

# **STING agonist induced-monocyte death combines apoptosis, pyroptosis and caspase 8 activation**

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## **Abstract**

### Objectives

The cyclic-GMP-AMP synthase – stimulator of interferon genes (cGAS-STING) pathway is important for recognition of double-stranded DNA, a marker of infection or cell damage. The cGAS-STING pathway activation leads to production of proinflammatory cytokines by immune cells that then modulate subsequent immune responses with antiviral and antitumoral properties. Consequently, compounds that activate the cGAS-STING pathway are of therapeutic interest.

We recently described that STING agonists also trigger cell death of monocytes. Therefore, we characterized monocyte cell death in terms of apoptosis, pyroptosis and necroptosis.

### Methods

Monocyte cell death was characterized by monitoring the activation of caspases 3, 7, 1 and 8, along with kinases RIP1, RIP3 and MLKL. The induction was assayed either in peripheral blood mononuclear cells (PBMCs) using multiparametric flow cytometry-based immunophenotyping with FAM-FLICA staining of active caspases, or phospho-flow, or in enriched monocytes (to demonstrate the direct effect of STING agonists) using reporter-based assays and western blot. The secretion of cytokines was analyzed using multiplex assay with Luminex technology.

### Results

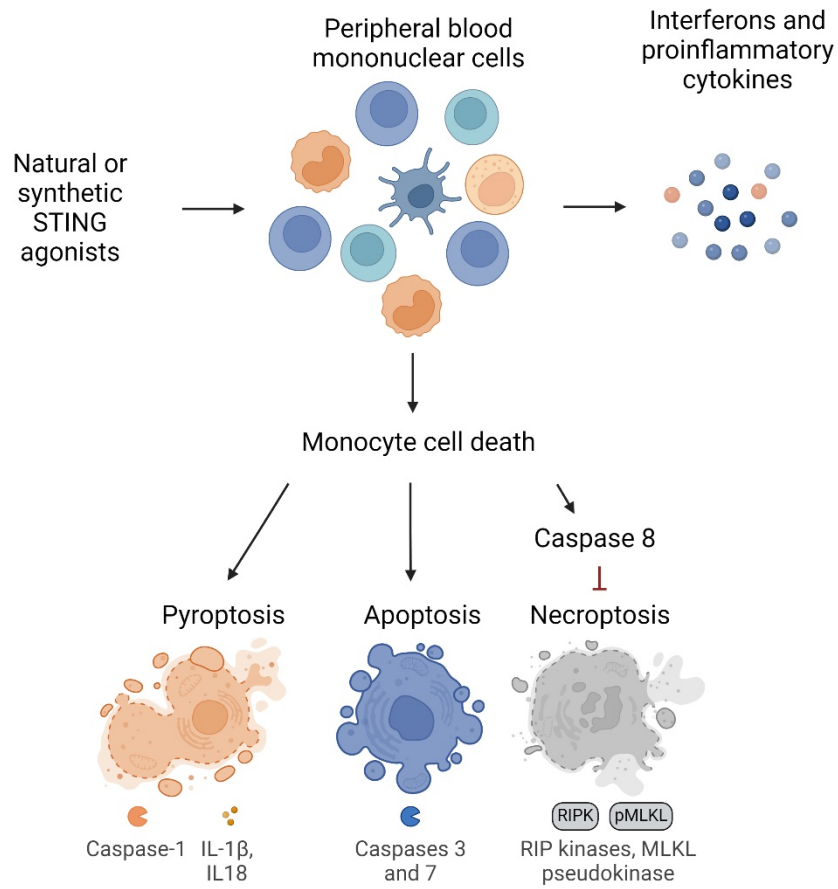
STING agonists trigger secretion of a broad cytokine portfolio in PBMCs. STING agonists induced activation of apoptotic caspases 3 and 7, and of pyroptotic caspase 1 already 4 hours after treatment. However, necroptotic cascade was not involved as no phosphorylation of RIP kinases or MLKL pseudokinase was detected. We suggest that necroptosis was blocked by the activation of caspase 8, as we detected cleavage of RIP1 kinase upon STING agonist treatment.

### Conclusions

The cGAS-STING pathway activation in PBMCs leads to proinflammatory cytokine secretion accompanied by a rapid monocyte cell death. The monocytes undergo regulated cell death featuring activation of both apoptosis and pyroptosis, while necroptosis is likely inhibited by activation of caspase 8. We propose that such immunogenic cell death may be an important immunoregulatory feedback loop for rapid inhibition of proinflammatory cytokine secretion and activation of subsequent immune processes.

### keywords

cGAS, STING, cytokines, regulated cell death, monocytes



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