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Inflammasomes as regulators of non-infectious disease

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Abstract

Inflammasomes are cytoplasmic organelles that stimulate inflammation upon cellular detection of infectious or non-infectious stress. While much foundational work has focused on the infection-associated aspects of inflammasome activities, recent studies have highlighted the role of inflammasomes in non-infectious cellular and organismal functions. Herein, we discuss the evolution of inflammasome components and highlight characteristics that permit inflammasome regulation of physiologic processes. We focus on emerging data that highlight the importance of inflammasome proteins in the regulation of reproduction, development, and malignancy. A framework is proposed to contextualize these findings.

Keywords

inflammasome; innate immunity; NLRs; interleukin-1; caspase; reproduction; development; malignancy; cancer; inflammation; pyroptosis

1. Introduction

Inflammation is a component of an organism's response to noxious or potentially noxious stimuli. Much of our knowledge in this area derives from studies of the immune response to infection, which results in a rapid, coordinated inflammatory assault designed to eradicate the invading microorganism. Inflammation is also associated with cellular responses to metabolic and endoplasmic reticulum stress, development, and malignancy[1–4]. A notable distinction between infection-induced and other inflammatory responses is the sensitivity of the inflammatory network to its trigger. Non-infectious stresses can be detected and resolved without inducing inflammation. For example, endoplasmic reticulum (ER) stress response pathways are activated in the absence of an inflammatory response within pancreatic acinar

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cells to promote digestive enzyme secretion[5]. If the ER stress response is overwhelmed, acinar cells induce inflammation via the secretion of chemokines that recruit macrophages to remove cells experiencing unresolved stress[6]. Thus, inflammatory activities in response to non-infectious stress are only initiated if existing homeostatic control systems are insufficient to restore homeostasis[7]. In contrast, the presence of a single microbe initiates an inflammatory response[8]. Within individual macrophages, single cell analysis revealed that innate immune pathways activated by bacteria operate in an all-or-nothing fashion (*i.e.*, macrophages either respond to a bacterial encounter or they do not)[8–10]. While detection of infection operates in a binary fashion, the output of macrophage activation varies based upon the number of microorganisms or concentration of pathogen associated molecular patterns, indicating that the inflammatory response is calibrated to the severity of the threat[11,12]. Regardless of the threshold associated with an inflammatory network, inflammation ultimately contributes to a restoration of homeostasis by altering the function of tissues throughout the organism. A key feature of this response is that it comes at the expense of normal tissue function and leads to a transient deviation from homeostasis[13]. For example, local inflammation increases vascular permeability to promote extravasation of leukocytes and serum proteins into the stressed, infected, or damaged tissue[14]. This process comes at the cost of impaired local circulation and hemostasis. The cost of this trade-off between host defense and homeostasis disruption may be proportional to the severity of the insult. Thus, the ability of an organism to survive a noxious stimulus depends on the severity of the stimulus and the organism's tolerance to inflammation-induced deviation from normal function[15,16]. The reduction in tolerance to homeostasis disruption that occurs with age may explain why older individuals are more likely to succumb to infections that are cleared by younger individuals[13,17].

Central to discussions of inflammation are the inflammasomes, which are key mediators of the inflammatory response to infection and injury[18]. Inflammasomes are cytoplasmic protein complexes that serve as the principal site of inflammatory caspase activities that promote the release of several interleukin-1 (IL-1) family cytokines[18]. Inflammasome activities are stimulated by pattern recognition receptors (PRRs) and guard proteins of the innate immune system (Figure 1). These proteins bind to microbial products directly (PRRs) or detect homeostatic disruptions (guards)[19,20]. Upon detection of either of these types of threats, an activated PRR or guard seeds the assembly of inflammasomes that can promote IL-1 release from either living or dead (*i.e.* pyroptotic) cells[21]. Diverse PRRs and guards can seed inflammasome assembly and activities, with well-described examples being members of the nucleotide-binding domain leucine rich repeat containing (NLR) family, the AIM2-like receptor family, the neuronal apoptosis inhibitor protein (NAIP) family, and the protein Pypin. PRRs include AIM2 and NAIP5, which respectively detect viral double stranded DNA or bacterial flagellin subunits in the host cytosol, and the guard protein Pypin, which detects dysregulation of Rho family GTPase activities[22–26]. NLRP3 is another guard protein which can be activated by diverse ion imbalances in the cell that lead to alterations in endosomal membrane dynamics[27]. PRRs and guards seed inflammasome assembly through the process of protein oligomerization, which results in the assembly of a micron-sized supramolecular organizing center (SMOC) that recruits and activates caspase-1 through auto-catalytic cleavage[19,28]. Activated caspase-1 cleaves the cytoplasmic protein

gasdermin D (GSDMD), liberating the N-terminal domain which then oligomerizes within the plasma membrane to form a pore[21,29]. These pores serve as membrane channels that facilitate ion exchange and the secretion of bioactive IL-1 family cytokines, which have been cleaved by caspase-1[30]. If GSDMD pores are not repaired by the cell, pyroptosis can ensue, resulting in membrane rupture of the cellular corpse via the membrane protein *Ninjurin1*[31,32].

