Characterization of STING agonist-induced monocyte cell death reveals combination of apoptosis, pyroptosis and caspase 8 activation

Marketa Pimkova Polidarova[1,2*], Andrea Brazdova[1,2], Ivan Hirsch[1,2], Klara Grantz Saskova[1,2]
[1]Department of Genetics and Microbiology, Faculty of Science, Charles University, Vinicna 5, 128 44 Praha, Czech Republic
[2]Institute of Organic Chemistry and Biochemistry of the Czech Academy of Sciences, Flemingovo namesti 2, 160 00 Praha, Czech Republic
marketa.polidarova@uochb.cas.cz

Purpose:

The cyclic-GMP-AMP synthase – stimulator of interferon genes (cGAS-STING) pathway senses double-stranded DNA in cytoplasm, a signal of invading pathogens or cell damage. The activation of the cGAS-STING pathway induces secretion of proinflammatory cytokines by immune cells that in turn modulate antiviral and antitumoral responses. Therefore, synthetic activators of the cGAS-STING pathway, such as STING agonists, are of therapeutic interest.

We have recently demonstrated that STING agonists not only trigger cytokine production in peripheral blood mononuclear cells (PBMCs) but also induce cell death of monocytes. Here we investigated the mechanisms of monocyte death in terms of apoptosis, pyroptosis and necroptosis.

Methods:

STING agonist-induced monocyte cell death was assayed in PBMCs using multiparametric flow cytometry-based immunophenotyping combined with FAM-FLICA staining of active caspases, or phospho-flow for kinase activation. Additionally, we demonstrated the direct effect in enriched monocytes using reporter-based assays and western blot. We investigated the activation of caspases 3, 7, 1 and 8, along with kinases RIP1, RIP3 and pseudokinase MLKL. The cytokine secretion was analyzed with multiplex assay.

Results:

PBMCs secreted a broad cytokine portfolio in response to STING agonist treatment. STING agonists triggered activation of apoptotic caspases 3 and 7 and pyroptotic caspase 1 in monocytes already upon 4-hour treatment. However, phosphorylation of RIP kinases or MLKL pseudokinase was not detected. STING agonists also induced caspase 8 activation and cleavage of RIP1 kinase.

Conclusion:

Activation of the cGAS-STING pathway induces proinflammatory cytokine secretion in PBMCs, yet it is linked with a rapid monocyte cell death. STING agonist-induced monocyte cell death combines both apoptotic and pyroptotic processes, while necroptosis is not involved. We propose that necroptosis is blocked by active caspase 8, as RIP1 kinase cleavage fragment was detected upon STING agonist treatment. We suggest that the immunogenic monocyte cell death could be an important immunoregulatory mechanism for inhibition of proinflammatory cytokine secretion, and subsequently activation of secondary innate and adaptive immune processes.

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