

Spotlight

NLRC5: back to innate immunity

Jessica Guerra¹ and
Greta Guarda^{1,*}



NLRC5 is a transcriptional regulator of genes governing T cell responses. Most characterized NLRs are instead innate immune sensors forming complexes leading to pyroptosis. Raising exciting questions, Sundaram and colleagues now demonstrate that NLRC5 forms large complexes and causes PANoptosis (immunogenic cell death), in response to heme in inflammatory contexts.

The NOD-like receptor (NLR) caspase recruitment domain (CARD)-containing (NLRC) 5 is a key transcriptional regulator of major histocompatibility complex class I (MHC-I) and human *BTN3A* genes, thus regulating conventional and unconventional T cell responses in mammals [1,2]. NLRC5, which is highly expressed in lymphocytes and induced by interferons (IFNs), is mostly a cytosolic protein with a small fraction shuttling to the nucleus [1]. There, by interacting with a complex of proteins known as the ‘enhanceosome’, NLRC5 is recruited to the SXY motif found in the promoter of target genes and binds to factors involved in chromatin remodeling, as well as transcription initiation and elongation, thus promoting the transactivation of target genes [1,2].

Most characterized NLR family members, such as NLR pyrin domain-containing (NLRP) 3, are instead crucial innate immune sensors forming ‘inflammasomes’ upon cellular perturbations [3]. Inflammasomes are wheel-like protein structures formed by NLRs that lead, through the adaptor protein ASC, to the recruitment and

activation of caspase-1. Caspase-1, in turn, proteolytically activates inflammatory cytokines such as IL-1 β . Moreover, it frees the N-terminal part of gasdermin D (GSDMD) to form pores at cellular membranes causing a lytic cell death called pyroptosis and enabling IL-1 β release [3,4].

Opening new research prospects, Sundaram and colleagues now demonstrate that NLRC5 is crucial for inflammatory cell death induced by free heme, which can be released by erythrocytes during hemolysis and can lead to significant inflammation and organ damage in pathologic conditions (e.g., hemolytic diseases) [5]. NLRC5-dependent death was thoroughly investigated in mouse bone marrow-derived macrophages (BMDMs) after concomitant exposure to heme and Toll-like receptor (TLR) 2/4 agonists or TNF [5]. This process not only involved mediators of pyroptosis, but also of apoptosis and necroptosis, a phenomenon coined ‘PANoptosis’. Apoptosis, a cell death type devised to minimize the release of inflammatory mediators, is induced by intrinsic or extrinsic stimuli, such as TNF. These signals trigger the activation of initiator caspases, for example caspase-8, which then unleash effector caspases [4]. Necroptosis is instead a programmed type of necrosis induced, for example, by TNF stimulation with caspase-8 blockade, favoring the receptor-interacting protein kinase 3 (RIPK3)-mediated phosphorylation of the executor mixed lineage kinase domain like pseudokinase (MLKL) [4]. Sundaram *et al.* show that BMDMs stimulated by heme together with the TLR2 agonist Pam3CSK4 exhibit activation of caspase-1, -3, -7, -8, and phosphorylated MLKL, and, furthermore, that NLRC5 becomes a part of the PANoptosome, co-immunoprecipitating with ASC, NLRP3, caspase-8, and RIPK3, 28–36 hours after stimulation [5].

Functional data further implicated NLRC5-NLRP12 in the induction of cell death and

NLRP3-NLRP12 in the activation of caspase-1 and IL-1 β [5,6]. Despite this neat division of labor, in the absence of NLRC5 (*Nlrc5*^{−/−} BMDMs), ASC did not co-immunoprecipitate with NLRP3 [5]. This discrepancy between the dispensability of NLRC5 in the caspase-1-IL-1 β axis and its requirement for ASC-NLRP3 interaction within the PANoptosome hints to the possibility that this complex does not mirror inflammasome activity.

Because NLRP12 plays a role in both the inflammasome and the cell death pathways activated by heme/Pam3CSK4, it might represent an upstream sensor [5,6]. However, the molecular details of heme sensing [7] as well as the modes of action of NLRP12 (with both pro- and anti-inflammatory roles) [5,8], remain to be fully elucidated.

Sundaram and coworkers concomitantly exposed BMDMs to heme/Pam3CSK4, resulting in oxidized nicotinamide (NAM) adenine dinucleotide (NAD⁺; a molecule that is essential for energy production) depletion. This led to NLRC5 upregulation, reactive oxygen species (ROS) production, and cell death after 30 hours [5]. While this approach is of high translational value, it opens questions on the sequence of events leading to PANoptosis. In the inflammasome field, reconstitution of cells with individual components expressed at controlled levels has helped to distinguish the ‘priming’ step, needed to increase the amounts of, for instance, NLRP3, from the actual inflammasome activation step [9].

