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NLRP6 in Infection and Inflammation

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Abstract

NLRs play fundamental roles in host-defense and inflammatory disorders. NLRP6 is a newly characterized member of this family that inhibits NF-κB and MAP-kinase dependent immune signaling to hamper anti-microbial defense. Further, NLRP6 regulates intestinal inflammation by maintaining gut microbiota composition. In this review, we examine the recent studies and emphasize the key functions regulated by NLRP6.

Keywords

NLRP6; NLR; Innate Immunity; Infection; Microbiota; Intestinal Homeostasis

1. Introduction

Members of the Nod-Like Receptor (NLR) family play fundamental roles in immunity and host defense. Gain-of-function mutations in NLRs are correlated with several inflammatory disorders. Further, certain NLRs play critical roles in pathogen recognition and activate downstream signaling cascades resulting in anti-microbial defense [1-3]. However, a functional role for most of the family members is yet to be elucidated. While pattern-recognition receptors such as Toll-like receptors (TLRs) are well defined, the biology of NLRs as a family is only beginning to be understood. In contrast to TLRs that mostly sense extracellular microbes, NLRs sense microbial or danger stimuli in the cytoplasm [1, 2]. Remarkably, pattern recognition receptors (PRRs) recognize pathogen-associated molecular patterns (PAMPs), which are conserved among pathogens and non-pathogens alike. As such, they are unable to differentiate microbes based on their pathogenicity [4, 5]. However, invasion of the host-cytoplasm is a more serious threat and NLRs are aptly localized to respond to these insults.

NLRs are comprised of 23 family members in humans and 34 in the mouse genome [6, 7]. Structurally, NLRP6 resembles the well-studied member NLRP3 consisting of a N-terminal Pyrin domain, a central NOD domain and C-terminal leucine-rich repeats. Following activation, NLRP3, as also NLRC4 and NLRP1, forms a multimeric structure known as the 'inflammasome' typically leading to oligomerization of the adaptor protein ASC and activation of the cysteine protease pro-caspase-1 [8], which further cleaves precursors of IL-1β and IL-18 to their biologically active forms [6, 9-12]. NLRP3 is activated in response

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to numerous diverse stimuli by equally distinct mechanisms that include changes in ion flux, generation of reactive oxygen species and lysosomal disruption. In contrast to a prior belief of direct sensing, NLRC4 was recently shown to attain specificity through distinct NAIP proteins that recognize a functional bacterial type III or IV secretion system or flagellin and subsequently activate NLRC4 [13, 14]. While it is beginning to be deciphered for a limited set of NLRs, the specific ligand and activation mechanisms for most of the family members remains unknown.

Characterization of the NLR family members is essential to gain deeper understanding of the functions of these receptors in immunity and inflammatory disorders. Recently, NLRC5 was described to act as a transactivator of MHC class I genes (CITA) [15]. NLRP10 was proposed to initiate adaptive immunity by dendritic cells and its ablation resulted in increased susceptibility to *Candida albicans* infection [16, 17]. NLRP7 was demonstrated to assemble an inflammasome in response to microbial diacylated lipopeptides in human macrophages [18]. In the last few years, a number of NLRs were described that function to restrain immune signaling. NLRX1 inhibited inflammatory cytokine production via the MAVS-RIG-I pathway [19]. NLRC3 and NLRP12 inhibited TLR-dependent activation of NF-κB [20-22]. Similarly, our lab described role for NLRP6 in inhibiting TLR-dependent NF-κB and MAP-kinase signaling [23]. Below, we will discuss in detail the newly discovered roles of NLRP6 in inflammation and host-defense.

2. NLRP6 protects against colitis and colitis-associated carcinogenesis

Ulcerative colitis is an inflammatory disorder of the gastrointestinal tract affecting 1.4 million people in the United States [24]. During DSS-induced experimental colitis in mice, tight junctions between epithelial cells in the colon are disrupted allowing permeability of commensal bacteria and bacterial ligands into the underlying lamina propria thereby promoting gratuitous inflammation. In this model, *Nlrp6*-deficient mice displayed enhanced susceptibility accompanied with reduced serum IL-18 levels [25]. Interestingly, this was correlated to altered fecal microbiota composition in *Nlrp6*-deficient mice as demonstrated by increased representation of the bacterial phyla Bacteroidetes (family Prevotellaceae) and TM7. These microbial communities also displayed enhanced expansion in $Asc^{-/-}$ and $Casp1^{-/-}$ mice that demonstrated equal susceptibility to colitis thereby implying a role for NLRP6 in inflammasome formation. Furthermore, the colitogenic gut microbiota could establish increased representation in WT mice when co-housed with any of the above-mentioned knock-out animals [25]. The expansion of Prevotellaceae stimulated elevated levels of CCL5 (RANTES) in colons of $Asc^{-/-}$, $Nlrp6^{-/-}$ and in susceptible co-housed WT mice [25]. However, despite comparable acquisition of Prevotellaceae, $Ccl5^{-/-}$ mice exhibited significant resistance to DSS-induced colitis [25] thus implying a downstream function for CCL5 in enhancing disease (Fig. 1). Finally, in agreement with a role for IL-18, mice deficient in *il18* also displayed expansion of Prevotellaceae and thus enhanced colon CCL5 expression. These findings thus demonstrate a critical role for inflammasome-dependent IL-18 production in maintaining composition of the intestinal microbiota (Fig. 1). However, certain differences exist in the fecal microbiota composition of $Asc^{-/-}$ and $Nlrp6^{-/-}$ mice compared to $il18^{-/-}$ mice [25] suggesting the presence of additional IL-18-independent mechanisms of microbiota regulation. Altered microbiota composition was also demonstrated to result in exacerbated non-alcoholic fatty liver disease and non-alcoholic steatohepatitis in *Nlrp6*- or *il18* - deficient mice [26]. However, whether IL-18 directly regulates the growth of bacteria belonging to family Prevotellaceae needs to be examined.

Chronic inflammation during colitis predisposes individuals to an increased risk of colorectal cancer, the second leading cause of death due to cancer in the US. In 2009, 136,000 people were diagnosed with colorectal cancer resulting in 50,000 deaths [27]. By

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employing the AOM/DSS model, Chen et al. demonstrated enhanced susceptibility of *Nlrp6*-deficient mice to colitis-associated carcinogenesis (CAC). This was associated with reduced serum and colonic IL-18 levels [28]. However, no difference in IL-1β and IL-18 transcripts or a role for IL-1R signaling was observed [29]. Indeed, *Nlrp6* expression has been proposed as a diagnostic marker in colon adenocarcinoma detection [30].

