NOD-LIKE RECEPTORS (NLRs)

Introduction

In mammals, germ-line encoded pattern recognition receptors (PRRs) detect the presence of pathogens either directly through recognition of pathogen-associated molecular patterns (PAMPs) or indirectly through sensing host-derived danger signals (DAMPs). The innate immune system comprises several classes of PRRs that allow the early detection of pathogens at the site of infection. The membrane-bound toll-like receptors (TLRs) detect microbes on the cell surface and in endosomes. The RIG-1 (retinoic acid-inducible gene I)-like receptors (RLRs; RLHs) and the Nod-like receptors (NLRs; nucleotide-binding domain leucine-rich repeat containing receptors) detect microbial components in the cytosol.

NLR Proteins

The intracellular NLR proteins organize signaling complexes such as inflammasomes and NOD signalosomes. These proteins have been first identified due to their homology with the plant R proteins, a large family of proteins involved in resistance to phytopathogens. NLRs contain three characteristic domains:

1) a C-terminal leucine-rich repeat (LRR) domain responsible for ligand sensing and autoregulation;
2) a central NACHT (NOD) domain that has similarity to the NB-ARC motif of the apoptotic mediator APAF1 and is required for nucleotide binding and self-oligomerization; and
3) an N-terminal effector domain responsible for signal transduction and activation of the inflammatory response. To date, four different N-terminal domains have been identified: acidic transactivation domain, caspase-recruitment domain (CARD), pyrin domain (PYD), and baculoviral inhibitory repeat (BIR)-like domain.

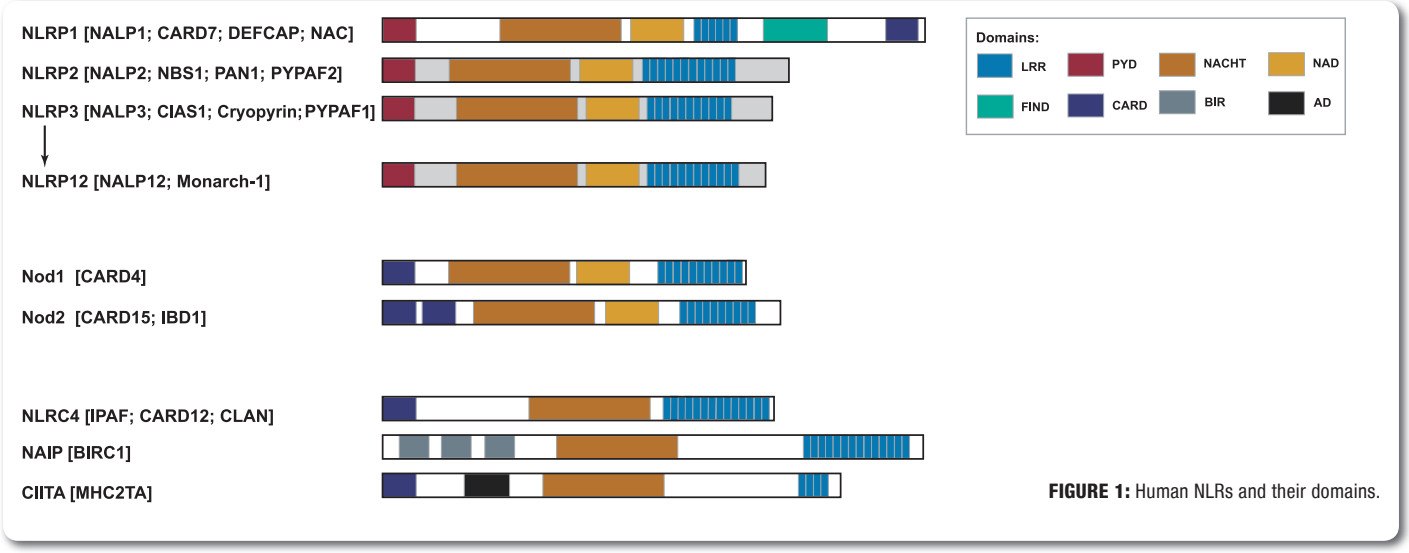

FIGURE 1: Human NLRs and their domains.

In 2008, J.P. Ting, et al. proposed a unified standard nomenclature for the NLR gene family based on the N-terminal effector domain (see Table 1).

