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INVITED REVIEW

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The microbiome and cytosolic innate immune receptors

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Abstract

The discovery of innate immune sensors (pattern recognition receptors, PRRs) has profoundly transformed the notion of innate immunity, in providing a mechanistic basis for host immune interactions with a wealth of environmental signals, leading to a variety of immune-mediated outcomes including instruction and activation of the adaptive immune arm. As part of this growing understanding of host-environmental cross talk, an intimate connection has been unveiled between innate immune sensors and signals perceived from the commensal microbiota, which may be regarded as a hub integrating a variety of environmental cues. Among cytosolic PRRs impacting on host homeostasis by interacting with the commensal microbiota are nucleotide-binding domain, leucine-rich repeat-containing protein receptors (NLRs), together with a number of cytosolic DNA sensors and the family of absent in melanoma (AIM)–like receptors (ALRs). NLR sensors have been a particular focus of research, and some NLRs have emerged as key orchestrators of inflammatory responses and host homeostasis. Some NLRs achieve this through the formation of cytoplasmic multiprotein complexes termed inflammasomes. More recently discovered PRRs include retinoic acid-inducible gene-I (RIG-I)–like receptors (RLRs), cyclic GMP-AMP synthase (cGAS), and STING. In the present review, they summarize recent advancements in knowledge on structure and function of cytosolic PRRs and their roles in host-microbiota cross talk and immune surveillance. In addition, we discuss their relevance for human health and disease and future therapeutic applications involving modulation of their activation and signaling.

KEYWORDS

immune system, inflammasome, innate immune receptors, microbiome, microbiota, pathogen, pattern recognition receptors

1 | INTRODUCTION

The immune system of mammals has traditionally been divided into an innate and an adaptive arm, each featuring separate but complementary functions.[1] Innate immune recognition is mediated by a large array of germline-encoded innate immune receptors, often referred to as "pattern recognition receptors" (PRRs). This concept implies that the specificity of each receptor is genetically predetermined, and has evolved to recognize highly conserved structures common to many microorganisms.[1] PRRs play an essential role in sensing pathogenic microbes, "pathogen-associated molecular patterns" (PAMPs, now also referred to as MAMPs for "microbe-associated molecular patterns"), and host-derived signals of danger (DAMPs

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for "damage-associated molecular patterns").[2] In addition, PRRs are implicated in activation and regulation of adaptive immunity in that antigen-presenting cells equipped with PRRs, such as dendritic cells (DCs) or macrophages, employ adaptive immune system elements upon signal recognition.[2] Mammals live in a close relationship with microbial communities in their immediate environment and colonizing their skin and mucosal surfaces.[3] Most notably, the human gut represents one of the most diverse and densely populated known habitats.[4] The commensal microbiota (microbial taxa associated with the host) and the catalogue of its genes ("microbiome") modify numerous aspects of host biology in health and disease, including complex mutual interactions with the host's immune system.[5,6] The importance of PRRs signaling in response to infectious pathogens has been extensively reviewed elsewhere.[7] However, microbial ligands recognized by PRRs are not unique to pathogens but are also expressed by commensals. As such, the traditional view of innate immunity as quiescent in the absence of infection and activated only upon pathogen recognition has been recently revised,[5,8] and innate immune receptors are increasingly appreciated to constitute important regulators of host-commensal microbiota interactions, thereby shaping systemic host responses toward these complex microbial communities leading to a variety of physiological consequences.[9,10]

Herein, we review current concepts and recent insights linking signaling by cytosolic innate immune receptors with the host microbiome. We highlight mechanistic knowledge on microbiome-immunity interplay mediated by cytosolic PRRs in homeostasis and disease. Moreover, we discuss current research challenges and perspectives on microbiome- and PRR-targeted therapeutic efforts. A comprehensive account of PRR biology and medical significance is beyond the scope of the present review. Instead, we focus on their crucial relationships with the microbiota, pathogens, and resulting impacts on mucosal homeostasis.

2 | STRUCTURE AND FUNCTION OF INNATE PATTERN RECOGNITION RECEPTORS

Germline-encoded innate pattern recognition receptors (PRRs) act as critical sensors of diverse PAMPs and DAMPs, which in turn trigger a multitude of signaling pathways and their downstream effector functions. Based on their molecular structures and functions, PRRs can be further categorized into six classes. Toll-like receptors (TLRs) are the best characterized membrane-bound PRRs involved in host defense against invasive extracellular pathogens.[11] TLR family members contain an N-terminal leucine-rich repeats (LRRs) domain responsible for PAMP binding, a transmembrane domain, and a cytoplasmic Toll/IL-1 receptor (TIR) region required for downstream signaling. TLRs elicit signaling through the recruitment of specific adapters (eg, MyD88) and activation of transcription factors (eg, NF-κB), leading to inflammatory cytokine expression and type I interferon production.[12] C-type lectin receptors (CLRs) are transmembrane PRRs that recognize carbohydrate structures derived from pathogens or self-proteins. Three groups of CLRs were identified based on their molecular structures: type I membrane-bound receptors (including DEC-205 and the macrophage mannose receptor) containing several carbohydrate-recognition domains (CRDs); type II membrane-bound receptors (Dectin-1, Dectin-2, DC-SIGN, DNGR-1, Mincle) carrying a single CRD; and type III soluble receptors (mannose-binding lectin) that also binds to various carbohydrates.[13] Upon ligand binding, CLRs are able to initiate complex signaling pathways through integral signaling motifs or recruitment of adapter molecules, and execute diverse cellular functions, such as anti-microbial responses, phagocytosis, and generation of inflammatory mediators.[14]

NOD-like receptors (NLRs) are the best studied cytoplasmic receptors, and sense a plethora of PAMPs and DAMPs. NLRs generally contain three domains: a central NBD (nucleotide-binding domain) common to all NLRs, a C-terminal leucine-rich repeat (LRR) in most NLRs, and an N-terminal domain that can vary. Some NLRs (eg, Nod1 and Nod2) can induce NF-κB signaling upon activation, while many other members are characterized by their ability to initiate inflammasome complex assembly, which leads to pro-inflammatory cytokine maturation and inflammatory cell death.[15]

In mammalian cells, nucleic acids of microbiome and host origin represent important signals for innate immunity. Cytosolic PRRs, including RIG-I-like receptors (RLRs) that detect RNA, AIM2-like receptors (ALRs), and cyclic GMP-AMP synthase (cGAS) which sense DNA, play critical roles in inducing signaling cascades to mediate host defenses.[16] Membrane-bound PRRs, including TLRs and CLRs, are extensively reviewed elsewhere,[11,17,18] Cytosolic receptors, including NLRs, RLRs, ALRs, RLRs, cGAS, and STING, will be further discussed in the present review.

