates and growth conditions.** Bacteria were cultured in Mueller–Hinton broth and on Mueller–Hinton agar (Oxoid) and incubated at 37°C in a variable-atmosphere incubator (VAIN; Don Whitley) under microaerophilic conditions in an atmosphere of 6% hydrogen, 5% carbon dioxide, 5% oxygen and 84% nitrogen. All isolates and mutants were subcultured every 24–48 h from a Microbank stock stored at −80°C. Details of wild-type isolates and mutants are detailed in Table 1. Wild-type clinical isolates and mutants in putative virulence determinants (potentially involved in initiating inflammation within an infected host) were used for this study. Mutants were obtained from Dr Andrey Karlyshev and Dr Dennis Linton at the London School of Hygiene and Tropical Medicine, UK (11168 kpsM, cdtA, tlyA, peb3 and 11168/G1pddA, pglH) and Professor Julian Ketley at the University of Leicester, UK (11168 pglI, pglJ). Mutants were constructed using allelic replacement and insertion of a selectable marker (van Vliet et al., 1998).

**Caco-2 polarized adherence and invasion assay.** Adherence and invasion assays were performed with Caco-2 cells. These assays have been described elsewhere (Everest et al., 1992). Briefly, for the invasion assay, Caco-2 cells were seeded at 1 × 10^5 cells per well in 12-well plates (Costar) and allowed to grow until they reached confluence. The confluent monolayers were washed and inoculated with 10 μl bacterial suspension, containing varying numbers of bacteria for different experiments (between 100 and 10 000 c.f.u.). For most experiments the m.o.i. was between 100 and 1000 bacteria per eukaryotic cell. The infected monolayers were incubated for 2, 4, 8 and 24 h, respectively (Western blotting), or 24 h only (kinase experiments) for different experiments (between 100 and 10 000 c.f.u.). For most experiments the m.o.i. was between 100 and 1000 bacteria per eukaryotic cell. The infected monolayers were incubated for 2, 4, 8 and 24 h, respectively (Western blotting), or 24 h only (kinase assay) at 37°C in a 6% CO_2 humidified atmosphere to allow the bacteria to adhere to and invade the cells. To kill extracellular bacteria, 250 μg gentamicin ml⁻¹ was added after various incubation time points and the cells reincubated for 2 h before cell lysis and viable plate counts. Uninfected cells were included in the experiment as a control for kinase assays and Western blotting.

<table>
<thead>
<tr>
<th>Strain</th>
<th>Description</th>
<th>Reference</th>
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<tbody>
<tr>
<td>11168</td>
<td>Sequenced strain</td>
<td>Parkhill et al. (2000)</td>
</tr>
<tr>
<td>L115</td>
<td>Strain from child with severe colitis</td>
<td>Everest et al. (1992, 1993)</td>
</tr>
<tr>
<td>G1</td>
<td>Strain causing diarrhoea and Guillain–Barre syndrome</td>
<td>Linton et al. (2000)</td>
</tr>
<tr>
<td>NCTC 12189</td>
<td>Non-colonizing mutant of mouse and rabbit intestines</td>
<td>Dolby &amp; Newell (1986); Everest et al. (1993)</td>
</tr>
<tr>
<td>81-176</td>
<td>Clinical isolate causing inflammatory diarrhoea in human volunteer studies</td>
<td>Black et al. (1988)</td>
</tr>
<tr>
<td>11168kpsM</td>
<td>Capsule-minus mutant</td>
<td>Karlyshev et al. (2000)</td>
</tr>
<tr>
<td>11168cdaA</td>
<td>Cytotoxic distending toxin mutant (mutation in A subunit of toxin)</td>
<td>–</td>
</tr>
<tr>
<td>11168tlyA</td>
<td>Mutant in what is characterized as a hemolysin in other pathogenic bacteria but may be a possible methylase or regulator in <em>C. jejuni</em></td>
<td>Zhang et al. (2002); Martino et al. (2001)</td>
</tr>
<tr>
<td>11168/G1pddA</td>
<td>Mutant in contact-dependent phospholipase D</td>
<td>–</td>
</tr>
<tr>
<td>11168pglH</td>
<td>N-linked bacterial protein glycosylation</td>
<td>Karlyshev et al. (2004)</td>
</tr>
<tr>
<td>11168pglI</td>
<td>N-linked bacterial protein glycosylation</td>
<td>Larsen et al. (2004)</td>
</tr>
<tr>
<td>11168pglJ</td>
<td>N-linked bacterial protein glycosylation</td>
<td>Pei et al. (1991); Linton et al. (2002)</td>
</tr>
<tr>
<td>11168peb3</td>
<td>Major antigenic protein, transport protein/colonization factor</td>
<td>–</td>
</tr>
<tr>
<td>11168H</td>
<td>Hypermotile variant of 11168</td>
<td>Dr Andrey Karlyshev (London School of Hygiene and Tropical Medicine, UK)</td>
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Preparation of cell lysates. After the incubation period, the medium was removed and monolayers were washed twice with ice-cold PBS. Cells were solubilized using ice-cold cell lysis buffer A or B (see below) and scraped into 1·5 ml microcentrifuge tubes. Cell lysates were homogenized using a needle and syringe then centrifuged at either 18,000 g for 10 min at 4 °C (Western blotting) or 25,000 g for 20 min at 4 °C (kinase assay). The soluble fraction was transferred to a new microcentrifuge tube and stored at −80 °C for later analysis.