Approved Name		Other Names and Aliases	NLR Family	Domain Organization
CIITA	class II, major histocompatibility complex, transactivator	NLRA; MHC2TA; C2TA	NLRA	(CARD)-AD-NACHT-NAD-LRR
NAIP	NLR family, apoptosis inhibitory protein	NLRB1; BIRC1; CLR5.1	NLRB	BIR3x-NACHT-LRR
NOD1	nucleotide-binding oligomerization domain containing 1	NLRC1; CARD4; CLR7.1	NLRC	CARD-NACHT-NAD-LRR
NOD2	nucleotide-binding oligomerization domain containing 2	NLRC2; CARD15; CD; BLAU; IBD1; PSORAS1; CLR16.3	NLRC	CARD2x-NACHT-NAD-LRR
NLRC3	NLR family, CARD domain containing 3	NOD3; CLR16.2	NLRC	CARD-NACHT-NAD-LRR
NLRC4	NLR family, CARD domain containing 4	CARD12; CLAN; CLR2.1; IPAF	NLRC	CARD-NACHT-NAD-LRR
NLRC5	NLR family, CARD domain containing 5	NOD27; CLR16.1	NLRC	CARD-NACHT-NAD-LRR
NLRP1	NLR family, pyrin domain containing 1	NALP1; DEFCAP; NAC; CARD7; CLR17.1	NLRP	PYD-NACHT-NAD-LRR-FIIND-CARD
NLRP2	NLR family, pyrin domain containing 2	NALP2; PYPAF2; NBS1; PAN1; CLR19.9	NLRP	PYD-NACHT-NAD-LRR
NLRP3	NLR family, pyrin domain containing 3	CIAS1; PYPAF1; Cryopyrin; CLR1.1; NALP3	NLRP	PYD-NACHT-NAD-LRR
NLRP4	NLR family, pyrin domain containing 4	NALP4; PYPAF4; PAN2; RNH2; CLR19.5	NLRP	PYD-NACHT-NAD-LRR
NLRP5	NLR family, pyrin domain containing 5	NALP5; PYPAF8; MATER; PAN11; CLR19.8	NLRP	PYD-NACHT-NAD-LRR
NLRP6	NLR family, pyrin domain containing 6	NALP6; PYPAF5; PAN3; CLR11.4	NLRP	PYD-NACHT-NAD-LRR
NLRP7	NLR family, pyrin domain containing 7	NALP7; PYPAF3; NOD12; PAN7; CLR19.4	NLRP	PYD-NACHT-NAD-LRR
NLRP8	NLR family, pyrin domain containing 8	NALP8; PAN4; NOD16; CLR19.2	NLRP	PYD-NACHT-NAD-LRR
NLRP9	NLR family, pyrin domain containing 9	NALP9; NOD6; PAN12; CLR19.1	NLRP	PYD-NACHT-NAD-LRR
NLRP10	NLR family, pyrin domain containing 10	NALP10; PAN5; NOD8; PYNOD; CLR11.1	NLRP	PYD-NACHT-NAD
NLRP11	NLR family, pyrin domain containing 11	NALP11; PYPAF6; NOD17; PAN10; CLR19.6	NLRP	PYD-NACHT-NAD-LRR
NLRP12	NLR family, pyrin domain containing 12	NALP12; PYPAF7; Monarch1; RNO2; PAN6; CLR19.3	NLRP	PYD-NACHT-NAD-LRR
NLRP13	NLR family, pyrin domain containing 13	NALP13; NOD14; PAN13; CLR19.7	NLRP	PYD-NACHT-NAD-LRR
NLRP14	NLR family, pyrin domain containing 14	NALP14; NOD5; PAN8; CLR11.2	NLRP	PYD-NACHT-NAD-LRR
NLRX1	NLR family member X1	NOD9; CLR11.3	NLRX	X-NACHT-NAD-LRR

TABLE 1: New standard nomenclature for the human NLR family members.

The following abbreviations are used: AD, acidic activation domain; CARD, caspase activating and recruitment domain; LRR, leucine-rich repeat; NACHT, domain present in NAIP, CIITA, HET-E, and TP-1; BIR, baculovirus inhibitor of apoptosis repeat; PYD, pyrin domain; NAD, NACHT-associated domain.

Adapted from: The NLR gene family: a standard nomenclature: J.P. Ting, et al.; Immunity ***28,*** *285 (2008)*

Selected Latest Review Articles

NLR, the nucleotide-binding domain leucine-rich repeat containing gene family: Z. Ye & J.P. Ting; Curr. Opin. Immunol. **20,** 3 (2008) ▪ Detection of immune danger signals by NALP3: F. Martinon; J. Leukoc. Biol. **83,** 507 (2008) ▪ Linking inflammasome activation and phagosome maturation: V. Lazarevic & F. Martinon; Cell Host Microbe **3,** 199 (2008) ▪ NOD-like receptors (NLRs): bona fide intracellular microbial sensors: M.H. Shaw, et al.; Curr. Opin. Immunol. **20,** 377 (2008) ▪ Molecular regulation of inflammation and cell death: G. Yeretssian, et al.; Cytokine **43,** 380 (2008) ▪ The microbial and danger signals that activate Nod-like receptors: S. Benko, et al.; Cytokine **43,** 368 (2008) ▪ The caspase-1 inflammasome: a pilot of innate immune responses: H.B. Yu & B.B. Finlay; Cell Host Microbe **4,** 198 (2008) ▪ The role of NLRs and TLRs in the activation of the inflammasome: M.G. Netea, et al.; Expert Opin. Biol. Ther. **8,** 1867 (2008) ▪ Inflammasomes: guardians of cytosolic sanctity: M. Lamkanfi & V.M. Dixit; Immunol. Rev. **227,** 95 (2009) ▪ Function of Nod-like receptors in microbial recognition and host defense: L. Franchi, et al.; Immunol. Rev. **227,** 106 (2009) ▪ The inflammasomes: guardians of the body: F. Martinon, et al.; Annu. Rev. Immunol. **27,** 229 (2009) ▪ Sensing pathogens and danger signals by the inflammasome: J.H. Pedra, et al.; Curr. Opin. Immunol. **21,** 10 (2009) ▪ The inflammasome: a caspase-1-activation platform that regulates immune responses and disease pathogenesis: L. Franchi, et al.; Nat. Immunol. **10,** 241 (2009) ▪ NLR-mediated control of inflammasome assembly in the host response against bacterial pathogens: I.E. Brodsky & D. Monack; Semin. Immunol. **21,** 199 (2009)

Latest Insight

T cells regulate innate immune responses through inhibition of NLRP1 and NLRP3 inflammasomes

A tight regulation of the inflammatory response is essential to avoid tissue damage and related diseases such as arthritis and diabetes type 2. Jürg Tschopp and colleagues examined the role of T cells in the regulation of innate immune responses. They showed that mouse effector and memory $CD4^+$ T cells abolish macrophage inflammasome-mediated caspase-1 activation and subsequent IL-1β release.