3 | NOD-LIKE RECEPTOR FAMILY

3.1 | Structure and signaling of NLRs

Members of the nucleotide-binding oligomerization domain (Nod)–like (NLR) family are expressed by a variety of cell types and play a pivotal role in innate immunity, orchestrating immune responses upon sensing of PAMPs and DAMPs.[19] NLRs can activate immune signaling via the NF-κB pathway.[20,21] Certain NLRs serve as scaffolds for the assembly of intracellular multiprotein complexes termed "inflammasomes," which promote the cleavage of the pro-inflammatory cytokines IL-1β and IL-18 into their bioactive states, and can trigger an inflammatory type of cell death termed pyroptosis.[22,23] During activation, the inflammasome-forming components oligomerize into a complex macromolecular wheel-shaped structure. Inflammasomes operate as activation platforms of caspases, which are cysteine proteases initiating or executing cellular programs upon activating cleavage by the inflammasome complex and act as master switches of inflammation in the context of inflammasome activation. Pro-inflammatory caspases are represented by caspase-1, caspase-11,

and caspase-12 in mice and caspase-1, caspase-4, and caspase-5 in humans.[23] Pyroptosis induction depends on the protein gasdermin D (GsdmD), which is activated upon cleavage by pro-inflammatory caspases.[24] The study of inflammasomes is rapidly evolving and is covered extensively by other recent reviews.[24-26]

In humans, the NLR family consists of 22 proteins, and 34 NLR genes have been identified in mice to date.[27] NLRs are primarily expressed by different immune cell lineages, but were also described in multiple non-hematopoietic cells.[15] The defining feature of NLR family members are the nucleotide-binding domain, the NACHT domain (acronym standing for NAIP (neuronal apoptosis inhibitor protein), CIITA (class II transcription activator), HET-E and TP-1 (telomerase-associated protein) and an additional domain containing a leucine-rich repeat motif.[28]

NLRs are capable of sensing a wide array of signals originating from microbes, endogenous danger signals or exogenous perturbations of homeostasis. Examples include peptidoglycan originating from Gram-negative bacteria, resulting in activation of the NF-κB pathway,[29] bacterial flagellin and TTSS apparatus components,[30,31] molecular processes associated with uric acid crystals (gout),[32] or perturbation of ion balance.[33] Indeed, inflammasome functions are important not only in the context of anti-microbial defense but also in the prevention of "sterile inflammation," such as those implicated in autoimmune and other chronic inflammatory disorders.[34]

3.2 | Nod1 and Nod2

Nod1 and Nod2 were the first members of the NLR family to be described.[15] Both receptors recognize muropeptides from bacterial peptidoglycan, although they sense distinct molecular motifs within peptidoglycan.[35,36] Nod1 is involved in the recognition and defense against a wide array of mainly bacterial pathogens, for example, *Helicobacter pylori* through recognition of peptidoglycan delivered from its Cag pathogenicity island,[37] *Pseudomonas aeruginosa*,[38] *Listeria monocytogenes*,[39] but also the intracellular protozoan parasite *Trypanosoma cruzi* (causative agent of Chagas disease).[40] Additionally, the microbiota constitutes a source of peptidoglycan that systemically primes innate immunity in a Nod1-dependent manner.[8] The serial peptidoglycan concentration correlates with neutrophil function. *Nod1−/−* mice show increased susceptibility to pneumococcal sepsis, underscoring the crucial role of the Nod1-microbiota axis in protective innate immunity.[8] Nod1 has been implicated in protecting the integrity of the intestinal epithelial barrier and to protect from microbiota-triggered tumorigenesis in a model of inflammation-related colon cancer.[41] In Nod1-deficient mice, a greater disruption of the intestinal epithelial barrier following chemical injury and increased susceptibility to inflammation-mediated colon cancer is observed. Depletion of the gut microbiota can suppress tumor development associated with Nod1 deficiency, highlighting the detrimental role of the microbiota on intestinal health in the context of a perturbed gut barrier.[41] Nod1 signaling upon sensing of commensals in mesenchymal stromal cells contributes to the maintenance of steady-state hematopoiesis in mice.[42] Nod1 signaling governs circulating myeloid cell lifespan incited by commensal-derived peptidoglycan.[43] Similarly, signaling through Nod1 promotes competitive survival of mature B cells.[44] Moreover, it was postulated that gut commensals fuel non-infectious pancreatic inflammation via Nod1 signaling in pancreatic acinar cells in experimental pancreatitis.[45]

Compared to Nod1, Nod2 is regarded as an even more general bacterial sensor since its peptidoglycan moiety muramyl dipeptide (MDP) is more widely expressed by both Gram-negative and Gram-positive bacteria (Figure 1).[2] It has been implicated in host responses to infections with mycobacteria,[46] *Listeria monocytogenes*,[47] and *Toxoplasma gondii*.[48] The Nod2 protein is a critical regulator of anti-bacterial immunity within the intestine, as *Nod2*-deficient mice were demonstrated to be susceptible to bacterial infection via the oral route but not through intravenous or peritoneal delivery.[47] During the past decade, a critical role of Nod2 emerged in maintaining intestinal and immune homeostasis in dialogue with the commensal microbiota. Nod2 sensing of the intestinal microbiota allows nuclear translocation of NF-κB that initiates transcription of inflammatory cytokines.[49] Nod2 signaling maintains the homeostasis of intestinal intraepithelial cells via recognition of gut microbiota and IL-15 production in mice.[50] Deletion of *Nod2* led to reduced subsets of intraepithelial lymphocytes (IELs), in particular, TCRγδ+ and CD8αα+ TCRαβ+ IELs, in the intestine but not in the thymus. Interestingly, the loss of IELs associated with Nod2 deficiency led to increased susceptibility to TNBS-induced colitis, which could be rescued by adoptive transfer of IELs form wildtype mice.[50] This highlights the protective role of Nod2 in maintaining intestinal homeostasis through immune system stimulation. Nod2 was implicated in a commensal bacteria-dependent lysozyme-sorting process in Paneth cells, an intestinal cell population critical to gut homeostasis, which serves protection of the host from enteric infection.[51] However, the evidence whether Nod2 is critical to Paneth cell anti-microbial function is conflicting.[52] *Nod2* was the first susceptibility gene identified for Crohn's disease (CD),[53,54] a major form of inflammatory bowel disease (IBD), which is characterized by alterations of the gut microbiota and perturbed intestinal homeostasis.[55,56] Nod2 was reported to prevent inflammation of the small intestine by restricting expansion of the commensal *Bacteroides vulgatus* in mice.[57] Moreover, Nod2 counter regulates inflammation by preventing commensal translocation into Peyer's patches (PP) by modulating the cross talk between CD4 + T cells and PP-associated epithelium in mice.[58] In contrast to wildtype Nod2, variants of the gene associated with CD are characterized by altered MDP sensing, which may result in microbiota dysbiosis.[49] One study reported that in intestinal resection specimens from human patients with CD the proportion of abnormal Paneth cells was associated with the number of CD-associated Nod2 risk alleles.[59] Moreover, both Nod1 and Nod2, alongside several TLRs, promote intestinal angiogenesis stimulated by microbiota-derived bacterial ligands in mice.[60] This innate immunity-mediated response was suggested to be implicated in expansion of the mucosal microvascular network, which in turn

