Aggregatibacter actinomycetemcomitans (*Aa*) Leukotoxin (LtxA) Dependent Pyroptosis and Cytokine Release In Macrophages

Aleena Defreitas; Kathleen Battaglia; Taewan Kim; Anuradha Dhingra
University of Pennsylvania School of Dental Medicine

INTRODUCTION

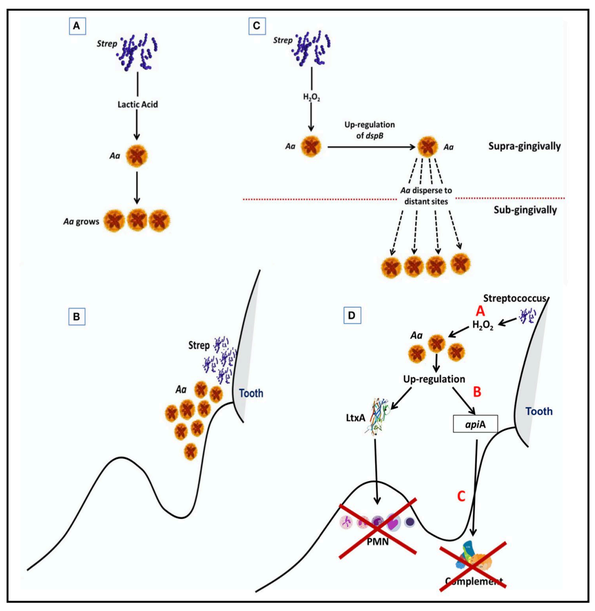


Figure 1. How *Aa* is activated to subvert host defense [1].

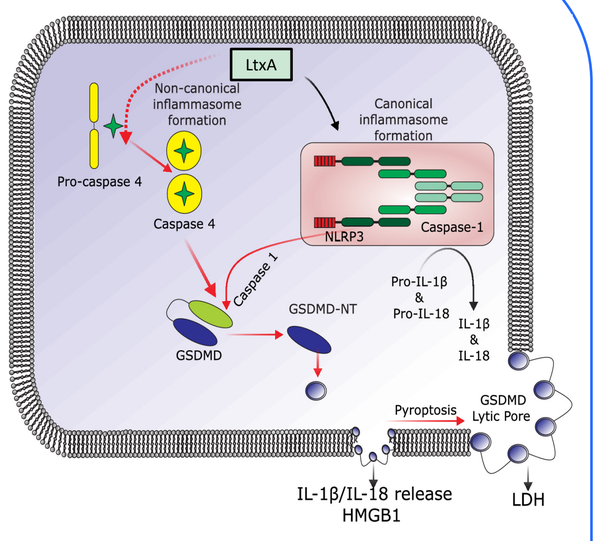
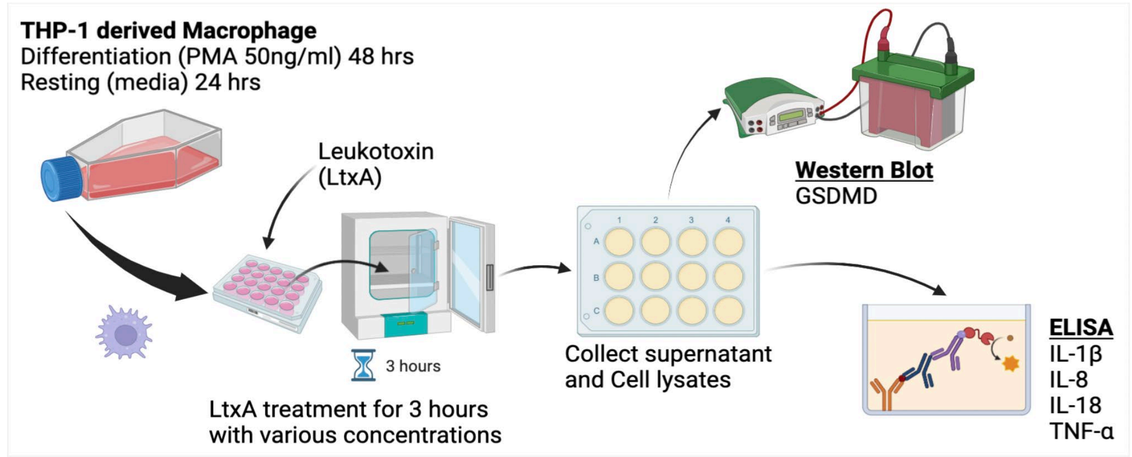


