

## COMMENT



# Dysregulation of innate immune sensors and autoinflammation: insights from an NLRC4 mouse AIFEC model

Jaewoo Park <sup>1</sup> and SangJoon Lee <sup>1,2</sup>✉

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The immune system is a complex protective network that is tightly controlled to protect and defend the host. Inflammation is a precisely regulated response that is crucial for host defense, while dysregulation can lead to tissue damage and systemic diseases. Defining the mechanisms that initiate, amplify, and resolve inflammation is crucial for understanding our complex immune system. The inflammasome, a multiprotein complex that functions as a sensor, plays a key role in regulating this inflammatory response. Inflammasomes act as molecular platforms that integrate upstream danger signals, catalyze the activation of caspase-1, and drive the maturation and secretion of proinflammatory cytokines such as IL-1 $\beta$  and IL-18. These inflammatory cytokines are released through pyroptosis, a lytic form of programmed cell death that eliminates infected or damaged cells while simultaneously propagating inflammation through the release of cytokines or chemokines [1].

Within this context, the study by Wang et al. provides a highly detailed and mechanistically rigorous examination of the pathological consequences arising from aberrant activation of NLRC4, a nucleotide-binding oligomerization domain-like receptor (NLR) protein that functions as a cytosolic sensor of specific bacterial components [2]. NLRC4 specifically recognizes conserved elements of the bacterial type III secretion system (T3SS), including needle and rod proteins, as well as flagellin, via interaction with NLR family apoptosis inhibitory protein (NAIP) proteins (NAIP1, NAIP2, NAIP5/6) [3–5]. Upon ligand recognition, NLRC4 oligomerizes to form an inflammasome, recruiting and activating caspase-1, which in turn processes pro-IL-1 $\beta$  and pro-IL-18 into their mature forms, IL-1 $\beta$  and IL-18, and initiates pyroptotic cell death. This molecular architecture underscores the pivotal role of NLRC4 in the early detection of intracellular bacterial infection and the maintenance of tissue integrity. The absence or dysfunction of NLRC4 compromises host defense [6–8], whereas gain-of-function mutations within the NLRC4 NACHT domain result in constitutive activation, provoking autoinflammatory disease [9].

A particularly compelling aspect of Wang et al.'s study is the creation of a knock-in (KI) mouse model harboring human autoinflammation with infantile enterocolitis (AIFEC)-associated V341A mutation in NLRC4 [2]. This mutation, which is localized within the NACHT domain, locks the protein in an ATP-bound, active conformation, thereby mimicking the constitutive inflammasome activation observed in patients. The strategic choice of this mutation was based on prior evidence indicating that alternative NACHT-domain variants, such as T337S, failed to reproduce the full spectrum of systemic and intestinal

inflammation [10], highlighting the specificity of V341A in driving autoinflammatory pathology. KI mice effectively model infantile enterocolitis and macrophage activation syndrome (MAS), both of which are hallmarks of human AIFEC, at just six days of age. Importantly, while the severity of the phenotype decreased in adulthood, the persistence of MAS-like features confirmed the fidelity of the model for studying the systemic consequences of NLRC4 hyperactivation.