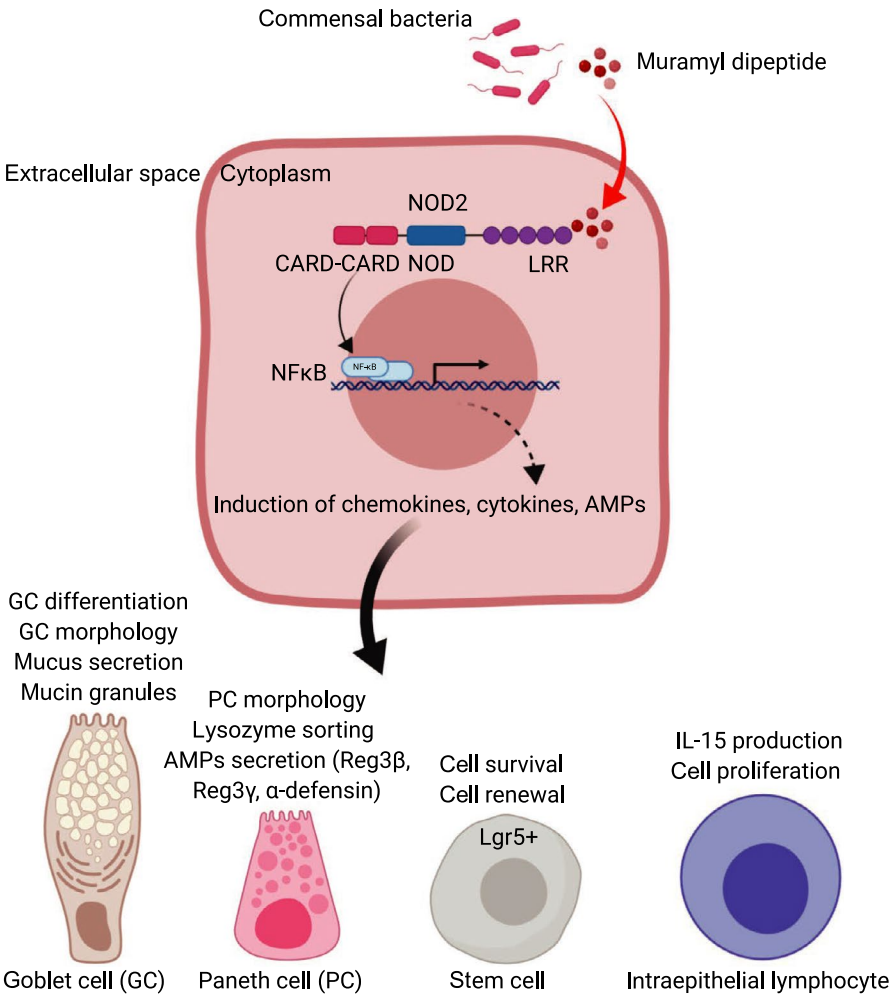

FIGURE 1 Role of cytosolic NOD2 in sensing commensal bacteria and regulation of intestinal homeostasis. NOD2 is an intracellular PRR that detects muramyl dipeptide (MDP) moiety of peptidoglycan of both Gram-positive and Gram-negative commensal bacteria. Sensing of MDP entering the cytosol by the leucine-rich repeat (LRR) domain of NOD2 induces the production of cytokines, chemokines, and antimicrobial peptides (AMPs) through NF-κB signaling. NOD2 sensing and signaling regulates the intestinal homeostasis by maintaining the physiological functions of several crucial intestinal cell populations. In goblet cells (GCs), NOD2 is a critical regulator of mucus secretion, the number of mucin granules, as well as physiological GC numbers and cell morphology. In Paneth cells, NOD2 was postulated to be required for lysozyme sorting, production of certain types of AMPs (Reg3β, Reg3γ, α-defensin) and possibly maintenance of normal Paneth cell morphology. NOD2 is strongly expressed in the Lgr5 + stem cells of the gut and regulate cell survival and stem cell self-renewal. NOD2 also plays an important role in maintaining the proliferation of intraepithelial lymphocytes and their IL-15 production

fosters immune cell recruitment and contributes to chronic intestinal inflammation.[60] Further studies are required to gain deeper insights into the mechanisms governing the interactions between Nod1 and Nod2 receptors and the microbiota, and their implications for human IBD pathophysiology.

3.3 | Nlrp1

Nlrp1 serves as an inflammasome sensor. Unlike human Nlrp1 (also known as Nalp1), mice possess 3 paralogues of Nlrp1 (a, b, and c).[61] In mice, Nlrp1a acts as a cellular sentinel forming an ASC-independent inflammasome to alert caspase-1 to hematopoietic and infectious stress.[62] Variations in *Nlrp1* have been reported to associate with several autoimmune conditions in humans. Interestingly, certain variations in *Nlrp1* were linked to celiac disease and glucocorticoid resistance in human pediatric CD, providing a possible link between Nlrp1 signaling and intestinal homeostasis.[63,64] Nlrp1 inflammasome signaling has been reported to aggravate DSS-induced colitis in mice by limiting beneficial butyrate-producing members of the bacterial order Clostridiales in the gut.[65] Loss of *Nlrp1* was shown to suppress clinical features associated with DSS-induced colitis in mice, whereby bone marrow reconstitution experiments showed that Nlrp1 activity from both the hematopoietic and non-hematopoietic compartments influences this phenotype, with a predominant role of Nlrp1 from non-hematopoietic tissues.[65] In the same study, the authors showed that increased levels of Nlrp1 in inflamed regions of the colon in human patients with ulcerative colitis (UC), another

major form of IBD, are associated with increased IFN-γ levels.[65] However, contradictory evidence has been reported showing that the Nlrp1 inflammasome attenuates colitis and colitis-associated tumorigenesis, demonstrated by amplified disease in Nlrp1-deficient mice.[66]

Recently, the notion that the phenotypic effects of altered *Nlrp1* are conferred by a regulatory impact on the microbiome has been challenged by murine studies.[67] Knowledge on the role of Nlrp1 in intestinal and gut microbiome homeostasis is by far incomplete and the debate still open. Such conflicting results highlight the importance of critical study design and careful breeding and housing strategies in evaluating host-microbiome interactions.

3.4 | Nlrp3

Nlrp3 (also termed Nalp3) is a cytosolic receptor assembling an inflammasome and is abundant in various hematopoietic and non-hematopoietic cell types. Assembly of the Nlrp3 inflammasome leads to caspase 1–mediated release of IL-1β and IL-18, and to GsdmD-mediated pyroptotic cell death.[25] The Nlrp3 inflammasome is regarded as unique in the class of NLRs since it senses ligands of highly diverse origins, including host-derived compounds such as uric acid, pathogen-derived motifs, and xenobiotic compounds (Figure 2).[68] The Nlrp3 inflammasome is subject to complex mechanisms of activation and regulation which have been recently reviewed in detail elsewhere.[25] A protective role has been postulated for the Nlrp3 inflammasome in enteric infection, as it was shown to limit pathogen colonization and to constrain inflammation during infection with the attaching/effacing murine intestinal pathogen *Citrobacter rodentium*. Akin to *Nod2* variations, polymorphisms in *Nlrp3* have been associated with susceptibility to CD.[69,70] It is increasingly clear that Nlrp3 plays a crucial role in maintenance of intestinal homeostasis. Mice lacking *Nlrp3* are susceptible to experimental colitis, which is associated with reduced levels of IL-1β, IL-10 levels, and the growth factor TGF-β.[71] Moreover, the Nlrp3 counter regulates tumorigenesis during colitis-associated cancer.[72] Deficiency of Nlrp3, its adapter ASC or caspase-1, results in increased commensal dispersion and increased mortality during experimental colitis.[73] This phenotype was shown to be a consequence of lack of IL-18 associated with Nlrp3 deficiency. These results highlight that the Nlrp3 inflammasome plays a critical role maintaining intestinal health.[73] However, conflicting evidence has been published on the impact of Nlrp3

