International Journal of Medical Microbiology 304 (2014) 1209–1217

Contents lists available at ScienceDirect

International Journal of Medical Microbiology

journal homepage: www.elsevier.com/locate/ijmm

Arcobacter butzleri induces a pro-inflammatory response in THP-1 derived macrophages and has limited ability for intracellular survival

Jennifer zur Bruegge[a,*], Carlos Hanisch[a], Ralf Einspanier[a], Thomas Alter[b], Greta Gölz[b], Soroush Sharbati[a,*]

[a] Institute of Veterinary Biochemistry, Department of Veterinary Medicine, Freie Universität Berlin, Oertzenweg 19b, 14163 Berlin, Germany
[b] Institute of Food Hygiene, Department of Veterinary Medicine, Freie Universität Berlin, Königsweg 69, 14163 Berlin, Germany

ARTICLE INFO

Article history:
Received 25 April 2014
Received in revised form 29 August 2014
Accepted 30 August 2014

Keywords:
Arcobacter butzleri
Macrophages
Caspase acivation
Inflammation
Intracellular survival

ABSTRACT

Recent case reports have identified *Arcobacter* (*A.*) *butzleri* to be another emerging pathogen of the family *Campylobacteraceae* causing foodborne diseases. However, little is known about its interaction with the human immune system. As macrophages act as first defense against bacterial infections, we studied for the first time the impact of *A. butzleri* on human macrophages using THP-1 derived macrophages as an *in vitro* infection model. Our investigations considered the inflammatory response, intracellular survival and activation of caspases as potential virulence mechanisms employed by *A. butzleri*. Induction of IL-1α, IL-1ß, IL-6, IL-8, IL-12ß and TNFα demonstrated a pro-inflammatory response of infected macrophages towards *A. butzleri*. gentamycin protection assays revealed the ability of *A. butzleri* strains to survive and resist the hostile environment of phagocytic immune cells for up to 22 h. Moreover, initial activation of intitiator- (CASP8) as well as effector caspases (CASP3/7) was observed without the onset of DNA damage, suggesting a potential counter regulation. Intriguingly, we recognized distinct strain specific differences in invasion and survival capabilities. This suggests the existence of isolate dependent phenotype variations and different virulence potentials as known for other intestinal pathogens such as *Salmonella enterica* ssp.

Introduction

Severe gastroenteritis has many origins. However, certain subspecies of *Campylobacteraceae* such as *Campylobacter (C.) jejuni* are recognized to be predominant pathogens in the etiology of bacterial gastroenteritis in industrialized countries (Acheson and Allos, 2001). Recent case reports suggest *Arcobacter* (*A.*) *butzleri* to be another emerging pathogen of this family causing food- and waterborne diseases (Lappi et al., 2013), (Lehner et al., 2005) with watery and persistent diarrhea as typical characteristics of infection. Contaminated and undercooked meat products, mainly poultry and pork, as well as polluted water constitute the main source of infection (Collado and Figueras, 2011; Rivas et al., 2004). *Arcobacter* spp. are gram-negative, spiral shaped motile bacteria which were first isolated from aborted bovine fetuses in 1977 (Ellis et al., 1977) and redefined to a new genus in 1991 (Vandamme and Ley, 1991). Since then different *Arcobacter* spp. have been isolated from stool samples of human patients suffering from diarrhea with *A. butzleri* being the predominant one associated with disease (Vandenberg et al., 2004). Additionally, there are single reports of peritonitis and bacteremia (Lau, 2002; On et al., 1995). However, the role of *A. butzleri* in the pathogenesis of bacterial gastroenteritis is still not clear and due to the lack of routine diagnostic and standardized isolation and identification methods the incidence of *A. butzleri* associated diseases cannot be properly evaluated (Vandenberg et al., 2004; Collado and Figueras, 2011). The prevalence of *Arcobacter* spp. among diarrheic patients was investigated in different studies throughout the world and was reviewed by Figueras et al. It ranged from 0.1 to 13%, with *A. butzleri* being the most prevalent one, and highly depended on the identification method (0.1–1.25% with culturing methods, 0.4–13% with PCR techniques) (Figueras et al., 2014). Houf and Stephan examined the presence and characteristics of *Arcobacter* spp. in feces of asymptomatic humans in Switzerland and found *A. cryoaerophilus* to be the only *Arcobacter* species present (1.4%

