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RESEARCH HIGHLIGHT

Cleaving an epithelial path to food tolerance

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The intestine is in constant contact with antigenically foreign dietary and microbial products, but must distinguish between innocuous and pathogenic antigens to maintain tissue homeostasis. In a recent study published in *Cell*, He et al. demonstrate that dietary antigens in the small intestine trigger unique cleavage of gasdermin-D in epithelial cells that promotes MHCII expression and food tolerance.

Ingestion of antigenically foreign dietary products necessitates that food antigens are recognized as harmless and rendered non-immunogenic. Disruption of this process likely contributes to development of food allergies, however, mechanisms that enable food tolerance remain poorly understood. Previous work has established that tolerance to dietary peptides requires induction of intestinal regulatory T cells (Tregs)[1] or promotion of hyporesponsive non-regulatory $CD4^+$ T cells.[2] Major histocompatibility complex class II (MHCII)-expressing antigen presenting cells (APCs) discern whether an antigen is harmless or harmful and instruct the appropriate T cell response. Intestinal epithelial cells (IECs) are uniquely positioned at the interface with foods digested in the stomach. IECs are capable of antigen processing and presentation, and can calibrate intestinal $CD4^+$ T cell responses.[3–9] However, we have a limited understanding of how IECs regulate immune tolerance to dietary antigens.

The pyroptosis protein gasdermin-D (GSDMD) is cleaved into active N-terminal fragments that canonically induce cell lysis. This cleavage process has been extensively studied in myeloid cells. In a recent work, He et al.[10] demonstrated that GSDMD was highly expressed in non-hematopoietic IECs. Interestingly, IECs in the upper small intestine expressed a unique smaller 13 kD non-pyroptotic GSDMD N-terminal fragment, GSDMD-N_{13}. The authors demonstrated that commensal-derived cues did not induce production of GSDMD-N_{13}. Given the localized accumulation of GSDMD-N_{13} in the duodenum, the authors hypothesized that dietary stimuli may instead be involved. Indeed, mice fed normal chow had elevated GSDMD-N_{13} relative to mice fed a diet lacking proteinaceous antigens. The authors then employed comprehensive and elegant molecular and biochemical approaches to make the exciting discovery that dietary peptides bound and activated caspase-3/7, leading to GSDMD cleavage, independent of inflammasome activation (Fig. 1). The exact peptide type or length required to activate caspase-3/7, and the mechanisms by which dietary antigen promotes this enzymatic cleavage, remain to be defined.

In contrast to pyroptotic GSDMD fragments that localize to the cell membrane, He et al. found that GSDMD-N_{13} translocated to the nucleus. To determine whether GSDMD-N_{13} regulated the transcriptome of IECs, the authors created a novel mutant mouse model that was unable to cleave GSDMD to generate GSDMD-N_{13} ($GSDMD^{SICR}$). Transcriptome and flow cytometric analyses on IECs isolated from $GSDMD^{SICR}$, and mice deficient in full-length GSDMD, demonstrated decreased expression of MHCII and its master regulator, CIITA in both models. The authors further found that GSDMD-N_{13} interacted with the transcription factor STAT1 to induce CIITA and MHCII expression, and that GSDMD-N_{13} enhanced STAT1 binding to the *Ciita* promoter. While some analyses employed GSDMD constructs expressed in immortalized cell lines, these compelling and unexpected findings collectively support that dietary antigens induce generation of a distinct GSDMD fragment that facilitates STAT1-dependent MHCII expression in IECs (Fig. 1). These data reveal an exciting new function for GSDMD proteins in non-hematopoietic cells and open new avenues for future investigation into how these proteins alter gene expression.

Next, by employing single-cell RNA-sequencing, the authors found that $GSDMD^{SICR}$ mice had reduced IL-10-expressing type-1 regulatory (Tr1) cells, specifically in the duodenum where GSDMD-N_{13} localized. These findings are consistent with the results by Tuganbaev et al. that described reduced IL-10-expressing $CD4^+$ T cells in the small intestine of mice lacking epithelial MHCII.[5] Interestingly, loss of GSDMD-N_{13} had no impact on intestinal $FoxP3^+$ Tregs. In addition, He et al. demonstrated that in vitro antigen-primed IECs promoted $CD4^+$ T cell activation in a GSDMD- and MHCII-dependent manner. While IEC processing of dietary antigens is not yet fully understood, these data align with studies showing that IECs are capable of antigen processing and activating antigen-specific $CD4^+$ T cells.[8,9]

