Campylobacter jejuni-infected human epithelial cell lines vary in their ability to secrete interleukin-8 compared to in vitro-infected primary human intestinal tissue

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Campylobacter jejuni causes symptoms of acute inflammatory diarrhoea in man. C. jejuni interaction with epithelial cells elicits interleukin-8 (IL-8) production, and IL-8 recruits neutrophils to sites of infection. Cell culture models of bacterial interaction with epithelium are useful to define bacteria–host interaction and are used because it is thought they mimic the same bacteria–host cell interaction in the natural disease. This study looks at the ability of C. jejuni strains to elicit IL-8 production from a variety of cell lines previously used for investigating the interaction of C. jejuni with host cells. A spectrum of IL-8 responses was observed, with minimal IL-8 elicited from Caco-2 cells and more marked responses elicited from HeLa and T84 cells. These in vitro-infected cell line responses were compared to IL-8 production from in vitro C. jejuni-infected human colonic and ileal tissue. The in vitro-infected tissue elicited the highest IL-8 responses and the cytokine was manifested earlier compared to the infected cell lines.

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 (Ketley, 1997; Konkel et al., 2001; Skirrow & Blaser, 2000; Skirrow, 1986; Wassenaar & Blaser, 1999). The mechanisms by which C. jejuni causes disease in the host are not fully understood; however, host inflammatory responses to the organism are likely to contribute to a large extent to the pathology observed (Everest et al., 1993a, b; Hickey et al., 2003; Bakhiet et al., 2004; Hickey et al., 1999, 2000; Hu & Hickey, 2005). Like other human enteric bacterial pathogens, C. jejuni infects humans by colonizing the mucus layer of the intestine followed by adherence to and invasion of intestinal epithelial cells (Everest et al., 1992; Harvey et al., 1999; Kopecko et al., 2001). These cells are likely to initiate the host inflammatory response, since they constitute one of the first physical barriers to the pathogen (Everest, 2005; Mellits et al., 2002; Sansonetti, 2002; Strober, 1998). In response to infection, the intestinal epithelial cells release pro-inflammatory cytokines which recruit neutrophils, macrophages and other cells involved in the immune response to the site of injury. A number of previous studies have employed an in vitro cell invasion model to study C. jejuni-induced pro-inflammatory cytokine secretion. Caco-2 cells (human colon carcinoma) are generally accepted as a model for colonization and invasion by C. jejuni; however, a detailed study of their role in the cytokine response has not previously been undertaken. In fact, a detailed comparison of cytokine secretion from different human cell lines infected with C. jejuni has not been performed and in order to determine their relevance to what is occurring in vivo in a C. jejuni-infected host in terms of eliciting inflammation, we undertook the current study. Interleukin-8 (IL-8) is a potent chemo-attractant and immune cell activator and likely to be very important in the host immune response to C. jejuni in vivo. Furthermore, it has been shown to be secreted by Int407 cells in response to C. jejuni exposure in vitro, in a dose-dependent manner (Al-Salloom et al., 2003; Bakhiet et al., 2004; Hickey et al., 1999, 2000; Hu & Hickey, 2005).

In this current study, IL-8 secretion from a number of human cell lines infected with C. jejuni was measured, in order to determine which cell line(s) provide a relevant model (reflecting what occurs in vivo) for the study of host cell cytokine responses to the organism. In addition, for comparison, we included in the study primary human intestinal tissue obtained from healthy ileum and colon removed during elective surgery. This allowed us to examine host inflammatory responses to C. jejuni in a whole-tissue...
system, and to compare infected in vitro cell lines with in vitro C. jejuni-infected human intestinal tissue. We wished to determine the validity of the cell culture models used and ascertain if they reflect cytokine responses induced by infected intestinal tissue, the site of C. jejuni-mediated inflammation and disease.

METHODS

Bacterial isolates and growth conditions. Bacteria were grown in Mueller–Hinton broth and 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 were subcultured every 24–48 h from a Microbank stock stored at −80 °C. The strains of C. jejuni used in this study were 11168 and L115. Both are wild-type strains, 11168 being fully sequenced (Parkhill et al., 2000) and L115 being derived from a child with severe colitis (Everest et al., 1992, 1993a).