* Corresponding authors at: Freie Universität Berlin, Institute of Veterinary Biochemistry, Department of Veterinary Medicine, Oertzenweg 19b, 14163 Berlin, Germany. Tel.: +49 30 838 62225.
E-mail addresses: Jennifer.zur.Bruegge@fu-berlin.de (J.z. Bruegge), Carlos.Hanisch@fu-berlin.de (C. Hanisch), Ralf.Einspanier@fu-berlin.de (R. Einspanier), Thomas.Alter@fu-berlin.de (T. Alter), Greta.Goelz@fu-berlin.de (G. Gölz), soroush.sharbati@fu-berlin.de (S. Sharbati).

http://dx.doi.org/10.1016/j.ijmm.2014.08.017
1438-4221/

of the samples). *A. butzleri* was not isolated (Houf and Stephan, 2007).

Miller et al. (2007) published the whole genome sequence of an *A. butzleri* human clinical strain and revealed the presence of putative virulence factors. But if and to what extent these virulence factors enhance *A. butzleris* pathogenicity still remains unknown. Previous *in vitro* studies focused on the interaction of *A. butzleri* with intestinal epithelial cells to unravel underlying mechanisms of host colonization. The ability to adhere to and invade host cells has been demonstrated by several authors (Karadas et al., 2013; Ho et al., 2007; Levican et al., 2013). Another study showed that *A. butzleri* induces epithelial barrier dysfunction by changes in tight junction proteins and induction of epithelial cell apoptosis, which are mechanisms that are consistent with a leak flux type of diarrhea in *A. butzleri* infection (Bücker et al., 2009) and indicate a high pathogenic potential. On one hand, further evidence of pathogenicity has been provided by proving cytotoxicity and induction of inflammation in epithelial cells mediated by IL-8 (Villarruel-López et al., 2003; Ho et al., 2007). On the other hand, *A. butzleri* does not possess any known gene for the cytolethal-distending toxin which is found in *C. jejuni* and is known to cause cytotoxicity of host cells by directly damaging eukaryotic DNA (Miller et al., 2007; Lee et al., 2003; Jinadasa et al., 2011).

However, little is known about the cellular innate immune response towards *A. butzleri* infection. Invasive microorganisms which are able to overcome anatomic host barriers such as epithelial surfaces are immediately recognized by phagocytic and antigen-presenting cells of the innate immune system such as macrophages or dendritic cells. Macrophages therefore have a fundamental role in this first line of defense during infection. They are resident in almost all tissues (Murphy et al., 2012) and their different abilities make them crucial for a successful elimination of invading pathogens. Macrophages are able to phagocytize and therefore eradicate pathogens but also play a key role in connecting innate and adaptive immune response via antigen presentation. Bacterial pathogens have co-evolved with their hosts and therefore developed mechanisms of escaping host immune defense. Survival mechanisms are diverging and often depend on the specific molecular pathogenesis of the microbe. Some pathogens are able to invade eukaryotic cells and replicate intracellularly, others are able to prevent their own uptake by phagocytes (Moine and Abraham, 2004). Another efficient way to escape host defense is to modulate the apoptotic machinery of mononuclear phagocytic cells. It is commonly known that different bacteria act in both ways and are either able to inhibit or to induce apoptosis to enhance their virulence potential. Intracellular bacteria such as *Mycobacterium tuberculosis* profit from decreased host cell death (Häcker et al., 2006) whereas bacteria such as *Salmonella* spp. induce apoptosis of macrophages to lower down numbers of phagocytic host cells (Navarre and Zychlinsky, 2000). Host cell apoptosis is an evolutionarily ancient mechanism to contain infection at the expense of infected cells. As a consequence, intracellular bacteria are hindered from replicating inside by revoking the pathogen's requirement of an intracellular environment (Denes et al., 2012). In other cases, it is reasonable for the host to lower down apoptotic processes in macrophages to prevent tremendous loss of phagocytic cells which would facilitate pathogen invasion.

