

Review

NLRP6: A Multifaceted Innate Immune Sensor

Maayan Levy,^{1,2} Hagit Shapiro,^{1,2} Christoph A. Thaiss,^{1,2} and Eran Elinav^{1,*}

NLRP6, a member of the nucleotide-binding domain, leucine-rich repeat-containing (NLR) innate immune receptor family, regulates inflammation and host defense against microorganisms. Similar to other NLRs, NLRP6 not only participates in inflammasome formation, but is also involved in nuclear factor- κ B (NF- κ B) and mitogen-activated protein kinase (MAPK) signaling regulation and facilitation of gastrointestinal antiviral effector functions. Additionally, NLRP6 contributes to the regulation of mucus secretion and antimicrobial peptide production, thereby impacting intestinal microbial colonization and associated microbiome-related infectious, autoinflammatory, metabolic, and neoplastic diseases. However, several of the mechanisms attributed to the functions of NLRP6 remain debatable, leaving open questions as to the relevant molecular mechanisms and interacting partners, and putative human relevance. We herein discuss recent findings related to NLRP6 activity, while highlighting outstanding questions and future perspectives in elucidating its roles in health and disease.

NLRs in the Innate Immune System

The mammalian innate immune system is a central regulatory element of organismal homeostasis. An array of signals from exogenous pathogens (pathogen-associated molecular patterns, PAMPs) and endogenous signals from damaged or dying cells (damage-associated molecular patterns, DAMPs) activate immune responses through germ line-encoded **pattern recognition receptors** (PRRs, see [Glossary](#)) that trigger downstream signaling cascades to instigate an inflammatory response. While the role of innate immunity and its PRR in the initiation of host defense responses against microbial pathogens has been established over several decades of research, cells of the innate immune system have only recently been appreciated as pivotal orchestrators of tissue function in almost every organ system.

The tasks performed by PRR signaling range from the detection of microorganisms and their products, the initiation of inflammation and regulation of adaptive immunity, to tissue repair, metabolic adaptation, and energy homeostasis. To accomplish this range of tasks, several fundamental prerequisites must be met by PRRs with regard to their mechanisms of action: (i) a strategic pattern of expression to ensure both the local and systemic detection of microorganisms; (ii) a range of recognition mechanisms to ensure a broad coverage of the rapidly evolving microbial repertoire, while receptor evolution is slower and more limited; (iii) the ability to perceive a spectrum of commensal and harmful microbial activity and interpret this spectrum with regard to the elicitation of appropriate host responses; and (iv) a context- and tissue-specific repertoire of downstream effector mechanisms that integrates signals evaluating both microbial presence and localization to ensure a controlled immune response.

Among several groups of structurally related PRRs, the NLR family of innate immune receptors stands out with regard to the wide range of triggers and functions associated with more than

Trends

NLRP6 is highly expressed in the small and large intestine, and has both inflammasome-dependent and -independent roles in the maintenance of intestinal homeostasis.

NLRP6 inflammasome-induced interleukin (IL)-18 is modulated by microbial metabolites, and downstream IL-18 secretion induces an antimicrobial peptide program in intestinal epithelial cells that is critical to prevent dysbiosis.

NLRP6 in sentinel goblet cells is required for mucus production, thereby preventing the invasion of enteric bacteria into the mucosal layer in an inflammasome-dependent, but IL-18-independent manner.

In association with the helicase DHX15, NLRP6 is involved in antiviral responses upon viral RNA sensing, in an inflammasome-independent manner.

In myeloid cells, NLRP6 negatively regulates NF- κ B signaling and thereby suppresses inflammatory responses.

¹Immunology Department, Weizmann Institute of Science, Rehovot, Israel

²These authors contributed equally.

*Correspondence: eran.elinav@weizmann.ac.il (E. Elinav).

20 members discovered to date, with the ligands and function of most NLR family members remaining unknown. Broadly, the activity of the best-understood NLRs can be classified into three categories: the first group of NLRs mediates the assembly of a multiprotein complex termed the '**inflammasome**', which regulates the post-transcriptional activation of the cytokines interleukin (IL)-1 β and IL-18 through caspase-1 [1]. Caspase-1, along with caspase-11, which is activated by noncanonical inflammasome, also initiates an inflammatory cell death, termed 'pyroptosis', by catalytic activation of gasdermin D and membrane pore formation [2–5]. Other NLRs are involved in the negative regulation of common intracellular signaling pathways, such as the NF- κ B and MAPK pathways, which influences downstream cytokine and chemokine expression [6]. Finally, NLRs participate in the regulation of antiviral immunity, including the modulation of antiviral effector functions and antigen presentation on MHC class I molecules [7].

While several NLRs have been found to belong to each of these categories, only one member to date, NLRP6, has been suggested to impact all three functional groups. In fact, the initial report identifying NLRP6, at that time still called PYPAF5, had already implicated a dual role for this protein as a regulator of both caspase-1 and NF- κ B [8]. Recently, NLRP6 has emerged as a central regulator of mucosal host–**microbiota** interactions, through mechanisms ranging from goblet cell mucus production to the orchestration of antibacterial and antiviral immunity. In this review, we summarize the mechanisms of action of this multifaceted NLR and suggest a conceptualization of the roles of its tissue and cell type specificity, while highlighting remaining controversies and open questions related to its mechanisms of activity and roles in mammalian disease regulation.

NLRP6 Regulates Host–Microbiome Interactions

Among all tissues, NLRP6 is highly expressed in kidney, liver, lung, and small and large intestines [9–11]. The function of NLRP6 has primarily been studied in the latter two organs, which are home to trillions of commensal microorganisms colonizing the mucosal surface and luminal content of the gut. The importance of the commensal community for human health and disease, and the teleological paradox of having to eradicate pathogenic infections in the scenario of tolerating trillions of mutualistic microbes, are a fundamental challenge for innate immunity and necessitate fail-safe mechanisms acting at the host–microbiome interface. Studies in both humans and mice have localized the expression of NLRP6 to exactly this interface, by showing high and specific expression in intestinal epithelial cells, both enterocytes [9,12] and colonic goblet cells [13]. Within the epithelial layer, NLRP6 is co-expressed with the inflammasome components apoptosis-associated speck-like protein containing a CARD (ASC) and caspase-1. NLRP6 deficiency is associated with low levels of intestinal IL-18 and caspase-1 activation, and *in vitro* experiments suggest that NLRP6 forms a complex with ASC and caspase-1 [14]. However, whether NLRP6 initiates the formation of an inflammasome and resultant ASC specks in intestinal epithelial cells, or alternatively impacts other inflammasome formation and associated IL-18 secretion, remains to be tested. Additionally, colonic myofibroblasts have been also suggested to express NLRP6 [10]. Interestingly, components of the NLRP6 inflammasome and its downstream cytokines are upregulated in response to microbial colonization of the intestine during infancy [14,15], indicating a role for the microbiome in inducing the NLRP6-dependent antimicrobial response. Given its expression in the gastrointestinal tract at high levels primarily within the cells of the epithelial lining, NLRP6 is strategically localized to participate in mediation of the communication networks involving the intestinal immune system and the gut microbiota.

