RESEARCH HIGHLIGHT

Microbial DNA regulates intestinal homeostasis via the AIM2 inflammasome

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Absent in melanoma 2 (AIM2) is a cytosolic DNA sensor which upon activation assembles a multiprotein complex called the inflammasome. Previous studies have shown that several inflammasome-forming pattern recognition receptors exhibit a protective function against inflammatory bowel disease and colorectal cancer. However, the role of AIM2 in sensing intestinal microbial DNA and regulating inflammatory responses therein was unknown. In a recent study published in Cell Reports, we demonstrated that *Aim2*$^{-/-}$ mice are highly susceptible to experimental colitis which was associated with a defect in the inflammasome activation as indicated by reduced caspase-1 cleavage and decreased production of IL-1β and IL-18. We also studied the underlying mechanism of AIM2 inflammasome-mediated protection against intestinal injury and inflammation. We found that the inflammasome downstream cytokine IL-18 contributes to intestinal homeostasis via induction of antimicrobial peptides, such as Reg3β, Reg3γ, Lcn2, S100A8, and S100A9 in intestinal epithelial cells. As a consequence of the defective production of antimicrobial peptides, *Aim2*$^{-/-}$ and other inflammasome-deficient mice harbor altered microbiota in the intestine as characterized by significantly higher burden of *Escherichia coli.* This research highlight will provide an overview of our findings and discuss how sensing of microbial DNA by AIM2 maintains intestinal homeostasis.

Keywords: AIM2; intestinal homeostasis; E. coli; antimicrobial peptides; colitis; inflammasome

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The recognition of pathogen-associated molecular patterns (PAMPs) by host's pattern recognition receptors (PRRs) is the first key step in host defense responses against pathogens. The participation of PRR in host-pathogen interaction is not only necessary for an effective immune response to infection, but also for immune homeostasis in healthy individuals. There are many evolutionary conserved PRRs in our immune system, such as Toll-like receptors (TLRs) [1, 2], C-type lectin-like receptors (CLRs) [3, 4], NOD-like receptors (NLRs) [5, 6], Retinoic acid-inducible gene (RIG)-I-like receptors (RLRs) [7], and HIN200 family receptors or AIM2-like receptors (ALRs) [8]. While TLRs and CLRs recognize PAMPs at the cell surface and endosomal compartment, NLRs, RLRs, and ALRs sense their respective ligands in the cytoplasm [9]. Increasing evidence points to the critical roles of several PRRs in immune homeostasis of healthy intestines where trillions of microbes reside without any harmful pathological consequence. Perturbation in pathogen sensing due to defects in PRRs, therefore, leads to intestinal inflammatory disorders such as inflammatory bowel disease

and colorectal cancer. Despite major progress in research on the role of several TLRs and NLRs in intestinal disorders, our understanding of other cytosolic sensors in the regulation of intestinal homeostasis and inflammation is poor.

We recently investigated the role of cytosolic dsDNA sensor AIM2 in intestinal homeostasis using a mouse model of inflammatory bowel disease or colitis induced by dextran sulfate sodium (DSS). Our study demonstrated that *Aim2*$^{-/-}$ mice are highly susceptible to DSS-induced colitis with increased loss of body weight and higher scores for diarrhea and rectal bleeding [10]. Histopathological analyses also revealed an increased loss of epithelial crypt, ulceration, edema, and infiltration of inflammatory cells in the lamina propria of *Aim2*$^{-/-}$ mice as compared to wild-type mice. Activation of inflammatory signaling pathways, such as NF-κB and ERK, leading to the production of cytokines, chemokines, and inflammatory mediators, precedes destructive inflammatory responses in the colon [11, 12]. Consistently, we observed a significantly higher production of inflammatory cytokines and chemokines, such as IL-1α, IL-6, KC, CXCL10, and CCL2, as well as higher activation of ERK in the colons of *Aim2*$^{-/-}$ mice at day 5 and 8 following DSS administration [10].