Cell lysis buffer. The following buffers were used to solubilize the cells: cell lysis buffer A was used to lyse the cells for analysis by Western immunoblotting (20 mM Tris pH 7·5, 150 mM sodium chloride, 1 mM EDTA, 1 mM EGTA, 1 % Triton-X 100, 2·5 mM sodium pyrophosphate, 1 mM β-glycerophosphate, 1 mM sodium orthovanadate, 1 μg leupeptin ml−1, 1 mM PMSF). Cells were lysed in buffer B for analysis by kinase assay (10 mM Tris, 150 mM sodium chloride, 2 mM EDTA, 2 mM DTT, 1 mM orthovanadate, 1 mM PMSF, 10 μg leupeptin ml−1, 10 μg aprotinin ml−1, pH 7·4 measured at 4 °C).

Immunoprecipitation. Cell lysates (200 μl) were incubated with primary antibody (1:50 to 1:100) (New England Biolabs) overnight with rocking at 4 °C. Samples were pre-cleared with Protein A agarose beads (Sigma) for 1 h at 4 °C. Samples were microcentrifuged for 30 s at 4 °C and the pellets were washed twice with 500 μl 1× cell lysis buffer A, being kept on ice throughout. The pellets were resuspended with 20 μl 2× Laemmli SDS-sample buffer (Sigma), vortexed and microcentrifuged for 30 s. The primary antibody used for immunoprecipitation was the same antibody used for Western blotting experiments.

SDS-PAGE and Western blotting. Samples were boiled for 5 min and lysate proteins were separated by SDS-PAGE on 12 % acrylamide gels. Proteins were transferred onto Immobilon-P membranes (Millipore). Non-specific binding sites on the membrane were blocked using 5 % non-fat dry milk in TBS/T (50 mM Tris, 150 mM NaCl, 0·1 % Tween 20). Membranes were incubated overnight with antibody specific to phosphorylated or total MAPK activity from activated phosphorylated MAPKs. Antibody specific to MAPK activity was determined to distinguish total cell MAPK activity from activated phosphorylated MAPKs. Antibody used for immunoprecipitation was the same antibody used for Western blotting. This tissue was precious and at a premium in terms of amount used; hence only one strain was used for explant infection.

MAPK inhibitors. Caco-2 cells were infected with C. jejuni 11168 and L115, with and without the presence of MAPK inhibitors. Cells were pre-treated for 1 h with inhibitors prior to infecting with bacteria. The inhibitors used in this experiment were PD98059 (ERK inhibitor) and SP600125 (JNK inhibitor). PD98059 was added at 50 μM and SP600125 was added at 60 μM. A p38 MAPK inhibitor (SB203580) was also used in this experiment (50 μM); however, problems with solubility were experienced, as the inhibitor precipitated in culture media. All inhibitors were obtained from Calbiochem.

Data analysis. Statistical analysis was performed using a Student’s t-test (two-tailed) and a P value <0·05 was considered to be significant. All data are expressed as the mean ± standard error of the mean.