Modulators of NLRP6 Activity

The specific expression pattern of NLRP6 suggests a role for the commensal microbiome in regulating its function. Indeed, induction of intestinal IL-18 by the microbiota is NLRP6

Glossary

Dysbiosis: an abnormal microbiome community, impacting the taxonomic composition as well as the metagenomic and metabolic function of the microbial community, that is linked to disease development. Compared with the healthy state, dysbiosis typically features blooms of pathobionts, and loss of commensals and diversity. Once the microbiota configuration is shifted, dysbiosis persists as a stable state and can assume various compositional manifestations, depending on the trigger. Several factors can drive the development of dysbiosis, including infection by a pathogen, diet, and xenobiotics, familial transmission, as well as genetics.

Inflammasome: a protein complex that functions as a sensor of the innate immune system, recognizing a diverse set of stimuli. Inflammasomes regulate the activation of caspase-1 and the production of the proinflammatory cytokines interleukin-1 β (IL-1 β) and IL-18. PRRs are important components of the inflammasome complex, among them NLRs and ALRs. Upon activation, the inflammasome complex oligomerizes to activate caspase-1, with or without the adaptor protein apoptosis-associated speck-like protein containing a CARD (ASC). A non-canonical inflammasome formed by caspase-11 can activate caspase-1, detect intracellular lipopolysaccharide (LPS) and intracellular bacteria, and mediate pyroptotic cell death and IL-1 α secretion, but not IL-1 β secretion.

Microbiota: the mammalian host harbors a dense microbial community, termed the 'microbiota'. This complex community of microorganisms colonizes the gastrointestinal tract, respiratory system, skin, and urogenital system. The microbiota comprises bacteria, viruses, and eukaryotic microbes.

Pattern recognition receptors (PRRs): cells of the innate immune system utilize germ line-encoded PRRs to sense the presence of pathogen-associated molecular patterns (PAMPs) and damage-associated molecular patterns (DAMPs). Several classes of PRR exist and can be classified according to their ligand, the downstream signaling pathway as well as the type

dependent [14]. An unbiased metabolomics approach recently identified the bile acid-derivative taurine as a microbiota-modulated metabolite that positively regulates NLRP6 signaling and IL-18 production. By contrast, the polyamine spermine and the amino acid histamine dampen NLRP6 activity [14]. Hence, NLRP6 signaling integrates information on the colonization state and metabolic function of the microbiota into its downstream signaling (Figure 1). The microbial contributors of these small molecules, the mechanisms by which they impact NLRP6 signaling, and whether these involve direct binding to NLRP6 or indirect signaling events merit further studies. Interestingly, the commensal protist *Trichomonas muscivorus* has been recently discovered to induce epithelial IL-18 [16], suggesting that protozoan members of the microbiota might similarly contribute to the modulation of epithelial inflammasome activity. Colonization with *T. muscivorus* resulted in alterations of the intestinal immune landscape, including myeloid cells, innate lymphoid cells (ILCs), as well as T_H1 and T_H17 cells [16]. The identity of the specific epithelial inflammasome responsible for the described induction of IL-18, and whether it involves NLRP6 signaling, was not addressed in this study.

Interestingly, NLRP6 expression may be also regulated by non-microbial mechanisms, such as stress-induced corticotrophin-releasing hormone (CRH) (Figure 1). Under water deprivation-associated stress, wildtype mice show elevated expression of CRH, which in turn down regulates the expression of intestinal NLRP6 inflammasome components and drives intestinal permeability [17]. Evidence for transcriptional regulation of NLRP6 comes from a transcription factor-binding analysis that showed that the NLRP6 promoter region harbors binding sites for both peroxisome proliferator-activated receptor- γ (PPAR γ) and retinoid X receptor [15]. *In vivo*, this stress-induced inflammatory response was abolished by administration of a PPAR γ agonist [17], which was further shown *in vitro* to induce NLRP6 expression in human intestinal epithelial cells [15]. However, the *in vivo* effect of PPAR γ on NLRP6 inflammasome formation and activation requires further investigation.

of immune response modulation. PRRs include Toll-like receptors (TLRs), C-type lectins (CTLs), NOD-like receptors (NLRs), RIG-I-like receptors (RLRs), AIM2-like receptors (ALRs), and OAS-like receptors (OLRs).

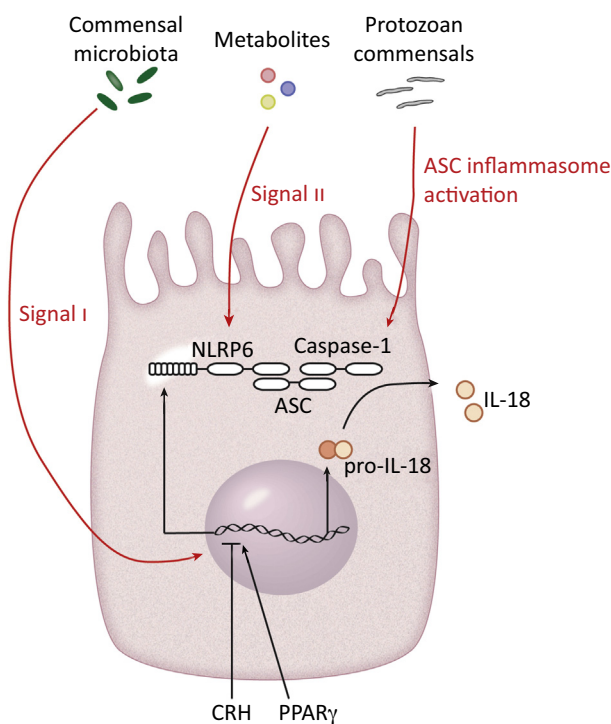


Figure 1. Activation of the NLRP6 Inflammasome. Commensal bacteria provide two signals for the activation of the NLRP6 inflammasome. The first signal is provided in the form of Toll-like receptor (TLR) ligands and promotes the transcription of inflammasome components and pro-interleukin (IL)-18. The second signal comprises microbial metabolites that modulate NLRP6 inflammasome activation. Commensal protozoans also regulate epithelial IL-18 secretion through a yet unknown mechanism. Corticotrophin-releasing hormone (CRH) has been implicated in the negative transcriptional regulation of NLRP6 inflammasome components. Abbreviations: ASC, apoptosis-associated speck-like protein containing a CARD; PPAR, peroxisome proliferator-activated receptor.

Collectively, these observations suggest a complex network of microbial and non-microbial regulators of NLRP6 activity. However, the molecular mechanisms by which these regulators influence the function of NLRP6 remain elusive.

