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Pyrin enhances IL-1 β processing and release in response to *Francisella*M. A. Gavrilin¹, S. Mitra¹, S. Seshadri¹, F. Berhe¹, M. D. Wewers¹¹The Ohio State University, Columbus, United States

Aims: Bacterial recognition by TLR and NLR sensors of mononuclear cells often initiates assembly of an inflammasome resulting in activation of caspase-1 and processing of IL-1 β , a key pro-inflammatory cytokine. However, the *Francisella*-sensing inflammasome remains uncharacterized. The purpose of this study was to investigate pyrin as a potential sensor of *Francisella* and its effect on caspase-1 activity.

Methods: Human monocytes (Mo), monocyte-derived macrophages (MDM), THP-1 and HEK293 cell lines, stably expressing either siRNA against pyrin or YFP-pyrin, were infected with *F. novicida* and analyzed for inflammasome activation. To confirm the effect of pyrin on caspase-1 activation, recombinant pyrin was analyzed for its effect on caspase-1 using an *in vitro* inflammasome.

Results: Using *F. novicida* as a model organism, we have shown that *Francisella* efficiently activates the inflammasome and IL-1 β release in Mo but not in MDM. In this context expression profiling showed a dramatic down regulation of pyrin levels in MDM, as compared to Mo (270 fold). Suppression of pyrin levels in Mo and THP-1 cells reduced caspase-1 activation and ability to process and release IL-1 β by *Francisella* challenge. In contrast, induction of pyrin in MDM (with M-CSF) and THP-1 (by overexpression) corrected the robust IL-1 β response to *Francisella*. Confocal microscopy showed a co-localization of YFP-pyrin with the inflammasome adaptor CFP-ASC and RFP-caspase-1 after cell stimulation, where ASC formed a central core and pyrin forms ring outside of the speck. Finally, *in vitro* pyrin inflammasome assembly on ASC cross-linked to maltose beads, showed that pyrin and caspase-1 formed a complex with ASC and that pyrin directly enhanced caspase-1 activity.

Conclusions: Intracellular pyrin upregulates mononuclear cell IL-1 β processing and release. Thus, pyrin is potential intracellular sensor for *Francisella*.