General Strategies in Inflammasome Biology

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Abstract The complementary actions of the innate and adaptive immune systems often provide effective host defense against microbial pathogens and harmful environmental agents. Germline-encoded pattern recognition receptors (PRRs) endow the innate immune system with the ability to detect and mount a rapid response against a given threat. Members of several intracellular PRR families, including the nucleotide-binding domain and leucine-rich repeat containing receptors (NLRs), the AIM2-like receptors (ALRs), and the tripartite motif-containing (TRIM) protein Pyrin/TRIM20, nucleate the formation of inflammasomes. These cytosolic scaffolds serve to recruit and oligomerize the cysteine protease caspase-1 in filaments that promote its proximity-induced autoactivation. This oligomerization occurs either directly or indirectly through intervention of the bipartite adaptor protein ASC, apoptosis-associated speck-like protein containing a caspase recruitment domain (CARD), which is needed for the domain interaction. Caspase-1 cleaves the precursors of the inflammatory cytokines interleukin (IL)-1β and IL-18 and triggers their release into the extracellular space, where they act on effector cells to promote both local and systemic immune responses. Additionally, inflammasome activation gives rise to a lytic mode of cell death, named pyroptosis, which is thought to contribute to initial host defense against infection by eliminating replication niches of intracellular pathogens and exposing them to the immune system. Inflammasome-induced host defense responses are the subject of intense investigation, and understanding their physiological roles during infection and the regulatory circuits that are involved is becoming increasingly detailed. Here, we discuss current understanding of the activation mechanisms and biological outcomes of inflammasome activation.

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1 Introduction: PRRs, PAMPs, and DAMPs

The complementary qualities of the innate and adaptive immune systems allow vertebrates to mount a highly tailored and efficacious host defense against intruding pathogens, while avoiding immunopathology. The efficacy of such combined immune response for defense against pathogens and harmful environmental agents is illustrated by the presence of innate and adaptive immune subsystems in early vertebrates such as the lamprey and mammals alike (Boehm 2012). The innate immune system detects and mounts the initial response to the threat. In mammals, the innate immune system encompasses a diversity of physical and chemical barriers. This includes mucosal membranes that interface the extracellular environment, a complement system that tags invading agents for removal, and professional phagocytes that clear the infectious agent. Phagocytes such as macrophages and neutrophils also release inflammatory mediators to recruit additional immune cells into the affected area (Palm and Medzhitov 2009a). Adaptive immunity is directed by dendritic cells and other antigen-presenting cells (APCs) that relay information about the harmful agent to lymphocytes. Pathogen-derived peptide antigens are presented to T-lymphocytes in association with major histocompatibility complex (MHC) proteins, whereas multiple mechanisms may govern how B-lymphocytes encounter antigens (Cyster 2010; Palm and Medzhitov 2009b). The highly specific adaptive immune system produces B- and T-lymphocytes with diverse antigen receptors, and clonal expansion of the cells recognizing foreign material culminates in its targeted removal. In addition, the adaptive immune system is capable of immune memory that provides protection from reinfection with the same pathogen (Koch and Radtke 2011).

While antigen receptor gene rearrangements in lymphocytes enable the adaptive immune system to recognize seemingly any antigen, innate immune cells rely on only a fixed set of germline-encoded ‘pattern recognition receptors’ (PRRs) to detect pathogens (Takeuchi and Akira 2010). PRRs are expressed on many cell types that may come in contact with microbes, including hematopoietic cells, fibroblasts, endothelial cells, and epithelial cells that line mucosal membranes.

Given the limited repertoire of PRRs that is available to the host, it may be unsurprising that—rather than signaling out a particular microbe—PRRs guard conserved microbial signatures termed 'pathogen-associated molecular patterns' (PAMPs) that may signal infection by a certain class of pathogens. Microbial nucleic acids, bacterial secretion systems, and components of the microbial cell wall that are not produced by eukaryotes are the examples of such conserved microbial factors that are sensed by PRRs. Nevertheless, damaged host cells as well may trigger PRR activation by releasing danger-associated molecular patterns (DAMPs) such as uric acid crystals, ATP, high-mobility group box 1 (HMGB1), and the heat-shock proteins Hsp70 and Hsp90 (Takeuchi and Akira 2010). Detection of DAMPs by PRRs is thought to primarily promote tissue repair, although excessive release might elicit a severe inflammatory response exacerbating tissue damage in several infectious and autoinflammatory and autoimmune diseases (Lotze et al. 2007).