Enhanced susceptibility to colitis and colorectal tumorigenesis has also been demonstrated before in mice lacking components of the NLRP3 inflammasome owing to reduced IL-18 production in these mice [31-33]. NLRP3-inflammasome dependent IL-18 production maintained colonic epithelial cell integrity in these studies (Fig. 1). Although, whether this was accompanied with altered intestinal microbiota is not known. However, dysregulated colonic β-defensin expression and consequently reduced antimicrobial ability in *Nlrp3*$^{-/-}$ mice was shown to be associated with altered gut flora represented by enhanced expansion of bacterial groups belonging to several families including Enterobacteriaceae and Clostridiaceae [34]. Further, co-housing experiments between WT and *Nlrp3*$^{-/-}$ mice performed by Elinav et al. to equilibrate microflora resulted in slightly attenuated colitis in the knockout mice [25]. Taken together, these studies propose that NLRP3-inflammasome mediates protection against colitis by multiple mechanisms and that the relative role of each is dependent upon several factors including differences in experimental conditions and microflora variations among mice harbored in different laboratories. Further, if established, it would be interesting to clarify whether *Asc*$^{-/-}$ and *Casp1*$^{-/-}$ mice also show identical alterations as *Nlrp3*$^{-/-}$ mice in their gut flora. Similarly, a recent report demonstrated enhanced susceptibility to colitis and colitis-associated carcinogenesis in *Nod2*-deficient mice owing to altered gut microbial communities [35]. However, in agreement with the established role of NOD2, this was independent of IL-18 levels but surprisingly resulted due to enhanced IL-6 levels. Nonetheless, the susceptibility of several different mice lacking distinct NLRs suggests non-redundant roles for these receptors in maintaining intestinal homeostasis.

The mechanisms that promote intestinal repair and wound healing lead to tumorigenesis upon dysregulation [29, 36]. Certainly, the features shared between wound healing and tumour development are similar enough to lead to the view that wounds incompetent to heal develop into tumors because of enhanced cell migration and proliferation in that area [37]. Normand et al. demonstrated that NLRP6 regulates self-renewal of the intestinal epithelium and its ablation lead to defective wound healing thereby promoting colitis associated-colorectal carcinogenesis [29]. Deficiency in *Nlrp6* resulted in enhanced expression of several genes including *Csnk1ε* (casein kinase 1 epsilon) and *Smarcc1* (member of SWI/SNF family), which are associated with enhanced proliferation of the dysplastic epithelium [29]. Notably, Csnk1ε is known to phosphorylate proteins involved in several key pathways of tumour progression such as β-catenin and *Csnk1ε* inhibition results in growth arrest [38]. Therefore production of numerous paracrine factors in *Nlrp6*$^{-/-}$ mice may promote tumour development through activation of the Wnt/β-catenin pathway that is critical in cell proliferation during development and healing.

Consistent with a role in regulating wound healing, another study proposed that NLRs NLRP6 and NLRP3 modulate levels of IL22 neutralizing receptor IL22BP (IL22 binding protein) secreted by CD11c^{+} dendritic cells at steady-state levels [36]. Upon AOM/DSS exposure, the induction of intestinal damage induces IL-22 levels, probably by innate lymphoid cells [39]. IL-22 activity promotes cell proliferation and is necessary during the early phase for colonic epithelial cell repair; however, IL-22 needs to be neutralized in the later phases by IL-22BP. Counterintuitive to this is the data that inflammasome-mediated IL-18 production down-regulates IL-22BP thereby increasing IL-22/IL-22BP ratio (Fig. 1); yet ablation of *il18* or *il18r1* results in enhanced susceptibility to colitis-associated colon

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cancer [40, 41]. Apparently, this can be explained by the fact that IL-18 protects against intestinal damage by maintaining epithelial barrier integrity [40, 41]. Thus, delayed colon repair in *il18*$^{-/-}$ mice may allow bacterial translocation and hence intestinal inflammation, thereby promoting tumorigenesis. Indeed, elevated production of IL-6 family cytokines and STAT3 phosphorylation were observed in *il18*$^{-/-}$ mice upon AOM/DSS treatment [41]. Furthermore, IL-18 administration can lower tumour burden in *Casp1*$^{-/-}$ mice possibly through its role in IFN-γ-mediated antitumour activity of cytotoxic T and Natural Killer cells [42]. These studies thus suggest that IL-18 plays distinct roles and that regulating IL-22BP levels is one of the mechanisms by which IL-18 maintains intestinal homeostasis.

3.NLRP6 negatively regulates host-defense signaling

Recently, we described a detrimental role for NLRP6 in microbial infection and demonstrated that ablation of *Nlrp6* results in profound resistance to intracellular pathogens *Listeria monocytogenes* and *Salmonella typhimurium* [23]. According to estimates from the Centers for Disease Control, 48 million people get hospitalized owing to food borne illnesses every year. *Listeria* and *Salmonella* are among the top-three pathogens for these illnesses that eventually lead to death [43]. Mice deficient in *Nlrp6* exhibited significantly reduced bacterial burden by day 1 post-infection and more than 70% of the deficient mice survived lethal *Listeria* infection [23]. These data propose NLRP6 as a highly compelling drug target for protection again bacterial infections. However, the activity of NLRP6 can only be neutralized selectively since its deficiency is also associated with disruption of gut homeostasis. Particularly, in recent years the therapeutic efficacy of some of the commonly used antibiotics has decreased extensively because of their excessive misuse leading to the emergence of multi-drug resistant strains. Therefore, there is an urgent need to discover newer therapies to combat infectious diseases. Here, neutralization of NLRP6 in conjunction with conventional antimicrobials may provide protection in addition to removing selective pressure and thereby the emergence of drug-resistant strains [44]. However, if *Nlrp6*-deficiency will be protective against a broad-range of pathogenic microbes needs to be examined.