NLRP6 in the Regulation of Goblet Cell Function

Given the centrality of microbial molecules in the regulation of NLRP6 activity, it is interesting to consider the downstream consequences of NLRP6 function at the host–microbiome interface. Intestinal epithelial cells coordinate the microbial colonization niche via a variety of mechanisms. Throughout the gastrointestinal tract, most microorganisms do not reside in direct physical contact with the epithelium, but are spatially separated from the host through a mechanism that requires the mucin MUC2, the antimicrobial peptide (AMP) REGIII γ , and the glycoprotein LYPD8, and potentially other mechanisms [18–20]. The intestinal mucus is a primary layer of defense against invasion of pathogens and protects the epithelium from physical damage. It comprises an outer mucus layer that is loose in its structure and contains microbiota, and an inner layer, which is firmly attached to the epithelium, densely packed with mucins, and, therefore, devoid of bacteria [18,21]. Epithelial goblet cells generate granules of mucins that are secreted into the lumen, where they form the intestinal mucus layer [22]. MUC2 is the primary mucin in the inner mucus layer and MUC2-deficient mice display loss of the inner mucus layer and consequent bacterial translocation to the intestinal epithelium, resulting in enhanced inflammation and carcinogenesis [18,23]. Interestingly, NLRP6 appears to have a role in the orchestration of a mucus-dependent barrier, since *Nlrp6*^{−/−} mice show dysfunctional mucus granule exocytosis [13]. A possible mechanism for NLRP6-driven mucus secretion may involve an effect of NLRP6 on autophagy (Figure 2). NLRP6-deficient mice exhibited disrupted autophagosome formation in the intestinal epithelium, and autophagy was found to be essential for mucus secretion by goblet cells. Despite these observations, a direct causative link between NLRP6, mucus secretion, and autophagy merits further investigation. In contrast to *Nlrp6*^{−/−} mice and mice lacking ASC or caspase-1, deficiency of the downstream cytokines IL-1 β or IL-18 did not affect mucus layer formation, suggesting that mucus secretion requires cell autonomous activity of the NLRP6 inflammasome [13] (Figure 2).

Furthermore, a recent study further deciphered the pathway of NLRP6-dependent mucus secretion by identifying Toll-like receptor (TLR) ligand-responsive ‘sentinel’ goblet cells localized to the upper crypt that are expelled from the epithelium upon activation and trigger mucus

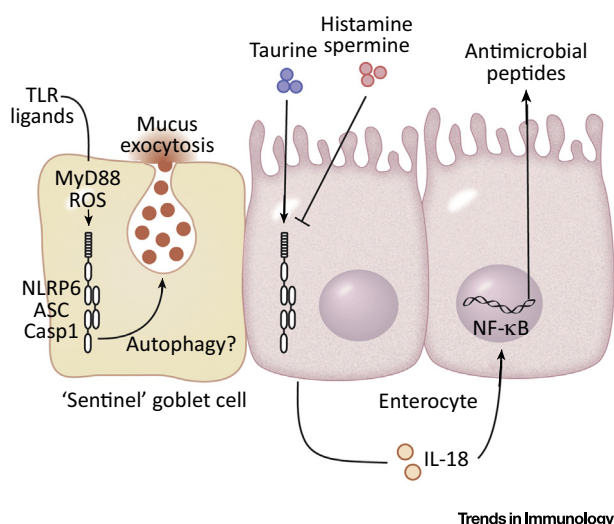


Figure 2. NLRP6 Activity in Intestinal Epithelial Cells. NLRP6 controls mucus granule exocytosis in ‘sentinel’ goblet cells in response to Toll-like receptor (TLR) ligands. Interleukin (IL)-18 released by enterocyte NLRP6 inflammasome activation promotes the secretion of antimicrobial peptides. Abbreviations: Casp1, Caspase-1; MyD88, myeloid differentiation primary response 88; NF-κB: nuclear factor-κB; ROS, reactive oxygen species.

release from neighboring goblet cells in a calcium-dependent manner [24]. These sentinel goblet cells required the components of the NLRP6 inflammasome for mucus release downstream of TLR ligand endocytosis and reactive oxygen species (ROS) production in colonic explants. Importantly, sentinel goblet cells might mediate the mucus response to bacterial invasion into the inner mucus layer in an NLRP6-dependent manner, but are not necessary for the formation of a normal mucus layer under homeostatic conditions; in addition, *Nlrp6*^{-/-} mice featured an intact inner mucus layer in this study [24].

Together, these results suggest a model in which the NLRP6 inflammasome in sentinel goblet cells is important for ‘emergency’ mucus production against bacterial invasion, a scenario whose frequency and importance for steady-state mucus production may depend on local microbiome configurations and vary among animal facilities. Further support for the notion that sentinel goblet cells could be important for protecting the lower crypt from bacterial invasion comes from the finding that NLRP6-deficient mice infected with enteric pathogens displayed enhanced attachment of bacteria to the epithelium [13]. Thus, further elucidation of NLRP6 function in mucus layer maintenance in steady-state and infectious/inflammatory contexts would be valuable.

NLRP6 in Orchestrating the Antimicrobial Peptide Landscape

In addition to the mucus barrier, epithelial cells regulate microbial colonization through the secretion of microbicidal peptides, including members of the defensin family, cathelicidins, and angiogenins [25,26]. NLRP6 initiates the formation of an ASC-dependent inflammasome and triggers the release of intestinal IL-18 in response to the microbiota [14]. IL-18, in turn, acts in an autocrine manner on the epithelium to promote the transcription of the AMPs angiogenin-4, intelectin-1, and resistin-like beta, among others [14]. Thus, while the role of NLRP6 in goblet cell mucus secretion may be inflammasome dependent but IL-18 independent, activation of AMPs through NLRP6 requires IL-18 (Figure 2), potentially reflecting the cell autonomous versus paracrine effects of epithelial NLRP6 signaling. Importantly, in the above studies, an involvement of inflammasome components was concluded based on the fact that genetic deficiencies in ASC, caspase-1, and IL-18 phenocopy the diminished levels of AMPs in mice lacking NLRP6. IL-18 released by intestinal epithelial cells also induces AMPs through an indirect mechanism. IL-18 downregulates the expression of IL-22-binding protein (IL-22BP) in intestinal myeloid cells, thereby increasing the levels of bioactive IL-22, which is a potent inducer of the epithelial antimicrobial response [27]. IL-22, in turn, upregulates the expression of pro-IL-18 transcripts and thereby provides a substrate for an activated inflammasome [28]. Dysfunctions in this NLRP6-mediated epithelial circuit result in distortion of the antimicrobial peptide repertoire and leads to **dysbiosis** (i.e., alterations in the composition and function of the intestinal microbial community [9]), which can partially be prevented by restoring IL-18 levels [14].

Thus, while NLRP6-dependent mucus secretion by goblet cells prevents abnormal epithelial adherence by intestinal bacteria, which would otherwise lead to local invasiveness, the regulation of AMPs through NLRP6 is involved in controlling the composition of the microbial community colonizing the intestinal niche. Further details on NLRP6-dependent development and maintenance of dysbiosis are detailed in Box 1.

Box 1. NLRP6 Prevents the Development and Persistence of Dysbiosis

The impact of NLRP6 on shaping intestinal mucus production and antimicrobial peptide secretion is centrally involved in microbial community structuring, and a dysbiotic taxonomic configuration arises in the absence of NLRP6 inflammasome signaling [9]. The dysbiotic microbiota observed in mice lacking NLRP6 as well as the downstream inflammasome components ASC or caspase-1 is present across different animal vivaria both at the compositional level as well as the functional capacity [14]. 16S rDNA sequencing and shotgun metagenomic sequencing studies of wildtype C57Bl/6 mice, *Nlrp6*^{-/-} mice, and mice lacking the adapter ASC at different animal facilities revealed a common dysbiotic structure that differed from the microbiota harbored by locally bred wildtype controls. While the relative abundance of bacterial taxa was comparable in *Nlrp6*-deficient mice among different facilities, C57Bl/6 mice differed from one another, such that the relative changes in taxonomic composition varied between facilities (Figure 1A–C). The disease-driving dysbiotic signature found in mice lacking NLRP6 includes mucosal-proximal

Prevotellaceae and Helicobacteriaceae species [9], which have been found to trigger a mucosal IgA response and bacterial coating [31]. Highly restrictive animal facilities with reduced microbial diversity that do not feature these bacterial triggers are expected to display different NLRP6 activation and dysbiosis manifestations. Additional possible explanations for interfacility differences include the colonization by commensal protists, recently shown to contribute to epithelial inflammasome activation and downstream modulation of intestinal immunity [16].