RESULTS

Adherence and invasion of C. jejuni strains

Bacteria were left either extracellularly and intracellularly for some experiments, or representative monolayers were lysed after gentamicin treatment to determine if bacteria were intracellular in these assays. C. jejuni L115 adhered and invaded in numbers similar to those previously described for this strain (Everest et al., 1992). C. jejuni 11168 adhered at 5 × 103 c.f.u. and invaded at 5 × 102 c.f.u. Mutants derived from this strain (11168ΔcdaT and 11168ΔpI2A) adhered and invaded to similar levels. A hypermotile variant of 11168, 11168H, adhered and invaded in greater numbers compared with the sequenced strain (103-fold adhesion, 104-fold invasion; Karlyshev et al., 2004). Mutant strains 11168ΔpEB3 and 11168ΔpgfH had decreased adhesion and invasion compared with the parent strain, and 11168ΔkpsM had increased adhesion (8 × 104 c.f.u.) and culture plates and infected with wild-type or mutant strains of C. jejuni or uninfected as a control, for 24 h. Following the incubation period, the monolayers were washed with PBS and solubilized in cell lysis buffer, and the lysates analysed for kinase activity according to the assay protocol. The assay was based on measuring the degree of phosphorylation of a substrate highly selective for ERK1/2 to determine relative kinase activity in lysates from both the infected and uninfected cells.

Infection of human colonic tissue. Primary human colonic tissue explants were obtained from healthy sections of colon removed during surgery for colonic resection. Tissue explants (2 cm²) were cut into sections and divided into three pieces/sample for infection with C. jejuni and uninfected controls. They were cultured initially in DMEM with 10 % FCS and with added antibiotics for 1 h after arrival in the laboratory (penicillin/streptomycin; Gibco). After this period, tissue explants were washed, inoculated with similar numbers of bacteria as described above (or uninfected as a control) and incubated for 12 h at 37 °C, 6 % CO2 (medium was changed to antibiotic-free DMEM, 10 % FCS). Tissue was perfused with oxygen intermittently for maintenance of viability over the course of the experiment. At the end of the time points, portions of both infected and uninfected tissue were examined histologically (by fixation in formalin, wax embedding, sections cut by microtome and sections stained using haematoxylin and eosin) and both exhibited normal colonic morphology (data not shown). Explants were homogenized in cell lysis buffer A (as described above) for analysis by Western blotting. This tissue was precious and at a premium in terms of amount used; hence only one strain was used for explant infection.
markedly decreased invasion (10^3) compared with 11168. Strains G1, 11168pgll and pgfJ were not tested for adhesion and invasion ability (data not shown). MAPK activity did not differ markedly for monolayers where bacteria were both extracellular and intracellular or intracellular only.

**ERK1/2 (p44/p42 MAPK) kinase is activated in Caco-2 cells infected with *C. jejuni***

To detect ERK1/2 (p44/p42) MAPK activity in *C. jejuni*-infected Caco-2 cells, a kinase enzyme assay was performed. Radioactive counts varied between assays because of the varying specific activity of different batches of radioactivity, but uninfected controls at each time point allowed comparison within individual experiments and experimental replicates. Kinase activity was increased in *C. jejuni*-infected cells compared with uninfected controls. Results are shown in Fig. 1. Values shown are the means ± standard errors for eight replicates (n = 8). Results were statistically analysed using a paired t-test (P < 0.05 is significant). ERK1/2 activity was significantly higher in the *C. jejuni*-infected Caco-2 cells compared with the uninfected controls. Killed bacteria had no effect on kinase levels (data not shown).

This increase was independent of the strain of *C. jejuni* infecting the monolayers, since kinase activity was found to be increased in cells infected with all wild-type and mutant bacteria included in the experiment (Fig. 1). This increase was more than twofold in wild-type *C. jejuni* G1-infected cells. Strain 11168 showed a significant increase, as did strains L115 and 81-176 (data not shown). All mutants also had a significant effect on kinase activity (paired t-test); this was most marked with strain 11168tlyA and 11168H. Infection with the mutant strains 11168 kpsM, cdaA, pgllH, pgllJ, pglI, peb3 were not tested for adhesion and invasion ability (data not shown). MAPK activity did not differ markedly for monolayers where bacteria were both extracellular and intracellular or intracellular only.

**Phosphorylated ERK1/2 (p44/p42 MAPK) is increased in Caco-2 cells infected with *C. jejuni***

Immunoblot analysis of lysates from *C. jejuni* 11168- and L115-infected Caco-2 cells (although not a direct measure of kinase activity) detected differences in the amount of phosphorylated ERK1/2 in the infected cells compared with uninfected controls. Phosphorylation of ERK1 (p44) was upregulated at the later time points of 8 and 24 h (compared with uninfected controls) with strain L115 but only at 24 h with strain 11168 (Fig. 2a) compared with uninfected control cells. ERK2 (p42) phosphorylation was greatest at 2 h in uninfected cells, with an actual down-regulation of p42 activity in Caco-2 cells infected with L115 at this time point compared with uninfected controls (Fig. 2a). However, increased p42 phosphorylation was evident in L115-infected cells at 8 but not 24 h post-infection compared with uninfected Caco-2 cells. Total ERK1/2 levels (Fig. 2b) were compared with phosphorylated ERK1/2 to demonstrate that there was little change in total levels compared with phosphorylated forms.

