

Inflammasomes in the gastrointestinal tract: infection, cancer and gut microbiota homeostasis

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Abstract | Inflammasome signalling is an emerging pillar of innate immunity and has a central role in the regulation of gastrointestinal health and disease. Activation of the inflammasome complex mediates both the release of the pro-inflammatory cytokines IL-1 β and IL-18 and the execution of a form of inflammatory cell death known as pyroptosis. In most cases, these mediators of inflammation provide protection against bacterial, viral and protozoal infections. However, unchecked inflammasome activities perpetuate chronic inflammation, which underpins the molecular and pathophysiological basis of gastritis, IBD, upper and lower gastrointestinal cancer, nonalcoholic fatty liver disease and obesity. Studies have also highlighted an inflammasome signature in the maintenance of gut microbiota and gut–brain homeostasis. Harnessing the immunomodulatory properties of the inflammasome could transform clinical practice in the treatment of acute and chronic gastrointestinal and extragastrointestinal diseases. This Review presents an overview of inflammasome biology in gastrointestinal health and disease and describes the value of experimental and pharmacological intervention in the treatment of inflammasome-associated clinical manifestations.

The immune system has an extraordinary capacity to recognize and respond to a range of microbial patterns and danger signals. In the gastrointestinal tract, trillions of microorganisms colonize the mucosal surface and lumen and constantly release immunomodulatory molecules that interact with and shape the immune system¹. Most of these microorganisms are symbionts or so-called commensals and do not generally evoke a detrimental inflammatory response. However, pathogens have the ability to invade the mucosal barrier and underlying tissue, inducing the production of cytokines, chemokines and antimicrobial molecules.

A central and rapid mechanism triggering an inflammatory response occurs through activation of the innate immune signalling complex called the inflammasome^{2,3}. The inflammasome is formed in a variety of immune and non-immune cells when a subset of cytosolic pattern-recognition receptors known as inflammasome sensors recognizes pathogen-associated molecular patterns (PAMPs) or danger-associated molecular patterns (DAMPs)⁴. To date, members from three families of pattern-recognition receptors are known to have an established role in the assembly of an inflammasome: NLRP1, NLRP3, NLRC4 and neuronal apoptosis inhibitory proteins (NAIPs) from the NOD-like receptor (NLR) family; absent in melanoma 2 (AIM2) from

the AIM2-like receptor (ALR) family; and pyrin from the tripartite motif-containing protein (TRIM) family⁵ (FIG. 1). Several other sensors have been suggested to activate the inflammasome, including the NLR family members NLRP6, NLRP7, NLRP9 (NLRP9b in mice) and NLRP12, the DNA sensor IFN γ -inducible protein 16 (IFI16) and the RNA sensor retinoic acid-inducible gene I protein (RIG-I; also known as DDX58); however, the ability of these sensors to form inflammasome complexes is still uncertain⁶. On activation, inflammasome sensors recruit the adaptor protein apoptosis-associated speck-like protein containing a caspase activation and recruitment domain (ASC; also known as PYCARD) and the cysteine protease caspase 1, and form a single cytoplasmic aggregate known as the inflammasome speck⁶. Caspase 1 is activated in this complex, inducing proteolytic cleavage of the pro-inflammatory cytokines pro-IL-1 β and pro-IL-18 and the pore-forming protein gasdermin D, which results in the release of bioactive IL-1 β and IL-18 into the extracellular milieu and pyroptosis, respectively⁷. Bioactive IL-1 β and IL-18 and certain DAMPs can be secreted through the gasdermin D pores before rupture of the host cell membrane, cell lysis and cell death^{8–10}.

Inflammation and cell death responses triggered by the inflammasome contribute to the pathogenesis of a

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Key points

- Inflammasomes are expressed in both immune and non-immune cells, contributing to their functional ties to infection, IBD, cancer, autoinflammation and autoimmune conditions.
- Inflammasome sensors function by recognizing and responding to a pathogen (lipopolysaccharide, microbial DNA or bacterial flagellin) or to a danger signal (ion flux, self-DNA or ATP).
- Inflammasomes control the magnitude of inflammation and cell death in response to pathogen-associated molecular patterns or danger-associated molecular patterns, which, in part, determines a protective or detrimental outcome in the host.
- Experimental and pharmacological interventions have yielded success in the treatment of inflammasome-mediated disorders, such as autoinflammatory enterocolitis.
- The inflammasome–gut microbiota axis and its relevance to health and disease are influenced by genetic, environmental and experimental factors.
- The deep and complex relationship between inflammasomes, pathogens and the microbiota provides an exciting platform for basic and clinical research with which to understand health and disease.

spectrum of acute and chronic diseases in the gastrointestinal tract, including infection, gastritis, IBD, cancer, nonalcoholic fatty liver disease (NAFLD) and obesity^{11,12}. Inflammasomes might also support a healthy gut microbiota and regulate metabolic and gut–brain homeostasis, with a majority of studies focusing on bacteria of the gut microbiota^{13,14}. This Review describes the central roles of inflammasome signalling in the gastrointestinal tract, highlighting its major functionalities in shaping the outcome of acute and chronic gastrointestinal diseases in the context of infectious agents, genetic susceptibilities, physiological aberrations and the changing landscape of the bacterial gut microbiota. I also discuss the potential translation and efficacies of pharmacological interventions targeting the inflammasome pathways in gastrointestinal diseases.

Inflammasome biology and activation

An inflammasome complex is generally categorized into a canonical or non-canonical inflammasome. The inflammasome sensors NLRP1, NLRP3, NLRC4, NAIPs, AIM2 and pyrin are capable of assembling a canonical inflammasome complex¹⁵ (FIG. 1). The canonical inflammasome is a protein complex comprising one or more inflammasome sensors, ASC and caspase 1. The non-canonical inflammasome refers to an activation pathway of the NLRP3 inflammasome that specifically requires mouse caspase 11, human caspase 4 or human caspase 5 (REF.¹⁶). The current conceptual view is that inflammasome sensors either directly bind and interact with a ligand, such as flagellin, DNA or lipopolysaccharide (LPS), or indirectly sense a physiological aberration, such as ion fluxes and mitochondrial dysfunction¹⁷. The former case is exemplified by AIM2, caspase 4, caspase 5, caspase 11 and NAIPs. Direct binding between double-stranded DNA (dsDNA) and AIM2, independently of the DNA sequence, via electrostatic interaction initiates activation of the AIM2 inflammasome^{18–21}. Similarly, direct binding between cytosolic LPS and caspase 4, caspase 5 or caspase 11 initiates activation of the non-canonical NLRP3 inflammasome pathway²². Activation of the NLRC4

inflammasome requires initial recognition of the ligand by NAIP^{23–27}. The single NAIP encoded in the human genome is capable of recognizing the needle and inner rod components of the type 3 secretion system (T3SS) and flagellin subunits of bacteria^{28–30}. Of the mouse NAIPs, NAIP5 and NAIP6 directly bind flagellin subunits, and NAIP1 and NAIP2 bind the needle and inner rod components of the T3SS, respectively^{28,31–34}. Ligand-bound NAIPs then recruit and induce oligomerization of NLRC4 in order to catalyse the assembly of a functional NAIP–NLRC4 inflammasome complex^{35,36} (FIG. 1).

The inflammasome sensors NLRP1, NLRP3 and pyrin do not bind directly to a ligand but respond to a subset of cellular events triggered by PAMPs and DAMPs. Human NLRP1, rat NLRP1 and mouse NLRP1b are activated by any protease capable of mediating site-specific proteolytic cleavage at the amino terminus or function to find domain (FIIND) of NLRP1 (REFS^{37–40}), such as the lethal factor component of the anthrax lethal toxin⁴¹. NLRP3 indirectly responds to a multitude of PAMPs and DAMPs, including pathogens, toxins, crystalline substances and host and microbial metabolites¹⁴. These NLRP3 activators trigger physiological aberrations in the cell, signified by potassium efflux^{42–44}, lysosomal rupture⁴⁵, mitochondria disruption and release of either mitochondrial reactive oxygen species (ROS) or oxidized mitochondrial DNA^{46–49} or calcium influx and reduction in cAMP levels⁵⁰. These signals converge on a pathway that drives activation of the NLRP3 inflammasome, which is mediated by serine/threonine-protein kinase NEK7 (FIG. 1).

The most recently described inflammasome sensor, pyrin, responds to inactivation of host small GTPases of the RHO family⁵¹ (FIG. 1). RHO-inactivating events occur as a result of infection by bacteria capable of secreting RHO-inactivating toxins, such as infection by the diarrhoeal pathogen *Clostridium difficile*^{51,52}, or as a result of host genetic mutations, such as mutations S208A and S242R in *MEFV* (which encodes pyrin), which impair either actin dynamics or phosphorylation of pyrin^{53,54}.

On activation, inflammasome components assemble a single speck of 1 µm in diameter^{55–59}. Cryoelectron microscopy analysis revealed that ASC and caspase 1 act in a prionoid-like fashion to form filamentous structures and catalyse formation of the inflammasome speck^{59–62}. Although the use of cryoelectron microscopy analysis of overexpressed inflammasome proteins can provide potentially ground-breaking insights into inflammasome structures and assembly, these techniques can lead to artefactual observations and would require further experimental support.