Furthermore, given the importance of animal husbandry and housing conditions in shaping the microbiota in animal vivaria [46], as well as the impact of vertical transmission on mouse line-specific microbiomes [47], solid conclusions about the deterministic impact of genetic deficiency on microbial community development requires stringent experimental proof. In the case of NLRP6, germ-free *Nlrp6*^{-/-} mice allowed to spontaneously colonize in a specific pathogen-free (SPF) vivarium acquired dysbiotic microbiota that was indistinguishable from that of SPF-raised *Nlrp6*^{-/-} mice, but markedly different from spontaneously colonizing wildtype controls [14], supporting *de novo* acquisition of an altered community structure in the absence of NLRP6. When germ-free C57Bl/6 and *Nlrp6*^{-/-} mice were directly colonized with the microbiota from SPF C57Bl/6 mice, or when *Nlrp6*^{-/-} mice and their littermates obtained from heterozygous breeding were separated after weaning, the microbiota configuration between wildtype and *Nlrp6*^{-/-} mice became distinct 15 weeks after colonization (Figure ID-F). These observations suggest a role for NLRP6 in controlling the development of intestinal microbial ecology.

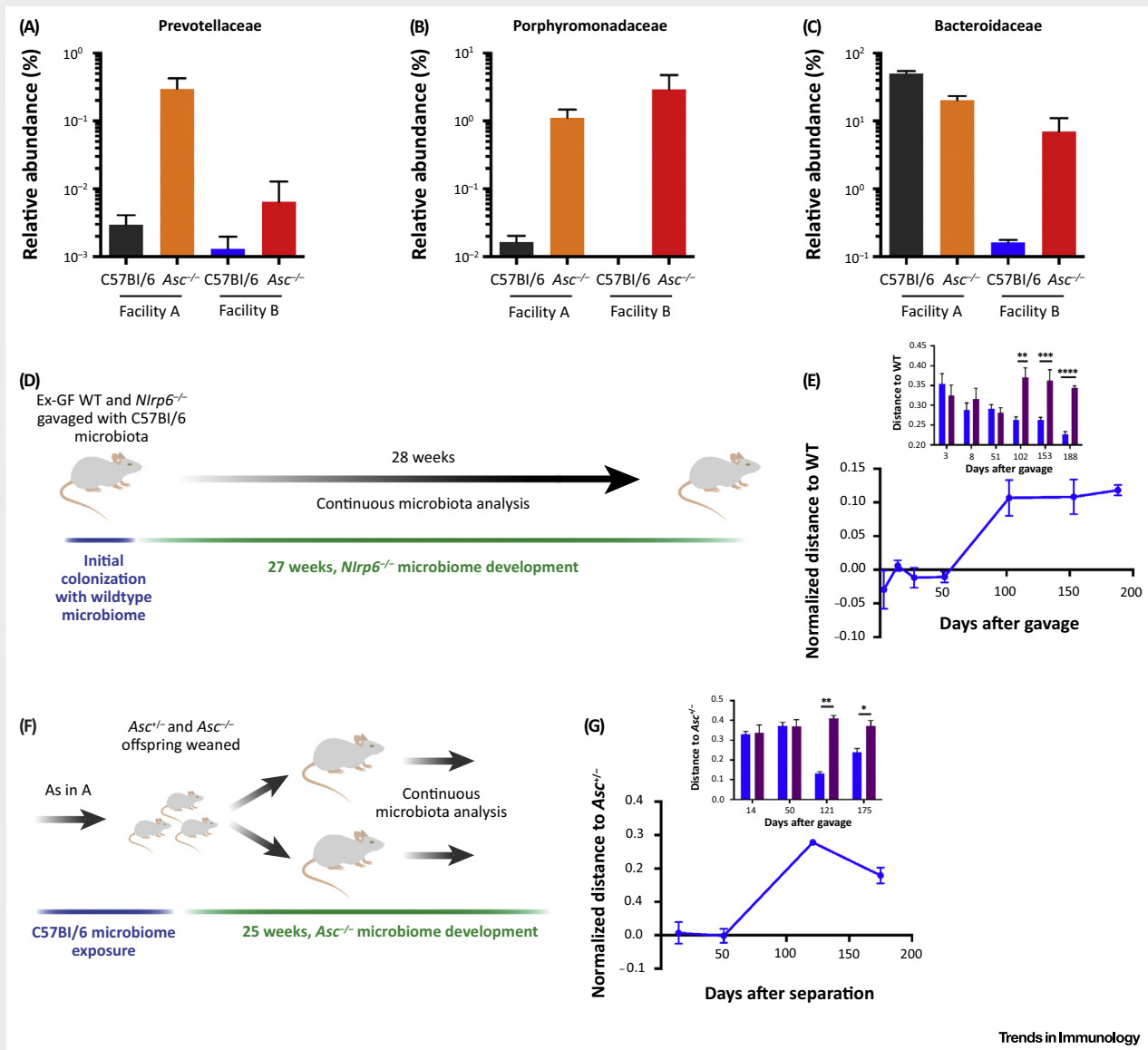


Figure 1. De Novo Development of Dysbiosis in Inflammasome-Deficient Mice. (A–C) Relative abundances of bacterial families in *Asc*-deficient mice vary between different animal facilities and wild-type controls. (D,E) Divergence of microbial communities can be observed in germ-free wild-type and NLRP6-deficient mice colonized by gavage with the microbiota from C57Bl/6 mice. Shown is a schematic and UniFrac-based distance quantification of the microbiota over time. (F,G) Divergence of microbial communities can be observed in *ASC*-deficient mice and heterozygous littermates that were followed over time after littermate separation. Shown is a schematic and UniFrac-based distance quantification of the microbiota over time. Abbreviation: WT, wildtype.

NLRP6 in the Regulation of Viral Infection

In addition to the above role in the response to intestinal bacteria, NLRP6 was recently discovered to have a critical role in response to viral infection [29]. Epithelial NLRP6 was found to bind to viral RNA via the helicase DHX15 and to regulate the expression of a large number of interferon (IFN)-stimulated genes that are critical for antiviral immunity (Figure 3). This induction of gene expression occurs through signaling via the mitochondrial adapter protein mitochondrial antiviral signaling protein (MAVS), but does not require caspase-1 [29]. Interestingly, the expression of NLRP6 itself can be induced by viral infection and type I IFNs, in a manner that is dependent on the IFN- α receptor and the transcription factors IRF3 and IRF7. Consequently, NLRP6-deficient mice are more susceptible to enteric infection with RNA viruses, such as encephalomyocarditis virus and murine norovirus. Of note, mortality after systemic infection with these viruses was not affected by the absence of NLRP6 [29], which highlights the important and specific role of this NLR in mediating intestinal mucosal host defense. Whether NLRP6, in addition to its role in orchestrating the bacterial microbiome, is involved in controlling the homeostatic composition of the enteric virome remains to be determined, but is an intriguing possibility to be explored in future studies.

