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Review

The multifaceted nature of NLRP12

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ABSTRACT

NLRs are a class of cytoplasmic PRRs with various functions, ranging from pathogen/damage sensing to the modulation of inflammatory signaling and transcriptional control of MHC and related genes. In addition, some NLRs have been implicated in preimplantation and prenatal development. NLRP12 (also known as RNO, PYPAF7, and Monarch-1), a member of the family containing an N-terminal PYD, a NBD, and a C-terminal LRR region, is one of the first described NLR proteins whose role remains controversial. The interest toward NLRP12 has been boosted by its recent involvement in colon cancer, as well as in the protection against some severe infections, such as that induced by *Yersinia pestis*, the causative agent of plague. As NLRP12 is mainly expressed by the immune cells, and its expression is down-regulated in response to pathogen products and inflammatory cytokines, it has been predicted to play a role as a negative regulator of the inflammatory response. Herein, we present an overview of the NLR family and summarize recent insights on NLRP12 addressing its contribution to inflammatory signaling, host defense, and carcinogenesis. *J. Leukoc. Biol.* 96: 991–1000; 2014.

Abbreviations: AD=activation domain, ASC=apoptosis-associated speck-like protein containing a caspase recruitment domain, BIR=baculoviral inhibitor of apoptosis repeat domain, CARD=caspase recruitment domain, CIITA=MHC class II transcriptional activator, DAMP=damage-associated molecular pattern, Hsp=heat-shock protein, IRAK=IL-1R-associated kinase, LRR=leucine-rich repeat, NACHT=neuronal apoptosis inhibitor protein, MHC class 2 transcription activator, incompatibility locus protein from *Podospora anserina,* and telomerase-associated protein, NAIP=neuronal apoptosis inhibitory protein, NBD=nucleotide-binding domain, NIK=NF-κB-inducing kinase, NLR=nucleotide-binding and oligomerization domain-like receptor, NLRC=nucleotide-binding and oligomerization domain-like receptor caspase recruitment domain, NLRC5=nucleotide-binding domain and leucine-rich repeat containing 5, NLRP=nucleotide-binding oligomerization-like receptor protein, *Nlrp6/12*$^{-/-}$=*Nlrp6/12*-deficient, NLRP12AD=nucleotide-binding oligomerization-like receptor protein 12-associated disorder, NOD=nucleotide-binding and oligomerization domain, p=phospho, p50/52/65=members of the NF-κB transcription factor family, p100=NF-κB2 precursor, Pam3Cys4=tripalmitoyl-S-glycerol-cysteine, PAMP=pathogen-associated molecular pattern, PRR=pattern recognition receptor, PYD=pyrin domain, PYPAF=pyrin-containing apoptotic protease-activating factor 1-like protein, RelA/B=members of the NF-κB transcription factor family, RIP2=receptor-interacting protein kinase 2, RLR=retinoic acid-inducible gene I-like receptor, RNO=regulated by NO, S. *typhimurium*=*Salmonella enterica* serovar Typhimurium, TRAF3=TNFR-associated factor 3

Introduction

Innate receptors are able to detect and respond quickly to highly conserved microbial components, as well as to host-derived molecules, released following stress or tissue injury. This strategy is called "pattern recognition" and relies on a germline-encoded, limited set of PRRs, which are expressed by cells of innate immunity, including monocytes, macrophages, dendritic cells, endothelial cells, and neutrophils, as well as cells of the adaptive immune system [1–3]. PRRs have different cellular locations: on the cell surface, they recognize the microbes and their derivatives present in the extracellular environment; in the cytosol, they sense intracellular and extracellular pathogens that have damaged vacuolar or phagosomal membranes; whereas in the endosomes, PRRs interact with microbes that have entered the phagolysosomal degradation pathway [1, 4]. The recognition of PAMPs and DAMPs by the PRRs initiates an inflammatory response, leading to cytokine and chemokine secretion, production of host defense peptides, pyroptotic cell death, recruitment of phagocytes, and induction of autophagy [2, 3, 5, 6].

PRRs in the cells of the innate immune system can be classified as C-type lectin receptors, RLRs, cytosolic DNA sensors, TLRs, and nucleotide-binding LRRs containing receptors also known as NLRs [6]. Each PRR family has a distinct structure and acts differently to detect pathogens and to generate the appropriate immune response.

THE NLR FAMILY

NLRs are evolutionarily conserved intracellular PRRs playing an important role in host defense and physiology [7]. Mammalian NLRs share structural homology to plant disease resistance R proteins, which mediate the plant's defense response against infection. The similarity between plant R and animal NLR proteins hints that the NLRs represent an ancient family of immune defense genes [8, 9].

The NLR proteins, in general, have a tripartite domain organization: they consist of a central NACHT domain (also known as NBD), which enables the activation of the signaling complex via ATP-dependent oligomerization, a C-terminal LRR

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domain that functions in ligand sensing and autoregulation, and a variable N-terminal interaction/effector domain that mediates homotypic protein–protein interactions for downstream signaling [10, 11]. The mammalian NLRs can be divided into four subfamilies, based on different N-terminal effector domains. The effector domains found in NLRs are: CARD, PYD, BIR, and AD. A standardized nomenclature system categorizes the NLR family into four subfamilies based on the initial of the domain name: NLRA, NLRB (formerly known as NAIP or baculovirus inhibitor of apoptosis repeat-containing protein), NLRP (formerly known as NLRs with PYD), and NLRC (formerly known as NODs) [12, 13] (**Fig. 1**).

