NLRP3 Inflammasome in Inflammatory Bowel Disease: Friend or Foe?

Ling Liu¹ · Xuhang Li²

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Introduction

Inflammasomes are large multimeric protein complexes (Fig. 1) that actively regulate cellular immunity and homeostasis through sensing and responding to either intracellular or extracellular stimuli [1, 2]. They are grouped on the basis of their sensor proteins, including nucleotide-binding domain (NBD) and leucine-rich repeat (LRR)-containing receptors (NLRs), absent in melanoma-2 receptor (AIM2), and pyrin (Fig. 1a). The NLR family of intracellular innate immune receptors has more than 20 known members, including NLRP1, NLRP3, NLRP6, NLRP12, and NLRC4. The NLR family has two subfamilies, NLRP and NLRC, based on their N-terminal domains: NLRP contains a pyrin domain (PYD), whereas NLRC contains a caspase activation and recruitment domain (CARD) domain (Fig. 1a, b). Homotypic interactions between the pyrin domain of NLRP3 and the adaptor molecule apoptosis-associated speck-like protein containing CARD (ASC) bridge the association of caspase-1 to NLRP3 in a large protein complex termed the NLRP3 inflammasome (Fig. 1b). The NLRP3 inflammasome is one of the most studied subfamily members of inflammasomes, sensing and activated by a sizeable spectrum of pathogen-associated patterns (PAMPs) and damage-associated molecular patterns (DAMPs). Little is known about the

negative regulator of the NLRP3 inflammasome, except that a recent report has suggested interleukin (IL)-10 functions as a potent suppressor in cultured macrophages and in mice [3]. Activated caspase-1 converts the cytosolic precursors of proinflammatory cytokine IL-1 β or IL-18 into biologically active forms (Fig. 1c), which substantially contribute to the pathogenesis of several autoinflammatory diseases, including Muckle–Wells syndrome, familial cold autoinflammatory syndrome, chronic infantile neurological cutaneous and articular (CINCA) syndrome, gouty arthritis, Alzheimer’s disease, silicosis, and alcoholic liver disease.

Inflammasomes and Inflammatory Bowel Diseases (IBD), a Friend or Foe?

IBD, encompassing ulcerative colitis (UC) and Crohn’s disease (CD), is associated with dysregulation of the mucosal immune system of the gastrointestinal tract. The gut luminal contents, including commensal microbes and their metabolites, pathogens, and food antigens, substantially affect gut immune homeostasis. Considering the functions of inflammasomes in sensing microbial or other danger signals, it is conceivable that intestinal inflammasomes must be a key component of the mucosal immune system that mediates host-microbial and host-environmental interactions. Indeed, a considerable number of reports suggest that NLR family inflammasomes, including NLRP1, NLRP3, NLRC4, and AIM2, are involved, one way or another, in the intestinal inflammation characteristic of murine models of experimental colitis [3, 4]. Nevertheless, how these different inflammasomes contribute toward IBD pathogenesis remains controversial, as both pathogenic and protective effects have been reported.

✉ Xuhang Li
xuhang@jhmi.edu

¹ Department of Gastroenterology, West China Hospital, Sichuan University, Chengdu, China

² Division of Gastroenterology, Department of Medicine, Johns Hopkins University School of Medicine, 720 Rutland Ave, Ross 918, Baltimore, MD 21205, USA

Fig. 1 Structures and functions of the NLR family inflammasomes. **a** The components of the NLR family inflammasomes. Each inflammasome contains numerous copies of three different protein components, including NLRP or NLRC, ASC, and pro-caspase-1. Each component consists of unique domains as illustrated (see article for detailed description). **b** Two representatives of the NLR family inflammasome complexes: the NLRP3 inflammasomes and NLRC4 inflammasomes. Activation of these inflammasomes activates pro-

caspase-1. *NLRC4 has also been reported to form complexes directly with pro-caspase-1 through their CARD domains without participation of ASC. **c** Activation of pro-forms of IL-1β or IL-18. Activated caspase-1 cleaves the pro-IL-1β or pro-IL-18, converting it to a biologically active form, which contributes to either the pathogenesis of specific immunological diseases via proinflammatory responses, or the resolution of infection through antimicrobial responses