Caspase 1 contains a CARD. Inflammasome sensors that do not carry their own CARD must first recruit ASC — a bipartite protein composed of a pyrin domain and a CARD, the former can interact with the pyrin domain of CARD-less inflammasome sensors, and the latter can subsequently bind the CARD of caspase 1 (REF.⁶³). Indeed, inflammasome sensors that have a CARD, such as NLRC4 and NLRP1, can activate inflammasome responses or induce pyroptosis without

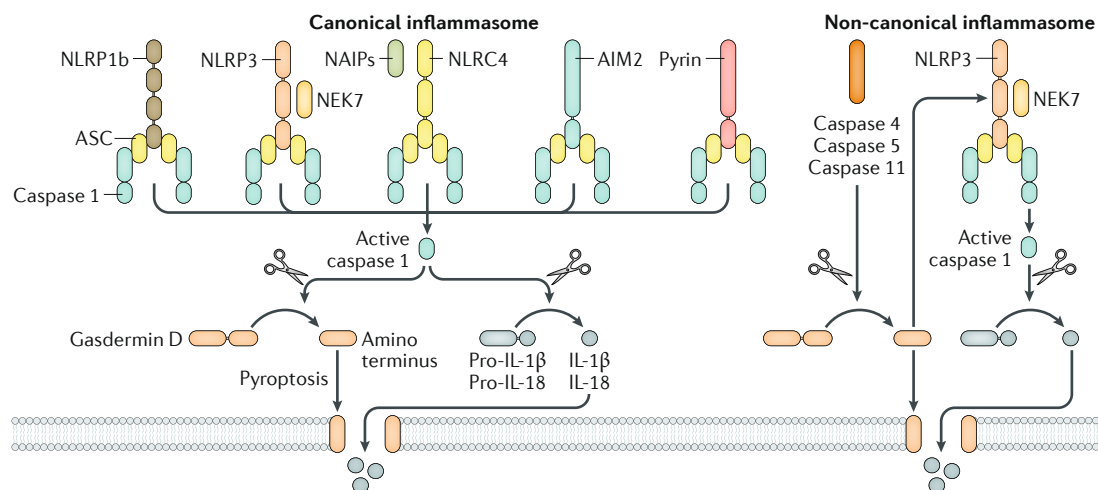


Fig. 1 | Inflammasome complexes. The inflammasome sensors NLRP1b, NLRP3, NLRC4, AIM2 and pypin are all capable of forming a canonical inflammasome complex containing the adaptor protein ASC and the cysteine protease caspase 1. NLRP1b and NLRC4 also recruit caspase 1 without ASC owing to the presence of a CARD domain in their structure^{64–66}. Activation of NLRP3 and NLRC4 requires the kinase NEK7 and the NLR family members neuronal apoptosis inhibitory proteins (NAIPs), respectively. Caspase 1 cleaves the precursor cytokines pro-IL-1 β and pro-IL-18 and the pore-forming protein gasdermin D. The active fragment of gasdermin D oligomerizes and forms pores on the cell membrane, resulting in pyroptosis^{73–80}. These pores also allow passive release of biologically active IL-1 β and IL-18 from the cell. The non-canonical inflammasome is defined by a requirement for human caspase 4, human caspase 5 or mouse caspase 11 for the activation of the NLRP3 inflammasome complex¹⁶. Activation of these caspases leads to cleavage of gasdermin D and pyroptosis^{73–75}. The pore-forming fragment of gasdermin D activates the NLRP3 inflammasome and caspase 1-dependent maturation of IL-1 β and IL-18 (REFS^{73,74}).

ASC, whereas inflammasome sensors that have a pypin domain but not a CARD, such as NLRP3 and AIM2, require ASC for inflammasome activation^{23,51,56,64–66}.

Although it has been known for decades that caspase 1 induces proteolytic cleavage of the substrates pro-IL-1 β and pro-IL-18 (REFS^{67–70}), how caspase 1 mediates pyroptosis was unknown^{71,72}. Studies have now identified the pore-forming protein gasdermin D as a substrate of inflammatory caspases^{73–75}. Caspase 1 and caspase 11 cleave gasdermin D between its Asp276 and Gly277 residues^{73,74}, yielding an active 30–31 kDa amino-terminal fragment that oligomerizes and forms pores on the host cell membrane^{76–80} (FIG. 1). The pores are 10–21 nm in diameter, and biologically active and pleiotropic forms of the cytokines IL-1 β and IL-18 and certain DAMPs are released through them, even before lytic demise of the cell^{8–10,76–80} (FIG. 1). These cytokines and DAMPs collectively regulate intestinal inflammation, host defence, gut barrier functions and host–microbiota homeostasis^{81,82}. Thus, secretion of cytokines and execution of cell death via the inflammasome are critical to the functional activity of the gastrointestinal tract.

Infectious diseases

Microbial pathogens from all domains of life are capable of colonizing and infecting the mammalian gut. These pathogens continue to pose serious concerns to public health and cause substantial morbidity and mortality worldwide⁸³. The inflammasome operates in intestinal cells and immune cells as a cytosolic sentinel that serves to protect the host from invading microorganisms (FIG. 2).

Bacteria

Bacteria carry an array of PAMPs, some of which are delivered into the host cytoplasm, where they activate the inflammasome (FIG. 3). A thematic overview of the mechanisms of inflammasome activities in the recognition of bacteria and host defence, with a focus on gastrointestinal pathogens, is provided herein.

NAIP–NLRC4 and caspase 11 — direct sensors of pathogen-associated molecular patterns.

Flagellin, T3SSs and LPS of bacteria are some of the most potent activators of the innate immune system⁵. Flagellated bacteria that are of clinical importance in the gut and are recognized by the NAIP–NLRC4 inflammasome include *Salmonella enterica* subsp. *enterica* serovar Typhimurium, *Escherichia coli*, *Shigella flexneri* and *Listeria monocytogenes* (FIG. 4a). Flagellin subunits of bacteria delivered into the cytoplasm by T3SSs are recognized directly by NAIP sensors, triggering activation of the NLRC4 inflammasome^{24–27}. Indeed, studies have shown that mice lacking NAIPs or NLRC4 are highly susceptible to infection by *S. Typhimurium*^{55,84–87}, *S. flexneri*^{26,88,89}, *L. monocytogenes*⁹⁰, the mouse-specific enteropathogen *Citrobacter rodentium*^{91,92} and other extragastric pathogens that carry flagellin and/or the T3SS⁶³, highlighting the physiological importance of the NAIP–NLRC4 inflammasome in the host defence against bacterial infection. The release of IL-1 β as a result of NAIP–NLRC4 inflammasome activation drives IL-1 receptor (IL-1R) signalling to promote neutrophil recruitment to the intestine and prevent colonization of gastrointestinal bacteria such as *S. Typhimurium*^{85,93,94}.

NAIPs and NLRC4 are expressed in both immune cells and enterocytes (FIG. 2). Infection of intestinal macrophages with *S. Typhimurium*, but not with the commensal bacteria *Bacteroides fragilis*, *Enterococcus faecalis* or *Lactobacillus plantarum*, triggers activation of the NAIP–NLRC4 inflammasome⁸⁵, suggesting that innate immune detection of flagellin and the T3SS enables the host immune cell to, in part, discriminate between pathogenic and commensal bacteria (FIG. 4b). In addition, intestinal macrophages, unlike bone marrow-derived macrophages, are relatively unresponsive to activators of the NLRP3 inflammasome owing to rapid proteasomal degradation of NLRP3 and pro-IL-1 β ⁹⁵, potentially representing a mechanism to restrain activation of an inflammasome sensor in a cell type that normally exposes and responds to a plethora of signals.

In addition to the NAIP–NLRC4 inflammasome, LPS from Gram-negative bacteria including *S. Typhimurium*, *E. coli* and *C. rodentium* have been found to be recognized by caspase 4, caspase 5 and caspase 11 on the basis of cell culture and mouse studies¹⁶. LPS from these bacteria is liberated in the cytoplasm through a host-mediated process requiring interferon-inducible GTPases⁹⁶. These interferon-inducible effector proteins rupture the membrane of the pathogen-containing vacuole and/or the bacterial cell membrane⁹⁶. Cytosolic Gram-negative

bacteria that naturally escape the pathogen-containing vacuole, such as *S. flexneri* and *Burkholderia thailandensis*, transport their LPS into the cytoplasm of macrophages, where the hexa-acylated lipid A portion of the LPS binds and activates caspase 4, caspase 5 and caspase 11 (REFS^{22,97,98}). Indeed, the naturally cytosolic bacteria *Burkholderia pseudomallei*, *B. thailandensis* and a genetically engineered strain of *S. Typhimurium* that are incapable of remaining in the pathogen-containing vacuole of macrophages and thus enter the host cytoplasm aberrantly, are rapidly cleared from the host in a caspase 11-dependent manner^{99,100}. Furthermore, outer membrane vesicles carrying LPS are shed by *E. coli* and most Gram-negative bacteria¹⁰¹. These outer membrane vesicles are phagocytosed by macrophages, leading to LPS-induced activation of caspase 11 and the non-canonical NLRP3 inflammasome even in the absence of bacterial invasion of the host cell¹⁰².

Emerging evidence indicates that pyroptosis executed by NAIP–NLRC4 or caspase 11 is an effective host-protective mechanism that either drives bacteria out of infected host cells or removes an infected cell in its entirety from the host⁶³. In the former scenario, the expelled bacteria released from an infected macrophage could be free or trapped within a cellular corpse composed of pyroptotic debris, both of which are cleared by neutrophils^{84,103}. In the latter scenario, enterocytes in the mouse intestine infected with bacteria, such as *S. Typhimurium*, can be physically extruded in their entirety from the gut epithelium into the lumen for removal^{93,104} (FIG. 4c). Rearrangement of the cytoskeleton in enterocytes is required for epithelial cell extrusion, and caspase 8 has been implicated in this extrusion process during *S. Typhimurium* infection owing to the ability of NLRC4 to engage caspase 8-dependent cell death^{58,93,94,104}.

Activation of the NAIP–NLRC4 inflammasome can also prevent further bacterial uptake and enhance intracellular bactericidal activity in macrophages¹⁰⁵ (FIG. 4d). Unlike macrophages and enterocytes, neutrophils are resistant to pyroptosis despite expressing all essential components of the inflammasome, including gasdermin D^{10,106} (FIG. 2). This cell-intrinsic resistance to pyroptosis enables neutrophils to phagocytose extracellular bacteria or bacteria entrapped in pyroptotic debris, secrete an abundant amount of IL-1 β to amplify local inflammation and generate ROS to facilitate intracellular pathogen killing^{84,106} (FIG. 4d).