The NLR family is the most recently discovered among PRRs, and the research field is very dynamic. The research on agonists, interaction partners, and signaling pathways of NLRs is increasing rapidly. However, different studies report contradictory results, and many aspects related to the divergent functions of NLRs are not fully understood as yet [14].

Mechanism of action of NLR proteins

The NLR family consists of 22 members, and only about one-half of them has been characterized in some details [15]. Although it is well known that NLRs play a critical role in host defense, it remains unclear how the NLR family of receptors recognizes specific ligands and how they are activated by upstream signaling. A further level of complication is given by the fact that NLRs might interact with additional sensors to execute their functions [7, 10].

NLRs can be broadly categorized into four groups, depending on their functions: 1) transcriptional transactivators, 2) activators of NF-κB and MAPK, 3) activators of inflammasome, and 4) inhibitors of inflammatory signaling [15, 16]. It should also be considered that some NLRs are likely to have overlapping functions, and some of them might have cell type-specific roles and/or can be activated by multiple mechanisms with distinct downstream effects. Furthermore, several NLRs function in preimplantation and prenatal development, indicating that this family of receptors can play multiple roles [17–79] (**Table 1**).

CIITA and NLRC5 as transactivators of MHC expression. CIITA is the sole member of the NLRA family known to act as a transcriptional transactivator at the promoter of MHC class II and MHC class II-linked antigen-presentation accessory genes, such as the invariant chain and the HLA-DM. CIITA is expressed in macrophages, B and T lymphocytes, and dendritic cells [17, 18]. The N-terminal AD of CIITA is responsible for its recruitment to the enhanceosome, a protein complex consisting of several nuclear factors, including CREB-binding protein, regulatory factor X5, NF Y, and CREB that binds cis-acting elements in the MHC class II promoter [19]. Type II Bare Lymphocyte Syndrome, a severe immunodeficiency disorder, is caused by the lack of MHC class II expression on the cell surface, which is a consequence of a "loss-of-function" mutation in CIITA [20].

It has recently been discovered that the NLR family member NLRC5 transcriptionally activates essential components of MHC class I antigen processing and presentation, such as MHC class I genes themselves or β2-microglobulin, transporter associated with antigen processing 1, and low molecular mass polypeptide 2 [21, 22].

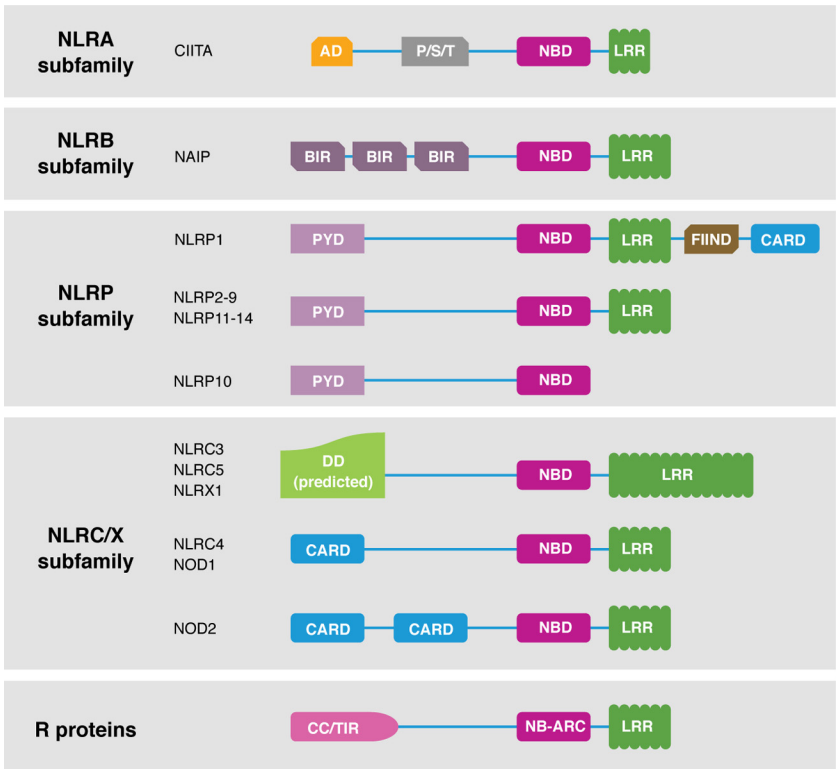

Figure 1. Schematic representation of the human NLR family. There are 22 human NLRs characterized by a central NBD. The NLR family can be classified further into four subfamilies, depending on the protein's N-terminal domain. Plant disease resistance R proteins are shown at the bottom. P/S/T, Proline/serine/threonine-rich region; FIIND, Find domain; DD, death domain; CC, coiled coil domain; TIR, TLR/IL-1R homology domain; NB-ARC, nucleotide-binding adaptor shared by apoptotic protease-activating factor 1, certain R genes, and cell death protein 4 (CED4).