The molecular details of how distinct microbial products, virulence factors, and disruptions of homeostasis promote inflammasome and anti-infective activities have been reviewed elsewhere and will not be described in detail here[18–20,25,27,33,34]. In this review, we will discuss the contribution of inflammasomes to the regulation of physiologic processes beyond infection and across the species. We begin by discussing the evolution of inflammasome regulatory proteins (NLRs and caspases) and highlight how features conserved over evolutionary time prime these proteins to regulate homeostasis outside of their role in immunity. We will then discuss examples of the inflammasomes in the non-infectious contexts that span the stages of life, from development to reproduction to malignancy.

2. Diversity of NLRs and Caspases Across the Species

Proteins of the NLR family are present in several domains of life[19,35–38]. NLRs in plants and metazoans appear to have arisen separately during evolutionary time from domains that originated in bacteria[37]. Metazoan and plant NLRs contain C-terminal leucine-rich repeat (LRR) domains and an N-terminal effector domain but differ in the central regions between these domains[39]. Several metazoan NLRs contain a central NACHT domain (NAIP, major histocompatibility complex [MHC] transactivator [CIITA], HET-E, and TP1) whereas plant NLRs contain a central NB-ARC (Nucleotide-Binding, Apaf1, Resistance, CED4) domain[39]. In plants the N-terminal effector domain is comprised of either the Toll/IL-1 receptor/resistance (TIR) or coiled-coiled (CC) domain, while in metazoans it is comprised of either a caspase activation and recruitment domain (CARD) or Pyrin domain (PYD). The functional consequences of using different nucleotide-binding domains in plants and metazoans is unclear but may reflect evolution from distinct lineages[39].

Caspases are the effector proteins downstream of metazoan NLRs. Caspases are a class of cysteine-dependent proteases that cleave target proteins at conserved sequences containing aspartic acid and are required for the regulation of programmed cell death (PCD) and induction of inflammation[40]. Caspases are categorized based upon their ability to initiate or execute apoptotic cell death and their involvement in inflammation[40]. A related group of proteins, termed metacaspases, exist in bacteria, plants, fungi, and other eukaryotes, and are postulated to fill the same regulatory function as metazoan caspases, however this idea remains debated[41,42]. Metacaspases share with metazoan caspases a conserved cysteine-histidine catalytic site but differ in that they do not require dimerization for activation and cleave after arginine or lysine residues[41]. Despite these functional differences, metacaspases are involved in PCD in plants and protists, although it remains unknown if the mechanisms are shared with metazoan caspases[43].

Apoptotic caspases are also required for development independent of their ability to induce PCD. The most convincing evidence exists for caspases being required for differentiation and maintenance of stem cell state[43]. Importantly, this developmental role shares a key feature with the role in PCD, namely that caspases regulate irreversible cell fate decisions. For example, caspase-3 is critical for differentiation of embryonic stem cells and hematopoietic stem cells in mice[44,45]. Similarly, drICE (the *Drosophila* caspase-3 homolog) is necessary for spermatid maturation and reproduction, the metacaspase Yca1 regulates proteostasis in yeast, and CED-3 (a *C. elegans* caspase-3 homolog) regulates asymmetric division of seam cells (neuroectodermal stem cells)[46–49]. Furthermore, transient activation of caspase-3 and caspase-9 is required for the differentiation of myotubes and formation of murine skeletal muscle[50–52]. Notably, the kinetics and intensity of caspase activation during differentiation is different than what is observed during PCD. Whereas caspase activation in PCD is rapid and maximal, the same caspase is only transiently and mildly activated during development[43]. The mechanisms of caspase regulation are just being uncovered but so far appear to center on posttranslational modification (PTM) of caspases and their target proteins alongside spatial control of caspase activation[43]. Thus, caspase activation occurs along a spectrum, from most intense which is responsible for PCD to least intense which is important for regulating the functions of living cells.

Inflammatory caspases are the primary effector enzymes within inflammasomes, which cleave select IL-1 family members to bioactive components and promote GSDMD pore formation[21]. While activation of inflammatory caspases was initially described to result in pyroptosis, recent work has indicated that inflammasomes can process inflammatory cytokines in the absence of cell death[53]. Cells that use inflammasomes to secrete IL-1 β while remaining viable are potent inducers of adaptive immunity[54]. These cells are referred to as hyperactive, as compared to inflammatory cells that release cytokines other than IL-1 β (which are commonly referred to as active)[53,55–60]. The discovery of inflammasome activities in living cells indicates that while activation of the inflammasome may be digital, the output is not. Whether inflammasome activities lead to pyroptosis or cell hyperactivation remains an active area of research, and there are at least four possible underlying mechanisms. First, posttranslational modifications of inflammasome seed proteins may allow diverse cellular processes to regulate inflammasome output [27]. Alternatively, a secondary process may regulate the threshold of inflammasome signaling, allowing the output to be tuned to the specific activating signal. Third, differential regulation of membrane repair could determine the balance between pyroptosis and hyperactivation[32,61]. Finally, it is possible that, like apoptotic caspases that operate in living cells during development, the kinetics, localization, and activities of inflammasome-associated caspase activities dictates pyroptosis versus cell hyperactivation. Overall, despite notable gaps in our mechanistic understanding of these processes, caspases have the capability to control cellular function in cell deathdependent and -independent manners. In the next section, we will discuss three physiologic processes whereby inflammasomes and their effector caspases regulate restoration of homeostasis after non-infectious stress.