NLRP3 and pyrin — sensors of physiological aberrations. Bacteria that either evade or fail to activate the NAIP–NLRC4 inflammasome or caspase 11 can be recognized by other inflammasome complexes^{22,98} (FIG. 4). Of clinical importance in this bacterial category are the Gram-negative bacterium *Helicobacter pylori* and several toxin-producing foodborne bacteria, such as *Staphylococcus aureus*. *H. pylori*, the most common bacterial cause of gastric cancer¹⁰⁷, encodes flagella and flagellin A (FlaA) subunits whose structures evade detection by the NAIP–NLRC4 inflammasome¹⁰⁸. Instead, *H. pylori* infection induces ROS production, potassium efflux and lysosomal destabilization in the

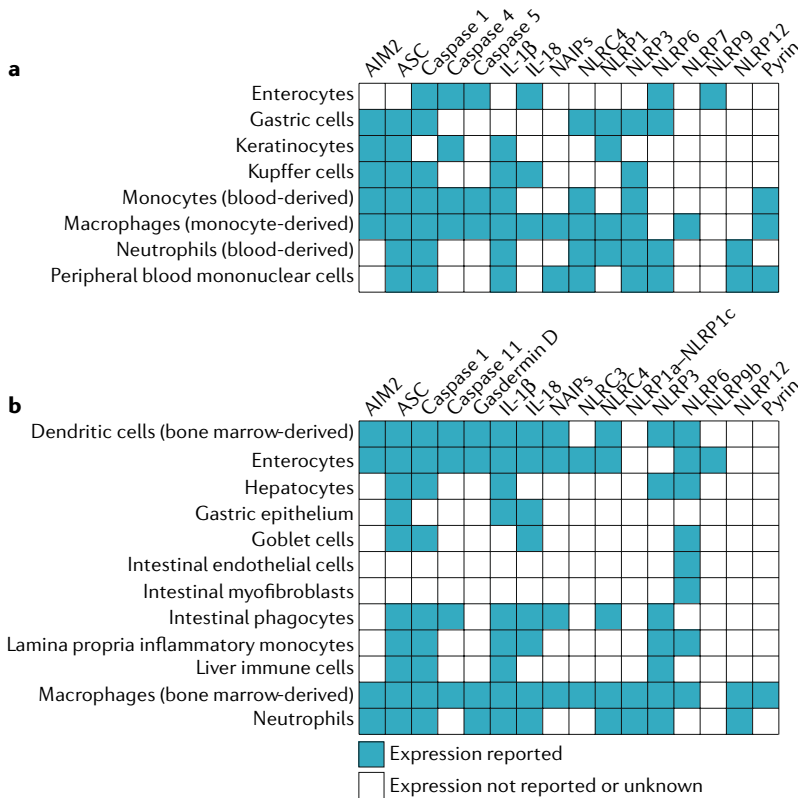


Fig. 2 | Expression of inflammasome sensors and related molecules by cell type. **a** | Expression in humans. **b** | Expression in mice. References for expression data are given in Supplementary table 1. AIM2, absent in melanoma 2; ASC, apoptosis-associated speck-like protein containing a caspase activation and recruitment domain (CARD); NAIP, neuronal apoptosis inhibitory protein; NLRC, nucleotide-binding domain, leucine-rich repeat-containing protein (NLR) family CARD domain-containing protein; NLRP, NACHT, LRR and PYD domains-containing protein.

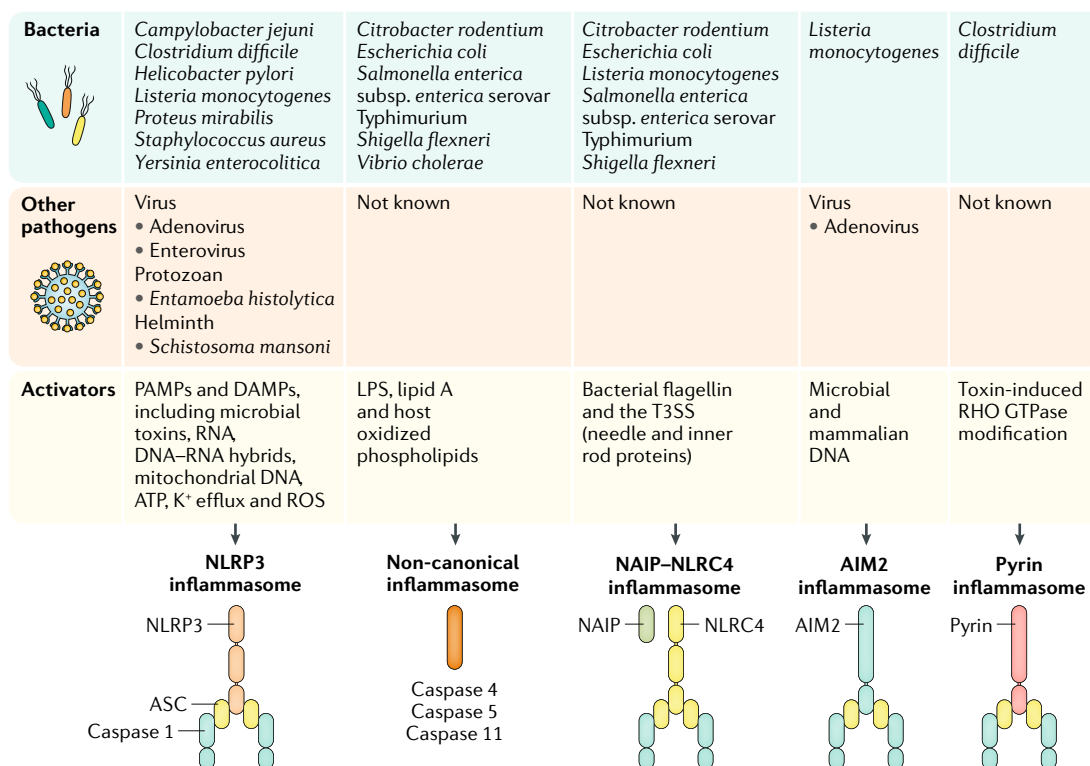


Fig. 3 | Inflammasomes recognize gastrointestinal bacteria, viruses, protozoa and helminths. Pathogens carry a plethora of pathogen-associated molecular patterns (PAMPs). These PAMPs either directly bind and activate an inflammasome sensor or induce a physiological change in the cell that is sensed by an inflammasome sensor⁵. Pathogens also cause substantial damage to the host cell, a process that induces the liberation of danger-associated molecular patterns (DAMPs) that activate the inflammasome¹⁵. AIM2, absent in melanoma 2; ASC, apoptosis-associated speck-like protein containing a caspase activation and recruitment domain (CARD); LPS, lipopolysaccharide; NAIP, neuronal apoptosis inhibitory protein; NLRC4, nucleotide-binding domain, leucine-rich repeat-containing protein (NLR) family CARD domain-containing protein 4; NLRP3, NACHT, LRR and PYD domains-containing protein 3; ROS, reactive oxygen species; T3SS, type 3 secretion system.

host cell, ultimately leading to activation of the canonical NLRP3 inflammasome^{109–113}. Further studies have shown that the *H. pylori* virulence factors cag pathogenicity island and urease B subunit potentiate activation of the NLRP3 inflammasome and secretion of IL-1 β ^{109,112,113}. Excessive production of IL-1 β could be a vital link between *H. pylori* infection and tumorigenesis in the stomach. Indeed, a study found that a transgenic mouse strain engineered to overexpress human IL-1 β in the stomach is prone to the development of gastric cancer, either spontaneously or after colonization with *Helicobacter felis*, a model organism for *H. pylori*¹¹⁴. The specific inflammasome involved in the development of gastric cancer is not known; however, an association between polymorphisms in the genes encoding NLRP3 and caspase 1 and patients with gastric cancer has been reported¹¹⁵. Furthermore, *H. pylori* infection suppresses the expression of the microRNA molecule miR-22 in gastric epithelial cells, which leads to elevated expression of NLRP3 and cellular proliferation¹¹⁶. These studies suggest that activation of the inflammasome by *Helicobacter spp.* infection contributes to the molecular basis of gastritis and gastric cancer.

Toxins secreted by bacteria can induce a state of intracellular physiological aberration that is sensed by

inflammasome sensors. The Gram-positive bacterium *C. difficile* is a causative agent of diarrhoea associated with the use of antibiotics, toxic megacolon and colonic perforation¹¹⁷. The toxins toxin A (TcdA) and toxin B (TcdB) secreted by *C. difficile* can inactivate host transforming protein RHOA at the GTPase switch I region; this RHO-inactivating activity is sensed by pyrin, resulting in the assembly of the pyrin inflammasome^{51,52}. The physiological function of pyrin in the gastrointestinal tract remains unexplored, and further studies are required to clarify its role. However, wild-type mice treated with the IL-1R antagonist anakinra are protected from *C. difficile*-toxin-induced intestinal injury¹¹⁸. This finding indicates that activation of the pyrin inflammasome in response to *C. difficile* infection might lead to excessive IL-1-mediated inflammation and pathology that is detrimental to the host. Pharmacological inhibitors of microtubules are efficacious in blocking the pyrin inflammasome in cell culture systems^{54,119,120}, but their clinical applications against pyrin-mediated pathology have not been tested in animal models.

