

NOD-like Receptors (NLRs) and Inflammasomes

In mammals, germ-line encoded pattern recognition receptors (PRRs) detect the presence of pathogens through recognition of pathogen-associated molecular patterns (PAMPs) or endogenous danger signals through the sensing of danger-associated molecular patterns (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) and C-type lectin receptors (CTRs) detect PAMPs in extracellular milieu and endosomal compartments. TLRs and CTRs cooperate with PRRs sensing the presence of cytosolic nucleic acids, like RNA-sensing RIG-I (retinoic acid-inducible gene I)-like receptors (RLRs; RLHs) or DNA-sensing AIM2, among others. Another set of intracellular sensing PRRs are the NOD-like receptors (NLRs; nucleotide-binding domain leucine-rich repeat containing receptors), which not only recognize PAMPs but also DAMPs.

PAMPs			
FUNGI/PROTOZOA	BACTERIA	VIRUSES	MOLECULES
<i>C. albicans</i> <i>Plasmodium</i> <i>S. cerevisiae</i>	<i>A. hydrophila</i> <i>B. brevis</i> <i>E. coli</i> <i>L. monocytogenes</i> <i>L. pneumophila</i> <i>N. gonorrhoeae</i> <i>P. aeruginosa</i> <i>S. aureus</i> <i>S. flexneri</i> <i>S. hygroscopicus</i> <i>S. typhimurium</i>	Adenovirus Encephalomyo- carditis virus Herpes simplex virus Influenza virus Sendai virus	Bacillus anthracis lethal toxin (LeTx) Bacterial pore-forming toxins Cytosolic dsDNA Cytosolic flagellin MDP meso-DAP
DAMPs			
MOLECULES	PARTICLES	OTHERS	
DNA Extracellular ATP R837 Irritants Glucose Hyaluronan	Uric acid CPPD Asbestos Silica Alum Amyloid- β Hemozoin Nanoparticles	UVB Mutations Cytosolic dsDNA	

FIGURE 1: Overview on PAMPs and DAMPs recognized by NLRs.

NLR Family	Approved Symbol	Approved Name	Alternative Names
NLRA	CIITA	class II, major histocompatibility complex, transactivator	NLRA; MHC2TA; C2TA
NLRB	NAIP	NLR family, apoptosis inhibitory protein	NLRB1; BIRC1; CLR5.1
NLRC	NOD1	nucleotide-binding oligomerization domain containing 1	NLRC1; CARD4; CLR7.1
NLRC	NOD2	nucleotide-binding oligomerization domain containing 2	NLRC2; CARD15; CD; BLAU; IBD1; PSORAS1; CLR16.3
NLRC	NLRC3	NLR family, CARD domain containing 3	NOD3; CLR16.2
NLRC	NLRC4	NLR family, CARD domain containing 4	IPAF; CARD12; CLAN; CLR2.1
NLRC	NLRC5	NLR family, CARD domain containing 5	NOD4; NOD27; CLR16.1
NLRP	NLRP1	NLR family, pyrin domain containing 1	NALP1; DEFCAP; NAC; CARD7; CLR17.1
NLRP	NLRP2	NLR family, pyrin domain containing 2	NALP2; PYPAF2; NBS1; PAN1; CLR19.9
NLRP	NLRP3	NLR family, pyrin domain containing 3	NALP3; CIAS1; PYPAF1; Cryopyrin; CLR1.1
NLRP	NLRP4	NLR family, pyrin domain containing 4	NALP4; PYPAF4; PAN2; RNH2; CLR19.5
NLRP	NLRP5	NLR family, pyrin domain containing 5	NALP5; PYPAF8; MATER; PAN11; CLR19.8
NLRP	NLRP6	NLR family, pyrin domain containing 6	NALP6; PYPAF5; PAN3; CLR11.4
NLRP	NLRP7	NLR family, pyrin domain containing 7	NALP7; PYPAF3; NOD12; PAN7; CLR19.4
NLRP	NLRP8	NLR family, pyrin domain containing 8	NALP8; PAN4; NOD16; CLR19.2
NLRP	NLRP9	NLR family, pyrin domain containing 9	NALP9; NOD6; PAN12; CLR19.1
NLRP	NLRP10	NLR family, pyrin domain containing 10	NALP10; PAN5; NOD8; PYNOD; CLR11.1
NLRP	NLRP11	NLR family, pyrin domain containing 11	NALP11; PYPAF6; NOD17; PAN10; CLR19.6
NLRP	NLRP12	NLR family, pyrin domain containing 12	NALP12; PYPAF7; Monarch1; RNO2; PAN6; CLR19.3
NLRP	NLRP13	NLR family, pyrin domain containing 13	NALP13; NOD14; PAN13; CLR19.7
NLRP	NLRP14	NLR family, pyrin domain containing 14	NALP14; NOD5; PAN8; CLR11.2
NLRX	NLRX1	NLR family member X1	NOD5; NOD9; CLR11.3

