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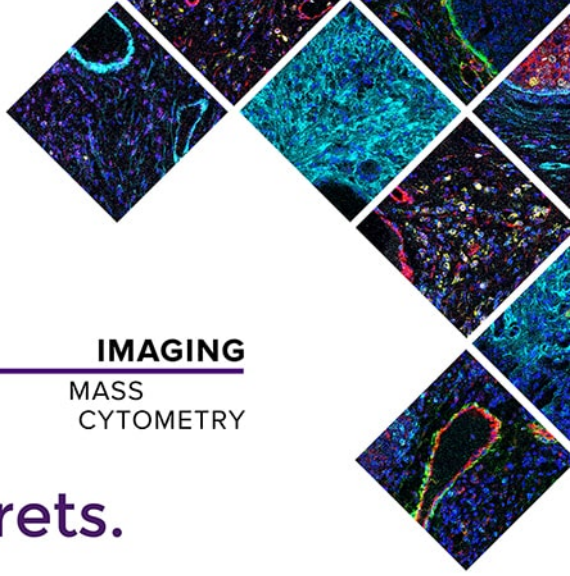

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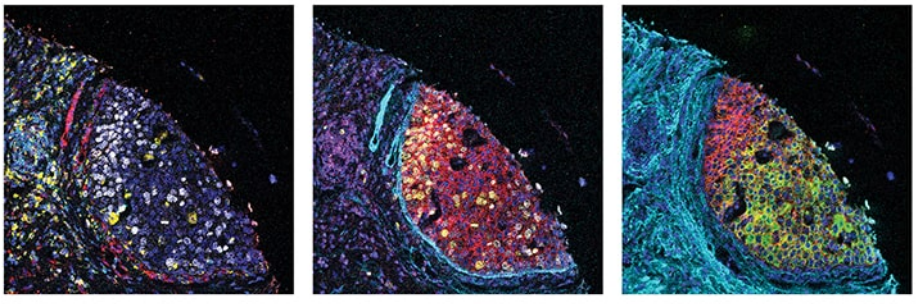

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EDITORIAL COMMENTARY

Inflammasome activation: Neutrophils go their own way

See corresponding article on Page 437

Neutrophils are innate immune cells that rapidly and in large numbers reach tissues infected with microbes. As a result, they are considered the first line of defense against many pathogens such as bacteria, fungi, and viruses. Neutrophils have many weapons to eradicate infectious agents including activation of NADPH oxidase to produce radical oxygen species (ROS) and release of antimicrobial proteins including serine proteases and antibiotic proteins. In addition, neutrophils can promote inflammation by the secretion of cytokines and chemokines. In particular, due to their large number compared with monocytes/macrophages, neutrophils could be a relevant source of proinflammatory IL-1β.[1]

In monocytes/macrophages, IL-1β secretion relies on the activation of multiprotein complexes called inflammasomes. The NOD-like receptor family, pyrin domain containing 3 (NLRP3) (NALP3/cryopyrin) inflammasome, consisting of the intracellular sensor NLRP3, the adaptor apoptosis-associated speck-like protein containing a caspase-recruitment domain (ASC), and pro-Caspase-1, is the most studied inflammasome. It assembles in response to stress or danger signals and leads to the activation of Caspase-1-mediated processing and secretion of the proinflammatory cytokines IL-1β and IL-18. Moreover, activated Caspase-1 cleaves the pore-forming gasdermin D (GSDMD). N-terminal cleaved GSDMD proteins form pores at the plasma membrane resulting in the release of intracellular content and subsequent inflammatory cell death called pyroptosis.[2]

In human monocytes, three pathways of activation of the NLRP3 inflammasome have been reported. Full NLRP3 inflammasome activation by the canonical pathway requires both a NF-kB-mediated transcriptional priming (induced, for example, by the engagement of TLR4 by LPS of Gram-negative bacteria), and an activation step performed by a wide spectrum of infectious and stress-associated signals (like pore-forming bacterial toxins, ATP, and crystals) (Fig. 1A, left panel).[2] On the other hand, intracellular LPS from phagocytosed enteropathogen bacteria can activate a non-canonical NLRP3 inflammasome. This signaling pathway requires human Caspase-4/5 (or the equivalent murine Caspase-11), which directly cleave GSDMD leading to intracellular potassium efflux and consequent activation of the canonical NLRP3 inflammasome, as well as pyroptosis (Fig. 1A, middle panel).[2–4] The latter has been proposed to confer cell defense against cytosolic Gram-negative bacteria as bacteria released in the extracellular compartment from lytic cells are exposed to bactericidal activity of neutrophils (mediated by proteases, ROS, and extrusion of neutrophil extracellular traps [NET]). Finally, LPS alone can trigger an alternative NLRP3 inflammasome pathway in human, but not murine, monocytes.[5] This alternative inflammasome involves a RIPK1 (receptor-interacting serine/threonine-protein kinase)–Fas-associated death domain (FADD)–Caspase-8 signaling occurring upstream of the classical NLRP3-ASC-Caspase-1 signaling. Moreover, it occurs in absence of potassium efflux and pyroptosis (Fig. 1A, right panel).