Previous data showed that NLRC5 is induced by TLR agonists largely through auto/paracrine type I IFN signaling [1], in line with the reported role for IFN regulatory factor 1 for heme/Pam3CSK4-induced cell death [6]. Heme also increases NLRC5 protein expression via TLR4 in a ROS-independent, but NAD⁺ depletion-dependent manner in BMDMs, as shown via NAM supplementation [5]. Compatible

with recent observations [10], these data suggest that NAD⁺ depletion downstream of TLR engagement contributes to IFN-dependent NLRC5, and most probably NLRP12, priming [5,6].

Albeit unlikely, a transcriptional contribution by NLRC5 to the observed cell death phenotype cannot be excluded. Reconstitution experiments (e.g., with a nuclear

localization sequence-mutated NLRC5) might also be valuable to address this point.

Consistent with minor pyroptotic features (compared with canonical inflammasome activators) and with the negligible role of the NLRP3-caspase-1-GSDMD axis in cell death induced by heme/Pam3CSK4 [5,6], another study showed that BMDMs

treated for 6 hours with heme following lipopolysaccharide (LPS) priming exhibited undetectable caspase-1 but substantial poly(ADP-ribose) polymerase (PARP) cleavage, indicative of heme toxicity [11]. Focusing on early time-points following macrophage heme exposure, previous work showed that cell death was not blocked by pan-caspase inhibition, but rather involved the effects of RIPKs, ROS,