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

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

Selected Latest Review Articles

- The inflammasomes: guardians of the body: F. Martinon, et al.; *Annu. Rev. Immunol.* **27**, 229 (2009)
- The NLRP3 inflammasome: a sensor of immune danger signals: S. L. Cassel, et al.; *Semin. Immunol.* **21**, 194 (2009)
- Molecular mechanisms involved in inflammasome activation: C. Bryant & K. A. Fitzgerald; *Trends Cell Biol.* **19**, 455 (2009)
- Inflammasomes in infection and inflammation: C. R. McIntire, et al.; *Apoptosis* **14**, 522 (2009)
- The innate immune response to DNA: A. Vilaysane & D. A. Muruve; *Semin. Immunol.* **21**, 208 (2009)
- Sensing pathogens and danger signals by the inflammasome: J. H. Pedra, et al.; *Curr. Opin. Immunol.* **21**, 10 (2009)
- Function of Nod-like receptors in microbial recognition and host defense: L. Franchi, et al.; *Immunol. Rev.* **227**, 106 (2009)
- The inflammasome: a caspase-1-activation platform that regulates immune responses and disease pathogenesis: L. Franchi, et al.; *Nat. Immunol.* **10**, 241 (2009)
- The inflammasomes: K. Schroder & J. Tschopp; *Cell* **140**, 821 (2010)
- NLR-containing inflammasomes: central mediators of host defense and inflammation: K. A. Fitzgerald; *Eur. J. Immunol.* **40**, 595 (2010)
- Sterile inflammatory responses mediated by the NLRP3 inflammasome: S. L. Cassel & F. S. Sutterwala; *Eur. J. Immunol.* **40**, 607 (2010)
- Inflammasomes as microbial sensors: L. Franchi, et al.; *Eur. J. Immunol.* **40**, 611 (2010)
- Signaling by ROS drives inflammasome activation: F. Martinon; *Eur. J. Immunol.* **40**, 616 (2010)

