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RESEARCH HIGHLIGHT

Gasdermin D as a cellular switch to orientate immune responses via IL-33 or IL-1β

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Interleukin (IL)-33 is a nuclear cytokine in the IL-1 family that is constitutively expressed in the epithelial cells of environmentally exposed tissues (skin, gastrointestinal tract and lungs) and endothelial cells. IL-33 is involved in type 2 innate immunity via the activation of eosinophils, basophils, mast cells, group 2 innate lymphoid cells and macrophages through its receptor ST2 (also called IL1RL1) [1]. Due to the absence of a secretory signal sequence, IL-33 was thought to be passively released during cell necrosis, physical stress or tissue damage and was accordingly considered an alarmin [2]. However, Chen et al. recently demonstrated that in response to exposure to allergen proteases, IL-33 is transported from the nucleus to the cytosol via stress granule (SG) assembly followed by subsequent release of the active cytokine through membrane pores formed by the unusual p40 N-terminal fragment of gasdermin D (Gsdmd) [3]. Importantly, these events were not associated with any signs of cell death, thus uncovering a new pathway of IL-33 release distinct from the "alarmin" archetype (Fig. 1).

The role of Gsdmd has been well described in pyroptosis, which is a form of cell death associated with tissue damage. During canonical inflammasome-induced pyroptosis, Gsdmd is proteolytically cleaved by activated caspase-1 to generate an active fragment with membrane pore-forming abilities, which allows the release of IL-1β and IL-18 through unconventional protein secretion [4]. In contrast, Chen et al. observed that following stimulation with the allergen protease papain, the formation of a novel p40 or p35 N-terminal (NT) Gsdmd fragment in murine MLE-12 or human A549 epithelial cell lines, respectively, occurred [3]. The appearance of these neofragments was concomitant with the delocalization of IL-33 from the cell nucleus and its release into the supernatant without apparent cell death or caspase activation. This effect was reversible after the removal of papain from the medium. To differentiate this observation from the conventional inflammasome-dependent pyroptosis mediated by Gsdmd, Chen et al. compared the effect of inflammasome activation and papain stimulation in murine bone marrow-derived macrophages (BMDMs). While activation of the inflammasome with lipopolysaccharide (LPS) and ATP or nigericin led to the cleavage of caspase-1 and the generation of a conventional pyroptotic Gsdmd fragment (35-kDa) plus lactate dehydrogenase (LDH) and IL-1β release in these cells, stimulation with papain promoted the appearance of p40 NT-Gsdmd and the secretion of IL-33. The lack of caspase involvement in papain-induced IL-33 secretion was confirmed with casp-1/casp-11-deficient BMDMs and the use of the pan-caspase inhibitor Z-VAD-FMK. On the other hand, the protease activity of papain and other allergen proteases was indispensable for IL-33 secretion. However, proteases from *Alternaria alternata* failed to induce p40 NT-Gsdmd but induced IL-33 release in vitro, suggesting an alternative pathway for IL-33 secretion via this protease.

The essential role of SGs in this newly described mechanism of IL-33 secretion was determined by visualising the formation of G3BP1-positive SG puncta in response to papain stimulation, which permitted the nuclear-cytosol translocation of IL-33. SGs are dynamic compartments assembled in the cytoplasm for the transport of RNA, ribosomal subunits and various proteins following translational arrest in response to stress [5]. Although SG assembly induced by arsenite and papain resulted in the nucleocytoplasmic translocation of IL-33, papain stimulation exclusively triggered the secretion of IL-33 into the supernatant, suggesting that SG assembly was an independent prerequisite event in IL-33 secretion.

Chen et al. then explored the potential cleavage sites that generated the p40 NT fragment responsible for IL-33 secretion. Among the in silico predicted fragments and the caspase-induced pyroptotic fragments they generated, only the Gsdmd$^{1-311}$ and pyroptotic Gsdmd$^{1-276}$ fragments induced efficient IL-33 secretion when cotransfected with mature IL-33 lacking the nuclear localization signal peptide to bypass the need for nucleocytoplasmic translocation. However, the Gsdmd$^{1-311}$ fragment triggered less LDH release than the pyroptotic Gsdmd$^{1-276}$ fragment. The introduction of site-specific mutations further identified residues 309–313 and 288–292 as putative cleavage sites to obtain the murine p40 NT and human p35 NT-Gsdmd fragments, respectively.