3. Physiologic Roles of Inflammasomes

3.1. NLRs in Reproduction

There are several NLR family members for which the upstream activators and downstream effectors remain unknown. A group of these orphan NLRs (NLRP2, -5, -7, -9, -14) regulate reproduction in mammals (Figure 2A)[62]. These reproductive NLRs are the result of a tandem duplication event that phylogenetically separates them from other NLR proteins[63]. Each reproductive NLR can be classified by which gamete expresses it: oocytes express NLRP2, -5, -7, and -9 while developing spermatocytes express NLRP14[62]. Deficiency of either NLRP2 or NLRP5 is associated with embryonic arrest at the 2-cell stage in mice and humans[64–69]. Embryonic arrest in NLRP5 deficient ova has been linked to increased mitochondrial activity and generation of reactive oxygen species (ROS)[70]. NLRP9 underwent gene duplication during rodent evolution such that there are three isoforms in rodents and one isoform in humans[71]. In humans, NLRP9 is expressed in oocytes, and knock down leads to embryonic arrest at the 2 or 4-cell stage[72]. Oocytes isolated from mice deficient for all three isoforms of NLRP9 similarly undergo embryonic arrest at the 2- or 4-cell stage[73]. Intriguingly, the gene duplication event in rodent evolution permitted the diversification of NLRP9 function, and mice deficient in NLRP9b are more susceptible to rotavirus infection[74]. Rotaviral RNA binds to DHX9 in the cytosol of infected cells, which then triggers NLRP9b oligomerization and activation[74]. However, it is unclear if this mechanism is conserved in humans, as crystal structures of the human NLRP9 pyrin domain suggests that it is unlikely to oligomerize[75,76]. Despite this uncertainty, the ability of viral RNA to activate NLRP9b in mice suggests that specific RNA species could be sensed by the NLRP9 inflammasome to control development.

NLRP7 is unique to primates and has been linked to the development of molar pregnancies[77]. Multiple non-synonymous mutations in NLRP7 have been linked to the development of hydatiform mole, potentially due to disruption of oligomerization[78–80]. How NLRP7 regulates pregnancy remains uncertain, however it appears important for both oocyte development and placental development[77]. One possibility is that mutations in NLRP7 impair oligomerization in response to an endogenous or exogenous stimulus. However, the ability of NLRP7 to activate the inflammasome remains unclear, with disparate studies reporting distinct mechanisms of regulation[81–84].

NLRP14 is uniquely expressed in the testes and mutations in it are linked to failure of spermatogenesis[85]. NLRP14 appears to inhibit cytosolic nucleic acid sensing by interfering with the activity of a central kinase that regulates interferon expression, TBK1[86]. It is unclear how nucleic acid production and the consequent TBK1 signaling are important in spermatogenesis and subsequent fertilization, however this is reminiscent of the ability of the *Drosophila* caspase-3 homolog drICE to control spermatogenesis[46]. We do not know if NLRP14 can oligomerize and form a functional inflammasome.

How and why are NLR proteins essential for normal reproduction? It is unlikely NLRs control reproduction through production of IL-1 or induction of pyroptosis, as mice deficient in MyD88 and GSDMD are viable. The existing data suggest the possibility that cytosolic nucleic acid sensing may be the upstream activator of these diverse NLRs (Figure 2A).

Whether there is specificity beyond the type of nucleic acid or if this is a purposefully controlled process remains unknown. Additionally, there is no evidence supporting the role of inflammatory caspases as essential downstream effectors of NLR activation during reproduction, nor have ASC or IL-1 β been implicated. It is known that mice deficient in caspase-1 and caspase-11 have normal fecundity, however subtler developmental differences may not manifest in mice raised in a vivarium.

3.2. Inflammasomes in Development

Development is characterized by exponential cell division. This process results in the generation of multiple byproducts including ATP, dsDNA, and mitochondrial ROS, all of which regulate inflammasomes[18]. Production of these inflammasome regulatory factors serves as a readout of a specific cellular function, with ATP and ROS indicating the health of mitochondrial function and dsDNA indicating nuclear integrity. Substantial data indicate the evolutionarily conserved role of apoptotic caspases in normal development[43–46,50–52]. However, it is unknown if byproducts of development activate the inflammasome to regulate developmental programs.

