Campylobacter induces diverse kinetics and profiles of cytokine genes in human and swine intestinal epithelial cell lines

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INTRODUCTION

Campylobacter spp. are considered to be the most common causative agent of foodborne illnesses. Campylobacter is a Gram-negative bacterium that infects the gastrointestinal tract and is usually transmitted by drinking water or contaminated animal food. Of the 17 species within the genus Campylobacter, C. jejuni and C. coli are the most important from a food safety point of view. In addition to being associated with acute cases of bacterial diarrhea, Campylobacter spp. also contribute to post-infection traumatic risks of acquiring immune-mediated neuropathies and inflammatory bowel diseases. Despite that Campylobacter colonizes the intestinal mucosa of pigs and humans causing similar disease symptoms in both species, very little is known about bacterial pathogenesis or bacterium-host interactions. Therefore, the aim of this study was to examine the interaction between C. jejuni and C. coli and two porcine intestinal epithelial cell lines (IPEC-J2 from jejunum and IPI-21 from ileum) by analyzing the expression of proinflammatory cytokines and chemokines and to compare their response to the human intestinal epithelial cell line INT407.

MATERIAL AND METHODS

Porcine and human intestinal epithelial cells (IPEC-J2, IPI-21 and INT407) were stimulated with C. jejuni and C. coli employing a bacterium-cell ratio of 1:100 during 3h of infection. The samples of RNA and supernatant were harvested after incubation time of 4, 6, 8, 12 and 24h. Total RNA was extracted using RNeasy Min kit (QIAGEN). The method was slightly modified by inclusion of a DNA digestion step with RNase-Free DNase (QIAGEN). Then, RNA samples were reverse transcribed using cDNA Synthesis Kit (Quanta) according to the manufacturer’s instructions. Quantitative real time PCR assays were carried out using iQ5Cycler (BioRad) and PerleCta SYBR Green SuperMix for IQ (Quanta). All experiments were performed at least three times and duplicate samples were analyzed in each experiment. The relative expression ratio of each gene was calculated based on primers efficiencies and threshold cycle number deviation of each sample versus a control (non-infected cells), and expressed in comparison to housekeeping genes (xine cyclophilin-A or human GAPDH, respectively).

The results indicated that in response to Campylobacter infection, porcine and human intestinal epithelial cells showed a similar gene expression profile to those described for other pathogens such as Salmonella typhimurium, and Helicobacter pylori.

Overall, our results showed that C. jejuni and C. coli are capable of inducing a similar pattern of gene expression in IPEC-J2, IPI-21 and INT407 during the time course of the in vitro infection.

RESULTS AND DISCUSSION

This is the first approach to the study of the response of porcine and human intestinal epithelial cells to Campylobacter infection.

The expression of pro-inflammatory cytokine IL6 decreased gradually in IPEC-J2, whereas IPI-21 presented two peaks expression at 4 and 24 hours incubation of bacteria. These IPEC-J2 data are consistent with those obtained in human and chicken (Figure1).

The chemokines CCL28 and CXCL2 has been analyzed in the IPEC-J2, IPI-21 and INT407 (Figure2).

The induction of MyD88 is moderated, close to the baseline and not coming in any case at least twice a term over time. The low expression could be explained because signal infection transduction occurs alternative TLR signalling (Figure1).

Higher levels of expression for pro-inflammatory cytokines IL1β, IL8 and TNF-α are similar with peak expression of 4 hours incubation of bacteria. IPI-21 and INT407 present a similar response pattern between the detected molecules, evidenced a different profile of against both pathogens with IL8 and IL1β (Figure2).

The signal transducer molecule NFκB is activated in intestinal epithelial cells in response to Campylobacter infection. IPEC-J2 and INT407 increased mRNA level between 4 and 6 hours of infection with C. jejuni and C. coli (Figure2).

The production of IL-8 showed up a higher release of this protein along the time of incubation with the bacteria in the three cells types. IPEC-J2 and IPI-21 reaching values of up 3000 pg/ml and 2000 pg/ml to 24 h of incubation ,respectively, although in the case of IPI-21 the increase is not as spectacular. Secretion of IL-8 protein could be found in all samples reaching values of up 900 pg/ml with C. coli at 24h (Figure3).

The secretion of IL-8 protein during time course showed up that the expression of mRNA does not necessarily result in translation to protein.

CONCLUSIONS

We demonstrate here that porcine epithelial cells transcribe and secrete chemokines and cytokines essential to the activation of the host’s inflammatory response when exposed to C. jejuni and C. coli.

The different expression profile between IPEC-J2 and IPI-21/INT407 for some molecules could be explained by nature line cells from different intestinal segments or activation of other alternative signaling route in response to Campylobacter.

The similar gene expression profile between human and porcine epithelial cells suggests the potential usefulness of porcine intestinal cell lines for studying enteric infections in man.