The contribution of Gsdmd to the development of type 2 inflammation was confirmed in asthmatic patients and in a mouse model of asthma induced by house dust mites (HDMs). In asthmatic patients, Gsdmd expression in the lung airway epithelium correlated with IL-33 secretion in bronchoalveolar lavage (BAL) fluid and elevated serum IgE levels. Similarly, in mice that were intranasally challenged with HDM, inflammatory infiltration in the lungs was associated with a pulmonary increase in Gsdmd levels. When mice were challenged with papain, IL-33 was significantly increased, while the levels of the other type 2 inflammatory cytokines IL-25 and thymic stromal lymphopoietin

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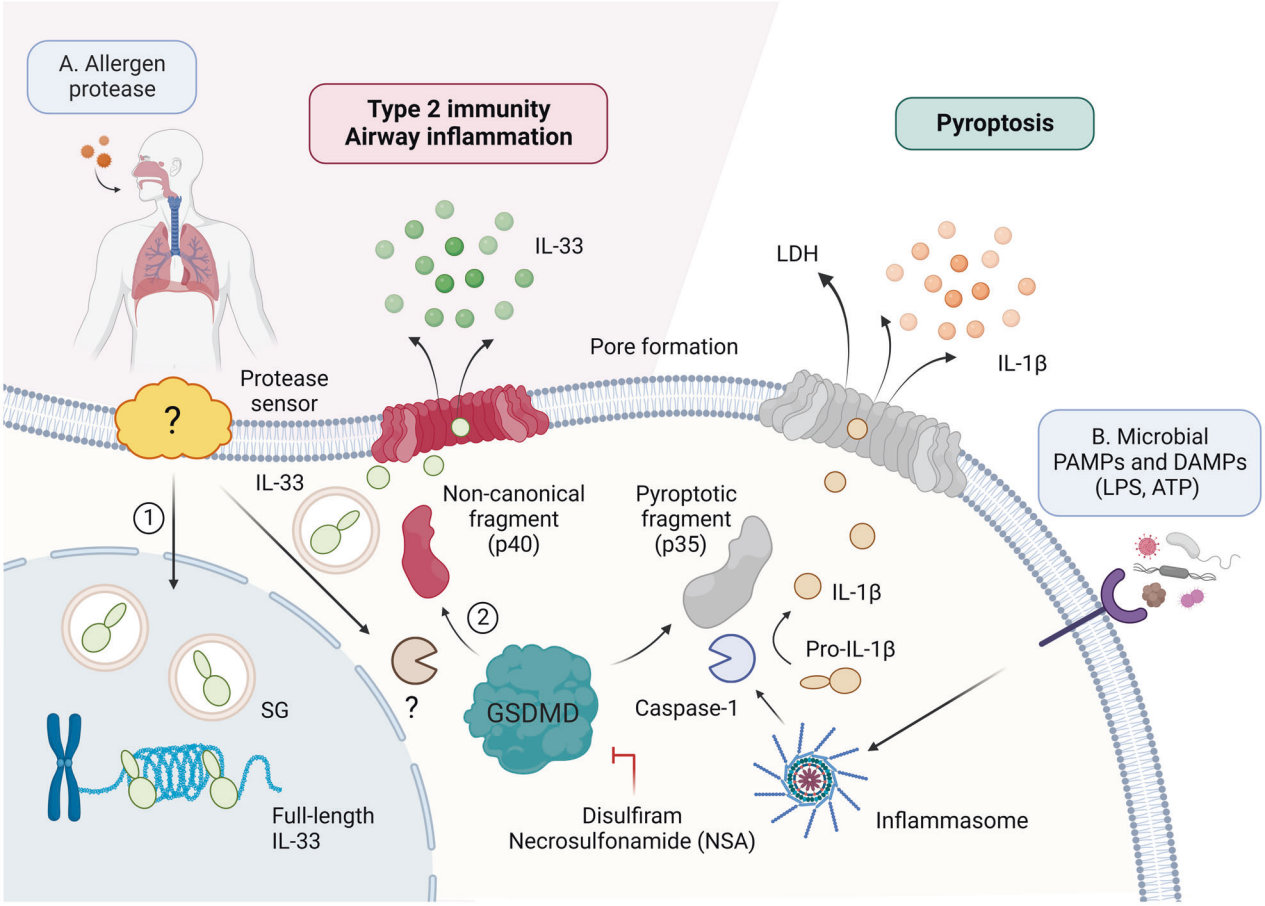

Fig. 1 Context-dependent role of Gasdermin D (Gsdmd) in the release of IL-33 and IL-1β. In response to allergen protease stimulation (A), nuclear IL-33 is transported into the cytosol via dynamic SG assembly (1). The subsequent generation of pores by the newly described fragment of Gsdmd (p40 NT in mouse/p35 NT in human) (2) leads to the active release of IL-33 into the extracellular space, which is independent of the canonical inflammasome pathway that is activated in response to microbial pathogen-associated molecular patterns (PAMPs) such as LPS and molecules associated with the endogenous danger-associated molecular pattern (DAMP) ATP (B). Drugs targeting Gsdmd could be used as therapeutic agents to curb type 2 inflammation. However, the nature of the sensing receptor of the allergen protease and the identity of the enzyme that generates the p40 NT-Gsdmd need to be investigated. Figure created at BioRender.com

