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Campylobacter Jejuni Increases Transcribed Il-1B and Causes Morphometric Changes in the Ileal Enterocytes of Chickens

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Campylobacter jejuni, chicken, explants, TGF-β3, IL-1β.

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ABSTRACT

Campylobacteriosis is a worldwide foodborne zoonosis disease caused by *Campylobacter jejuni*. This microorganism is considered a commensal bacterium in chicken hosts. *C. jejuni* produces epithelial cell modifications and induces a cytokine gene transcription innate immunity repertoire. In the present study, we describe the invasiveness, morphological cellular modifications, and transcript level expressions of innate immune cytokines from *C. jejuni*-inoculated chicken ileum explants. *C. jejuni* was internalized by epithelial ileum cells at 15 minutes postinoculation (p.i.) and was detected intracellularly for 4hs (p.i.). Inoculated explants displayed significant increases in cell height. *C. jejuni* induced a significant elevation of Transforming Growth Factor Beta 3 (TGF-β3) and Interleukin-1β (IL-1β) transcripts. In conclusion, *C. jejuni* is internalized in explanted epithelial ileum cells, produces morphological cell modifications, and induces gene transcription of both anti-inflammatory and pro-inflammatory cytokines.

INTRODUCTION

Campylobacteriosis is a worldwide foodborne bacterial zoonosis caused by *Campylobacter jejuni* (De Perio *et al.*, 2013; Guyard-Nicodème *et al.*, 2015). Contaminated chicken meat is the most common source of human infection by *C. jejuni* bacteria (De Carvalho *et al.*, 2013). The human disease is characterized by a robust intestinal inflammatory response with bloody, watery diarrhoea (Sjöling *et al.*, 2015). Despite its commensal behaviour in chicken hosts, *C. jejuni* is able to invade the cytoplasm of poultry enterocytes and survive in the mucus layer at the brush border of epithelia (Van Deun *et al.*, 2008). *C. jejuni*-induced modifications of the enterocyte microvillus or mucosal crypts were described after experimental infection (Awad *et al.*, 2015).

It has been demonstrated that both the addition of *C. jejuni* antigens to primary cell cultures of chicken embryo intestines (Li *et al.*, 2008) and inoculation of these antigens in live chickens (Barjesteh *et al.*, 2013; Humphrey *et al.*, 2014) cause inflammatory response and early elevation of the innate immune response of cytokine mRNA expression. Among chicken cytokines, Interleukin-1β (IL-1β) has pro-inflammatory effects, while the Transforming Growth Factor-β (TGF-β) has anti-inflammatory properties (Schat, Kaspers, & Kaiser, 2014).

Animal intestinal explants have been used to investigate the relationship between luminal bacteria and the gastrointestinal immune system (Low *et al.*, 2006; Skoczek *et al.*, 2014). Human explant models were used for the determination of cell damage and the expression of pro-inflammatory cytokines in colitis studies and for the investigation of the interaction between enteropathogenic bacteria, such as *Salmonella typhimurium* and *C. jejuni,* and the host immune system (Haque *et al.*,

2004; Harvey *et al.*, 2014). *C. jejuni* behaviour and the chicken innate immune response to this agent have been described by the examination of the intestinal sections of experimentally-infected live chickens (Lamb-Rosteski *et al.*, 2008). Attempts to investigate the most significant age of the chicken host for *C. jejuni* infection (2 to 3 weeks old) could be relevant for focusing on 3-week-old chickens (Chaloner *et al.*, 2014).

In this study, we investigated whether *C. jejuni* could be internalized by the host cell, cause morphological alterations in epithelial cells, and induce the transcription of cytokine genes of the innate immunity repertoire in the experimentally *C. jejuni*-infected chicken ileum explants.

