Investigating the interaction between *Campylobacter jejuni* and intestinal epithelial cells resulting in activation of the unfolded protein response

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Background

Campylobacter jejuni is a leading cause of foodborne bacterial gastroenteritis worldwide [1]. *C. jejuni* adheres to and invades intestinal epithelial cells (IECs) then can reside within campylobacter containing vacuoles (CCVs). *C. jejuni* can survive within CCVs and deviate from the canonical endocytic pathway preventing fusion with lysosomes [2, 3]. However the exact process at cellular level leading to diarrhoeal disease is poorly understood. Studies have linked intestinal inflammation to the unfolded protein response (UPR) [4, 5]. The UPR is a conserved pathway to relieve ER stress and restore homeostasis in the endoplasmic reticulum (ER) [6]. There are three ER-membrane bound sensors which detect unfolded proteins in the ER, i) protein kinase R-like ER kinase (PERK), ii) inositol-requiring enzyme 1α (IRE1α), and iii) activating transcription factor 6 (ATF6). Downstream signalling of each sensor induces transcription of target genes involved in recovery of ER-homeostasis [7]. Recent data has shown that *C. jejuni* activates the UPR through the IRE1α pathway [8]. We have investigated *C. jejuni* activation of the UPR through the PERK, IRE1α, and ATF6 pathways.

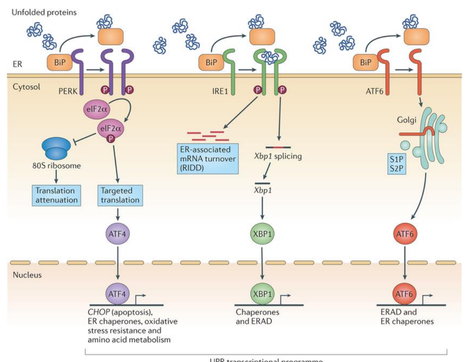

Figure 1. The UPR pathways under ER stress [6].

Results

C. jejuni up-regulates genes involved in PERK, IRE1α, and ATF6 pathways in T84 intestinal epithelial cells.

- *C. jejuni* 11168H, 81-176 and 488 wild-type strains induce PERK and IRE1α pathways in T84 IECs.
- *C. jejuni* 81-176 wild-type strain induces ATF6 pathway in T84 IECs after 6-hour infection.
- *bip* was down-regulated after 6-hour infection with *C. jejuni* 11168H. 81-176 and 488 wild-type strains.