TABLE 1. Functions of Human and Murine NLR Proteins

NLR protein	Function	References
CIITA	Induction of de novo MHC class II and MHC class II-linked antigen-presentation accessory gene transcription, enhancement of constitutive MHC class I gene expression	[17–22]
NAIP	Increasing of neuronal survival in pathological conditions, modulation of the inflammasome assembly	[23–25]
NLRP1	Inflammasome component	[26, 27]
NLRP2	Inflammasome activation, inhibition of NF-κB signaling, embryonic development	[28–30]
NLRP3	Inflammasome component	[31, 32]
NLRP4	Inhibition of NF-κB signaling, negative regulation of RLR signaling, autophagy inhibition	[33–35]
NLRP5	Regulation of caspase activation and apoptosis in injured neurons, involvement in embryonic development	[36–39]
NLRP6	Suppression of NF-κB signaling, inflammasome activation	[40–42]
NLRP7	Regulation of inflammasome, inhibition of NF-κB activation; mutations associate with at higher risk of pregnancy complications and perinatal mortality	[43–46]
NLRP10	Dendritic cell migration, negative regulation of NF-κB activation and cell death, inhibition of IL-1β secretion, enhancement of antibacterial proinflammatory response	[47–49]
NLRP12	Regulation of NF-κB signaling, inflammasome activation, dendritic cell migration, transcription of MHC class I genes (details in text)	
NLRP14	Spermatogenesis involvement	[50]
NLRC3	Inhibition of NF-κB activation	[16, 51]
NLRC4	Inflammasome component	[52]
NLRC5	Transcriptional activation of MHC class I and related genes involved in antigen presentation; regulation of innate immune responses through TLR-mediated NF-κB activation, type I IFN production, and inflammasome activation	[21,22, 53–57]
NLRX1	Mitochondrial localization; negative regulation of RLR signaling, positive regulation of the immune system by triggering the generation of reactive oxygen species, inhibition of NF-κB activation, autophagy enhancer	[58–62]
NOD1 and NOD2	Activation of NF-κB and MAPK signaling pathways; interaction with other NLRs important for caspase-1 activation; function in autophagy and integration with antigen receptor-driven T cell activation. Additionally, NOD1 regulates B cell activation and NOD2 plays a role in viral recognition, T cell activation, and in the survival of regulatory T cells.	[63–79]
NLRP8, -9, -11, -13	?	

NLR proteins regulate inflammation, apoptosis, autophagy, embryonic development, as well as transcriptional reprogramming of immune genes.

NLRs as activators of NF-κB and MAPKs. NOD1 (NLRC1 or CARD4) and NOD2 (NLRC2 or CARD15) are the two NLR members that regulate NF-κB and MAPK pathways [65]. NOD1 and NOD2 recognize the diaminopimelic acid and muramyl dipeptide bacterial peptidoglycans, respectively [77, 78]. Upon ligand binding, NOD1 and NOD2 undergo conformational changes and self-oligomerization via the central NOD domain, followed by the recruitment and activation of the serine threonine kinase RIP2 by CARD–CARD interactions [65, 79]. The assembly of NOD1 and NOD2 signalosomes results in the activation of NF-κB and MAPKs, which drives the up-regulation of proinflammatory genes [80].

Besides the well-characterized role in RIP2-dependent NF-κB activation, NOD2 has been described recently as having other functions. It has been demonstrated that NOD2 can also function as a cytoplasmic viral PRR that responds to cytosolic RNA during viral infection and potentiates antiviral signaling [71]. In addition, NOD2 has been shown to interact with the proto-oncogene c-Rel in T lymphocytes, resulting in decreased nuclear accumulation of c-Rel and impaired IL-2 transcription [75]. NOD1 and NOD2 also stimulate autophagy in response to invasive bacteria, independently from the adaptor protein RIP2 and NF-κB signaling [68].

Consistent with their role in mediating inflammatory processes, NOD1 and NOD2 have been implicated in a number of chronic inflammatory diseases, such as Crohn's disease, Blau's syndrome, sarcoidosis, inflammatory bowel disease, asthma, atopic eczema, and graft-versus-host disease [81–86].

NLRs and the inflammasome. Sensing of PAMPs and DAMPs by certain NLR proteins can result in the assembly of a multiprotein complex, referred to as the inflammasome, which causes the autoactivation of caspase-1 and ultimately results in the secretion of the potent proinflammatory cytokines, IL-1β and IL-18 [1]. This generates a loop that amplifies the NF-κB, JNK, and p38 MAPK signaling pathways, leading to a higher expression of proinflammatory cytokines and chemokines, whose effect is the recruitment of immune cells to the site of inflammation. The final step is the removal of the danger signal, resulting in the resolution of infection and/or cell death [87].