NLRP6 specifically inhibited TLR2 and TLR4-dependent activation of the canonical NF-κB (Fig. 2) and MAP-kinase pathways [23]. Furthermore, there was increased production of cytokines and chemokines such as TNFα, IL-6 and KC by *Nlrp6*$^{-/-}$ macrophages either infected with *Listeria* or exposed to TLR2 or TLR4 ligands (Fig. 1). Indeed, NF-κB dependent cytokines such as TNFα and IL-6 were also markedly elevated in *Nlrp6*$^{-/-}$ mice *in vivo* after exposure to AOM/DSS [28]. Elevated production of TNFα and IL-6 can promote tumorigenesis (Fig. 1) through activation of the STAT3 pathway specifically in the intestinal epithelial cells [45, 46]. In agreement, mice lacking TNF-Rp55 or IL-6 exhibited decreased colitis associated cancer [46, 47]. Consistent with enhanced levels of chemokines, increased frequency of monocytes and neutrophils were observed in the circulation of *Listeria* infected *Nlrp6*-deficient mice (Fig. 1). Although, these cells did not exhibit enhanced pathogen killing *in vitro,* their enhanced numbers may contribute to early clearance of bacteria [23]. Taken together, these studies convincingly support a negative role for NLRP6 in regulating the NF-κB pathway. However, NLRP6 did not affect NOD1 and NOD2 dependent activation of NF-κB and MAP-kinase pathways thereby proposing specificity for TLR-induced signaling and implying an upstream function for NLRP6 in the TLR pathway [23]. More likely, we believe, NLRP6 acts as a checkpoint for enhanced unwarranted inflammation. Although, how NLRP6 is activated and whether it plays any significant role during TLR3 or TLR7 signaling, mainly activated upon viral infection, still needs to be investigated.

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As mentioned earlier, naïve *Nlrp6*-KO mice show alterations in microbiota composition with expanded representation of Prevotellaceae [25]. However, co-housing experiments between WT and *Nlrp6*$^{-/-}$ mice, which resulted in similar gut microbiota composition, did not alter the resistance of *Nlrp6*$^{-/-}$ mice to *Listeria* [23]. One explanation here could be that altered microbial communities calibrate responses only in the gastrointestinal models and do not regulate immunity upon systemic bacterial infection. Further, in the colitis model, *Nlrp6*$^{-/-}$ mice showed expansion of Prevotellaceae that resulted in elevated expression of the proinflammatory chemokines CCL5 [25]. Collectively, these studies suggest that irrespective of the upstream trigger, the development of colitis and CAC is accompanied with, and perhaps dependent upon enhanced cytokine and chemokine production in *Nlrp6*$^{-/-}$ mice.

CCL5 is secreted by many cells including epithelial cells, macrophages, fibroblasts and T cells [48] and is a potent chemoattractant of a variety of innate and adaptive immune cells [49]. Earlier studies demonstrated a detrimental role for CCL5 in both TNBS- and DSS-induced colitis [50, 51]. However, a protective role has been exhibited for CCL5 upon challenge with various viral and bacterial pathogens. *Ccl5*-deficient mice showed poor CD8 T-cell responses to LCMV accompanied with increased viral load [52] and its expression was actively reduced by human cytomegalovirus to evade immune responses [53]. Upon *Chlamydia* infection, the mRNA expression of CCL5 and its receptor CCR5 were elevated in the spleen and lymph nodes and its inhibition was associated with delayed bacterial clearance [54]. Likewise, its expression was also associated with increased resistance to *Leishmania major* infection [55]. Interestingly, both NOD1 and NOD2 stimulate CCL5 production from murine macrophages [56]. As *Listeria* signals through NLRs NOD1 and NOD2 [3, 57], it would be interesting to investigate whether CCL5 has any role in immunity to *Listeria* infection in *Nlrp6*$^{-/-}$ mice.

4. NLRP6 expression and signaling compartment

NLRP6 is expressed in immune cells such as granulocytes, dendritic cells, CD4 and CD8 T-cells and macrophages [23, 28, 29, 58]. Also, it is highly expressed in duodenum, ileum, colon, small intestine and liver [28]. Further, *Nlrp6* was also abundantly expressed in human PBMCs [59] and exhibited enhanced levels in tissue samples from colon cancer patients [30]. *Nlrp6* expression increased gradually during the late gestation from embryonic days E16 to E20 in the rat fetal intestine [60]. Analysis of the promoter region demonstrated an abundance of peroxisome proliferator-activated receptor γ (PPAR-γ) and retinoid × receptor-α (RXR-α) binding motifs in the *Nlrp6* gene. PPAR-γ is a member of the nuclear hormone receptor superfamily and heterodimerizes with RXR-α to play a critical role in glucose homeostasis [61, 62]. Consequently, the transcriptional expression of *Nlrp6* increased in Caco2 cells exposed to PPAR-γ agonist rosiglitazone [60]. *Nlrp6* expression was also enhanced in human and mouse colon epithelial cell lines treated with rosiglitazone but dampened when exposed to corticotropin-releasing hormone (CRH) [63]. Serum levels of CRH elevated in mice subjected to water-avoidance stress model and the associated reduction in *Nlrp6* resulted in altered microbiota and severe intestinal pathology [63]. However, reduced inflammasome activity observed in this model was because of lower transcriptional expression of *Nlrp6* and pro-IL-1β. Interestingly, treatment with either probiotics or PPAR-γ agonist reduced intestinal pathology.

Similar to NLRP6, PPAR-γ maintains intestinal homeostasis [64] and rosiglitazone administration reduced the severity of colitis [61]. In addition, PPAR-γ also appears to compromise immunity against bacterial infections both *in vitro* and *in vivo* [65]. siRNA knock-down of PPAR-γ in THP1 macrophages resulted in reduced intracellular *Mycobacterium tuberculosis* load [66]. Furthermore, in parallel with *Nlrp6*-ablation, loss of

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PPAR-γ (myeloid cell specific) resulted in increased production of inflammatory cytokines coupled with enhanced monocyte recruitment, thereby promoting increased protection against *Listeria* infection [65]. These results unambiguously agree with NLRP6 function and raise the possibility that PPAR-γ functions in an NLRP6-dependent manner to impede bacterial clearance.

NLRP6 signaling was found to be important in both the radiosensitive (hematopoietic) and radioresistant (stromal) cells in the bacterial infection model [23]. This is consistent with previous reports highlighting a critical role for cytokine and chemokine production by both the compartments during bacterial infection. However, contrasting results were reported in the colitis and colitis-associated cancer model with reports suggesting a role for either epithelial cells or both the hematopoietic and non-hematopoietic compartment [25, 28, 29]. In one study, NLRP6 signaling in the radiosensitive colon myofibroblasts was associated with self-renewal of the intestinal epithelium [29]. Furthermore, NLRP6 has been suggested to regulate inflammasome-dependent IL-18 production in the epithelial cells, but was dispensable for this function in macrophages or neutrophils [23, 25, 28]. Clearly, NLRP6 signaling plays distinct roles in different cell types and studies in myeloid cell specific or epithelial cell specific conditional knock-outs will further clarify its role in these discrete compartments.