Neurodevelopment is characterized by high rates of replicative stress resulting in DNA damage and the generation of cytosolic dsDNA[87]. Several neurologic diseases are caused by mutations in genes involved in DNA damage repair[88,89]. AIM2 is predominantly expressed myeloid cells and is activated by cytosolic dsDNA larger than 80 base pairs without sequence specificity[22–24,90]. AIM2 deficient mice develop increased anxious behaviors and decreased associative memory[91]. These behavioral differences are associated with increased dendrite length and branching, suggestive of inadequate neuronal pruning during development[91]. Subsequent work demonstrated that formation of AIM2 inflammasomes in neurons accompanies normal development[92]. Neuronal AIM2 may detect DNA damage during neurodevelopment to control cell pruning, a process that requires caspase-1 activation and GSDMD pore formation (Figure 2B)[92]. Neither microglial AIM2 nor total body IL-1 signaling are required for control of anxious behaviors and associative memory, suggesting that inflammation is not responsible for the observed impairment in neurodevelopment[92]. The inflammation-independent role of AIM2 in control of anxious behaviors and memory is distinct from the described importance of TLR signaling, cytokines, and microglia in the control of other neurodevelopmental processes (reviewed in [93–95]). In addition to its role in neurodevelopment, AIM2-deficiency protects against the development of Parkinson's Disease in mice and reduces the severity of experimental autoimmune encephalomyelitis independent of inflammasome activation[96,97]. However, AIM2 may contribute to the pathology of Alzheimer's Disease and to worsened outcomes after ischemic and hemorrhage stroke[98]. Thus, AIM2 may support beneficial developmental processes but be accompanied by a subsequent vulnerability to certain acute or chronic injuries that manifest later in life. A prediction would be that AIM2 serves other important roles in development outside of the nervous system.

Pyrin is another inflammasome seed protein that regulates development. Pyrin is encoded by the *MEFV* gene, mutations in which cause Familial Mediterranean Fever[99].

Under homeostatic conditions, Rho GTPases activate protein kinases N1 and N2 which phosphorylate and inactivate Pyrin (Figure 1)[100]. Upon disruption of Rho GTPase function, Pyrin becomes dephosphorylated, reducing its interaction with 14-3-3 proteins and permitting oligomerization and formation of an active inflammasome[26,101–105]. These findings indicate Pyrin is a sensor of Rho GTPase function while other studies have indicated Pyrin also senses cytoskeletal function[106]. Rho GTPases regulate numerous cellular processes including the actin cytoskeleton, cell polarity, and gene transcription, and thus their activity is controlled by over 130 proteins[107]. Rho GTPase activity can thus serve as a proxy for a variety of cellular functions important for development. Furthermore, migration is a key feature of the developmental program for neural crest cells and depends on a functional cytoskeleton[108]. These observations raise the question of whether the Pyrin inflammasome contributes to development. No studies have specifically evaluated this question, and Pyrin deficient mice appear phenotypically normal. However, caspase-1 deficient mice also initially appeared phenotypically normal until subtle neurologic developmental defects were identified in these animals[92]. Thus, we suggest that Pyrin may serve an important developmental role due to its ability to detect changes in Rho GTPase function (Figure 2B).

3.3. Inflammasomes in Malignancy

There is an intimate relationship between the immune system and the development of malignancy. While the immune system can exert anti-tumorigenic effects, local and systemic inflammation can promote tumorigenesis[1,109–111]. For example, in the azoxymethane (AOM) and dextran sodium sulfate (DSS) model of colitis-associated colorectal cancer (CAC) in mice, AOM triggers DNA damage, resulting in cellular transformation[112]. Recurrent exposure to DSS induces chronic inflammation and accelerates tumorigenesis[112]. This AOM-DSS model recapitulates the phenotypic appearance of human colorectal cancer (CRC)[113].

The inflammasome is a central mediator of the inflammatory component driving tumorigenesis[109,111]. Inflammatory activities are a likely link between inflammasomes and cancer, and chemical induction of pyroptosis directly in tumor cells can trigger a robust anti-tumor immune response[114,115]. However, inflammasome signaling independent of inflammation also contributes to controlling tumorigenesis. Below, we will discuss the roles of inflammasomes in the regulation of tumorigenesis and integrate that into our understanding of inflammasome biology.