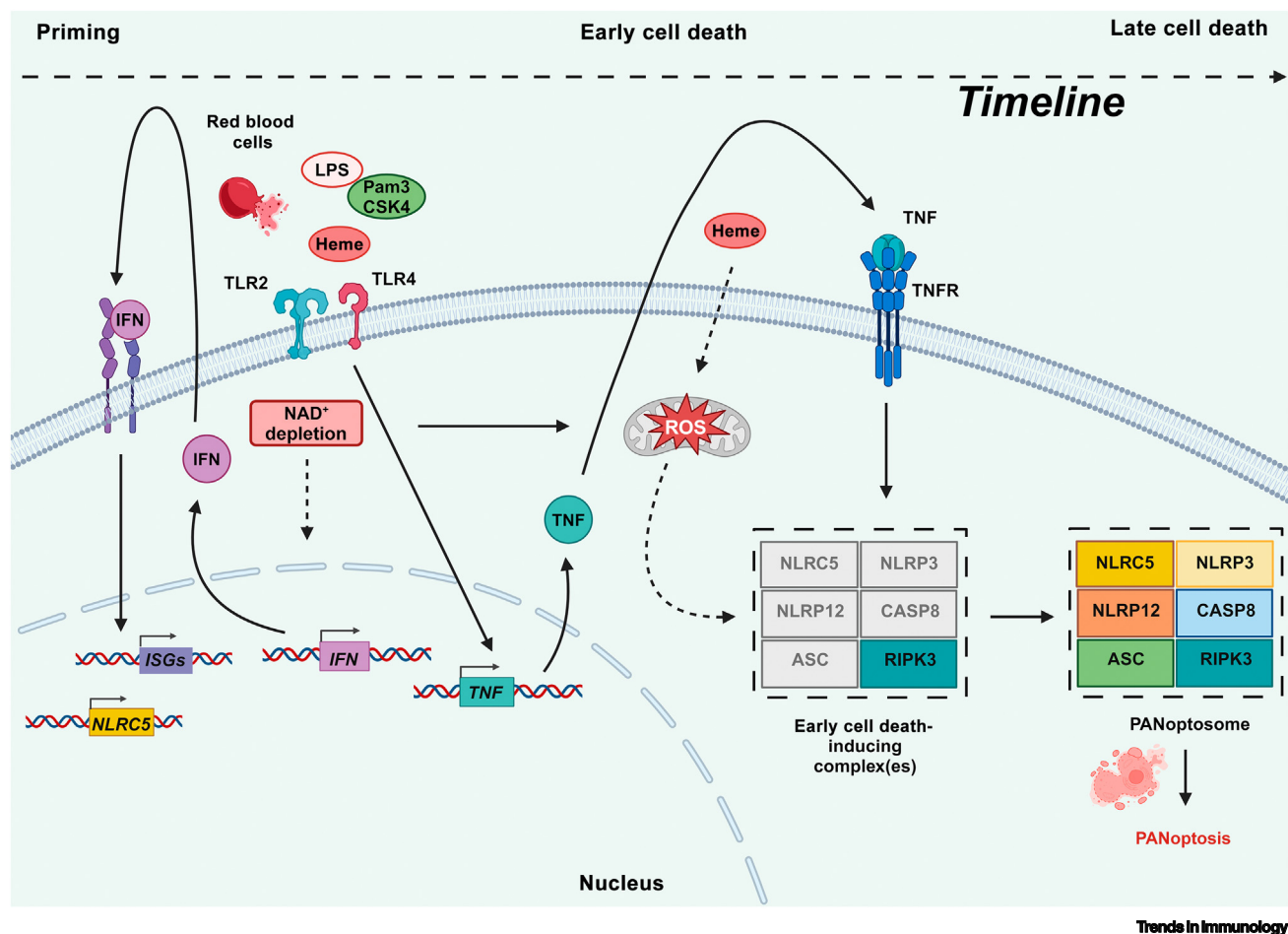


Figure 1. Integrated model of NLRC5-dependent PANoptosis in murine macrophages. In the priming step, TLR2/4 signaling following exposure of BMDMs to heme, together with Pam3CSK4 or LPS, leads to upregulation of NLRC5, an NLR best known for regulating MHC-I-encoding gene transcription. Integrating published data [1,10], we infer that after heme/Pam3CSK4 exposure, *Nlrc5* induction depends on an IFN response that is enhanced by NAD⁺ depletion. Furthermore, Sundaram *et al.* propose that NAD⁺ depletion regulates the generation of ROS and the formation of the PANoptosome – a complex comprising various cell death pathway mediators [5]. Considering previous data [7], we also surmise that, early after heme sensing, death by primed cells involves RIPKs, ROS, and TLR4-dependent TNF, but not caspases. This suggests the existence of an early cell death complex functionally relying on RIPKs and possibly encompassing fewer mediators compared with the NLRC5-dependent PANoptosome. At later time points, the described complex includes NLRC5, NLRP3, ASC, caspase-8, RIPK3, and, as shown upon overexpression, NLRP12, corresponding to the PANoptosome. While this is a hypothetical model that remains to be further investigated, it puts forward possible early events which, being more upstream, are important. Abbreviations: ASC, apoptosis-associated speck-like protein containing a caspase recruitment domain; BMDM, bone marrow-derived macrophage; CASP8, caspase-8; IFN, interferon; ISGs, IFN-stimulated genes; MHC, major histocompatibility complex; NAD⁺, nicotinamide (NAM) adenine dinucleotide; NLRC, NOD-like receptor (NLR) caspase recruitment domain (CARD)-containing; Pam3CSK4, Pam3CysSerLys4; RIPK, receptor-interacting serine/threonine-protein kinase; ROS, reactive oxygen species; TLR, Toll-like receptor; TNFR, tumor necrosis factor receptor. This figure was created with BioRender.com.

and TLR4-dependent TNF, thus eliciting a non-pyroptotic type of programmed necrosis [7]. Therefore, resolving the NLRC5-dependent process(es) and complex(es) that mediate heme toxicity over time represents an exciting question to address.

Sundaram and colleagues show compelling *in vivo* data in hemolytic disease and inflammation mouse models, where NLRC5-deficient mice are significantly protected from lethality, implicating NLRC5 in pathogenesis [5]. A first model based on treatment with TLR3 and 4 agonists poly(I:C) and LPS, respectively, a second one on a hemolytic agent (phenylhydrazine) with LPS to cause kidney injury, and a third one on dextran sodium sulfate-induced colitis. Given the acuteness of these models, particularly the first two, it is unlikely that the effects are mediated by T cells, supporting the newly described role of NLRC5 in innate immune responses to danger signals and inflammation (Figure 1). These novel findings may be highly relevant for the treatment of hemolytic diseases such as sickle cell anemia or malaria [5,6].

The data by Thirumala-Devi Kanneganti's group suggest that NLRC5 forms complexes reminiscent of inflammasomes. This idea is compatible with the notion that NLRC5 exhibits the typical domain organization of NLRs. Thus, determining how the cell death and the transcriptional roles of NLRC5 are reciprocally regulated is not only a timely aspect to address, but also raises other important questions to better understand the potential of NLRC5 targeting in a growing number of pathologies.

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Declaration of interests

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¹Università della Svizzera italiana (USI), Faculty of Biomedical Sciences, Institute for Research in Biomedicine, 6500 Bellinzona, Switzerland

*Correspondence:

greta.guarda@irb.usi.ch (G. Guarda).

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