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REVIEW

Inflammasomes and the microbiota—partners in the preservation of mucosal homeostasis

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Abstract Inflammasomes are multiprotein complexes that serve as signaling platforms initiating innate immune responses. These structures are assembled upon a large array of stimuli, sensing both microbial products and endogenous signals indicating loss of cellular homeostasis. As such, inflammasomes are regarded as sensors of cellular integrity and tissue health, which, upon disruption of homeostasis, provoke an inflammatory response by the release of potent cytokines. Recent evidence suggests that in addition to sensing cellular integrity, inflammasomes are involved in the homeostatic mutualism between the host and its indigenous microbiota. Here, we summarize the involvement of various inflammasomes in host–microbiota interactions and focus on the role of commensal as well as pathogenic bacteria in inflammasome signaling.

Keywords Inflammasome · Colitis · Microbiota · NLRs

The innate immune system utilizes an array of germline-encoded pattern-recognition receptors (PRRs) to detect conserved microbial patterns and host-derived signals of cellular stress. PRRs include the membrane-bound Toll-like receptors (TLRs) and C-type lectins (CTLs). Additionally, intracellular PRRs sense foreign nucleic acid including RIG-like helicases (RLHs) and the DNA sensors, DAI and AIM2. In addition, NOD-like receptors (NLRs) recognize pathogen-derived molecules and host-derived damage signals. The mammalian NLR family contains a C-terminal leucine-rich repeat domain, a central nucleotide-binding NACHT domain, and an N-terminal protein–protein interaction domain composed of a caspase activation and recruitment domain (CARD) or Pyrin domain [1]. The NLR family can be subdivided into three distinct subfamilies, NODs, NLRPs, and the IPAF subfamily.

The group of Jurg Tschopp first demonstrated that NLR family members can form, upon certain stimuli and under tightly regulated conditions, a multiprotein complex termed inflammasome [2]. This complex consists of an upstream NLR, in some cases the adapter protein ASC, and caspase-1. Upon assembly, active caspase-1 cleaves the pro-forms of IL-1β and IL-18 into their bioactive forms. Additionally, inflammasome activation is associated with a peculiar form of cell death called pyroptosis [3]. The large amount of different endogenous and exogenous stimuli that have subsequently been described to activate the inflammasome has led Tschopp and colleagues to propose a function for inflammasomes as "guardians of the body" [4], suggesting that they form a surveillance system controlling the integrity of various vital cellular functions and reacting to metabolic and cellular stress with a strong "last resort" pro-inflammatory response.

Over the last decade, it has been recognized that the homeostasis of the mammalian "meta-organism" requires mutualistic interaction between the host and its indigenous microbiota [5]. The microbiota contains trillions of microbes, which colonize mucosal surfaces. We propose to extend the concept of inflammasomes as guardians of cellular integrity to an additional role as a surveillance system controlling the host–microbiota cross-talk in health and disease. In the following review, we will summarize the involvement of inflammasome-forming NLRs in host control of the commensal microbiota and of intestinal pathogenic infections.

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NLRP3

In terms of assembly, stimuli, and biochemical details, the NLRP3 inflammasome is the best-characterized representative of this class of innate immune receptors. A large range of both microbial and non-microbial ligands lead to NLRP3 activation and cytokine secretion. Over the last decade, many potential mechanisms have been discussed that may lead to NLRP3 inflammasome assembly downstream of these stimuli. Recently, it has been proposed that the reduction of intracellular potassium ions might be common to all NLRP3 activating agents [6]. What remains equally controversial is the role of NLRP3 in mediating the inflammatory response to commensal bacteria. Since the role of NLRP3 in colitis has recently been discussed in several excellent reviews [7, 8], we focus here on potential explanations for differences in the observed phenotypes.

