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INFLAMMASOMES

Acting out

Inflammasomes assemble intracellularly in response to a multitude of signals, such as infection and stress, but whether inflammasomes have a role in the extracellular space has been unclear. Two studies published in *Nature Immunology* now show that inflammasome components are released from cells and function as danger signals to amplify the inflammatory response.

Inflammasomes are protein complexes that are composed of an inflammasome sensor molecule, such as NOD-, LRR- and pyrin domain-containing 3 (NLRP3), that connects to caspase 1 via the adaptor molecule ASC (which is encoded by *PYCARD*). The assembly of the inflammasome leads to activation of caspase 1, which then proteolytically activates the pro-inflammatory cytokines interleukin-1β (IL-1β) and IL-18, and this leads to pyroptotic cell death.

After inflammasome activation, both Baroja-Mazo *et al.* and Franklin *et al.* found inflammasome components in cell-free supernatants from monocyte and macrophage cultures, and extracellular ASC was found in oligomers that aggregated with NLRP3 into so-called ASC specks. The time-dependent accumulation of extracellular ASC specks after the activation of different inflammasomes in macrophages correlated with the release of intracellular proteins, which indicates the loss of plasma membrane integrity. Of note, caspase 1 was required for the extracellular accumulation of ASC specks but not for the formation of intracellular ASC specks. This suggests that the formation of ASC specks precedes cell death and that their release into the extracellular space is controlled by caspase 1.

> “released ASC specks can function as an endogenous danger signal”

Next, both research groups investigated whether the ASC specks remained active in the extracellular space. Indeed, ASC specks could activate pro-caspase 1 and pro-IL-1β that were released from macrophages in response to inflammasome activation. By contrast, supernatants from *Nlrp3*$^{-/-}$ macrophages (which do not release ASC or pro-caspase 1 after inflammasome stimulation) and *Pycard*$^{-/-}$ macrophages could not process pro-caspase 1 and pro-IL-1β into their mature forms. Interestingly, macrophages ingested extracellular ASC specks and this induced the activation of caspase 1. Thus, extracellular ASC specks can activate both pro-caspase 1 and pro-IL-1β, and can function as an endogenous danger signal that could propagate inflammatory signals to surrounding cells.

Could extracellular ASC specks have a role in inflammatory disease? Franklin *et al.* used a mouse model of smoke-induced chronic obstructive pulmonary disease (COPD) to test whether ASC specks accumulate during chronic lung inflammation. Mice that had inhaled cigarette smoke for 8 weeks were shown to have increased numbers of ASC specks in their bronchoalveolar lavage fluid compared with mice that were exposed to normal air. Interestingly, bronchoalveolar lavage fluid from patients with COPD and pneumonia contained extracellular ASC specks, whereas samples from healthy donors did not. Thus, ASC specks may contribute to chronic inflammation in response to smoke-induced damage to cells and tissues.

Baroja-Mazo *et al.* investigated whether ASC specks could be found in serum from patients with cryopyrin-associated periodic syndromes (CAPS), which is a spectrum of autoinflammatory syndromes that are associated with mutations in *NLRP3*. Indeed, extracellular ASC specks were more frequent in serum that was taken from patients with CAPS during acute inflammatory episodes compared with serum from healthy donors. Of note, there was not a substantial difference in the amount of ASC specks in the serum of healthy donors and serum that was taken from patients with CAPS during symptom-free intervals. The authors conclude that ASC specks might be involved in the pathogenesis of CAPS by activating surrounding macrophages.

In summary, these studies provide evidence that the activation of inflammasomes leads to the release of functional ASC specks that promote the activation of caspase 1 extracellularly and also in surrounding macrophages after being internalized. The fact that extracellular ASC specks appear after inflammasome activation could be important when designing novel therapies for inflammatory diseases.

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ORIGINAL RESEARCH PAPERS
Baroja-Mazo, A. *et al.* The NLRP3 inflammasome is released as a particulate danger signal that amplifies the inflammatory response. *Nature Immunol.* http://dx.doi.org/10.1038/ni.2919 (2014) | Franklin, B. S. *et al.* The adaptor ASC has extracellular and ‘prionoid’ activities that propagate inflammation. *Nature Immunol.* http://dx.doi.org/10.1038/ni.2913 (2014)
FURTHER READING Latz, E. *et al.* Activation and regulation of the inflammasomes. *Nature Rev. Immunol.* **13**, 397–411 (2013)