Figure 2. Schematic of possible activation of the noncanonical inflammasome and pyroptosis by LtxA [3]

Hypothesis

- LtxA stimulates a pyroptotic cascade leading to cytokine release in macrophages
- LtxA stimulates macrophage pyroptosis and inflammatory cytokine release requires caspase-1



METHODS & MATERIAL

- THP-1 derived macrophages were treated 6.25, 12.5, 25, 50, 100ng/ml of LtxA or 200 ng/ml of LPS (control) for 3 hrs assayed for various cytokines (IL-8, IL-1β, IL-18, and TNF-α) by ELISA.
- THP-1 macrophages (THP-1^{WT}), and cells deficient in caspase-1 (THP-1^{Casp1-}) or caspase-4 (THP-1^{Casp4-}), with 100 and 50 ng/ml of LtxA and assayed for different cytokines (IL-18, IL-1β, IL-8, and TNF-α), cell viability by lactate dehydrogenase (LDH) assay, and (Gasdermin D) GSDMD cleavage by Western blotting.

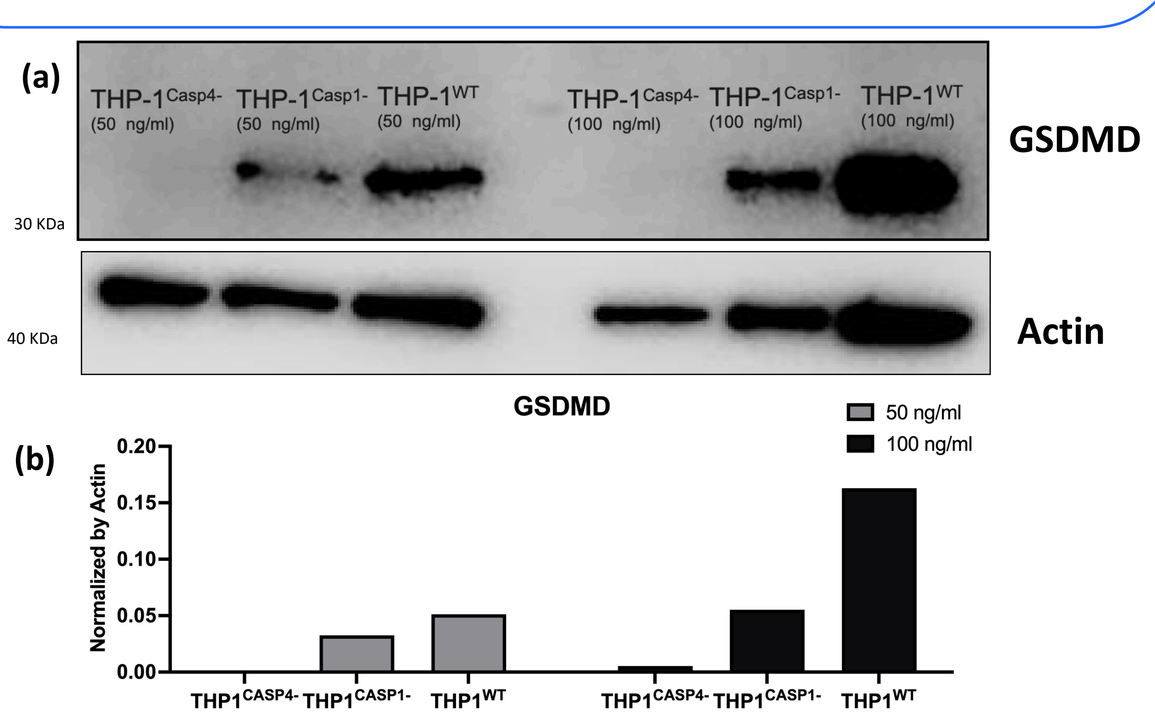


Figure 4. Inflammatory response by LtxA is driven by caspase-1 and caspase-4 activity. THP-1^{WT}, THP-1^{casp1-}, and THP-1^{casp4-}, cells treated with 100 and 50 ng/ml of LtxA exhibit GSDMD cleavage. (A) show a representative blot. (B) shows data normalized with actin show a representative blot.