A number of NLR family members have been shown to take part in the multiprotein complex, forming the inflammasome in vitro, but only few NLR proteins have clear in vivo functions in the inflammasome assembly [12]. The particular NLR involved in the process appears to be stimulus specific. For example, the NLRC4 inflammasome is formed in response to multiple Gram-negative bacteria expressing flagellin, whereas

the NLRP3 inflammasome has been shown to respond to a variety of pathogens and host-derived molecules [31, 32, 48]. An inflammasome complex consisting of NOD2, NLRP1, and caspase-1 has also been reported [66]. With the consideration of the well-known role of NOD2 in NF-κB activation, it has been hypothesized that heterogeneous inflammasome couples transcriptional activation of inflammatory genes with IL-1β production [66]. More recently, NOD1 has also been shown to induce ASC-independent IL-1β secretion in *Chlamidia*-infected trophoblasts [76]. The existence of other heterogeneous inflammasomes and the interactions between certain elicitors and NLR proteins represent an intense field of research [85].

A breakthrough in the field has recently come from the demonstration that the NLRP3 inflammasome is released by macrophages as extracellular oligomeric particulate complexes, which also contain the adaptor ASC. These complexes would amplify inflammation by promoting caspase 1 activation in the extracellular milieu, as well as within bystander cells upon internalization [88]. ASCs are also released by cells undergoing pyroptosis and assume a "prion-like" activity, aggregating soluble ASCs in the microenvironment, as well as in the cytosol of receiving cells [89]. Accordingly, such complexes have been found in the serum of cryopirin-associated periodic syndrome patients, and ASC autoantibodies are present in patients and mice with autoimmune diseases [88, 89]. Whether other NLRP proteins can give rise to such structures will surely be the object of future research.

NLRs with inhibitory functions. In contrast to NOD1, NOD2, and some of the inflammasome-forming NLRs, little is known about the other NLR proteins. Among the members of the NLR family, NLRP2, NLRC3, NLRP4, NLRP6, NLRP7, NLRP10, NLRP12, and NLRX1 have been suggested to play inhibitory roles during inflammation by controlling caspase-1-mediated IL-1β secretion or by suppressing NF-κB signaling, but how these proteins are activated and perform their inhibitory action is not fully understood as yet [15, 28, 33, 40, 46, 48, 49, 51, 60, 90–92]. It is intriguing, however, that under different circumstances, the same family members can play opposite roles as inflammasome triggers or inhibitors. This is the case for NLRP7, which has been suggested to act as negative regulator of inflammasomes, whereas more recently, it has been shown to be part of the inflammasome formed in response to microbial-acylated lipopeptides [43]. The same is true for NLRP12, as discussed in the next section.

It is conceivable that the type of stimulus, the route by which it is delivered, as well as the genetic and epigenetic background of the recipient cells determine which components of the NLR family members are required for the inflammasome activation in response to the danger signals. Many questions remain to be answered, and the field is moving rapidly.

A number of excellent reviews discussing the role of NLR proteins during inflammation have been published [1–16]. Here, we will focus on NLRP12, which despite being one of the first discovered, remains somewhat enigmatic as a result of the contradictory messages coming from different experimental settings.

NLRP12

Identification and expression

NLRP12, also named RNO, PYPAF7, and Monarch-1, is a pyrin-containing NLR protein. The gene was first identified and partially characterized in the HL60 human leukemic cell line [92]. It was first named RNO, as it was found to be up-regulated in HL60 cells upon NO stimulation. Subsequently, the full-length gene was independently cloned by two groups and named as Monarch-1 and PYPAF7 [93, 94].

NLRP12 is an intracellular protein consisting of an N-terminal PYD, a central NBD, and a C-terminal LRR region. The full-length human NLRP12 cDNA encodes for a 1062-aa protein with an estimated molecular weight of ~120 kDa [95] (**Fig. 2A**). Alternative splicing results in multiple transcript variants of NLRP12 (Fig. 2B and **Table 2**). Whether these variants are differentially expressed and/or serve functions distinct from the full-length product has not yet been determined.

Human NLRP12 is expressed predominantly in cells of myeloid lineage, such as neutrophils, eosinophils, monocytes, macrophages, and immature dendritic cells, and its expression is down-regulated in response to pathogens, pathogen products, and inflammatory cytokines [93, 95, 96]. Partial down-regulation of NLRP12 transcription is achieved after TLR stimulation by the binding of B lymphocyte-induced maturation protein-1 to the promoter [97]. NLRP12 has been shown to interact with Hsp70 and Hsp90 chaperones, and these interactions are important for its stability [98].

Function

As the expression of NLRP12 is restricted to the immune cells and down-regulated in response to pathogen products and inflammatory cytokines, this protein has been predicted to regulate inflammation and immunity [91, 94, 99].

Regulation of NF-κB. One of the earliest reports indicates that when overexpressed in nonimmune cells, NLRP12 colocalizes with ASC and activates NF-κB and caspase-1, leading to IL-1β secretion [94]. Although these findings demonstrated a role for NLRP12 in the activation of NF-κB, subsequent publications indicated NLRP12 as a damper of the inflammatory loop. In one of these studies, NLRP12 was shown to antagonize NF-κB signaling, acting as a negative regulator of TLR and TNFR signaling [91]. The silencing of NLRP12 in the human monocytic cell line THP-1 increased NF-κB activation and the proinflammatory cytokines in response to TLR agonists, TNF-α, and *Mycobacterium tuberculosis* [91, 99].