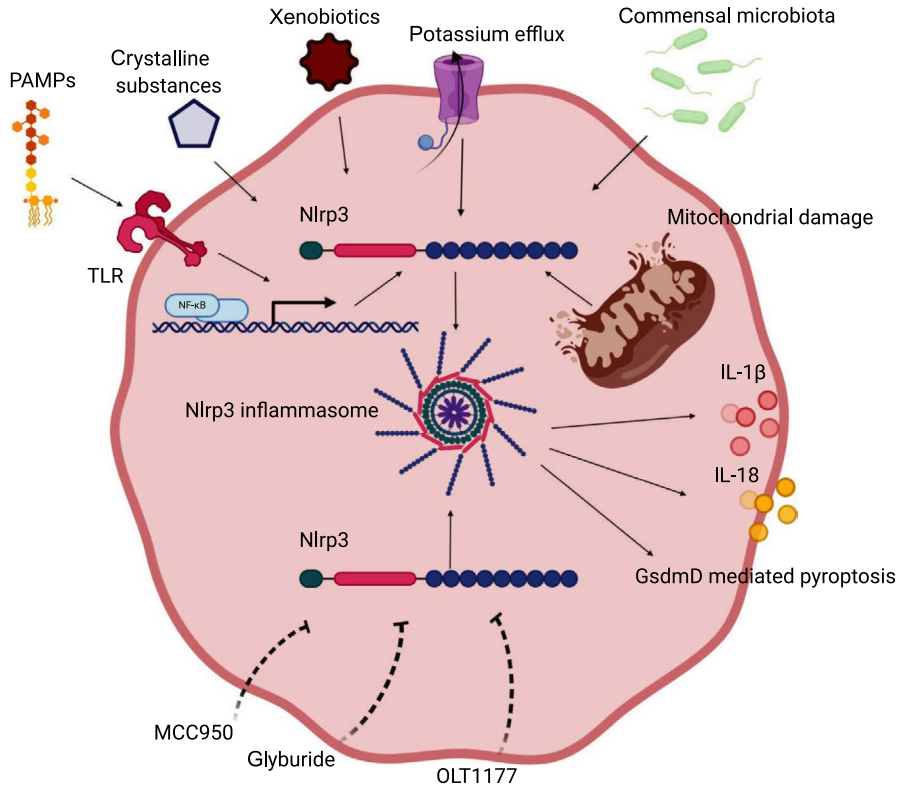

FIGURE 2 Nlrp3 signaling activation and pharmacological inhibition. Nlrp3 assembles an inflammasome upon recognition of a multitude of signals, including (but not limited to) crystalline particles (uric acid), pathogen-derived motifs (PAMPs), xenobiotics, potassium efflux, endogenous danger signals (DAMPs) such as those associated with mitochondrial damage, and signals originating from the commensal microbiota. One way of microbe recognition by Nlrp3 is mediated via toll-like receptor (TLR) sensing and employment of the pro-inflammatory transcription factor NFκB ("signal 1"). The Nlrp3 inflammasome releases via activating cleavage the bioactive forms of the pro-inflammatory cytokines IL-1β and IL-18, and promotes pyroptosis via cleavage of GsdmD. Recently, small molecular inhibitors of the Nlrp3 inflammasome emerged with promising therapeutic potential, such as MCC950, the anti-diabetic drug glyburide, and OLT1177

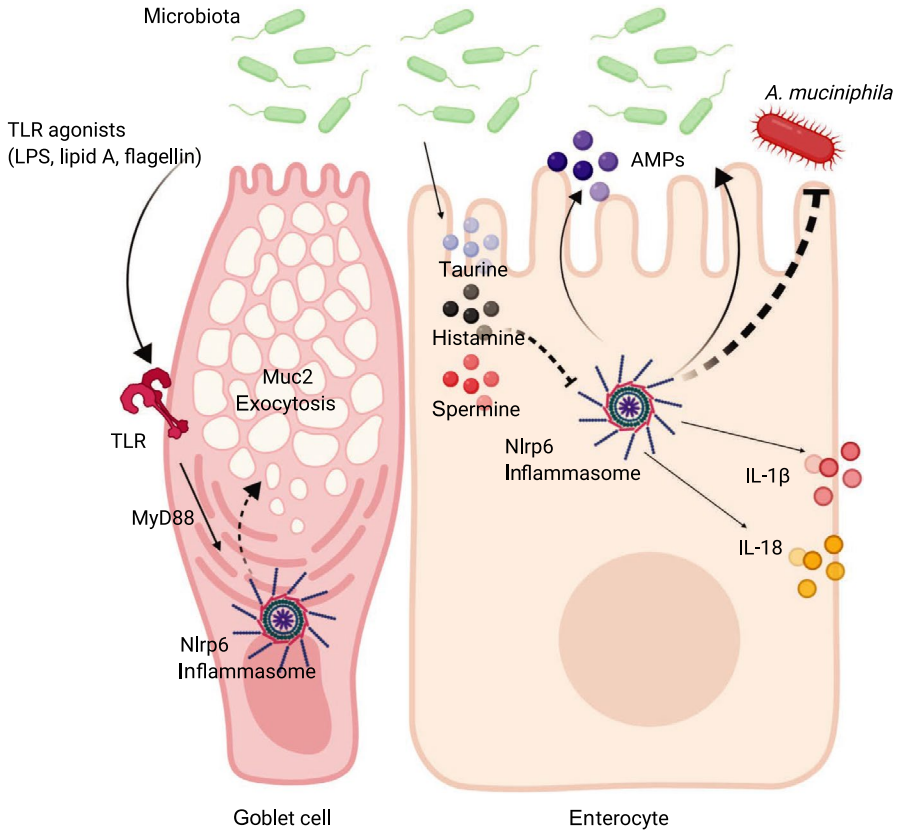

FIGURE 3 Interactions between the Nlrp6 inflammasome and the microbiota. Nlrp6 assembles an inflammasome and cleaves both pro-IL1β and IL-18 into their bioactive states. Microbe-derived TLR agonists, including lipopolysaccharide (LPS), lipid A, or flagellin, promote Nlrp6 inflammasome signaling in a MyD88-dependent manner. In intestinal sentinel goblet cells, the Nlrp6 inflammasome promotes secretion of Muc2 and enteric mucus formation to protect the gut barrier. The intestinal microbiota is natural source of small microbe-derived ligands inhibiting the Nlrp6 inflammasome, including taurine, histamine, and spermine. The Nlrp6 inflammasome contributes to intestinal homeostasis, for example, by stimulating production of anti-microbial peptides (AMPs). At least in the context of certain microbiota configurations, the Nlrp6 inflammasome shapes the gut microbiota composition. Moreover, it can limit the growth of specific species, such as *Akkermansia muciniphila*, to protect the host from intestinal inflammation

in intestinal inflammation, as another research group showed that *Nlrp3* deficiency resulted in protection against experimental colitis.[74] In addition, upon intestinal injury certain members of the gut microbiota stimulate newly recruited monocytes to induce Nlrp3-dependent IL-1β release, which further fuels intestinal inflammation.[75] For decades, diets rich in fermentable fibers have been postulated to provide many health benefits. Recently, the microbiota fiber fermentation has been suggested to modulate intestinal inflammation in a Nlrp3-dependent manner.[76] In *Mdr2*-deficient mice, a model of cholestatic liver disease and translocation of bacterial ligands via the gut-liver axis were shown to amplify liver injury through stimulating the Nlrp3 inflammasome.[77] Although most studies on Nlrp3-microbiota interactions focused on the intestine, there is some evidence of Nlrp3-microbiota communication regulating other important process, for example, an activating mutation of *Nlrp3* was demonstrated to be implicated in skin inflammation driven by mast cells and dysregulated IL-1β production.[78]

Nlrp3 inflammasome signaling is postulated to play a role in a growing array of inflammatory diseases, ranging from IBD,[79] rheumatic diseases,[80] and pancreatitis[81] to the neurodegenerative disorder Alzheimer's disease.[82] This renders it a promising therapeutic target.[25] Several small molecular agents were introduced showing promising in vitro and in vivo effects in animal disease models. For example, the anti-diabetic drug glyburide was shown to inhibit the Nlrp3 inflammasome and thus to delay lipopolysaccharide-induced lethality in mice.[83] In addition, the small molecule MCC950 blocks both canonical and non-canonical Nlrp3 activation at nanomolar concentrations, in turn inhibiting IL-1β production and attenuating the severity of experimental autoimmune encephalomyelitis (EAE), a murine model for the neuroinflammatory disorder multiple sclerosis.[84] Recently, a favorable effect of Nlrp3 blockade by MCC950 has been demonstrated in experimental acute pancreatitis.[81] Moreover, OLT1177, an orally active β-sulfonyl nitrile molecule, inhibits Nlrp3 inflammasome signaling and significantly reduces the metabolic perturbation associated with inflammation.[85]

To conclude, the study of Nlrp3 inflammasome activation is an exciting and rapidly developing field in immunology. The rise of inflammatory disorders due to the increasing global number of individuals adopting a "Western life style" and aging populations highlights

the need for novel anti-inflammatory treatments. Refinement of the knowledge on Nlrp3 activation and regulation in dialogue with the microbiota spurs progress in therapeutic applications for various inflammatory disorders. Despite the progress achieved in identification and repurposing of Nlrp3 inhibitors in preclinical studies, more clinical research proving the efficacy and safety of such compounds in patients is warranted.