5. NLRP6 regulates inflammasome - dependent and – independent functions

The earliest studies on NLRP6 demonstrated its role in inflammasome assembly [58]. Overexpression of inflammasome components and NLRP6 in COS7L cells lead to the cleavage of pro-caspase-1 and release of IL-1β. Similarly, overexpression studies in 293T cells resulted in NLRP6 co-localization with ASC that was blunted in the absence of a functional PYRIN domain [58]. However, the authors were unable to demonstrate this interaction by co-immunoprecipitation experiments therefore making it difficult to interpret contrasting results obtained by microscopy and biochemical assays. Subsequent studies by employing *Nlrp6*-deficient mouse macrophages suggested no role in caspase-1 activation or mature IL-1β release in response to diverse inflammasome activating ligands, including LPS and ATP, in two different studies [23, 28]. Further, NLRP6 did not regulate inflammasome-dependent IL-1β production in human PBMCs [59]. However, studies with colitis and CAC models in mice observed reduced IL-18 production in serum and colon lysates [25, 28, 67]. These studies suggest that NLRP6 may mediate inflammasome assembly in colon epithelial cells and that this NLR accomplishes inflammasome-independent roles in other cell types such as macrophages and neutrophils. Nonetheless, direct biochemical evidence demonstrating an NLRP6 inflammasome in colonic epithelial cells is still awaited and further studies are required to decipher activating ligands of NLRP6.

6. Concluding remarks

The physiological function of NLRP6 seems to be to dampen detrimental inflammatory responses. Particularly in the case of intracellular cytoplasmic pathogens, multiple pattern recognition receptors such as TLRs and NLRs are activated at different stages of bacterial invasion, at the cell surface or in the cytosol. Prolonged activation of these multiple receptors may lead to robust pro-inflammatory responses that might be damaging to the host-tissue (Fig. 2). Hence, NLRP6 acts as a molecular rheostat to dampen TLR induced pathways activated by the extracellular recognition of bacterial ligands and thus maintains immune homeostasis (Fig. 2). Indeed, *Nlrp6*-deficiency elevated production of cytokines and chemokines and resulted in enhanced susceptibility to colitis and colitis-associated carcinogenesis. Taken together, this suggests that NLRP6 signaling is beneficial during

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conditions where an inflammatory response is unwarranted, while its activity is detrimental when an inflammatory response is vital. Thus, although the role of NLRP6 seems to be either protective or detrimental depending on the context of the model used, the underlying mechanisms in these studies appear to be the same.

As with other NLR family members, activation mechanisms of NLRP6 are still unclear. It is reasonable to believe that NLRP6 recognizes select host-derived factors during intestinal damage. Recognition of certain conserved bacterial PAMPs would explain the role of NLRP6 in both infection and gastrointestinal models, where commensal bacteria too play a large role. However, another possibility would be that just like NLRP3, NLRP6 does not have any direct ligand binding and is activated in response to certain upstream biochemical alterations that signal enhanced unwarranted inflammation. Clearly, NLRP6 activity can be modulated for therapeutic purposes. However, future studies are required to answer several of these outstanding questions.

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References

[1]. Creagh EM, O LA. TLRs, NLRs and RLRs: a trinity of pathogen sensors that co-operate in innate immunity. Trends Immunol. 2006; 27:352–357. [PubMed: 16807108]

[2]. Kawai T, Akira S. The roles of TLRs, RLRs and NLRs in pathogen recognition. Int Immunol. 2009; 21:317–337. [PubMed: 19246554]

[3]. Anand PK, Tait SW, Lamkanfi M, Amer AO, Nunez G, Green DR, Kanneganti TD. TLR2 and RIP2 pathways mediate autophagy of *Listeria monocytogenes* via ERK activation. J Biol Chem. 2011; 286:42981–42991. [PubMed: 22033934]

[4]. Franchi L, Kamada N, Nakamura Y, Burberry A, Kuffa P, Suzuki S, Shaw MH, Kim YG, Nunez G. NLRC4-driven production of IL-1beta discriminates between pathogenic and commensal bacteria and promotes host intestinal defense. Nat Immunol. 2012; 13:449–456. [PubMed: 22484733]

[5]. Franchi L, Munoz-Planillo R, Nunez G. Sensing and reacting to microbes through the inflammasomes. Nat Immunol. 2012; 13:325–332. [PubMed: 22430785]

[6]. Kanneganti TD. Central roles of NLRs and inflammasomes in viral infection. Nat Rev Immunol. 2010; 10:688–698. [PubMed: 20847744]

[7]. Rathinam VA, Vanaja SK, Fitzgerald KA. Regulation of inflammasome signaling. Nat Immunol. 2012; 13:333–332. [PubMed: 22430786]

[8]. Martinon F, Burns K, Tschopp J. The inflammasome: a molecular platform triggering activation of inflammatory caspases and processing of proIL-beta. Mol Cell. 2002; 10:417–426. [PubMed: 12191486]

[9]. Kanneganti TD, Lamkanfi M, Nunez G. Intracellular NOD-like receptors in host defense and disease. Immunity. 2007; 27:549–559. [PubMed: 17967410]

[10]. Kanneganti TD, Ozoren N, Body-Malapel M, Amer A, Park JH, Franchi L, Whitfield J, Barchet W, Colonna M, Vandenabeele P, Bertin J, Coyle A, Grant EP, Akira S, Nunez G. Bacterial RNA and small antiviral compounds activate caspase-1 through cryopyrin/Nalp3. Nature. 2006; 440:233–236. [PubMed: 16407888]

[11]. Anand PK, Malireddi RK, Kanneganti TD. Role of the nlrp3 inflammasome in microbial infection. Front Microbiol. 2011; 2:12. [PubMed: 21687408]

[12]. Lupfer C, Thomas PG, Anand PK, Vogel P, Milasta S, Martinez J, Huang G, Green M, Kundu M, Chi H, Xavier RJ, Green DR, Lamkanfi M, Dinarello CA, Doherty PC, Kanneganti TD.