Biochemical studies showed that NLRP12 suppressed the production of proinflammatory cytokines and chemokines by interfering with canonical and noncanonical NF-κB pathways [100, 101]. In NLRP12-overexpressing THP-1 cells, while the nuclear translocation of the canonical NF-κB subunits RelA (p65) and p50 proceeded normally after stimulation with the TLR2 agonist, the pretreatment with the TLR2 ligand, followed by CD40 ligand stimulation, caused a reduction in p52 processing and nuclear translocation as a result of the ability of NLRP12 to associate with NIK through its NOD and LRR domains, leading to NIK proteasome-mediated degradation [99]. A recent study has highlighted the interaction of

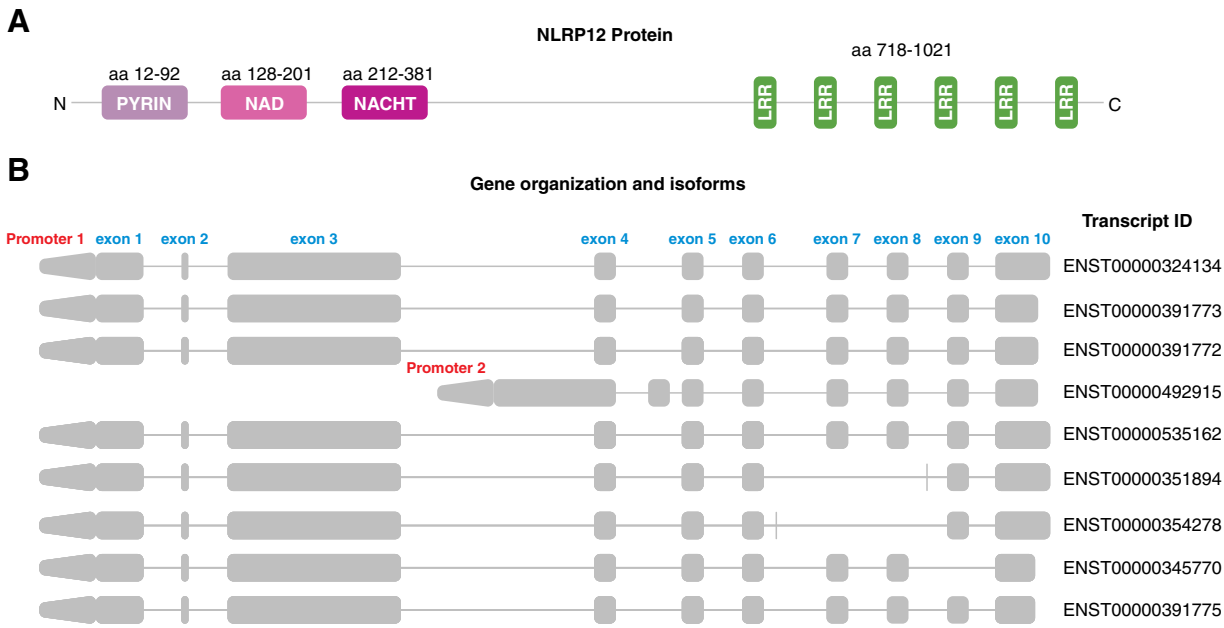

Figure 2. Human NLRP12 gene and protein. (A) NLRP12 protein domains. The NLRP12 protein is 1062 residues long, encoded by 10 exons. Exon 1 codes for a PYD. The largest exon, Exon 3, encodes for the NBD, which consists of a NACHT domain and a NACHT-associated domain (NAD). Exons 4–9 of the gene encode for the LRRs. Pfam database, release 27.0 (http://pfam.xfam.org/), was used to assign the domain boundaries. (B) Transcripts of human NLRP12. NLRP12 has nine transcripts. The transcripts are shown separately by drawing linear sequences of exons and splice junctions. To obtain a condensed view, introns did not necessarily reflect their original length. The image was obtained by TViewer, suite v3.1 (Genomatix software, Ann Arbor, MI, USA; http://www.genomatix.de/). The splicing variants are from Ensembl database (http://www.ensembl.org/).

NLRP12, not only with NIK but also with TRAF3, which is directly involved in NIK degradation [101]. Therefore, NLRP12 would keep noncanonical NF-κB activation in check by preventing TRAF3 degradation and consequently, NIK activation (**Fig. 3**). Accordingly, *Nlrp12*$^{-/-}$ cells have constitutively elevated NIK, p100 processing to p52, and reduced TRAF3 levels. By means of different mechanisms, several NLRs, including NLRP12, have been shown to converge their action by dampening the canonical NF-κB activation pathway. In particular, NLRP12 prevents IRAK1 phosphorylation and ATP binding to the NBD of NLRP12, which appears to be critical for the inhibition to occur [16, 91, 95, 100] (Fig. 3).