3.5 | Nlrp6

Nlrp6 (previously referred to as Pypaf-5) possesses a pyrin as a C-terminal effector domain that interacts with ASC and caspases assembling an inflammasome.[86] Notably, Nlrp6 recruits both caspase-1 and caspase-11 to form an inflammasome.[87] The Nlrp6 inflammasome was postulated to promote epithelial regeneration, exerts anti-tumorigenic effects, and preserves the epithelial barrier (Figure 3).[88] Nlrp6 was initially reported to be a negative regulator of inflammatory signaling.[89] Nlrp6 was shown to shape the gut microbiota, with *Nlrp6*-deficient mice exhibiting a transmissible colitogenic microbiota dysbiosis.[90] Moreover, microbiota alterations associated with *Nlrp6* deficiency were linked to transmissible colon cancer in mice.[91] And it was shown to protect *IL-10*-deficient mice from colitis by limiting the colonization with *Akkermansia muciniphila*.[92] The microbiota-derived metabolites taurine, histamine, and spermine were shown to shape the host-microbiome interface by co-modulating Nlrp6 inflammasome signaling,[93] potentially highlighting the notion of "postbiotic" metabolomic interventions targeting Nlrp6 as a modulator of dysbiosis-driven diseases. The recognition of pathogens by the Nlrp6 inflammasome differs from that of commensals, in that lipoteichoic acid from Gram-positive pathogens binds and activates Nlrp6 which subsequently induced the activation of caspase-11.[94] Cohousing experiments suggested that microbial modulation of Nlrp6 signaling operates through influencing inflammasome activation ("signal II") rather than transcription of inflammasome components ("signal I").[93] Nevertheless, conflicting results have been reported regarding the impact of Nlrp6 on microbiota configuration in different facilities,[95,96] with the impact of the Nlrp6 inflammasome on microbiota composition being dependent on the pre-existing community structure in the respective vivarium. Strowig and colleagues showed that the presence of pathobionts, for example, certain *Helicobacter* spp., leads to dysbiosis in mice lacking Nlrp6, while *Nlrp6*-deficient mice in "enhanced pathogen free" condition showed no differences in microbiota structure compared to wildtype mice.[97] Mucus production by intestinal goblet cells is considered a crucial protective anti-microbial mechanism. Similarly, under some microbiome conditions *Nlrp6* deficiency leads to impaired autophagy in goblet cells and defective mucus secretion into the gut lumen, thereby impairing mucosal pathogen clearance.[98] Furthermore, this function is likely mediated at the outer crypt entrance, where Nlrp6 acts as a sentinel gatekeeper.[99] Interestingly, the same group recently showed that the mucus-related functions of Nlrp6 are dispensable under a "clean" steady-state conditions and are promoted by a second TLR-activating hit (under pathogenic or diverse commensal conditions), which triggers its mucus-promoting gatekeeper impacts.[100] Recently, a dietary flavone compound was demonstrated to confer communicable protection against colitis through Nlrp6 signaling in an inflammasome-independent manner.[101] For further reading, we refer to a recent in-depth review dedicated to the Nlrp6 inflammasome.[87]

To conclude, the Nlrp6 inflammasome has a wide range of microbiome-dependent and independent functions, some of which are vivarium and microbial context-specific and require further verification in littermate experiments.[102] More detailed mechanistic information is warranted on how the Nlrp6 recruits caspase-11 in addition to caspase-1. Importantly, evidence for the role of Nlrp6 in extra intestinal contexts is widely lacking. Future studies on cell-, tissue-, and organ-specific functions of Nlrp6 are warranted. A formidable challenge is the translation of insights on Nlrp6 biology into therapeutic applications aiming at attenuating inflammatory disorders.

3.6 | Nlrp12

The expression of Nlrp12, previously known as monarch-1, is largely restricted to DCs and neutrophils.[103] The ability of Nlrp12 to assemble an inflammasome is controversial. Few studies reported the inflammasome-dependent role of Nlrp12 as an innate immune sensor in recognizing *Yersinia pestis*,[104] malaria parasites[105] or herpes simplex virus type 1 (HSV-1).[106] Others suggested that Nlrp12 as a negative regulator of inflammatory responses, acting independent of inflammasome activation. It was implicated in the suppression of colonic inflammation and inflammation-associated carcinogenesis, mainly through inhibiting both canonical and non-canonical NF-κB signaling in inflammasome-independent manner.[107,108] Nlrp12 also negatively regulates the TLR and tumor necrosis factor α pathways.[109] Nlrp12 ameliorates the disease severity and inflammation in a murine model of multiple sclerosis, indicating its anti-inflammatory role in autoinflammatory disorders.[110] However, the immunosuppressive functions of Nlrp12 may be exploited by pathogens. Nlrp12-dependent suppression of host immune responses facilitates the infection and persistence of invasive agents, including *Salmonella Typhimurium*,[111] *Brucella abortus*,[112] and vesicular stomatitis virus.[113]

The emerging role of Nlrp12 in regulating the gut microbiome was put forth in several recent studies. Mice with *Nlrp12* deficiency host an altered gut microbiome, characterized by less beneficial gut commensals belonging to the family Lachnospiraceae and increased abundance of colitogenic and obesity-associated bacteria from the family Erysipelotrichaceae. Consequently, *Nlrp12*-deficient mice exhibit increased susceptibility to chemically induced colitis, which can be rescued by treatment with protective commensal Lachnospiraceae isolates or by antibodies targeting inflammatory cytokines.[114] Furthermore, *Nlrp12*-deficient mice fed a high fat diet were shown to be prone to obesity, which can also be reversed by

administration of Lachnospiraceae or short chain fatty acids.[115] The protective role of Nlrp12 in maintaining gut homeostasis through regulation of gut microbiota provides another promising target for microbiota-directed therapies in intestinal inflammation and metabolic diseases. However, the mechanisms ruling Nlrp12 signaling and its impacts on the microbiota require further elucidation.

3.7 | Nlrc4

Nlrc4, which is mainly expressed in myeloid cells, is extensively investigated for its sensing role in the context of bacterial infections. Nlrc4 belongs to the NLRs assembling an inflammasome. The Nlrc4 inflammasome drives the production of IL-1β in intestinal phagocytes, which is critical to discriminate between pathogenic bacteria and commensals in the gut.[116] However, the role of Nlrc4 in recognizing commensal bacteria seems to be redundant, since flagellin derived from non-pathogenic *Escherichia coli* shows markedly lower efficiency in activating Nlrc4 compared to flagellin derived from *Salmonella Typhi*.[117] In macrophages, the Nlrc4 inflammasome can be activated by cytosolic flagellin derived from Salmonella species.[118] *Salmonella* type III secretion system (T3SS) components are also important Nlrc4 activators.[119] In addition to *Salmonella*, Nlrc4 is capable of identifying other Gram-negative bacteria possessing either flagellin or T3SS factors, including *Pseudomonas aeruginosa*,[120] *Legionella pneumophila*,[121] and *Shigella* spp.[122] Expression of Nlrc4 in intestinal epithelial cells confers protection against *Citrobacter rodentium*, while the mechanism remains unknown.[123]

Different from other NLRs, the assembly and activation of the Nlrc4 inflammasome requires interaction with other NLR family members, the NLR apoptosis inhibitory proteins (NAIPs). Mouse NAIPs directly recognize bacterial ligands in a ligand-specific manner, mediated by the NBD-associated helical domains of NAIPs.[124] Specifically, NAIP5 and NAIP6 sense the cytosolic bacterial flagellin, while NAIP1 and NAIP2 mainly activate NLRC4 in response to the bacterial T3SS apparatus.[30,125] Structural analyses of the purified PrgJ-NAIP2-Nlrc4 inflammasome[126] or flagellin-NAIP5-Nlrc4 inflammasome[127] indicate that one single NAIP is sufficient to activate Nlrc4, leading to self-propagation of multiple Nlrc4 to form a 10 to 12 spoke wheel-like complex. In addition to the maturation and secretion of pro-inflammatory cytokines IL-18 and IL-1β, other downstream effectors of NAIP/Nlrc4 inflammasomes have recently been discovered, including the pore-forming protein GsdmD and caspase-7, which protect against *Legionella pneumophila*,[128] as well as caspase-8-dependent cell apoptosis in response to *Salmonella infection*.[129]

