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## Assembling the Inflammasome, Piece by Piece

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## Assembling the Inflammasome, Piece by Piece

Fiachra Humphries and Katherine A. Fitzgerald

An evolutionarily conserved cysteine protease, IL-1 $\beta$ -converting enzyme (ICE), later called caspase-1, was identified in 1992 as the enzyme controlling the proteolytic cleavage and maturation of IL-1 $\beta$ , a proinflammatory cytokine controlling inflammation. In this *Pillars of Immunology* article, published a decade later, Tschopp and colleagues (1) identified the inflammasome: a large multiprotein complex controlling the activation of caspase-1 and maturation of IL-1 $\beta$  [this article may be viewed at [https://doi.org/10.1016/S1097-2765\(02\)00599-3](https://doi.org/10.1016/S1097-2765(02)00599-3)]. Prior to this seminal 2002 study, caspase-1 was known to control IL-1 $\beta$  and IL-18 maturation. However, the mechanisms controlling caspase-1 activity remained elusive (2). Pycard, now commonly known as ASC, was a bipartite CARD/PYD domain protein that aggregated and interacted with caspase-1 (3, 4). Using coimmunoprecipitation and an *in vitro* cell-free system, Tschopp and colleagues demonstrated that IL-1 $\beta$  maturation was preceded by the formation of an ASC, caspase-1/5, and Nalp1 (NLRP1)-containing complex, which he and his coauthors called the inflammasome. This large complex was formed through highly specific, bridging interactions of the caspase-1 CARD and NLRP1 PYD domains via ASC. Ab depletion of ASC from cell lysates attenuated caspase-1-mediated IL-1 $\beta$  cleavage. Notably, the authors also detected active caspase-1 in the extracellular medium following inflammasome activation, an observation later explained by the discovery of pyroptotic cell death (5). Interestingly, disruption of the plasma membrane was sufficient to trigger assembly of the inflammasome complex, which retrospectively highlighted how loss of plasma membrane integrity is a key driver of inflammasome assembly. The authors likened the inflammasome to the cognate APAF1/caspase-9 apoptosome activated via mitochondrial cytochrome C release.

Although this *Pillars of Immunology* article first characterized the NLRP1 inflammasome, continued work from Tschopp and other groups later defined the now more widely studied inflammasome, NLRP3, among others (6–8). Like NLRP1, NLRP3 forms an ASC-dependent inflammasome with caspase-1 (6, 9, 10). In the 17 y since the discovery of the inflammasome, we now appreciate that multiple distinct

inflammasomes exist, each activated by different microbial or endogenous danger signals. These foundational observations established a new field in inflammation research that has collectively uncovered key mechanisms driving inflammasome activation and function in both health and disease. Multiple upstream stimuli have been implicated in controlling NLRP3 activation, including potassium efflux, chloride ions, calcium flux, lysosomal disruption, mitochondrial dysfunction, and *trans*-Golgi disassembly (11–15), homeostatic perturbations that are all sensed indirectly by NLRP3. However, the exact mechanisms controlling NLRP3 activation remains to be fully elucidated. Tschopp and colleagues (6) also identified gain-of-function mutations in NLRP3 and speculated that patients harboring these mutations would have uncontrolled activation of caspase-1 and mature IL-1 $\beta$ . Indeed, patients with these mutations are treated with IL-1-blocking therapeutics.

Since the early discovery of the NLRP1 and NLRP3 inflammasomes, additional NLR family members were linked to caspase-1 activation. Cytosolic dsDNA was found to engage the AIM2 inflammasome complex (16), whereas cytosolic LPS engages the related protease caspase-11 to form a noncanonical inflammasome complex (17). Another discerning insight from the Tschopp study also forecasted what we now understand about the mechanisms controlling NLRP1 activation. Indeed, Tschopp proposed a means of basal self-regulation for NLRP1, as deletion of the NLRP1/LRR domain triggered its constitutive activation. More recently, elegant studies on NLRP1 activation have highlighted the importance of proteasomal degradation of the N-terminal region, a key signal required for NLRP1 inflammasome assembly. *Bacillus anthracis* lethal toxin induces cleavage of the murine NLRP1 and proteasomal degradation of the NLRP1 N terminus. These events then cause release of the bioactive C-terminal, CARD-containing fragment to form the active NLRP1 inflammasome (18, 19).

Since the initial discovery of the inflammasome, additional substrates of caspase-1 have been identified, broadening the inflammatory potential of these complexes through the proteolytic cleavage of gasdermin-D and induction of pyroptosis (17, 20, 21). Caspase-1 or caspase-11 activation leads to the generation of an N-terminal fragment of gasdermin-D that inserts into the membrane, leading to pore formation, release of IL-1 $\beta$ , potassium efflux, and secondary activation of NLRP3. Thus, from the initial discovery of the NLRP1 inflammasome, our understanding of inflammasomes now encompasses complex integrated cell signaling mechanisms that respond to pathogen- and danger-associated molecular patterns.

Given the diversity of danger signals as activators of inflammasomes and the proinflammatory potential of IL-1 $\beta$  and

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pyroptosis, these pathways now represent a major target for therapeutic development. Inflammasomes have been implicated in a number of inflammatory diseases, such as arthritis, type II diabetes, cardiovascular disease, Parkinson disease, and Alzheimer disease (22). Enormous efforts in the biotechnology and pharmaceutical sectors are now focused on the development of NLRP3 targeting small molecule drugs for the treatment of inflammatory diseases.

In summary, the study by Tschopp and colleagues first defined formation of the inflammasome complex and pioneered a new field of cell signaling that represents a major arm of the innate immune and inflammatory response. Since its inception, our understanding of inflammasomes and the molecular and physiological events that drive inflammasome assembly has continued to grow. The significance of this *Pillars in Immunology* article continues to be highlighted with new studies uncovering novel mechanistic insights into inflammasome biology and their role in inflammatory diseases.

## Disclosures

K.A.F. is a consultant for two companies focused on inflammasomes and holds stock interests in both (Quench Bio and NodThera). The other author has no financial conflicts of interest.

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