RESULTS

- LtxA stimulates a dose-dependent increase in pro-inflammatory cytokines including IL-8, IL-1β, IL-18, and TNF-α.
- Decrease in the release of IL-18, IL-1β, IL-8 and TNF-α in THP-1^{Casp1-}, THP-1^{Casp4-} compared to THP-1^{WT} cells treated with 100 and 50 ng/ml LtxA.
- THP-1^{Casp1-} released lower IL-1β and TNF-α compared to THP-1^{Casp4-} suggesting while LtxA may mediate its pathogenesis in macrophages through the use of both caspase-1 and caspase-4, caspase-1 is the primary mechanism used for IL-1β release.
- LtxA mediates a decrease in level of cleaved GSDMD in THP-1^{Casp1} cells and an even larger decrease in THP-1^{Casp4-} cells.

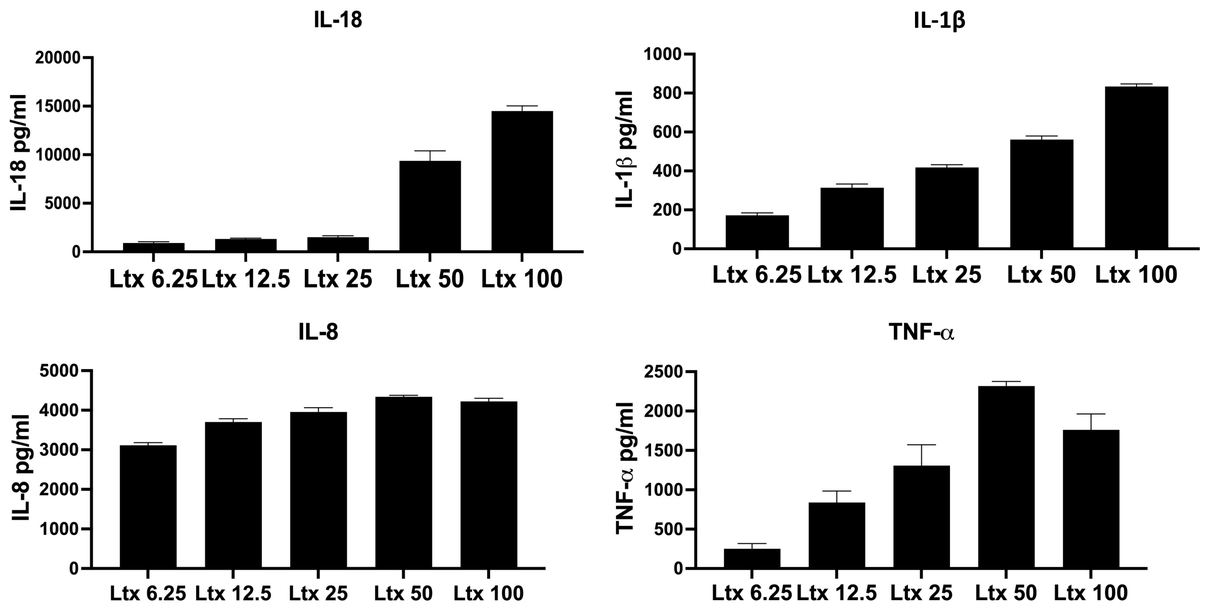


Figure 5. Dose-dependent increase in pro-inflammatory cytokines with LtxA treatment. Differentiated THP-1 macrophages treated with increasing concentrations of LtxA for 3 hours to stimulate inflammation. Supernatant was collected and ELISA was done for IL-1β, IL-8, IL-18 and TNF-α. Data represent 3 independent experiments with technical triplicate for ELISA.