Independent from bacterial virulence sensing, Nlrc4 is involved in the context of different inflammatory diseases. The "sterile" inflammatory stimulator lysophosphatidylcholine can induce Nlrc4 inflammasome activation and IL-1β secretion, which enhances microglial accumulation and astrogliosis in the central nervous system during demyelination.[130] The role of Nlrc4 in regulating acute colonic inflammation and chronic inflammation-associated tumorigenesis has been studied with inconsistent results,[72,131,132] possibly stemming from the inherent differences in facility-dependent murine gut microbiota. In obese mice, Nlrc4 inflammasome activation in the tumor microenvironment promotes the progression of breast cancer through stimulation of angiogenesis, indicating that blocking the Nlrc4 inflammasome might be protective for obese cancer patients.[133]

4 | NON-CANONICAL INFLAMMASOMES

Microbiome sensing by non-canonical inflammasomes is gaining increasing attention. Non-canonical inflammasome activation is mainly mediated by murine caspase-11, corresponding to human caspase-4 or caspase-5,[134] which are innate sensor and receptor for intracellular lipopolysaccharide in immune cells.[135,136] Specifically, caspase-11 directly binds to the lipid A component of lipopolysaccharide through its CARD domain, resulting in pyroptotic cell death.[135] In particular, hexa-acylated lipid A of lipopolysaccharide shows an optimal binding affinity to caspase-11.[137] As such, non-canonical inflammasome signaling can be activated by a wide range of Gram-negative bacteria, including *Escherichia coli*, *Citrobacter rodentium*, *Vibrio cholerae*, and *S Typhimurium*.[134,138] Indeed, non-canonical inflammasomes play critical roles in host anti-microbial defense, as mice lacking caspase-11 are more susceptible to specific mutants of *S Typhimurium* and *Legionella pneumophila* that aberrantly escape vacuolization and invade the cytosol.[139] Caspase-11 also protects the mice against *L pneumophila* infection in the lung by promoting fusion of lysosomes with pathogenic bacteria-containing phagosomes.[140] In addition to immune cells, non-canonical inflammasome activation involving caspase-4 or caspase-11 also exists in intestinal epithelial cells, mediating host defense against both intracellular (*S Typhimurium*) and extracellular (*Citrobacter rodentium*) enteric pathogens.[138] Beyond exogenous bacterial ligands, caspase-11 can be activated by the endogenous ligand oxPAPC in DCs, an oxidized phospholipid released massively from damaged and dying cells.[141] Unlike bacterial ligands, oxPAPC, which can be understood as a lipopolysaccharide mimic, binds specifically to the catalytic domain of caspase-11 and ignites caspase-11-dependent interleukin-1β release.

One direct effector function downstream of non-canonical inflammasome activation is pyroptosis, and this essentially requires direct cleavage of GsdmD by caspase-11, caspase-4, and caspase-5.[142,143] After cleavage, the N-terminal fragment of GsdmD binds specifically to membrane lipids with pore-forming activity, which directly leads to cell lysis and bacterial killing.[144-146] Another effector function is the activation of Nlrp3 inflammasome downstream of either GsdmD or caspase-11.[147] On the one hand, active caspase-11 is required for Nlrp3 inflammasome activation by enhancing potassium efflux in the presence of lipopolysaccharide.[148] Upon Gram-negative bacterial infection, TRIF signaling activates caspase-11 through type I interferon, which subsequently drives Nlrp3 inflammasome activation and caspase-1-dependent effector functions.[147] On the other hand, cleaved GsdmD can promote Nlrp3-dependent activation of

caspase-1 and IL-1β secretion against Gram-negative bacterial infection.[142] A third effector function involves anti-microbial protein secretion, as activation of non-canonical caspase-4/5 inflammasome results in secretion of multiple proteins by human primary macrophages, including S100A8 and prothymosin-α, which represent TLR4 ligands,[149] while the exact contribution of these secreted proteins in host defense against bacterial invaders remains to be investigated.

5 | RIG-I-LIKE RECEPTORS

RIG-I-like receptors (RLRs) are a group of PRRs initially characterized by their roles in sensing cytoplasmic viral RNA in most cell types. The RLR family consists of 3 members: RIG-I (Retinoic Acid-Inducible Gene-I), MDA5 (Melanoma-Differentiation-Associated gene 5), and LGP2 (Laboratory of Genetics and Physiology 2). Structurally, RIG-I and MDA5 contain an N-terminal caspase activation and recruitment domains (CARD) and a DEAD box helicase, both of which mediate detection of viral RNA, fuel cell-intrinsic innate immune signaling, and lead to type I interferon production.[150] A wide variety of RNA virus can be detected by both RIG-I and MDA5, ranging from flaviviruses (eg, West Nile virus), dengue virus, reoviruses, and paramyxoviruses, which have been reviewed in detail elsewhere.[151] Despite this, RIG-I and MDA5 preferentially bind to short and long dsRNAs, respectively, and RIG-I also senses dsRNAs without an uncapped 5' triphosphate end.[152] In contrast, LGP2 is currently conceptualized to act as a regulator of RLR signaling due to the lack of a N-terminal domain.[153] Added to viral RNA, accumulating evidence highlights the role of RLRs in sensing of host-derived RNA during virus infection, which has been reviewed in detail elsewhere.[154,155] For example, the 5S rRNA pseudogene 141 (*RNA5SP141*) binds to RIG-I and initiates antiviral immunity upon HSV-1 infection.[156] Other host-derived RNAs that can be recognized by RLRs include misprocessed triphosphorylated non-coding RNAs during Kaposi's sarcoma-associated herpesvirus infection (30451863),[157] and RNAs containing 5'-monophosphates.[158]

In addition to RNA virus, the role of RLRs in recognizing DNA virus is emerging. In fact, the DNA virus-encoded RNA contains necessary structures and biochemical moieties that can be recognized by RLRs. For instance, small RNAs encoded by Epstein-Barr virus (EBV) are detected by RIG-I, which can initiate signaling pathways related to the induction of type I interferon,[159] or can trigger IL10 activation through RIG-I-mediated IRF-3 signaling.[160] Similar to EBV, RNAs encoded by adenovirus virus are also recognized by RIG-I leading to type I interferon expression.[161] Another mechanism by which RIG-I detects cytosolic DNA is through the RNA polymerase III (RNAPIII),[162] and examples include the sensing and restriction of the DNA virus HSV-1 in murine microglia and astrocytes via RIG-I-RNAPIII axis.[163] Sensing of DNA virus by RLRs and the underlying mechanisms have been reviewed recently.[164]

While type I interferon–related pathway remains the most studied antiviral signaling downstream of RLR activation, recent studies have uncovered other effector functions. It has been recently shown that RLRs drive inflammatory macrophage polarization and suppress wound healing macrophages against West Nile virus infection.[165] MDA5 and RIG-I are involved in the TLR3-mediated IL-6 signaling in human glomerular endothelial cells.[166] More interestingly, in vivo delivery of therapeutically active RIG-I mimetics leads to RIG-I activation in breast cancer, triggering apoptosis and pyroptosis for immunogenic killing of tumor cells.[167] Better understanding of RLR-mediated antiviral or anti-tumor signaling pathways will greatly facilitate the discovery of new therapeutic targets. Additional studies are of interest to explore potential roles of microbiome (including the virome) and microbiota-derived ligands in RLRs signaling in health and disease.