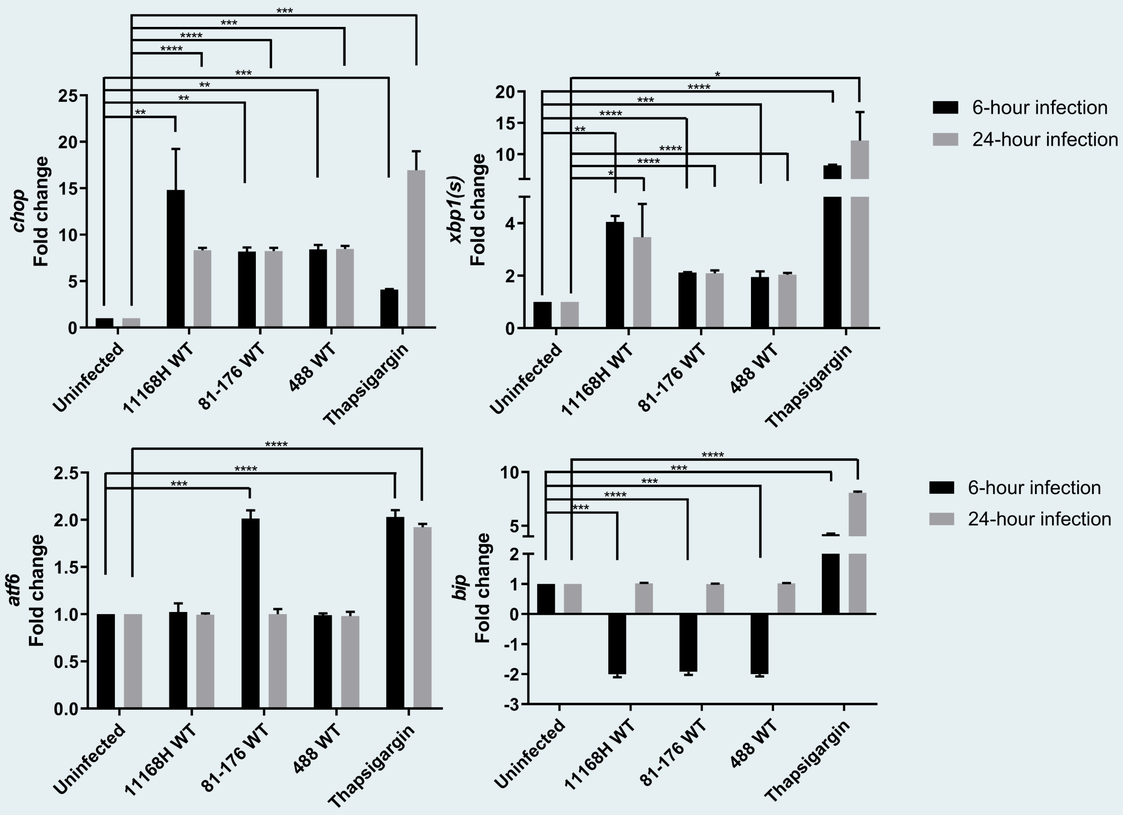

Figure 2. T84 IECs were infected with *C. jejuni* 11168H, 81-176, and 488 wild-type strains for 6 and 24 hours (MOI 200:1). Quantitative PCR (qPCR) was performed to investigate the transcriptional level of *chop*, spliced *xbp1*, *atf6* and *bip*. *gapdh* was used as an internal control. T84 IECs were treated with 2 μM thapsigargin as a positive control. (** = p < 0.01; *** = p < 0.001; **** = p < 0.0001).

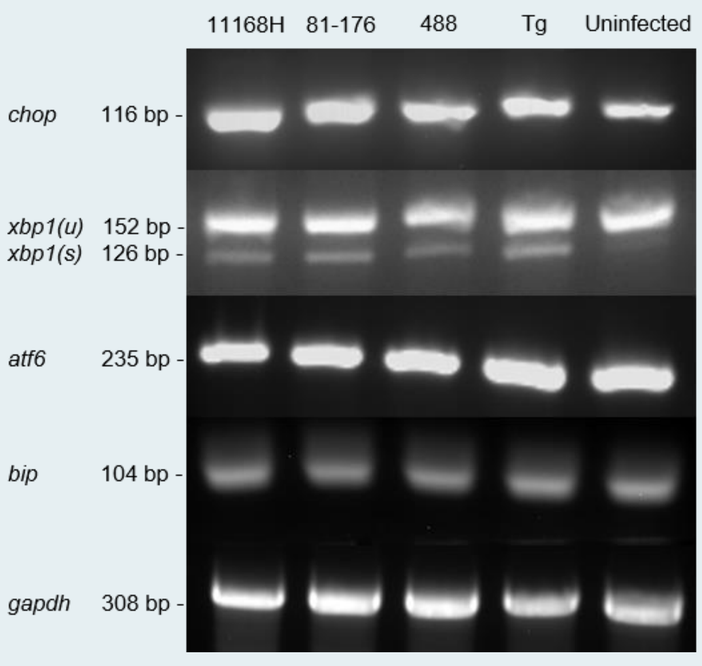

Figure 3. RT-PCR showing expression of *chop*, spliced *xbp1*, unspliced *xbp1*, *atf6* and *bip* in T84 IECs infected with *C. jejuni* wild-type strains for 24 hours. *gapdh* was used as an internal control. T84 IECs were treated with 2 μM thapsigargin (Tg) as a positive control.

Thapsigargin-mediated UPR does not affect adhesion and invasion of *C. jejuni* in T84 IECs.