LIT: T cells dampen innate immune responses through inhibition of NLRP1 and NLRP3 inflammasomes: G. Guarda, et al.; Nature **460,** 269 (2009)

Nod Signalosomes

Nod1 (CARD4; NLRC1) and Nod2 (CARD15; NLRC2) are intracellular pattern-recognition molecules (PRMs) of the NLR (Nod-like receptor) family. Nod1 is ubiquitously expressed, while Nod2 expression is restricted to monocytes, macrophages, dendritic cells and intestinal Paneth cells. Both proteins are implicated in the detection of bacterial peptidoglycan (PGN). Nod1 senses mesodiaminopimelic acid (meso-DAP)-containing peptidoglycan found in the cell wall of Gram-negative bacteria. Nod2 seems to be a general sensor which is activated by muramyl dipeptide (MDP), the minimal motif common to all PGNs of Gram-negative as well as Gram-positive bacteria. Upon activation, Nod1 and Nod2 initiate a pro-inflammatory response through recruitment of the receptor-interacting protein 2 (RIP-2; RICK; CARDIAK). K^{63}-linked ubiquitination of RICK leads to the recruitment of TAK1 and the IKK complex. The interaction between RICK and NEMO ultimately leads to the activation of IKKs and NF-κB by TAK1 (see Figure 2). Both Nod1 and Nod2 are key receptors in epithelial cells where they control infections via the gastro-intestinal system. Mutations in Nod1 have been reported to confer susceptibility to several inflammatory disorders including inflammatory bowel disease, atopic eczema and asthma. Similarly, mutations in Nod2 have been linked to Crohn's disease.

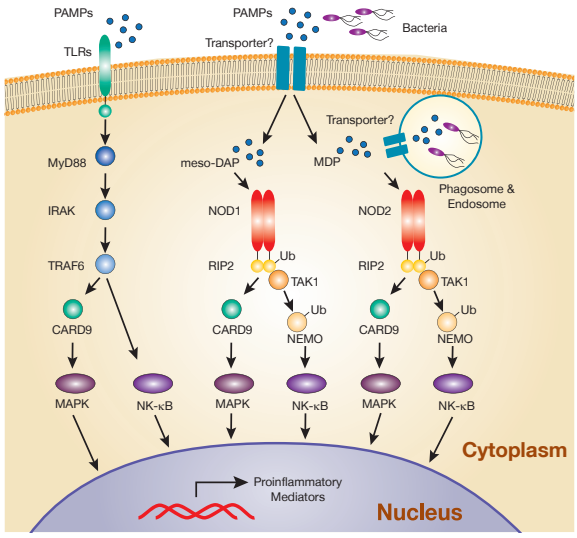

FIGURE 2: Model for PGN recognition by Nod1 and Nod2.

The NLR proteins Nod1 and Nod2 sense intracellular meso-DAP and MDP, respectively, leading to recruitment of the adaptor proteins RIP2. Extracellular PAMPs are recognized by TLRs, which signals through MyD88, IRAK protein, and TRAF members. For clarity, the TLR pathway has been simplified. The subsequent activation of NF-κB and MAP kinases results in the transcriptional upregulation of proinflammatory genes.

Adapted from: Intracellular NOD-like receptors in host defense and disease: T.D. Kanneganti, et al.; Immunity ***27,*** *549 (2007)*

Nod1 [CARD4] & Nod2 [CARD15]

Nod1 (human), pAb (AL184)

ALX-210-918-C050 — 50 µg

From rabbit. **IMMUNOGEN:** Synthetic peptide corresponding to aa 2-31 (E^2EQGHSEMEIIPSESHPHIQLLKSNRELLV^{31}) of human Nod1 (CARD4). **SPECIFICITY:** Recognizes human Nod1. **APPLICATION:** WB.

Nod2 (human), mAb (2D9)

ALX-803-307-1 — 1 Vial

CLONE: 2D9. **ISOTYPE:** Mouse IgG1. **IMMUNOGEN:** Recombinant human Nod2 (CARD15) (aa 28-301). **SPECIFICITY:** Recognizes human Nod2. **APPLICATION:** IHC, IP, WB.

Nod2 (human), pAb

ALX-210-373-1 — 1 Vial

From rabbit. **IMMUNOGEN:** Recombinant human Nod2 (CARD15) (aa 28-301). **SPECIFICITY:** Recognizes human Nod2. **APPLICATION:** IP, WB.

RIP2 [RICK]

RIP2 (human), mAb (Nick-1)

ALX-804-139-C100 — 100 µg

CLONE: Nick-1. **ISOTYPE:** Rat IgG2. **IMMUNOGEN:** Recombinant human RIP2 (receptor-interacting protein 2) (aa 1-322). **SPECIFICITY:** Recognizes human RIP2. **APPLICATION:** WB.

LIT: Participation of Rip2 in Lipopolysaccharide signaling is independent of its kinase activity: C. Lu et al.; J. Biol. Chem. **280,** 16278 (2005) ▪ PIDD mediates NF-κB activation in Response to DNA damage: Janssens, et al.; Cell **123,** 1079 (2005) ▪ Participation of Rip2 in lipopolysaccharide signaling is independent of its kinase activity: C. Lu, et al.; J. Biol. Chem. **280,** 16278 (2005)

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Latest Insight

A new role for Nod2 in antiviral immunity

Recent studies identified a second pathway of Nod2 activation through viral ssRNA. A. Sabbah, et al. showed that ssRNA viruses activate Nod2 and subsequently lead to the production of interferon and anti-viral immunity. This response requires MAVS, a mitochondrial membrane-anchored CARD protein that is a potent activator of IRF3.

LIT: Activation of innate immune antiviral responses by Nod2: A. Sabbah, et al.; Nat. Immunol. **10,** 1073 (2009) ▪ Beyond peptidoglycan for Nod2: P.J. Murray; Nat. Immunol. **10,** 1053 (2009)

Inflammasomes

By definition, an inflammasome represents a high molecular weight complex that activates inflammatory caspases and the cytokines of the IL-1 family. Several inflammasomes have been described and are defined by the NLR protein that they contain: the NLRP1 (NALP1) inflammasome, the NLRP3 (NALP3) inflammasome and the NLRC4 (IPAF) inflammasome. Inflammasomes can be activated through multiple signals including live bacteria, microbial molecular patterns, microbial toxins, xenocompounds and endogenous danger signals (for a complete overview see Page 6). Upon sensing of their respective ligands, NLPR1 and NLPR3 recruit the adaptor protein ASC (apoptosis-associated speck-like protein containing a CARD) through homophilic PYD-PYD interactions. ASC contains an N-terminal PYD and a C-terminal CARD that allows the recruitment of inflammatory caspases through CARD-CARD interactions. The oligomerization of NLRPs is believed to bring inflammatory caspases into close proximity, leading to their activation within the inflammasome. In contrast, NLRC4 does not recruit an adaptor molecule but directly activates caspase-1 via its CARD domain (see Figure 3).