6 | AIM2-LIKE RECEPTORS

The absent in melanoma 2 (AIM2)–like receptors (ALRs) are mainly involved in the recognition of pathogenic and cytosolic DNA. Two members of the ALR family have been identified: AIM2 and interferon-inducible protein 16 (IFI16). AIM2 senses dsDNA and forms an inflammasome platform with ASC and caspase-1, leading to enzymatic cleavage of the pro-inflammatory cytokines IL-1β and IL-18 (19131592, 19158675).[168,169] Structurally, both AIM2 and IFI16 contain an N-terminal Pyrin domain (PYD) and a C-terminal HIN domain which directly binds to dsDNA backbone through electrostatic attraction, whereby DNA must be over 80 base pairs in length to be bound.[170] Activation of AIM2 through DNA binding triggers inflammasome assembly via polymerization of PYD and ASC.[171]

AIM2 is an innate immune sensor of microbial DNA originating from a broad range of pathogenic bacteria, including but not limited to *Francisella tularensis*, *Listeria monocytogenes*, *Mycobacterium tuberculosis*, and *Streptococcus pneumoniae*, which is reviewed elsewhere.[172] The mechanisms of how bacterial DNA activates AIM2 have been extensively investigated in the context of murine *Francisella* infection. AIM2 activation in host cells essentially requires the ability of *F novicida* and *F tularensis* to escape vacuoles into the cytosol, which can be regulated by the presence of *Francisella* Pathogenicity Island in dendritic cells,[173] signaling via type I interferon, or guanylate-binding proteins (GBPs).[174,175] Similarly, the release of *Listeria monocytogenes* DNA into host cytoplasm by bacteriolysis is the prerequisite to trigger AIM2 inflammasome-mediated pyroptosis.[176] The role of type I interferon in regulating AIM2 activation is bacteria-dependent. In *Francisella* infection, type I interferon drives the expression of the transcription factor IRF1 and further induces the expression of GBPs, which finally leads to bacterial DNA release that can be sensed by AIM2.[174] Type I IFN signaling also regulates the activation of the AIM2 inflammasome in macrophages during murine *S pneumoniae* infection[177] but not in *Mycobacterium tuberculosis* infection.[178] In addition to bacteria, AIM2 inflammasome mediates recognition and host defense against cytosolic DNA derived from some but not all viruses, such as mouse cytomegalovirus[179] and human papillomaviruses.[180] Furthermore, AIM2 confers protection in mice against the protozoon *Plasmodium berghei*[181] and the fungal

pathogen Aspergillus[182] by forming a single inflammasome with Nlrp3. Sensing of DNA is not the sole mechanism underlying the restriction of infectious agents by AIM2. For instance, AIM2 mediates host defense against *Salmonella typhimurium* by regulating intestinal barrier integrity via activation of Protein kinase B, also known as Akt.[183] AIM2 recognizes host-derived DNA accumulated in the lung microenvironment during influenza virus infection.[184] The underlying molecular and regulatory mechanism of AIM2 sensing of other viral, fungal, and parasitic pathogens remains to be investigated.

In addition to defense against pathogens, growing evidence supports the notion of regulation of the intestinal microbiome by AIM2. Sensing of microbial DNA by AIM2 activates the inflammasome, which restricts the growth of intestinal *Escherichia coli* in mice, promotes the production of anti-microbial peptides in intestinal epithelial cells, and protects mice from chemically induced colitis.[185] In another study, AIM2 was required to restrict the susceptibility to colorectal tumorigenesis, partly through modulating the gut microbiota, as *AIM2−/−* mice hosted higher abundance of *Akkermansia muciniphila* and *Anaeroplasma* compared to wildtype mice, while cohousing of *AIM2−/−* and wildtype mice was sufficient to rescue the phenotype.[186]

Another dsDNA sensor of the ALR family, human IFI16, or its mouse ortholog p204, is directly associated with IFN-β-inducing motifs of viral DNA, and transcriptionally induce genes encoding IFN-β in response to HSV-1 infection.[187] IFI16 also acts as a nuclear pathogen sensor of the nuclear replicating Kaposi Sarcoma-associated herpesvirus in endothelial cells, and is capable of forming an inflammasome complex with ASC and procaspase-1 in the nucleus and perinuclear area.[188] In hepatic nuclei infected with hepatitis B virus (HBV), IFI16 binds to and epigenetically suppresses the nuclear-located covalently closed circular DNA (cccDNA) of HBV by targeting an interferon-stimulated response element of cccDNA, ultimately resulting in downregulation of cccDNA transcription and inhibition of HBV replication.[189] IFI16 also exert its antiviral function by inhibiting human cytomegalovirus DNA synthesis.[190] However, in human immunodeficiency virus (HIV) infection, IFI16 sensing of the incomplete HIV DNA transcripts in lymphoid CD4 + T cells is key to trigger caspase-1 activation and pyroptosis, which contributes to HIV progression. These results indicate pleiotropic effects of IFI16 in pathogen-dependent contexts. It is intriguing to study the unexplored potential role of IFI16 in regulating the intestinal microbiome and gut homeostasis.

7 | CGAS AND STING

The most recently identified cGAS-STING pathway, consisting of the DNA-binding protein cyclic GMP-AMP synthase (cGAS), the messenger product cyclic GMP-AMP (cGAMP), and the downstream effector stimulator of interferon genes (STING), forms another major DNA-sensing apparatus in mammalian cytoplasm that activates the type I interferon pathway.[191] A recent study showed that the eukaryotic cGAS-STING immune pathway may actually phylogenetically originate from bacterial defense against bacteriophages, as the presence of cGAS and the production of cGAMP in bacteria confer protection against phage infection.[192] Structurally, human and mouse cGAS binds to DNA directly in a sequence-independent manner, and the conformational alterations of the catalytic domain of cGAS further induce enzymatic activity.[193-195] The human cGAS-DNA-sensing system is adapted for enhanced specificity to favor longer DNA binding, due to two amino acid substitutions in the DNA-binding surface of cGAS.[196]

DNA of various origin released into the cytosol can bind and activate cGAS, including but not limited to viral DNA, tumor DNA, and host-derived DNA. Moreover, cGAS is required for type I interferon responses against various DNA viruses, such HSV-1 and gamma herpesviruses.[191,197] Genetic deletion of murine cGAS was demonstrated to impair antiviral immunity to HSV-1 and infection with gamma herpesvirus 68, as well as West Nile virus, a ssRNA virus.[198] In human HSV-infected fibroblasts and keratinocytes, cGAS is partially nuclear, interacts with, and mediates the stabilization of IFI16, thus contributing to innate antiviral signaling cooperatively with IFI16.[199] In addition to DNA/RNA virus, the cGAS-STING axis can be activated by cytosolic RNA:DNA hybrid molecules in vitro, while this observation needs to be validated in vivo.[200] It should be noted that a number of DNA and RNA viruses have evolved different strategies to counteract the cGAS-STING pathway to evade the immune surveillance, which have been reviewed elsewhere.[201,202]

The role of cGAS-STING system in sensing bacterial DNA has discovered recently, among which cyclic dinucleotides (CDNs) produced by bacteria are a novel target for cGAS sensing.[203] Detection of extracellular CDNs by mammalian cells requires a highly ordered process, including internalization of CDNs through endocytosis, directly binding of internalized CDNs to cytosolic cGAS, the assembly of the cGAS/STING complex and activation of innate immune responses.[204] Beyond CDNs, bacterial DNA resulting from bacteriolysis of *Listeria monocytogenes*[205] is able to induce IFNβ expression dependent on the cGAS-, STING-, and IFI16-related pathways. Interestingly, *Listeria* DNA from infected cells can be sorted into extracellular vesicles and delivered to bystander cells to stimulate the cGAS-STING signaling, a process mediated by the multivesicular body protein MVB12b.[206] The mechanism of how cGAS senses the nucleic acid products from other pathogenic and commensal bacteria and how bacteria exploit this pathway to evade immune surveillance remains to be further determined.