PRR families may be subdivided into genuine transmembrane receptors that survey the extracellular environment and endosomes for DAMPs and PAMPs, and those that reside in cytosolic compartments. Toll-like receptors (TLRs) and C-type lectin receptors (CLRs) are prime examples of the first class, while the RIG-I-like receptor (RLR), the AIM2-like receptor (ALR), and the nucleotide-binding domain and leucine-rich repeat containing (NLR) proteins all respond to pathogens at intracellular compartments (Takeuchi and Akira 2010). PRRs are also frequently classified according to the PAMPs and DAMPs they sense, or the immune signaling pathways they control. For instance, members of several PRR families may detect microbial DNA or RNA molecules and engage signaling cascades that culminate in activation of members of the nuclear factor-kappa B (NF-κB), activator protein 1 (AP1), and interferon regulatory factor (IRF) transcription factor families (Battistini 2009; Kim and Choi 2010; Vallabhapurapu and Karin 2009). The concerted activities of these key inflammatory transcription factors lead to the production of type I interferons (IFN-I), inflammatory cytokines, and other pro-inflammatory or microbicidal proteins (Takeuchi and Akira 2010). In addition to these comprehensive transcriptional reprograming events, innate immune cells are equipped with PRRs that may assemble cytosolic multi-protein complexes called 'inflammasomes.' Inflammasomes are regarded as key elements in the innate immune response of mammalian hosts in providing protection against invading micro-organisms. By definition, inflammasomes are scaffolds for activation of the inflammatory cysteine-dependent aspartate-specific protease caspase-1. This protease is chiefly known for its key role in maturation and secretion of the inflammatory cytokines interleukin IL-1β and IL-18 (Lamkanfi and Dixit 2014). Additionally, caspase-1 activation may result in a programmed, lytic cell death of myeloid cells that has been named 'pyroptosis' (Cookson and Brennan 2001). Based on a wealth of experimental evidence gathered in the past 2 decades, these two inflammasome-dependent biological responses (cytokine production and pyroptosis) contribute importantly to the immune system's ability to resolve the threat and restore homeostasis. Here, we will review and discuss current understanding of inflammasome biology with an emphasis on recent developments in

control of microbial infections. A brief introduction of the different inflammasomes characterized to date will be followed by a discussion of the roles of particular inflammasome complexes in microbial infections. Finally, mechanisms regulating inflammasome activation will be discussed along with specific examples illustrating the importance of tight regulation of inflammasome activation.

2 PRRs as Inflammasome Scaffolds

Selected members of the 'Hematopoietic interferon-inducible nuclear protein family' (Hin200) and the 'Tripartite Motif Family' (TRIM) PRR families assemble inflammasomes in their own right (Fig. 1), but the majority of inflammasomes relies on NLR proteins for pathogen sensing and scaffold assembly. NLRs are defined by the combined presence of a nucleotide-binding and oligomerization domain (NACHT/NBD) and leucine-rich repeat (LRR) motifs, typically located in the central and most carboxy-terminal regions of the proteins (Kanneganti et al. 2007). Most NLRs in addition contain an amino-terminal protein interaction domain of the baculovirus inhibitor repeat (BIR), Pyrin (PYD), and caspase recruitment domain (CARD) types, thus allowing their subclassification based on domain architecture (Fig. 1). The NLR family consists of 22 human and 34 murine members that play diverse roles in the mammalian immune and reproductive systems (Kanneganti et al. 2007; Van Gorp et al. 2014). NLRs are considered an evolutionary ancient PRR family as supported by the identification of over 200 NLR genes in the genome of the sea urchin *Strongylocentrotus purpuratus* (Rast et al. 2006), and the presence of NLRs in zebrafish (Laing et al. 2008; Stein et al. 2007) and tetrapods (Hansen et al. 2011). However, NLR genes appear to have been lost during speciation of particular animal species. For instance, NLRs are absent from the genome of the model nematode species *Caenorhabditis elegans* and the insects *Drosophila melanogaster* (fruit fly) and *Apis mellifera* (honey bee). However, they are present in other insects such as *Culex quinquefasciatus* (southern house mosquito) and *Aedes aegypti* (yellow fever mosquito) (Lange et al. 2011). Although genuine NLR genes have so far only been identified in animals, homologs with functionally related domains and motifs have been described in *Hydra*, fungi, and plants as well (Lange et al. 2011). The comparable domain architecture of the pathogen-resistant (R-) proteins of higher plants in which carboxy-terminal LRR motifs combine with a centrally located NB-ARC domain that is structurally related to the NACHT ATPase of NLRs represents an nice example of convergent evolution (Chisholm et al. 2006). Moreover, also R-proteins are central mediators of antimicrobial resistance mechanisms that are collective referred to as the 'hypersensitive response' in plants (Hulbert et al. 2001).

The physiological roles of most mammalian NLRs are largely obscure, but some members have well-defined roles in the regulation of inflammatory gene transcription. Among the best characterized examples are the intracellular peptidoglycan