NLRP3 was first reported to be protective against microbiota-driven colitis, and mice lacking NLRP3 featured enhanced susceptibility to experimentally induced intestinal inflammation and tumorigenesis [9–11]. These differences were attributed to a role of NLRP3 in maintaining epithelial integrity. However, in a different study, the opposite outcome of *Nlrp3* deficiency was observed, as these mice exhibited ameliorated colitis severity [12]. These differences can most likely be attributed to housing and microbiota differences, as it was observed that co-housing of *Nlrp3*-deficient and control mice equalized the severity of colitis [13].

Similarly, the contribution of NLRP3 in human inflammatory bowel disease (IBD) is confusing. While two studies have reported an association of variants in the human Nlrp3 gene region with Crohn's disease [14, 15], this was not confirmed in a third study [16]. Despite the fact that some of these studies included rather large cohorts, the contribution of Nlrp3 thus remains controversial and requires further investigation.

The question why common genetic deficiency can result in such different phenotypic outcomes is intriguing. We discuss here two, not mutually exclusive, mechanistic possibilities that may reconcile these contrasting findings. First, several cell types have been found to express the NLRP3 inflammasome and to facilitate the secretion of IL-1β and IL-18. These include several myeloid cell types [17, 18] and intestinal epithelial cells [19]. What remains unresolved is the relative contribution of NLRP3 activation in different cell types as a means to control intestinal microbial composition and colitis severity. Potentially, different cellular compartments are activated by distinct signals during the inflammatory response [20, 21]. Another possibility is that the distribution of cytokine receptor expression for IL-1β and IL-18 is what is driving different roles of cellular compartments in the inflammasome response. Future insight into the cell type specificity of inflammasome activation and regulation, as well as the receptor expression and downstream signaling, may lead toward a better understanding of the impact of NLRP3 on microbiota-driven inflammatory disease.

A further complication pertains to the contrasting effect of IL-1β and IL-18, both downstream targets of the NLRP3 inflammasome. While a barrier-protective role has been ascribed to IL-18 [9–11], IL-1β exacerbated intestinal inflammation in different mouse models through the induction of pathogenic T_H17 cells [22], and blockage of IL-1 receptor signaling was effective against chronic IBD in mice [23]. These contrasting effects raise the question whether there is an additional layer of regulation that determines the cell type specificity of the cytokines released and the pattern of receptor expression on target cells.

Second, the role of NLRP3 might be a prime example for the co-evolution of host and microbial genes. Hence, the NLRP3 inflammasome may be involved in the control of intestinal microbial composition, by shaping niche availability in the intestine and the niche construction ability of certain members of the commensal microbiota [24]. We discuss below that such a mechanism might be involved in other NLR family members and their role in the predisposition to colitis and other inflammatory diseases, as well.

NLRP6

NLRP6 is a member of the NLR family that contains the characteristic LRR, NACHT, and Pyrin domains. More than a decade ago, NLRP6 (at that time still called PYPAF5) has been found to regulate caspase-1 and NF-κB activity [25]—two themes that still remain the major focus of NLRP6 research. Substantial evidence has accumulated so far for a dual role of NLRP6 in both inflammatory pathways and the downstream consequences. Nonetheless, the mechanistic details on the molecular level remain unknown, i.e., NLRP6 has not been shown biochemically to form an inflammasome, and neither has a common binding partner in the NF-κB signaling cascade been identified. *Nlrp6*-deficient mice feature both lower levels of colonic IL-18 [26] and aberrantly high activation of NF-κB signaling in myeloid cells [27]. In line with these observations, NLRP6 expression has been detected in various cell types, including intestinal epithelial cells [26], myofibroblasts [28], lymphoid cells [25], and myeloid cells [27]. This points toward the intriguing possibility that NLRP6 performs distinct functions in different cellular compartments—a characteristic that might be central for NLRP6's role in the orchestration of inflammatory responses.