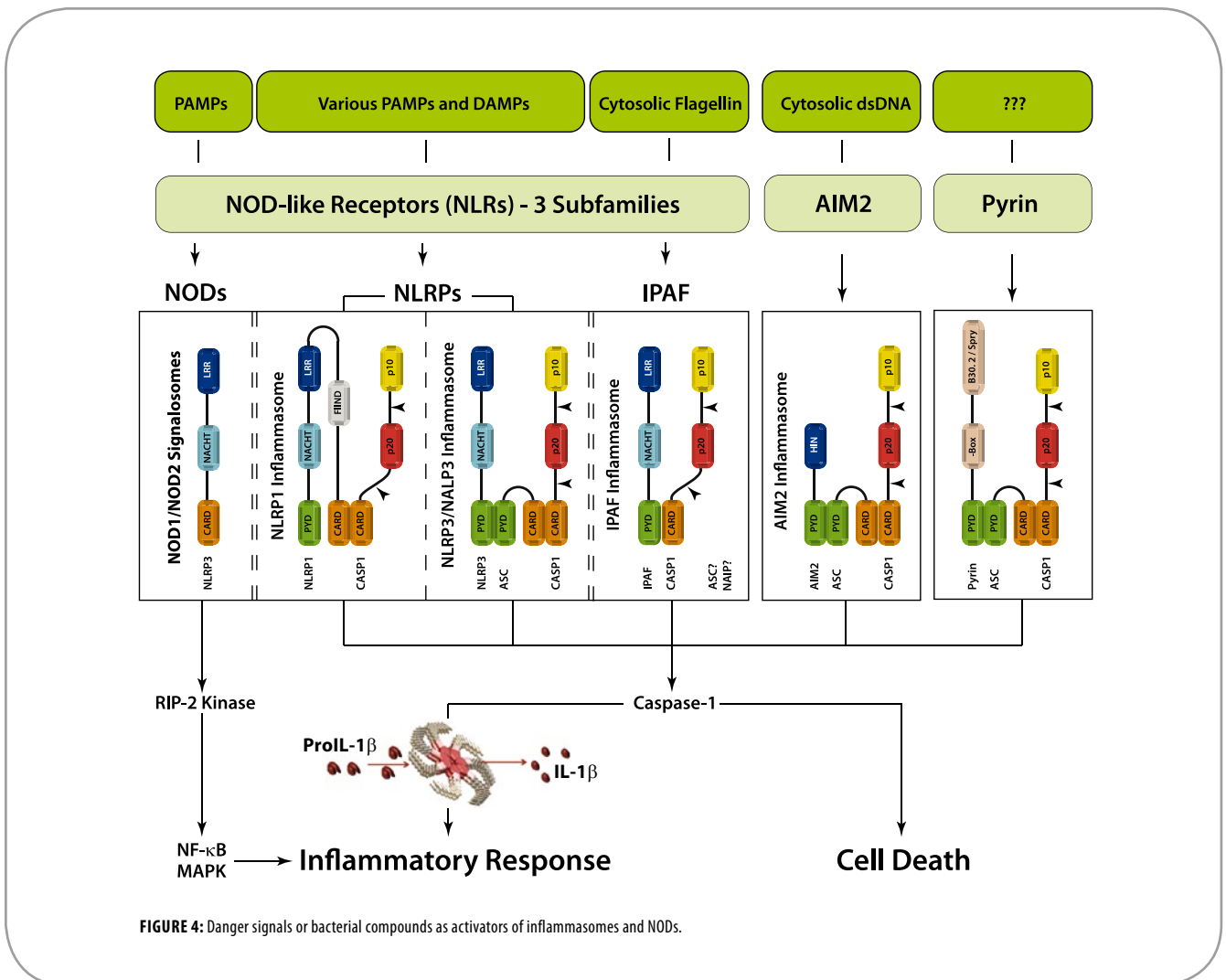
NOD Signalosomes

NOD1 (CARD4; NLRC1) and NOD2 (CARD15; NLRC2) are both cytosolic pattern recognition molecules (PRRs) of the NLR (NOD-like receptor) family. Whereas NOD1 is widely expressed by many cell types, the expression of NOD2 is restricted to macrophages, dendritic cells, Paneth cells, keratinocytes and epithelial cells of the oral cavity, intestine and lung. NOD1 and NOD2 are both implicated in sensing the presence of bacterial peptidoglycan (PGN) fragments. NOD1 senses meso-diaminopimelic acid (meso-DAP)-containing PGN fragments, which are present in most Gram-negative bacteria and certain Gram-positive ones, like the *Bacillus* species. Interestingly, the dipeptide γ -D-glutamyl-meso-DAP (iE-DAP) was shown to be sufficient for NOD1 activation. NOD2 however, seems to be a general sensor which becomes activated by muramyl dipeptide (MDP), the minimal motif common to nearly all Gram-positive as well as Gram-negative bacteria.

Upon activation, NOD1 and NOD2 initiate a pro-inflammatory response through the recruitment of the recep-

tor interacting protein 2 (RIP2; RICK; CARDIAK). Binding of K63-ubiquitinated RIP2 leads to the direct binding to NEMO (IKK γ), which then gets ubiquitinated and degraded in a proteasome-dependent manner. Ultimately, this leads to the activation of the catalytic subunits IKK α and IKK β . The activated IKK complex phosphorylates the inhibitor I κ B, leading to its proteasomal degradation and allowing NF- κ B to translocate into the nucleus and exerting its function. Additionally, K63-polyubiquitinated RIP2 recruits TAK1, which is also essential for IKK complex activation. Furthermore, NOD1 and NOD2 mediated MAPK activation is also dependent on RIP2 and TAK1.

Both, NOD1 and NOD2 are key receptors in epithelial cells where they control infections via the gastrointestinal system. Mutations in NOD1 have been reported to confer susceptibility to several inflammatory disorders including inflammatory bowel disease, atopic eczema and asthma.