Thus, the mechanisms leading to IL-1β secretion by monocytes/macrophages have been extensively investigated in many studies focusing on the role of inflammasomes. By contrast, the scientific community's interest in the mechanisms contributing to IL-1β secretion by neutrophils is still in its infancy, although neutrophils are present at the site of inflammation and therefore represent a potential source of IL-1β in many infectious diseases. In the past years, two non-mutually exclusive mechanisms accounting for the processing and secretion of IL-1β by neutrophils have emerged. Neutrophil serine protease-mediated maturation and secretion of IL-1β were first described by Greten et al.[6] Subsequent studies have implicated the inflammasomes, drawing on discoveries made in monocytes/macrophages.[7] As observed in monocytes, both canonical (induced by nigericin, Gram-positive *Streptococcus pneumonia*, pneumolysin, ATP, and so on) and non-canonical (induced by Gram-negative bacteria) NLRP3 inflammasome activation were reported to induce IL-1β secretion by neutrophils. But in contrary to monocytes, no alternative NLRP3 inflammasome activation (induced by extracellular LPS) was described in neutrophils.[1,8,9] Another difference between monocytes/macrophages and neutrophils is that although classical NLRP3 inflammasome activation leads to both IL-1β secretion and pyroptosis of monocytes/macrophages, it does not induce neutrophil pyroptosis.

In this issue, Kremserova and Nauseef showed that ultrapure human neutrophils that have ingested community-associated methicillin-resistant *Staphylococcus aureus* (CA-MRSA) secreted IL-1β independently of the NLRP3 inflammasome activation. They described a novel mechanism of IL-1β secretion involving active RIPK3, but not RIPK1, paving the way for the search for a new "signalosome" complex.

In monocytes/macrophages, it is assumed that RIPK3 can orchestrate IL-1β secretion, although the molecular signaling is still not fully elucidated. It was proposed that in the absence of inhibitors of

Abbreviations: ASC, apoptosis-associated speck-like protein containing a caspase-recruitment domain; GSDMD, gasdermin D; IAPs, inhibitors of apoptosis; MLKL, mixed-lineage kinase-like protein; NE, neutrophil elastase; NLRP3, NOD-like receptor family, pyrin domain containing 3; PR3, proteinase 3; RIPK1, receptor-interacting serine/threonine-protein kinase

