Effects of Caspase-1 Activation by NLRP-3 Inflammasome

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INTRODUCTION

•Dengue is a mosquito-borne disease caused by infection of the dengue virus (DENV) which has four serotypes (1 - 4).
•Incidence rate of dengue hemorrghagic fever in Indonesia has been increasing for the past 50 years.
•Dengue infection triggers NLRP3 inflammasome assembly in immune cells, resulting in IL-1 release and increased vascular permeability.
•Increased vascular permeability is correlated to dengue hemorrghagic fever.

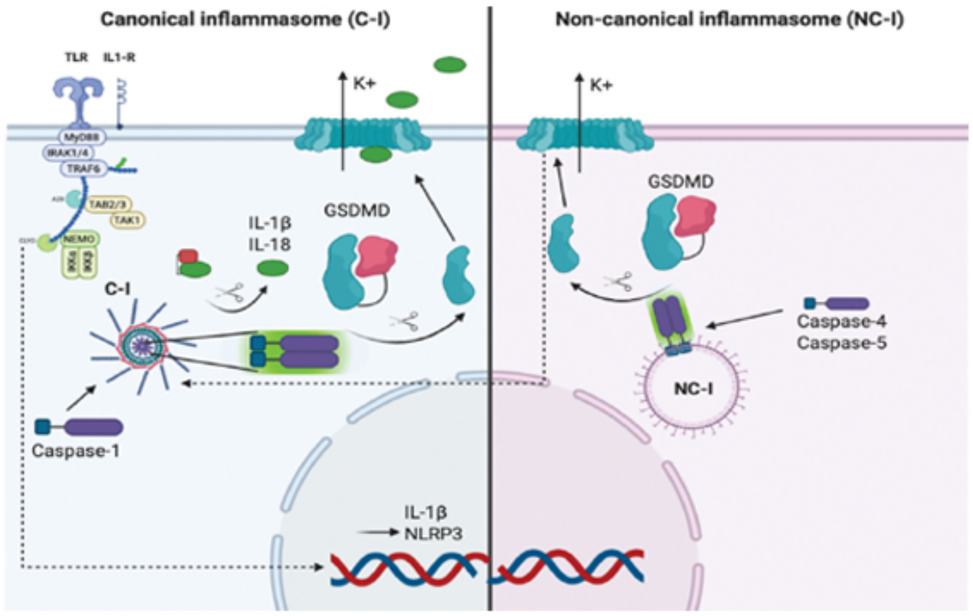


Fig 1. Activation of NLRP3 inflammasome can be achieved by two pathways; canonical and non-canonical.

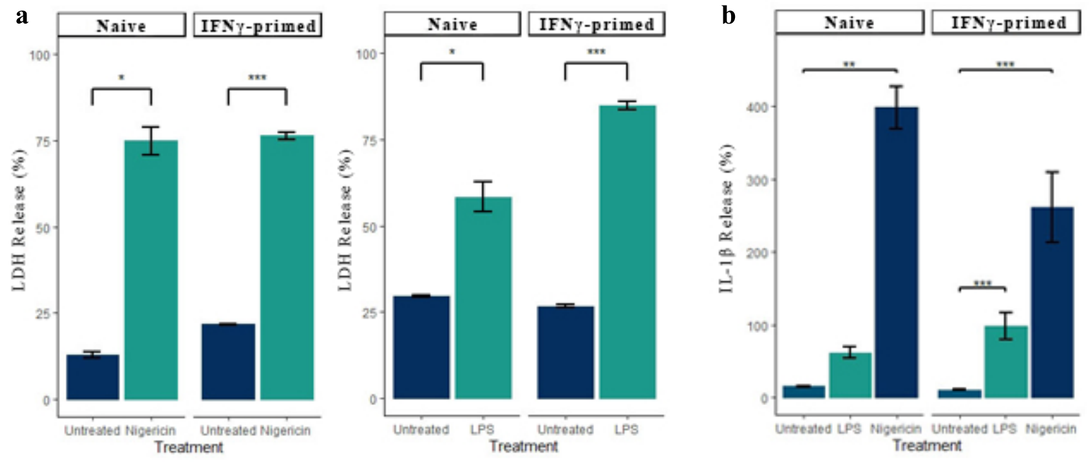
•NLRP3 inflammasome results in caspase-1 activation in canonical pathway and is activated downstream of the non-canonical pathway (caspase-4/5).
•These inflammatory caspases cleave gasdermin-D, an executor of pyroptosis required for IL-1β secretion.
•We will be looking at two possible substrates of caspase-1, transport protein 1 (T1) and splicing protein (S1)