Involvement in cancer. The inhibitory role of NLRP12 on NF-κB signaling and inflammation was also studied in in vivo models of colon inflammation and colorectal cancer [100, 101]. Bone marrow-derived macrophages from *Nlrp12*$^{-/-}$ mice exhibited increased pp105/NF-κB1 in response to LPS stimulation, which supports a role for NLRP12 in regulating the canonical NF-κB pathway. In accordance, *Nlrp12*$^{-/-}$ mice were highly susceptible to colon inflammation and showed a dramatic increase in the production of proinflammatory cytokines and chemokines via NF-κB and ERK activation. Intriguingly, *Nlrp12*$^{-/-}$ mice exhibited a higher susceptibility to tumor formation in the

TABLE 2. Gene Splicing Variants of Human NLRP12

Name	Location (19q13.4; reverse strand)	Ensembl transcript ID	Length (bp)	Ensembl protein ID	Length (aa)
NLRP12-001	54,296,857–54,327,597	ENST00000324134	3801	ENSP00000319377	1061
NLRP12-002	54,296,997–54,327,648	ENST00000391773	3715	ENSP00000375653	1062
NLRP12-007	54,296,997–54,327,648	ENST00000391772	3205	ENSP00000375652	892
NLRP12-005	54,296,997–54,311,959	ENST00000492915	2479	No protein product	–
NLRP12-203	54,296,857–54,327,648	ENST00000535162	3681	ENSP00000438030	1004
NLRP12-201	54,296,857–54,327,648	ENST00000351894	3516	ENSP00000340473	949
NLRP12-202	54,296,857–54,327,648	ENST00000354278	3345	ENSP00000346231	892
NLRP12-004	54,296,997–54,327,648	ENST00000345770	3547	ENSP00000341428	1006
NLRP12-003	54,296,996–54,327,571	ENST00000391775	3465	ENSP00000375655	1004

Data were obtained from the Ensembl database (http://www.ensembl.org/).