MATERIAL AND METHODS

Chicken and ileum explants

Four 25-day-old specific pathogen free (SPF) broilers, tested negative for *C. jejuni*, were euthanized for the collection of ileum explants. Explants were inoculated with the *C. jejuni* strain IAL2383 (Fonseca *et al.*, 2014) at a dose level of $2x10^7$ CFU, diluted in saline solution at a final volume of 100μL. The control was inoculated with 100μL of the saline solution. All the experiments were performed in triplicate. This study was approved by the Ethics Committee on the Use of Animals (CEUA) of the Universidade Federal de Uberlândia (Minas Gerais, Brazil), number 057/09 and number 323/09.

Invasiveness

The invasiveness assay was carried out as previously described (Van Deun *et al.*, 2008). *C. jejuni*-inoculated ileum explants were evaluated 15, 30, and 60 minutes (min.) and 2 and 4hs post-inoculation (p.i.). Non-internalized bacteria were removed by successive washing procedures or by treatment with the antibiotic gentamicin (100μg/mL). Internalized bacteria were recovered by the permeabilization of the cytoplasmic membrane with 1% Tritom-X-100. *C. jejuni* quantification was performed using real-time PCR (BaxÒSystem, DuPont, USA).

Morphological epithelial cell alterations

Morphological epithelial cell alterations were detected in *C. jejuni*-inoculated ileum explants at 15 and 60 minutes p.i. Explants were processed by histology and stained with eosin-haematoxylin. Enterocyte morphology and morphometry were evaluated by optical microscopy at 1000x magnification (Olympus BX 40 microscope, USA). Morphometric analyses were performed using Image HL software (Western Vision Software, USA).

Cytokine transcripts

Transcripts of cytokines and chemokines (IL-1β, IL-8, IL-6, Transforming Growth Factor-2 [TGF-2] and CXCL2) were quantified using quantitative real-time PCR (qPCR) and reverse transcription at 15, 30, and 60 minutes, and 2 and 4hs (p.i.). For detection of complementary DNA (cDNA) from the RNA transcript, PCR was performed in real time for each gene using as a reference to the endogenous gene beta actin.

Statistical analysis

The data were statistically analysed using one-way analysis of variance (ANOVA) and Tukey's test. A p-value ≤ 0.05 was considered significant.

RESULTS AND DISCUSSION

C. jejuni bacteria were internalized by the epithelial cells of the *C. jejuni*-inoculated chicken ileum explants during the first 15 min (p.i.) and the concentration of intracellular bacteria (log CFU/g) was unaltered throughout the invasiveness experiment (Figure 1). The dynamics of *C. jejuni* invasion in cultured human cells (De Melo, Gabbiani, & Pechère, 1989) includes bacterium adherence to the host cell surface and internalization of bacteria around 1h (p.i.), as well as intracytoplasmic bacteria during the interval of time from 3h to 9h (p.i.). Additionally, it was demonstrated that *C. jejuni* has the ability to invade different cultured cell lines and that its

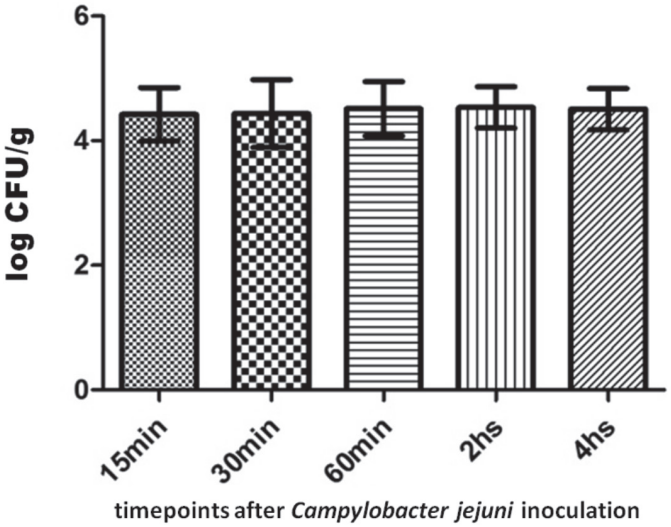

Figure 1 – Dynamics of bacterium invasiveness in Campylobacter jejuni-infected chicken ileum explants at different times post infection.

