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Research paper

Interaction between *Campylobacter* and intestinal epithelial cells leads to a different proinflammatory response in human and porcine host

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ABSTRACT

Campylobacter jejuni and *Campylobacter coli* are recognized as the leading causes of human diarrheal disease throughout the development world. Unlike human beings, gastrointestinal tract of pigs are frequently colonized by *Campylobacter* to a high level in a commensal manner. The aim of this study was to identify the differences underlying the divergent outcome following *Campylobacter* challenge in porcine *versus* human host. In order to address this, a comparative *in vitro* infection model was combined with microscopy, gentamicin protection assay, ELISA and quantitative PCR techniques. Invasion assays revealed that *Campylobacter* invaded human cells up to 10-fold more than porcine cells ($p < 0.05$). In addition, gene expression of proinflammatory genes encoding for IL1α, IL6, IL8, CXCL2 and CCL20 were strongly up-regulated by *Campylobacter* in human epithelial cell at early times of infection, whereas a very reduced cytokine gene expression was detected in porcine epithelial cells. These data indicate that *Campylobacter* fails to invade porcine cells compared to human cells, and this leads to a lack of proinflammatory response induction, probably due to its pathogenic or commensal behavior in human and porcine host, respectively.

1. Introduction

Campylobacteriosis remains the most frequently reported zoonotic disease in humans in the European Union with 220,209 cases in 2011(EFSA and ECDC, 2013). The illness, associated to infections produced by bacteria of the genus *Campylobacter*, ranges from mild to severe diarrheal disease and has been identified as an important risk factor for the development of inflammatory bowel disease (Spiller, 2007). *Campylobacter* can sometimes spread beyond the intestinal tract, resulting in severe and sometimes life-threatening infection of other organs, particularly in children, elder and immunocompromised people (Ganan et al., 2012). In addition, other extra-intestinal manifestations such as reactive arthritis or the Guillain–Barré syndrome might occur (Nachamkin et al., 1998; Yuki et al., 2004). The most common source of *Campylobacter* in humans is contaminated food, especially raw/undercooked chicken and pork (Wilson et al., 2008). The most common pathogenic *Campylobacter* species are *Campylobacter jejuni* (*C. jejuni*) and *Campylobacter coli* (*C. coli*), which account for up to 95% of all human cases (Wilson et al., 2008). *C. jejuni* is most often found in chickens, but it is found in pigs as well, although pigs are predominantly colonized by *C. coli* (Horrocks et al., 2009). Although *Campylobacter* has now become the

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most common cause of food-borne disease, the bacterial pathogenesis is not yet well understood (Epps et al., 2013). Nevertheless, several studies have shown that bacterial invasion appears to be crucial in the infectious process and that *Campylobacter* plays a role in initiating the inflammatory response in the intestinal epithelial tissue (Everest et al., 1992; Hu and Hickey, 2005).

Despite its pathogenicity in humans, *Campylobacter* asymptomatically colonize the intestine of many food production animals (Man, 2011), and it is therefore considered a commensal in the gastrointestinal tract of pigs, even if this is not part of the normal porcine microbiota (Isaacson and Kim, 2012). The underlying mechanisms of this commensal behavior are not well known although appears to be related with the absence of any host response to the bacteria. Thus, it has been observed that broilers fail to mount an efficient immune response to clear the bacteria from their intestines, carrying the infection right up until slaughter (Smith et al., 2008). Also, immunocompetent pigs exposed early in life but generally asymptomatic, shed bacteria in feces, making it difficult to control *Campylobacter* at farm level by standard hygienic measures, especially in open systems of organic pig production (Jensen et al., 2006; Bratz et al., 2013).

The effort to reduce *Campylobacter* infections is directly linked, not only to a better understanding of the pathological mechanisms of the disease in humans, but also to a good understanding of the host–pathogen biology in food animals that commonly carry *Campylobacter* as intestinal commensal. As a bacterial infection, human campylobacteriosis is a multifactorial process mediated by interaction of bacteria to host epithelial cells and the response of the host immune system. Nevertheless, it is currently not known why *Campylobacter* is pathogenic to humans and not to pigs. In order to provide insight into the different behavior of *Campylobacter* in human and pigs, in this work we use an *in vitro* model of infection to examine both issues in the interaction of *C. jejuni* and *C. coli* with human and porcine intestinal epithelial cells.