METHODS

•THP-1 cells were differentiated using PMA
•Select lines (THP-1 and HeLa) were primed with IFNγ.
•Cells were treated with either Nigericin, transfected with LPS, or left untreated.
•Inhibitors were used in several cell lines: VX-765, a caspase-1 inhibitor; MCC950, a NLRP3 inflammasome inhibitor; MG-132, proteasome inhibitor; dimethyl fumarate (DMF) and disulfiram (DS), Gasdermin-D inhibitors.
•Cytotoxicity assays were carried out by measuring LDH extracellular release.
•ELISA assay was carried out to measure IL-1β release.
•Western blots were carried out to identify cleaveage and presence of following proteins: actin, gasdermin-D, caspase-1, T1, and S1
•Wilcoxon and Kruskal-Wallis test was used to perform statistical analysis for cytotoxicity assays and ELISA.

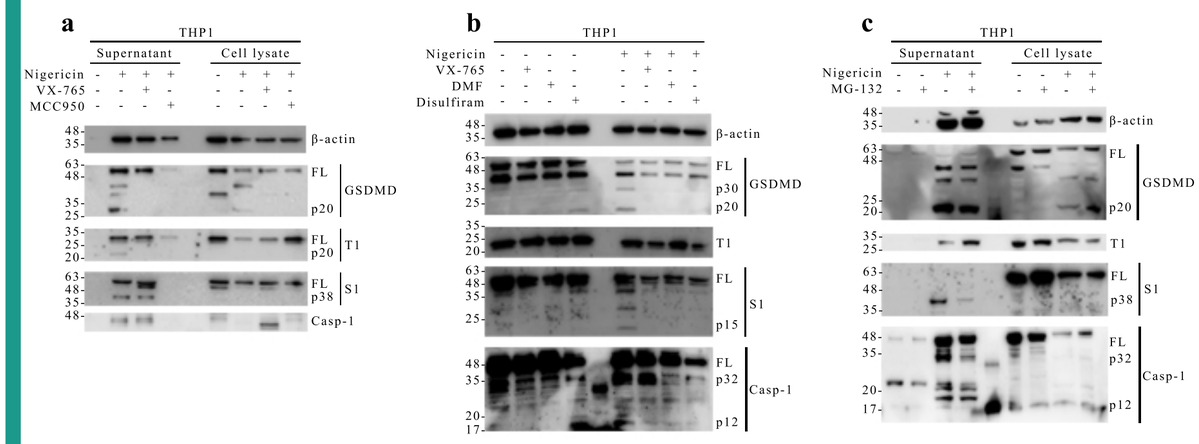
RESULTS

Fig 2. Nigericin and LPS induces LDH and IL-1β release through pyroptosis in THP1 cells



(a) Cell death measured by release of LDH from naive or IFNγ-primed THP1 cells, at 6-h post infection, ***p<0.001, *p<0.05, Wilcoxon test. **(b)** IL-1β release from naive or IFNγ-primed THP1 cells, at 6-h post infection, ***p<0.001, **p<0.01, Kruskal-Wallis test. Graphs show the mean ± SEM.

Fig 3. Nigericin induces cleavage of Gasdermin-D(GSDMD), T1 protein, S1 protein, and caspase-1(Casp-1) by caspase-1. Several inhibitors prevent this cleavage.



(a - c) Immunoblots for β-actin, GSDMD, T1 protein, S1 protein in THP1 cells treated with nigericin and **(a)** VX-765 or MCC950, **(b)** VX-765, or dimethyl fumarate(DMF), or disulfiram, **(c)** MG-132. GSDMD, T1 protein, and S1 protein cleavage is inhibited by VX-765, DMF, and disulfiram. GSDMD and T1 protein cleavage is also inhibited by MCC950.

CONCLUSIONS

•Results suggest that T1 protein and S1 protein are both caspase-1 substrates downstream of NLRP3 inflammasome activation.
•Further research could give insight on the role of these proteins in Dengue infection pathogenesesis and potentially any target for drugs or medication.

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