NLRP6 controls microbial composition and biogeography

Microbial metagenomic sequencing has revealed an aberrant composition of the intestinal microbiota in Nlrp6-deficient mice [26, 27], hinting toward the possibility of NLRP6 being

involved in the homeostatic maintenance of intestinal microbiota ecology. In particular, dysbiosis in *Nlrp6*-deficient mice is characterized by the outgrowth of *Prevotellaceae* and TM7, which show a highly elevated abundance in the fecal microbiota obtained from knockout mice. Dysbiosis can also be observed in mice with genetic deficiency in the canonical inflammasome pathway, including ASC, caspase-1, and IL-18 [26]. In addition, the aberration in colonic microbial ecology in the absence of NLRP6 is characterized by another feature: the accumulation of commensal microorganisms in the colonic crypt. The colonic crypt normally features a paucity of microorganisms compared to the area of surface enterocytes that face the intestinal lumen. It harbors LGR5-expressing stem cells and the accompanying stem cell niche. In the crypts of *Nlrp6*-deficient mice, an accumulation of bacteria was observed whose morphology suggests their classification as *Prevotellaceae*. Together, the maintenance of both composition and biogeographical distribution of commensal bacteria requires NLRP6.

Consequence of dysbiosis 1: transmissible colitis

The intestinal microbiota is in close physical contact with a single layer of intestinal epithelial cells, which separate the microbial from the mammalian part of the superorganism. As such, intestinal epithelial cells and the underlying lamina propria are directly exposed to changes in microbial ecology and are the first tissues involved in the inflammatory response to aberrant microbiota composition and location. Indeed, manifestations of inflammatory bowel disease (IBD, comprised of Crohn's disease and ulcerative colitis) have long been associated with intestinal bacterial colonization.

The aberrant microbial community in *Nlrp6*-deficient mice predisposes the host to low-grade intestinal inflammation and exacerbated chemically induced colitis and inflammation-induced tumorigenesis [29]. Epithelial regeneration is massively impaired in the setting of inflammatory colitis [28], indicating an involvement of NLRP6 in the intestinal self-renewal process. The enhanced inflammatory response can also be transmitted to genetically normal recipients by microbiota transfer [26]. This inflammasome deficiency-driven dysbiosis provokes epithelial reprogramming and elevated production of the chemokine CCL5. Consequently, the amount of inflammatory cell populations recruited to the intestine is enhanced, and a pro-inflammatory milieu is generated in response to the bacteria. CCL5 deficiency rescues from the pro-inflammatory effects of the dysbiotic microbiota, suggesting a central involvement of this mediator downstream of the microbial community.

Together, several studies suggest an involvement of NLRP6 in regulating host susceptibility to intestinal inflammation, indicating that the combination of aberrant microbiota composition or localization with host genetic susceptibility predisposes to IBD.

Consequence of dysbiosis 2: transmissible cancer susceptibility

As mentioned above, the enhanced inflammatory response to dysbiosis in *Nlrp6*-deficient mice leads to increased susceptibility to colorectal tumorigenesis [28, 29]. This is in concordance with data indicating an involvement of NLRP6 in epithelial repair and renewal upon injury. Interestingly, this susceptibility is also communicable. The CCL5-mediated recruitment of inflammatory populations to the intestinal lamina propria in response to the aberrant microbiota leads to an accumulation of the pro-inflammatory cytokine IL-6. IL-6, in turn, acts on intestinal epithelial cells to induce a proliferative response, which predisposes to insipient neoplasia [30]. This suggests that the microbiota not only invokes an inflammatory response but may also promote the neoplastic consequences of chronic deviation from tissue homeostasis.

Together with more recent data [31], these studies indicate that there might be a transmissible element involved in the etiology of colorectal cancer. Again, a two-hit scenario might apply, in which host genetic susceptibility or the accumulation of pre-neoplastic mutations promotes a propensity to transformation and dysbiosis provides an inflammatory insult that cumulates in carcinogenesis.

Consequence of dysbiosis 3: transmissible metabolic syndrome

In addition to its local effect in shaping the inflammatory milieu, the microbiota has also been recognized to exert its effects beyond the intestine and to influence systemic metabolic and inflammatory processes [32, 33]. The liver represents the first pass organ for bacterial molecules, metabolites, and dietary products, receiving the majority of its blood supply through the intestine-draining portal vein. As such, the liver represents an important hub in the decision-making process between systemic immunity or tolerance induction against microbial and food antigens that have passed the intestinal barrier. Indeed, the liver has recently been recognized as an anti-microbial firewall, analogous to the mesenteric lymph node system [34].

