Trends in
Immunology

CellPress

Spotlight

Chopped! Newfound GSDMD cleavage facilitates tolerance to food allergens

Liam B. Healy, [1,2] Gang Du, [1,2] and Hao Wu [1,2,*]

In a recent article, He *et al.* report that, in response to dietary protein antigens, mouse intestinal epithelial cells (IECs) accumulate a newfound 13-kDa N terminus of gasdermin D (GSDMD-N13), cleaved by caspase-3/7. Unlike the pyroptotic 30-kDa fragment, GSDMD-N13 translocates to the nucleus, inducing CIITA and major histocompatibility complex class II (MHCII) expression to promote type 1 regulatory T (T1r) cell development, thus revealing its role in balancing immunity and food tolerance.

IECs are specialized cells lining the inner surface of the intestine, forming a barrier between the digestive contents in the intestinal lumen and underlying tissues. They have a vital role in the absorption of nutrients, secretion of mucus and enzymes, and maintenance of the intestinal barrier function [1]. Certain food antigens, despite being non-self, do not usually induce abnormal immune responses, unless certain pathological conditions break immune tolerance to induce inflammation and tissue damage in the gastrointestinal tract [2,3]. Of note, GSDMD, the primary executioner of pyroptosis, is highly expressed in mammalian IECs, especially from the upstream small intestine [1,4,5].

In a recent article in *Cell* [6], He *et al.* isolated various mouse intestinal tissues and identified, via immunoblotting, full-length GSDMD and the inflammatory caspase-cleaved N terminus of GSDMD (GSDMD-N30) in small intestine and bone marrow tissues. In small intestine IEC samples, the unexpected GSDMD-N13 was detected, and was expressed more prominently compared with inflammatory GSDMD-N30. To confirm the identity of the 13-kDa band, the authors generated knock-in mice containing N-terminally FLAG- or C-terminally HA-tagged GSDMD. IEC samples collected from these mice confirmed the 13-kD band as GSDMD-N13 (cleaved at D88) and a corresponding C-terminal fragment (GSDMD-C42). Notably, the signals for GSDMD-N13 were especially dominant in upper small intestine IECs, while GSDMD-N30 was more abundant in whole-tissue samples from bone marrow and intestine, leading the authors to speculate that GSDMD might have a nonpyroptotic role in IECs.

To identify the triggers that generated the unique 13/42 cleavage of GSDMD, the authors systematically depleted various environmental components from the small intestine. For instance, the removal of commensal bacteria and viruses, or inflammasome activators including microbial signaling molecules or bile acids, did not reduce GSDMD-N13 induction. Considering the abundance of undigested nutrients in the upper small intestine, the authors interrogated whether food components might be involved in inducing GSDMD-N13. Supporting this hypothesis, mice fed standard chow containing proteinaceous dietary antigens exhibited significantly higher expression of GSDMD-N13 in the upper small intestine compared with mice fed an amino acid diet devoid of intact proteins.

Moreover, to identify the enzymes responsible for the 13/42 GSDMD cleavage, purified tagged GSDMD was used to immunoprecipitate proteins in IEC or HEK293T lysates, followed by immunoprecipitation–mass spectrometry (IP-MS). The analysis revealed caspase-1/3/7 as the top candidates binding GSDMD. However, caspase-1 was only weakly activated in IECs, whereas caspase-3 and-7 were strongly activated in IECs but not in bone marrow cells. Additionally, caspase-3/7 activation occurred in upper small intestine IECs, but not in distal small intestine or colon IECs. In addition, overexpression of caspase-3 or -7 generated 13/42 GSDMD cleavage at D88 in HEK293T cells, and cleavage site mutation or inhibition of caspase-3/7 abolished 13/42 GSDMD processing in cells, as well as *in vivo* in mice, without affecting pyroptotic cleavage.

Next, the authors explored the mechanism by which protein-based dietary antigens triggered GSDMD-N13 cleavage. Using biotin-labeled full-length ovalbumin (OVA-FL) or an ovalbumin peptide harboring an H-2^b-restricted MHCII epitope and streptavidin beads, they pulled down OVA-interacting proteins from lysates of the upper small intestine. They discovered that caspase-3/7, but not caspase-1, interacted with the ovalbumin peptide, but not OVA-FL, suggesting a potential correlation of antigen binding with the antigen presentation process. Furthermore, the authors found that certain OVA peptides induced cleavage and activated the catalytic activity of caspase-3/7.