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Receptor interacting protein kinase 2-mediated mitophagy regulates inflammasome activation during virus infection. Nat Immunol. 2013; 14:480–488. [PubMed: 23525089]

[13]. Kofoed EM, Vance RE. Innate immune recognition of bacterial ligands by NAIPs determines inflammasome specificity. Nature. 2011; 477:592–595. [PubMed: 21874021]

[14]. Zhao Y, Yang J, Shi J, Gong YN, Lu Q, Xu H, Liu L, Shao F. The NLRC4 inflammasome receptors for bacterial flagellin and type III secretion apparatus. Nature. 2011; 477:596–600. [PubMed: 21918512]

[15]. Meissner TB, Li A, Biswas A, Lee KH, Liu YJ, Bayir E, Iliopoulos D, van den Elsen PJ, Kobayashi KS. NLR family member NLRC5 is a transcriptional regulator of MHC class I genes. Proc Natl Acad Sci U S A. 2010; 107:13794–13799. [PubMed: 20639463]

[16]. Joly S, Eisenbarth SC, Olivier AK, Williams A, Kaplan DH, Cassel SL, Flavell RA, Sutterwala FS. Cutting edge: Nlrp10 is essential for protective antifungal adaptive immunity against *Candida albicans*. J Immunol. 2012; 189:4713–4717. [PubMed: 23071280]

[17]. Eisenbarth SC, Williams A, Colegio OR, Meng H, Strowig T, Rongvaux A, Henao-Mejia J, Thaiss CA, Joly S, Gonzalez DG, Xu L, Zenewicz LA, Haberman AM, Elinav E, Kleinstein SH, Sutterwala FS, Flavell RA. NLRP10 is a NOD-like receptor essential to initiate adaptive immunity by dendritic cells. Nature. 2012; 484:510–513. [PubMed: 22538615]

[18]. Khare S, Dorfleutner A, Bryan NB, Yun C, Radian AD, de Almeida L, Rojanasakul Y, Stehlik C. An NLRP7-containing inflammasome mediates recognition of microbial lipopeptides in human macrophages. Immunity. 2012; 36:464–476. [PubMed: 22361007]

[19]. Lei Y, Wen H, Yu Y, Taxman DJ, Zhang L, Widman DG, Swanson KV, Wen KW, Damania B, Moore CB, Giguere PM, Siderovski DP, Hiscott J, Razani B, Semenkovich CF, Chen X, Ting JP. The mitochondrial proteins NLRX1 and TUFM form a complex that regulates type I interferon and autophagy. Immunity. 2012; 36:933–946. [PubMed: 22749352]

[20]. Schneider M, Zimmermann AG, Roberts RA, Zhang L, Swanson KV, Wen H, Davis BK, Allen IC, Holl EK, Ye Z, Rahman AH, Conti BJ, Eitas TK, Koller BH, Ting JP. The innate immune sensor NLRC3 attenuates Toll-like receptor signaling via modification of the signaling adaptor TRAF6 and transcription factor NF-kappaB. Nat Immunol. 2012; 13:823–831. [PubMed: 22863753]

[21]. Zaki MH, Vogel P, Malireddi RK, Body-Malapel M, Anand PK, Bertin J, Green DR, Lamkanfi M, Kanneganti TD. The NOD-like receptor NLRP12 attenuates colon inflammation and tumorigenesis. Cancer Cell. 2011; 20:649–660. [PubMed: 22094258]

[22]. Allen IC, Wilson JE, Schneider M, Lich JD, Roberts RA, Arthur JC, Woodford RM, Davis BK, Uronis JM, Herfarth HH, Jobin C, Rogers AB, Ting JP. NLRP12 suppresses colon inflammation and tumorigenesis through the negative regulation of noncanonical NF-kappaB signaling. Immunity. 2012; 36:742–754. [PubMed: 22503542]

[23]. Anand PK, Malireddi RK, Lukens JR, Vogel P, Bertin J, Lamkanfi M, Kanneganti TD. NLRP6 negatively regulates innate immunity and host defence against bacterial pathogens. Nature. 2012; 488:389–393. [PubMed: 22763455]

[24]. Centers for Disease Control and Prevention. Inflammatory Bowel Disease (IBD). 2013. http://www.cdc.gov/ibd/

[25]. Elinav E, Strowig T, Kau A, Henao-Mejia J, Thaiss C, Booth C, Peaper D, Bertin J, Eisenbarth S, Gordon J, Flavell R. NLRP6 inflammasome regulates colonic microbial ecology and risk for colitis. Cell. 2011; 145:745–757. [PubMed: 21565393]

[26]. Henao-Mejia J, Elinav E, Jin C, Hao L, Mehal W, Strowig T, Thaiss C, Kau A, Eisenbarth S, Jurczak M, Camporez J-P, Shulman G, Gordon J, Hoffman H, Flavell R. Inflammasome-mediated dysbiosis regulates progression of NAFLD and obesity. Nature. 2012; 482:179–185. [PubMed: 22297845]

[27]. Centers for Disease Control and Prevention. Colorectal (Colon) Cancer. 2013. http://www.cdc.gov/cancer/colorectal/

[28]. Chen G, Liu M, Wang F, Bertin J, Nunez G. A functional role for Nlrp6 in intestinal inflammation and tumorigenesis. J Immunol. 2011; 186:7187–7194. [PubMed: 21543645]

[29]. Normand S, Delanoye-Crespin A, Bressenot A, Huot L, Grandjean T, Peyrin-Biroulet L, Lemoine Y, Hot D, Chamaillard M. Nod-like receptor pyrin domain-containing protein 6

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(NLRP6) controls epithelial self-renewal and colorectal carcinogenesis upon injury. Proc Natl Acad Sci U S A. 2011; 108:9601–9606. [PubMed: 21593405]

[30]. Ahmed FE, Vos P. Molecular markers for human colon cancer in stool and blood identified by RT-PCR. Anticancer Res. 2004; 24:4127–4134. [PubMed: 15736463]

[31]. Allen IC, TeKippe EM, Woodford RM, Uronis JM, Holl EK, Rogers AB, Herfarth HH, Jobin C, Ting JP. The NLRP3 inflammasome functions as a negative regulator of tumorigenesis during colitis-associated cancer. J Exp Med. 2010; 207:1045–1056. [PubMed: 20385749]