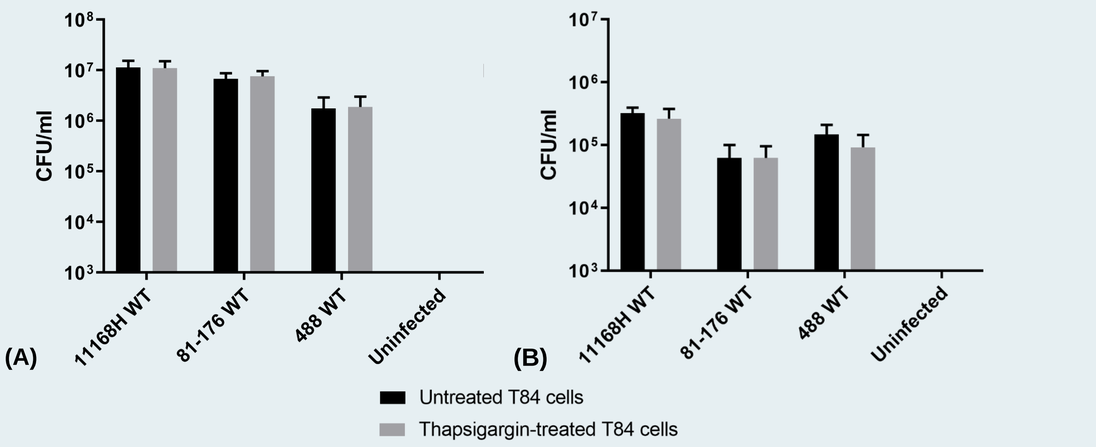

Figure 4. T84 IECs were pre-treated with 2 μM of thapsigargin for 6 hours and infected with *C. jejuni* 11168H, 81-176 and 488 wild-type strains for 3 hours (MOI 200:1). Then T84 cells were washed with PBS three times and lysed and the numbers of interacting bacteria were assessed (A) or were incubated with gentamicin (150 μg/ml) for 2 hours to kill extracellular bacteria and then lysed, and the numbers of intracellular bacteria were assessed (B).

Thapsigargin-mediated UPR reduces intracellular survival of *C. jejuni* in T84 IECs.

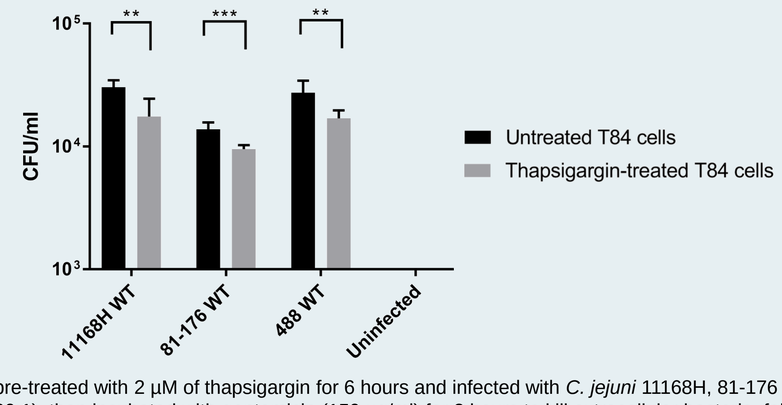

Figure 5. T84 IECs were pre-treated with 2 μM of thapsigargin for 6 hours and infected with *C. jejuni* 11168H, 81-176 and 488 wild-type strains for 3 hours (MOI 200:1), then incubated with gentamicin (150 μg/ml) for 2 hours to kill extracellular bacteria, followed by further incubation with gentamicin (10 μg/ml) for 18 hours. The cells were lysed and the numbers of intracellular bacteria were assessed. (** = p < 0.01; *** = p < 0.001).