Consequences of Dysbiosis in the Absence of NLRP6

A remarkable feature of dysbiosis in *Nlrp6*-deficient mice is its ability to transfer and establish itself in wildtype mice [9,30]. Upon cohousing or cross-fostering of wildtype mice with *Nlrp6*^{-/-} mice, the dysbiotic microbiota dominates the intestinal community and stably persists. Intriguingly, this is partially contributed by metabolite-mediated modulation of NLRP6 signaling in the wildtype recipients [14]. Combinatorial levels of taurine, histamine, and spermine, while

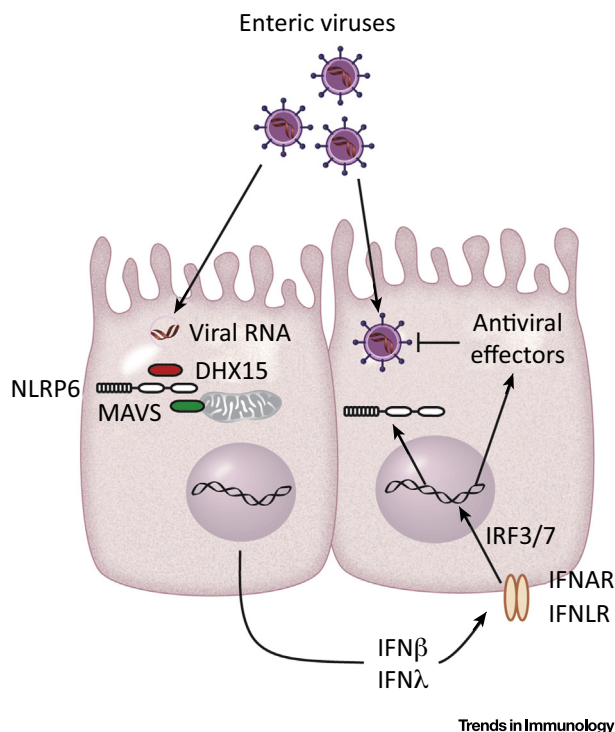
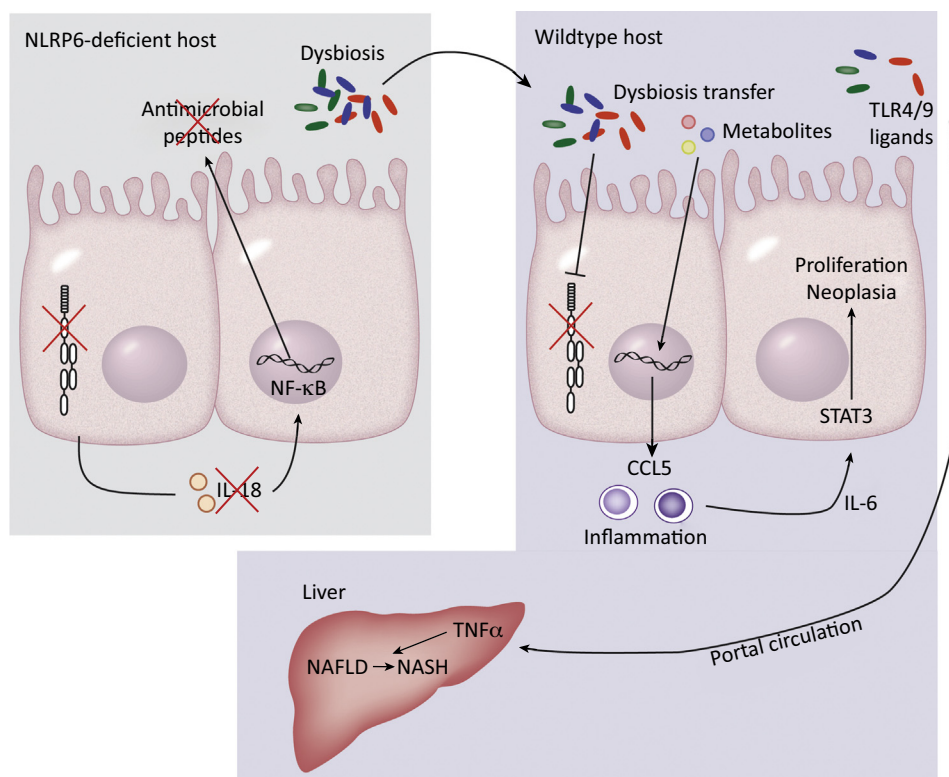


Figure 3. NLRP6 in Intestinal Antiviral Immunity. NLRP6 binds to viral RNA through DEAD-box helicase 15 (DHX15) and triggers the induction of type I/III interferons (IFNs) through mitochondrial antiviral signaling protein (MAVS). Type I/III IFNs then stimulate the expression of antiviral effector genes, including *Nlrp6* itself. Abbreviations: IFNAR/IFNLR, IFN- α /IFN- λ receptor; IRF, IFN regulatory factor.



Trends in Immunology

Figure 4. Consequences of Dysbiosis Associated with NLRP6 Deficiency. Aberrations in NLRP6 inflammasome signaling lead to dysbiosis development. The dysbiotic microbiota can dominantly establish itself in a wildtype host by metabolite-mediated suppression of NLRP6 inflammasome signaling. Dysbiosis also leads to inflammatory responses dependent on the chemokine CCL5 and interleukin (IL)-6 secretion, which in turn drive epithelial proliferation and susceptibility to cancer. Influx of Toll-like receptor-4 (TLR4) and TLR9 ligands from the dysbiotic microbiota to the liver drives tumor necrosis factor (TNF)- α -dependent progression from nonalcoholic fatty liver disease (NAFLD) to nonalcoholic steatohepatitis (NASH). Abbreviation: STAT, signal transducer and activator of transcription.

activating NLRP6 signaling in the homeostatic situation, are distorted in the dysbiotic setting, leading to dampening of NLRP6 activation. Thereby, the dysbiotic microbiome renders the host functionally deficient in IL-18 as well as in downstream antimicrobial peptide production, thereby facilitating the perpetuation of aberrant microbial colonization (Figure 4).

The disease-driving dysbiotic signature found in mice lacking *Nlrp6* includes mucosal-proximal Prevotellaceae and Helicobacteriaceae species, as documented in one animal facility [9]. While the causative role of NLRP6 in driving gut community structure, and the family and vivarium-driven modulatory roles of this microbial effect remain to be conclusively determined (Box 1), spontaneous colonization of *Nlrp6*-deficient mice, or wildtype microbiome transfer into germ-free *Nlrp6*-deficient mice both resulted in the *de novo* formation of dysbiosis (see Figure 1 in Box 1). This suggests that, in a diverse enough microbial environment, NLRP6 signaling is involved in the regulation of microbiome composition and function. As such, it is plausible that local microbiome configurations and diversity may influence the level and type of dysbiosis that occurs in the absence of NLRP6. Furthermore, in the vivarium in which both Prevotellaceae and Helicobacteriaceae were associated with NLRP6 deficiency-induced dysbiosis, both taxa were found to trigger a mucosal IgA response and bacterial coating [31], suggesting that they are locally invasive and actively contribute to colitis induction in the setting of that particular vivarium.