[32]. Dupaul-Chicoine J, Yeretssian G, Doiron K, Bergstrom KS, McIntire CR, LeBlanc PM, Meunier C, Turbide C, Gros P, Beauchemin N, Vallance BA, Saleh M. Control of intestinal homeostasis, colitis, and colitis-associated colorectal cancer by the inflammatory caspases. Immunity. 2010; 32:367–378. [PubMed: 20226691]

[33]. Zaki MH, Boyd KL, Vogel P, Kastan MB, Lamkanfi M, Kanneganti TD. The NLRP3 inflammasome protects against loss of epithelial integrity and mortality during experimental colitis. Immunity. 2010; 32:379–391. [PubMed: 20303296]

[34]. Hirota SA, Ng J, Lueng A, Khajah M, Parhar K, Li Y, Lam V, Potentier MS, Ng K, Bawa M, McCafferty DM, Rioux KP, Ghosh S, Xavier RJ, Colgan SP, Tschopp J, Muruve D, MacDonald JA, Beck PL. NLRP3 inflammasome plays a key role in the regulation of intestinal homeostasis. Inflamm Bowel Dis. 2011; 17:1359–1372. [PubMed: 20872834]

[35]. Couturier-Maillard A, Secher T, Rehman A, Normand S, De Arcangelis A, Haesler R, Huot L, Grandjean T, Bressenot A, Delanoye-Crespin A, Gaillot O, Schreiber S, Lemoine Y, Ryffel B, Hot D, Nunez G, Chen G, Rosenstiel P. Chamaillard M, NOD2-mediated dysbiosis predisposes mice to transmissible colitis and colorectal cancer. J Clin Invest. 2013; 123:700–711. [PubMed: 23281400]

[36]. Huber S, Gagliani N, Zenewicz L, Huber F, Bosurgi L, Hu B, Hedl M, Zhang W, O'Connor W, Murphy A, Valenzuela D, Yancopoulos G, Booth C, Cho J, Ouyang W, Abraham C, Flavell R. IL-22BP is regulated by the inflammasome and modulates tumorigenesis in the intestine. Nature. 2012; 491:259–263. [PubMed: 23075849]

[37]. Villanueva T. Tumorigenesis: Wound-up tumours. Nat Rev Cancer. 2011; 11:235. [PubMed: 21548398]

[38]. Yang WS, Stockwell BR. Inhibition of casein kinase 1-epsilon induces cancer-cell-selective, PERIOD2-dependent growth arrest. Genome Biol. 2008; 9:R92. [PubMed: 18518968]

[39]. Sonnenberg GF, Fouser LA, Artis D. Border patrol: regulation of immunity, inflammation and tissue homeostasis at barrier surfaces by IL-22. Nat Immunol. 2011; 12:383–390. [PubMed: 21502992]

[40]. Zaki MH, Vogel P, Body-Malapel M, Lamkanfi M, Kanneganti TD. IL-18 production downstream of the Nlrp3 inflammasome confers protection against colorectal tumor formation. J Immunol. 2010; 185:4912–4920. [PubMed: 20855874]

[41]. Salcedo R, Worschech A, Cardone M, Jones Y, Gyulai Z, Dai RM, Wang E, Ma W, Haines D, O'HUigin C, Marincola FM, Trinchieri G. MyD88-mediated signaling prevents development of adenocarcinomas of the colon: role of interleukin 18. J Exp Med. 2010; 207:1625–1636. [PubMed: 20624890]

[42]. Terzic J, Grivennikov S, Karin E, Karin M. Inflammation and colon cancer. Gastroenterology. 2010; 138:2101–2114. e2105. [PubMed: 20420949]

[43]. Centers for Disease Control and Prevention. 2010. http://www.cdc.gov/foodborneburden/2011-foodborne-estimates.html

[44]. Anand PK, Kanneganti TD. Targeting NLRP6 to enhance immunity against bacterial infections. Future Microbiol. 2012; 7:1239–1242. [PubMed: 23075441]

[45]. Grivennikov SI, Karin M. Dangerous liaisons: STAT3 and NF-kappaB collaboration and crosstalk in cancer. Cytokine Growth Factor Rev. 2010; 21:11–19. [PubMed: 20018552]

[46]. Grivennikov S, Karin E, Terzic J, Mucida D, Yu GY, Vallabhapurapu S, Scheller J, Rose-John S, Cheroutre H, Eckmann L, Karin M. IL-6 and Stat3 are required for survival of intestinal epithelial cells and development of colitis-associated cancer. Cancer cell. 2009; 15:103–113. [PubMed: 19185845]

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[47]. Popivanova BK, Kitamura K, Wu Y, Kondo T, Kagaya T, Kaneko S, Oshima M, Fujii C, Mukaida N. Blocking TNF-alpha in mice reduces colorectal carcinogenesis associated with chronic colitis. J Clin Invest. 2008; 118:560–570. [PubMed: 18219394]

[48]. Makino Y, Cook DN, Smithies O, Hwang OY, Neilson EG, Turka LA, Sato H, Wells AD, Danoff TM. Impaired T cell function in RANTES-deficient mice. Clin Immunol. 2002; 102:302–309. [PubMed: 11890717]

[49]. Mantovani A, Sica A, Sozzani S, Allavena P, Vecchi A, Locati M. The chemokine system in diverse forms of macrophage activation and polarization. Trends Immunol. 2004; 25:677–686. [PubMed: 15530839]

[50]. Ajuebor MN, Hogaboam CM, Kunkel SL, Proudfoot AE, Wallace JL. The chemokine RANTES is a crucial mediator of the progression from acute to chronic colitis in the rat. J Immunol. 2001; 166:552–558. [PubMed: 11123336]

[51]. Andres PG, Beck PL, Mizoguchi E, Mizoguchi A, Bhan AK, Dawson T, Kuziel WA, Maeda N, MacDermott RP, Podolsky DK, Reinecker HC. Mice with a selective deletion of the CC chemokine receptors 5 or 2 are protected from dextran sodium sulfate-mediated colitis: lack of CC chemokine receptor 5 expression results in a NK1.1+ lymphocyte-associated Th2-type immune response in the intestine. J Immunol. 2000; 164:6303–6312. [PubMed: 10843684]

[52]. Crawford A, Angelosanto JM, Nadwodny KL, Blackburn SD, Wherry EJ. A role for the chemokine RANTES in regulating CD8 T cell responses during chronic viral infection. PLoS Pathog. 2011; 7:e1002098. [PubMed: 21814510]