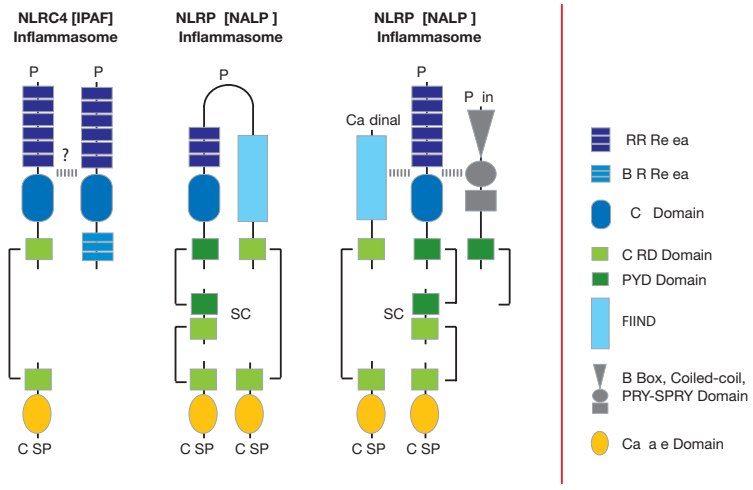


FIGURE 3: Schematic models of proposed caspase-1-activating inflammasomes.

Adapted from: Inflammatory caspases and inflammasomes: master switches of inflammation: F. Martinon & J. Tschopp; Cell Death. Differ. ***14,*** *10 (2007).*

The assembly of the different inflammasomes leads to a common outcome, namely the activation of an inflammatory caspase. In mammals, these include human and mouse caspase-1 and -14, human caspase-4 and -5 as well as mouse caspase-12. These caspases all have a CARD domain followed by a domain containing the catalytic residue and are called inflammatory caspases because their main substrates are cytokines (such as IL-1β, IL-18 and possibly IL-33). IL-1β, inflammatory caspases and the inflammasomes may play an important role in several diseases including auto-inflammatory diseases, cancer and neurodegenerative diseases. In addition, inflammasome activation can lead to host cell death in certain cell types, which might be important in restricting the intracellular replication of invasive bacterial pathogens.

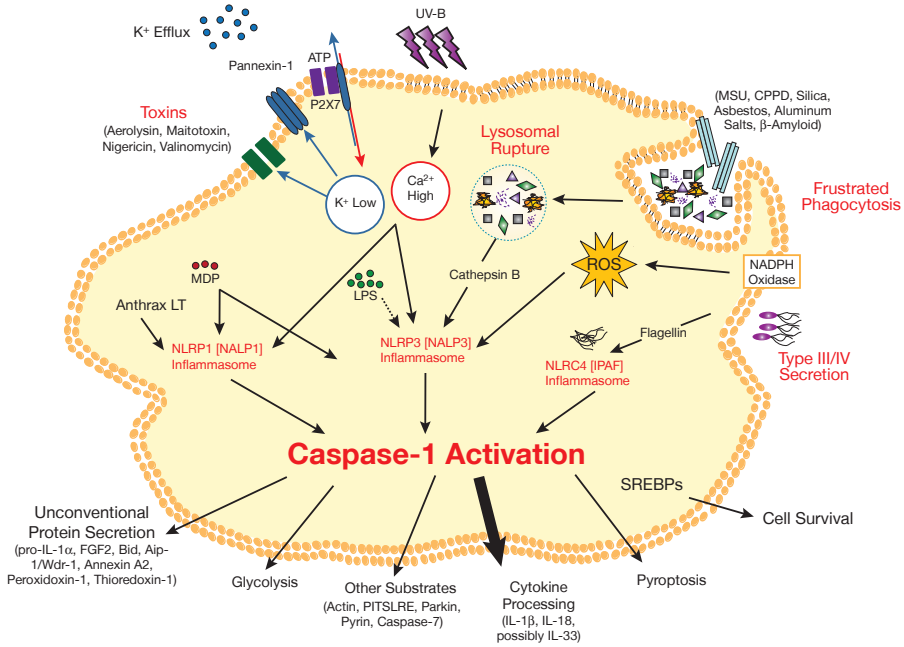


FIGURE 4: Pathways for activation of the inflammasome by PAMPs and DAMPs.

Multiple pathways are responsible for the activation of the caspase-1 inflammasomes. Pores formed in the host cell membrane by pore-forming toxins and ATP-P2X7-activated pannexin-1 lead to K^+ efflux, Ca^{2+} influx and entry of extracellular PAMPs into the host cytosol, which activate NLRP (NALP) inflammasomes. *S. typhimurium*, *S. flexneri* and *L. pneumophila* require a functional type III or type IV secretion system to secrete flagellin into the host cell cytosol, which activates the NLRC4 (IPAF) inflammasome. Phagocytosis of MSU, CPPD, silica, asbestos and aluminum salts leads to phagocytosis of the crystals followed by lysosomal rupture, which results in the release of cathepsin B into the cytosol and activates the NLRP3 (NALP3) inflammasome. Crystals, that are too large to be ingested, remain at the cell surface where they induce membrane perturbations or "frustrated phagocytosis". Reactive oxygen species (ROS), produced through the actions of membrane-bound NADPH oxidase, are implicated in transducing the signal from the membrane to the NLRP3 inflammasome. Inflammasome assembly stimulates the activity of caspase-1, which mediates processing of cytokines, glycolysis enzymes and other substrates, pyroptosis, unconventional protein secretion, and promotion of cell survival through activation of SREBPs in response to pore-forming toxins

Adapted from: Inflammasomes in infection and inflammation: C.R. McIntire, et al.; Apoptosis **14,** 522 (2009)

Antibodies to NALPs / NLRPs

NALP1 (human), mAb (Nalpy1-4)

ALX-804-803-C100 100 µg

CLONE: Nalpy1-4. **ISOTYPE:** Mouse IgG1. **IMMUNOGEN:** Recombinant human NALP1 (NACHT-, LRR- and PYD-containing protein 1) (pyrin domain). **SPECIFICITY:** Recognizes the pyrin domain (PYD) of human NALP1. **APPLICATION:** IHC (FS, PS), ICC, IP, WB.

