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UNDERSTANDING THE MOLECULAR BASES OF INNATE IMMUNE RESPONSES TO FOREIGN OR MISLOCALIZED SELF-DNA

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We previously described that Murine polyomavirus MPyV induces type I interferon (IFN) responses via the sensors, p204 (human IFI16 homolog) and cGAS which sense viral genomes and micronucleus-like bodies generated during infection[1]. Sensing by both DNA sensors leads to canonical STING activation. In the canonical pathway, STING recruits and activates TBK1, which phosphorylates STING and IRF3 transcription factor to induce IFN and other cytokines. During MPyV infection, there are additional cell responses activated, i) DNA damage response and ii) low levels of apoptosis – the so-called sub-lethal apoptosis. Importantly, in the last years, DNA damage induced by different stimuli, among them sub-lethal apoptosis (in which caspase-activated Dnase (CAD) generates DNA breaks) has been shown to induce IFN responses[2,3]. Moreover, a novel, noncanonical pathway for IFN production was proposed in the responses to DNA damage. In this pathway, DNA sensing is mediated by IFI16 and DNA damage response factors. After sensing, STING signaling complex that includes the p53 and TRAF6 is assembled. TRAF6 ubiquitinylates STING, leading to the activation of the transcription factor, NF-κB[3]. Here, we investigated a possible contribution of the STING non-canonical pathway and the role of sublethal apoptosis in the IFN and other cytokine responses to the infection by MPyV. For the study, we followed innate immune responses in the mouse embryo fibroblasts (MEF) with knockout (KO) of *TRAF6* or *CAD* gene.

We found that TRAF6 is essential for immune responses to MPyV, since the absence of TRAF6 impairs the translocation of NF-Kβ to the nucleus and leads to the downregulation of IFN and pro-inflammatory cytokines, IL-6 and CXCL10. Furthermore, our results suggest that sublethal apoptosis is responsible at least in part for the immune responses to MPyV since CAD-KO displayed lower levels of IL-6 and CXCL10.

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REFERENCES

1. Ryabchenko B., Soldatova I., Sroller V., Forstova J., Huerfano S.: FEBS J. *288(20)*, 5964 (2021).
2. Dunphy G., Flannery S., Almine J., Connolly D., *et al.*: Mol Cell. *71*, 745, e5 (2018).
3. Brokatzky D., Dorflinger B., Haimovici A., *et al.*: EMBO J. *38(11),* e100907 (2019).