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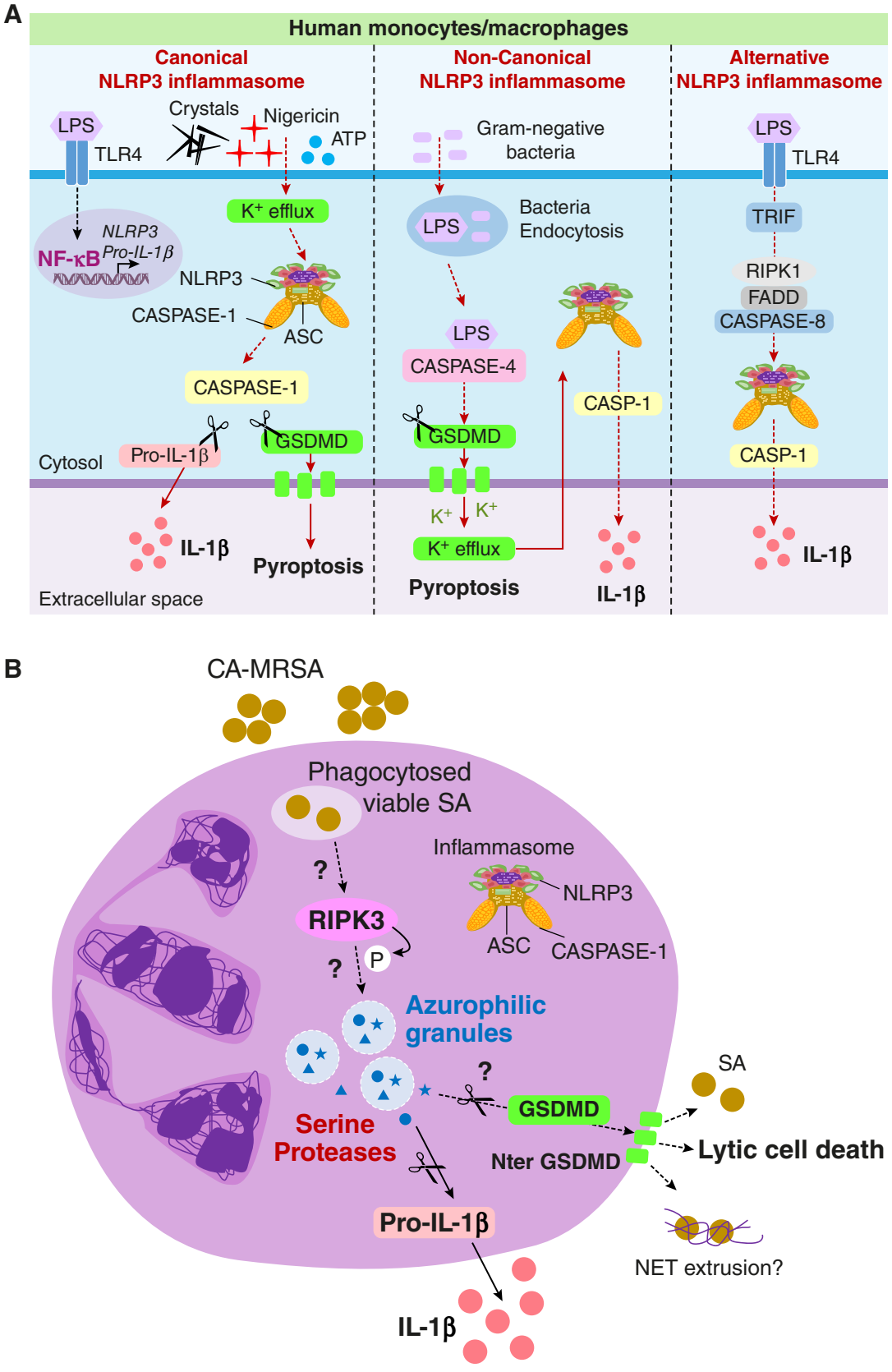

FIGURE 1 **Distinct signaling pathways are involved in IL-1β secretion in neutrophils and in monocytes.** (A) Activation pathways of the NLRP3 inflammasome in human monocytes/macrophages. Left panel: full canonical NLRP3 inflammasome requires both a NF-κBmediated transcriptional priming and a danger- or pathogen-associated molecules-mediated activation. Activated CASPASE-1 accounts for both IL-1β secretion and pyroptosis. Middle panel: Non-canonical NLRP3 inflammasome requires CASPASE-4 to cleave gasdermin D (GSDMD) upon detection of cytosolic LPS. GSDMD pore formation creates a K^+ efflux activating the NLRP3 inflammasome. Right panel: Alternative NLRP3 inflammasome requires

(continued on the next page)

apoptosis (IAPs), the RIPK3 scaffold can activate Caspase-8, which can in turn either directly process pro-IL-1β into IL-1β or activate the NLRP3 inflammasome by a yet undefined mechanism.[10] However, the results from Kremserova and Nauseef showed that IL-1β secretion by neutrophils fed CA-MRSA was inhibited by blocking the kinase activity of RIPK3, but did not involve its scaffold activity. Because RIPK3 kinase activity was necessary for IL-1β secretion by neutrophils fed CA-MRSA, we can exclude that RIPK3 scaffold mediates the activation of Caspase-8 that in turn processes IL-1β. Moreover, they showed that neither NLRP3 nor Caspase-1 were required for IL-1β secretion, ruling out the possibility of a RIPK3-Caspase-8-NLRP3 inflammasome signaling pathway.

When both IAPs and Caspase-8 are inactive in monocytes/macrophages, RIPK3 kinase can phosphorylate and activate mixed-lineage kinase-like protein (MLKL) resulting in necroptotic cell death and subsequent inflammasome activation.[10] However, as previously said, the NLRP3 inflammasome was not required for IL-1β secretion by neutrophils fed CA-MRSA. Moreover, previous work from the same research group provided evidence of that CA-MRSA triggered a non-apoptotic cell death and found a pathway dependent on RIPK3 but independent of MLKL and RIPK1 activities, differentiating it from necroptosis.[11] Thus, these studies propose central non-canonical role for RIPK3 in both cell death and IL-1β release in neutrophils phagocytosing CA-MRSA. Whether cell death and IL-1β secretion are inextricably linked under these circumstances remains to be clarified but the absence of IL-18 secretion suggests that IL-1β release may not be a simple consequence of cell death.