LIT: Inflammasome components NALP 1 and 3 show distinct but separate expression profiles in human tissues suggesting a site-specific role in the inflammatory response: J.A. Kummer, et al.; J. Histochem. Cytochem. **55,** 443 (2007)

NALP1 (human), pAb (AL176)

ALX-210-904-R100 100 µl

From rabbit. **IMMUNOGEN:** Synthetic peptide corresponding to aa 2-25 (A^{2}GGAWGRLACYLEFLKKEELKEFQ^{25}) of N-terminal human NALP1 (NACHT-, LRR- and PYD-containing protein 1). **SPECIFICITY:** Recognizes human NALP1. **APPLICATION:** WB.

LIT: The inflammasome: a molecular platform triggering activation of inflammatory caspases and processing of proIL-beta: F. Martinon; Mol. Cell. **10,** 417 (2002)

NALP1 (human) (CT), pAb (Bur 242)

ALX-210-017-R050 50 µl

From rabbit. **IMMUNOGEN:** Synthetic peptide corresponding to aa 1058-1077 (C^{1058}VPSPASQGDLHTKPLGTDD^{1077}) of C-terminal human NALP1 (NACHT-, LRR- and PYD-containing protein 1). **SPECIFICITY:** Recognizes human NALP1. **APPLICATION:** IHC (PS), IP, WB.

LIT: A novel enhancer of the Apaf1 apoptosome involved in cytochrome c-dependent caspase activation and apoptosis: Z.L. Chu, et al.; J. Biol. Chem. **276,** 9239 (2001)

NALP1 (human) (NT), pAb (Bur 241)

ALX-210-018-R050 50 µl

From rabbit. **IMMUNOGEN:** Synthetic peptide corresponding to aa 161-180 (P^{161}SSPDHESPSQESPNAPTST^{180}) of N-terminal human NALP1 (NACHT-, LRR- and PYD-containing protein 1). **SPECIFICITY:** Recognizes human NALP1. **APPLICATION:** IHC (PS), IP, WB.

LIT: A novel enhancer of the Apaf1 apoptosome involved in cytochrome c-dependent caspase activation and apoptosis: Z.L. Chu, et al.; J. Biol. Chem. **276,** 9239 (2001)

NALP3 (human), mAb (Nalpy3-a)

ALX-804-818-C100 100 µg

CLONE: Nalpy3-a. **ISOTYPE:** Mouse IgG1. **IMMUNOGEN:** Recombinant human NALP3 (NACHT-, LRR- and PYD-containing protein 3) (pyrin domain). **SPECIFICITY:** Recognizes human NALP3. Detects endogenous protein by ICC and WB. **APPLICATION:** ICC, WB.

LIT: NALP3 forms an IL-1beta-processing inflammasome with increased activity in Muckle-Wells autoinflammatory disorder: L. Agostini, et al.; Immunity **20,** 319 (2004)

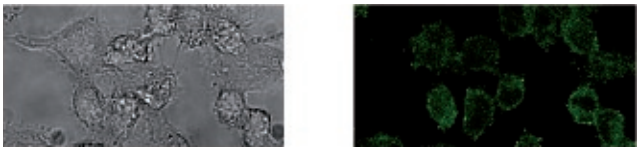


FIGURE: Immunofluorescence microscopy showing the detection of endogenous NALP3 in THP1 cells with the NALP3 (human), mAb (Nalpy3-a) (Prod. No. ALX-804-818). Method: Following fixation with paraformaldehyde 3.7%, THP1 cells were incubated with the NALP3 (human), mAb (Nalpy3-a) (Prod. No. ALX-804-818) at a 1:100 dilution. An anti-mouse IgG antibody conjugated with Alexa Fluor 488 was used for detection.

NALP3 (human), mAb (Nalpy3-b)

ALX-804-819-C100 100 µg

CLONE: Nalpy3-b. **ISOTYPE:** Mouse IgG1. **IMMUNOGEN:** Recombinant human NALP3 (NACHT-, LRR- and PYD-containing protein 3) (pyrin domain). **SPECIFICITY:** Recognizes human NALP3. Detects endogenous protein by IP and WB. **APPLICATION:** IHC (FS), IP, WB.

LIT: NALP3 forms an IL-1beta-processing inflammasome with increased activity in Muckle-Wells autoinflammatory disorder: L. Agostini, et al.; Immunity **20,** 319 (2004) ▪ Inflammasome components NALP 1 and 3 show distinct but separate expression profiles in human tissues suggesting a site-specific role in the inflammatory response: J.A. Kummer, et al.; J. Histochem. Cytochem. **55,** 443 (2007)

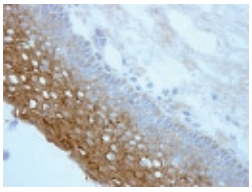


FIGURE: Staining of endogenous NALP3 in epithelial layer of human tonsil (frozen section) using NALP3 (human), mAb (Nalpy3-b) (Prod. No. ALX-804-819).

NALP12 (human), pAb (AL236)

ALX-210-958-C100 100 µg

From rabbit. **IMMUNOGEN:** Synthetic peptide corresponding to aa near the N-terminus of human NALP12. **SPECIFICITY:** Recognizes human NALP12. **APPLICATION:** IP, WB.

NALP12 (mouse), pAb (AT141)

ALX-210-967-C100 100 µg

From rabbit. **IMMUNOGEN:** Recombinant mouse NALP12 (aa 1-100). **SPECIFICITY:** Recognizes mouse NALP12. **APPLICATION:** WB.