Inflammasomes – Caspase-1 Activation Scaffolds

An inflammasome represents a high molecular weight complex that activates inflammatory caspases and activates cytokines of the IL-1 family. Several inflammasomes have been described and were so far defined by the NLR protein that they contain - the NLRP1 (NALP1) inflammasome, the NLRP3 (NALP3) inflammasome and the IPAF (NLRC4) inflammasome. Interestingly, the AIM2 (absent in melanoma 2) inflammasome, which has been described recently, does not contain any member of the NLR family. Inflammasomes can be activated through multiple signals including live bacteria, microbial toxins, xeno-compounds, PAMPs and DAMPs (for an overview see page 1). It is suggested that the LRR domains of NLRP3 mediate autorepression, probably by SGT1 and HSP90 chaperones that maintain NLRP3 in an inactive, but signal-competent state. Upon sensing of their respective ligands, NLRP1 or NLRP3 oligomerize via the NACHT domain, what leads to PYD clustering and to the recruitment of the adapter protein ASC (apoptosis-associated speck-like protein containing a CARD) due to homotypic PYD-PYD interactions. In the case of AIM2, oligomerization is likely mediated by clustering upon multiple binding sites within dsDNA and not by a central oligomerization domain like NACHT. However, AIM2 oligomerization also leads to the recruitment of ASC via homotypic PYD-PYD interactions. ASC contains an N-terminal PYD domain and a C-terminal CARD domain that allows the recruitment of inflammatory caspases through homotypic CARD-CARD interactions. Thus inflammatory

caspases are brought into close proximity what permits autoactivation and formation of the active caspase. In case of procaspase-1, a p10/p20 tetramer is formed after autocleavage. In addition to caspase-1, NLRP1 can also recruit caspase-5 to the complex but the role of caspase-5 is still under debate. Contrary to NLRP1, NLRP3 and AIM2, IPAF does not recruit an adapter molecule but directly interacts with procaspase-1 via its CARD domain (see FIGURE 4). However, depending on the IPAF stimulus, maximal caspase-1 activation downstream of IPAF can require ASC or NAIP.

The assembly of the different inflammasomes elicits a common downstream cascade, namely the activation of inflammatory caspases. These include caspase-1, -4 and -5 in human and caspase-1, -11 and -12 in mouse. However, caspase-1 appears to be the most dominant inflammatory caspase associated with inflammasomes. The inflammatory caspases all have a CARD domain followed by a domain containing the catalytic residue cysteine and are called inflammatory caspases because their main substrates are cytokines (such as pro-IL-1 β , pro-IL-18 and eventually pro-IL-33) which are cleaved to their active and secreted form. In addition, inflammasome activation can lead to host cell death in certain cell types, known as pyroptosis. Pyroptosis is thought to be important in restricting the intracellular replication of invasive pathogens.

Inflammasomes – Activity Regulation

Even though mechanisms that regulate inflammasome activity remain elusive, various proteins were identified that may interfere with inflammasome activation and inflammasome dependent inflammatory caspase processing. In general, two subtypes of inflammasome regulators can be distinguished – those containing a CARD domain and

those with a PYD domain. Such proteins encompass not only host derived inflammasome regulators, but also various bacterial virulence factors inhibiting caspase-1 activation and viral PYD proteins.

Inflammasomes – Therapeutic Implications

As IL-1 β and other cytokines are key players in the inflammatory response, it is tempting to speculate that IL-1 β , inflammatory caspases and inflammasomes play an important role in several diseases (see FIGURE 5). Indeed, some human hereditary or acquired diseases have been linked to elevated IL-1 β , some of which can be treated by antagonists against IL-1 β or its receptor. A number of diseases, known as cryopyrin-associated periodic syndroms (CAPS), have been directly linked to NLRP3 mutations.

Furthermore, gout, an autoinflammatory disease characterized by severe joint inflammation, is associated with the deposition of MSU crystals in joints, among other characteristics. As MSU is a potent NLRP3 inflammasome agonist, it is believed inflammasome-regulated IL-1 β exerts a pathogenic role in gout. Furthermore, IL-1 β secretion by the NLRP3 inflammasome is triggered by high extracellular glucose in β -cells. Elevated IL-1 β is a risk factor for the development of Type 2 Diabetes Mellitus (T2DM) and contributes to insulin resistance.

Thus by functioning as a sensor for metabolic stress, like in the form of monosodium urate (MSU) or hyperglycemia, the NLRP3 inflammasome likely contributes to the pathogenesis of gout or T2DM, respectively.

In addition, several inflammasome regulators have a significant relevance in diseases. In patients with FMF (familial Mediterranean fever), pyrin was shown to be mutated. Elevated IL-1 β levels in the autoinflammatory disease PAPA (pyogenic arthritis, pyoderma gangrenosum, and acne) are associated with mutations in PSTPIP1, a pyrin interacting protein. Together, this indicates the importance of pyrin and inflammasome regulators in autoinflammatory diseases and may allow novel entry points for disease treatment.