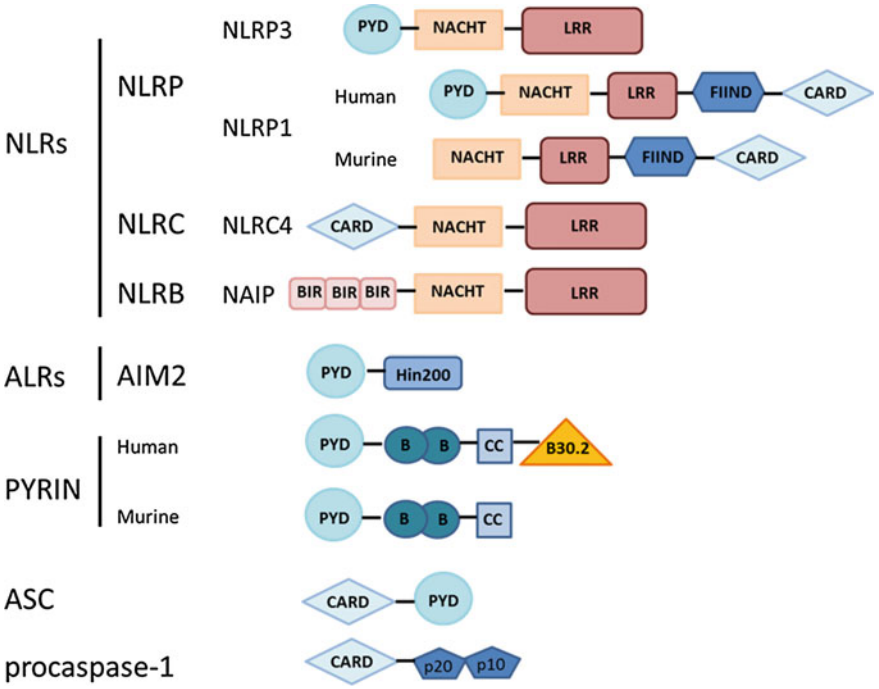

Fig. 1 Inflammasome components and their domain architecture. Members of the nucleotide-binding domain and leucine-rich repeat containing receptors (NLR) family contain a central 'neuronal apoptosis inhibitor protein (NAIP), CIITA (MHC class II transcription activator), HET-E (incompatibility locus protein from *Podospora anserina*) and TP1 (telomerase-associated protein)' (NACHT) ATPase domain, and leucine-rich repeat (LRR) motifs. NLR family members may be further subdivided into NLRP, containing a Pyrin domain (PYD); NLRC, containing a caspase recruitment domain (CARD); and NLRB, containing a Baculovirus inhibitor repeat (BIR) domain. The latter subset is frequently referred to as the NAIP proteins. The AIM2-like receptor (ALR) family is composed of a PYD and a dsDNA-binding 'hematopoietic interferon-inducible nuclear protein with a 200 amino acid repeat' (Hin200) domain. Finally, the inflammasome scaffold protein Pyrin consists of PYD, B-Box-type zinc finger (BB), and coiled-coil (CC) domains that are followed by a carboxy-terminal B30.2 domain in the human, but not its murine ortholog. Inflammasome assembly involves homotypic CARD and PYD interactions between its components

receptors NOD1 and NOD2 (nucleotide-binding oligomerization domain containing proteins 1/2) that promote RIPK2- and CARD9-dependent transcription of NF-κB- and AP1-target genes (Wilmanski et al. 2008). Additionally, transcription of major histocompatibility class II (MHC II) genes in antigen-presenting cells requires the NLR protein class II trans-activator (CIITA) (Wilmanski et al. 2008). Another subset of NLRs—namely NLRP1, NLRP3, NLRC4, and NAIP—promotes immune responses at the post-translational level by initiating inflammasome signaling (Lamkanfi and Dixit 2014).

Signal-induced PRR clustering is thought to recruit and promote the nucleation of procaspase-1 filaments in which the protease zymogens undergo proximity-induced autoactivation. Caspase-1 may be recruited in these inflammasome scaffolds through direct homotypic interactions involving the CARD motifs of procaspase-1 and that of the nucleating PRR (NLRC4, NLRP1b), or indirectly through homotypic interactions with the bipartite PYD/CARD inflammasome adaptor protein ASC in the case of PYD-containing NLRP3, AIM2, and Pyrin receptors. Notably, inflammasome-induced activation of caspase-1 by each of these inflammasomes is associated with ASC-dependent autoproteolytic cleavage of caspase-1, but automaturation does not appear essential for pyroptosis induction in the context of the NLRP1b and NLRC4 inflammasomes (Broz et al. 2010b; Van Opdenbosch et al. 2014). Nevertheless, ASC does contribute to efficient secretion of bioactive IL-1β and IL-18 in response to triggers of these respective inflammasomes.