Asc

Asc, pAb (AL177)

ALX-210-905-R100 100 µl

From rabbit. **IMMUNOGEN:** Synthetic peptide corresponding to aa 2-27 (G^{2}RARDAILDALENLTAEELKKFKLKL^{27}) of N-terminal human Asc (apoptosis-associated speck-like protein containing CARD; Pycard). **SPECIFICITY:** Recognizes human and mouse Asc. **APPLICATION:** ICC, IP, WB.

LIT: The inflammasome: a molecular platform triggering activation of inflammatory caspases and processing of proIL-beta: F. Martinon, et al.; Mol. Cell. **10,** 417 (2002) ▪ NALP3 forms an IL-1beta-processing inflammasome with increased activity in Muckle-Wells autoinflammatory disorder: L. Agostini, et al.; Immunity **20,** 319 (2004) ▪ P2X7 Receptor Differentially Couples to Distinct Release Pathways for IL-1{beta} in Mouse Macrophage: P. Pelegrin, et al.; J. Immunol. **180,** 7147 (2008)

IPAF [CARD12]

IPAF (human), mAb (Luna-1)

ALX-804-848-C100 100 µg

CLONE: Luna-1. **ISOTYPE:** Mouse IgG2. **IMMUNOGEN:** Recombinant human IPAF (ice protease-activating factor) (aa 9-494). **SPECIFICITY:** Recognizes human IPAF. **APPLICATION:** WB.

Pyrin

Pyrin (human), pAb (AL196)

ALX-210-946-C100 100 µg

From rabbit. **IMMUNOGEN:** Synthetic peptide corresponding to aa 2-29 (A^{2}K-TPSDHLLSTLEELVPYDFEKFKFKLQ^{29}) of human pyrin (MEFV; mediterranean fever protein). **SPECIFICITY:** Recognizes human pyrin. **APPLICATION:** IP, WB.

LIT: The SPRY domain of Pyrin, mutated in familial Mediterranean fever patients, interacts with inflammasome components and inhibits proIL-1beta processing: S. Papin, et al.; Cell Death Differ. **14,** 1457 (2007)

Nod-like Receptors & Their Ligands

NLR	Elicitor	
hNOD1		
	Microbial motifs	*meso*-Lanthionine, *meso*-DAP
		γ-D-Glu-*meso*-DAP (iE-DAP)
		L-Ala-γ-D-Glu-meso-DAP (TriDAP)
		D-Lactyl-L-Ala-γ-Glu-*meso*-DAP-Gly (FK156)
		Heptanoyl-γ-Glu-*meso*-DAP-D-Ala (FK565)
	Bacterial extracts	*Bacillus* species[a], *B. anthracis* spores, *L. pneumophila*, *S. typhimurium*, *M. tuberculosis*
	Live bacteria	*S. flexneri* (G^- Intra Ep), *H. pylori* (G^- Extra Ep), Enteroinvasive *E. coli* (G^- Intra Ep), *Pseudomonas* species[b] (G^- Intra Ep, F), *Chlamydia* species[c] (G^- Intra Ep, En, F), *L. monocytogenes* (G^+ Intra Ep, En)
mNOD1		
	Microbial motifs	GlcNAc-(anhydro)MurNAc-L-Ala-γ-D-Glu-*meso*-DAP-D-Ala (TCT)
		D-Lactyl-L-Ala-γ-Glu-*meso*-DAP-Gly (FK156)
NOD2		
ALX-151-035	Microbial motifs	MurNAc-L-Ala-D-isoGln (muramyldipeptide)
		MurNAc-L-Ala-γ-D-Glu-L-Lys (M-triLys)
	Bacterial extracts	*Bacillus* species[a], *B. anthracis* spores, *Lactobacillus* species[d], *Corynebacterium xerosis, E. coli*, *Pseudomonas* species[e], *L. pneumophila* , *M. tuberculosis*
	Live bacteria	*L. monocytogenes* (G^+ Intra Ep), *S. pneumoniae* (G^+ Intra Ep, M), S. *typhimurium* (G^- intra Ep), S. *flexneri* (G^- intra Ep)
NLRP1 (NALP1)		
	Microbial motifs	MurNAc-L-Ala-D-isoGln (muramyldipeptide; MDP)
	Microbial toxins	Lethal factor from *B. antracis*
NLRP3 (NALP3)		
ALX-151-035	Microbial motifs	MurNAc-L-Ala-D-isoGln (muramyldipeptide; MDP)
		Bacterial RNA
ALX-420-038 / -039		Imidazoquinoline Compounds (R837, R848)
ALX-581-007 to 020		LPS
ALX-746-021		Poly I:C
	Live bacteria	*S. aureus* (G^+ Intra M) , *L. monocytogenes* (G^+ Intra M)
	Viruses	Influenza virus, Sendai virus
	Microbial toxins	Aerolysin (*A. hydrophila*)
		Listeriolysin O
		Maitotoxin (Marine dinoflagellates)
BML-CA421		Nigericin (*Streptomyces hygroscopicus*)
BML-KC140		Valinomcyin
	DAMPs	Alum
		Asbestos
ALX-480-021		ATP, NAD^+ (P2X7 receptors)
		β-Amyloid
		Calcium pyrophosphate dihydrate (CPPD) deposition (CPPD)
		Hemozoin (*Plasmodium* species)
ALX-420-038 / -039		Imidazoquinoline Compounds (R837, R848)
		Reactive oxygen species (ROS)
		SDS
		Silica
		TNCB, TNP-Cl
ALX-400-047		Uric Acid Crystals (Monosodium Urate)
		UVB
NLRC4 (IPAF)		
ALX-522-058	Microbial motifs	Cytosolic Flagellin
	Live bacteria	*S. typhimurium* (G^- Intra M), *P. aeruginosa, S. flexneri, L. pneumophila*
	Microbial secretion apparatus	Type III secretion apparatus (*P. aeruginosa*)
NAIP5		
ALX-522-058	Microbial motifs	Cytosolic Flagellin
	Live bacteria	*L. pneumophila* (G^- Intra Ep M)

a) *Bacillus* species: *cereus, simplex, subtilis, megaterium, pumilus.*
b) *Pseudomonas* species: *aeruginosa, putida.*
c) *Chlamydia* species: *pneumoniae, trachomatis, muridarum.*
d) *Lactobacillus* species: *plantarum, pentosus.*
e) *Pseudomonas* species: *aeruginosa, putida.*

Nod-like Receptor Ligands

Ac-muramyl-Ala-D-Glu-amide

[MDP-LD; N-Acetylmuramyl-L-alanyl-D-isoglutamine]

ALX-151-035-M001 1 mg

ALX-151-035-M005 5 mg

Synthetic. Specific ligand for NLRP3 (NALP3). For inactive control compound see MDP-DD (Prod. No. ALX-151-036).

