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CELL DEATH

Pathways for cross-priming

> immunogenicity of necroptosis does not rely on DAMPs but involves RIPK1

Immunogenic cell death promotes adaptive immunity through CD8$^+$ T cell cross-priming by dendritic cells (DCs) that have received antigen from and are activated by dying cells. Reporting in *Science*, Albert and colleagues show that cross-priming does not depend on the form of cell death per se but requires receptor-interacting protein kinase 1 (RIPK1) signalling and nuclear factor-κB (NF-κB)-mediated transcription within the dying cells.

Necrosis results in the release of damage-associated molecular patterns (DAMPs) and is thus inflammatory in nature, whereas apoptosis was thought to be immunologically silent. The death effector proteins RIPK3 and caspase 8 initiate necroptosis (a regulated form of necrosis) and apoptosis, respectively. These proteins are incorporated into large signalling modules termed ripoptosomes, which can facilitate the crosstalk between innate immune and cell death signalling pathways. The scaffold protein RIPK1 also has an important role in ripoptosome formation.

In this study, the authors generated cell lines in which oligomerization of RIPK3 or dimerization of caspase 8 can be selectively induced by treatment with a dimerizer (referred to as acR3 and acC8 cells, respectively). The authors also generated a cell line that contained a mutant form of RIPK3 (acR3ΔC cells) that does not recruit RIPK1 or induce ripoptosome formation but still results in RIPK3-dependent necroptotic cell death.

Using these cell lines, the authors confirmed that necroptotic acR3 and acR3ΔC cells, but not apoptotic acC8 cells, released DAMPs, activated DCs *in vitro* and promoted immune cell recruitment *in vivo*. Mice immunized with necroptotic acR3 cells expressing a non-secretable form of ovalbumin (OVA) showed significantly higher levels of CD8$^+$ T cell cross-priming than mice immunized with apoptotic OVA$^+$ acC8 cells. The CD8$^+$ T cells primed by immunization with necroptotic OVA$^+$ acR3 cells produced multiple effector cytokines, were cytolytic *in vivo* and protected mice from tumour challenge. However, necroptotic OVA$^+$ acR3ΔC cells did not promote robust CD8$^+$ T cell activation in immunized mice. These data suggest that necroptosis is an efficient inducer of cross-priming; however, the immunogenicity of necroptotic cells does not rely on DAMPs but involves RIPK1, independently of its role in cell death.

Further analysis showed that NF-κB signalling was rapidly induced following RIPK3 oligomerization in acR3 cells but not in acR3ΔC cells (nor in treated acC8 cells). NF-κB activation in necroptotic acR3 cells resulted in the upregulation of several immune-related genes and the production of newly transcribed interleukin-6 and CXC-chemokine ligand 1, despite rapid membrane permeabilization and cell death. Cross-priming was greatly reduced in mice that received necroptotic acR3 cells lacking *Ripk1*, with defective NF-κB signalling or in which transcription was inhibited.

Interestingly, cells undergoing necrosis (induced by repeated freeze–thaw) or secondary necrosis were poor inducers of CD8$^+$ T cell responses *in vivo*. By contrast, cells transfected with polyinosinic–polycytidylic acid (polyI:C), which induces apoptosis, promoted significant cross-priming *in vivo* in a RIPK1-dependent manner. Furthermore, the loss of RIPK1 expression in polyI:C-transfected colon carcinoma cells rendered them poorly immunogenic *in vivo* compared with control cells.

Together, these data indicate that RIPK1 and NF-κB signalling in dying cells is crucial for CD8$^+$ T cell cross-priming, regardless of the form of death the cells undergo. The authors highlight the necessity of characterizing the underlying signalling pathway(s) when investigating the complex relationship between cell stress, cell death and immunity.

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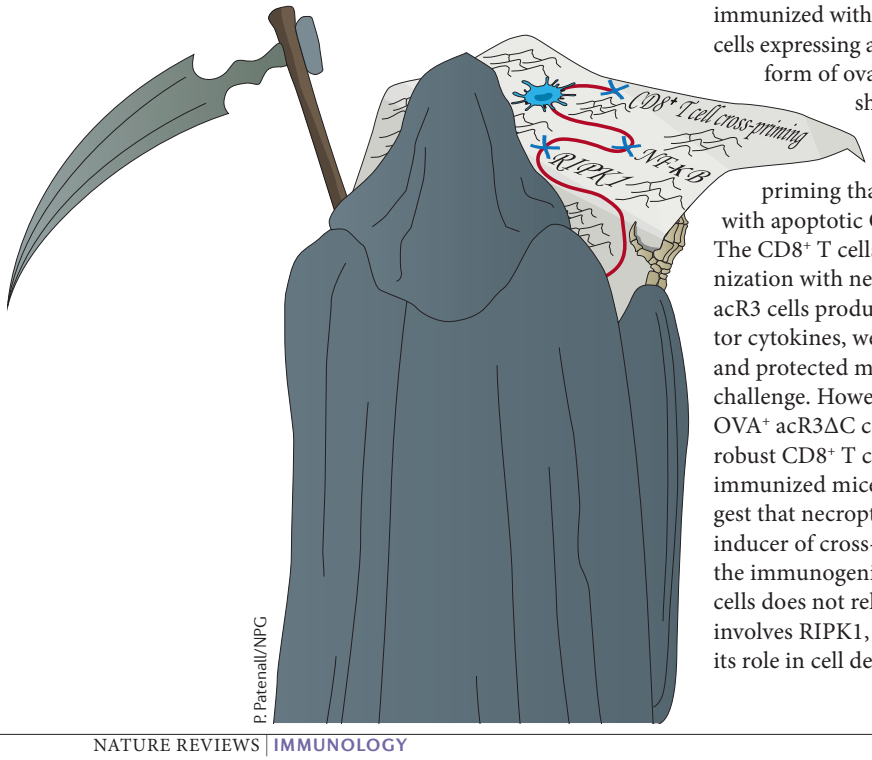

P. Patenall/NPG

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