IL-1β is responsible for generation of fever, lymphocyte activation, and guiding the transmigration of leukocytes into the stress location (Dinarello 2009). As such, IL-1β induces both a systemic and a local response to infection and injury. IL-18 does not have this pyrogenic activity, but it orchestrates IFN gamma production, leading to control over the Th1 population. Depending on the cytokine environment, IL-18 can as well orchestrate the Th2 population. Furthermore, IL-18 regulates ROS production, expression of cell adhesion molecules and expression of other chemokines/cytokines (Dinarello 2009). Considering the broad impact of these proinflammatory cytokines on the hosts' immune responses, a strict control of their activity is needed. One mechanism by which this is accomplished involves transcriptional control of their expression levels. ProIL-1β is virtually absent in naïve myeloid cells, but its mRNA levels are highly responsive to NF-κB-dependent transcriptional upregulation. By contrast, proIL-18 is constitutively present in the cytosol of naïve macrophages and dendritic cells. The differential regulation of proIL-1β and proIL-18 at the transcriptional level is illustrated by the observation that the NLRP1b and NLRC4 inflammasomes can be induced to secrete mature IL-18—but not IL-1β—in the absence of prior TLR engagement (Nystrom et al. 2013; Van Opdenbosch et al. 2014). In this context, the requirement for cytokine maturation may be regarded as another safeguard against accidental release of IL-1β and IL-18. Indeed, unlike most other cytokines that are secreted through the classical secretory pathway, proIL-1β and proIL-18 are produced as biologically inactive cytosolic precursors that await caspase-1-dependent cleavage for release of their bioactive forms (Gu et al. 1997; Lamkanfi 2011). Several mechanisms have been proposed by which the latter cytokines may be released, the most recent being another prominent outcome of inflammasome activation, namely pyroptosis. Pyroptosis is a lytic form of cell death characterized by cytoplasmic swelling and early rupture of the plasma membrane that requires the protease activities of either caspase-1 or caspase-11 (Cookson and Brennan 2001; Kayagaki et al. 2011). Pyroptosis is thought to constitute a defensive innate immune strategy of the host that eliminates the replicative niche of intracellularly replicating pathogens and exposes them to other immune cells (Aachoui et al. 2013; Casson

et al. 2013; Miao et al. 2010a). Additionally, pyroptosis-associated cell lysis further releases DAMPs such as IL-1α and HMGB1 into the extracellular environment (de Gassart and Martinon 2015; Lamkanfi et al. 2010).

3 Inflammasome Activation Mechanisms

3.1 The NLRP3 Inflammasome

The NLRP3 inflammasome is by far responding to the largest set of activating agents (Lamkanfi and Dixit 2014). It is also rather unique among inflammasomes in that it requires an NF-κB-mediated signal prior to its activation. This so-called 'signal 1' or 'priming' step involves transcriptional upregulation of NLRP3 together with proIL-1β (Bauernfeind et al. 2009) and is defective in mice lacking the NF-κB regulator A20/TNFAIP3 (Vande Walle et al. 2014). Non-transcriptional mechanisms that involve its de-ubiquitination and/or IL-1 receptor-associated kinase (IRAK-1) kinase activity have also been proposed to additionally control NLRP3 inflammasome activation (Juliana et al. 2012; Lin et al. 2014; Lopez-Castejon et al. 2013; Py et al. 2013). Together, these priming mechanisms establish effective checkpoints that prevent accidental NLRP3 inflammasome activation.

In the presence of such priming signals, NLRP3 inflammasome assembly and activation is induced when the host cell is exposed to a 'signal 2'. NLRP3 inflammasome activation may be induced by the components of bacterial [*Staphylococcus aureus, Streptococcus pneumoniae, Listeria monocytogenes* (Mariathasan et al. 2006; McNeela et al. 2010; Wu et al. 2010)]; viral [Influenza A virus (IAV); Encephalomyocarditis virus (EMCV); Vesicular stomatitis virus (VSV)] (Allen et al. 2009; Kanneganti et al. 2006; Rajan et al. 2011); as well as fungal (*Candida albicans, Aspergillus fumigatus, Cryptococcus neoformans*) (Gross et al. 2009; Guo et al. 2014; Hise et al. 2009; Said-Sadier et al. 2010) origin. In addition, DAMPs such as millimolar concentrations extracellular ATP, calcium pyrophosphate dehydrate and monosodium urate (Mariathasan et al. 2006; Martinon et al. 2006, 2009), environmental crystals (alum, silica, asbestos) (Dostert et al. 2008; Eisenbarth et al. 2008; Hornung et al. 2008; Martinon et al. 2006), β-fibrils and aggregates (β-amyloid, β-glucans) (Halle et al. 2008; Kumar et al. 2009), and ionophores (nigericin, maitotoxin) (Mariathasan et al. 2006; Perregaux and Gabel 1994) all engage the NLRP3 inflammasome. The NLRP3 inflammasome may also be engaged by intracellular LPS. Unlike for the 'canonical' stimuli above, caspase-11 and its human orthologs caspases 4 and 5 are required for cytosolic LPS-induced NLRP3 inflammasome activation (Baker et al. 2015; Hagar et al. 2013; Kayagaki et al. 2011; Kayagaki et al. 2013; Schmid-Burgk et al. 2015; Shi et al. 2014). In this 'non-canonical' signaling pathway, caspase-11 induces pyroptosis independently of the NLRP3 inflammasome, while cleavage of proIL-1β and proIL-18 is relayed through the NLRP3 inflammasome (Kayagaki et al. 2011).