The intestinal microbiota has long been associated with liver inflammation by clinical evidence. Non-alcoholic fatty liver disease (NAFLD) is the hepatic manifestation of the metabolic syndrome [35]. Clinical observations have linked NAFLD with the outgrowth of Gram-negative bacterial species in the small intestine, leading to choline deficiency and ethanol accumulation, all factors involved in the etiology of NAFLD [35]. The disease starts with the accumulation of fat in the liver and low levels of hepatic inflammation and

progresses to non-alcoholic steatohepatitis (NASH) in about 30 % of all patients. NLRP3 and NLRP6 inflammasome deficiency results in exacerbated progression from NAFLD to NASH, which in humans can progress to steatosis and hepatocellular carcinoma. The susceptibility of inflammasome-deficient mice to NASH is also mediated by the aberrant microbiota composition and can be transferred to genetically normal mice upon microbiota transmission [36]. This demonstrates that dysbiosis not only exerts its inflammatory consequences on the local tissue environment of the intestine but also affects more distal, systemic inflammatory and metabolic responses. Mechanistically, the aberrant composition and localization of commensal bacteria in inflammasome-deficient mice cause an increased translocation of microbially derived molecules to the intestinal lamina propria and an enhanced flux through the portal vein into the liver. There, microbial products interact with TLRs, which are expressed on a wide array of cells in the liver [37]. The triggering of hepatic TLRs leads to increased release of pro-inflammatory cytokines, especially TNF, which propagates hepatic inflammation and the transition from NAFLD to NASH. Mice deficient in TLR signaling or TNF are protected from the effects of dysbiosis on the exacerbation of hepatic inflammation [36].

Furthermore, the aberrant microbiota associated with inflammasome deficiency also worsens other extra-intestinal manifestations of the metabolic syndrome, including obesity and glucose intolerance. Together, these studies suggest that inflammasome deficiency predisposes to exacerbated metabolic disease because of an inability to control the intestinal microbial community [3].

Linking NLRP6 and microbial biogeography

Given the amount of physiological and pathological consequences of dysbiosis in the absence of NLRP6, the mechanisms by which this NLR controls intestinal microbial ecology are of special interest. Generally, the immune system has several ways by which it can potentially shape the microbial community colonizing the intestine, including the secretion of anti-microbial peptides, the release of anti-microbial antibodies, and the luminal migration of leukocytes. Localization of *Nlrp6* expression in the mouse intestine revealed a high abundance in goblet cells of the large intestine. These secretory cells specialize in the secretion of mucus, thereby forming a spatial barrier between the epithelial layer and the majority of microorganisms living in the intestinal lumen [38]. Goblet cells perform their function by synthesizing large mucus-filled granules, which are subsequently released into the intestinal lumen, where they form a continuous layer covering the enterocytes. Indeed, goblet cell function seems to be impaired in the absence of NLRP6 due to an inability to exocytose mucus granules. Instead, inflammasome-deficient mice feature intact mucus granules at the apical side of goblet cells, resulting in a failure to generate a protective mucus barrier. Autophagy in Goblet cells is involved in the generation and secretion of mucus granules [39], and this mechanism is impaired in the absence of NLRP6, ASC, or caspase-1. Of note, these phenotypes were not transmissible by microbiota transfer, indicating that they might represent a cause, rather than an effect, of the aberrant microbial biogeography and crypt localization.

These observations provide an explanation for the abovementioned aberrant spatial distribution of commensal bacteria in mice lacking NLRP6. In addition, the lack of a functional mucus layer also allows enteric pathogens to adhere to the epithelial layer and causes exacerbated inflammation [38]. Whether there are additional mechanisms by which NLRP6 controls the community composition of the microbiota remains an open question.