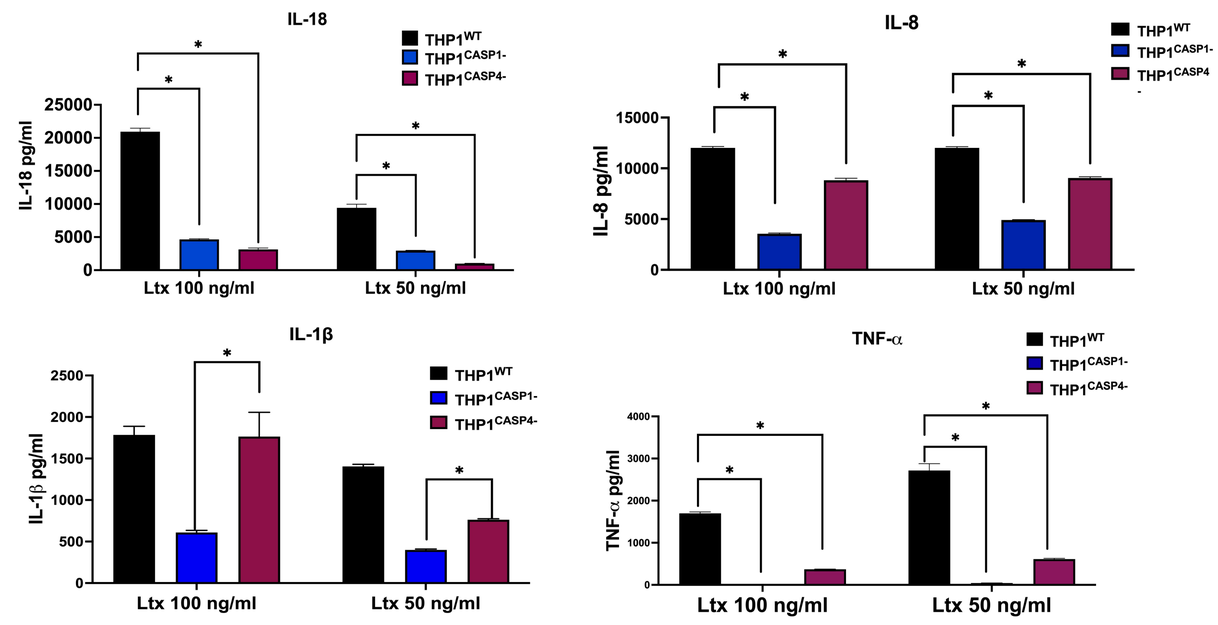


Figure 6. LtxA Inflammatory response in wildtype vs caspase-1 or -4 deficient macrophages. Differentiated THP-1 wildtype, caspase-1 deficient, and caspase-4 deficient macrophages were treated with different concentrations of LtxA for 3 hours to stimulate inflammation. Supernatant was collected and ELISA was done for IL-1β, IL-8, and IL-18. Data represent 3 independent sets with technical triplicate for ELISA. Student's t-test. *, p-value <0.05 wildtype vs deficient (caspase-1 or -4).

CONCLUSION

- LtxA stimulates release of inflammatory cytokines including IL-8, IL-1β, TNF-α, and IL-18 suggesting pyroptotic cell death
- Treatment of cells with LtxA results in the cleavage of GSDMD in a caspase dependent manner.
- LtxA mediates release of pro-inflammatory cytokines in a caspase-1 and caspase-4 dependent manner.

Presented at the 99th Annual Session of the Greater New York Dental Meeting in 2023.