NLRC4 is expressed in intestinal epithelial cells (IECs)[116]. Upon activation by NAIPs during *Salmonella enterica* serovar Typhimurium infection, the NAIP-NLRC4 inflammasome induces rapid extrusion of infected IECs into the intestinal lumen[116,117]. NLRC4 activation triggers polymerization of actin around the infected cell which is required for cell extrusion from the epithelial monolayer[117]. IEC extrusion does not require cell death[117]. The control of IEC survival and residence within the epithelial layer suggests a role for the NAIP-NLRC4 inflammasome as an epithelial cell quality control sensor. In the AOM-DSS CAC model, NLRC4 deficiency leads to increased tumor burden without changes in inflammation[118]. The precise activator of NLRC4 in the AOM-DSS

model is unclear, however NAIPs are required for the anti-tumorigenic process of NLRC4. Mice deficient in all 6 NAIPs develop increased tumor burden in the AOM-DSS CAC model[119]. NAIPs may play a protective role early in tumorigenesis, as NAIP deficient mice are protected from AOM induced tumorigenesis in the absence of DSS[119]. NAIP deficiency within the IEC compartment leads to increased tumorigenesis in the AOM-DSS CAC model, suggesting a cell-intrinsic effect of the NAIP-NLRC4 inflammasome in CRC tumorigenesis[119]. Additionally, NLRC4 has been shown to reduce growth in a cutaneous melanoma model independent of caspase-1, however recent studies have shown conflicting results[120–122]. As in the studies on AOM-DSS CAC, the agonist of NLRC4 in melanoma remains unknown. Taken together, these data suggest that NAIPs respond to changes in cellular homeostasis that occur along the path to tumor formation and activate NLRC4 to eliminate the potentially tumorigenic focus (Figure 2C). It remains unknown whether the tumor suppressive role of NLRC4 always requires caspase-1 or if the cell extrusion seen during *Salmonella* infection is the underlying mechanism. One clue may reside in a study demonstrating that NLRC4 has antitumorigenic effects independent of caspase-1[123]. This study found that NLRC4 was required for p53-mediated cell death, suggesting that p53 senses genotoxic stress and activates the NLRC4 inflammasome to initiate cell extrusion[123]. One prediction of these observations is that NAIPs and NLRC4 are important for tumor surveillance in addition to their role protecting against enteric pathogens. A few studies demonstrate that lower expression of NLRC4 correlates with CRC development and mortality; however further mechanistic studies are needed to confirm these findings[124,125].

AIM2 was identified originally as a tumor suppressor in melanoma and has additionally been found to suppress tumor growth in hepatocellular carcinoma, renal cell carcinoma, breast cancer, prostate cancer, and colon cancer[126,127]. Detailed examination of 394 human CRC samples revealed that expression of AIM2 within colorectal tumors is correlated with survival[128]. In this study, over 75% of patients had reduction in AIM2 expression in CRC cells compared to adjacent normal tissue[128]. Additionally, complete loss of AIM2 expression within the malignant cell was associated with a 3-fold increase in mortality[128]. Two studies have suggested a mechanism by which AIM2 can regulate CRC tumorigenesis. In the murine AOM-DSS model of CAC, mice lacking AIM2 develop more numerous, larger, and higher-grade tumors[129]. This process occurs independently of inflammasomes, as caspase-1 cleavage and secretion of IL-1 β and IL-18 are unchanged in the absence of AIM2[129]. The authors suggest that AIM2 suppresses enterocyte proliferation through suppression of AKT phosphorylation on serine 473 and consequent promotion of cell-intrinsic apoptosis (Figure 2C)[129]. Intriguingly, AIM2 expression in bone marrow-derived and stromal cells appears to be important[129]. A second study demonstrated an identical phenotype in AIM2 deficient animals using the AOM-DSS model and confirmed their findings in the *Apc*^{Min/+} model of CRC[130]. Mechanistic insights from this study suggest that AIM2 suppresses DNA-dependent protein kinase phosphorylation of AKT on serine 473 to regulate cellular growth[130]. Inhibition of AKT in AIM2 deficient animals reduced tumor burdens in the AOM-DSS model without affecting tumorigenesis in wild type mice[130]. In both studies, the assumption is that AIM2 detects cytosolic dsDNA produced during the transformation of a cell from benign to malignant. It is unknown

whether AIM2 activation of caspase-1 within the malignant cell is required for its tumor suppressive function or if there are caspase-independent functions for AIM2 in CRC.

NLRP6 is predominantly expressed in non-hematopoietic tissues and is reportedly activated by myriad stimuli[131,132]. NLRP6 is most highly expressed at mucosal barrier tissues and appears to control secretion of IL-18 *in vivo*, as animals deficient in NLRP6 have lower circulating IL-18 levels at baseline and in response to DSS colitis[131,133]. Notably, IL-1 β levels are unchanged to slightly increased in NLRP6 deficient animals, possibly due to compensatory upregulation of NLRP3 activity[132]. A key function of NLRP6 is to control of the composition of the gut microbiota. Studies from three laboratories indicated that NLRP6 deficient animals have abnormal gut microbial communities and develop more severe colitis after DSS treatment due to impaired caspase-1 activation and IL-18 secretion[131,133,134]. NLRP6 may detect microbe-derived small molecules to regulate IL-18 secretion[135]. NLRP6 triggers secretion of IL-18 independent of cell death, suggesting that hyperactivation is not restricted to myeloid cells[135]. However, our understanding of the role of NLRP6 in regulation of the gut microbiota remains incomplete, as subsequent work from two laboratories found no difference in microbiota composition or DSS colitis severity in NLRP6 deficient mice[136,137]. The reasons for these differential observations are unclear. More work is needed, especially to ensure that any phenotypes observed are not linked to passenger mutations associated with distinct lines of the same murine colonies[138]. NLRP6 also has functions independent of caspase-1. NLRP6 deficient animals are more susceptible to *Citrobacter rodentium* infection due to impaired goblet cell mucus secretion[139]. In this context, NLRP6 regulates autophagy to control mucus secretion from goblet cells through an unknown mechanism[139]. Alternatively, NLRP6 deficient mice are resistant to infections with *Listeria*, *Salmonella*, and *E. coli*[140]. During infection with these organisms, NLRP6 negatively regulates ERK1/2 and NF- κ B signaling, impairing the host response to infection[140]. These observations point to important caspase-dependent and caspase-independent functions of NLRP6 in the regulation of colonic microbial communities. Thus, through two distinct pathways, NLRP6 curates the gut microbial community and controls responses to infection.