LIT: Minimal structural requirements for adjuvant activity of bacterial peptidoglycan derivatives: F. Ellouz, et al.; BBRC **59,** 1317 (1974) ▪ Nod2 is a general sensor of peptidoglycan through muramyl dipeptide (MDP) detection: S.E. Girardin, et al.; J. Biol. Chem. **278,** 8869 (2003) ▪ Host recognition of bacterial muramyl dipeptide mediated through NOD2. Implications for Crohn's disease: N. Inohara, et al.; J. Biol. Chem. **278,** 5509 (2003)

Adenosine 5'-triphosphate . 2Na

[ATP . 2Na]

ALX-480-021-G001 1 g

ALX-480-021-G005 5 g

Specially crystallized and tested on biological activity.

LIT: Gout-associated uric acid crystals activate the NALP3 inflammasome: F. Martinon, et al.; Nature **440,** 237 (2006) ▪ Cryopyrin activates the inflammasome in response to toxins and ATP: S. Mariathasan, et al.; Nature **440,** 228 (2006)

Flagellin (high purity)

ALX-522-058-C010 10 µg

Isolated from *Salmonella typhimurium* strain 14028. Binds to human and mouse TLR5 (Toll-like receptor 5). Activates the NLRC4 (IPAF) inflammasome.

LIT: Pathophysiological role of Toll-like receptor 5 engagement by bacterial flagellin in colonic inflammation: S.H. Rhee, et al.; PNAS **102,** 13610 (2005) ▪ Cytosolic flagellin requires Ipaf for activation of caspase-1 and interleukin 1beta in salmonella-infected macrophages: L. Franchi, et al.; Nat. Immunol. **7,** 576 (2006) Flagellin-deficient Legionella mutants evade caspase-1- and Naip5-mediated macrophage immunity: T. Ren, et al.; PLoS Pathog. **2,** e18 (2006) ▪ **For a comprehensive bibliography please visit our website.**

Imiquimod

[R-837]

ALX-420-039-M100 100 mg

ALX-420-039-M250 250 mg

NLRP3 (NALP3) inflammasome activator.

LIT: Bacterial RNA and small antiviral compounds activate caspase-1 through cryopyrin/Nalp3: T.D. Kanneganti, et al.; Nature **440,** 233 (2006) ▪ **For a comprehensive bibliography please visit our website.**

Monosodium urate (crystals)

[MSU Crystals; Uric Acid Crystals]

ALX-400-047-M002 2 mg

NALP3 inflammasome activator. Specially crystallized and tested on biological activity.

LIT: Gout-associated uric acid crystals activate the NALP3 inflammasome: F. Martinon, et al.; Nature **440,** 237 (2006)

Nigericin . Na

[Antibiotic K 178]

BML-CA421-0005 5 mg

Isolated from *Streptomyces hygroscopicus*. Acts as an H^+, K^+, Pb^{2+} ionophore. NLRP3 (NALP3) inflammasome activator.

LIT: Nigericin, a new crystalline antibiotic from an unidentified streptomyces: R. L. Harned, et al.; Antibiot. Chemother. **1,** 594 (1951) ▪ Nigericin-induced Na+/H+ and K+/H+ exchange in synaptosomes: effect on [3H]GABA release: R. Rodriguez & M. Sitges; Neurochem. Res. **21,** 889 (1996) ▪ Nigericin inhibits insulin-stimulated glucose transport in 3T3-L1 adipocytes: C.Y. Chu, et al.; J. Cell. Biochem. **85,** 83 (2002) ▪ Cryopyrin activates the inflammasome in response to toxins and ATP: S. Mariathasan, et al.; Nature **440,** 228 (2006) ▪ **For a comprehensive bibliography please visit our website.**

R-848

ALX-420-038-M005 5 mg

ALX-420-038-M025 25 mg

Selective ligand for Toll-like receptor 7 (TLR7) in mouse and for TLR7 and TLR8 in human. NLRP3 (NALP3) inflammasome activator.

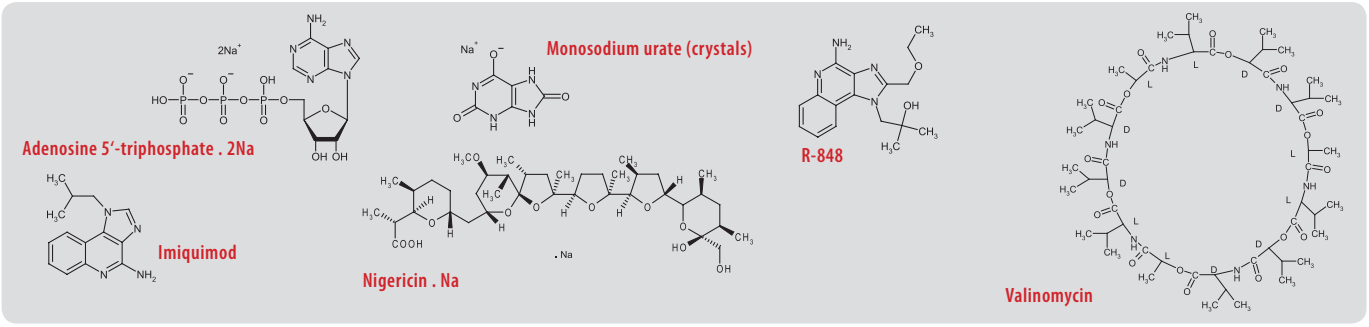
LIT: Small anti-viral compounds activate immune cells via the TLR7 MyD88-dependent signaling pathway: H. Hemmi, et al.; Nat. Immunol. **3,** 196 (2002) ▪ Bacterial RNA and small antiviral compounds activate caspase-1 through cryopyrin/Nalp3: T.D. Kanneganti, et al.; Nature **440,** 233 (2006) ▪ **For a comprehensive bibliography please visit our website.**

Valinomycin

BML-KC140-0025 25 mg

Isolated from *Streptomyces fulvissimus*. Potassium ionophore. Activates the NLRP3 (NALP3) inflammasome.