To understand the functional consequences of 13/42 GSDMD cleavage, He and co-workers expressed and examined the subcellular localization of GSDMD-N13 and GSDMD-C42. GSDMD-N13 localized to the nucleus, while GSDMD-C42 localized to the cytosol. Through IP-MS analysis of small intestinal cell extracts using GSDMD-N13 as bait, the authors identified several nucleoporins, components of the nuclear pore complex, that interacted with GSDMD-N13. Further experiments confirmed the interaction between GSDMD-N13 and nucleoporins and showed that knockdown of these nucleoporins altered the nuclear distribution of GSDMD-N13.

Furthermore, to examine the physiological impact of nuclear GSDMD-N13 in IECs, He *et al.* generated GSDMD-knockout mice, IEC-specific GSDMD-deficient mice, and mice harboring a GSDMD mutant that was resistant to 13/42 cleavage. Transcriptome analysis revealed the differential regulation of genes related to antigen processing and presentation, particularly MHCII proteins and CIITA, which is a key regulator of MHCII expression. Indeed, MHCII proteins are essential for the initiation of antigen-specific $CD4^+$ T cell immune responses [7]. Thus, mice that were either fed a specific amino acid diet or treated with a caspase-3/7 inhibitor, or mice harboring a GSDMD mutant resistant to 13/42 cleavage, were assessed; the authors reported decreased expression of transcripts encoding MHCII and

