INTERLEUKIN-1β INDUCTION IN THE HUMAN MONOCYTIC CELL LINE THP-1 BY LIPOOLIGOSACCHARIDES OF *CAMPYLOBACTER* SPP.

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BACKGROUND

- *Campylobacters* are motile bacteria; commensal in the chicken gut but a food-borne pathogen in the human gut.[1]
- Lipooligosaccharides (LOS) are the sugar containing molecules on the *Campylobacter* cell surface which are synthesised at the genetic level. The particular LOS structures on the *Campylobacter* cell surface help them to evade the host immune system and modulate disease development and progression.[1]
- The activation of NLRP3 inflammosome in a human macrophage is a two step process. Initial signalling event occurs via Toll-like receptors which triggers the NF-kβ to initiate the transcription of NLRP3 and pro interleukin-1β. In the second step, assembled inflammasome elicit the Caspase-1, which further catalyses the pro interleukin-1β into mature interleukin-1β (IL-1β) (Figure 1)[2].
- Aim of the present study was to investigate whether live *Campylobacter coli*, similar to *Campylobacter jejuni,* elicits the NLRP3 inflammasomes mediated IL-1β in macrophages . This study also aimed at investigating the impact of *C. jejuni* and *C. coli* LOS on IL-1β secretion from human THP-1 cells. For this purpose, THP-1 cells were either infected with live cells of *C. coli* RM1875, wild-type (WT) *C. jejuni* 11168 and mutant *C. jejuni* 11168 or treated with LOS of these strains.
- *C. jejuni* 11168 mutant was deficient of LOS biosynthesis genes and therefore, it had a lack of core oligosaccharides, modified lipid A, and an addition of a Kdo (Figure 2)[3].

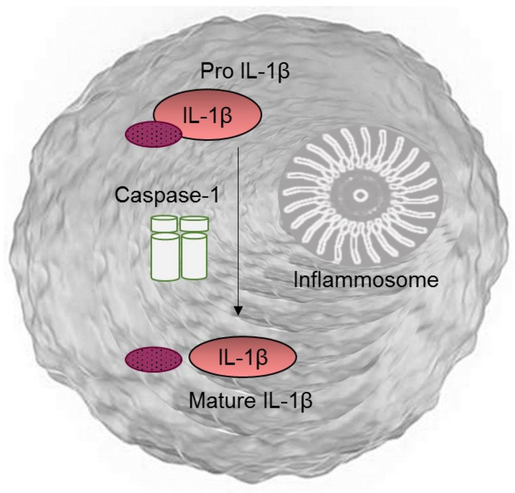

Fig. 1: Formation of mature IL-1β within the macrophage cytosol . Intracellular cleavage of pro IL-1β (31 KDa) leads to the formation of a mature, biologically functional IL-1β (17 KDa). It requires an inflammosome (a complex of NLRP3, ASC, and pro Caspase-1 proteins) and a caspase-1[2]

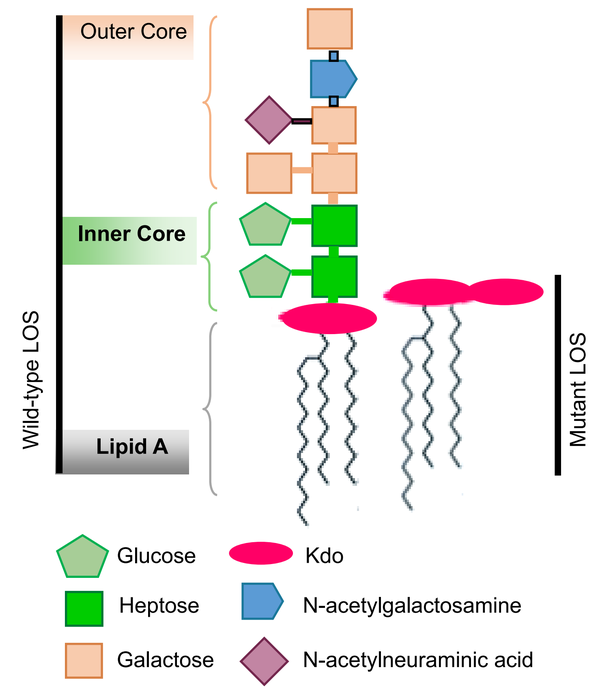

Fig. 2: Difference between wild-type *C. jejuni* 11168 LOS and mutant *C. jejuni* 11168 LOS. Wild-type LOS contains core oligosaccharides and lipid A with a Kdo molecule whereas mutant LOS has only lipid A with two Kdo molecules[2, 3]