NLRP12

NLRP12 (also known as Nalp12 and Pypaf-7) was one of the first NLRs which was shown in biochemical co-expression assays to co-localize and interact with the adaptor protein ASC to form an inflammasome and promote caspase-1 activation and production of IL-1β [40]. In some later studies, however, a role for NLRP12 in inflammasome signaling has been questioned, as detailed below.

The role of NLRP12 in inflammation and tumorigenesis is controversial, with both stimulatory and inhibitory functions proposed. It has been shown in several independent works that NLRP12 negatively regulates both canonical and non-canonical NF-κB signaling in biochemical assays, colon cancer and colitis models [40–43]. *Nlrp12*-deficient mice were found to have higher non-canonical activation of NF-κB and were more susceptible to colitis and colon cancer [42, 44]. However, it remained unclear whether NRLP12 acts primarily on the canonical or non-canonical pathway. On the contrary, it has been shown that co-expression of NLRP12 and ASC results in a potent synergistic activation and non-inhibition of NF-κB and caspase-1 [40]. On the other hand, Arthur et al. demonstrated in a mouse model of allergic dermatitis that inflammasome activation and cytokine production were unaffected in *Nlrp12*-deficient mice [45].

The function of NLRP12 during bacterial infection has been studied in several infectious models. NLRP12 has been described to have a pro-inflammatory role during bacterial infection as an important regulator of IL-1β and IL-18 release. Like the intestine, the mucosal surface of the lung is exposed to bacterial colonization. Allen et al. [46] investigated the possible involvement of NLRP12 in infectious models of acute airway inflammation. In these models, it was found that

airway exposure to *Klebsiella pneumoniae* results in subtle differences in cytokine levels and reduction of monocytes and lymphocytes in *Nlrp12*-deficient mice, although no differences in disease severity were observed.

NLRP12 was shown to play an important role following *Yersinia pestis* infection, the causative agent of plague. *Nlrp12*-deficient mice have increased mortality following infection by an attenuated strain of *Y. pestis* in comparison to wild-type (WT) mice with increased bacterial load [47]. The levels of IL-1β and IL-18 are not completely eliminated in this setting, indicating that there might be redundancy between several inflammasomes in this model. To date, the specific bacterial ligand that activates the NLRP12 inflammasome remains unknown.

Moreover, during *Salmonella enterica* sv. Typhimurium infection, NLRP12 was suggested to inhibit host defense, independent of the inflammasome, as *Nlrp12*-deficient mice are more resistant to *S.* Typhimurium infection compared with WT controls and present lower inflammatory cytokine levels [48].

Ataide et al. suggested that NLRP12 is mediating systemic production of IL-1β and hypersensitivity to secondary bacterial infection during malaria [49]. *Plasmodium* infection was shown to trigger inflammasome formation and caspase-1 activation and requires both NLRP3 and NLRP12 [49]. Notably, an in vivo assembly of NLRP3 and NLRP12 specks and ASC oligomerization can be seen in febrile malaria patients [49].

In addition, NLRP12 was shown to maintain intestinal homeostasis. NLRP12 plays an important role in protecting against the development of dextran sulfate sodium (DSS)-induced colitis and inflammation-associated tumorigenesis in the AOM/DSS model [42, 50]. Unlike *Nlrp6*-deficient mice, following microbiota transfer by cohousing, *Nlrp12*-deficient mice did not affect the susceptibility of cohoused WT mice to develop colitis [26]. *Nlrp12*-deficient mice were shown to be highly susceptible to colon inflammation and tumorigenesis, associated with increased production of inflammatory cytokines due to a failure to dampen NF-κB and ERK activation in macrophages [50, 42]. Zaki et al. [50] reported that NLRP12 functions in hematopoietic cells to suppress tumorigenesis. Conversely, Allen et al. [42] described that it is not the hematopoietic but the non-hematopoietic compartment which is central for limiting tumor numbers, although NLRP12 function in both the hematopoietic and non-hematopoietic compartments is important for early disease manifestations of DSS-induced colitis [42]. The discrepancy in findings regarding whether NLRP12 is important in the hematopoietic or non-hematopoietic compartments might be explained by differences in the facilities of *Nlrp12*-deficient mice that result in microbiota differences. Nonetheless, both studies suggest an important role for NLRP12 in controlling inflammatory responses in the colon.