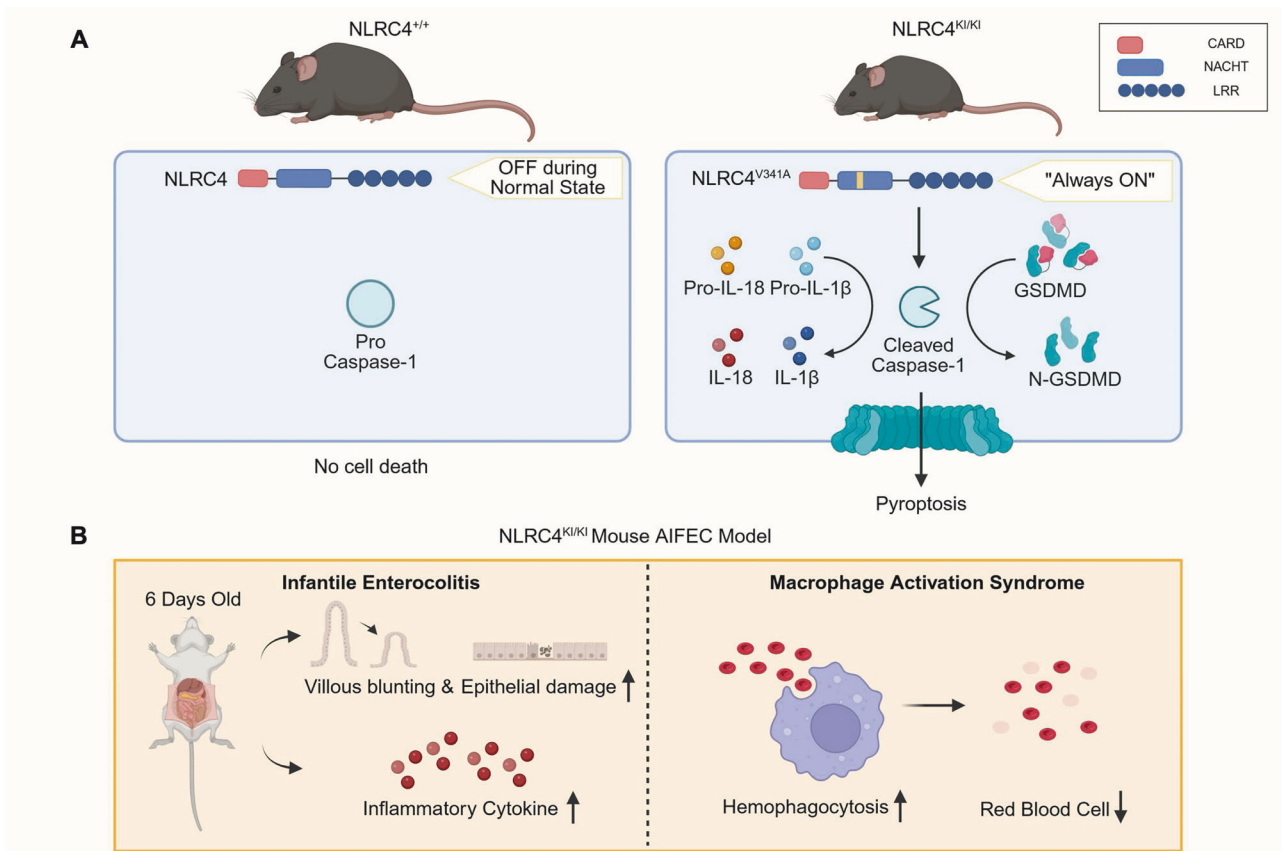
In parallel with phenotypic characterization, this study provides a comprehensive examination of the cellular and molecular mechanisms underlying NLRC4-driven pathology [2]. At the molecular level, NLRC4 KI mice displayed robust upregulation of proinflammatory cytokines, including IL-1 $\beta$ , IL-18, TNF, and IL-6, alongside elevated caspase-1 and gasdermin D cleavage, providing direct evidence that pyroptosis is a mechanistic driver of tissue injury (Fig. 1A). Histological analyses revealed villous blunting and epithelial disruption of the colon and small intestine. In addition, flow cytometry of colon tissues revealed increased infiltration of inflammatory macrophages and neutrophils within the intestinal lamina propria. Flow cytometry further confirmed alterations in immune cell composition, with reductions in T-cell populations and the expansion of myeloid subsets. Furthermore, NLRC4-KI mice presented increased hemophagocytosis, a key marker of the MAS (Fig. 1B). These findings underscore a central tenet of autoinflammatory disease, highlighting that the constitutive activation of a single innate immune sensor is sufficient to precipitate systemic inflammation, tissue damage, and organ dysfunction.