NLRP6 deficient mice develop more numerous and larger colorectal tumors in the AOM-DSS model of CAC[133,134]. This process depends on caspase-mediated secretion of IL-18 (Figure 2C)[133,141]. Intriguingly, the importance of NLRP6 regulating CRC may partially explain the sexual dimorphism seen in colon cancer in humans. Females have reduced incidence of CRC compared to males[142]. Additionally, mortality is lower in women aged 18–44 compared to men of the same age or women older than 50, suggesting that estrogen may play a protective role in the development and progression of CRC[143]. Reduction in circulating estrogens due to oophorectomy increases CRC risk while taking exogenous estrogens in the form of hormone replacement therapy reduces CRC risk and mortality[143]. Furthermore, loss of estrogen receptor isoform β (ER β) in CRC is correlated with lower survival[143]. Thus, systemic estrogen levels and local estrogen signaling are important for regulation of CRC. A mechanistic explanation of this association was unclear until a recent paper showed that ER β induces NLRP6 expression, reducing the severity of DSS colitis in mice[144]. These data suggest that NLRP6 plays a central role in curation of a pro-health microbiota, the disturbance of which contributes to multiple diseases.

The NLRP3 inflammasome predominantly controls tumorigenesis through its pro-inflammatory actions. Multiple reviews have detailed the role of NLRP3-derived inflammation in tumorigenesis[109,111]. Emerging research suggests that the NLRP3 inflammasome can regulate tumorigenesis independent of inflammation. Non-hematopoietic NLRP3 has been proposed to suppress colitis in response to DSS[145]. Additionally, non-hematopoietic NLRP3 may contribute to an intermediate suppression of tumorigenesis in the AOM-DSS CAC model, however another study did not find an effect of NLRP3 on AOM-DSS induced CAC[118,146]. Interestingly, in a CRC metastases model, both hematopoietic and non-hematopoietic NLRP3 were shown to inhibit metastases[147]. Furthermore, NLRP3 appears to directly regulate growth of hepatocellular carcinoma independent of inflammation [148]. We are just beginning to appreciate the role of NLRP3 in the regulation of tumorigenesis independent of its ability to induce inflammation.

Taken together, multiple inflammasome regulatory proteins impact tumorigenesis independent of their role in triggering inflammation. These data suggest that inflammasome biology extends beyond the ability to induce secretion of IL-1 family members. Of note, most studies have been performed in mice with total body deficiency of a gene of interest. Future work examining the tissue specific function of inflammasome regulators will be important to identify the relative roles of the inflammasome in the immune and stromal cells. While NLRP6 regulates tumorigenesis through secretion of IL-18, the precise activating signals and downstream effectors for the remaining proteins remain to be elucidated. One intriguing possibility is that inflammasomes in tumorigenesis are acting analogously to apoptotic caspases during differentiation. Tumorigenesis is an irreversible, stepwise change in cellular function that is akin to the stepwise cellular changes that occur during differentiation, and thus inflammatory caspases could regulate tumorigenesis by sensing inappropriate cell fate decisions.

4. Conclusions and Perspectives

The field is just beginning to understand how inflammasomes regulate physiologic processes in addition to infection and injury. In this review, we discussed examples where inflammasomes contribute to the control of reproduction, development, and tumorigenesis. Below, we propose a few key principles that will help guide our understanding and future research.