invasiveness is dependent on the host species (Aguilar *et al.*, 2014). Our results corroborate the occurrence of bacterium internalization in cultured primary chicken cells, as previously described (Byrne, Clyne, & Bourke, 2007), and may suggest that chicken ileum explants could be an alternative method to investigate *C. jejuni* invasiveness. Curiously, although mucin proteins in chicken intestines reduces *C. jejuni* internalization by the host cell (Alemka *et al.*, 2010), that phenomenon was apparently not impaired in the *C. jejuni*-inoculated chicken ileum explants.

There was a significant increase in the height of epithelial cells in the *C. jejuni*inoculated ileum explants (Figure 2). We did not detect disruptions in the structure of the microvilli or crypts (Figure 2). *In vitro*, the inoculation of *C. jejuni* in the cell monolayer has been shown to increase paracellular permeability and the transepithelial flux of the cultured cells (Lamb-Rosteski *et al.*, 2008). Additionally, *C. jejuni* disrupts intercellular junctions, increasing the width of the infected cells compared with the controls (Wine, Chan, & Sherman, 2008). Cytolethal Distending Toxin (CDT) induces a cytoplasmic distension and leads to cell death (Jeon, Itoh, & Ryu, 2005). It seems that the epithelial cell modification observed in the inoculated chicken ileum explants was an effect of bacterium on cells.

In the present study, we showed a significant elevation of TGF-β3 and IL1β mRNA transcript

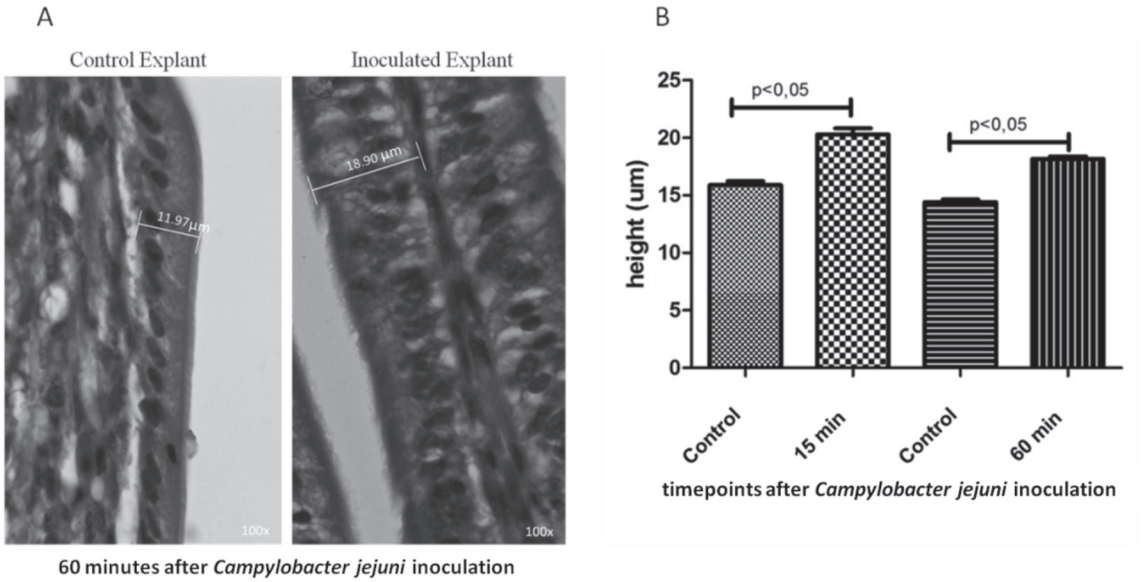

Figure 2 – Morphometry from inoculated explants: (A) micrography of Campylobacter jejuni-inoculated chicken ileum explants at 60 minutes postinfection; (B) average of epithelial cell height from C. jejuni-inoculated chicken ileum explants at two different times.