As cells of the innate immune system hold this crucial position during bacterial infection and are essential for a successful elimination of invading pathogens, we were interested in *A. butzleris* impact on human macrophages and the molecular mechanisms of infection. We therefore investigated the influence of *A. butzleri* on the inflammatory response, activation of initiator as well as executioner caspases and intracellular survival as possible virulence mechanisms modulating host defense. For this purpose, THP-1 derived macrophages served as an *in vitro* infection model.

Table 1
Bacterial strains used in this study.

Strain	Source	Supplier
A. butzleri CCUG30485 (type strain)	Human isolate reference strain	Culture Collection University of Göteborg, Sweden
A. butzleri F0 also ref. to as FR1, H2	Human isolate	NRZ *Helicobacter*, National Reference Centre for *Helicobacter pylori*; University Medical Center Freiburg, Germany
A. butzleri 88	Chicken meat isolate	Bavarian Health and Food Safety Authority, Oberschleißheim, Germany
A. butzleri 89	Chicken meat isolate	Bavarian Health and Food Safety Authority, Oberschleißheim, Germany
A. butzleri 94	Chicken meat isolate	Bavarian Health and Food Safety Authority, Oberschleißheim, Germany
A. butzleri 102	Pork isolate	Bavarian Health and Food Safety Authority, Oberschleißheim, Germany
C. jejuni 81-176		ATCC, # BAA-2151

Our findings provide a better insight into the pathophysiology of the infection which is important for evaluating the pathogenicity of isolates and to develop strategies to combat *A. butzleri* induced diseases.

Material and methods

Bacterial strains and culture conditions

Employed bacterial strains are listed in Table 1. *C. jejuni* was grown at 37 °C, all *A. butzleri* strains were grown at 30 °C on Mueller–Hinton blood agar for 48 h (MHB; Oxoid) before being transferred in *Brucella* Broth to grow an overnight liquid culture at 37 °C and 30 °C, respectively. This liquid culture was then adjusted to an OD_{600} of 0.01 and cultured for another 24 h (*A. butzleri*) and 48 h, respectively (*C. jejuni*). Microaerobic atmosphere (5% O_2, 10% CO_2) was generated using the Anoxomat system (Mart Microbiology). To be used for infection experiments, cultures were centrifuged, washed with PBS (Sigma-Aldrich) and resuspended in cell culture medium (RPMI + 10% FCS superior, both Biochrom). For quantification of bacteria the number of colony forming units was determined by plating serial dilutions on MHB agar plates which were incubated for 48 h at 30 °C (*A. butzleri*) and 37 °C, respectively (*C. jejuni*).

Cell culture

The monocytic cell line THP-1 (DSMZ ACC 16) was cultured as described earlier (Sharbati et al., 2012). Cells were used up to passage 20. To perform infection experiments, cells were differentiated into macrophages by stimulation with phorbol-12-myristate-13-acetate (PMA, Sigma-Aldrich) 48 h prior to infection. For that purpose, cells were seeded in a 10 μM PMA-solution in RPMI and 10% FCS without antibiotics at a density of $4–6 \times 10^5$ cells applying a 1.5 ml volume together with a 6-well cell culture plate (TPP). After 6 h of PMA stimulation the stimulus was removed by washing the adherent cell-layer with PBS and fresh RPMI + 10% FCS without antibiotics was provided for another 42 h before being used for infection experiments.