The importance of non-canonical NLRP3 inflammasome signaling in Gram-negative infections is highlighted by the resistance of caspase-11-deficient mice to LPS-induced lethality (Kayagaki et al. 2011; Wang et al. 1998), and their increased susceptibility to enteric pathogens (Broz et al. 2012; Gurung et al. 2012; Knodler et al. 2014). Recent work revealed cleavage of the gasdermin D as a central commonality of pyroptosis induction by caspases 1 and 11 (Kayagaki et al. 2015; Shi et al. 2016, 2015). Gasdermin D cleavage also links LPS-induced caspase-11 activation with engagement of the NLRP3 inflammasome, but how it integrates with mechanisms of canonical inflammasome activation is not fully clarified.

Since the diverse canonical NLRP3 inflammasome-activating agents listed above are structurally and chemically unrelated, a direct ligand sensing model for the NLRP3 inflammasome is highly unlikely. Instead, NLRP3 activation is thought to involve a defined cellular event or secondary messenger that is commonly and selectively triggered by these NLRP3-activating agents. In this respect, K^+ is among the most frequently cited ions in regulation of NLRP3 activation. A drop in intracellular K^+ levels is regarded as a prerequisite for activation of NLRP3 because it accompanies NLRP3 inflammasome activation by a diversity of agents, and because preventing K^+ efflux by cultivating cells in media containing high extracellular K^+ concentrations prevents caspase-1 activation by the NLRP3 inflammasome (Franchi et al. 2007; Munoz-Planillo et al. 2013; Perregaux and Gabel 1994; Petrilli et al. 2007). However, also activation of the Nlrp1b inflammasome was reported to be sensitive to high K^+ concentrations (Fink et al. 2008; Wickliffe et al. 2008). Na^+, Ca^{2+}, and Cl^- are other candidates for ion flux-mediated NLRP3 activation. For instance, exchanging Na^+ for Li^+, choline or K^+ in iso-osmotic media of LPS-primed macrophages was shown to prevent ATP-induced caspase-1 activation (Perregaux and Gabel 1998). The specific role of intracellular Ca^{2+} is a matter of debate, with reports arguing against its requirement but rather correlating its involvement with simultaneous K^+ efflux induction (Katsnelson et al. 2015; Munoz-Planillo et al. 2013); or implicating Ca^{2+} fluxing as a critical step in NLRP3 inflammasome activation (Lee et al. 2012; Yaron et al. 2015).

Mitochondrial dysfunction—sometimes coupled with ionic flux deregulation—has also been proposed to control NLRP3 activation, with potential involvement of cardiolipin release, oxidized mitochondrial DNA, and loss of the mitochondrial membrane potential as secondary messengers for NLRP3 activation (Iyer et al. 2013; Nakahira et al. 2011; Shimada et al. 2012). Despite the ambiguities surrounding the molecular events regulating NLRP3 activation, Nek7 was recently established as a key NLRP3-binding partner that selectively controls activation of the NLRP3 inflammasome (He et al. 2016; Schmid-Burgk et al. 2016; Shi et al. 2016, 2015). This finding may provide a novel grasping point for further dissection of NLRP3 activation mechanisms.

The need for tight regulation of NLRP3 inflammasome activation is best highlighted by the existence of autoinflammatory diseases caused by gain-of-function mutations in NLRP3. These cryopyrin-associated periodic syndromes (CAPS) cover three autoinflammatory diseases. Familial cold autoinflammatory syndrome (FCAS) is associated with cold-induced fevers, rash, and constitutional symptoms.

Muckle–Wells syndrome (MWS) is not triggered by exposure to cold and additionally features the possible occurrence of hearing loss (Hoffman et al. 2001). Fever, chronic meningitis, eye inflammation, hearing loss, skin rash, and a deforming arthropathy all are clinical aspects of neonatal-onset multisystem inflammatory disease (NOMID) (Aksentijevich et al. 2002). Over 80 disease-associated NLRP3 mutations have been reported, most of them being situated within or in the close vicinity of the central NACHT domain. The effectiveness of anti-IL-1 therapies in CAPS patients illustrates the pathogenic role of IL-1β in these diseases (Yu and Leslie 2011). The identification of small-molecule inhibitors that prevent NLRP3 inflammasome activation might one day enable therapeutic strategies that selectively prevent cytokine secretion by this inflammasome only (Coll et al. 2015; Lamkanfi et al. 2009).