The haemolysin of the Enterobacteriaceae member *Proteus mirabilis* can activate NLRP3 and drive inflammation in the mouse intestine¹²¹. Similarly, α -haemolysins, β -haemolysins and γ -haemolysins of

the Gram-positive foodborne bacterium *S. aureus* mediate activation of the NLRP3 inflammasome in mouse macrophages and mice^{122,123}, and the pore-forming cytolysin listeriolysin O of the Gram-positive foodborne bacterium *L. monocytogenes* potentiates activation of the NLRP3, NAIP–NLRC4 and AIM2 inflammasomes^{90,124–128}. Infection with either *L. monocytogenes* or *S. aureus* activates caspase 1 in human macrophages via NLRP3 and NLRP7 (REF.¹²⁹). NLRP7 (encoded in

the human, but not the mouse, genome) is a sensor of bacterial lipopeptides; its activation in macrophages impairs intracellular replication of *L. monocytogenes* or *S. aureus*¹²⁹. Further studies have shown that, in macrophages or embryonic fibroblasts, inflammasome components accumulate on the phagosome and promote phagosome–lysosome fusion and phagosomal acidification^{27,105,130–132}. The functional diversity of the inflammasome in both immune and non-immune cells

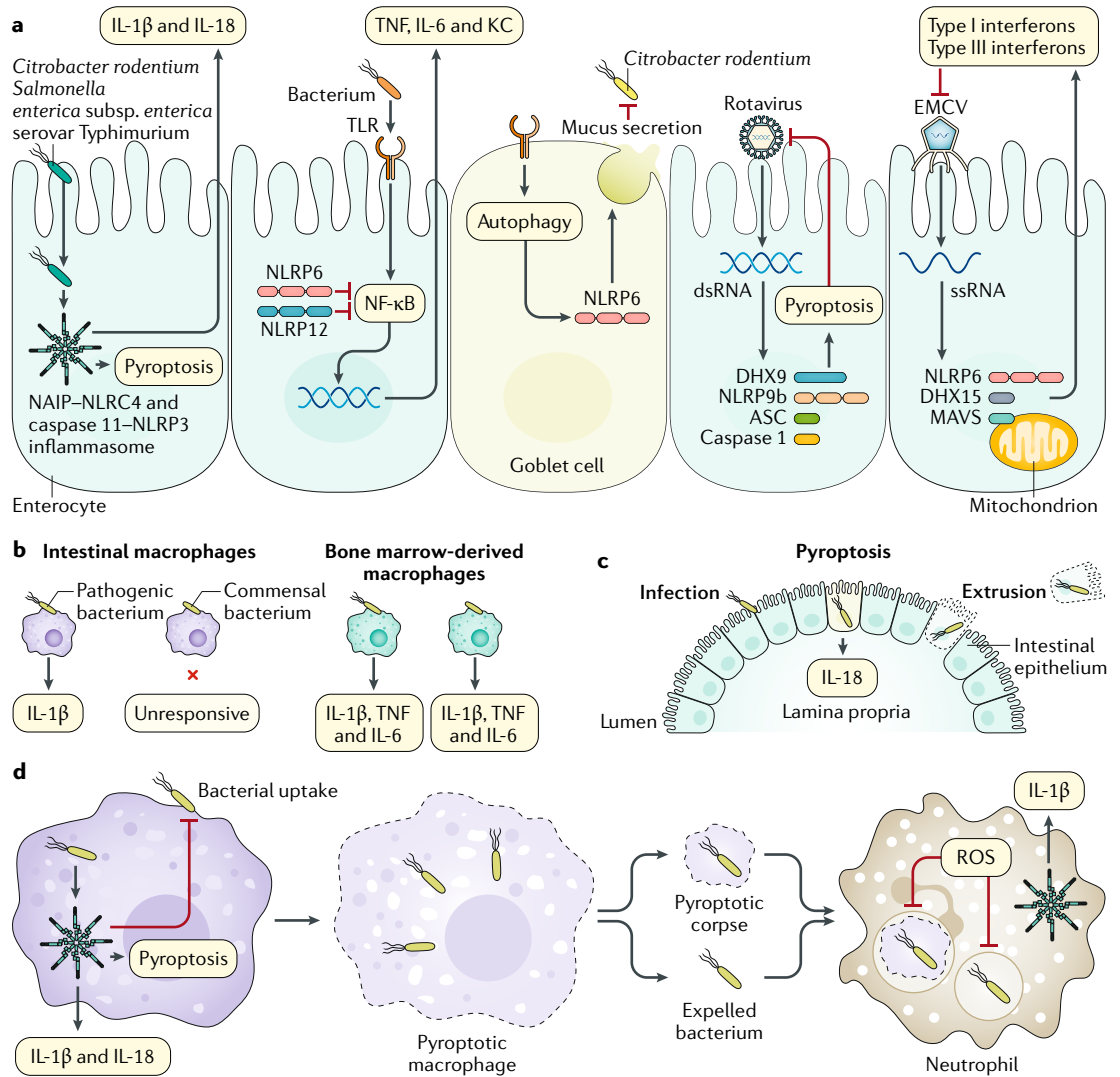


Fig. 4 | Inflammasomes and related molecules contribute to the killing and clearance of gastrointestinal pathogens in intestinal cells and immune cells. a | Inflammasomes mediate host protection against Gram-negative bacteria by inducing the secretion of IL-1 β and IL-18 and pyroptosis^{55,59,84,85,91,92,254,255}. NLRP6 and NLRP12 negatively regulate inflammation^{133,134,256,257}. NLRP6 responds to Toll-like receptor (TLR)-induced autophagy in goblet cells and mediates secretion of mucus¹³⁷. RNA-bound DEAH box protein 9 (DHX9) interacts with NLRP9b, inducing the assembly of an inflammasome complex¹⁶¹. The NLRP6–DHX15 complex binds to viral RNA and induces the production of type I and type III interferons¹⁶⁵. **b** | Intestinal macrophages can discriminate pathogens from commensals⁸⁵. **c** | Activation of caspase 1, caspase 8 or caspase 11 leads to cell death, which removes and extrudes the infected enterocyte from the epithelium^{93,94,104}. **d** | The inflammasome can reduce bacterial load by inhibiting bacterial uptake, which limits macrophage movement and stiffness, and can promote the production of reactive oxygen species (ROS)¹⁰⁵. Pyroptotic macrophages liberate either whole bacteria or bacteria entrapped within pore-induced intracellular traps¹⁰³. These entities are phagocytosed by neutrophils^{84,106}. ASC, apoptosis-associated speck-like protein containing a caspase activation and recruitment domain (CARD); dsRNA, double-stranded RNA; EMCV, encephalomyocarditis virus; KC, keratinocyte chemoattractant (also known as CXCL1); MAVS, mitochondrial antiviral-signalling protein; NAIP, neuronal apoptosis inhibitory protein; NF- κ B, nuclear factor- κ B; NLRC4, nucleotide-binding domain, leucine-rich repeat-containing protein (NLR) family CARD domain-containing protein 4; ssRNA, single-stranded RNA.

ensures host protection against a range of bacteria with preferential tropisms and virulence strategies.

NLRP6 and NLRP12 — enigmatic sensors. In line with the functional versatility of other inflammasome sensors, NLRP6 and NLRP12 have multiple functions during bacterial infection. The functions of these sensors are enigmatic owing to the lack of expert consensus on their biological functions. Both NLRP6 and NLRP12 can negatively regulate activation of the nuclear factor- κ B (NF- κ B) and mitogen-activated protein kinase (MAPK) pathways in macrophages infected with *L. monocytogenes*, *S. Typhimurium*, *E. coli* or other non-gastrointestinal bacteria (FIG. 4a), resulting in a state of reduced inflammation that can be readily exploited by bacterial pathogens during infection of a cell or a mouse^{133–136}. Others have proposed that NLRP6 and NLRP12 mediate activation of caspase 1 in response to infection by *C. rodentium* and *Yersinia pestis*, respectively^{137,138}, and that NLRP12 functions in dampening the cell-intrinsic migratory capacity of neutrophils¹³⁹. A further study suggests that the C57Bl/6J mice that are used widely in research harbour a missense mutation in the *Nlrp12* gene that impairs the ability of NLRP12-expressing macrophages to draw neutrophils to the site of inflammation in response to bacterial infection¹⁴⁰. These conflicting studies highlight that the biological functions of NLRP6 and NLRP12 should be revisited and should take into account the genetic background of the mouse strains, cell types, bacterial agents and the experimental approaches used.

Detrimental roles of the inflammasome. Overt activation of the inflammasome is detrimental, especially during systemic bacterial dissemination, which might arise from intestinal perforation. Mice lacking caspase 11 or the pyroptosis-inducing effector gasdermin D are remarkably resistant to LPS-induced or *E. coli*-induced endotoxaemia compared with wild-type mice^{16,73,97,98,141–144}, suggesting that caspase 11-mediated pyroptosis drives lethality in endotoxaemia. Deletion of the *Nlrp3* gene in mice offers protection against polymicrobial endotoxaemia following caecal ligation and puncture^{145,146}. In this model of systemic inflammation, the lack of inflammasome activity impairs the production of pro-inflammatory and endogenous lipid mediators but increases the levels of pro-resolving lipid mediators¹⁴⁵. Moreover, LPS-induced endotoxaemia promotes secretion of bile acids from hepatocytes in mice; bile acids are a form of DAMP that either synergize with ATP to activate the NLRP3 inflammasome¹⁴⁷ or signal through the membrane receptor G protein-coupled bile acid receptor 1 (GBAR1) to inhibit the NLRP3 inflammasome¹⁴⁸.

In other models of systemic inflammation, activation of the NAIP–NLRC4 inflammasome by systemic delivery of cytosolic flagellin to mice results in the production of inflammatory lipid mediators called eicosanoids¹⁴⁹. Synthesis of eicosanoids initiates fluid loss into the mouse intestine and peritoneal cavity and ultimately causes rapid host death within 30 min¹⁴⁹. The presence of a multidrug-resistant *E. coli* pathobiont in the gut can also trigger activation of the NAIP–NLRC4 inflammasome

and increase susceptibility to sepsis¹⁵⁰, whereas certain strains of *E. coli*, such as strain O21:H⁺, engage activation of the NAIP–NLRC4 inflammasome to inhibit the body wasting induced by *S. Typhimurium* infection¹⁵¹. Thus, the inflammasome influences the magnitude of inflammation towards bacterial infection and, in part, determines either a protective or detrimental outcome in the host.