NLRC4

Another extensively studied NLR is NLRC4, also known as IPAF. This NLR is expressed in myeloid cells and is primarily characterized for its protective role against bacterial infections. As such, it is of great interest to determine how this NLR discriminates between "friend and foe," that is, between pathogenic bacteria and harmless commensals, and the answers to this question are now beginning to unravel.

The first study to demonstrate a role for NLRC4 in host defense against bacteria examined the pathogen *S.* Typhimurium. In the case of *S.* Typhimurium, NLRC4 has been shown in vitro to be essential for caspase-1 activation and pyroptosis by sensing flagellin in the cytosol [51, 52]. Nlrc4-deficient macrophages are defective in their ability to activate caspase-1 and secrete IL-1β and IL-18 and induce macrophage death [53]. In addition, it has been shown that IFN-γ secretion by NK cells in response to *S.* Typhimurium requires flagellin-sensing NLRC4 inflammasomes [54]. NLRC4 deficiency in BALB/c mice results in increased susceptibility to oral challenge with *Salmonella* but not in C57Bl/6 mice, probably due to their reduced susceptibility to *Salmonella* infection [55]. In addition to *Salmonella*, several studies have demonstrated a clear role for NLRC4 in host defense against the flagellated Gram-negative human pathogen *Pseudomonas aeruginosa* [56–58]. *Nlrc4*-deficient macrophages infected with *P. aeruginosa* exhibited a marked delay in cell death and reduced IL-1β secretion compared with WT macrophages [57]. Additionally, in both pulmonary and peritoneal in vivo models of *P. aeruginosa* infection, Nlrc4-deficient mice are more susceptible to infection [56] and reduced levels of IL-18 are detected. Although flagellin is sensed by both NLRC4 and TLR5, induction of caspase-1 activation and cell death is independent of TLR5 in *P. aeruginosa*-infected macrophages [56]. *Pseudomonas* may also activate caspase-1 through NLRC4-dependent, but flagellin-independent, pathway. While several studies have suggested that cytosolic flagellin can activate the NLRC4 inflammasome, Sutterwala et al. found that *P. aeruginosa* activation of NLRC4 and caspase-1 is independent of flagellin but does require a functional type III secretion system (T3SS) [57]. It has been shown that infection with either a non-flagellated strain or flagellin-deficient mutant strain resulted in the robust activation of caspase-1 and secretion of IL-1β. This result suggests that *P. aeruginosa* flagellin may act to enhance T3SS-dependent caspase-1 activation by supporting bacterial adhesion to the host cell.

In addition to flagellin, NLRC4 activation can stem from virulence factor injection into the cytosol via bacterial type III and IV secretion systems [59–61]. The NLRC4 inflammasome activators identified to date are Gram-negative bacteria that have either a type III or type IV secretion

system and include *Salmonella*, *Shigella*, *Legionella*, and *Pseudomonas* [53, 56–58, 61–63]. It has been demonstrated that macrophages derived from *Nlrc4*-deficient mice are permissive to intracellular *Legionella* replication. In the absence of NLRC4 or caspase-1 activation, the *Legionella*-containing phagosomes do not fuse with the lysosome supporting *Legionella* replication. Moreover, *Legionella* mutants lacking flagellin do not activate caspase-1 in macrophages [63].

Both NLRC4 and NLRP3 form well-characterized inflammasomes, especially with respect to their responses to pathogenic bacteria. Man et al. [64] have shown that *Salmonella* activates both NLRC4 and NLRP3, which results in ASC focus formation and recruitment of caspases to the inflammasome. Moreover, it was shown that endogenous NLRC4 and NLRP3 co-localize in the same speck in a concentric organization [64].