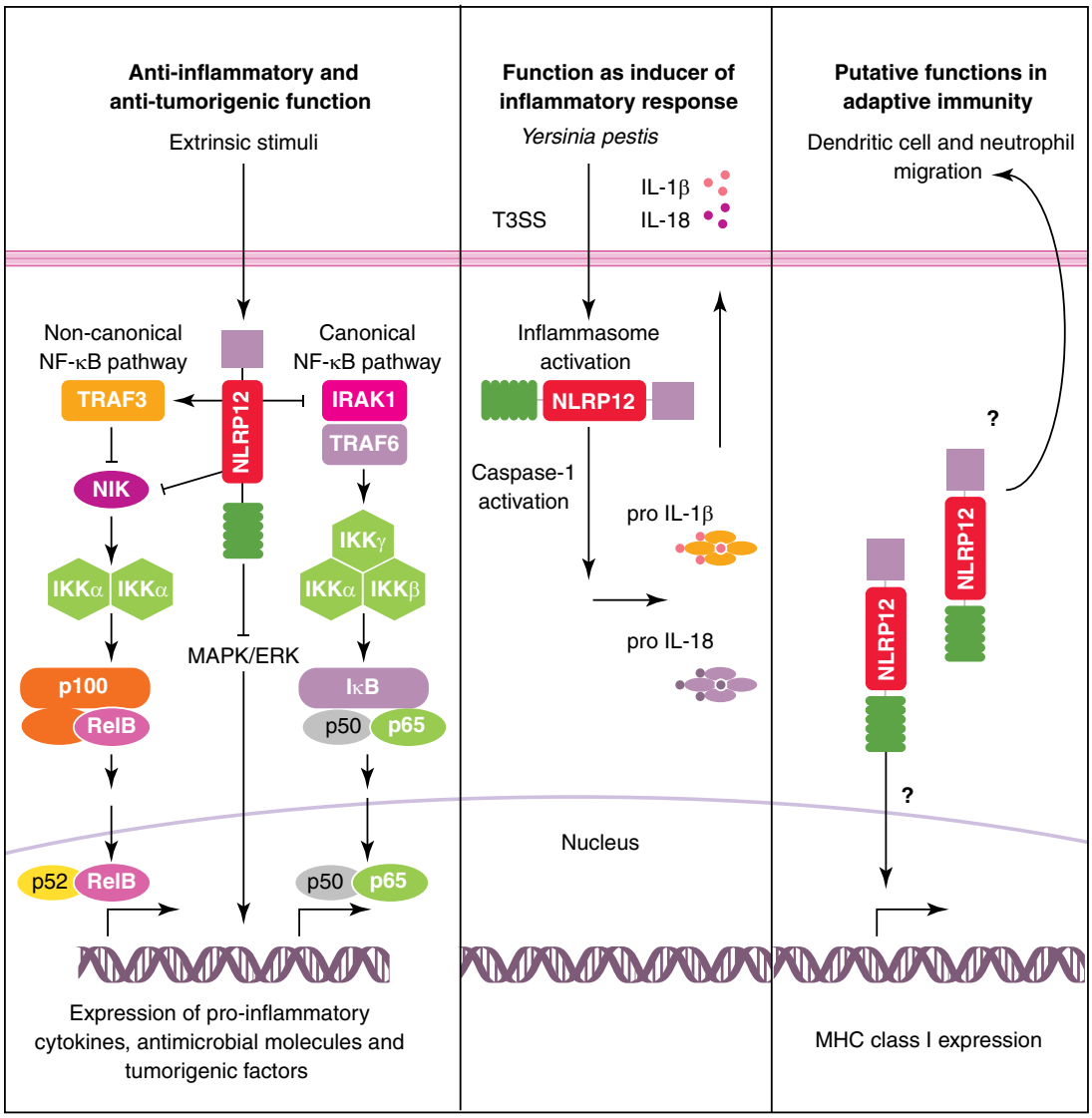

Figure 3. Functions of NLRP12. NLRP12 is supposed to function as a negative regulator of inflammatory signaling and colon tumorigenesis by suppressing canonical and noncanonical NF-κB signaling and the MAPK/ERK pathway of activation. However, NLRP12 can also be involved the inflammasome signaling, as shown in the *Y. pestis* infection, by driving caspase-1 activation and proinflammatory cytokine release. NLRP12 has also been indicated as having a role in adaptive immunity by controlling migration of dendritic cells and neutrophils and classical and nonclassical MHC class I expression. IKK, IκB kinase; T3SS, type III secretion system.

azoxymethane/dextran sodium sulfate colitis-associated colorectal tumorigenesis model [100].

The role of NLRP12 in maintaining intestinal homeostasis and providing protection against colitis-associated colon tumorigenesis was also supported by another report [101]. In this case, treatment with the TLR1/2 agonist, Pam3Cys4, gave rise to a small increase in pp65 and pIκBα in dendritic cells of *Nlrp12*$^{-/-}$ mice. However, an extensive comparison of cytokine production from the wild-type and *Nlrp12*$^{-/-}$ dendritic cells showed that most cytokines associated with canonical NF-κB signaling were not altered following Pam3Cys4 stimulation, which agrees with the modest change in canonical NF-κB [101]. Indeed, the higher susceptibility to colitis and colitis-associated colon cancer of *Nlrp12*$^{-/-}$ mice was rather attributed to the higher activation of the noncanonical NF-κB, ERK, and protein kinase B (AKT) signaling and an increased expression of cancer-associated, NIK-dependent target genes, including *Cxcl12* and *Cxcl13*. It has been suggested that this happens via interaction of NLRP12 with TRAF3, which normally targets NIK for degradation to prevent aberrant noncanonical NF-κB activation [101, 102].

In conclusion, findings by Zaki et al. [100] and Allen et al. [101] point to NLRP12 as a pivotal checkpoint element of NF-κB signaling, intestinal inflammation, and tumorigenesis. However, whereas the first study reports NLRP12 as playing its regulatory role on the canonical NF-κB signaling pathway, the second endorses a major effect on the noncanonical pathway. The inconsistency of the two reports could be attributed to the different experimental settings, i.e., cells analyzed (macrophages vs. dendritic cells), stimuli inducing NF-κB activation, and different read-outs (analysis of distinct NF-κB cascade and complex components). A further relevant difference comes from experiments with bone marrow chimeric mice: Zaki and colleagues [100] identify the NLRP12 signaling in the hematopoietic compartment as crucial for protection against colitis and tumorigenesis, whereas Allen's group [101] is in favor of a combined effect of both the hematopoietic and nonhematopoietic compartments. The reason for such discrepancy is not

straightforward; it is possible, however, that differences in the *Nlrp12*$^{-/-}$ mice colonies and housing conditions can impact on the gut microbiota and on the threshold of the inflammatory signaling.

Like NLRP12, NLRP6 has been implicated in the maintenance of gut homeostasis, as demonstrated by the fact that *Nlrp6*$^{-/-}$ mice are more susceptible to colitis and colitis-induced tumorigenesis than their wild-type counterpart [40–42]. However, the two knockout mouse models diverge during cohousing experiments; unlike *Nlrp6*$^{-/-}$, *Nlrp12*$^{-/-}$ mice do not transfer colitis susceptibility to wild-type mice, suggesting that *Nlrp6*$^{-/-}$ allows the outgrowth of a pathogenic microbiota, whereas *Nlrp12*$^{-/-}$ appears not to affect the microbiota composition [42].

These results hint at a distinct mechanism for the way the two molecules negatively control inflammation, with NLRP6 playing a protective role more focused on the regulation of the interplay between the epithelial barrier functions and the gut microbiota [40–42].

Further studies, exploiting additional experimental models of colitis, will be pivotal to gain insights into the overlapping and/or unique functions of NLRP12 versus NLRP6 in protecting against gut inflammation, dysbiosis, and carcinogenesis.

Role in fighting infections. Other studies have addressed the role of NLRP12 in host resistance to infectious agents. NLRP12 has been implicated as an inflammasome component, recognizing *Y. pestis*, the causative agent of plague. *Nlrp12*$^{-/-}$ mice showed higher mortality and bacterial load after *Y. pestis* infection, where the NLRP12 inflammasome was shown to be a central regulator of IL-18 and IL-1β production, mediated by caspase-1 activation. Furthermore, NLRP12 also induced IFN-γ production via IL-18; however, *Nlrp12*$^{-/-}$ had minimal effect on NF-κB signaling after infection with *Y. pestis* strains [103].