NLRP3 Inflammasomes and IBD

Among all studies addressing the intersection of inflammasomes and IBD, the NLRP3 inflammasome has perhaps attracted the most attention. Earlier reports in 2010 reported conflicting evidence and conclusions regarding the contribution of the NLRP3 inflammasome to IBD pathogenesis. Zaki et al. [5] showed that mice deficient in NLRP3 or ASC and caspase-1 manifested loss of epithelial integrity and aggravated inflammation in DSS-induced colitis. Similarly, Allen et al. [6] demonstrated that loss of *Nlrp3* increased DSS-induced intestinal injury and inflammation, suggesting that the NLRP3 inflammasome protected against colitis. Nonetheless, Bauer et al. [7] revealed that murine macrophages incubated in vitro with DSS secreted high levels of IL-1β in a NLRP3/ASC/caspase-1-dependent manner. After oral administration of DSS, NLRP3^{-/-} mice developed a less severe colitis and

produced lower levels of colonic proinflammatory cytokines than WT mice [7].

The unsettling connection of the NLRP3 inflammasome toward IBD pathogenesis has persisted in the recent literature. A 2016 report demonstrated a protective effect of the NLRP3 inflammasome against oxazolone-induced colitis [8]. Two other reports, both using IL-10^{-/-} mice but by different experimental approaches, provided consistent evidence supporting a pathogenic function of the NLRP3 inflammasome in IBD [3, 9]: One report in 2014 showed that IL-1β was increased in IL-10^{-/-} mice and that an IL-1β receptor antagonist or caspase-1 inhibitors ameliorated colitis [9]. The other report published in 2017 using both IL-10^{-/-} mice and human mucosal explant culture demonstrated that chemically inhibiting the NLRP3 inflammasome potently suppressed the intestinal mucosal inflammation of both mice and humans [3].

The reason for the inconsistency is unknown. It is highly likely due to the well-recognized variations of the gut microbiota at different facilities where experiments were performed, or variations in either the murine genetic backgrounds or models of experimental colitis. Nevertheless, these conflicting data emphasize the need for further investigation of how the NLRP3 inflammasome contributes to IBD pathogenesis, particularly in area of clinical studies.

In this issue of *Digestive Diseases and Sciences* [10], Lazaridis and colleagues examined NLRP3 inflammasome activation in isolated human peripheral blood mononuclear cells (PBMCs) and its potential association with disease characteristics and ATG16L gene polymorphisms. They reported that the NLRP3 inflammasome in human PBMCs is significantly activated in CD patients but not in UC patients. By activation of PMBCs from CD, UC patients and healthy controls with lipopolysaccharide (LPS) in the absence or presence of the NLRP3 stimulus, uric acid crystals (MSU), the authors investigated NLRP3 inflammasome activation in IBD patients. NLRP3 inflammasome activation, defined as >30% increase in IL-1 β production after MSU addition, was observed in 60% of CD patients (15/25) compared to 28.6% of controls (6/21); no significant difference was detected between UC and controls (11/20 or 55% vs 6/21 or 28.6%). The IL-1 β release was significantly higher in CD patients in comparison with controls at the post-transcriptional level. This is supported by the observation of no differences in *NLRP3*, *caspase-1* and *IL-1 β* gene expression and pro-IL-1 β production between CD patients and controls. The NLRP3 inflammasome activation appeared to be associated with longstanding disease (>1.5 years) in UC patients. Yet, NLRP3 inflammasome activation in CD patients was not associated with any clinical characteristics such as disease activity, severity, or location. Since the rs2241880 allele of ATG16L gene, a major risk allele in CD, has been reported to activate the NLRP3 inflammasome via mitochondrial damage and excessive reactive oxygen species (ROS) production, the authors also investigated the possible association of this ATG16L gene allele with the NLRP3 inflammasome activation. They found that IL-1 β production was similar between carriers of wild type and of single-nucleotide polymorphisms of the ATG16L1 gene. The authors concluded that ex vivo NLRP3 inflammasome in PBMCs is activated in patients with CD and UC with longstanding disease.