The consequences of intestinal community alterations in *Nlrp6*-deficient mice and wildtype recipients of this microbiota are manifold, ranging from intestinal inflammation to metabolic disease [32]. Microbial-triggered epithelial CCL5 enhances both recruitment of inflammatory immune cells to the intestine and the susceptibility to chemically triggered colitis [9–11] and impaired intestinal wound healing [10]. Similar microbially transmissible phenotypes were observed in mice lacking the inflammasome adapter ASC, caspase-1, and the downstream cytokine IL-18 [9]. Consequently, antibiotic treatment as well as exogenous supplementation of IL-18 ameliorates the disease [14]. The inflammatory state associated with dysbiosis in the absence of *Nlrp6* also led to enhanced intestinal tumorigenesis in a mouse model of inflammation-induced colorectal cancer [10,11,33]. This susceptibility to intestinal neoplasia was microbially transmissible and driven by IL-6 signaling in the intestinal epithelium and resultant aberrant proliferation [33].

Furthermore, dysbiosis and enhanced epithelial-proximal colonization in NLRP6 inflammasome-deficient mice triggers exacerbated translocation of microbial products and their entry into the portal vein. In particular, TLR4 and TLR9 agonists were found in the portal circulation of NLRP6 inflammasome-deficient mice and wildtype recipients of dysbiosis [34]. These TLR ligands triggered the production of tumor necrosis factor (TNF)- α in the liver. Under conditions of nonalcoholic fatty liver disease (NAFLD) induced by feeding of a methionine choline-deficient diet (MCDD) to mice, this hepatic dysbiosis-mediated inflammation facilitated the progression to nonalcoholic steatohepatitis (NASH), a condition that in humans may lead to cirrhosis and development of life-threatening complications, such as hepatocellular carcinoma and portal hypertension [35]. Furthermore, leptin-deficient genetically obese mice and diet-induced obese mice featured enhanced steatosis and inflammation when exposed to dysbiosis arising in NLRP6 inflammasome-deficient mice [34]. Thus, NLRP6 is pivotal in preventing aberrant microbiota–host interactions, which in turn are required to prevent adverse metabolic hepatic consequences (Figure 4). Furthermore, a recent study reported enhanced expression of hepatic IL-1 β upon exposure to a high-fat diet and lipopolysaccharides (LPS) that induced obesity and metabolic complications, including NAFLD development [36]. Hepatic cell lines treated with palmitic acid and LPS displayed enhanced expression of NLRP6 inflammasome, which was abrogated by treatment with a PPAR δ agonist. However, a direct causative role between PPAR δ and NLRP6 inflammasome sensing in NAFLD development and progression has not been investigated to date.

Nlrp6 as a Regulator of Immune Signaling

In addition to epithelial cells of the gastrointestinal tract, NLRP6 was recently shown to be expressed in infiltrating intestinal Ly6C^{hi} inflammatory monocytes and neutrophils upon chemical induction of colitis [37]. Adoptive transfer of Ly6C^{hi} monocytes from wildtype mice to *Nlrp6*-deficient mice resulted in protection from colitis, a reduction in barrier permeability, lower bacterial translocation, and increased survival of the mice [37]. Characterization of Ly6C^{hi} monocytes in mice with *Nlrp6* deletion showed attenuated ROS and IL-18-induced TNF- α production, suggesting that NLRP6-dependent activation of inflammatory monocytes leads to TNF- α production and amelioration of intestinal inflammation (Figure 5).

Conversely, NLRP6 was also shown to have a fundamental role in regulating the canonical NF- κ B and MAPK pathways in myeloid cells [38]. In macrophages and neutrophils, NLRP6 was found to specifically inhibit TLR2 and TLR4-dependent activation of downstream signaling pathways, leading to enhanced production of inflammatory cytokines, such as TNF- α and IL-6, in the absence of NLRP6 regulation (Figure 5). Consequently, *Nlrp6*-deficient mice featured enhanced inflammatory responses, myeloid cell recruitment, and bacterial clearance in response to systemic infection with the intracellular pathogens *Listeria monocytogenes* and *Salmonella typhimurium* [38]. How the opposing roles of NLRP6 in promoting TNF- α in Ly6C^{hi}

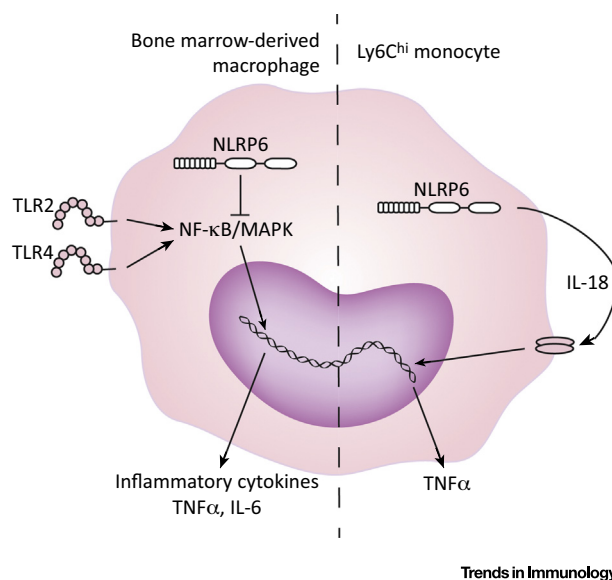


Figure 5. Control of Inflammatory Signaling by NLRP6 in Myeloid Cells. NLRP6 functions as a negative regulator of Toll-like receptor (TLR)-induced nuclear factor- κ B (NF- κ B) and mitogen-activated protein kinase (MAPK) signaling, thereby suppressing cytokine secretion in bone marrow-derived macrophages. By contrast, NLRP6-mediated IL-18 drives tumor necrosis factor (TNF)- α in monocytes.

monocytes versus suppressing TNF- α in bone marrow-derived macrophages can be reconciled awaits further studies, but likely involves differential regulation in the different cell types studied.

NLRP6 in Humans

While most insights into the expression and function of NLRP6 have derived from studies in mice, data on the activity of NLRP6 in humans remain limited. Nonetheless, RNA-sequencing analyses of human intestinal tissue found high expression of NLRP6, which was specifically located to intestinal epithelial cells, recapitulating the findings in mice [12]. However, while the expression of NLRP6 in mice was found to be high in both the small intestine and colon, in healthy humans the expression of NLRP6 transcripts and protein was primarily detected in duodenum, jejunum, and ileum [12].

With regard to a potential disease relevance of NLRP6 in humans, adipose tissue NLRP6 and circulating levels of IL-18 were significantly upregulated in patients with NASH and portal fibrosis compared with patients without portal fibrosis [39]. Moreover, while NLRP6 was suggested to have a role in protection from colorectal cancer in mice, changes in NLRP6 expression were observed in neither human colorectal cancer samples compared with healthy controls [40,41] nor patients with HIV compared with healthy individuals. Intriguingly, a single nucleotide polymorphism in *NLRP6* has been linked to mean platelet volume in a large genome-wide association study [42], a result that warrants the closer examination of *NLRP6* expression in human platelets and a potential involvement of this NLR in platelet function.