LIT: Opposing interactions of ionophores (valinomycin and monensin) on calcium ion uptake in rat retinal preparations: J.B. Lombardini; Neurochem. Res. **10,** 77 (1985) ▪ Caspase-1 activation of lipid metabolic pathways in response to bacterial pore-forming toxins promotes cell survival: L. Gurcel, et al.; Cell **126,** 1135 (2006) ▪ **For a comprehensive bibliography please visit our website.**



Latest Insight

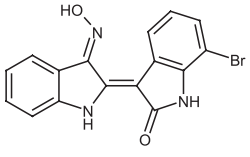
Recently, H. Li. et al. reported that 7BIO (7-bromoindirubin-3'-oxime), a potent inducer of caspase-independent necrosis, activates the inflammasome and triggers the release of the proinflammatory cytokines IL-1β and IL-18.

7BIO

ALX-430-149-M005 5 mg

ALX-430-149-M025 25 mg

LIT: Cutting Edge: Necrosis activates the NLRP3 Inflammasome: H. Li, et al.; J. Immunol. **183,** 1528 (2009) ▪ **For a comprehensive bibliography please visit our website.**



AIM2 – An Inflammasome Sensing Cytoplasmic DNA

In 2008, D.A. Muruve, et al. reported that the inflammasome recognizes cytosolic microbial and host DNA and triggers an inflammatory response [1]. Four new reports now identified the HIN-200 family member AIM2 (absent in melanoma 2) as a sensor of double-stranded cytoplasmic DNA [2-5]. Their results suggest that AIM2 directly binds to cytoplasmic DNA, triggers the assembly of an AIM2 inflammasome, and results in caspase-1 activation. In addition cytoplasmic DNA also triggers the activation of the apoptotic caspase-3 in an AIM2 dependent way [5].

LIT: [1] The inflammasome recognizes cytosolic microbial and host DNA and triggers an innate immune response: D.A. Muruve, et al.; Nature **452,** 103 (2008) ▪ **[2]** An orthogonal proteomic-genomic screen identifies AIM2 as a cytoplasmic DNA sensor for the inflammasome: T. Burckstummer, et al.; Nat. Immunol. **10,** 266 (2009) ▪ **[3]** AIM2 activates the inflammasome and cell death in response to cytoplasmic DNA: T. Fernandes-Alnemri, et al.; Nature **458,** 509 (2009) ▪ **[4]** AIM2 recognizes cytosolic dsDNA and forms a caspase-1-activating inflammasome with ASC: V. Hornung, et al.; Nature **458,** 514 (2009) ▪ **[5]** The innate immune response to DNA: A. Vilaysane & D.A. Muruve; Semin. Immunol. **21,** 208 (2009)

Poly(dA:dT) (endotoxin-free) (synthetic)

[Poly(deoxyadenosine:deoxythymidine) (endotoxin-free) (synthetic)]

ALX-746-022-C050 50 µg

Synthetic. Potent inducer of IFN-α/β. AIM2 inflammasome activator.

Oligo(dA:dT) (endotoxin-free) (synthetic)

[Oligo(deoxyadenosine:deoxythymidine) (endotoxin-free) (synthetic)]

ALX-746-023-C050 50 µg

Synthetic. Potent inducer of IFN-α/β.

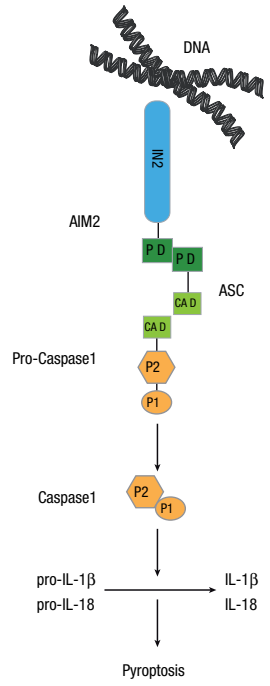


FIGURE 5: AIM2 inflammasome.

The HIN200 family member AIM2 binds DNA via its HIN200 domain. The oligomerization of the protein with the adaptor ASC recruits pro-caspase-1 via a homotypic CARD interaction to form the AIM2 inflammasome. Activated caspase-1 mediates processing and secretion of pro-IL-1β and pro-IL-18 in addition to triggering pyroptosis.

Adapted from: The innate immune response to DNA: A. Vilaysane & D.A. Muruve; Semin. Immunol. ***21,*** *208 (2009)*

Inflammasomes – Related Caspases

Caspase-1 (human) (rec.)

BML-SE168-5000 5000 U

Produced in *E. coli.*

Caspase-5 (human) (rec.)

BML-SE171-5000 5000 U

Produced in *E. coli.*

Caspase-1 Assay Kit for Drug Discovery

BML-AK701-0001 1 Kit

INCLUDES BOTH COLORIMETRIC AND FLUOROGENIC SUBSTRATES! This kit is a complete assay system to measure protease activity of recombinant caspase-1. Cleavage of a tetrapeptide substrate is monitored colorimetrically at 405 nm or fluorometrically (Ex. 360 nm/Em. 460 nm). Assays are performed in a convenient 96-well plate format.

QUANTITY: 96 assays.

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