First, inflammasome seed proteins evolved to surveil the cytoplasmic environment. The cytoplasmic location of inflammasome seed proteins created evolutionary constraints on which specific signals they can detect. As such, two strategies to detect perturbation of cellular homeostasis evolved in inflammasomes: direct detection of microbial molecules or guarding specific cellular processes[39]. In the first strategy, inflammasome seed proteins detect the presence of microbial molecules directly (*e.g.*, LPS) or cellular molecules associated with tissue injury (*e.g.*, cytoplasmic DNA)[21]. These inflammasome seeds operate as PRRs. Many of the molecules detected by PRRs are also produced by physiologic cellular processes. For example, dsDNA is generated as part of the DNA damage response or cell death, creating the opportunity for inappropriate activation of the immune response[40,87]. Other PRRs that detect DNA, such as Toll-like receptor (TLR)

9, are compartmentalized into endosomes or phagosomes[149]. Compartmentalization is a strategy employed by the immune system to control the inflammatory response, yet the cytoplasmic location of inflammasomes evades this locus of control. In the second strategy, inflammasome seeds function as guard proteins and detect perturbation of specific cellular functions (*e.g.*, ion concentration or the cytoskeleton)[20]. These cellular functions are altered by an array of stimuli besides infections. For example, neurons and myocytes see cyclical change in ion concentration while migrating cells see dramatic reorganization of the cytoskeleton. Thus, the presence of inflammasomes in the cytosol permits detection of molecules and activities that serve as a readout of cellular processes.

Second, caspases can be activated without resulting in cell death. Apoptotic caspases can regulate cell differentiation while inflammatory caspases can permit secretion of IL-1 cytokines from living (hyperactive) cells. The ability to dissociate caspase-1 activity from cell death allows for more robust T cell activation and anti-tumor immunity[54]. What leads one cell with active caspase-1 to die while another to live is unknown, but a clue from research on the control of apoptotic caspases suggests that PTMs may play a role[43]. Inflammasome seed proteins are the target of multiple kinases, phosphatases, E3 ligases, and deubiquitinating enzymes[27,150–152]. The accumulation of PTMs could thus serve as an integration of cellular function, allowing physiologic processes to fine tune inflammasome activity. Furthermore, it remains unclear if the sole effect of inflammatory caspase activation in living cells is secretion of IL-1 cytokines or if another output is contributing.

Third, each cell type appears to have a distinct mechanism of inflammasome activation. Macrophages and dendritic cells (DCs) undergo inflammasome activation with pyroptosis[19]. Hyperactivation appears to be most common in DCs, although reports of this process in macrophages and neutrophils exist[53,55,57,58,153,154]. Additionally, human monocytes secrete IL-1 β in response to LPS alone while murine monocytes do not[59]. Furthermore, living non-immune cells such as adipocytes secrete IL-1 to control differentiation[155].

Taken together, these three principles suggest a model by which inflammasomes can be activated by physiologic processes. The ability of inflammasomes to detect endogenously generated signals and activate caspase-1 without inducing cell death in a cell specific manner likely underpins this capability. Many questions remain unanswered: what other processes or activities are sensed by inflammasomes, what are the other outputs of caspase-1 activation besides IL-1, and what controls whether a cell lives or dies when the inflammasome is activated? The answers to these questions promise to improve our understanding of inflammasome biology and provide the basis for design of novel therapies for diseases of dysregulated homeostasis.

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Abbreviations

| | |
|--------------------------------|--|
| AIM2 | Absent in melanoma 2 |
| AOM | Azoxymethane |
| CAC | Colitis-associated colorectal cancer |
| CARD | Caspase activation and recruitment domain |
| CC | Coiled-coiled |
| CRC | Colorectal cancer |
| DC | Dendritic cell |
| DHX9 | DEXH-box helicase 9 |
| drICE | Death related ICE-like caspase |
| DSS | Dextran sodium sulfate |
| ER | Endoplasmic reticulum |
| ERβ | Estrogen receptor β |
| ERK | Extracellular signal-related kinases |
| GSDMD | Gasdermin D |
| HET-E | Heterokaryon incompatibility E |
| IEC | Intestinal epithelial cell |
| IL | Interleukin |
| LPS | Lipopolysaccharide |
| MHC | Major histocompatibility complex |
| NAIP | Neuronal apoptosis inhibitor protein |
| NB-ARC | Nucleotide-binding Apaf1 Resistance CED4 |
| NF-κB | Nuclear factor Kappa B |
| NLR | Nucleotide-binding domain leucine rich repeat containing |
| NLRC | NLR family CARD domain containing |
| NLRP | NLR family Pyrin domain containing |

| | |
|-------------|---------------------------------|
| PCD | Programmed cell death |
| PRR | Pattern recognition receptor |
| PTM | Post-translational modification |
| PYD | Pyrin domain |
| ROS | Reactive oxygen species |
| TBK1 | TANK-binding kinase 1 |
| TIR | Toll/Interleukin-1 receptor |
| TLR | Toll-like receptor |
| TP1 | Telomerase associated protein 1 |