EXPERIMENTAL PROCEDURE

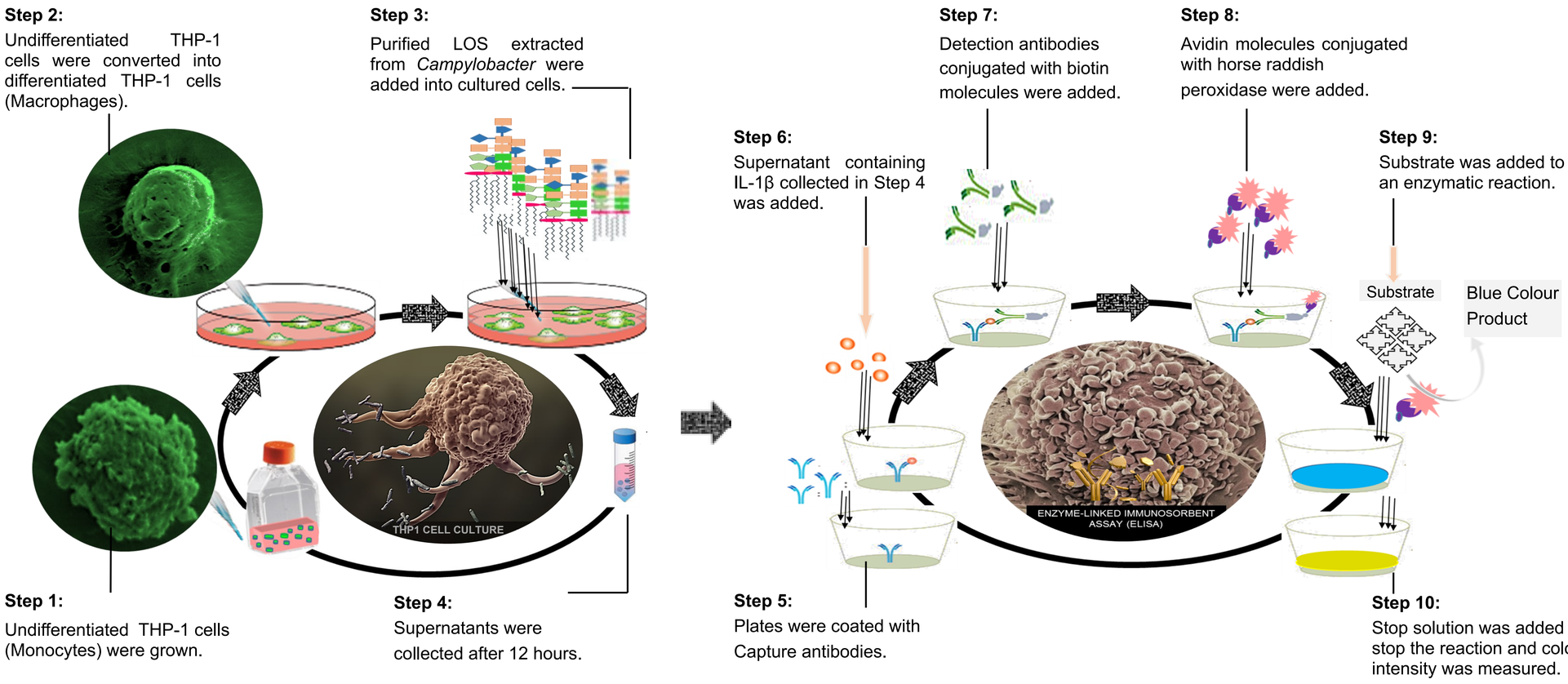

Fig. 3: TEN STEPS OF EXPERIMENTAL PROCEDURE

Caspase-1 ELISA was performed with the same procedure (step 6 - 10) with Caspase-1 specific primary and secondary antibodies.

RESULTS

- Compared to untreated THP-1 cells (negative control), the quantity of secreted IL-1β and Caspase-1 significantly increased in positive control (0.1 µg LPS) and *Campylobacter* LOS (1mg) treated THP-1 cells. The LOS extracted from mutant 11168 induced significantly less IL-1β and negligible Caspase-1 from THP-1 cells in comparison to the WT 11168 LOS (Figure 4).
- THP-1 cells treated with commercially available *E. coli* LPS (~0.1 µg) and *C. jejuni* 11168 live cells were used as positive controls in all THP-1 cell infection experiments. THP-1 cells culture treated with PBS alone was used as a negative control.
- In comparison to uninfected THP-1 cells, a significant increase in IL-1β production was observed when cells were infected with live cells of *C. coli* RM1875 and *C. jejuni* 11168 LOS mutant. The level of secreted IL-1β in THP-1 cells raised with increase in MOI (multiplicity of infection) or number of live *Campylobacter* cells (Figure 5A).
- In addition to IL-1β, Caspase-1 was also induced in *C. coli* RM1875 and *C. jejuni* 11168 LOS mutant infected THP-1 cells (Figure 5B). The secreted IL-1β and Caspase-1, both significantly reduced in the presence of Z-VAD-FMK (Caspase-1 inhibitor), indicating that IL-1β induction in THP-1 cells was Caspase-1 dependent (Figure 5B & 5C).
- In the presence of glyburide (K^+ channel inhibitor), a significant decrease in IL-1β was observed in *C. jejuni* 11168 mutant infected THP-1 cells, unlikely to WT *C. jejuni* 11168 and *C. coli* RM1875 infection. Blocking of K^+ channels by glyburide and alteration in LOS structure at the same time decreased the IL-1β production in THP-1 cells, suggesting that K^+ efflux and LOS structures are both associated with NLRP3 inflammosome activation in human macrophages (Figure 5C).