Activation of cGAS upon DNA binding converts ATP and GTP into cGAMP. DNA binding to cGAS also induces liquid phase separation to activate cGAS by forming liquid-like droplets, inside which the increased concentrations of the enzyme and reactants can robustly promotes cGAMP production.[207] cGAMP is an endogenous second messenger that in turn binds to the STING, an adapter protein localized to the endoplasmic reticulum (ER) membrane, leading to its oligomerization and activation.[208] Following activation, STING is translocated from the ER to perinuclear regions including the Golgi apparatus and triggers the production of type I interferons dependent on the TANK-binding kinase 1 (TBK1)-interferon

regulatory factor 3 (IRF3) pathway.[209,210] In addition to interferon production, autophagy emerges as a major function downstream of the cGAS pathway, as activated STING induces autophagy through TBK1- and interferon-independent mechanism, which is important for clearance of pathogenic DNA from the cytoplasm.[211] Beyond cGAS, STING itself also functions as an innate immune sensor that can directly binds to bacterial ligands CDNs to initiate type I IFN response,[212] or can sense vita-PAMPs from viable Gram-positive bacteria to mediate an ER stress response.[213]

Special attention has been paid to the recognition of non-infectious, tissue damage-associated DNA by cGAS-STING pathway and its important implications in autoimmunity, inflammation, and cancer biology, which has been reviewed in detail elsewhere.[214,215] Moreover, STING has been implicated in experimental pancreatitis, in which it senses acinar cell death by detecting DNA from dying acinar cells and activates a signaling pathway that promotes inflammation.[216] Given this evidence, it is vital to "fine tune" the cGAS-STING system in order to differentiate self-DNA from foreign DNA in the context of infectious, inflammatory, and autoimmune disorders. Studies are required to uncover potential microbiome-derived signals that can be sensed by the cGAS-STING apparatus in homeostasis and disease.

8 | CHALLENGES AND LIMITATIONS

The discovery of PRRs and inflammasomes has changed the perspective on innate immunity and greatly promoted research in this area in the two past decades. Unraveling of molecular mechanisms governing activation and regulation of innate immune receptor signaling uncovered the close interconnection between innate and adaptive immunity[217] and the eminent role of elements of innate immunity at the host-microbiome interface. Regardless of the remarkable progress achieved in PRR and inflammasome biology, numerous unknowns and challenging questions remain to be solved. First, much of the research focused on PRR-related outcomes in the intestine. However, emerging evidence points toward critical roles in multiple other organs and tissues, which warrants substantial additional research. Although crucial insights have been gained into activation of innate immune sensors in response to bacterial motifs, eukaryotic activation originating from fungi and protozoa, which not only account for important pathogens but constitute an integral and important part of the host's microbiome,[218] is largely unexplored. The same limitations apply to the host's virome, which accounts for the most abundant and fastest mutating genetic elements on Earth.[219] The lack of mechanistic insights related to non-bacterial members of the microbiota is a significant part explained by their scarcity in conventionally housed laboratory mice. A promising new strategy to overcome these hurdles is represented by the new concept of "wildling" mice, which result from the transfer of C57BL/6 embryos into wild mice, thus harboring "natural" wild-like microbiota against a defined genetic background.[220] This approach appears to more accurately model human responses,[220] and thus has the potential enhance the overall success of immunological microbiota studies. Whereas much of the previous research on innate immune receptor signaling focused on cytokine secretion and cell death outcomes, other downstream processes such as extra intestinal barrier function, autophagy or mucus production are also of great interest. Moreover, microbiota-derived ligands activating innate immune sensors warrant deeper characterization. Additionally, commensal microbes may be capable of not only activating but also dampen or fine tune innate immune sensor signaling, which requires further investigation. Many studies on microbiome-immune receptor interactions relied on 16S rRNA sequencing. Given that species and strain level resolution and functional insights are better addressed by metagenomic shotgun metagenomic sequencing, the field is expected to greatly transition to these more advanced methods, in addition to increased adoption of other "omics" techniques. Functions of many PRR proteins remain to be determined to date. Moreover, while most previous research pursued a reductionist approach, it is expected that many complex interactions between different PRRs and inflammasomes exist, which have to be unraveled yet in order to gain a systemic understanding. Given the numerous contradictory results in the field, strategies are urgently required to more stringently account for inter-facility microbiome differences in order to ensure robustness and reproducibility of immunological research. Here, it is imperative to carefully consider breeding and housing in the experimental design and data analysis. Finally, it must be kept in mind that many inter-species divergences between murine and human PRRs and inflammasomes exist, which hamper translation of murine studies into the human condition. Thus, future efforts must be directed at studying innate immune sensors in the context of human health and disease.

9 | PROSPECTS FOR FUTURE THERAPEUTIC APPLICATIONS

In the sections above, we highlighted that PRRs are vital for the host's homeostasis and survival. Nevertheless, inappropriate PRR activation, in particular dysregulated inflammasome signaling, has been implicated in numerous diseases. Thus, there is a growing interest and great anticipation toward translation of innate immune sensor biology into therapeutic applications.[25,221] Therapies attempting to neutralize IL-1β signaling, which is downstream of signaling by many innate immune receptors[2] and has a crucial role as a gatekeeper of inflammation,[222] recently gained prominence. The Canakinumab Anti-Inflammatory Thrombosis Outcome Study (CANTOS) was initiated in 2011.[223] It was based on the now popular hypothesis that chronic low-grade inflammation underlies the onset and progression of metabolic and cardiovascular diseases. This large phase 3 clinical trial aimed at studying the cardiovascular outcomes of canakinumab-mediated IL-1β blockade. The trial showed that IL-1β neutralization reduced the incidence of cardiovascular disease.[224] However, it did not reduce the incidence of new-onset diabetes mellitus,[225] highlighting that

pathophysiological conclusions drawn from preclinical research are in some instances too simplistic, and may neglect the more nuanced aspects of innate immune sensor regulation and simultaneous contributions of various immune components. Surprisingly, an exploratory subset analysis of the CANTOS trial indicated a protective effect of IL-1β neutralization from lung cancer.[226] Together, these data highlight the complex and context-specific roles of IL-1 in preventing or promoting disease and the need for further studies. PRRs have been shown to engage in mutual cross-regulation.[2] Given that PRRs show different expression patterns in a tissue or even cell type–specific manner, the nature of their interactions (activating vs inhibitory) and downstream immunological outcomes will likely depend on the specific context of their activation. This imposes an additional layer of complexity on efforts toward therapeutic translation. At the same time, deciphering of those complex mechanisms holds great medical promise. Most of existing preclinical interventional studies focused on the Nlrp3 inflammasome.[25] Small molecular inhibitors of the Nlrp3 inflammasome showed varying degrees of specificity, whereby a high specificity is a priori desirable given the crucial functions of PRRs/inflammasomes in host protection. Although the high specificity of compounds such as MCC950 is promising,[227] their safety profiles remain an open and critical question, given the centrality of their treatment targets in a large array of vital immune responses to a wide spectrum of potential threads.

10 | CONCLUDING REMARKS

The delicate balance between homeostasis and disease is orchestrated by a fine-tuned network of innate immune signaling molecules and cascades. This sophisticated signaling network coordinates a vital defense against pathogens and other sources of host damage. The research community has only recently begun to uncover the intricate relationships between these signaling networks and the host microbiota and their medical implications. It is increasingly evident that aberrations in these innate immune responses promote the development of a wide spectrum of inflammatory diseases. Remarkable advances have been achieved in recent years in the understanding of innate immune sensor regulation and related therapeutic potentials. However, further basic and clinical research is urgently required in this dynamic and exciting field of immunology.

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CONFLICT OF INTEREST

EE is a salaried scientific consultant for DayTwo and BiomX. TL and DZ have nothing to declare.

AUTHOR CONTRIBUTIONS

All authors performed an extensive literature research, contributed substantially to discussion of the content, and wrote and edited the manuscript.

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