Inflammasomes and intestinal homeostasis: regulating and connecting infection, inflammation and the microbiota

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Abstract

Inflammasomes are large cytosolic protein complexes that detect infection and stress-associated signals and promote immediate inflammatory responses. In the intestine, activation of the inflammasome leads to an inflammatory response that is important for controlling enteric infections but can also result in pathological tissue damage. Recent studies have suggested that the inflammasome also regulates intestinal homeostasis through its effects on the intestinal microbiota. Notably, many conflicting studies have been published regarding the effect of inflammasome deficiencies on intestinal homeostasis. Here, we attempt to reconcile these contrasting data by highlighting the many ways that the inflammasome contributes to intestinal homeostasis and pathology and exploring the potential role of alterations in the microbiota in these conflicting studies.

Keywords: IBD, infection, inflammasome, intestine, microbiota

Introduction

Inflammasomes are a large cytosolic protein complexes that are a crucial component of the innate immune response to cytosolic perturbations (1). Inflammasome formation and activation can be triggered by a variety of infection- and stress-associated signals and facilitates the autocatalytic cleavage of pro-Caspase-1 into its active form (2, 3). Active Caspase-1 subsequently mediates host defense and acute inflammation via multiple mechanisms. First, Caspase-1 is responsible for the activation and secretion of the pre-made, ‘leaderless’ pro-inflammatory cytokines IL-1β and IL-18. In so doing, activated inflammasomes are able to trigger a rapid, transcription-independent initiation of potent inflammatory responses. Second, Caspase-1 can induce an inflammatory form of cell death referred to as ‘pyroptosis’, which limits intracellular pathogen replication by destroying their niche and, by secreting IL-1β and IL-18 in the process, attracting immune cells to attack the released pathogens (4).

Inflammasomes typically consist of a ‘sensor’ protein, an adaptor protein and Caspase-1 (1). The largest family of sensor proteins is referred to as the nucleotide-binding domain (NBD) and leucine-rich repeat (LRR)-containing (NLR) receptor family (also sometimes referred to as NOD-like receptors). NLRs typically contain three domains: (i) an LRR domain, which is thought to be involved in ligand-binding; (ii) a central NBD (also sometimes referred to as NACHT); and (iii) either a Caspase recruitment domain (CARD), which allows for direct recruitment of Caspase-1, or a pyrin domain (5) (Fig. 1). NLRs that contain a pyrin domain are able to recruit and activate Caspase-1 through their interactions with the adaptor protein apoptosis-associated speck-like protein containing a CARD (ASC). Recently, activation of Caspase-11 has been identified as a crucial prerequisite in the activation of Caspase-1 following the detection of bacterial LPS in the cytosol, in a pathway referred to as non-canonical inflammasome activation (6–8).

In addition to the NLRs, the AIM2-like receptor (ALR) and Rig-I-like receptor (RLR) families, which are largely involved in sensing of bacterial and viral nucleic acids, have also been shown to form inflammasomes (9, 10). In this review, we will mainly focus on the NLRs since their role in intestinal homeostasis has been most broadly studied.
Inflammasomes control intestinal homeostasis

Fig. 1. Schematic overview of inflammasome formation. Following a cytosolic stimulus, inflammasome complexes are assembled via homotypic domain–domain interactions, resulting in the autoproteolytic activation of Caspase-1 and the processing and subsequent secretion of IL-1β and IL-18. Protease, proteolytic domain; PYR, pyrin domain.

Inflammasome-mediated responses to enteric bacterial infections

NLRs can detect bacterial infections in a variety of ways; for example, some NLRs have been shown to specifically detect the presence of cytosolic bacterial components (e.g. NLRC4 detects bacterial flagellin and AIM2 detects bacterial DNA), whereas other NLRs appear to detect alterations in cellular homeostasis that occur as a result of cytosolic bacterial infection (e.g. NLRP3 is proposed to detect mitochondrial damage and potassium efflux after pore formation) (3). Notably, many inflammasome components, including Caspase-1, ASC and a variety of NLRs, are widely expressed in both hematopoietic and non-hematopoietic cell types in the intestine (11). Therefore, it is not surprising that a large number of studies have revealed an important role for the inflammasome in host defense against enteric pathogens.