Cell culture. Caco-2 (human colon carcinoma) cells were maintained in Dulbecco’s minimal essential medium (DMEM) with Glutamax-1 (Gibco) and supplemented with 10% fetal calf serum (FCS; Gibco). Int407 and HeLa (human cervical carcinoma) cells were grown in MEM supplemented with 2 mM glutamine (Gibco), 1% non-essential amino acids (NEAA; Gibco) and 10% FCS. HT29 (human colon carcinoma) cells were maintained in McCoy’s 5a medium (Sigma) with 2 mM glutamine and 10% FCS. T84 (human colon carcinoma) cells were grown in Ham’s F12 and DMEM (1:1), also supplemented with 2 mM glutamine and 10% FCS. All cell lines were grown routinely in a 75 cm² flask (Costar) at 37 °C in a 5% CO₂ humidified incubator. Confluent stock cultures were trypano-sedimented (1% trypsin/EDTA; Gibco) and new stock cultures were seeded at ~10⁶ cells per cm² in 12-well plates (Costar) and incubated until confluent. Antibiotics were not used. The final cell concentration at the time of infection was approximately 1 × 10⁶ cells per well.

Infection of human cell lines. Cell monolayers were inoculated with 50 μl bacterial suspension, containing ~1 × 10⁶ c.f.u. per well. The amount of bacteria added was standardized between experiments by measuring the optical density of the bacterial suspension, ensuring that the same number of bacteria was added each time. The infected monolayers were incubated for 2, 4, 8, 24 and 48 h at 37 °C in a 6% CO₂ humidified atmosphere to allow the bacteria to adhere to and invade the cells. Uninfected cells were included in the experiment as a control. At the end of each time point the cell supernatants were collected into Eppendorf tubes, particulate material was removed by centrifugation and the samples aliquoted and stored at −20 °C until analysis by cytokine ELISA.

IL-8 studies. The human Quantikine IL-8 ELISA kit from R&D systems (D8000C) was used to measure IL-8 secretion from infected and uninfected cells or tissue. The assay was based on the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for IL-8 was pre-coated onto a microplate. Standards and samples were pipetted into the wells and any IL-8 present was bound by the immobilized antibody. After washing away any unbound substances, an enzyme-linked polyclonal antibody specific for IL-8 was added to the wells. Following a wash to remove any unbound antibody-enzyme reagent, a substrate solution was added to the wells and colour developed in proportion to the amount of IL-8 bound in the initial step. The colour development was stopped and the intensity of the colour was measured using a microplate reader.

Data presentation. All data are expressed as the mean ± SEM. All values shown are the mean of at least three replicates. Statistical analysis was performed using a Student’s t-test and P < 0.05 was considered to be significant.

RESULTS

C. jejuni-infected Caco-2 cells are low secretors of IL-8

Basal levels of IL-8 secretion from this cell line were very low (50–100 pg ml⁻¹). Infected Caco-2 cells did not secrete significantly greater levels of IL-8 compared to the uninfected cells at any time point examined (data not shown).

C. jejuni-infected Int407 and HeLa cells demonstrate elevated IL-8 secretion

Overall basal levels of IL-8 secretion from Int407 cells were considerably higher than those detected for the Caco-2 cells (600–800 vs 50–100 pg ml⁻¹; Fig. 1). Levels of IL-8 secretion were significantly increased in the infected cells compared to the uninfected cells at 48 h (1.5-fold increase over uninfected) with one strain of C. jejuni (L115). Basal levels of IL-8 secretion from the HeLa cells were much lower than those observed for Int407 (50–100 vs 600–800 pg ml⁻¹). The effect of C. jejuni infection upon HeLa cells was striking, since infected cells produced significantly greater levels of IL-8 compared to the uninfected cells at four different time points (4, 8, 24 and 48 h) and with both strains of C. jejuni. This increase was most marked at 48 h, when the increase of infected over uninfected was 22-fold for L115 infected cells (50 vs 1100 pg ml⁻¹).
HT29 and T84 cells secrete increased levels of IL-8 in the presence of *C. jejuni*

Basal levels of IL-8 secretion from HT29 cells were between 300 and 350 pg ml$^{-1}$. Infected HT29 cells did demonstrate significantly higher levels of IL-8 secretion than uninfected (1.5- and 1.6-fold) at two time points (8 and 48 h), but this was with only one of the *C. jejuni* strains tested per time point (i.e. 11168 at 8 h and L115 at 48 h; Fig. 2). T84 cells demonstrated the greatest basal levels of IL-8 secretion (uninfected cells 750–850 pg ml$^{-1}$). However, significantly elevated IL-8 secretion was detected from the infected cells compared to uninfected at three time points (8, 24 and 48 h) and with both strains of *C. jejuni* (1.37–1.78-fold increase). T84 cells were therefore responsive in terms of IL-8 secretion and the effects of *C. jejuni* infection were clear for both strains at later time points.