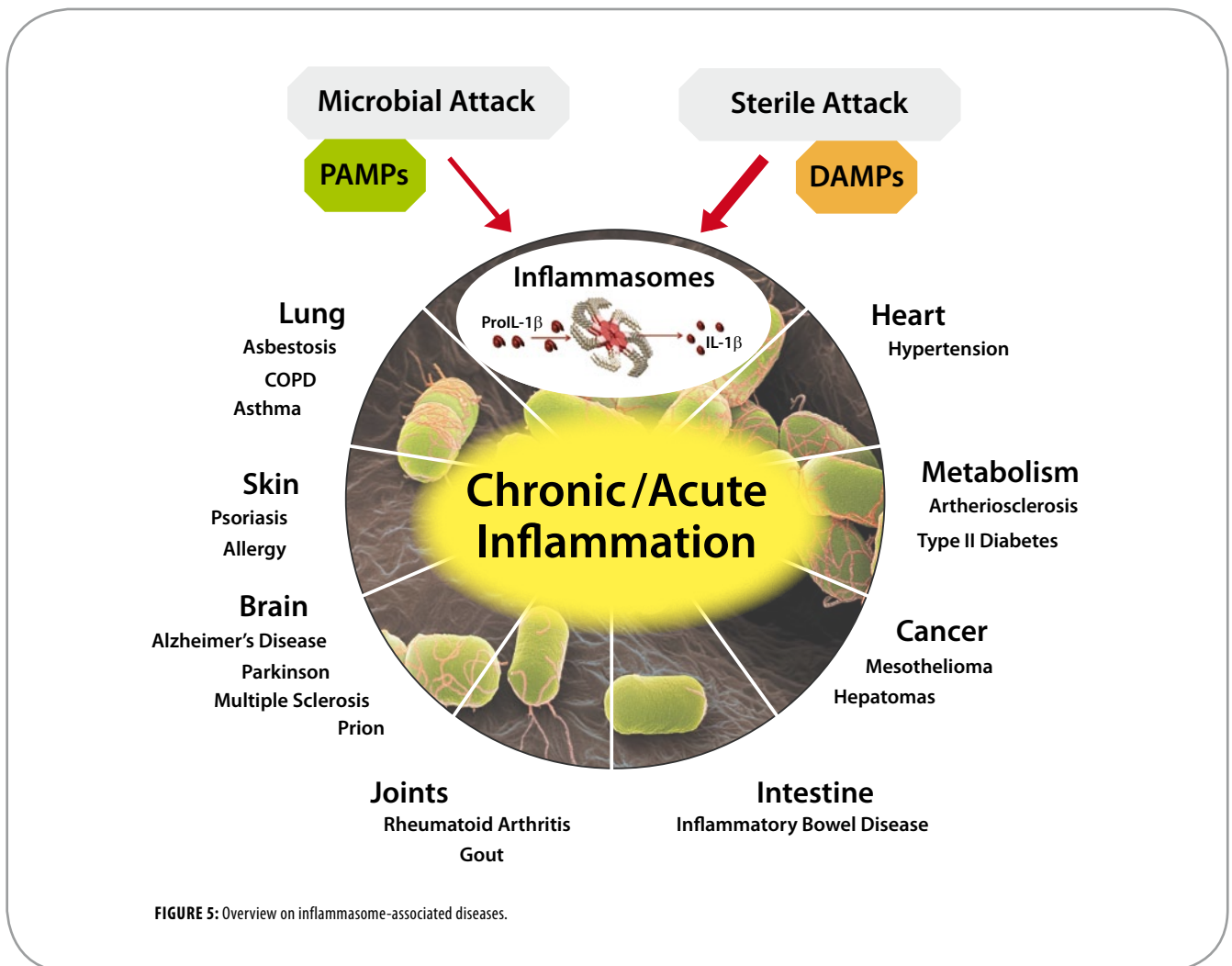
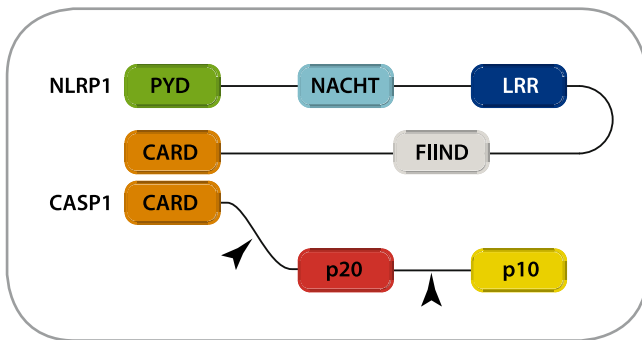


FIGURE 5: Overview on inflammasome-associated diseases.