Three NLRs seem to play primary roles in the detection of bacterial pathogens: NLRC4, NLRP3 and AIM2. For instance, Salmonella typhimurium, which invades macrophages and epithelial cells, is detected by NLRC4 through the release of bacterial flagellin and AIM2 detects bacterial DNA, whereas other NLRs appear to detect alterations in cellular homeostasis that occur as a result of cytosolic bacterial infection (e.g. NLRP3 is proposed to detect mitochondrial damage and potassium efflux after pore formation) (3). Notably, many inflammasome components, including Caspase-1, ASC and a variety of NLRs, are widely expressed in both hematopoietic and non-hematopoietic cell types in the intestine (11). Therefore, it is not surprising that a large number of studies have revealed an important role for the inflammasome in host defense against enteric pathogens.

For instance, Salmonella typhimurium, which invades macrophages and epithelial cells, is detected by NLRC4 through the release of bacterial flagellin and/ or components of its type III secretion system (T3SS) into the cytosol during or after cellular invasion (12). Furthermore, during extensive intracellular bacterial replication, the non-canonical Caspase-11-NLRP3 inflammasome pathway is activated after the detection of LPS leaking into the cytosol (13). Citrobacter rodentium, a non-invading intestinal pathogenic bacterium that adheres to the epithelial cell lining of the cecum and colon, has been shown to trigger both NLRC4 and NLRP3 activation in epithelial cells (14, 15). NLRC4 and NLRP3 also play a crucial role in the detection of Yersinia enterocolitica and Listeria monocytogenes infection (16, 17). In addition, Listeria has been shown to release small amounts of DNA into the cytosol, which are then sensed by AIM2 (17).

In general, activation of the inflammasome during the infections described above limits bacterial burden and systemic spread through the induction of IL-1β and IL-18 secretion and pyroptosis. The importance of the inflammasome as an evolutionarily adaptive defense mechanism against pathogens is also illustrated clearly by the prevalence of inflammasome-evasion strategies in enteric pathogens, which are necessary to retain virulence; for example, Salmonella downregulates flagellin and T3SS expression after cellular invasion, Listeria shuts off flagellin expression completely when entering the host and actively minimizes DNA release intracellularly, and Yersinia enterocolitica evolved effector proteins that limit the detection of T3SS components (12, 16). Nonetheless, the limited amount of bacterial stimuli that remains is often still enough to induce potent protective responses.

Inflammasomes, intestinal inflammation and inflammatory bowel disease

Given the important role of the inflammasome in intestinal immunity, it is not surprising that a large number of studies have examined the potential role of the inflammasome in the development and pathogenesis of inflammatory bowel disease (IBD). Even before the discovery of the inflammasome, many studies focused on the role of the inflammasome-dependent cytokines IL-1β and IL-18 in intestinal inflammation. Over 20 years ago, it was reported that IL-1β and IL-18 levels are elevated in the serum of IBD patients; furthermore, the levels of these cytokines correlated with the severity of intestinal inflammation, suggesting potential involvement of these cytokines in disease (18).

Later studies, mostly performed in murine models of colitis, revealed that IL-1β and IL-18 regulate intestinal inflammation and IBD through a number of mechanisms. IL-1β is thought to play a largely pro-inflammatory role in the intestine and, therefore, is thought to promote IBD. For example, IL-1β can alter intestinal permeability by influencing tight junctions (19) and can promote the development of pathogenic T17 cells, which are a distinct population of CD4 T cells that play a key role in driving intestinal inflammation (20). Accordingly, inhibition of IL-1β was shown to ameliorate dextran sulfate sodium (DSS)-induced colitis (21).

In contrast, the role of IL-18 in the maintenance of intestinal homeostasis is more complex: IL-18 is cytoprotective during the early stages of IBD, while chronic release of IL-18 exacerbates the disease (22). Also, while IL-18-deficient mice were resistant to 2,4,6-trinitrobenzene sulfonic acid (TNBS)-induced colitis (23), both IL-18- and IL-18R-deficient mice exhibited exacerbated DSS colitis and increased development of colorectal cancer (23, 24). Thus, it appears that IL-18 plays opposing roles in colitis development depending on the model used.

One explanation for these seemingly conflicting studies is that IL-18 exacerbates T-cell-dependent colitis (e.g. TNBS colitis) by supporting IFN-γ production by T cells but alleviates innate colitis induced via tissue injury (e.g. DSS colitis) by supporting tissue repair functions (23).