3.2 The NLRP1 Inflammasome

NLRP1 was one of the first NLRs reported to assemble an inflammasome (Martinon et al. 2002). NLRP1 undergoes autocleavage in a unique 'function to find' domain (FIIND) that lays between its carboxy-terminal CARD and LRR motifs (Chavarria-Smith and Vance 2013; Finger et al. 2012; Frew et al. 2012). Whereas humans encode a single NLRP1 gene, mice may express up to three different NLRP1 isoforms, known as Nlrp1a, Nlrp1b, and Nlrp1c (Boyden and Dietrich 2006). Moreover, human NLRP1 is equipped with both an amino-terminal PYD and a carboxy-terminal CARD, the former being absent in its murine homologs. Nevertheless, the murine paralogs appear to have non-redundant roles in immune signaling. Nlrp1b is critical for inflammasome assembly in macrophages upon exposure to lethal toxin (LeTx) of *Bacillus anthracis*, the causative agent of anthrax (Fig. 2). LeTx is a bipartite toxin consisting of a protective antigen (PA) and a lethal factor (LF) subunit, in which PA functions as a pore-forming unit enabling cytosolic delivery of the zinc metalloprotease LF subunit (Bann 2012). LF protease activity is required for Nlrp1b inflammasome activation because catalytically inactive mutants of LF fail to activate the Nlrp1b inflammasome (Levinsohn et al. 2012). This suggests that instead of directly sensing the presence of LF, Nlrp1b monitors LF protease activity in the cytosol. Notably, the inflammasome adaptor ASC is critical for LeTx-induced caspase-1 autocleavage, but the induction of pyroptosis proceeded unhampered in the absence of ASC specks (Guey et al. 2014; Van Opdenbosch et al. 2014). ASC was as well dispensable for Nlrp1b-dependent IL-1β and IL-18 secretion, although it could enhance this output. These observations suggest that direct recruitment of procaspase-1 to Nlrp1b platforms induces conformational changes in caspase-1 that suffice for its activation, while ASC-dependent caspase-1 autocleavage may serve to lock the protease in its active conformation. Next to its role in sensing LeTx activity, Nlrp1b was recently identified as a type 1 diabetes-susceptibility gene in the NOD mouse model (Motta et al. 2015), and implicated in protection from dextran sodium sulfate-induced

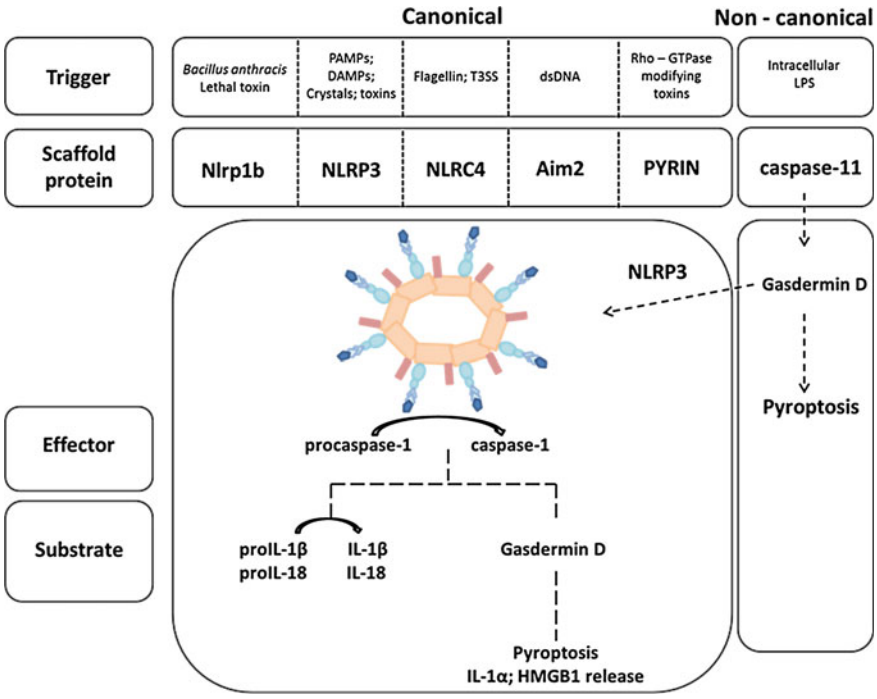

Fig. 2 Simplified scheme of inflammasome triggers and signaling events. AIM2, Pyrin, and inflammasome-assembling NLR family members detect—directly or through a secondary messenger—the presence of a pathogen and/or cellular damage. This results in their oligomerization and inflammasome assembly in which inactive procaspase-1 precursors are recruited directly or through the bipartite adaptor protein ASC. Oligomerization of procaspase-1 leads to its autoactivation, and active caspase-1 catalyzes the maturation and secretion of the inflammatory cytokines interleukin IL-1β and IL-18. Additionally, inflammasome activation may result in a lytic mode of programmed cell death termed pyroptosis. A non-canonical mode of inflammasome signaling is engaged upon detection of bacterial LPS in the cytosolic compartment by murine caspase-11 and its human orthologs caspases 4 and 5. Caspase-11 directly induces pyroptosis through cleavage of gasdermin D and promotes secretion of IL-1β and IL-18 through the NLRP3 inflammasome

colitis in mice (Williams et al. 2015). Also the Nlrp1a isoform was shown to induce inflammasome responses in vivo (Masters et al. 2012). Hematopoietic progenitor cells expressing gain-of-function mutations in Nlrp1a had ASC-independent, but caspase-1-mediated induction of pyroptosis, which prevented their proliferation and differentiation. These mice consequently suffered from leukopenia and anemia during hematopoietic stress (Masters et al. 2012). Identified single-nucleotide polymorphisms (SNPs) in the human NLRP1 locus were shown to confer an increased risk for the development of autoimmune diseases as well, with genetic variants being linked to Addison's disease, generalized vitiligo and type I diabetes (Jin et al. 2007; Spritz 2007; Zurawek et al. 2010). Other studies further linked

NLRP1 variants to systemic lupus erythematosus and rheumatoid arthritis (Levandowski et al. 2013; Magitta et al. 2009).