[53]. Billstrom Schroeder M, Worthen GS. Viral regulation of RANTES expression during human cytomegalovirus infection of endothelial cells. J Virol. 2001; 75:3383–3390. [PubMed: 11238864]

[54]. Sakthivel SK, Singh UP, Singh S, Taub DD, Igietseme JU, Lillard JW Jr. CCL5 regulation of mucosal chlamydial immunity and infection. BMC Microbiol. 2008; 8:136. [PubMed: 18700040]

[55]. Santiago HC, Oliveira CF, Santiago L, Ferraz FO, de Souza DG, de-Freitas LA, Afonso LC, Teixeira MM, Gazzinelli RT, Vieira LQ. Involvement of the chemokine RANTES (CCL5) in resistance to experimental infection with *Leishmania major*. Infect Immun. 2004; 72:4918–4923. [PubMed: 15271961]

[56]. Werts C, le Bourhis L, Liu J, Magalhaes JG, Carneiro LA, Fritz JH, Stockinger S, Balloy V, Chignard M, Decker T, Philpott DJ, Ma X, Girardin SE. Nod1 and Nod2 induce CCL5/RANTES through the NF-kappaB pathway. Eur J Immunol. 2007; 37:2499–2508. [PubMed: 17705131]

[57]. Corr SC, O'Neill LA. *Listeria monocytogenes* infection in the face of innate immunity. Cell Microbiol. 2009; 11:703–709. [PubMed: 19191786]

[58]. Grenier JM, Wang L, Manji GA, Huang WJ, Al-Garawi A, Kelly R, Carlson A, Merriam S, Lora JM, Briskin M, DiStefano PS, Bertin J. Functional screening of five PYPAF family members identifies PYPAF5 as a novel regulator of NF-kappaB and caspase-1. FEBS Lett. 2002; 530:73–78. [PubMed: 12387869]

[59]. Meixenberger K, Pache F, Eitel J, Schmeck B, Hippenstiel S, Slevogt H, N'Guessan P, Witzenrath M, Netea MG, Chakraborty T, Suttorp N, Opitz B. *Listeria monocytogenes*-infected human peripheral blood mononuclear cells produce IL-1beta, depending on listeriolysin O and NLRP3. J Immunol. 2010; 184:922–930.

[60]. Kempster SL, Belteki G, Forhead AJ, Fowden AL, Catalano RD, Lam BY, McFarlane I, Charnock-Jones DS, Smith GC. Developmental control of the Nlrp6 inflammasome and a substrate, IL-18, in mammalian intestine. Am J Physiol Gastrointest Liver Physiol. 2011; 300:G253–263. [PubMed: 21088234]

[61]. Su CG, Wen X, Bailey ST, Jiang W, Rangwala SM, Keilbaugh SA, Flanigan A, Murthy S, Lazar MA, Wu GD. A novel therapy for colitis utilizing PPAR-gamma ligands to inhibit the epithelial inflammatory response. J Clin Invest. 1999; 104:383–389. [PubMed: 10449430]

[62]. Kaul D, Anand PK, Khanna A. Functional genomics of PPAR-gamma in human immunomodulatory cells. Mol Cell Biochem. 2006; 290:211–215. [PubMed: 16633734]

[63]. Sun Y, Zhang M, Chen CC, Gillilland M 3rd, Sun X, El-Zaatari M, Huffnagle GB, Young VB, Zhang J, Hong SC, Chang YM, Gumucio DL, Owyang C, Kao JY. Stress-Induced Corticotropin-

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Releasing Hormone-Mediated NLRP6 Inflammasome Inhibition and Transmissible Enteritis in Mice. Gastroenterology. 2013; 144:1478–1487. e1478. [PubMed: 23470617]

[64]. Dubuquoy L, Rousseaux C, Thuru X, Peyrin-Biroulet L, Romano O, Chavatte P, Chamaillard M, Desreumaux P. PPARgamma as a new therapeutic target in inflammatory bowel diseases. Gut. 2006; 55:1341–1349. [PubMed: 16905700]

[65]. Abdullah Z, Geiger S, Nino-Castro A, Bottcher JP, Muraliv E, Gaidt M, Schildberg FA, Riethausen K, Flossdorf J, Krebs W, Chakraborty T, Kurts C, Schultze JL, Knolle PA, Klotz L. Lack of PPARgamma in myeloid cells confers resistance to *Listeria monocytogenes* infection. PLoS One. 2012; 7:e37349. [PubMed: 22629382]

[66]. Mahajan S, Dkhar HK, Chandra V, Dave S, Nanduri R, Janmeja AK, Agrewala JN, Gupta P. *Mycobacterium tuberculosis* modulates macrophage lipid-sensing nuclear receptors PPARgamma and TR4 for survival. J Immunol. 2012; 188:5593–5603. [PubMed: 22544925]

[67]. Chen GY, Nunez G. Inflammasomes in intestinal inflammation and cancer. Gastroenterology. 2011; 141:1986–1999. [PubMed: 22005480]