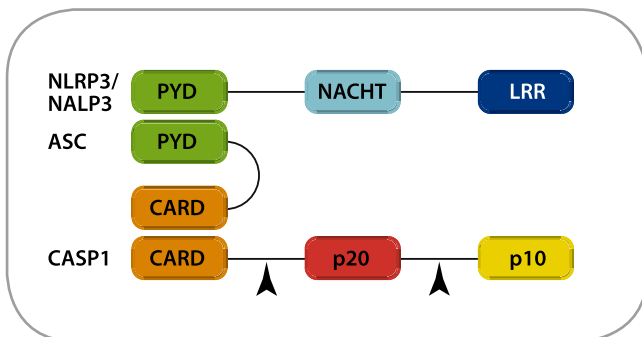
Detailed View on the Inflammasomes

NLRP1 Inflammasome



The anti-apoptotic proteins Bcl-2 and Bcl-X_L were recently shown to bind and inhibit NLRP1. Bcl-2 and Bcl-X_L inhibit ATP binding to NLRP1, what is required for oligomerization and furthermore, Bcl-X_L was shown to suppress NLRP1 oligomerization. Thus inflammatory caspases are not brought into close proximity what negatively interferes with caspase-1 autoactivation and subsequent processing of pro-inflammatory cytokines. In addition to those potential NLRP1 activity regulators, K⁺ efflux appears to be essential for NLRP1 activation.

NLRP3/NALP3 Inflammasome



Even though the exact mechanism of NLRP3/NALP3 inflammasome activation is still under debate, three models are favored among researchers:

1) The NLRP3 inflammasome agonist, extracellular ATP, binds to its receptor P2X7 what triggers K⁺ efflux and pannexin-1 membrane pore formation. The latter may enable the entry of extracellular factors, which can be direct NLRP3 activators. It is also suggested that NLRP3 may sense the K⁺ efflux or membrane integrity.

2) Phagocytosed crystalline or particulate NLRP3 ligands, such as MSU, alum, silica and asbestos, can cause lysosomal destabilization and rupture due to mechanical insult what leads to the release of lysosomal content into the cytosol. As this pathway is sensitive to the cathepsin B inhibitor CA-074-Me, it was suggested that cathepsin B, a lysosomal protease, is involved in the activation of a direct NLRP3 ligand. How-

ever, no altered IL-1 β secretion and caspase-1 cleavage in response to NLRP3 ligands, like MSU or alum, was observed in cathepsin B deficient macrophages. It is yet not known how NLRP3 senses cytoplasmic lysosomal content.

3) The third model involves the production of reactive oxygen species (ROS). All NLRP3 agonists tested lead to the production of ROS and furthermore, ROS scavengers suppress NLRP3 activation. The cellular source of ROS is currently unknown and although ROS seems to be necessary for NLRP3 inflammasome activation, it is not sufficient. In b-cells, TXNIP (thioredoxin-interacting protein; VDUP1), which is a ROS-sensitive NLRP3 ligand, was shown to be involved in triggering NLRP3 activation.

Another important parameter in NLRP3 activation, as for NLRP1, seems to be the cytoplasmic K⁺ concentration. The fact that macrophages cultured in high K⁺ concentration show a decreased NLRP3-dependent caspase-1 activation likely implies that K⁺ efflux is necessary upstream of NLRP3 activation.