Viruses

Norwalk and Norwalk-like viruses, rotavirus, astroviruses, adenoviruses, caliciviruses and coronaviruses are among the leading causes of acute gastroenteritis worldwide¹⁵². Evidence has emerged that inflammasomes respond to some of these gastrointestinal viruses. Earlier studies had demonstrated that the DNA virus adenovirus induces the production of IL-1 β in circulation and in hepatocytes and Kupffer cells of mice¹⁵³. Further functional studies have shown that adenovirus, specifically its virion component, triggers activation of the NLRP3 inflammasome and secretion of IL-1 β in human THP-1 macrophages and primary mouse macrophages¹⁵⁴. Adenovirus-induced secretion of IL-1 β in human monocyte-derived macrophages and peripheral blood mononuclear cells partially requires Toll-like receptor 9 (TLR9)^{155,156}, presumably owing to TLR9-mediated sensing of viral DNA as the virus penetrates the cell membrane and enters the endosome. Injection of an adenovirus vector into mice triggers NLRP3-dependent production of IL-1 β and other pro-inflammatory cytokines, highlighting a physiological role for the NLRP3 inflammasome in licensing an innate immune response to adenovirus in vivo¹⁵⁴. This finding is also consistent with the observation that IL-1R, which is activated downstream of inflammasomes, incites an acute and deleterious inflammatory signalling cascade in mice infected with adenovirus¹⁵³. The magnitude of the inflammatory response is reduced in mice lacking IL-1R or in wild-type mice treated with an anti-IL-1 antibody¹⁵³, suggesting that blockade of IL-1 signalling prevents overt inflammation that might otherwise be damaging to the host.

Similar to adenovirus, enterovirus EV71, a sporadic cause of diarrhoea and a common cause of hand, foot and mouth disease in humans¹⁵⁷, is sensed by the NLRP3 inflammasome^{158,159}. Inflammasome-mediated production of IL-18 is crucial for the protection against enterovirus EV71 in mice^{158,159}. Administration of IL-18 to infected mice reduces viral loads and the production of pro-inflammatory cytokines¹⁵⁹. The precise viral components of enterovirus EV71 that activate NLRP3 are unknown; it is possible that the single-stranded RNA (ssRNA) viral genome and/or the structural proteins of the capsid might trigger activation of the NLRP3 inflammasome.

The double-stranded RNA (dsRNA) virus rotavirus is one of the most common causes of gastroenteritis in children¹⁶⁰. The role of inflammasomes in the host defence against this virus was revealed when the poorly characterized NLR member NLRP9b was found to mediate sensing of rotavirus and drive the production of IL-18 and pyroptosis in organoids generated from the small intestine of mice¹⁶¹. Furthermore, mice lacking

NLRP9b are substantially more susceptible to rotavirus infection, harbour a higher viral load and develop more severe intestinal pathology than wild-type mice¹⁶¹. Mouse NLRP9b and its human counterpart, NLRP9, are strongly and specifically expressed in ileal epithelial cells and primary intestinal epithelial cells, respectively (FIG. 2). Both mouse NLRP9b and human NLRP9 cooperate with the RNA sensor DEAH box protein 9 (DHX9) to sense dsRNA¹⁶¹ (FIG. 4a), implicating the existence of an RNA-sensing NLRP9 inflammasome complex in the host defence against rotavirus infection. A protective role of inflammasomes was also observed in a previous study showing that administration of bacterial flagellin to mice — causing activation of NLRP4 and the membrane-bound flagellin sensor TLR5, which induced the production of IL-18 and IL-22, respectively — mediates clearance of rotavirus within 24 h (REF.¹⁶²). Recapitulating the beneficial effects of flagellin, coadministration of IL-18 and IL-22 similarly drives anti-rotavirus immunity in infected mice, supporting the view that inflammasome signalling can mediate protection against rotavirus infection.

Similar to NLRP9, NLRP6 is strongly expressed in the intestinal tract of humans and mice^{163,164} (FIG. 2). In concert with the RNA sensor DHX15, NLRP6 interacts with viral RNA and mediates the recognition of the ssRNA viruses encephalomyocarditis virus and murine norovirus¹⁶⁵ (FIG. 4a). In this case, NLRP6 does not assemble an inflammasome. Instead, the RNA-bound NLRP6–DHX15 complex migrates to the mitochondria, where it interacts with the signalling adaptor mitochondrial antiviral-signalling protein (MAVS) to induce the production of type I and type III interferons and viral clearance¹⁶⁵ (FIG. 4a). These studies underscore emerging roles of the inflammasome and associated signalling molecules in antiviral defence of the gastrointestinal tract. The relative contribution of IL-1 β and IL-18 secretion versus pyroptosis in driving immunity to viruses remains largely unknown. The current availability of mice lacking gasdermin D will enable exploration of this emerging research area.

Protozoa

Protozoal pathogens cause several neglected tropical diseases and are public health risks largely affecting the developing world¹⁶⁶. Inflammasomes are known to recognize major protozoal pathogens, including *Leishmania* spp., *Plasmodium* spp. and *Toxoplasma* spp.¹⁶⁷. However, information concerning the biological importance of inflammasomes in the context of gastrointestinal protozoa is limited. The foodborne protozoan *Entamoeba histolytica* causes amoebiasis, which is characterized by dysentery, amoebic colitis, amoeboma (the formation of fibrotic tissue masses in the intestinal wall) and invasive disease of the brain, lung and liver¹⁶⁸. There is evidence to support the idea that *E. histolytica* infection activates caspase 1 and induces secretion of IL-1 β and IL-18 in both human and mouse macrophages¹⁶⁹. Pharmacological blockade of potassium efflux inhibits activation of caspase 1, IL-1 β -release and pyroptosis in THP-1 macrophages or intestinal epithelial cells that have been infected with

*E. histolytica*¹⁷⁰. Further studies indicated that the parasite integrin-binding cysteine protease *E. histolytica* cysteine protease gene 5 (CP5) mediates activation of the host $\alpha 5\beta 1$ integrin during parasite–macrophage contact, leading to the release of ATP and subsequent activation of the canonical NLRP3 inflammasome¹⁷¹. The importance of the inflammasome in *E. histolytica* infection is partially inferred by the observation that recombinant IL-1 β and IL-18 are elevated and inactivated by *E. histolytica* CP5 (REFS^{172,173}).

Similar to pathogenic protozoa, the commensal gut protozoan *Trichomonas musculus* in mice also triggers activation of the inflammasome in intestinal epithelial cells in mice¹⁷⁴. In this context, *T. musculus*-induced activation of the inflammasome promotes IL-18 secretion, T helper 1 (T_H1) cell and T_H17 cell immunity and protection against *S. Typhimurium* infection¹⁷⁴, highlighting a favourable outcome of this host–protozoan engagement. However, intestinal colonization by *T. musculus* also drives sustained inflammation that elevates the likelihood of developing T cell-driven colitis and sporadic colorectal tumours in mice¹⁷⁴. The precise inflammasome complex triggered by *T. musculus* is unknown; however, it is likely that protozoa-induced activation of the inflammasome in the gastrointestinal tract is a double-edged sword that contributes to both health and disease.

Intestinal inflammation, IBD and cancer

The ability of inflammasomes to respond to danger signals broadens their clinical relevance to diseases other than infectious diseases^{175,176}. Genetic analyses in humans have pinpointed an association between mutations in genes encoding NLRs and intestinal inflammation, autoinflammatory conditions and cancer^{14,177}. The contribution of individual inflammasome sensors and related molecules in the pathogenesis of intestinal inflammation, IBD and cancer is discussed herein.

NLRP3

NLRP3 is a global sensor of PAMPs and DAMPs and is expressed in multiple cell types (FIG. 2), contributing to its links to a plethora of clinical manifestations. Genetic analysis identified an association between six polymorphisms located in a region 4.7 kb downstream of the *NLRP3* gene and the development of Crohn's disease in some, but not all, European populations^{178,179}. Peripheral blood mononuclear cells derived from patients with Crohn's disease who were homozygous for any one of these six polymorphisms have an impaired ability to express NLRP3 and secrete IL-1 β in response to LPS stimulation in vitro¹⁷⁸. A further study reported that a heterozygous *NLRP3* polymorphism, resulting in a mutation at residue Q705K, is associated with poor survival in patients with advanced colorectal cancer (CRC)¹⁸⁰. Global expression analysis of genes encoding cytosolic innate immune sensors revealed that the expression of genes encoding NLRP3 and other inflammasome sensors is substantially reduced in tumour tissues compared with non-tumour-associated tissues in patients with CRC¹⁸¹. Similar observations have been made in mice during colitis-associated CRC¹⁸².

However, how reduced expression of these genes is initially triggered or executed mechanistically is unknown.

Several groups have found that mice lacking NLRP3, ASC or caspase 1 and administered a combination of the DNA-damaging agent azoxymethane (AOM) and the chemical colitogen dextran sodium sulfate (DSS) are highly sensitive to the development of colitis and colitis-associated CRC^{183–188}. DSS-induced activation of the inflammasome in mice also causes a loss of enteric neurons and onset of abnormal gut motility¹⁸⁹. The activity of the NLRP3 inflammasome during DSS-induced colitis is controlled by the microRNA molecule miR-223, which binds the miR-223-binding site within the 3' untranslated region of *NLRP3* (REF.¹⁹⁰). However, the state of hyporesponsiveness of colonic macrophages to NLRP3 activators is lost during DSS-induced colitis⁹⁵, which might fuel inflammation. Indeed, studies in mice have also reported a deleterious or non-existent role for NLRP3 or caspase 1 and caspase 11 in colitis and colitis-associated CRC^{191–194}. The biological basis of these discrepancies is unclear; however, differences in gut microbiota, diet and/or housing conditions might affect the activity of the inflammasome (the differences in the microbiota are specifically discussed later). Indeed, a ketogenic diet or caloric restriction that increases the production of the ketone body β -hydroxybutyrate from the liver contributes to the inhibition of the NLRP3 inflammasome¹⁹⁵. Similarly, omega-3 fatty acids signal through the metabolite-sensing receptors G protein-coupled receptor 40 (GPR40; also known as FFAR1) and GPR120 (also known as FFAR4) to inhibit activation of the NLRP3 inflammasome and prevent diet-induced insulin resistance¹⁹⁶. By contrast, short-chain fatty acids from dietary fibres bind to the metabolite-sensing receptor GPR43 (also known as FFAR2) on enterocytes, stimulating potassium efflux that drives activation of the NLRP3 inflammasome and protection against colitis^{197,198}. A diet rich in cholesterol or saturated fatty acids also promotes activation of the NLRP3 inflammasome in mice^{199–203}, whereby a cholesterol-rich diet increases inflammation and tumour burden¹⁹⁹, but a high-fat diet reduces glucose tolerance and insulin sensitivity²⁰⁰. These studies provide evidence to indicate that dietary components, in association with the gut microbiota, are critical modulators of inflammasome activity and susceptibility to the development of intestinal inflammation, cancer and metabolic syndromes.