Although this study by Lazaridis et al. [10] appears to be straightforward and most of the results (such as associations of NLRP3 inflammasome activation with disease characteristics or ATG16L1 SNPs) appear to be negative, it is one of the few such reports using patient-derived cells or tissues. Currently, most of the reports on NLRP3 inflammasome are based on murine models of colitis. Although

animal model studies provide invaluable information and insights, the inconsistent results and conclusions based on data obtained using these models clearly warrant confirmation in studies using human or human-derived organs/tissues/cells, as mentioned earlier. Although human research is far more challenging to conduct than comparable experimental studies, it is the only means to assure the relevance of conclusions derived from data obtained in experimental models. A recent report in which the expression of the NLRP3 inflammasome by western blot analysis of the colonic mucosa from IBD patients and healthy controls reported that NLRP3, ASC, and IL-1 β were all significantly elevated in the mucosae of CD patients, compared with those obtained from of UC and healthy controls, but did not reach statistical significance in UC vs healthy controls [3]. Moreover, inhibition of the NLRP3 inflammasome with glyburide effectively suppressed the release of major proinflammatory cytokines/chemokines by cultured colonic mucosal explants obtained from Crohn's patients [3]. These data support the hypothesis that the NLRP3 inflammasome contributes toward the pathogenesis of CD, a conclusion supported by the data reported by Lazaridis et al. [10].

There are limitations and room for improvement in Lazaridis's study. One obvious issue is the limited cohort of patients used. For example, the data were highly variable (i.e., the standard errors of each cytokine measurement are roughly equal to the means). These variations, although not surprising considering the vast heterogeneities present in human populations, strongly suggest that a much larger pool of patients should be included in order to strengthen and further support the authors' conclusions. This is particularly true for further stratifying the "secondary endpoints" correlation of NLRP3 activation with patients' clinical characteristics or ATG16L gene polymorphisms. With only ~20 subjects in each group (CD, UC, and healthy controls) and substantial data variation, it is very likely that some important associations between NLRP3 inflammasome activation and clinical characteristics (such as the activity/severity, locations, duration of the disease) and ATG16L1 genotypes are either spurious or underestimated. Moreover, intestinal lamina propria mononuclear cells (LPMCs) should have been included in the study (or in a future study) in addition to the peripheral PBMCs used, since LPMCs are the major source of IL-1 β in the intestinal mucosa of Crohn's patients.

NLRP3 is present in many different cell types or different tissues/organs, although its functional differences in these different locations remain conjectural. In addition to innate immune cells such as macrophages and dendritic cells, NLRP3 inflammasomes are also expressed in colonic epithelial cells [3, 11]. Interestingly, Song-Zhao et al. [11] reported that NLRP3 activation in the intestinal epithelium

protects against *Citrobacter rodentium*, an intestinal pathogen used as a model of enteropathogenic *Escherichia coli* infections in humans. Surprisingly, this protective function is independent of IL-1 β or caspase-1, suggesting a novel mechanism of the NLRP3 inflammasome in maintaining intestinal epithelial homeostasis through sensing of commensal microbiota. The study further showed that, if the epithelial barrier is disrupted, the microbiota gain access to the lamina propria and mucosal immune cells; inflammasome activation may have a proinflammatory and deleterious effect on intestinal inflammation.

Conclusions

Based on the limited data in the human study, elevated expression or activation of the NLRP3 inflammasome in CD patients, either in PMBCs, colonic epithelial cells, or in colonic macrophages, suggests a pathogenic function of the NLRP3 inflammasome in human IBD. Therefore, targeting the NLRP3 inflammasome and its downstream signaling may potentially be an important strategy for developing new IBD therapies. Conversely, the continuing inconsistent reports of data obtained from animal studies would strongly suggest an ever-greater need for more clinical studies aimed at elucidating the exact function and clinical relevance of the NLRP3 inflammasome activation in IBD. The work of Lazaridis is a plausible example or step toward achieving these goals.

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