3.3 The NLRC4 Inflammasome

The upstream mechanisms promoting assembly and activation of the NLRC4 inflammasome probably are the best understood among inflammasome scaffolds (Fig. 2). NLRC4 has an amino-terminal CARD motif that allows direct recruitment of procaspase-1 in the absence of ASC (Broz et al. 2010b; Van Opdenbosch et al. 2014). Also the molecular constituents by which bacterial pathogens such as *Salmonella enterica* serovar Typhimurium (Lara-Tejero et al. 2006; Mariathasan et al. 2004; Miao et al. 2006), *Legionella pneumophila* (Amer et al. 2006; Zamboni et al. 2006), *Pseudomonas auruginosa* (Miao et al. 2008; Sutterwala et al. 2007), *Shigella flexneri* (Suzuki et al. 2007, 2014), and *Listeria monocytogenes* (Wu et al. 2010) trigger NLRC4 inflammasome activation have been mapped. The virulence of these bacteria strongly depends on flagellin-mediated motility and the delivery of pathogenic effector molecules in the host cell cytosol through bacterial type III and IV secretion systems (T3SS and T4SS, respectively). Intracellular detection of flagellin and/or T3SS components is what engages the NLRC4 inflammasome. Notably, NLRC4 does not detect the presence of these virulence factors directly, but their unwarranted presence in the cytosol is signaled by NLR family apoptosis inhibiting protein (NAIP) isoforms that act upstream of NLRC4 in the pathway. A full-length human NAIP isoform as well as mouse Naip5 and Naip6 serve as cytosolic flagellin receptors (Kofoed and Vance 2011; Kortmann et al. 2015; Miao et al. 2006). A shorter isoform of human NAIP and its murine Naip1 ortholog recognizes T3SS needle proteins (Miao et al. 2010b; Zhao et al. 2011), whereas Naip2 binds T3SS rod proteins (Kofoed and Vance 2011; Suzuki et al. 2014; Zhao et al. 2011). Notably, biochemical studies with chimeric Naip fusions pointed to the region surrounding the central NACHT as the bacterial ligand-binding domain of Naip proteins (Tenthorey et al. 2014). In addition to NAIP-mediated detection of flagellin and the bacterial T3SS, phosphorylation of NLRC4 at Ser533 was shown to be required for inducing NLRC4 inflammasome activation upon *S.* Typhimurium infection (Qu et al. 2012). Subsequent studies in Naip5-deficient macrophages showed that flagellin-dependent NLRC4 phosphorylation proceeds independently of Naip5, and that flagellin mutants which were unable to induce NLRC4 inflammasome activation retained their ability to induce potent NLRC4 Ser533 phosphorylation (Matusiak et al. 2015). Together, these findings suggest a two-step Nlrc4 activation mechanism in which Ser533 phosphorylation primes NLRC4 for subsequent Naip5-dependent activation of the NLRC4 inflammasome.

Notably, the NLRC4 inflammasome is not only linked to in vivo host defense against bacterial pathogens (Broz et al. 2010a; Miao et al. 2010a), but also may lead to the development of autoinflammatory disease in patients. Recently, three de novo SNPs in the NACHT domain of the NLRC4 gene were described to associate with

autoinflammatory disease: Nlrc4-MAS (NLRC4^{T337S}) (Canna et al. 2014), SCAN4 (NLRC4^{V341A}) (Romberg et al. 2014), and FCAS-like syndrome (NLRC4^{H443P}) (Kitamura et al. 2014). Although the three identified missense mutations induce somewhat distinct clinical syndromes, they have spontaneous activation of the NLRC4 inflammasome as the unifying underlying etiology. Nlrc4-MAS and SCAN4 patients further produce extremely high levels of circulating IL-18, and increased inflammasome-induced macrophage cell death was observed in SCAN4 patients (Canna et al. 2014; Romberg et al. 2014).

3.4 The AIM2 Inflammasome

Absent in melanoma 2 (AIM2) is a member of the HIN200 family/AIM-2-like receptor (ALR) family of PRRs that are characterized by an N-terminal PYD and the presence of one or two DNA-binding hematopoietic IFN inducible nuclear protein with 200 amino acid (Hin200) domains (Hornung et al. 2009). AIM2 assembles an inflammasome scaffold when dsDNA of bacterial or viral origin is bound to its Hin200 domain (Choubey 2012; Fernandes-Alnemri et al. 2009; Jin et al. 2012; Rathinam et al. 2010; Sauer et al. 2010). As such, AIM2 endows myeloid cells with the ability to produce IL-1β and IL-18 and induce pyroptosis upon recognition of bacterial and viral nucleic acids in the cytosol of infected macrophages (Rathinam et al. 2010; Sagulenko et al. 2013). The AIM2 inflammasome was shown to be critically involved in controlling infection by *Francisella tularensis*, the causative agent of tularemia (Fernandes-Alnemri et al. 2010; Jones et al. 2010; Rathinam et al. 2010), and further mediates host defense against *Listeria monocytogenes* (Tsuchiya et al. 2010; Wu et al. 2010) and the viral pathogens mouse cytomegalovirus (MCMV) (Alnemri 2010; Kanneganti 2010; Rathinam et al. 2010) and vaccinia virus (VV) (Fernandes-Alnemri et al. 2010; Rathinam et al. 2010).