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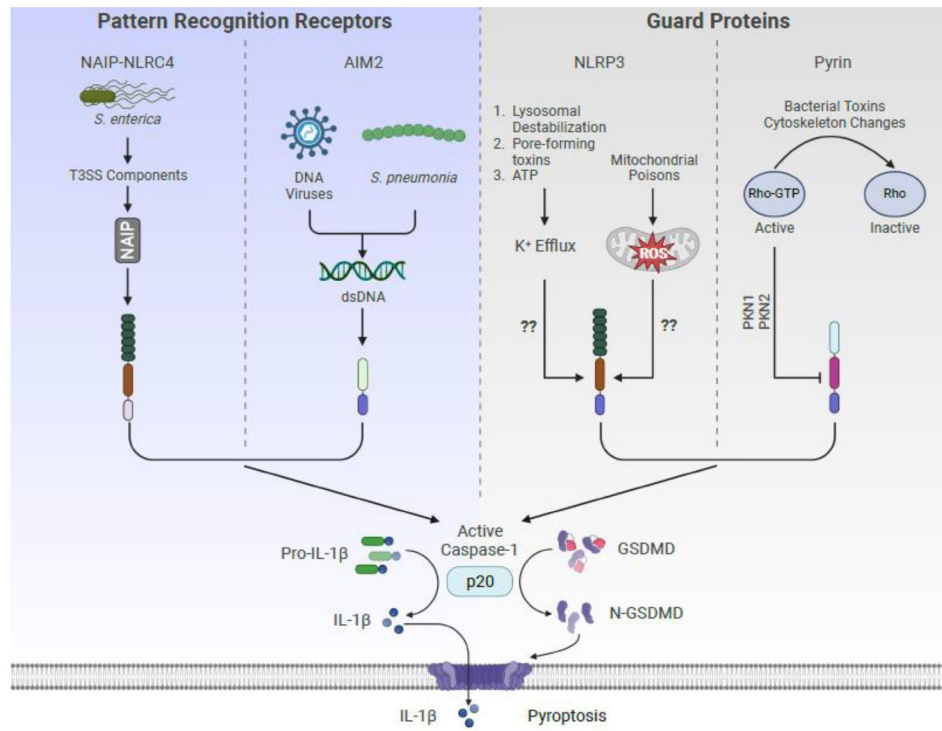


Figure 1. Pattern recognition receptors and guard proteins activate the inflammasome. Two classes of proteins can activate the inflammasome. Pattern recognition receptors (*e.g.*, NAIP-NLRC4 or AIM2) directly detect microbial products while guard proteins (*e.g.*, NLRP3 or Pypin) detect perturbation in specific cellular processes.

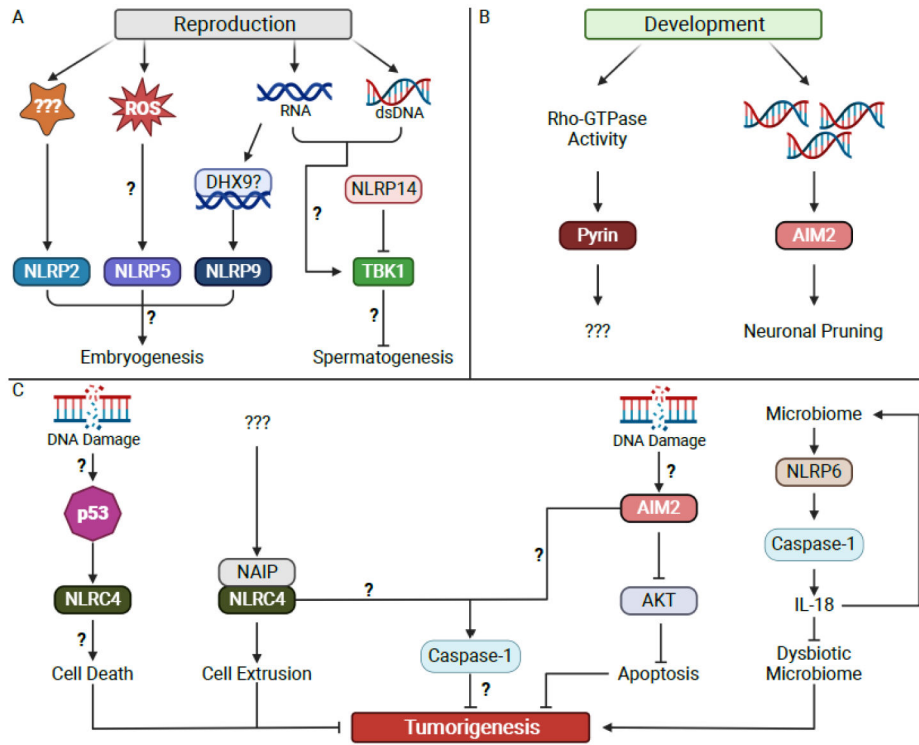


Figure 2. Inflammation regulates physiologic processes.

(A) Embryogenesis is regulated by NLRP2, NLRP5, and NLRP9, while spermatogenesis is regulated by NLRP14. Sensing of cytosolic nucleic acids may serve as a central regulator of both reproductive processes.

(B) Development generates cytosolic dsDNA which signals through AIM2 to control neuronal pruning. It is proposed that Pyrin senses changing Rho-GTPase activity to regulate unknown features of development.

(C) Multiple inflammasomes are proposed to regulate tumorigenesis through diverse pathways, some of which may be independent of inflammatory caspase activity.