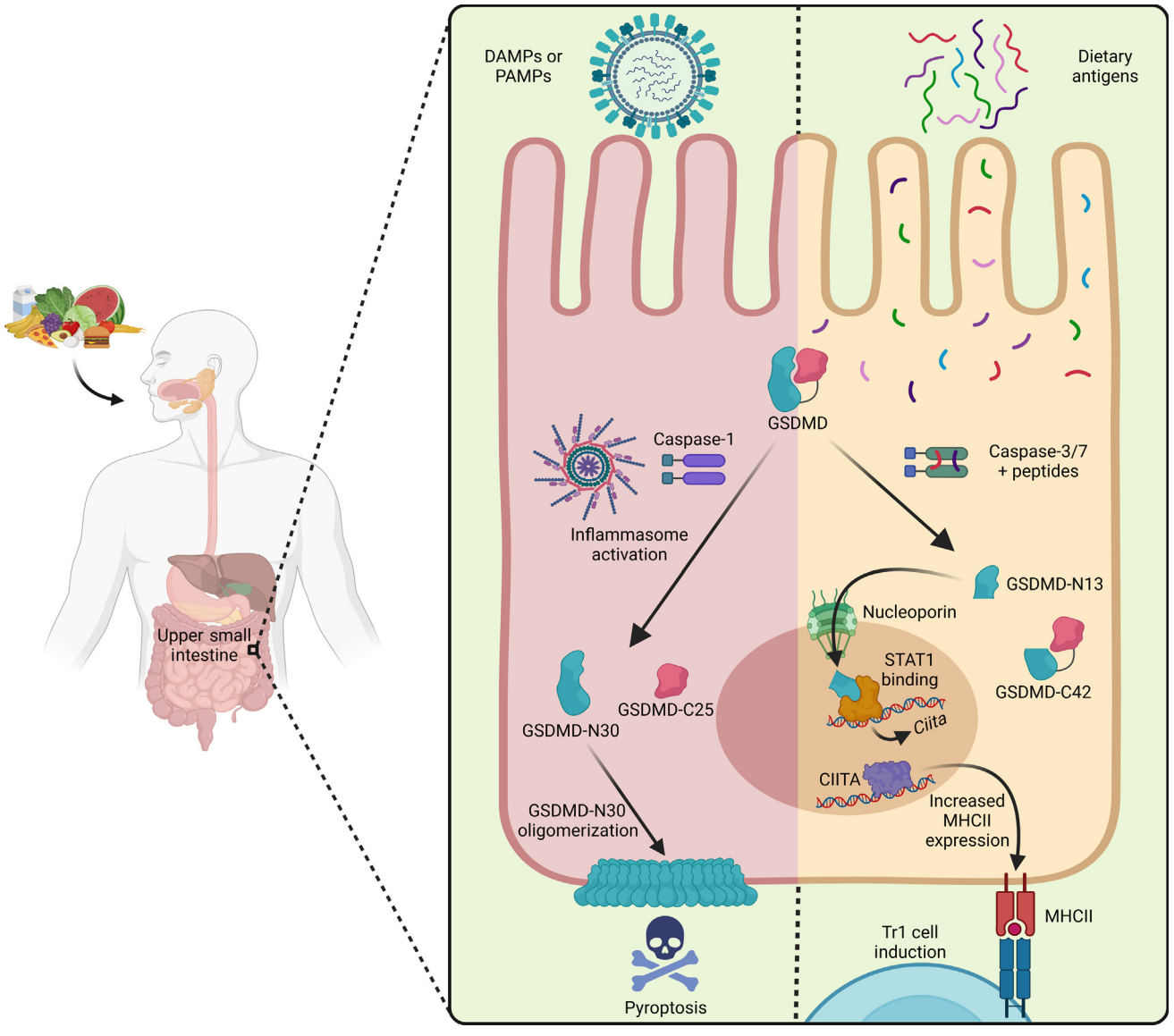

Figure 1. Newfound gasdermin D (GSDMD) cleavage can facilitate food tolerance in mice. In intestinal epithelial cells (IECs), food antigens trigger caspase-3/7 activation, leading to the production of the 13-kDa N terminus of gasdermin D (GSDMD-N13). GSDMD-N13 subsequently translocates to the nucleus via nucleoporins, interacts with STAT1, and enhances STAT1 binding to the *Ciita* promoter, ultimately promoting *Ciita* transcription. This cascade results in elevated major histocompatibility complex II (MHCII) expression and the induction of type 1 regulatory T (Tr1) cells, increasing immune tolerance to food [6]. Abbreviations: DAMPs, damage-associated molecular patterns; PAMPs, pathogen-associated molecular patterns. Figure created with BioRender (biorender.com).

CIITA in upper small intestine IECs in these mice, compared with controls, hence establishing a cause-and-effect relationship [6].

The role of GSDMD-N13 in transcriptional activation was further examined, revealing an interaction of this gasdermin with STAT1, a transcription factor involved in CIITA expression; indeed, this was one of the top hits from IP-MS using GSDMD-N13. Moreover, chromatin-IP analyses indicated that GSDMD-N13 significantly promoted the binding of STAT1 to one of the *Ciita* promoters, and enhanced STAT1 binding was diminished in IEC-specific GSDMD-deficient mice or in mutant mice harboring the 13/42 cleavage-resistant GSDMD. These findings support a model in which GSDMD-N13 is produced by food antigen-activated caspase-3/7 in IECs, translocates to the nucleus via nucleoporins, interacts with STAT1, promotes STAT1 binding to the *Ciita* promoter, and enhances *Ciita* transcription, in turn upregulating MHCII expression (Figure 1).

Of note, antigen presentation by IECs can induce tolerogenic immune cell populations [8,9]. Thus, He *et al.* conducted single cell RNA-sequencing, uncovering a profound reduction in Tr1 cells ($CD4^+IL\text{-}10^+Foxp3^-$), but not regulatory T cells (Tregs) ($CD4^+Foxp3^+$), in the upper small intestine of mutant mice harboring the 13/42 cleavage-resistant GSDMD relative to wild-type littermate controls [6]. Using a peanut extract-induced food allergy mouse model, He and colleagues showed that mice lacking GSDMD-N13, MHCII in IECs, or Tr1 cells exhibited increased susceptibility to food allergies and displayed characteristic signs of an anaphylactic response compared with controls. Furthermore, mice bearing such genetic modifications also showed impaired immune tolerance to another food antigen, OVA. These findings suggested that GSDMD-N13 has a crucial role in regulating MHCII expression in IECs, influencing both antigen presentation in the intestine and the numbers of adaptive immune cell populations.

Questions remain concerning the precise details and overarching scenario of the mechanisms involved in this model. Indeed, how food peptides are presented in IECs, and how they activate caspase-3/7 is unclear. In addition, why intestinal tissue IECs show basal STAT1 activation and subsequent nuclear translocation that is required for its interaction with GSDMD-N13 is intriguing. Additionally, what is the function of basal pyroptotic processing of GSDMD outside IECs in the small intestine, and what is/are the mechanism(s) of food antigen-specific T cell induction? Finally, more studies are required to address whether children with food allergies present with defects in such a GSDMD-N13-mediated immune tolerance pathway. Nevertheless, Shu Zhu and Richard Flavell's groups have uncovered a new role for GSDMD and provided important insights to advance our understanding of immune tolerance to food, which might aid the development of food allergy treatments.

Acknowledgments

This work was supported by US National Institutes of Health grants R01AI139914 (H.W.), DP1HD087988 (H.W.), and R01AI124491 (H.W.).

Declaration of interests

No interests are declared.

[1]Department of Biological Chemistry and Molecular Pharmacology, Harvard Medical School, Boston, MA 02115, USA
[2]Program in Cellular and Molecular Medicine, Boston Children's Hospital, Boston, MA 02115, USA

*Correspondence:
wu@crystal.harvard.edu (H. Wu).

https://doi.org/10.1016/j.it.2023.06.006

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