C. jejuni down-regulates the expression of *nox1* in T84 IECs.

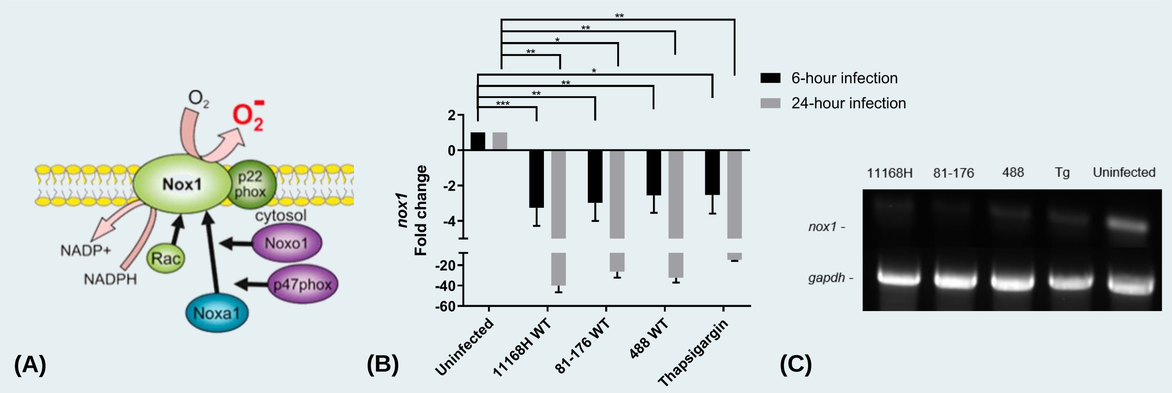

Figure 6. (A) Structure of Nox1 complex [9]. (B) qPCR showing expression of *nox1*. T84 IECs were infected with *C. jejuni* 11168H, 81-176, and 488 wild-type strains for 6 and 24 hours. *gapdh* was used as an internal control. (* = $p < 0.05$; ** = $p < 0.01$; *** = $p < 0.001$). (C) RT-PCR showing expression of *nox1* in T84 IECs infected with *C. jejuni* wild-type strains for 24 hours. *gapdh* was used as an internal control. T84 IECs were treated with 2 μM thapsigargin (Tg) as a positive control.

C. jejuni induces interleukin 8 in T84 IECs.

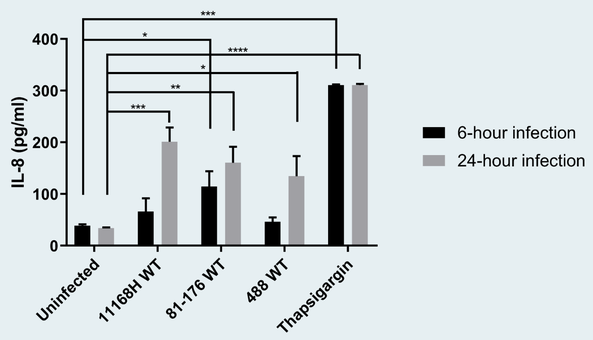

Figure 7. IL-8 ELISA using cell culture supernatants of T84 IECs infected with *C. jejuni* 11168H, 81-176, and 488 wild-type strains for 6 and 24 hours. Negative control is uninfected T84 cells and positive control is thapsigargin-treated cells. (* = $p < 0.05$; ** = $p < 0.01$; *** = $p < 0.001$; **** = $p < 0.0001$).

Conclusions

C. jejuni-induced UPR through the PERK, IRE1α and ATF6 pathways in T84 IECs was demonstrated using transcriptional methods. Also, the UPR significantly reduced *C. jejuni* intracellular survival in T84 IECs suggesting the UPR is activated as a host defence mechanism against *C. jejuni*. Down-regulation of *nox1* by *C. jejuni* and thapsigargin suggests the relationship between reactive oxygen species (ROS) generation and the UPR. In addition, *C. jejuni* and thapsigargin induced IL-8 in T84 IECs proposing the correlation of the UPR and inflammation. This study opens the way for improved understanding of the interaction between *C. jejuni* and IECs via UPR activation leading to intestinal inflammation and diarrhoeal disease.

References

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