2. Materials and methods

2.1. Intestinal epithelial cell lines

Small intestinal epithelial cell lines from human and pig origin were used to study interactions between bacteria and host. Two porcine cell lines, IPEC-J2 (from jejunum) and IPI-2I (from ileum), were used as previously described (Arce et al., 2010) to further characterize the bacterial–host interaction in cells from two different segments of the porcine small intestine. IPEC-J2 cells were cultured in Dulbecco's Modified Eagle Medium (DMEM)/Ham's F-12 (1:1) medium (Life Technologies, Carlsbad, CA, USA) supplemented with 5% FBS (PAA Laboratories GmbH, Austria), while IPI-2I cells were cultured in Dulbecco's Modified Eagle Medium DMEM/Ham's F-12 (1:1) medium (Life Technologies, Carlsbad, CA, USA) supplemented with 10% FBS (PAA Laboratories GmbH, Austria) and 4 mM L-glutamine (Life Technologies, Carlsbad, CA, USA). In addition, human intestinal epithelial cells INT-407 (human embryonic intestine, ATCC CCL-6) were cultured in RPMI-1640 medium (Lonza, Basel, Switzerland) and supplemented with 10% FBS (PAA Laboratories GmbH, Austria) and 2 mM L-glutamine (Life Technologies, Carlsbad, CA, USA). All cell lines were seeded in multiwell tissue culture plates (Thermo Fischer Scientific, Waltham, USA) on the day before an assay and allowed to reach confluence for *in vitro* infection. The cells were maintained in an atmosphere of 5% CO_2 at 37 °C.

2.2. Bacterial strains

C. jejuni and *C. coli* strains used in this study were isolated from chicken and pig feces, respectively, and further characterized by pulsed-field gel electrophoresis (PFGE). Both strains were recovered from stocks kept at −80 °C by plating on Columbia sheep blood agar (Oxoid, Basinstoke, Hampshire, UK) for 48 h at 37 °C under microaerobic conditions generated by the AnaeroGen system (Oxoid). For *in vitro* infections, colonies of *C. jejuni* and *C. coli* were cultivated as described above and bacteria were harvested from plates and re-suspended in fresh cell culture medium. The optical density (OD_{600}) was adjusted to 1 to achieve 10^8 CFU/ml. Bacterial suspensions were used immediately after preparation. All *in vitro* cell infections with bacteria was performed during 3 h of bacterial incubation at a multiplicity of infection (MOI) of 100/1.

2.3. Laser scanning confocal microscopy (LSCM)

Cells were grown on 0.1 mg/ml poly-L-lysine hydrobromide (Sigma–Aldrich, St. Louis, USA) treated glass coverslips (LabBox, Mataró, Spain) placed in 24-well cell culture plate. Prior to cell infection, *Campylobacter* strains were labeled with FITC (Sigma–Aldrich, St. Louis, MO, USA) following the method previously described by Pathirana et al. (2007) with minor modifications. After 3 h of bacterial incubation, the coverslips were washed with PBS to remove unbound bacteria and fixed in 4% paraformaldehyde buffered in PBS for 20 min. Then, the monolayer was permeabilized with 0.1% Triton X-100 (Boeringer Mannheim, Indianapolis, USA) in PBS for 5 min at room temperature, rinsed with PBS and incubated for 1 h in 1 μg/ml of tetramethylrhodamine B isothiocyanate (TRICT)-phalloidin (Sigma–Aldrich) and 2 min in 0.5 μg/ml 4′,6-diamidine-2′-phenylindole dihydrochloride (DAPI, Roche, Barcelona, Spain) to stain specifically actin filaments and nuclei, respectively. After three washes with PBS, the coverslips were mounted with Dako fluorescence mounting medium (Dako, Carpenteria, CA, USA). Images were captured using LSM 5 EXCITER confocal laser scanning microscope (Carl Zeiss, Jena, Germany) and analyzed using ImageJ software (National Institutes of Health, Bethesda, USA). To observe an entire *Z*-axis in one image, all images from one field were converted into a unique image making a *Z*-project. Additionally, *Campylobacter* was quantified by using the "analyze particles" tool of ImageJ in four fields of view selected randomly for each condition.

2.4. Scanning electron microscopy (SEM)

Confluent epithelial cells grown over glass coverslips were obtained as described above and after