Concluding Remarks and Future Perspective

While the *in vivo* function of NLRP6 had been completely unknown until a few years ago, this NLR has since been associated with a large number of immunological functions in the regulation of host-microbiome interactions, antiviral immunity, and the regulation of immune signaling in myeloid cells. One of the most interesting outstanding questions regarding the multifaceted biology of NLRP6 is how the precise context and cell type specificity of NLRP6

Outstanding Questions

Does NLRP6 form an inflammasome complex *in vivo*?

What are the regulators that determine the secretion of IL-18 but not IL-1 β in response to NLRP6 activation?

How does ligand recognition occur in the case of NLRP6? Are metabolite ligands bound directly, or is NLRP6 signaling modulated indirectly through unknown upstream mechanisms?

Is the intestinal virome shaped by the RNA-sensing and interferon-induction capabilities of NLRP6?

What is the role of different microbial compositions among animal vivaria in providing metabolite and viral nucleic acid ligands for NLRP6 activation, downstream signaling, mucus secretion, dysbiosis development, and the manifestation of inflammatory disease?

What determines the context-specific activity of NLRP6 as a metabolite sensor, a viral RNA sensor, and a negative regulator of NF- κ B? What determines the cell type specificity of these contexts?

What is the role of NLRP6 in humans?

activity is guaranteed (see Outstanding Questions). Recent studies have unraveled a remarkable range of NLRP6 functions, from the regulation of autophagy and mucus granule exocytosis in goblet cells, orchestration of antimicrobial peptide secretion, epithelial regeneration, and viral RNA recognition, to the regulation of inflammatory signaling in myeloid cells. While the versatile cellular functions of NLRP6 appear remarkably diverse, this may not yet be a comprehensive list, as suggested by the recent association of an inflammasome-independent function of NLRP6 with the recovery of peripheral nerves after injury [43]. Another yet unexplored finding is the sequence similarity of NLRP6 to the vasopressin receptor [44]. A unified model that comprises all of these cellular activities is lacking the regulatory mechanisms that determine NLRP6 function in each context. One plausible explanation is that each of these functions occurs in a different cell type. Indeed, while mucus and AMP secretion, as well as viral sensing, are *in vivo* epithelial functions of NLRP6, the regulation of NF- κ B and MAPK signaling has been described in bone marrow-derived macrophages. Likewise, the findings that mice lacking NLRP6 feature improved resistance to systemic infection with gram-negative Enterobacteriaceae [38] while being more susceptible to intestinal infection with members of the same bacterial family [13] emphasizes the concept that NLRP6 may perform highly differential functions in a cell type- and context-specific manner. In this regard, it is important to note that, while expression analyses and bone marrow chimera experiments were used to highlight the contribution of specific cellular compartments, all studies interrogating the activity of this NLR have made use of knockout mice lacking NLRP6 in the entire organism. Studies of mice harboring conditional NLRP6 deletion in intestinal epithelial cells, myeloid cells, and other cell types will be critical to elucidate the full spectrum of cell type-intrinsic and -extrinsic roles of NLRP6 in the orchestration of host-microbial interactions.

Additional layers of regulation, such as differential binding partners, may add to this complexity. In this regard, an interesting observation was made in yet another cell type, hepatocytes, in which transcripts of *Nlrp6* were found to be retained in the nucleus to a high degree [45]. The regulation of *Nlrp6* expression, as well as its potential role in the determining of NLRP6 function, is an exciting avenue for future study.

Similar complexity might also exist in the upstream regulators of NLRP6. While the microbial metabolites taurine, histamine, and spermine have been shown to influence NLRP6 activity in the context of inflammasome activation, binding to the helicase DHX15 and viral RNA appears to be the decisive step in triggering antiviral signaling through MAVS. Whether both regulations can occur simultaneously, and whether the antiviral and antibacterial functions of NLRP6 are cross-regulated remain to be determined.

With regard to the function of NLRP6 at the intestinal mucus secretion, it is remarkable that such a plethora of critical functions at the host-microbiome interface are orchestrated by a single innate immune receptor. One may speculate that NLRP6 has been critical during the coevolution of the host and its microbiota, given its central involvement in regulating the microbial colonization niche, spatial separation from the intestinal epithelium, and host defense against enteric infection with bacteria and viruses.

Finally, our understanding of the cellular and molecular functions of NLRP6 summarized here has been derived from studies in mice. By contrast, the role of NLRP6 in humans remains elusive. The finding that human NLRP6 is highly and specifically expressed in intestinal epithelial cells suggests that it recapitulates some of the functions discovered in mice with regard to regulation of host-microbiota interactions [12], but exploring the full spectrum of cellular functions of NLRP6 in humans, and its implications in human disease, will be part of the next chapter of research on this remarkable innate immune receptor.

Acknowledgments

We thank the members of the Elinav lab for discussions and apologize for authors whose work was not cited because of space constraints. C.A.T. is supported by a Boehringer Ingelheim Fonds PhD Fellowship. E.E. is supported by: Y. and R. Ungar; the Abisch Frenkel Foundation for the Promotion of Life Sciences; the Gurwin Family Fund for Scientific Research; the Leona M. and Harry B. Helmsley Charitable Trust; the Crown Endowment Fund for Immunological Research; the estate of J. Gitlitz; the estate of L. Hershkovich; the Benozio Endowment Fund for the Advancement of Science; the Adelis Foundation; J.L. and V. Schwartz; A. and G. Markovitz; A. and C. Adelson; the French National Center for Scientific Research (CNRS); D.L. Schwarz; the V.R. Schwartz Research Fellow Chair; L. Steinberg; J. N. Halpern; A. Edelheit; grants funded by the European Research Council; a Marie Curie Integration grant; the German-Israeli Foundation for Scientific Research and Development; the Israel Science Foundation; the Minerva Foundation; the Rising Tide Foundation; the Helmholtz Foundation; and the European Foundation for the Study of Diabetes. E.E. is the incumbent of the Rina Gudinski Career Development Chair and a senior fellow of the Canadian Institute For Advanced Research (CIFAR).

