Toll-like receptor 4-mediated inflammation triggered by extracellular IFI16 is enhanced by lipopolysaccharide binding

Andrea Iannucci[1,2,*], Valeria Caneparo[1], Stefano Raviola[1,2], Gloria Griffante[3], Santo Landolfo[3], Marisa Gariglio[1,2], and Marco De Andrea[1,3]
*(lead presenter): andrea.iannucci@med.uniupo.it
[1]CAAD - Center for Translational Research on Autoimmune and Allergic Disease, University of Eastern Piedmont, Novara, Italy;
[2]Department of Translational Sciences, University of Eastern Piedmont, Novara, Italy;
[3]Department of Public Health and Pediatric Sciences, University of Turin, Medical School, Turin, Italy.

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Background: Since its discovery in the early 90s, a cornucopia of biological activities has been attributed to the IFI16 protein, including cell cycle regulation, tumor suppression, apoptosis, DNA damage signaling, virus sensing, and virus restriction. In addition, aberrant IFI16 expression and release in the extracellular space has been reported in a series of inflammatory conditions. The current hypothesis is that overexpression of the IFI16 protein occurs in tissue compartments where it is not physiologically expressed during inflammation. The ensuing release of the IFI16 protein into the extracellular space may allow it to behave like a damage-associated molecular pattern (DAMP) that signals through the Toll-like receptor 4 (TLR4) triggering inflammation by itself or through interaction with exogenous molecules, e.g. lipopolysaccharide (LPS).
Methods: GST-pull down assays and ELISA were used to characterize IFI16 binding activity to LPS. The human monocytic cell line THP-1 and the renal carcinoma cell line 786-O were used as target cells to define IFI16-induced proinflammatory activity. Co-immunoprecipitation (co-IP), surface plasmon resonance (SPR), and silencing experiments were used to define IFI16 signaling.
Results: We show that the IFI16 HINB domain binds to the lipid A moiety of either high or weak TLR4 agonist LPS variants. Treatment of THP-1 or 786-O cells with IFI16 led to increased production of proinflammatory cytokines, which was further enhanced when IFI16 was pre-complexed with sub-toxic doses of high TLR4 agonist LPS but not low agonists. Silencing of TLR4/MD-2 or MyD88 abolished cytokine production. These findings alongside with other in vitro binding experiments indicate that IFI16 interacts and signals through TLR4.
Conclusions: Collectively, our data provide compelling evidence that: i) IFI16 is a DAMP that triggers inflammation through the TLR4/MD2-MyD88 pathway; and ii) its activity is strongly enhanced upon binding to LPS variants regarded as full TLR4 activators. These data strengthen the notion that extracellular IFI16 functions as DAMP and point to new pathogenic mechanisms involving the crosstalk between IFI16 and subtoxic doses of LPS.