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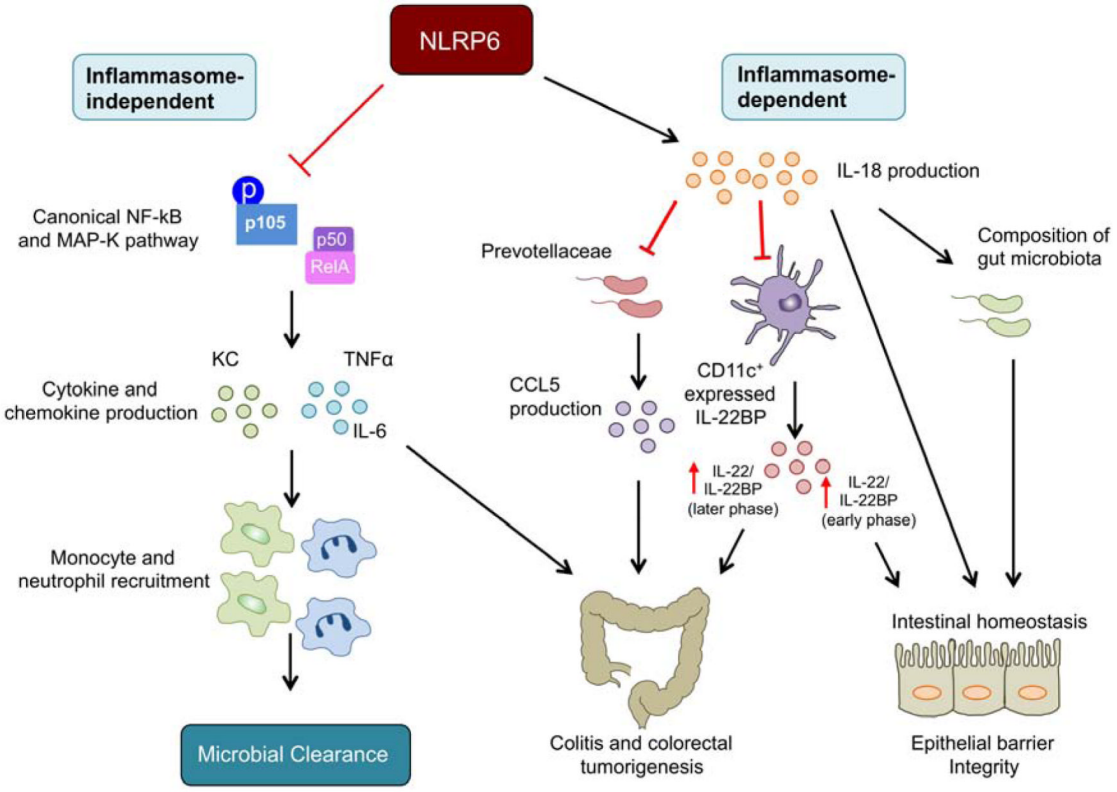

Figure 1. NLRP6 regulates inflammasome –dependent and inflammasome-independent functions

Left: NLRP6 inhibits canonical NF-κB and MAP-kinase signaling in an inflammasome-independent manner. Thus, deficiency in *Nlrp6* results in enhanced secretion of cytokines and chemokines. Consequently, increased monocytes and neutrophils are recruited thereby augmenting anti-microbial defense. Right: NLRP6 protects against intestinal homeostasis by maintaining gut microbiota composition and IL-18 production in an inflammasome-dependent manner. *Nlrp6* ablation results in an altered microbiota with enhanced expression of Prevotellaceae and enhanced production of CCL5 thus promoting colitis. IL-18 production also regulates epithelial barrier integrity. Further, IL-18 regulates the levels of IL-22BP released by dendritic cells to adjust IL-22/IL-22BP ratio. Increased IL-22/IL-22BP ratio in the early phase of intestinal damage promotes colonic epithelial cell repair while its increase in the later phase promotes colitis-associated carcinogenesis.

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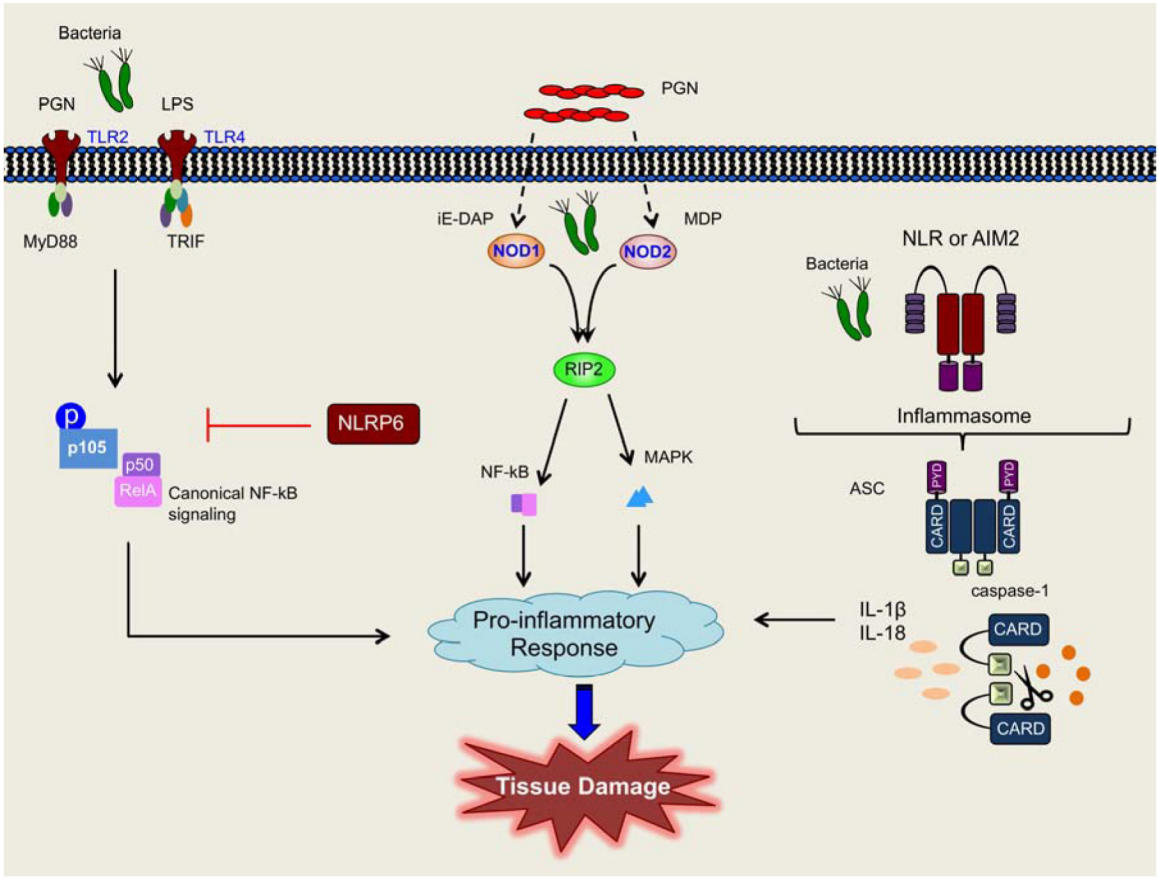

Figure 2. NLRP6 maintains immune homeostasis

Multiple pattern-recognition receptors such as TLRs and NLRs are activated at different stages of bacterial invasion, at the cell surface or in the cytoplasm. Prolonged activation of these receptors lead to enhanced unwarranted inflammation and may promote tissue-damage. NLRP6 inhibits canonical NF-κB signaling thus hindering the TLR pathway activated by the extracellular recognition of bacterial ligands and thus maintaining immune homeostasis.