References

- Henao-Mejia, J. *et al.* (2014) Inflammasomes and metabolic disease. *Annu. Rev. Physiol.* 76, 57–78
- He, W.T. *et al.* (2015) Gasdermin D is an executor of pyroptosis and required for interleukin-1 β secretion. *Cell Res.* 25, 1285–1298
- Kayagaki, N. *et al.* (2015) Caspase-11 cleaves gasdermin D for non-canonical inflammasome signalling. *Nature* 526, 666–671
- Liu, X. *et al.* (2016) Inflammasome-activated gasdermin D causes pyroptosis by forming membrane pores. *Nature* 535, 153–158
- Shi, J. *et al.* (2015) Cleavage of GSDMD by inflammatory caspases determines pyroptotic cell death. *Nature* 526, 660–665
- Thaiss, C.A. *et al.* (2016) Integration of innate immune signaling. *Trends Immunol.* 37, 84–101
- Lupfer, C. and Kanneganti, T.D. (2013) The expanding role of NLRs in antiviral immunity. *Immunol. Rev.* 255, 13–24
- Grenier, J.M. *et al.* (2002) Functional screening of five PYPAF family members identifies PYPAF5 as a novel regulator of NF- κ B and caspase-1. *FEBS Lett.* 530, 73–78
- Elinav, E. *et al.* (2011) NLRP6 inflammasome regulates colonic microbial ecology and risk for colitis. *Cell* 145, 745–757
- Normand, S. *et al.* (2011) Nod-like receptor pyrin domain-containing protein 6 (NLRP6) controls epithelial self-renewal and colorectal carcinogenesis upon injury. *Proc. Natl. Acad. Sci. U. S. A.* 108, 9601–9606
- Chen, G.Y. *et al.* (2011) A functional role for Nlrp6 in intestinal inflammation and tumorigenesis. *J. Immunol.* 186, 7187–7194
- Gremel, G. *et al.* (2015) The human gastrointestinal tract-specific transcriptome and proteome as defined by RNA sequencing and antibody-based profiling. *J. Gastroenterol.* 50, 46–57
- Wlodarska, M. *et al.* (2014) NLRP6 inflammasome orchestrates the colonic host-microbial interface by regulating goblet cell mucus secretion. *Cell* 156, 1045–1059
- Levy, M. *et al.* (2015) Microbiota-modulated metabolites shape the intestinal microenvironment by regulating NLRP6 inflammasome signaling. *Cell* 163, 1428–1443
- Kempster, S.L. *et al.* (2011) Developmental control of the Nlrp6 inflammasome and a substrate, IL-18, in mammalian intestine. *Am. J. Physiol. Gastrointest. Liver Physiol.* 300, G253–G263
- Chudnovskiy, A. *et al.* (2016) Host-protozoan interactions protect from mucosal infections through activation of the inflammasome. *Cell* 167, 444–456
- Sun, Y. *et al.* (2013) Stress-induced corticotropin-releasing hormone-mediated NLRP6 inflammasome inhibition and transmissible enteritis in mice. *Gastroenterology* 144, 1478–1487
- Johansson, M.E. *et al.* (2008) The inner of the two Muc2 mucin-dependent mucus layers in colon is devoid of bacteria. *Proc. Natl. Acad. Sci. U. S. A.* 105, 15064–15069
- Okumura, R. *et al.* (2016) Lypd8 promotes the segregation of flagellated microbiota and colonic epithelia. *Nature* 532, 117–121
- Vaishnav, S. *et al.* (2011) The antibacterial lectin RegIII γ promotes the spatial segregation of microbiota and host in the intestine. *Science* 334, 255–258
- Gill, N. *et al.* (2011) Roadblocks in the gut: barriers to enteric infection. *Cell Microbiol.* 13, 660–669
- Tytgat, K.M. *et al.* (1994) Biosynthesis of human colonic mucin: Muc2 is the prominent secretory mucin. *Gastroenterology* 107, 1352–1363
- Velcich, A. *et al.* (2002) Colorectal cancer in mice genetically deficient in the mucin Muc2. *Science* 295, 1726–1729
- Birchenough, G.M. (2016) A sentinel goblet cell guards the colonic crypt by triggering Nlrp6-dependent Muc2 secretion. *Science* 352, 1535–1542
- Hooper, L.V. *et al.* (2003) Angiogenins: a new class of microbicidal proteins involved in innate immunity. *Nat. Immunol.* 4, 269–273
- Salzman, N.H. *et al.* (2010) Enteric defensins are essential regulators of intestinal microbial ecology. *Nat. Immunol.* 11, 76–83
- Huber, S. *et al.* (2012) IL-22BP is regulated by the inflammasome and modulates tumorigenesis in the intestine. *Nature* 491, 259–263
- Munoz, M. *et al.* (2015) Interleukin-22 induces interleukin-18 expression from epithelial cells during intestinal infection. *Immunity* 42, 321–331
- Wang, P. *et al.* (2015) Nlrp6 regulates intestinal antiviral innate immunity. *Science* 350, 826–830
- Elinav, E. *et al.* (2013) Analysis of microbiota alterations in inflammasome-deficient mice. *Methods Mol. Biol.* 1040, 185–194
- Palm, N.W. *et al.* (2014) Immunoglobulin A coating identifies colitogenic bacteria in inflammatory bowel disease. *Cell* 158, 1000–1010
- Levy, M. *et al.* (2015) Inflammasomes and the microbiota-partners in the preservation of mucosal homeostasis. *Semin. Immunopathol.* 37, 39–46
- Hu, B. *et al.* (2013) Microbiota-induced activation of epithelial IL-6 signaling links inflammasome-driven inflammation with transmissible cancer. *Proc. Natl. Acad. Sci. U. S. A.* 110, 9862–9867
- Henao-Mejia, J. *et al.* (2012) Inflammasome-mediated dysbiosis regulates progression of NAFLD and obesity. *Nature* 482, 179–185
- Rinella, M.E. (2015) Nonalcoholic fatty liver disease: a systematic review. *J. Am. Med. Assoc.* 313, 2263–2273
- Lee, H.J. *et al.* (2015) Peroxisome proliferator-activated receptor- δ agonist ameliorated inflammasome activation in nonalcoholic fatty liver disease. *World J. Gastroenterol.* 21, 12787–12799
- Seregin, S.S. *et al.* (2016) NLRP6 function in inflammatory monocytes reduces susceptibility to chemically induced intestinal injury. *Mucosal Immunol.* 196 (1 Suppl), 126.14
- Anand, P.K. *et al.* (2012) NLRP6 negatively regulates innate immunity and host defence against bacterial pathogens. *Nature* 488, 389–393
- Mehta, R. *et al.* (2014) Expression of NALPs in adipose and the fibrotic progression of non-alcoholic fatty liver disease in obese subjects. *BMC Gastroenterol.* 14, 208
- Liu, R. *et al.* (2015) Expression profile of innate immune receptors, NLRs and AIM2, in human colorectal cancer: correlation with

- cancer stages and inflammasome components. *Oncotarget* 6, 33456–33469
41. Nasi, M. *et al.* (2015) Analysis of inflammasomes and antiviral sensing components reveals decreased expression of NLRX1 in HIV-positive patients assuming efficient antiretroviral therapy. *AIDS* 29, 1937–1941
42. Gieger, C. *et al.* (2011) New gene functions in megakaryopoiesis and platelet formation. *Nature* 480, 201–208
43. Ydens, E. *et al.* (2015) Nlrp6 promotes recovery after peripheral nerve injury independently of inflammasomes. *J. Neuroinflammation* 12, 143
44. Albrecht, M. *et al.* (2003) Identification of mammalian orthologs associates PYPAF5 with distinct functional roles. *FEBS Lett.* 538, 173–177
45. Bahar Halpern, K. (2015) Nuclear Retention of mRNA in Mammalian Tissues. *Cell Rep.* 13, 2653–2662
46. Stappenbeck, T.S. and Virgin, H.W. (2016) Accounting for reciprocal host-microbiome interactions in experimental science. *Nature* 534, 191–199
47. Ubeda, C. *et al.* (2012) Familial transmission rather than defective innate immunity shapes the distinct intestinal microbiota of TLR-deficient mice. *J. Exp. Med.* 209, 1445–1456