**Phosphorylation of SAPK/JNK and p38 MAPK is increased in Caco-2 cells infected with *C. jejuni***

Involvement of the phosphorylated forms of other MAPKs in signal transduction of *C. jejuni*-infected cells were assessed by Western blot with antibodies to the phosphorylated forms of p38 and SAPK/JNK. Western blotting was used to examine levels of phosphorylation of p38 MAPK and SAPK/JNK in *C. jejuni* 11168- and L115-infected Caco-2 cells. Phosphorylation of p38 MAPK was greater at all time points with wild-type strains 11168 and L115 (Fig. 2c). Since SAPK/JNKs respond by and large to the same agonists as p38 MAPK, phosphorylation of SAPK/JNK was also increased at all the time points measured with both strains (Fig. 2c). This experiment has shown that *C. jejuni* infection of Caco-2 cells induces increased levels of the phosphorylated forms of both p38 and SAPK/JNK. Total...
p38 and SAP/JNK levels (Fig. 2d, f) were compared with phosphorylated MAPKs to demonstrate that there was little change in total levels compared with phosphorylated forms. Killed bacteria had no effect on phosphorylation levels for all kinases studied (data not shown).

**Phosphorylation of ERK1/2 (p44/p42 MAPK), SAPK/JNK and p38 MAPK is increased in primary human colonic tissue infected with *C. jejuni***

Primary human colonic tissue explants were also infected with *C. jejuni* 11168, and phosphorylation of ERK1/2, SAPK/JNK and p38 MAPK was greater in the infected tissue, compared with uninfected tissue at this time point (12 h) (Fig. 3). This experiment demonstrates that *C. jejuni* infection increases the phosphorylated forms of MAPKs inside primary human colonic tissue.

**MAPK activity in *C. jejuni*-infected Caco-2 cells can be decreased or inhibited by MAPK inhibitors**

Fig. 4 shows phosphorylation activity for different kinases in infected cells and inhibition of this activity by specific MAPK inhibitors. This demonstrates that the phosphorylated kinases are activated by *C. jejuni* infection. Specific inhibitors of these kinases decreased the activity of ERK1/2 and JNK upon bacterial infection. The p38 inhibitor precipitated out of solution for these experiments so we were unable to determine specific inhibition for p38 MAPK.

**DISCUSSION**

In this study, we have provided evidence that *C. jejuni* infection of cultured intestinal epithelial cells and human colonic tissue results in the activation of the MAPKs ERK1/2, JNK and p38. Activation of these pathways could play...
an important role in the pathogenesis of Campylobacter infection.

ERK1/2 (p44/p42) kinase activity and phosphorylated forms are both increased inside infected cells at comparatively late time points. Thus, increased phosphorylation of ERK1 occurred at both 8 and 24 h in cells cultured with strain L115, but not until 24 h in those cultured with strain 11168. This activation is increased in time when compared with other enteric pathogens. Strain 11168 is known to be less motile than the original parent isolate (Gaynor et al., 2004), or hypermotile variants of this strain, and so it would take more time for the strain to adhere to host cell surfaces and invade compared with a more motile isolate. This delay in getting to the cell surface probably accounts for the delayed activation of phosphorylated ERK inside infected cells. The phosphorylation of p38 MAPK and JNK was upregulated at the earlier, as well as the later, time points with both strains 11168 and L115, perhaps because this particular pathway is involved in a more immediate response to stress. Thus, MAPK activity in infected Caco-2 cells has differential time expression for the different kinases, with ERK1/2 being activated later than both p38 and JNK. Assay conditions in terms of gaseous requirements favour the eukaryotic cells not the bacteria, again perhaps delaying MAPK activation. Caco-2 cell MAPK activation upon infection with C. jejuni correlated with demonstrated increases in the in vitro infected human primary colonic tissue. Although the time points were not the same, we have demonstrated clear increases in the phosphorylated forms of MAPKs in infected human tissue. These results show that in vivo tissue culture cell models mimic the interaction of C. jejuni with human colonic tissue and justify their use in studies of C. jejuni pathogenesis.