The molecular basis by which the inflammasome responds to specific stimuli is poorly studied. The activation of caspase-1 upon infection of macrophages by the intracellular pathogen, *Legionella pneumophila*, requires, in addition to NLRC4, a second NLR protein named Naip5 (Birc1e) [62]. Mice express multiple NAIP paralogs, which recognize distinct bacterial ligands, while sharing a high degree of amino acid identity. *Naip5*-deficient mice fail to activate NLRC4 either in response to infection with *L. pneumophila* or in response to the C-terminus of flagellin [65]. Additionally, NLRC4 activation by bacterial PrgJ requires NAIP2 while NAIP5 and NAIP6 activate NLRC4 in response to bacterial flagellin [66]. The biochemical function of NAIP5 is to promote NLRC4 oligomerization as the assembly of NLRC4 inflammasome was not observed in the absence of NAIP5 [66, 67].

Of special interest is the activity of NLRC4 in myeloid cells residing in the gut, where the immune system is facing the challenge of discriminating pathogenic bacteria from commensal bacteria. Unlike other tissues, in the intestinal lamina propria, macrophages and DCs are hyporesponsive to stimulation by the microbiota, and this can contribute to the immune tolerance to resident commensal bacteria [68] [69]. Franchi et al. [55] showed that mononuclear phagocytes (iMPs) are able to discriminate between pathogenic and commensal bacteria through activation of the NLRC4 inflammasome and production of IL-1β, which is not dependent on TLR signaling [70]. Consistent with several other works [69, 71, 72], Franchi et al. found that iMPs do not produce TNF or IL-6 in response to TLR ligands or commensal bacteria regardless of exposure to commensal bacteria [55]. iMPs originated from SPF or germ-free conditions are anergic to stimulation of TLRs, yet iMPs constitutively expressed pro-IL-1β. This enables iMPs to directly respond to pathogenic bacteria even in the absence of TLR priming. Infection of phagocytes with *Salmonella* or *P. aeruginosa* prompted IL-1β production by the NLRC4 inflammasome. Yet, infection with commensal bacteria did not result in the activation of the NLRC4 inflammasome. The molecular mechanisms by which *Salmonella* but not commensal bacteria induce the production of IL-1β in iMPs are dependent on NLRC4.

Together, the NLRC4 inflammasome has an important non-redundant role in host defense against bacterial pathogens and seems to be pivotal in the distinction between commensal and pathogenic intestinal organisms based on its ability to sense virulence-associated bacterial machineries.

New developments and future questions

Recently, the discovery of a non-canonical inflammasome pathway leading to the activation of caspase-11 has extended our knowledge of the intricate regulation of this pivotal cellular pathway [73–76]. Interestingly, similar to mice lacking both caspase-1 and caspase-11, mice lacking only caspase-11 feature enhanced intestinal inflammation and dysbiosis [77]. Nonetheless, colitis severity does not seem to stem from aberrant microbial communities, as cohousing of caspase-11-deficient and control mice does not equalize the degree of inflammation. In line with a critical role for IL-18 in regulating colonic microbial ecology and risk for colitis [26], this cytokine is not reduced, but rather elevated, in *caspase-11*-deficient mice. The mechanism by which the non-canonical inflammasome contributes to protection from colitis remains an intriguing future question.

The role of NLRs in the regulation of the intestinal microbiota has become a paradigm for the complexity of multifactorial diseases like IBD and the metabolic syndrome. Together with the well-documented role of NOD2 as a susceptibility gene for human IBD, research performed on inflammasome-forming NLRs and their involvement in host–microbiota interactions suggests a model in which genetic susceptibility and intestinal dysbiosis combine to determine disease manifestation. Solving the relative contribution of host-driven and environmentally driven shaping of the microbiota, as well as microbiota-dependent and microbiota-independent factors in these diseases, presents a formidable challenge. However, this challenge must be overcome in order to mechanistically understand common idiopathic disease and to design new therapeutic principles.

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