Another possible, and potentially related, explanation could be the link between IL-18 and IL-22. We recently found that IL-18 indirectly controls IL-22 activity by downregulating
the expression of IL-22-binding protein (IL-22BP), a soluble receptor that sequesters and neutralizes IL-22 (25). Like IL-18, IL-22 has been shown to have opposing roles in intestinal disease and can be either beneficial or detrimental. In the early phase of the immune response, IL-22 promotes wound healing and contributes to resolution of the immune response (26, 27). However, when chronically released or not properly controlled by IL-22BP, IL-22 promotes hyperplasia of intestinal epithelial cells, intestinal inflammation and colon cancer (25, 28). The interplay between IL-18 and IL-22, and their heterogeneous activities, continue to be interesting fields of study.

After the discovery of inflammasomes, many groups became interested in elucidating the role of the inflammasome in intestinal inflammation. Since inflammasomes regulate the activation and secretion of pro-inflammatory cytokines, genetic or pharmacological disruption of the inflammasome pathway was initially expected to reduce intestinal inflammation and ameliorate IBD. Indeed, Caspase-1-deficient mice were reported to be protected from both acute and chronic DSS colitis, and this was associated with reduced levels of IL-1β. IL-18 and IFN-γ (21). Furthermore, pharmacological inhibition of Caspase-1 was found to alleviate colitis in mice (29, 30). In addition, mice deficient in NLRP3 were protected from DSS colitis (31). Moreover, pharmacological inhibition of IL-1β or Caspase-1 reduced the expression of IL-17 by intestinal T cells and consequently ameliorated spontaneous colitis in IL-10-deficient mice (32).

Finally, in a mouse model of graft versus host disease (GvHD), NLRP3 and ASC activation in dendritic cells after total body irradiation led to IL-1β secretion, T17 cell development and systemic and intestinal inflammation that ultimately led to the death of the mice. In line with this, blockade of IL-1β reduced GvHD in mice, and elevated concentrations of IL-1β are found in the intestinal GvHD lesion of patients (33).

Taken together, the aforementioned studies seem to support the idea that the inflammasome plays a critical role in promoting the pathogenesis of IBD by regulating the secretion of inflammatory cytokines such as IL-1β. However, recent studies have revealed that the role of the inflammasome in intestinal inflammation and IBD might be more complicated than was initially expected. For example, while initial studies found that Caspase-1 deficiency ameliorated DSS colitis, later studies have shown the opposite result. First, Dupaul-Chicoine et al. found that both Caspase-1 and ASC-deficient mice developed a more severe DSS colitis, and that exogenous IL-18 administration could rescue this phenotype (34). At the same time, Zaki et al. found that NLRP3-, ASC- and Caspase-1-deficient mice were hypersensitive to both DSS and TNBS colitis; administration of exogenous IL-18 also could rescue this phenotype (35).

While these studies fit well with the putative role of IL-18 in tissue repair, it remained largely unclear why these results strongly contradicted previous studies suggesting at pathogenic role for the inflammasome in IBD. However, one potential hint came from the observation by Zaki et al. that the inflammasome-deficient mice used in their studies exhibited commensal bacterial overgrowth, and that antibiotic treatment could ameliorate the observed exacerbation of DSS colitis in these strains (35).

**Inflammasomes and the intestinal microbiota**

It is well known that the microbes that inhabit the intestine, i.e. the intestinal microbiota, have a dramatic effect on intestinal homeostasis and IBD. Development of IBD is microbiota-dependent, and alterations in the composition of the microbiota can determine susceptibility to and the severity of colitis.

Recent studies from our group have revealed that mice lacking various components of the inflammasome display prototypical alterations in their microbiota that predispose these animals to the development of a variety of diseases, including IBD. In other words, inflammasome deficiency is associated with the acquisition of a so-called ‘dysbiotic’ microflora. This was first observed in studies of mice lacking the previously poorly characterized NLR family member NLRP6 (11). In these studies, it was discovered that NLRP6-, ASC-, Caspase-1- and IL-18-deficient mice in our colony were hypersensitive to DSS colitis.

Most interestingly, this susceptibility to colitis was transferrable to wild-type mice through cohousing, suggesting that this phenotype was mediated by the intestinal microbiota. Indeed, 16S rRNA gene sequencing-based profiling revealed that NLRP6-, ASC-, Caspase-1- and IL-18-deficient mice displayed an altered microbiota as compared with wild-type mice, which is characterized by the presence of Prevotellaceae species. Importantly, cohousing of inflammasome-deficient mice and wild-type mice led to acquisition of the dysbiotic microbiota and susceptibility to DSS colitis by wild-type mice. Furthermore, antibiotic treatment reversed the hypersensitivity of inflammasome-deficient mice. Taken together, these data show that inflammasome-deficient mice possess a communicable dysbiotic microbiota that enhances susceptibility to colitis.