levels, which reached a peak at 15 min and 4h (p.i.), respectively (Figure 3). No statistical difference was found in the transcript levels of IL-6, IL-8, or of CXCL1 and CXCL2 mRNA transcripts. Bacterial pathogen-associated molecular patterns (PAMPs) recognized by pathogen recognition receptors (PRRs), which are expressed by intestinal epithelial cells, result in cytokine gene transcription (IL-1β, IL-6, IL-8 and chemokynes) from the innate immune repertoire (Keeler *et al.*, 2007; Wigley, 2013). Interestingly, disrupted or live *C. jejuni* bacteria have been shown to have different effects on the activation of the PRRs signalizing pathways (Al-Sayeqh *et al.*, 2010; De Zoete *et al.*, 2010). Smith *et al.* (2005) and Li *et al*. (2008) described an elevated expression of the proinflammatory cytokines IL-1β, IL-6, IL-8 transcript levels in cultured avian cells infected with *C. jejuni*. In this context, the live *C. jejuni* could apparently activate innate immunity signalling pathways in chicken ileum explants.

Commensal bacteria have the ability to suppress the expression of anti-inflammatory cytokine mRNA (i.e. TGF-β) and also reduce pro-inflammatory (i.e., IL-1β) cytokines, whereas pathogenic bacteria increase both pro-inflammatory and anti-inflammatory cytokine mRNA (Bahrami, Macfarlane, & Macfarlane, 2011). In this sense, *C. jejuni* cannot be regarded as merely a gut commensal bacterium because the infection of broiler chickens has been clearly associated with intestinal inflammation (Humphrey *et al.*, 2014). Additionally, the anti-inflammatory or pro-inflammatory cytokine mRNA levels display different concentrations according to the nature of the *C. jejuni* antigen preparation (Al-

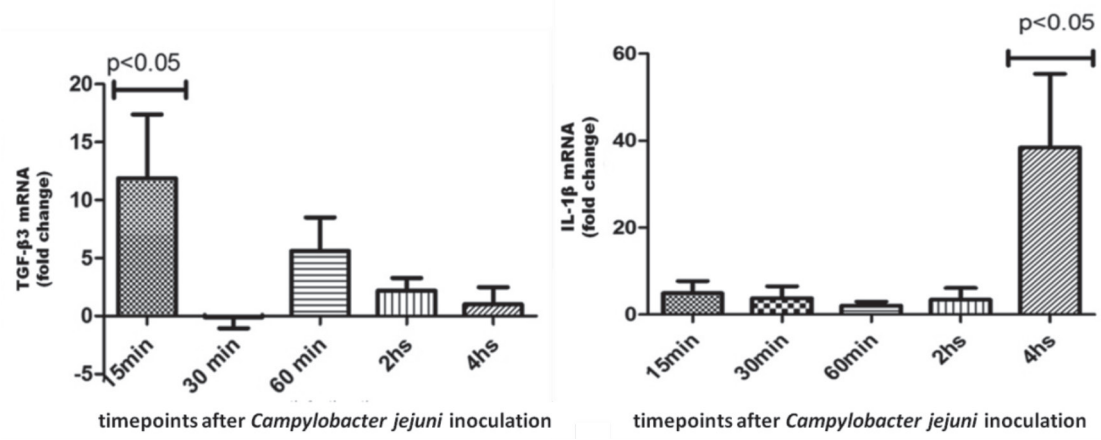

Figure 3 – Relative quantitation of transcript levels of TGF-β3 and IL-1β mRNA in *C. jejuni*-inoculated chicken ileum explants at different times.

Amri *et al.*, 2008). In the present study, live *C. jejuni* may have induced cytokine gene transcription similarly to pathogenic bacteria.

In conclusion, *C. jejuni* can be internalized by chicken ileum cells, causes morphological cell modifications, and induces both anti-inflammatory and pro-inflammatory cytokine transcripts.

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