To evaluate potential therapeutic interventions, Wang et al. systematically tested three strategies: IL-18 blockade, TNF neutralization, and glucose supplementation. The IL-18 blockade approach was predicated on the observation that excessive IL-18 promotes intestinal epithelial damage, goblet cell loss, and recruitment of inflammatory leukocytes. Treatment with the IL-18-binding protein improved survival to approximately 80% but failed to normalize all the inflammatory markers, suggesting residual inflammasome-driven pathology. TNF blockade, which utilizes anti-TNF antibodies, further increased survival to 90% and addressed systemic inflammation more broadly; however, IL-18-driven pathways remained partially active, highlighting the limitations of monotherapy. Finally, glucose supplementation, motivated by the profound hypoglycemia observed in NLRC4 KI neonates, provided metabolic support, increasing survival to 60%. While this approach does not directly mitigate inflammasome activation, it underscores the relevance of addressing secondary metabolic perturbations in the management of autoinflammatory

<sup>1</sup>Department of Biological Science, Ulsan National Institute of Science and Technology (UNIST), Ulsan, Republic of Korea. <sup>2</sup>Graduate School of Health Science and Technology, Ulsan National Institute of Science and Technology (UNIST), Ulsan, Republic of Korea. ✉email: sangjoon.lee@unist.ac.kr

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**Fig. 1** The NLRC4 V341A KI model recapitulates AIFEC. **A** Molecular mechanism comparison between the NLRC4<sup>+/+</sup> and NLRC4<sup>KI/KI</sup> V341A models. **B** Infantile enterocolitis and macrophage activation syndrome phenotypes in the NLRC4<sup>KI/KI</sup> AIFEC model. KI Knock-in, AIFEC Autoinflammation with infantile enterocolitis

syndromes. Collectively, these results suggest that multitarget therapeutic strategies addressing both upstream inflammasome signaling and downstream metabolic consequences may represent the most effective clinical approach.

In addition to its therapeutic implications, this study provides two critical conceptual insights into NLRC4-mediated autoinflammation. First, although the KI model employs constitutive activation of NLRC4 via the V341A mutation, it effectively recapitulates the biological context in which diverse physiological stimuli, including bacterial proteins recognized by NAIP sensors, activate NLRC4 in vivo. In this context, the model simulates the natural signals that activate NLRC4, allowing for a controlled investigation of downstream inflammatory responses without the variability inherent in microbial stimuli. Second, this study raises the fundamental question of how pyroptosis propagates tissue-level inflammation. Future research should integrate analyses of gene expression changes in neighboring cells with precise spatiotemporal mapping of cytokine release and inflammatory onset to identify critical "tipping points" at which localized pyroptotic cell death escalates into broader tissue inflammation. Such insights will be essential for elucidating the mechanistic links between single-cell death events and organismal pathology and for establishing a framework to design therapeutic interventions that target not only inflammasome activation but also the subsequent amplification of inflammatory signals across tissues.

In summary, the work by Wang et al. provides an exhaustive mechanistic framework for understanding NLRC4-mediated autoinflammation. This study highlights the profound consequences of dysregulated innate immune sensing, elucidates the cellular and molecular pathways that link pyroptosis to systemic inflammation,

and establishes a robust platform for testing targeted interventions. These findings not only deepen our understanding of AIFEC but also serve as a paradigm for investigating the interplay between genetic mutations in innate immune sensors and autoinflammatory disease pathogenesis, with broad implications for therapeutic development and clinical application.

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## AUTHOR CONTRIBUTIONS

J.P. and S.L. conceived this study, prepared the manuscript, and critically revised and approved the final submitted version of the manuscript. All the authors contributed to the manuscript and approved the submitted version.

## COMPETING INTERESTS

The authors declare no competing interests.

## ADDITIONAL INFORMATION

**Correspondence** and requests for materials should be addressed to SangJoon Lee.

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