3.5 The Pyrin Inflammasome

The human *MEFV* gene encodes Pyrin/TRIM20, a member of the TRIM family that is composed of an amino-terminal PYD motif that is followed by a centrally located coiled-coil and B-box region that mediates its oligomerization (Vajjhala et al. 2014; Yu et al. 2007). Unlike in rodents, this architecture is further extended to the carboxy-terminus with a B30.2 domain in human Pyrin (Fig. 1). The expression of Pyrin is largely restricted to myeloid cells with studies showing that monocyte differentiation toward macrophages significantly reduces the expression of Pyrin (Seshadri et al. 2007). Recently, Pyrin was shown to form an inflammasome that activates caspase-1 and mediates secretion of IL-1β in response to RhoA-inactivating bacterial toxins (Xu et al. 2014). As a result, the Pyrin

inflammasome is engaged in macrophages and monocytes that have been infected with *Clostridium difficile* or *Burkholderia cenocepacia* (Gavrilin et al. 2012; Xu et al. 2014). The former is a Gram-positive obligate anaerobic pathogen that is the primary cause of nosocomial diarrhea in hospitalized patients and elderly people. *B. cenocepacia* is an opportunistic pathogen that causes progressive respiratory inflammation in immunocompromised patients. The above studies suggest that Pyrin may indirectly respond to the enzymatic activity of RhoA-modifying toxins, but more studies are needed to clarify the molecular chain of events leading to Pyrin inflammasome activation.

Notably, more than 280 mutations have been identified in *MEFV* that cause familial Mediterranean fever (FMF), the most common monogenic autoinflammatory disease worldwide. FMF has a largely autosomal recessive inheritance, although patients with apparent dominant inheritance have also been documented (Balow et al. 1997; Consortium 1997). Notably, the majority of FMF-associated mutations is clustered in the carboxy-terminal B30.2 domain of human Pyrin that is absent in its mouse ortholog (Touitou et al. 2004). As the name suggests, FMF is particularly common in Southern Europe, the Mediterranean Basin, the Middle East, and the Caucasus, frequently affecting Jewish, Turkish, Armenian, Arab, and Italian populations. In these regions, the prevalence of FMF is between 1 in 500 and 1 in 1000, and *MEFV* mutations are very common, with the carrier rate reaching up to 1:5 in these endemic regions. This restricted geographical distribution suggests the existence of an as yet unknown selective advantage for heterozygous carriers in the Mediterranean Basin. Although the disease is less prevalent in Northern Europe (with estimated frequencies of 1:75,000), the disease has spread over the world with migrations of South European, North African, and Middle Eastern populations over the past centuries and in more recent times (Ozen and Bilginer 2014). FMF usually has a childhood onset and is characterized by recurrent attacks of fever associated with serositis. Its main long-term complication is amyloid A (AA) amyloidosis, a severe manifestation with poor prognosis. The microtubule polymerization inhibitor colchicine remains the therapeutic choice to prevent both FMF attacks and complications in most patients, and IL-1-targeting biologicals such as anakinra appear effective in patients with colchicine-refractory disease (Chae et al. 2006; Masters et al. 2009; Ng et al. 2010).

4 Concluding Remarks

Understanding of inflammasome biology is increasing at an impressive pace, with work during the past few years revealing the ligands and detailing the signaling mechanisms of the NLRC4, Pyrin, AIM2, and other inflammasomes. Activation of the different inflammasomes is increasingly recognized to be regulated by a variety of post-translational mechanisms, with each scaffold having its private peculiarities. In addition, disease-associated mutations in Pyrin, NLRP3, NLRC4, and NLRP1 have been shown to cause autoinflammatory diseases with variable clinical

presentations. This realization has also led to studies demonstrating the remarkable efficacy of anti-IL-1 therapies in these rare genetic syndromes. Despite the tremendous progress made on these and other fronts, quite some gaps remain in our understanding of inflammasome biology. Further progress is needed in understanding the molecular mechanisms promoting caspase-1 activation by the different inflammasome platforms. The mechanisms by which gasdermin D cleavage promotes pyroptosis warrant further investigation, and the physiological role of pyroptosis in release of cytokines and DAMPs in the context of infections, autoinflammation, and autoimmune diseases is an exciting area of continued study. Newly gained insight in these and other aspects of inflammasome biology likely is to offer novel opportunities for targeted treatment of inflammatory diseases.

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