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Campylobacter-induced interleukin-8 responses in human intestinal epithelial cells and primary intestinal chick cells

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Abstract

Campylobacter (*C.*) *jejuni* and *C. coli* can cause gastrointestinal disorders in humans characterized by acute inflammation. Inflammatory signals are initiated during interaction between these pathogens and human intestinal cells, but nothing is known about the stimulation of avian intestinal cells by *Campylobacter*. Interleukin-8 (IL-8) as a proinflammatory chemokine plays an important role in mobilizing cellular defence mechanism. IL-8 mRNA expression in both human intestinal cells (INT 407) and primary intestinal chick cells (PIC) was determined by quantitative real-time RT-PCR. The secretion of IL-8 protein by INT407 was measured using ELISA. Although *C. jejuni* and *C. coli* are considered to be harmless commensals in the gut of birds, the avian *Campylobacter* isolates investigated were able to induce the proinflammatory IL-8 in PIC as well as in INT407. In an in vitro system, *C. jejuni* as well as *C. coli* were able to induce IL-8 mRNA in PIC. Relation between the virulence properties like toxin production, the ability to invade and to survive in Caco-2 cells and the level of IL-8 mRNA produced by INT 407 and PIC after infection with *Campylobacter* strains was also investigated.
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1. Introduction

Campylobacter (*C.*) *jejuni* and its close relative *C. coli* are important human pathogens. They can cause diseases such as gastroenteritis characterized by severe inflammation of the intestinal mucosa with an influx of professional phagocytes (Ketley, 1997; Altekruse et al., 1999; Jones et al., 2003). Campylobacteriosis is described as a multifactorial process involving the intake of the *Campylobacter* strains in the gastrointestinal tract, followed by adherence to intestinal epithelial cells, secretion of virulence proteins and cell invasion (Raphael et al., 2005). Epithelial cells are able to secrete chemotactic mediators after contact with pathogenic bacteria as described for *Salmonella typhimurium*, *Helicobacter pylori* and others (Thorpe et al., 1999; Aubert et al., 2000; Gewirtz et al., 2000; Bäckhed et al., 2003). They

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deliver the initial signals for the immune response of the host (Eckmann et al., 1995). Chemotactic mediators belonging to the family of C-X-C chemokines, such as interleukin-8 (IL-8), play a major role in mobilizing cellular defence mechanisms to eliminate bacteria by recruiting and activating neutrophils and T cells (Kagnoff and Eckmann, 1997). It has been shown on a range of human-derived epithelial cell lines that *C. jejuni* is able to induce proinflammatory chemokine release, especially IL-8 (Hickey et al., 2000; Mellitis et al., 2002; Bakhiet et al., 2004; Watson and Galan, 2005). IL-8 as a potent stimulator of neutrophil activation and chemotaxis within the intestinal mucosa is associated with numerous acute and chronic inflammatory reactions (Sturm et al., 2005). It is undisputed that one of the main risk factors for human diseases is eating or handling of poultry meat contaminated by *Campylobacter* strains. Many reports describe that *Campylobacter* strains vary in their ability to resist environmental stressors during poultry processing (Alter et al., 2005; Newell et al., 2001). The relative proportion of *Campylobacter* subtypes changes during this processing, hence the surviving subtypes can reach the food chain. Normally, *C. jejuni* and *C. coli* colonize the gastrointestinal tract of many birds including chicken and turkeys and other animals as harmless commensals with little or no pathology (Newell and Fearnley, 2003; Hendrixson and DiRita, 2004). Some strains, however, were described to be invasive and/or toxigenic and may cause distension of the intestine, liver abnormalities and diarrhoea in chicken (Saleha et al., 1998). Nothing is known about the stimulation of avian intestinal cells by *Campylobacter*. Recently, the expression of proinflammatory cytokines in response to *Campylobacter* infection in avian primary chick kidney cells and in an avian macrophage cell line has been reported (Smith et al., 2005). However, the effect of *Campylobacter* strains on intestinal chick cells has not been reported until now.

The aim of our work was to investigate whether *Campylobacter* isolates from the end of poultry processing and characterized by specific virulence properties are able to induce IL-8 in primary intestinal chick cells (PIC cells) following in vitro incubation. The reactions of the chick cells were compared with responses of human intestinal epithelial cells (INT407) after stimulation with these strains.

2. Materials and methods

2.1. Bacteria strains

Six *C. jejuni* and two *C. coli* isolates (Table 1) from turkey carcasses at the end of the slaughter process were kindly provided by T. Alter (Institute of Food Hygiene, University of Leipzig, Germany). The isolates were routinely grown on Mueller–Hinton (MH) agar plates (Institut für Immunpräparate und Nährmedien, Germany) for 24 h at 37 °C under microaerophilic conditions (85% N_2, 10% CO_2, 5% O_2). The bacteria were harvested in phosphate buffered saline (PBS), pH 7.2 and adjusted to an optical density of 0.45 at 588 nm (Photometer CADAS 30, Lange GmbH, Germany) corresponding to about 5×10^8 bacteria/ml. Before the *Campylobacter* challenge, the bacterial suspensions were diluted 1:10 with cell culture medium containing 1% foetal calf serum (FCS, Gibco, Germany). The number of bacteria was determined by plate counting.

The putative virulence properties like survival in Caco-2 cells over 48 h as well as production of cytolethal distending toxin (CDT) and cytolethal rounding toxin (CRT) have been reported for these strains (Table 1, Hänel et al., 2007).

The *C. jejuni* strain 81–176 from human origin and its CDT-deficient mutant were kindly provided by C.L. Picket, University of Kentucky.

Table 1
Characterization of *Campylobacter* isolates

Strains	Reference number[a]	Survival in Caco-2 cells after 48 h	Toxin titer[b]
C. jejuni	av 245	+	CDT 1:16
C. jejuni	av 322 B	+	CDT 1:16
C. jejuni	av 67/3	–	CDT 1:8
C. jejuni	av 356	–	CRT 1:64
C. jejuni	av 347	–	CRT 1:32
C. jejuni	av 64/3	–	CDT 1:8
C. coli	av 352	+	None
C. coli	av 321 A	+	None
C. jejuni	81–176	n.d.	CDT 1:64
C. jejuni	81–176 *cdt* mutant	n.d.	None

CDT: cytolethal distending toxin; CRT: cytolethal rounding toxin; n.d.: not done.
[a] Abbreviation: (Alter et al., 2005).
[b] (Hänel et al., 2007).

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