MAPKs can also be activated via receptor tyrosine kinases and transduction of extracellular signals via clustering of integrins (Giancotti & Ruoslahti, 1999). This may be of particular relevance to enterocyte infection by C. jejuni. C. jejuni binds to fibronectin via CadF protein (Monteville & Konkel, 2002, Monteville et al., 2003), and this protein is involved in both adhesion and invasion of C. jejuni into epithelia (Monteville & Konkel, 2002, Monteville et al., 2003). Fibronectin is bound to cell-surface integrin molecules on the basolateral cell surface and integrin clustering is associated with tyrosine phosphorylation of cellular cytoplasmic contents leading to host cell cytoskeletal rearrangements, presumably facilitating bacterial entry into the cell (Giancotti & Ruoslahti, 1999). Tyrosine kinase inhibitors also decrease C. jejuni invasion inside host cells (Wooldridge & Ketley, 1997; Wooldridge et al., 1996). Thus, we have a link between campylobacter–host cell adhesion and invasion, integrin clustering, receptor tyrosine kinase activation and phosphorylation with MAPK cellular pathway activation in C. jejuni infection of host enterocytes.

Campylobacter is most likely to activate MAPKs by bacterial adhesion and invasion or a bacterial component, engaging a cell-surface receptor and the signal being transduced to the relevant signalling pathway via upstream regulators. Indeed, this ligand–receptor interaction has been demonstrated by C. jejuni JlpA surface protein binding to Hsp 90 resulting in p38 MAPK activation (Jin et al., 2003). Bacterial LPS can also trigger MAPK activation (Cario et al., 2000). Shigella flexneri invasion and intracellular LPS can also activate JNK (Girardin et al., 2001). JNK phosphorylates c-Jun, which is a component of the transcription factor AP-1, also considered to be an important regulator of the inflammatory response (Foletta et al., 1998). Previous studies have shown MAPK activation to be associated with infection of epithelial cells by various enteropathogens (Hobie et al., 1997; Warny et al., 2000; Meyer-ter-Vehn et al., 2000; Berin et al., 2002). A number of other bacteria or bacterial factors have been shown to modulate the activity of various MAPKs. For example, Escherichia coli LPS can activate p38 MAPK (Berin et al., 2002), Salmonella LPS can activate ERK via PI-3-kinase/phospholipase D pathways (Hobie et al., 1997), Clostridium difficile toxin A can activate p38, ERK and c-Jun (Warny et al., 2000), Helicobacter pylori stimulates ERK 1 and 2 (Meyer-ter-Vehn et al., 2000), and Yersinia YopJ/P inhibits p38 and JNK MAPKs (Orth et al., 1999). Inhibitors of MAPK pathways are being increasingly used to determine the role of MAPK pathways in the control of inflammation due to bacterial infection. After
reporting that *Listeria monocytogenes* stimulates ERK1/2 activity upon attachment to HeLa cells, Tang *et al.* (1998) have further shown that actual invasion of the cell requires the ERK2 pathway. *S. typhimurium* has been shown to activate ERK, JNK and p38 MAPKs in intestinal Henle-407 and HeLa cells. The authors have associated this with an increase in the production of the proinflammatory cytokine IL8 (Berin *et al.*, 2002). Enteropathogenic *E. coli* (EPEC) infection has also been shown to activate ERK1/2, this time in T84 cells, and again this was associated with the inflammatory response, since IL8 expression and the degradation of IkBz (the primary inhibitor of NF-κB) were stimulated (Czerucka *et al.*, 2001). The secretion of IL8 from the basal aspect of infected intestinal cells has been shown to be involved in the transepithelial migration of neutrophils during the inflammatory response to *C. jejuni* (Hickey *et al.*, 1999, 2000). In contrast, YopJ/P from *Yersinia* spp. has been found to inhibit MAPK activity (Orth *et al.*, 1999).

Our results indicate that the host enterocyte responds to *C. jejuni* infection by stimulation of MAPK signalling pathways. These responses may lead to host inflammatory reactions in terms of proinflammatory cytokine release and accumulation of neutrophils within the infected intestinal mucosa. Indeed such events characterize the microscopic histology of *C. jejuni* enterocolitis (Lambert *et al.*, 1979; Price *et al.*, 1979, 1984; van Spreeuwel *et al.*, 1985). Understanding the signalling events triggered by *C. jejuni* infection may lead to the development of novel therapeutic strategies to limit the clinical consequences of inflammatory diarrhoea.

**ACKNOWLEDGEMENTS**

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**REFERENCES**