AIM2 is a member of IFN inducible HIN200 family receptors (IFI16, AIM2, IFIX, and MNDA), which contains an N-terminal pyrin domain (PYD) and a C-terminal oligonucleotide binding HIN domain [13-16]. Upon binding of dsDNA to its HIN domain, AIM2 oligomerizes the apoptosis speck-like protein containing a caspase recruitment domain (ASC) by homotypic interaction with its N-terminal PYD domain. The adapter ASC subsequently recruits caspase-1 by the homotypic interaction of its caspase recruitment domain (CARD) with the CARD domain of caspase-1, leading to the formation of a large multi-protein complex called the inflammasome [13-16]. Inflammasome-mediated activation of caspase-1 is required for pyroptotic cell death and maturation of the proinflammatory cytokines IL-1β and IL-18 from their precursors. In agreement with the inflammasome function, there was reduced caspase-1 activation, and IL-1β and IL-18 production in *Aim2*$^{-/-}$ mouse colons at homeostasis (day 0) and during the early stage of colitis (day 3) [10]. Previous studies have demonstrated that dsDNA from bacteria such as *Francisella tularensis* [17, 18] and subspecies novicida (*F. novicida*) [19], *Streptococcus pneumonia* [20], *Listeria monocytogenes* [17, 21], or viruses such as vaccinia virus and mouse cytomegalovirus [17] can activate AIM2. However, the activation of the AIM2 inflammasome by microorganisms in the intestine has not been shown before. In our study, we found that caspase-1 activation and production of IL-1β and IL-18 in *Aim2*$^{-/-}$ mouse colons at homeostasis and initiation of colitis were remarkably attenuated [10]. To confirm whether AIM2 can be activated by DNA of gut microbiota, we transfected genomic DNA isolated from mouse feces into wild-type or *Aim2*$^{-/-}$ macrophages in vitro. Transfection of fecal DNA led to caspase-1 activation in wild-type, but not in *Aim2*$^{-/-}$ macrophages [10]. Overall, our study suggests that gut microbial DNA activates the inflammasome via AIM2 which then elicits a protective immune response to intestinal injury and inflammation.

In addition to AIM2, three NLR family members including NLRP1, NLRP3, and NLRC4 can activate the inflammasome. Also, NLRP6, NLRP7, and NLRP12 were recently described as inflammasome activating NLRs, although the precise PAMPs that activate these NLRs are yet to be identified [22-26]. Previous studies have shown that NLRP3, NLRP6, and NLRP1 protect mice from colitis and colorectal tumorigenesis in an inflammasome-dependent manner [27-31]. Questions remain as to why our intestinal immune system requires multiple inflammasome activating pathways and what their relative contribution is to intestinal homeostasis. Our study demonstrated that activation of the inflammasome in *Aim2*$^{-/-}$ mice is defective at homeostasis and early onset of the colitis (day 0 and day 3), but not during acute colitis (day 8) [10]. While this observation indicates that AIM2 is an important inflammasome-activating sensor in the gut, it also suggests that multiple inflammasome activating pathways exist and play a compensatory role during acute and chronic colitis. Notably, two recent studies have shown that *Aim2*$^{-/-}$ mice are susceptible to colorectal tumorigenesis induced by the carcinogen azoxymethane plus multiple cycles of DSS [32, 33]. However, both of these studies failed to find defective inflammasome activation in *Aim2*$^{-/-}$ mouse colon during tumorigenesis. A possible reason could be the time points when caspase-1 activation was measured in those studies. Since they measured caspase-1 activation in the tumors or in the colons during acute colitis, it is not surprising that other inflammasomes pathways, such as the NLRP3, NLRC4, and NLRP6 inflammasomes, were activated at those stages of the disease in *Aim2*$^{-/-}$ mice. Breaching of the intestinal epithelial barrier during acute and chronic colitis allows epithelial and lamina propria cells to be exposed to diverse microbiota and their PAMPs, resulting in the activation of multiple inflammasome pathways. Considering the fact that different inflammasome pathways are activated by pathogen- or PAMP-specific manner, a difference in the microbiota of the animals in our laboratory and others may also be the reason of inconsistent role of AIM2 in activating the inflammasome in the gut. Taken together, AIM2 is one of the major sensors in the intestine that participates in the inflammasome activation at homeostasis and during colitis.

