1 *Review*

2, 3 Regulation and sensing of inflammasomes and their impact on intestinal health

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11

12 **Abstract**

13 Pattern recognition receptors such as nucleotide-binding oligomerization domain (NOD)-containing
14 protein receptors (NLRs) and the pyrin and HIN domain (PYHIN) receptors initiate the inflammatory
15 response following cell stress or pathogenic challenge. When activated some of these receptors
16 oligomerize to form the structural backbone of a signalling platform known as the inflammasome.
17 The inflammasome promotes the activation of caspase-1 and the maturation of the proinflammatory
18 cytokines, interleukin (IL)-1β and IL-18. In the gut dysregulation of the inflammasome complex is
19 thought to be a contributing factor in the development of inflammatory bowel diseases (IBD), such
20 as ulcerative colitis (UC) and Crohn's disease (CD). The importance of inflammasomes to intestinal
21 health has been emphasized by various inflammasome deficient mice in dextran sulphate sodium
22 (DSS) models of intestinal inflammation and by the identification of novel potential candidate genes
23 in population based human studies. In this review we summarise the most recent finding with
24 regard to formation, sensing and regulation of the inflammasome complex and highlight their
25 importance in maintaining intestinal health.

26 **Keywords:** inflammasomes; ulcerative colitis; Crohn's disease; interleukin (IL)-1β; IL-18

27

28 **1. Introduction**

29 The gastrointestinal environment is a continuous system with dual function. Firstly, it provides the
30 human body with the energy it needs to grow and develop and aids in the elimination of waste
31 material. Secondly, it plays an important role in preventing infection by providing a vast array of
32 immune cells close to the mucosal surface to target environment toxins and potential pathogens.
33 Several disease are known to occur in the gastrointestinal tract, notably are ulcerative colitis (UC) and
34 Crohn's disease (CD). Both are characterised by chronic and relapsing inflammation of unknown
35 aetiology. In CD the inflammation is discontinuous, transmural and often associated with intestinal
36 wall thickening, ulcerations, bowel strictures, luminal narrowing and abscesses. While in UC the
37 inflammation is continuous usually spreading proximally from the anal verge and affecting only the
38 mucosa and submucosa layers [1]. For both diseases mechanisms that regulate the gastrointestinal
39 innate immune system have been highlighted as contributing to disease pathology.
40 At the mucosal/gut lumen interface surveillance for pathogen-associated molecular pattern
41 molecules (PAMPs) and damage-associated molecular pattern molecules (DAMPs) is carried out by

42 membrane bound pattern recognition receptors and intracellular pattern recognition receptors. Upon
43 activation many of these receptors promote the secretion of proinflammatory cytokines, transcription
44 mediators, and initiate pathways responsible for pathogen neutralisation and elimination. In
45 addition, some receptors are known to form the structural backbone of the multimolecular complex
46 known as the inflammasome. The inflammasome complex is a core component of the inflammatory
47 response and its activation enhances the maturation of pro-interleukin (IL)-1β and proIL-18 to their
48 biologically active IL-1β and IL-18 forms [2]. Transcription of proIL-1β is induced by Toll-like
49 receptor (TLR) and C-type lectins receptor (CLR) stimulation via the nuclear factor kappa-light-chain-
50 enhancer of activated B cells (NF-κB) transcription pathway, whereas proIL-18 is constitutively
51 expressed and its expression is increased after receptor activation [3, 4]. For activated macrophages
52 and monocytes of the lamina propria inflammasome maturation of IL-1β and IL-18 is crucial for
53 cytokine secretion [5].

54 In the intestine, the inflammasome can also promote an inflammatory form of cell death, known as
55 pyroptosis. Pyroptosis halts the replication of intracellular pathogens by destroying the infected
56 immune cell and exposing the surviving bacteria to circulating phagocytes and neutrophils [6]. Both
57 canonical (caspase-1) and non-canonical (caspase-11) inflammasome pathways are able to induce
58 pyroptosis, however caspase-11 does not produce mature IL-1β or IL-18. Caspase-11 induced
59 pyroptosis is thought to occur upstream of canonical inflammasomes in response to
60 lipopolysaccharides (LPS) sensed in Gram-negative bacteria. Both mechanisms are considered
61 important for microbial defences in the gut [6, 7].

62

63 **2. Formation of a NOD-like receptor protein (NLRP) inflammasome complex**
64 In general, the NLRP inflammasome complex consists of a nucleotide-binding oligomerization
65 domain (NOD)-like receptor (NLR) protein, a caspase and often an adaptor protein known as
66 apoptosis-associated speck-like protein containing a CARD (ASC) [2, 8]. Several receptors from the
67 NLR family, NLRP1, NLRP2, NLRP3, NLRC4, NLRP6, NLRP7 and NLRP12 (Figure 1) have all shown
68 the ability to form the structural backbone of an inflammasome complex. The ASC adaptor protein is
69 identical for all inflammasomes and contains two transduction domains, a pyrin domain (PYD)
70 domain and a caspase recruitment domain (CARD) domain [9].