Because NLRP3 inflammasome did not seem to be involved in the IL-1β secretion by human neutrophils fed CA-MRSA, Kremserova and Nauseef looked at the other components known to process IL-1β in neutrophils: serine proteases. They showed that serine proteases including proteinase 3 (PR3), neutrophil elastase (NE), and cathepsin G might account for the maturation and secretion of IL-1β, but not IL-18, in neutrophils fed CA-MRSA. By contrast, inhibition of serine proteases did not block IL-1β secretion in human monocytes-derived macrophages, supporting Caspase-1 as the mediator of IL-1β processing in these cells. These results underline that distinct signaling pathways are involved in IL-1β secretion in neutrophils and in monocytes.

Although it is already known that serine proteases, including PR3 and NE, can process pro-IL-1β into IL-1β, a connection between RIPK3 and neutrophil serine proteases signaling pathways is unexpected. Recently, NE released from cytosolic granules into the cytosol in aging neutrophils has been implicated in the cleavage of GSDMD, whereas Caspase-1 has not.[12] Although the cleavage site of the GSDMD by Caspase-1 and NE differs, cleavage by both enzymes produces a N-terminal GSDMD fragment forming pores at the plasma membrane.[12] Thus, GSDMD cleavage by cytosolic NE leads to lytic cell death of neutrophils with NET release. Results obtained by the Nauseef group allow us to imagine a scenario in which the phagocytosis of CA-MRSA by neutrophils would lead to the activation of a signaling pathway involving the RIPK3 kinase activity, which leads to the release of serine proteases into the cytosol. Once in the cytosol, the serine proteases would mature the pro-IL1β into IL-1β, and potentially cleave GSDMD thereby inducing the necroptosis-, RIPK1-, and MLKL-independent and RIPK3-dependent lytic cell death of neutrophils fed CA-MRSA (Fig. 1B).

It was recently demonstrated that activation of the non-canonical inflammasome in neutrophils by intracellular LPS or intracellular Gram-negative bacteria triggers Caspase-4/11-mediated cleavage of GSDMD that subsequently induces plasma membrane rupture and bacteria release.[13] Moreover, the GSDMD-induced cell death of neutrophils allows extrusion of NET and subsequent cytolysis leading the authors to propose that neutrophils could use this mechanism to fight cytosolic Gram-negative bacteria.[13] Interestingly, Kremserova and Nauseef demonstrated that neutrophils harboring viable CA-MRSA secreted IL-1β in a RIPK3- and serine proteases-dependent manner, whereas neutrophils fed heat-killed bacteria were unable. Moreover, neutrophils harboring viable CA-MRSA lyse in a RIPK3-dependent fashion.[11] Thus, RIPK3 engages an inflammatory, lytic signaling pathway in neutrophils fed viable CA-MRSA. Because Caspase-1 does not efficiently cleave GSDMD in neutrophils,[13] and because Gram-positive bacteria activate the canonical inflammasome (and thus are unable to activate Caspase-4/11 that efficiently cleave GSDMD), we can speculate that serine proteases might be the weapon that cleaves GSDMD in neutrophils fed CA-MRSA ultimately leading to an "inflammatory-type death" that is still not fully characterized.

The study from Kremserova and Nauseef is part of a rapidly expanding field of investigation aiming to identify new mechanisms used by neutrophils to promote inflammation and activate precise and specific death pathways that are adapted to a type of pathogen and are directly involved in their pathogenicity. Regarding the key role of neutrophils in infectious and autoinflammatory diseases,[14,15] the discovery of key molecules regulating the secretion of proinflammatory cytokines like IL-1β and neutrophil death might constitute valuable therapeutic targets.

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DISCLOSURE

The authors have no conflict of interest.

the TLR-adaptor TRIF to engage the RIPK1–FADD–CASPASE-8 complex leading to NLRP3 inflammasome activation. (B) Hypothetical mechanism accounting for RIPK3-mediated secretion of IL-1β and lysis of neutrophils harboring viable *Staphylococcus aureus*. Community-associated methicillin-resistant *S. aureus* (CA-MRSA) ingested by phagocytosis by human neutrophils can remain viable and trigger a RIPK3-mediated signaling pathway. RIPK3 kinase activity, through still unidentified molecular signaling, might induce release of the serine proteases from cytoplasmic granules into the cytosol of neutrophils. Serine proteases then induce processing and secretion of IL-1β, while canonical NLRP3 inflammasome is not involved. Serine proteases might also cleave GSDMD to generate N-terminal fragments (NterGSDMD). The pores formed by NterGSDMD at the plasma membrane might allow bacteria release into the extracellular compartment, lytic cell death of neutrophils, and possibly extrusion of neutrophil extracellular traps (NET) promoting inflammation

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