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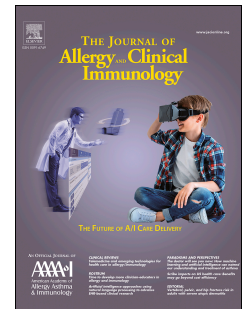
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Caspase 8 Sounds the Alarm for Allergic Inflammation

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Alarmins are innate cytokines released by damaged or dying cells that act to initiate inflammatory immune responses. One prototypical alarmin is IL-33, a member of the IL-1 family of cytokines that is enriched in epithelial cells and plays a key role in the initiation of allergic immune responses (1-3). The 270 amino acid (AA) IL-33 protein has three main regions: an N-terminal nuclear domain (AA 1-65), a protease sensitive central domain (AA 65-112), and a C terminal IL-1-like domain (AA 112-270) that binds to the IL-33 receptor ST2 and contains caspase cleavage sites that are thought to permit its degradation. The N-terminal nuclear domain localizes the full length 270 AA precursor form (IL-33₁₋₂₇₀) in the nucleus, where it is normally sequestered. Upon cellular damage or necrosis, IL-33₁₋₂₇₀ is released into the extracellular milieu, where it can either directly bind ST2 or it can be cleaved into its more active mature form (3). This extracellular cleavage step is mediated by endogenous proteases released from activated neutrophils and mast cells, which cleave at several sites in the central domain to yield C terminal mature IL-33 (mIL-33) fragments (IL-33₉₅₋₂₇₀, IL-33₁₀₇₋₂₇₀ and IL-33₁₀₉₋₂₇₀) that exhibit increased activity compared to IL-33₁₋₂₇₀. This cleavage step can also be performed by exogenous cysteine and serine proteases found in allergens such as house dust mite and *Alternaria alternata* (4). These observations have led to our current understanding of IL-33 function in which the full-length protein is released from the nucleus of necrotic cells into the extracellular milieu, where it has some minimal activity on its receptor ST2. Upon cleavage of its central domain by endogenous and exogenous proteases, free and highly active mIL-33 containing the intact C terminal IL-1-like domain is released. This pathway is central to the initiation of allergic immune responses, however, fundamental questions remain about the cleavage and release of IL-33. In response to allergen exposure, mIL-33 can be detected in the absence of necrosis and drives the *initiation* of allergic inflammation (3). If mIL-33 is detected prior to cell death and the influx of inflammatory cells, how is mIL-33 formed and released? Alternatively, is there another form of IL-33 that can initiate the inflammatory response?

In a recent issue of Nature Immunology, Brusilovsky et al illustrate a novel pathway for the allergen induced activation and release of $mIL-33$ (**Figure 1**). The authors found that incubation of the EPC2 epithelial cell line with a diverse group of animal and fungal, but not plant or food allergens induced the rapid intracellular production of $mIL-33$ (~21KDa). This occurred concurrently with RIPK1 phosphorylation and caspase 8 cleavage, suggesting that allergen stimulation of epithelial cells activates the ripoptosome (5). The ripoptosome is a spontaneously assembled macromolecular protein complex of RIPK1 (RIP1), pro-caspase 8, TRADD, FADD, and cFLIP. Activation of this complex by diverse stimuli including genotoxic stress, loss of cellular inhibitor of apoptosis proteins (cIAPs), and TLR3 stimulation leads to cleavage of procaspase 8 into its active form, ultimately culminating in caspase 8-mediated apoptosis and/or necrosis (6, 7). Despite the role of caspase 8 in cell death, $mIL-33$ production and release appeared to precede or occur independently of cell death, indicating an alternative mechanism for IL-33 release and cleavage. The authors found that caspase 8 activation led to downstream activation of caspases 3 and 7, which were capable of cleaving IL-33₁₋₂₇₀. Caspases 3 and 7 have previously been described to inactivate IL-33 by cleaving it in its IL-1-like cytokine domain after residue D175 or D178. Disruption of the C terminal, IL-1 like domain, would be expected to inactivate IL-33, but the authors showed instead that the IL-33₁₋₁₇₅ and IL-33₁₋₁₇₈ contained a minimal ST2 binding domain. As such, they were not only capable of binding to the ST2 receptor, but were as potent as the unprocessed IL-33₁₋₂₇₀ at promoting inflammatory cytokine and chemokine release from the HMC-1 human mast cell line and primary murine eosinophils. Intriguingly, the ST2-mediated activity of the IL-33₁₋₁₇₅ and IL-33₁₋₁₇₈ fragments was augmented on the presence of histones that remained bound to the N terminal chromatin-binding domain (5).