Following discoveries that GSDMD-N_{13} regulates epithelial MHCII and Tr1 cells, and that dietary antigens induce cleavage of GSDMD, He et al. next asked whether this unique GSDMD fragment was required for food tolerance. To answer this question, the authors sensitized and re-challenged a collection of tissue-specific knock-out and inhibitor-treated mice with peanut protein extracts. Mice lacking either GSDMD-N_{13}, epithelial MHCII, or Tr1 cells, as well as mice treated with caspase-3/7 inhibitor, all exhibited characteristics of an anaphylactic response, suggesting enhanced susceptibility to food allergies. These results are consistent with studies showing that epithelial-intrinsic MHCII controls antigen-specific $CD4^+$ T cell responses to ingested peptides.[4,5] The authors found that loss of MHCII in classical APCs also triggered a susceptible food allergy phenotype, suggesting essential and complimentary roles for classical and non-classical APCs in promoting food tolerance. Collectively,

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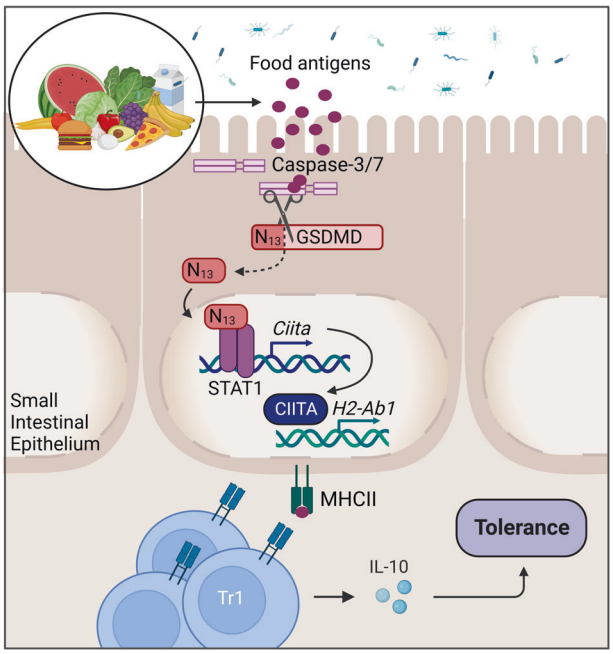

Fig. 1 Diet-induced cleavage of GSDMD directs intestinal epithelial MHCII expression and food tolerance. Dietary antigens bind to caspase-3/7 and activate cleavage of a novel 13-kD N-terminal fragment of GSDMD (N_{13}), specifically in small intestinal epithelial cells. GSDMD-N_{13} enters the nucleus and promotes STAT1-dependent regulation of MHCII expression. GSDMD-N_{13}-induced MHCII controls Tr1 cells and tolerance to food antigens. This figure was created using Biorender.com.

these data support a model in which dietary antigens promote immune tolerance by inducing caspase-directed cleavage of GSDMD, and that cleaved GSDMD interacts with STAT1 to drive epithelial MHCII expression and promote Tr1 cell accumulation (Fig. 1).

This work by He et al. employed extensive and sophisticated techniques to characterize a novel GSDMD fragment that enters the nucleus and promotes transcription of epithelial MHCII. Importantly, cleavage of GSDMD into GSDMD-N_{13} does not impact accumulation of the canonical pyroptotic GSDMD-N_{35} fragment in IECs. Therefore, these data provide a deeper appreciation for how innate mechanisms direct distinct pathways to balance inflammatory and tolerogenic immune responses. Consistent with other studies,[3–5,8] the authors confirm that microbial and dietary antigens coordinate to differentially regulate epithelial MHCII expression in the intestine. IEC expression of MHCII has long been known, however, its immunoregulatory function has been only recently interrogated in more depth. While initial studies describe potentially conflicting functions of epithelial MHCII, later work has consistently highlighted a key role for IEC-intrinsic MHCII in promoting tolerogenic immune cells and intestinal homeostasis.[4–8,10] Future investigation into the distinct cellular specificity of GSDMD-N_{13}, mechanisms directing its interactions with STAT1, and how this fragment alters global transcriptional programing may reveal new strategies to treat or prevent inflammatory conditions.

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ADDITIONAL INFORMATION

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