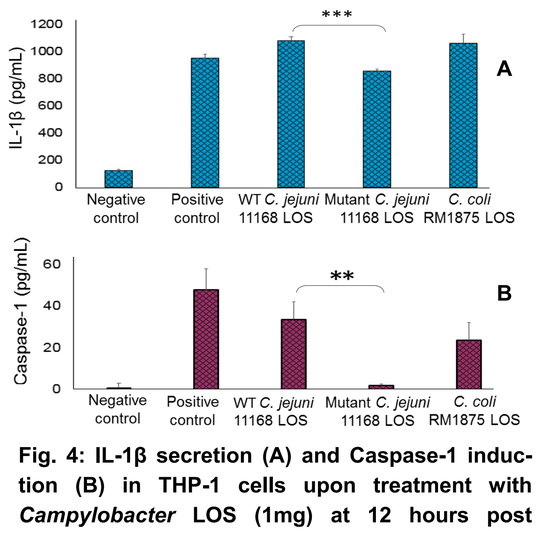

Fig. 4: IL-1β secretion (A) and Caspase-1 induction (B) in THP-1 cells upon treatment with *Campylobacter* LOS (1mg) at 12 hours post treatment. Values are the mean ± SD of three independent experiments performed in triplicate; ($p<0.01$**; $p< 0.001$***).

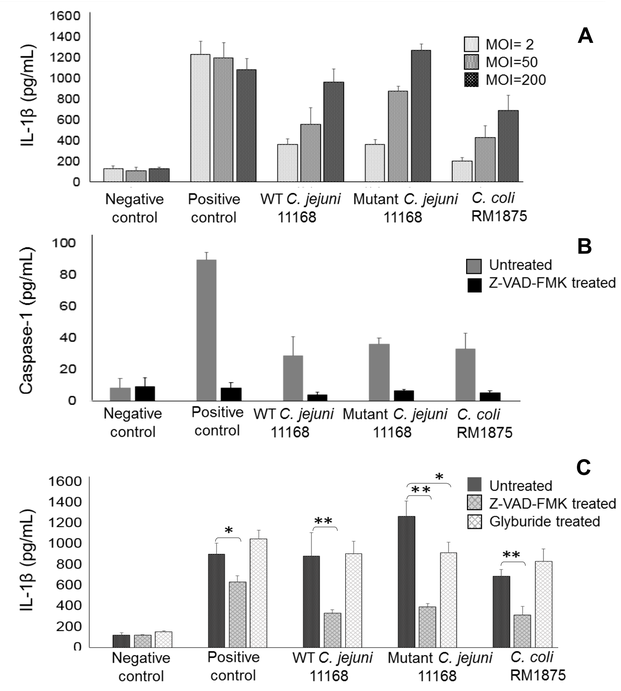

Fig. 5: (A) At 12 hours post treatment, IL-1β secretion by THP-1 cells infected with *Campylobacter* live cells (MOI= 2, 50, 200); (B) Caspase-1 secretion by THP-1 cells by *Campylobacter* live cells (MOI=200) and its reduction upon THP-1 cells treatment with Z-VAD-FMK; (C) Effect of Z-VAD-FMK and glyburide on IL-1β secretion in Campylobacter infected (MOI=200) THP-1 cells. Values are the mean ± SD of three independent experiments performed in triplicate; ($p<0.05$*; $p< 0.01$**).

CONCLUSION

- This study describes for the first time that *C. coli* is also included in the list of those bacteria which can activate the NLRP3 inflammasomes in the human macrophages.
- *C. jejuni* and *C. coli* LOS, similar to live cells, induce the Caspase-1 dependent IL-1β secretion in human macrophages. The extent of Caspase-1 and IL-1β induction may alter with variation in LOS structures.

It is also concluded that *Campylobacter*-associated molecular pattern (*Campylobacter* LOS) and danger-associated molecular pattern (K^+ efflux), both independently trigger the activation of NLRP3 inflammasomes during *Campylobacter* infection. This study is the first description which represents the association of *Campylobacter* LOS with NLRP3-mediated inflammatory response as well as provides new insight into the interaction of *Campylobacter* with human macrophages.

1). Karlyshev, A. V., J. M. Ketley, and B. W. Wren. (2005).The *Campylobacter jejuni* glycome. FEMS Microbiol. Rev. 29377-390.2).
2). Bouwman, L.I., de Zoete, M.R., Bleumink-Pluym, N.M., Fl vell, R.A., van Putten, J.P. (2014). Inflammasome activation by *Campylobacter jejuni*. J Immunol. 193(9):4548–57. 2399.
3). Marsden, G.L., Li, J., Everest, P.H., Lawson, A.J., Ketley, J.M. (2009) Creation of a Large Deletion Mutant of Campylobacter Jejuni Reveals That the Lipooligosaccharide Gene Cluster Is Not Required for Viability. Journal of Bacteriology. 191(7), 2392–2399.