The role of NLRP12 during the in vivo host immune response to *Klebsiella pneumoniae* and *M. tuberculosis* has also been investigated. Despite the known effects of *K. pneumoniae* and *M. tuberculosis* infections on the activation of canonical NF-κB signaling, no significant difference was observed between *Nlrp12*$^{-/-}$and wild-type mice after *K. pneumoniae* or *M. tuberculosis* infections [104]. Moreover, NLRP12 did not contribute significantly to the in vivo host innate immune response to LPS stimulation or *M. tuberculosis* infection, even though the in vitro findings implicate NLRP12 as a negative regulator of NF-κB signaling after LPS stimulation or *M. tuberculosis* infection [91, 104]. These results suggest that unlike *Yersinia* infection, NLRP12 does not contribute to inflammasome-mediated host innate responses during *M. tuberculosis* and *K. pneumoniae* infections and that conceivably, other NLRs come into play in place of NLRP12.

Similarly, NLRP12 does not play any role as a classical inflammasome activator during *S. typhimurium* infection, even though *Nlrp12*$^{-/-}$mice were highly resistant to salmonellosis [105]. For the efficient control of *S. typhimurium* infection, NLRP12 was shown to suppress the activation of canonical NF-κB and ERK signaling and to inhibit the production of inflammatory cytokines and NO [105]. Interestingly, *S. typhimurium* infection increased the level of NLRP12 mRNA transcripts in macrophages from wild-type mice, whereas *M. tuberculosis* infection of the THP-1 monocytic cell line had been shown previously to down-regulate NLRP12 transcription [91, 105]. More recently, a report has shown the presence of NLRP3 and NLRP12 in inflammasome complexes in monocytes derived from malaria patients, as well as in mouse models, prompting the authors to conclude that the NLRP3/NLRP12-dependent activation of caspase-1 is likely to be the key event regulating the systemic expression of IL-1β and mediating the hypersensitivity of malaria patients to secondary bacterial infections [106].

In conclusion, the role of the NLRP12 inflammasome in fighting infections appears sporadic. It is possible that the action of NLRP12 is context dependent and sensitive to the specific nature of stimuli, otherwise masked by the redundant action of other members of NLR family.

Role in autoinflammatory disorders. In accordance with a regulatory role of NLRP12 during inflammation, mutations in the NLRP12 gene have been found associated with a new class of autoinflammatory syndromes, called NLRP12AD, which include some forms of familial cold autoinflammatory syndrome [107]. Studies have shown how the nonsense mutation p.Arg284X, which is located within the NBD, is less effective in suppressing NF-κB activity compared with the wild-type NLRP12 [108]. In line with a loss of function causing inflammation, an insertion generating a splicing defect also induces a clear reduction of the inhibitory properties of NLRP12 on NF-κB signaling. In contrast to these findings, however, the missense mutation p.Asp294Glu, mapping within the evolutionarily conserved NBD, associates with an increased caspase-1 activation rather than with inhibition of NF-κB signaling [109].

Furthermore, another missense mutation involving a CpG site (p.Arg352Cys) has been identified in Exon 3 of NLRP12, which encodes the nucleotide-binding site of the protein, and this again does not show a direct effect on NF-κB signaling but rather, an increase in speck formation and activated caspase-1 signaling [109]. In addition, PBMCs of patients with NLRP12AD have been shown to release spontaneously a large amount of IL-1β compared with PBMCs from healthy individuals [110]. In conclusion, although the physiological relevance of NLRP12 in periodic fever syndrome is certain, the way it acts to regulate the NF-κB pathway remains to be defined.

Role in controlling migration. Besides its role as a regulator of inflammation, other roles are now emerging for NLRP12. In vivo studies have revealed an unexpected role for NLRP12 in cellular migration: dendritic cells and neutrophils from *Nlrp12*$^{-/-}$mice showed reduced migration (attenuated contact hypersensitivity) from the periphery to the draining lymph nodes in vivo and failed to respond to chemokines in vitro [111]. However, *Nlrp12*$^{-/-}$did not significantly affect the production of proinflammatory cytokines in murine dendritic cells in response to TLR stimulation [91].

Finally, and in addition to the indicated roles, NLRP12 has been suggested to control the expression of classical and nonclassical MHC class I genes in vitro [93]. These findings are reminiscent of CIITA and NLRC5, which are the transactivators of MHC class II and MHC class I genes, respectively.

CONCLUDING REMARKS

NLRs represent a heterogenous group of proteins that play roles in a number of functions, ranging from pathogen/damage sensing to the modulation of inflammatory signaling and antigen presentation. Furthermore, some members of this family are involved in embryonic development. NLRP12, although one of the earliest identified family members, remains one of the more elusive. Its expression is restricted to myeloid cells, and despite the increasing number of studies, its physiological function during microbial infection and its role in the modulation of canonical versus noncanonical NF-κB signaling are still puzzling (Fig. 3). In addition, the precise nature of the NLRP12 ligand(s) is currently unknown.

These conflicting results may arise from the fact that its role can be pathogen specific as well as time and cell dependent. Consequently, the in vitro and in vivo experiments are difficult to reconcile, and multiple factors can play a role in determining whether NLRP12 attenuates or activates inflammation in different experimental settings. Crucially, a role for NLRP12 has been established in colon cancer, highlighting the importance of determining how its expression and function are regulated.

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KEY WORDS:
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