In addition to its effects on DSS colitis, inflammasome-mediated dysbiosis was found to impact a number of other diseases. NLRP3-, NLRP6-, ASC- and IL-18-deficient mice all showed increased susceptibility to diet-induced development of non-alcoholic fatty liver disease and progression to non-alcoholic steatohepatitis (36). Furthermore, these mice all were also more susceptible to the development of other aspects of metabolic syndrome, including obesity and insulin resistance. These phenotypes were also attributable to alterations in the microbiota in inflammasome-deficient mice, as susceptibility to metabolic syndrome was transmissible to wild-type mice through cohousing, was reversed by antibiotic treatment and was characterized by prototypical changes in microbiota composition. Finally, in addition to its effects on DSS colitis and metabolic syndrome, inflammasome-mediated dysbiosis was found to affect the development of inflammation-driven colorectal cancer (37, 38).

**Role of inflammasomes in intestinal inflammation and IBD: current perspective on conflicting studies**

In this review, we tried to reconcile our findings that inflammasome-deficient mice are hypersensitive to colitis with apparently contrasting data in which inflammasome deficiency or inhibition ameliorated disease (Fig. 2). It is clear that inflammasomes sense intestinal microbes and regulate intestinal immunity. Inflammasome-mediated responses are central
to controlling the invasion of pathogenic bacteria, such as *Salmonella*, but they also appear to be able to regulate the composition of the commensal microbiota. The microbiota is composed of mutualistic commensal bacteria and potentially pathogenic bacteria, which are sometimes referred to as pathobionts. The *Prevotellaceae* species that we find in our inflammasome-deficient mice can potentially be considered pathobionts since these bacteria are not classically entero-colitic but can exacerbate DSS-induced colitis.

It thus appears that the environment may be the decisive factor in determining whether inflammasome-deficient mice are susceptible or resistant to development of colitis. When inflammasome-deficient mice have been exposed to pathobionts, such as *Prevotellaceae* species, they are highly prone to develop a dysbiosis that renders these mice hypersensitive to colitis. On the other hand, when inflammasome-deficient mice do not become exposed to pathobionts, the reduction in inflammasome-dependent cytokine production that results from inflammasome deficiency renders them less susceptible to colitis. This model also appears to fit with the results obtained in studies that used inhibitors rather than knockout mice to study the effects of the inflammasome on IBD. In these studies, inhibition of Caspase-1, IL-1β or IL-18 largely alleviated colitis (21, 29, 39). In contrast, studies that used Caspase-1- or IL-18-deficient mice gave variable results according, probably, to the composition of flora (11, 21, 23, 31, 35, 40).

**Conclusion**

Inflammasomes play a crucial role in defense against infections with traditional pathogens and in control of the intestinal microbiota. While the mechanisms by which the inflammasome regulates pathogen clearance are clear, the precise mechanisms of inflammasome-mediated control of the microbiota remain largely unknown. Beyond the regulation of IL-1β, IL-18 and pyroptosis, our group has recently identified another interesting mechanism by which the inflammasome may mediate antimicrobial defense and, thereby, regulate microbiota composition. These studies revealed that NLRP6 is a critical regulator of mucus production by goblet cells in the intestine (41). Since mucus is an important mediator of barrier defense in the intestine, this may render NLRP6-deficient mice more susceptible to persistent bacterial infection and, potentially, development of dysbiosis.

The precise ligands that activate many inflammasomes are another aspect of NLR biology that remains to be fully elucidated. Although NLRs that detect bacterial pathogens (for instance NLRC4 and NOD2) have well-defined bacterial ligands, no such ligands have currently been found for NLRs that are involved in controlling microbiota composition (e.g. NLRP6). Elucidation of these ligands may shed light on the bacterial classes that can be recognized by these NLRs and provide insight into why and how these bacteria elicit inflammatory responses. It will also be interesting to see which specific members of the microbiota are actively sensed by which inflammasomes. A more complete understanding of
the molecular basis of inflammasome activation by the microbiota might eventually enable the development of intervening therapeutics that would ameliorate intestinal inflammation either by reducing pathological inflammasome-mediated inflammation or by enhancing inflammasome-mediated control of microbiota composition.

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