Activation of the NLRP3 inflammasome leads to secretion of both IL-1 β and IL-18. Although injection of recombinant IL-1 β into mice has been shown to mediate protection against DSS-induced colitis²⁰⁴, studies have found variable levels of IL-1 β in the colon tissue of inflammasome-deficient mice in response to DSS^{163,184,185,204–211}. These findings would suggest that the role of IL-1 β is not always coupled with inflammasome-mediated protection of intestinal inflammation and colitis-associated CRC.

The protective role of the NLRP3 inflammasome in colitis and CRC is attributed in part to the secretion of IL-18 (FIG. 5a). Mice lacking IL-18 are highly susceptible to the development of colitis and CRC^{183,212,213}. Injection of recombinant IL-18 reduces intestinal inflammation and

tumour burden in inflammasome-deficient mice that had been administered AOM and DSS; the same treatment also ameliorated colitis in inflammasome-deficient mice exposed to the chemical colitogen oxazolone^{183,187,204,211,214}. IL-18 functionally exerts healing properties^{184,185,187,214} and triggers natural killer cell-mediated killing of colonic tumour cells that have metastasized to the liver²¹⁵. Another study challenged the view that IL-18 is beneficial in intestinal inflammation and reported that the IL-18 and IL-18 receptor signalling pathway drives depletion of mucus-secreting goblet cells, such that a loss of the mucous layer augments sensitivity to DSS-induced colitis in mice²¹⁶. Similarly, a transgenic mouse strain overexpressing IL-18 develops aggressive colitis in response to DSS owing to increased levels of infiltrating macrophages in the colon²¹⁷. One possibility that might reconcile the diametrically opposing roles of IL-18 is the ability of this cytokine to downregulate the soluble IL-22 receptor called IL-22-binding protein (IL-22BP)²¹⁸. IL-22BP modulates the bioavailability of IL-22, a cytokine capable of suppressing early intestinal damage but also promoting tumour development over time²¹⁸ (FIG. 5a).

Inappropriate activation of the NLRP3 inflammasome can result in damage to the small intestine²¹⁴. In mice, enteropathy induced by NSAIDs, such as indomethacin, can be attenuated through the use of neutralizing antibodies to IL-1 β or inhibitors of the NLRP3 inflammasome, including the ATP scavenger apyrase, the P2X purinoceptor 7 (P2X7) antagonist brilliant blue G and the cytoskeletal inhibitor colchicine²¹⁴. These studies support the view that unwarranted activation of the NLRP3 inflammasome is a catalyst for small intestinal damage. The availability of more specific inhibitors of the NLRP3 inflammasome, such as MCC950 and CY-09 (REFS^{219–221}), enables clinical trials to explore new options for the treatment of human diseases¹⁸².

NLRC4

Studies in humans have revealed that gain-of-function mutations in the gene encoding NLRC4 are associated with enterocolitis and autoinflammation^{222–224}. For example, a substitution mutation within the HD1 domain of the NLRC4 protein is linked to recurrent enterocolitis and autoinflammation in several members of the same family²²². A newborn baby from this family and carrying this mutation died 23 days after birth owing to severe gastrointestinal complications, fever and systemic inflammation²²². A further study identified that a heterozygous de novo substitution mutation in the nucleotide-binding domain of NLRC4, potentially causing destabilization and autoactivation of the protein, was linked to macrophage activation syndrome, gastrointestinal pathology, splenomegaly and systemic inflammation²²³. Monocytes or macrophages carrying the aforementioned disease-associated mutations undergo spontaneous activation of the inflammasome and pyroptosis and secrete increased levels of IL-1 β and IL-18 (REFS^{222–224}), suggesting that unchecked inflammasome activation contributes to the molecular basis of these autoinflammatory conditions. Of clinical importance is that the aforementioned patient with the substitution mutation in *NLRC4* responded to treatment with the recombinant

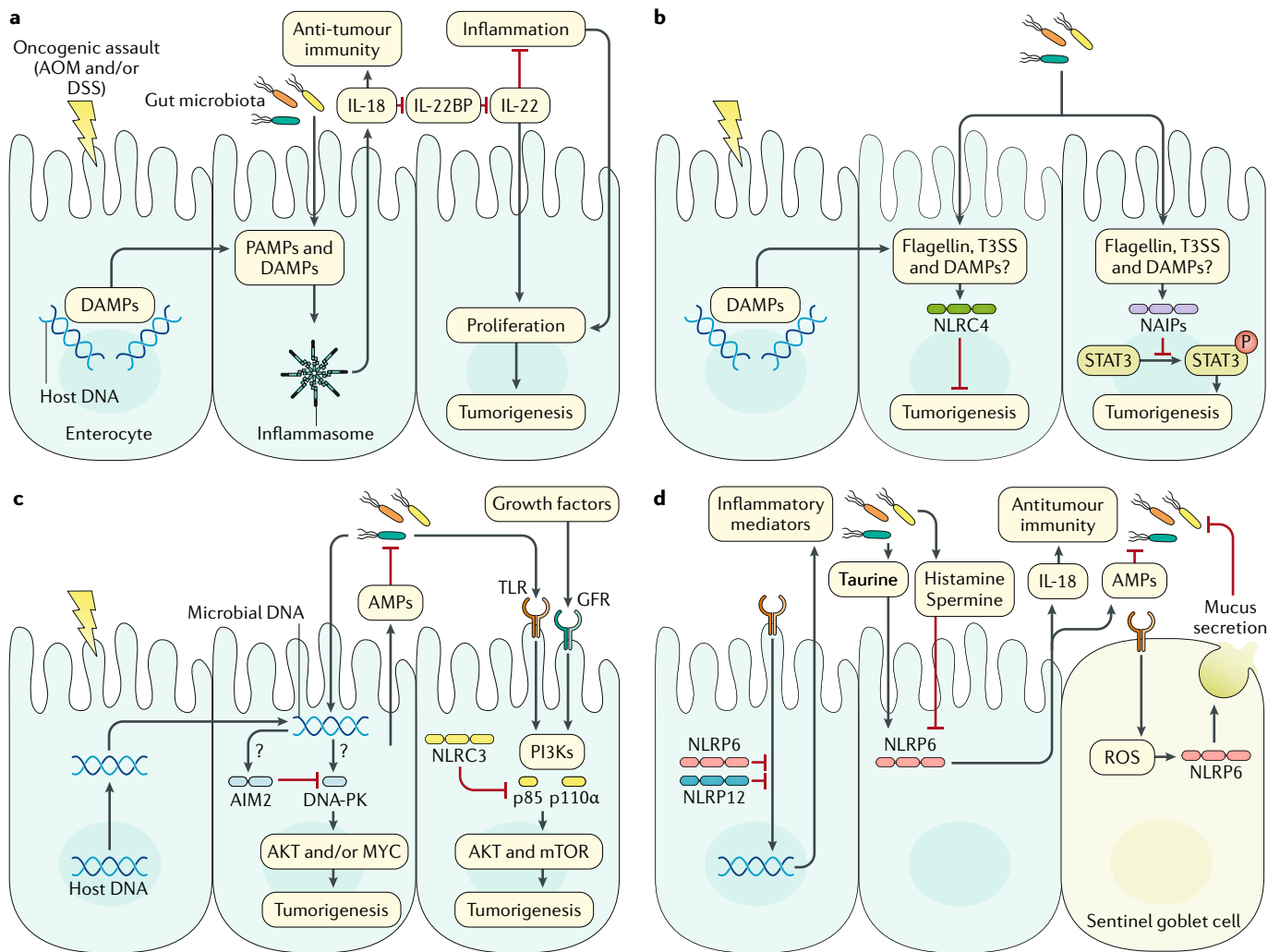


Fig. 5 | Development of intestinal inflammation and cancer is regulated by the inflammasome-microbiota axis. **a** | Oncogenic assaults such as azoxymethane (AOM) and dextran sodium sulfate (DSS) cause damage, leading to the release of danger-associated molecular patterns (DAMPs). Bacteria can invade enterocytes and introduce pathogen-associated molecular patterns (PAMPs) into the host cell. DAMPs and PAMPs are sensed by inflammasomes^{183–185,187,188,204}. IL-18 promotes downregulation of soluble IL-22-binding protein (IL-22BP), which controls the ability of IL-22 to suppress inflammation or induce tumorigenesis in the gut²¹⁸. **b** | Nucleotide-binding domain, leucine-rich repeat-containing protein (NLR) family CARD domain-containing protein 4 (NLRC4) and neuronal apoptosis inhibitory proteins (NAIPs) can block cellular proliferation and tumorigenesis^{192,226}. **c** | DNA-dependent protein kinase (DNA-PK) induces colorectal tumorigenesis via activation of AKT and the transcription factor MYC^{205,206}. This response is inhibited by absent in melanoma 2 (AIM2)^{205,206}. AIM2 also triggers the production of antimicrobial peptides (AMPs) in intestinal epithelial cells to modulate the gut microbiota^{207,208}. A similar negative regulatory role for NLRC3 has been described²²⁹. **d** | NLRP6 and NLRP12 contribute to the pathogenesis of gastrointestinal infection, acute colitis and colorectal cancer^{133,134,163,234–236,244,248,256,257}. Question marks denote unknown mediators. GFR, growth factor receptor; mTOR, mechanistic target of rapamycin; NLRP, NACHT, LRR and PYD domains-containing protein; PI3Ks, phosphoinositide 3-kinases; ROS, reactive oxygen species; STAT3, signal transducer and activator of transcription 3; T3SS, type 3 secretion system; TLR, Toll-like receptor.