*C. jejuni*-infected human tissue explants from the ascending colon, transverse colon and terminal ileum secrete increased levels of IL-8

IL-8 secretion from ascending colon tissue infected with *C. jejuni* L115 was significantly higher than that from uninfected tissue from the same section of the colon (top Transwell; 2278 ± 57 vs 1818 ± 48) (Fig. 3). Tissue obtained from the transverse colon secreted increased levels of IL-8 in the presence of both *C. jejuni* strains when compared to uninfected tissue from the same region (top Transwell; 11168, 3284 ± 193; L115, 4007 ± 271; vs uninfected, 2291 ± 53). Secretion of IL-8 from terminal ileum tissue showed a particularly marked increase over uninfected and this occurred with both strains of *C. jejuni*. This increase was sevenfold over the uninfected controls in media collected from the top Transwell (11168, 2957 ± 212; L115, 3286 ± 27;...
Fig. 3. IL-8 secretion from primary tissue (ascending or transverse colon or terminal ileum) infected with *C. jejuni* 11168 and L115 for 3 h. Supernatants from primary human colonic tissue infected with strains 11168 and L115 were analysed by ELISA for IL-8 concentration. Supernatants were collected from the top well of the Transwells (tissue was cultured in the top well). An uninfected control was included. Values are the mean ± SEM of three replicates. Results were statistically analysed using a Student’s *t*-test, where *P* < 0.05 is significant. Statistical significance is shown as an asterisk (*). Note the difference in scale from the other figures.

These increases in IL-8 secretion from the primary tissue occurred much earlier than that seen with the cell line models, since the increases were apparent only 3 h post-infection. The levels and differences in IL-8 secretion were most apparent in media collected from the top well of the Transwells, rather than from the bottom well.

**DISCUSSION**

In this study, five human cell lines were examined for the ability to respond to *C. jejuni* infection by the production of IL-8 and the findings showed that IL-8 secretion in response to *C. jejuni* infection varied markedly, depending on the cell line used. It is unclear why Caco-2 cells secreted low levels of IL-8 and did not show a significant IL-8 response to *C. jejuni*. It could be that Caco-2 cells produce little or no cytokines in the absence of polymorphonuclear leucocytes (PMNs), supporting previous suggestions that there is PMN–epithelial cell cross-talk co-ordinating the cytokine response in the gut mucosa (Strober, 1998). Previous studies examining the IL-8 response from Caco-2 cells in the presence of other bacteria have found that the cytokine response is conditioned by the natural bacterial product, butyrate, which exerts a stimulatory effect on IL-8 secretion and modulates its release in response to lipopolysaccharides. This has been demonstrated with *Desulfovibrio desulfuricans* (Weglarz *et al.*, 2003) and with *Salmonella enterica* serovar Enteritidis (Malago *et al.*, 2005). It appears then, that additional stimulation of Caco-2 cells may be required to cause higher levels of cytokine induction.

Int407 cells were originally derived from the jejunum and ileum of a 2-month-old Caucasian embryo; however, this cell line was subsequently contaminated by the HeLa cell line (Masters, 2002) and stocks from culture collections supplying Int407s contain HeLa cells. It is therefore classified as a human cervical carcinoma cell line rather than an intestinal epithelial cell line. However, because it is so widely used in studies of *C. jejuni* interaction with intestinal epithelium, we considered it essential to include this cell line in the study. Int407 cells are more responsive to *C. jejuni* in terms of IL-8 secretion than Caco-2 cells. When the experiments were repeated using the HeLa cell line itself, these cells were also very responsive in terms of IL-8 secretion when infected with *C. jejuni*. It is therefore unclear if the different response by the Int407 compared to the Caco-2 cells was because they were originally obtained from a different part of the intestine, or because they are HeLa contaminated. It is also unclear as to why the basal level of IL-8 from uninfected Int407 cells is much higher than for uninfected HeLa cells. Presumably the cells of intestinal origin still present in the Int407 monolayer are secreting much higher levels of background IL-8 compared to the HeLa contaminants.

The findings from the primary tissue explants suggest that the anatomical region of the intestine may play an important role in determining the level of the innate immune response to *C. jejuni*. It appears that tissue from the terminal ileum is particularly responsive in terms of IL-8 secretion, suggesting that the innate immune response may be strong in this part of the intestine. Tissue was taken from the terminal ileum, which may contain more antigen-sampling cells and hence be more immunologically responsive to infection. IL-8 secretion by the primary tissue (both ileum and colon) was greatly increased in terms of amount over that secreted by the cell lines and this increase occurred much earlier with the primary tissue at 3 h post-infection. This can probably be attributed to the full range of inflammatory cell types present in the primary tissue, allowing cross-talk of cells and enhancement of the innate immune response. Primary tissue is precious and difficult to get in large amounts but provides perhaps the 'gold standard' in terms of bacteria–host interaction because it contains all the cell types and tissue architecture encountered by *C. jejuni in vivo*. However, the anatomical site from which the tissue was originally obtained must be documented and considered in any observed response. Understanding the interaction between the intestinal epithelium and *C. jejuni* will allow us to understand more clearly *C. jejuni*-induced disease in the host.

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**REFERENCES**