remained unchanged. However, a slight increase in IL-1β was also detected in papain-challenged mice, suggesting residual activation of the canonical inflammasome pathway in these mice. Analysis of the BAL fluid from papain-exposed Gsdmd-deficient mice confirmed the requirement of Gsdmd for the secretion but not the de novo synthesis of IL-33 because the RNA levels in the transgenic mice were unaffected. Furthermore, when Gsdmd$^{-/-}$ and WT mice were exposed repetitively to HDM to mimic chronic asthma or were acutely stimulated with papain for 5 days, decreased levels of IL-5 and IL-13 were associated with reduced lung infiltration in Gsdmd$^{-/-}$ mice, confirming the involvement of Gsdmd in the development of type 2 inflammation.

The data described here explain a plausible alternative mechanism for the secretion of IL-33 in response to allergen proteases. However, several molecular players involved in this pathway remain to be elucidated, such as the direct sensing receptor of the allergen protease in this context and the pathway(s) that lead to p40 NT-Gsdmd fragmentation. Although it has been well established that the pyroptotic fragment is generated by the cleavage of Gsdmd by caspase-1, the enzyme that generates p40 NT-Gsdmd has not been identified. The ability of Gsdmd to create membrane pores that can have divergent physiological responses in distinct cell types or in response to diverse stimuli has been proposed [6]. Based on this work, we postulate that the pores formed by the pyroptosis fragment could be structurally dissimilar from the p40 NT-Gsdmd generated in response to different environmental cues. Gsdmd seems to be a molecular switch that orients the immune response depending on the trigger or stimuli. Moreover, the current data might help in identifying the mechanism(s) by which treatment with intravenous immunoglobulin, which is one of the commonly used immunotherapeutic drugs, leads to enhanced IL-33 in the circulation [7].

Chen et al. identified SG as a major player in IL-33 transport in the cytosol after allergen exposure. However, the trigger and the regulatory mechanisms of SG assembly are not known. IL-1β has been shown to induce SG assembly in human osteoarthritis chondrocytes [8]. IL-1β released from necroptotic cells could also regulate the formation of SGs and indirectly affect IL-33 secretion by epithelial cells and macrophages. The involvement of SG in the production of IL-1β in macrophages has also been suggested to modulate the Th1/Th17 balance [9], showing the importance of these dynamic compartments in the regulation of immune responses.

The allergen protease papain has also been shown to activate basophils, leading to the production of the Th2 cytokine IL-4. Despite previous attempts to identify the signaling pathways that are activated by papain in basophils, the specific molecular mechanisms are not yet clear. Studies using various knockout mice have revealed that the activation mechanism in basophils is independent of many common cell signaling pathways, including the caspase-1 inflammasome pathway [10]. In this context, a

pathway similar to p40 NT-Gsdmd could be envisioned in the case of basophils or other innate cells and mediate the release of cytokines.

In line with the findings of Chen et al., could targeting GSDMD be useful in curbing type 2 inflammation and airway inflammatory responses? Efforts targeting the Cys191/Cys192 site of Gsdmd, which is indispensable for Gsdmd oligomerization and pore formation, have shown success with the discovery of necrosulfonamide (NSA) [2] and the FDA-approved drug disulfiram [11] as an inhibitor of pyroptosis that blocks gasdermin pore formation (Fig. 1). In a recent study, pharmacological inhibition of Gsdmd by disulfiram prevented neutrophil extracellular trap (NET) formation and reduced inflammation and lung tissue damage in an experimental model of COVID-19, highlighting the importance of targeting this molecule in multiple inflammatory pathologies [12]. Of note, SARS-CoV-2 infection has been shown to induce IL-33 production in epithelial cells [13]. Similar small-molecule drug approaches targeting the cleavage site of the newly discovered p40 NT-Gsdmd fragment could be a viable therapeutic option for the benefit of patients with chronic airway inflammation. As another example, the necroptosis inhibitor GW80 attenuated lung inflammation in vivo in an IL-33-dependent *Aspergillus fumigatus* extract-induced asthma model [2]. These drugs have been examined in the context of proinflammatory forms of cell death that result in cell lysis. However, with the novel discovery of the involvement of Gsdmd in IL-33 secretion, it would be worth studying them in the context of allergen protease sensitization and type 2 inflammation.

Altogether, Chen et al. convincingly shed light on two uncoupled mechanisms that could explain the transport of IL-33 in the cytosol via SG assembly and its active secretion into the extracellular milieu through the generation of pores by a newly described fragment of Gsdmd. These results suggest many attractive possibilities to ameliorate type 2 inflammation.

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AUTHOR CONTRIBUTIONS

CC, SVR and JB performed the literature search and analyses and drafted the manuscript.

COMPETING INTERESTS

The authors declare no competing interests.

ADDITIONAL INFORMATION

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