IL-1R antagonist anakinra²²³. A newly identified patient with the heterozygous de novo mutation in *NLRC4*, who also experienced enterocolitis and skin rash, responded to a combined blockade therapy targeting IL-1 β and IL-18 (REF.²²⁵). These studies exemplify the success of experimental targeted therapies in the treatment of inflammasome-mediated disorders.

In animal studies, deletion of the genes encoding *NLRC4* or *NAIPs* heightens the susceptibility of mice to the development of colitis and colitis-associated CRC in

response to AOM and DSS^{192,226}. Mechanistically, *NLRC4* inhibits cellular proliferation and promotes apoptosis via an undefined mechanism¹⁹², whereas *NAIPs* inhibit proliferation in enterocytes by blocking phosphorylation and activation of the transcription factor signal transducer and activator of transcription 3 (STAT3) independently of *NLRC4* (REF.²²⁶) (FIG. 5b). A possible scenario is that either spontaneous activation or loss of function associated with the *NAIP* or *NLRC4* signalling axis could upset the equilibrium of the gut ecosystem

and drive intestinal inflammation and tumorigenesis. Immunization of mice with tumour cell lines expressing flagellin — to trigger activation of the NAIP–NLRC4 and TLR5 pathways — induces clearance of tumour cells by innate immune cells and tumour-specific CD4⁺ and CD8⁺ T cells²²⁷. Thus, modulation of the NAIP–NLRC4 and/or the IL-1–IL-18 pathways might provide a promising avenue for cancer immunotherapy.

AIM2 and other signalling molecules

The inflammasome-associated sensor AIM2 also contributes to the pathogenesis of colitis and CRC^{205–208}. The levels of AIM2 expression in tumour tissues often predict survival in patients with CRC²²⁸. Patients whose tumour cells lack AIM2 expression are threefold more likely to die within 5 years of diagnosis than patients whose tumour cells retain some level of expression²²⁸. Reflecting the scenario in humans, the expression of the *Aim2* gene is markedly reduced in inflamed and tumour-associated tissues of mice, and mice lacking AIM2 are hypersusceptible to both colitis-associated and spontaneous colorectal tumorigenesis^{205,206}. AIM2 has functions independent of the inflammasome that drive protection against both colitis-associated and spontaneous CRC, the latter of which arises from a mutation in the mouse homologue of the human *APC* gene or from aberrant β -catenin activation^{205,206}. AIM2 suppresses the ability of the DNA-binding kinase DNA-dependent protein kinase (DNA-PK) to activate RAC serine/threonine-protein kinase (AKT) and transcription factor MYC proto-oncogene protein, ultimately preventing excessive proliferation of stem cells in the colon^{205,206} (FIG. 5c). A similar negative regulatory effect of the orphan receptor NLRC3 on the AKT–mechanistic target of rapamycin (mTOR) pathways activated during colitis-associated CRC or spontaneous CRC has been described²²⁹ (FIG. 5c). Pharmacological inhibitors of the AKT–mTOR pathway are efficacious against the development of colitis-associated or spontaneous CRC in mice lacking either AIM2 or NLRC3 (REFS^{205,206,229}). The precise identity or source of the ligand(s) activating these sensors in the colon is not known. It is possible that self-DNA liberated from the damaged intestinal barrier or DNA derived from the gut microbiota might activate AIM2 (REF²³⁰) (FIG. 5c). Consistent with this hypothesis, AIM2 can localize to DNA in the nucleus of mouse intestinal epithelial cells and bone marrow cells following dsDNA breaks caused by ionizing radiation or chemotherapeutic agents²³¹. The generation of conditional mouse models that enable deletion or re-expression of AIM2 and other related sensors in all cells or in certain cell types once tumours are established would provide additional insights into the molecular and cellular mechanisms of the operation of these sensors. These studies would also aid further assessment of the translational potential of targeting these sensors in cancer immunotherapy.

Indeed, therapeutic inhibition of the AIM2 inflammasome might, in some cases, reduce the adverse gastrointestinal effects associated with chemotherapy²³². The chemotherapeutic and cytotoxic agent irinotecan

(CPT-11), a topoisomerase I inhibitor used in the treatment of a variety of cancers, elicits the release of self-DNA, subsequent activation of AIM2 and secretion of IL-1 β and IL-18 in enterocytes, leading to intestinal mucositis, late-onset diarrhoea and microbiota disturbance in mice^{232,233}. Deletion of *Aim2* or pharmacological inhibition of the AIM2 inflammasome in mice reduces the incidence of irinotecan-induced diarrhoea without dampening the anticancer efficacy of irinotecan²³².

Further studies have shown that NLRP6 (REFS^{137,163,234–237}), NLRP1b (REF²⁰⁴) and caspase 11 (REFS^{209–211}) are important innate immune rheostats of the gut, providing additional targets for experimental therapies. NLRP6 controls intestinal inflammation and tumorigenesis via multiple mechanisms (discussed further later), including modulation of IL-18 secretion^{163,234,235,237}, curtailing the colonization of pro-colitogenic gut bacteria¹⁶³ and promoting secretion of mucus in goblet cells^{137,236} (FIG. 5d). NLRP6 expression in enterocytes and inflammatory monocytes has been reported to mediate protection against DSS-induced colitis^{163,235,237}. NLRP1b regulates activation of the inflammasome and secretion of IL-1 β and IL-18 specifically in non-haematopoietic cells²⁰⁴, whereas caspase 11 triggers IL-1 β –IL-18-dependent and IL-1 β –IL-18-independent functions^{209–211}, with both sensors mediating protection against DSS-induced colitis. A side-by-side comparison revealed that, under isobiotic conditions with normalized gut microbiota across genotypes, mice lacking caspase 1, but not mice lacking caspase 11, were hypersensitive to DSS-induced colitis¹⁸⁸. Evidence from this study would seem to favour a role for canonical inflammasome complexes over the non-canonical inflammasome in this mouse model of intestinal inflammation. Although these studies point towards a protective role of inflammasomes in the context of intestinal inflammation and tumorigenesis, a study found that deletion of the genes encoding the mucous layer components core 1-derived and core 3-derived intestinal O-glycans in mice causes colonic mucous barrier breach, spontaneous colitis and the development of colorectal tumours, and further deletion of the genes encoding caspase 1 and caspase 11 reduces the propensity of colitis and colorectal tumorigenesis²³⁹. These findings illustrate that in the presence of pre-existing immunological dysregulation triggering overt inflammation, blockade of inflammasome signalling might inhibit an inflammatory circuitry that normally fuels disease progression. Development of safe and efficacious modulators of AIM2, NLRP1 and NLRP6 and investigations into the bilateral interaction between inflammasomes and the gut ecosystem would create new avenues for the treatment of intestinal inflammation and cancer.

Inflammasome–gut microbiota axis

The gut microbiota has an important role in the development and progression of a range of clinical manifestations, especially in association with the gastrointestinal tract. Evidence supporting this view includes the ability of postoperative diversion of the

faecal stream to prevent recurrent IBD in humans and the use of broad spectrum antibiotics in the amelioration of chemical-induced colitis in mice¹⁷⁷. The latest developments in the field have suggested that the inflammasome regulates gut microbiota composition, which would imply that the inflammasome–gut microbiota axis might be a centrepiece in the development of intestinal inflammation, cancer and metabolic syndromes^{177,238,240,241}.

Colitis and cancer

The concept of inflammasomes controlling the biogeography of intestinal bacteria was spearheaded by studies showing that mice lacking NLRP6 harbour a pro-colitogenic gut microbiota that renders these mice more susceptible to DSS-induced colitis than wild-type mice¹⁶³. This pro-colitogenic signature is defined by an increased relative abundance of Prevotellaceae and the phylum *Candidatus* Saccharibacteria, reduced relative abundance of *Lactobacillus* spp. and the transmissible nature of the pro-colitogenic microbiota through means of cross-fostering and cohousing techniques^{163,242}. Furthermore, NLRP6 dampens spontaneous colitis in mice lacking the anti-inflammatory cytokine IL-10 by reducing levels of the mucus-degrading bacterium *Akkermansia muciniphila*²⁴³. Carriage of a transmissible pro-colitogenic gut microbiota has also been described in mice lacking the inflammasome components AIM2, NLRP3, ASC, caspase 1 or caspase 11 or mice lacking the related NLR member NLRP12 (REFS^{163,186,205,207–209,244,245}). Similar to the findings with NLRP6, reciprocal exchange of the gut microbiota between wild-type mice and mutant mice lacking AIM2 or NLRP12 elevates the susceptibility of wild-type mice, but reduces the susceptibility of mutant mice, to colitis-associated tumorigenesis^{205,244}, suggesting that the colitogenic potential and transmissibility of the gut microbiota influence disease outcome.