To establish a role for the caspase 8-dependent N-terminal fragment of IL-33 in vivo, the authors used an *Alternaria alternata* induced murine model of allergic airway inflammation. Intratracheal exposure to *Alternaria alternata* led to caspase 8-dependent IL-33 release in the bronchial alveolar lavage (BAL), with concomitant neutrophil and eosinophil influx that was blocked by a caspase 8-specific inhibitor or in mice lacking airway caspase 8. Based on the molecular weight of IL-33 and its detection prior to inflammatory cell infiltration it is likely that this represented the IL-33_{1-175/178} fragment, although it is difficult to rule out the presence of the IL-33₁₀₇₋₂₇₀ C-terminal fragment using these assays. Likewise, it is unclear whether caspase 8 was required for IL-33₁₋₂₇₀ cleavage or cell death, but these data provide supporting in vivo evidence

for the caspase 8-mediated pathway. To link these murine observations with human disease, the investigators examined esophageal biopsies from patients in remission or with active eosinophilic esophagitis (EoE). Active EoE was associated with increased staining for caspase 8 and caspase 3, IL-33, and eosinophils, thereby providing correlative evidence for their in vitro pathway in human samples (5).

These data reveal an unexpected new ripoptosome-mediated pathway for IL-33 maturation and release. However, many questions remain. The mechanism by which allergens activate this process remains unknown. The authors show that fungal and animal allergens induce this pathway, while plant and food allergens did not. The inducing allergens include both protease rich (e.g., fungal and cockroach) and protease poor (e.g., cat dander) allergens, making protease activity an unlikely trigger. House dust mite extracts have been shown to contain dsRNA with activity on TLR3, but TLR3 deficiency had no effect on downstream caspase activation or IL-33_{1-175/178} production (8). Thus, the mechanism of direct allergen sensing and the activation of ripoptosome triggered by allergens in the epithelium remains unclear.

Beyond allergen sensing, this work raises many questions about the roles of different IL-33 cleavage products. Cleavage within the protease-sensitive central region of IL-33₁₋₂₇₀ generates and frees the highly active and mature form IL-33₁₁₂₋₂₇₀ containing the intact C terminal IL-1-like domain. If IL-33_{1-175/178} is also released after allergen exposure, what is the relative role or importance of each of these IL-33 cleavage products? The IL-33_{1-175/178} fragment appeared to have similar activity to the full length pIL-33, suggesting that IL-33_{1-175/178} is less active than the IL-33₁₁₂₋₂₇₀ fragment. Could these different IL-33 fragments play sequential roles in allergen-induced inflammation, with IL-33_{1-175/178} initiating and IL-33₁₁₂₋₂₇₀ amplifying local allergic inflammation? Alternatively, the IL-33 cleavage products may have different functions. IL-33_{1-175/178} was released in complex with histones, which have innate anti-microbial activities and activate alternative inflammatory pathways (9). It is tempting to speculate that allergen induced release of IL-33_{1-175/178} may have dual actions by activating innate inflammatory responses as well as by directly killing allergen-accompanying microbes. Given the similar size of both cleavage products (differing in size by 2-3 kDa) and their dual detection by commercial antibodies, determining the relative contribution of IL-33_{1-175/178} versus IL-33₁₁₂₋₂₇₀ may require the development of specific antibodies for detection. But as this work indicates, understanding

the complexity of IL-33 regulation and release may be central to understanding allergic immunity.

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Figure 1. Allergens trigger the caspase 8-rioptosome signaling pathway to activate and release IL-33. In lung epithelium, multiple allergens such as *Alternaria alternata* and house dust mite induce the activation of the ripoptosome. Following RIP1 (RIPK1) phosphorylation, pro-caspase 8 undergoes self-cleavage into active caspase 8, induces the generation of active caspases 3 and 7. Caspase 3 and caspase 7 cleave the histone-bound pIL-33₁₋₂₇₀ within the IL-1 cytokine domain, generating mIL-33_{1-175/178}. The mIL-33_{1-175/178} fragment is released bound to histones that potentiate its ST2-mediated activity and promote Type-2 or allergic inflammation.