Priming of the NLRP3 Inflammasome

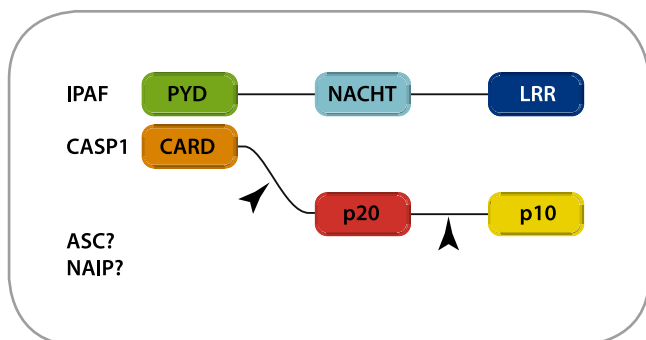
The most prominent function of the NLRP3 inflammasome is the processing and activation of pro-IL-1 β . Yet most cells do not express pro-IL-1 β and thus prior synthesis of pro-IL-1 β is required. This can be achieved by stimulating receptors such as TLRs, NODs or TNF-R that activate NF- κ B and initiate pro-IL-1 β transcription. This process of pro-IL-1 β induction is called priming (Signal 1). Priming also induces NF- κ B-dependent transcription of NLRP3.

An additional stimulus (Signal 2) results in the activation of the NLRP3 inflammasome and subsequent initiation of downstream signaling. In the absence of priming, NLRP3 inflammasome-dependent caspase-1 activation can also be observed, but IL-1 β secretion is absent.

FOR DETAILS SEE:

- Critical functions of priming and lysosomal damage for NLRP3 activation: V. Hornung & E. Latz; Eur. J. Immunol. **40**, 620 (2010)
- The inflammasomes: K. Schroder & J. Tschopp; Cell **140**, 821 (2010)

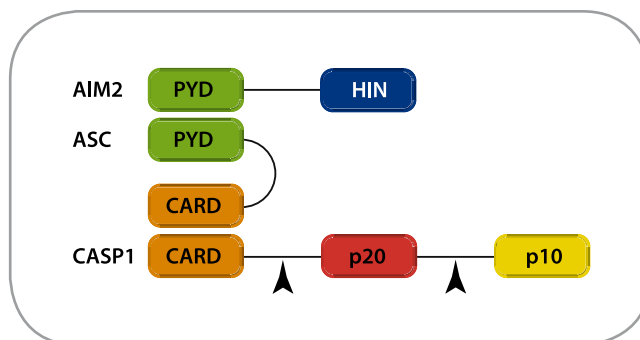
IAPF Inflammasome



The IAPF inflammasome becomes activated by gram-negative bacteria possessing a functional T3SS or T4SS, like *S. typhimurium*, *S. flexneri*, *L. pneumophila*, and *P. aeruginosa*. Initially, cytosolic flagellin was shown to trigger the IAPF inflammasome activity. However, as nonflagellated bacteria like *S. flexneri* also induce IAPF inflammasome activation, it is very likely that there exist other IAPF agonists. As NLRP3, IAPF was shown to bind SGT1 and HSP90. The inhibition of HSP90 by geldanamycin blocks the IAPF activity, indicating that HSP90 is somehow crucial for IAPF signaling.

Unlike NLRP1 and NLRP3, IAPF activation is not inhibited by high extracellular K^+ concentration, indicating that IAPF is not a sensor for ionic fluxes. However, no direct ligand receptor interaction was observed to date.

AIM2 Inflammasome



The HIN-200 family member, AIM2, acts as a sensor for cytosolic bacterial, viral and host dsDNA and triggers an inflammatory response through the formation of the AIM2 inflammasome. It is suggested that AIM2 binds directly to dsDNA via its C-terminal HIN-200 domain. As AIM2 recognizes also host dsDNA it might be involved in autoimmune diseases. The discovery of the AIM2 inflammasome is not only remarkable because AIM2 is the first non-NLR family member forming an inflammasome, but also because AIM2 is the first inflammasome receptor shown to directly interact with its ligand.

Product Highlights

anti-NLRP3/NALP3 (mouse), mAb (Cryo-1)	AG-20B-0006-C100	100µg
anti-NLRP3/NALP3, mAb (Cryo-2)	AG-20B-0014-C100	100µg
anti-Asc (human), pAb (AL177)	AG-25B-0006-C100	100µg

For more products please visit www.adipogen.com

AdipoGenTM
Connecting Immunology to MetabolismTM
www.adipogen.com

EUROPE/REST OF WORLD
Adipogen International
TEL +41-61-926-60-40
FAX +41-61-926-60-49
info@adipogen.com

NORTH & SOUTH AMERICA
Adipogen Corp.
TEL +1-858-457-8383
FAX +1-858-457-8484
info-us@adipogen.com

SOUTH KOREA/ASIA
Adipogen, Inc.
TEL +82-32-858-1470
FAX +82-32-831-1470
info-kr@adipogen.com

For local distributors please visit our website.