Other studies, however, challenged the idea that NLRP6 controls the biogeography of the gut microbiota^{246,247}. Phylogenetic analyses of wild-type mice and littermate-controlled mice lacking either NLRP6 or ASC failed to reveal any difference in the composition of the gut microbiota²⁴⁶. Lifetime separation of wild-type mice and mice lacking NLRP6 also does not seem to promote alterations of their gut microbiota profile or differential susceptibility to DSS-induced colitis²⁴⁶, casting doubt over the previously suggested role of NLRP6 and ASC in driving dysbiosis¹⁶³. This study also argued that any difference associated with the gut microbiota might be entirely dependent on the facilities used to house the animals²⁴⁶. Thus, food sources, gut microbiota and sterilization techniques used by different mouse vivarium across the globe can influence the activation status of inflammasomes. Future experimental approaches should be more tightly controlled to examine the role of the gut microbiota in any disease context, including the use of littermate controls and careful evaluation of cage-to-cage and room-to-room effects and the overall sterility of the facility.

Despite the uncertainty surrounding the nexus between NLRP6 and the gut microbiota, the mechanistic functions

of NLRP6 have been further characterized against the backdrop of intestinal inflammation, cancer and metabolic syndromes. NLRP6 senses the microbial metabolite taurine and drives the secretion of IL-18 and antimicrobial peptides to maintain a healthy gut microbiota and to promote antitumour immunity in mice^{163,248} (FIG. 5d). By contrast, the microbial metabolites histamine and spermine suppress NLRP6-dependent IL-18 secretion in mice, indicating that components of the gut microbiota can reciprocate and fine-tune the activities of NLRP6 (REF.²⁴⁸) (FIG. 5d). The NLRP6–gut microbiota axis modulates the expression of the chemokine CC-chemokine ligand 5 (CCL5) and the pro-inflammatory cytokines IL-6 and TNF and, therefore, the overall inflammatory state of the intestinal tract^{163,237,242}. In the absence of NLRP6, the dysbiotic gut microbiota developed as a result induces the expression of CCL5 in enterocytes; CCL5, in turn, mediates the secretion of IL-6 and drives proliferation of epithelial cells and tumour formation in mice^{163,242}. Deletion of the gene encoding CCL5 in mice prevents colitis and colitis-associated tumorigenesis despite these mice carrying a dysbiotic gut microbiota^{163,242}, placing dysbiosis upstream of CCL5-mediated immune dysregulation. Furthermore, inflammatory monocytes residing in the lamina propria promote NLRP6-dependent and IL-18-dependent secretion of TNF and feature a protective role in DSS-induced colitis and colitis-associated tumorigenesis²³⁷.

The relationship between NLRP6 and intestinal microorganisms is further exemplified by the ability of NLRP6 to respond to Toll-like receptor (TLR)-induced autophagy in goblet cells¹³⁷. This NLRP6 response mediates secretion of mucus via granule exocytosis and prevents colonization of the mouse gut by *C. rodentium*¹³⁷. Further studies have shown that sentinel goblet cells located at the colonic crypt entrance execute NLRP6-dependent secretion of mucin 2 (MUC2) to expel intruding bacteria found in the inner mucous layer²³⁶ (FIG. 5d). In the small intestine of mice, a loss of endogenous NLRP6 expression owing to stress is linked to the development of intestinal pathology and changes to the relative abundance of Bacteroidetes, Clostridiales, Firmicutes, Lachnospiraceae, Lactobacillaceae, Ruminococcaceae and Streptococcaceae²⁴⁹. The peroxisome proliferator-activated receptor- γ (PPAR γ) agonist rosiglitazone, among other functions, elevates the expression of NLRP6 in the small intestine and prevents stress-related intestinal pathology in mice^{164,249}. Whether this therapeutic effect is directly or specifically dependent on the ability of rosiglitazone to modulate the expression of NLRP6 remains unclear.

An additional function of NLRP6 is its ability to suppress activation of the canonical NF- κ B and MAPK pathways in response to infection by the gastrointestinal pathogens *L. monocytogenes*, *S. Typhimurium* and *E. coli*¹³³ (FIG. 5d). This immunomodulatory pathway operates in the presence or absence of an altered gut microbiota composition¹³³, highlighting that certain biological functions of NLRP6 can clearly be uncoupled from the gut microbiota and that previous studies identifying a connection with the gut microbiota should be interpreted with care and revisited.

Nonalcoholic steatohepatitis, obesity and diet

The influence of the inflammasome–gut microbiota axis extends to the development of nonalcoholic steatohepatitis (NASH) and obesity. Mice lacking NLRP3, NLRP6, ASC, caspase 1 or IL-18 have a gut microbiota profile that is associated with exacerbated hepatic steatosis and inflammation and NASH progression, phenotypes of which are transmissible to wild-type mice via gut microbiota transfer²⁴⁵. A further study found that mice lacking ASC are prone to hepatosteatosis and obesity induction when fed a high-fat diet²⁴⁵. These abnormalities are abrogated following treatment with the antibiotics ciprofloxacin and metronidazole²⁴⁵, suggesting a connection with the gut microbiota in the context of inflammasome signalling and obesity.

A role for inflammasome sensors and diet in regulating microbial diversity and disease is further demonstrated in mice lacking NLRP3, which when fed a high-fat and high-carbohydrate diet develop microbial dysbiosis compared with mice lacking NLRP3 fed with standard chow; the degree of dysbiosis between the two groups seems to be more substantial than the changes observed between wild-type mice that had been fed a high-fat, high-carbohydrate diet and wild-type mice that had been fed with chow²⁵⁰. Compared with wild-type mice on a high-fat and high-carbohydrate diet, the dysbiotic change in mice lacking NLRP3 on the same diet was associated with elevated levels of triglyceride in the liver and faeces and increased intestinal permeability, liver injury and inflammation of the adipose tissue²⁵⁰. Moreover, a diet composed of high fat and high cholesterol induces a reduction in the relative abundance of *Prevotella* spp., which is associated with reduced expression of pro-IL-1 β in the intestine of mice carrying a mutation that predisposes them to spontaneous inflammatory bone disease²⁵¹.

Gut–brain axis

The gut microbiota also operates at the interface between inflammasome signalling and the gut–brain axis. An example of this relationship is the observation that inhibition of the inflammasome-mediated IL-1 β signalling pathway in the small intestine in mice by *S. Typhimurium*-encoded E3 ubiquitin-protein ligase SlrP apparently prevents infection-induced anorexia owing to a blockade of signal transduction to the hypothalamus via the vagus nerve²⁵². Furthermore, it was reported that mice lacking caspase 1 or treatment of wild-type mice with a semisynthetic antibiotic minocycline, which potentially functions to inhibit caspase 1 and modulates the gut microbiota, are protected from developing depressive-like and anxiety-like behaviours, even when experiencing chronic stress²⁵³. The increased resistance to stress in these mice is associated with an increase in the relative abundance of species of *Akkermansia*, *Blautia* and *Lachnospiraceae* and a reduced abundance of *Allobaculum* spp., *Bifidobacterium* spp., *Turicibacter* spp. and *Clostridium* spp.²⁵³. These studies highlight a complex and potentially emerging link between inflammasomes, microorganisms and the gut–brain axis.

Conclusions

Inflammasome signalling is a central pillar of innate immunity that triggers inflammation and cell death. The antimicrobial function of the inflammasome is largely attributed to IL-1 β and IL-18 and pyroptosis: IL-1 β and IL-18 activate an inflammatory circuitry, whereas pyroptosis expels the entire infected cell from the body or liberates concealed pathogen from the infected host cell to facilitate pathogen elimination by other antimicrobial mechanisms. These diverse and multifaceted immune mechanisms are operated in both intestinal epithelial cells and immune cells. Although microorganisms across all kingdoms of life are detected by the inflammasome pathways, the mechanisms regulating activation of the inflammasome in response to many pathogens remain unclear, especially in the context of gastrointestinal viruses and protozoa.

Uncontrolled inflammation and cell death underpin the molecular basis of immunopathology and fuel the progression of chronic inflammatory conditions and cancer. Inflammasome activities largely prevent the development of colitis and gastrointestinal cancer owing to their functions in tumour immunosurveillance, mucus production, cell-renewal, suppression of proliferation of intestinal epithelial stem cells and antitumorigenic responses. The specific PAMPs and DAMPs that trigger activation of the inflammasome in the gut in non-infectious diseases remain largely unknown. What is clear is that the development of intestinal inflammation and cancer is intimately linked to dysregulated inflammasome signalling and/or dysbiosis. This connection is in part owing to the ability of the inflammasome and the gut microbiota to regulate one another. Although advanced sequencing technologies have been informative in providing a snapshot of the global changes of the gut microbiota in the presence of inflammasome dysregulation, the causative microbial species or population responsible for a specific clinical manifestation has not been identified. Furthermore, spatial and temporal changes to the gut microbiota over the length of the intestine and over time with respect to inflammasome dysregulation have not been investigated. Nevertheless, further studies are needed in light of new evidence suggesting that the role of the inflammasome in regulating the composition of the gut microbiota is not always apparent and is, in fact, heavily influenced by a multitude of genetic, environmental and experimental factors associated with animal models^{246,247}.

Given that experimental therapies have yielded success in the treatment of inflammasome-mediated disorders^{53,182,219,221,223,225}, strategies targeting distinct aspects of the inflammasome pathways will become an emerging area of research and development. Discovery of novel small-molecule compounds for use in pharmacological activation or inhibition of the inflammasome and in the prevention and treatment of disease in humans will probably take priority in the next decade of inflammasome research. The deep and complex relationship between inflammasomes, pathogens and the microbiota provides an exciting platform for basic and clinical research with which to understand health and disease.

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Review criteria

A detailed literature review was performed using the PubMed database using a combination of the following search terms: “inflammasome”, “NLRP1”, “NLRP3”, “NLRP4”, “AIM2”, “pyrin”, “caspase-1”, “caspase-4”, “caspase-5”, “caspase-11”, “pyroptosis”, “IL-1”, “IL-18”, “infection”, “bacteria”, “viruses”, “protozoa”, “colitis”, “IBD”, “cancer” and “microbiota”. Relevant English-language papers were evaluated.

Supplementary information

Supplementary information is available for this paper at <https://doi.org/10.1038/s41575-018-0054-1>.