Multiple mechanisms, such as epithelial cell proliferation,

goblet cell function, and the regulation of gut microbiota, have been proposed to describe the protective functions of the inflammasome against intestinal injury and inflammation [11, 29, 30, 34]. Our study made an interesting observation that defects in AIM2 and other inflammasome components lead to an overgrowth of *Escherichia coli* (*E. coli*), which is widely considered the most important bacteria associated with IBD pathogenesis [10]. The observation of higher level of *E. coli* in *Aim2*$^{-/-}$ mice may not be just an example of altered microbiota in the gut, but also an underlying cause of colitis susceptibility. This notion was supported by experimental results showing similar colitis pathogenesis in littermate control wild-type and *Aim2*$^{-/-}$ mice. Notably, *E. coli* counts in littermate wild-type and *Aim2*$^{-/-}$ mice were comparable. Similarly, colonization of germ-free (GF) mice with microbiota either from conventionally raised wild-type mice (GF-WT) or *Aim2*$^{-/-}$ mice (GF-*Aim2*$^{-/-}$) resulted in higher *E. coli* burden and colitis pathogenesis in GF-*Aim2*$^{-/-}$ mice as compared to GF-WT mice. However, since these approaches allow other gut microbiota of *Aim2*$^{-/-}$ mice to transfer to the littermate or GF wild-type mice, the possibility of other unknown microbiota in colitis pathogenesis of *Aim2*$^{-/-}$ mice can not be excluded.

A critical concern that was investigated in this study is how inflammasome signaling maintains healthy microbiota while suppressing the growth of harmful bacteria such as *E. coli* in the gut. The intestinal epithelial cells contribute to the shaping of intestinal microbiota by producing antimicrobial peptides (AMPs) [35-37]. In our study, the significantly reduced production of AMPs such as Reg3γ, Reg3β, S100A8, and S100A9 in *Aim2*$^{-/-}$ mouse colons as compared to those of WT mice suggests a possible mechanism for the regulation of *E. coli* and Enterobacteriaceae by the AIM2 inflammasome. The higher colonic burden of *E. coli* was also observed in other inflammasome defective mice including *Nlrp3*$^{-/-}$, *caspase-1*$^{-/-}$, *Il1β*$^{-/-}$ and *Il18*$^{-/-}$ mice. Consistently, the expression of Reg3γ, Reg3β, S100A8, and S100A9 was also significantly reduced in *caspase*-1$^{-/-}$ mice, reminiscent of the role of the AIM2 inflammasome in the induction of AMPs. Moreover, these AMPs were seen to be induced by in vitro stimulation of intestinal epithelial cells with IL-18 [10]. Recently, Levy *et al*. also have shown that the NLRP6 inflammasome regulates intestinal homeostasis via the regulation of AMPs in intestinal epithelial cells [38]. We and others previously demonstrated that caspase-1-deficient mice were rescued from exacerbated colitis by the treatment with recombinant IL-18 [27, 30]. In agreement, here we observed that IL-18 infusion causes a dramatic reduction of colonic *E. coli* burden and DSS- induced colitis in *Aim2*$^{-/-}$ mice [10].

In conclusion, this study demonstrated that the AIM2 inflammasome regulates intestinal microbial ecology, particularly *E. coli* growth, via the production of IL-18, which triggers induction of AMPs upon binding to the IL-18 receptor on the intestinal epithelial cells. Although inflammasome-mediated induction of AMPs provides a possible mechanism for suppression of *E. coli* growth, why *E. coli* is selectively inhibited by AMPs which are primarily non-selective in nature is less clear. Future studies should investigate further mechanistic insight of the regulation of *E. coli* by the inflammasome/IL-18 signaling axis.

Conflicting interests

The authors have declared that no conflict of interests exists.

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Abbreviations:

AIM2: Absent in melanoma 2; ALR: AIM2-like receptor; ASC: Apoptosis speck-like protein containing a caspase recruitment domain; CARD: caspase recruitment domain; CLR: C-type lectine-like receptor; DSS: Dextran sulfate sodium; GF: Germ-free; IBD: Inflammatory bowel diseases; NLR: Nod-like receptor; PAMPs: Pathogen-associated molecular patterns; PRR: Pattern recognition receptors; PYD: Pyrin domain; RLR: Retinoic acid-inducible gene (RIG)-I-like receptor; TLR: Toll-like receptor.

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