71 Formation of a NLRP inflammasome is initiated by ligand activation of the receptor protein and this
72 causes the NLR proteins to oligomerize through their nucleotide-binding and oligomerization
73 (NACHT) domains (Figure 2). This oligomerization creates a platform of NLR^{PYD} molecules at the N-
74 terminal and through NLR^{PYD}/ASC^{PYD} interactions nucleates helical ASC clusters to form an ASC
75 filament structure. The aggregation of multiple ASC^{CARD} molecules promotes ASC^{CARD}/caspase-1^{CARD}
76 interactions which in turn brings caspase domains into close proximity for dimerization, *trans*-
77 autocleavage and activation [9]. The binding of ASC to both the NLR protein and caspase-1 is
78 facilitated by a 23-residual linker which orientates ASC^{PYD} and ASC^{CARD} back to back hence
79 preventing steric interference of binding sites, while enhancing binding partner prospects [10]. ASC
80 is sequestered in the nucleus but rapidly translocates to the cytoplasm upon stimulation where it
81 participates in inflammasome formation [11]. Interestingly, inflammasome formation can be
82 abolished by preventing the cellular redistribution of ASC [11].

83

Subfamily	Protein names	Effector Domain	NACHT domain	Ligand sensing domain
NLRP	NLRP1			
	NLRP2 - NLRP9, NLRP11 - NLRP14			
	NLRP10			
NLRC	NOD1, (NLRC1), NLRC4			
	NOD2, (NLRC2)			
	NLRC3, NLRC5			
NLRB	NAIPs			
NLRA	CIITA			
NLRCX	NLRCX1			

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Legend:
- Nucleotide-binding and oligomerization domain (NACHT)
- Caspase recruitment domain (CARD)
- Pyrin domain (PYD)
- Leucine rich repeat (LRR) domain
- Baculoviral inhibitory repeat (BIR)
- Function to find domain (FIIND)
- Acid transactivation domain (AD)
- Undefined domain

85   **Figure 1: Structure of the human NOD-like receptor subgroups**

86   The NOD-like receptor (NLR) family comprises 23 human members [12, 13]. All NLR proteins contain
87   a central nucleotide-binding and oligomerization (NACHT) domain flanked by a C-terminal LRR
88   domain and N-terminal effector domain. The NACHT domain facilities self-oligomerization and has
89   ATPase activity. The N-terminal domain participates in protein-protein interactions while the LRR
90   domain is involved in ligand recognition. Subgroup classification is based on the structure of the N-
91   terminal effector region which generally comprises a CARD, PYD or BIR domain. The NLRP1,
92   NLRP2, NLRP3, NLRC4, NLRP6, NLRP7 and NLRP12 receptors have all shown the ability to form
93   inflammasome complexes.
94

95   Caspase-1 is synthesized as an inactive, monomeric zymogen (procaspase-1) and initially is cleaved
96   into a p35 fragment containing a CARD and p10 fragment. Autoproteolysis results in the generation
97   of a large p20 subunit and a small p10 subunit and the removal of the N-terminal CARD domain.
98   Dimerization of caspase molecules (p20 and p10) results in the catalytically active caspase-1 enzyme
99   (Figure 3) [14, 15]. Inflammasome activated caspase-1 cleaves its substrates, proIL-1β and proIL-18 at
100 recognition sites adjacent to aspartic acid residues, resulting in mature IL-1β and IL-18 [8].
101 In contrast to other members of the NLRP subfamily, NLRP1 contains both a function to find (FIIND)
102 and CARD domain at the C-terminal, and a PYD domain at the N-terminal [16] (Figure 1). Given that
103 NLRP1 contains two signal transduction domains (PYD and CARD) it can activate caspase-1 through
104 its C-terminal CARD domain without the need for the ASC adaptor protein, however ASC has been
105 shown to greatly enhance inflammasome formation and IL-1β processing [17]. The FIIND domain is
106 a highly conserved protein region and based on amino acid sequencing is only present in two human
107 proteins, NLRP1 and the caspase recruitment domain family, member 8 (CARD8) protein [18].
108 CARD8 is thought to function as an adaptor molecule that negatively regulates NF-κB activation,
109 caspase-1 dependent IL-1β secretion and apoptosis, and is often overexpressed in many types of
110 cancers [19-21].
111 NLRP1 inflammasome formation is strictly dependent on autolytic proteolysis within the FIIND
112 domain and after cleavage the two fragments remain associated to form a processed NLRP1. Dimers
113 of ASC joined by ASC[PYD]/ASC[PYD] are recruited to the C-terminal NLR[CARD] domain and bind via
114 NLR[CARD]/ASC[CARD] interactions. This is in contrast to other NLRP proteins which recruit ASC to the
115 N-terminal PYD domain and bind via NLR[PYD]/ASC[PYD] interactions to form the inflammasome
116 complex. Subsequently, caspase-1 through its CARD domain interacts with ASC[CARD] which leads to
117 dimerization, *trans*-autocleavage and activation of caspase-1 and IL-1β, IL-18 processing [22]. The
118 formation of ASC filaments in the activation of the NLRP1 inflammasome remain to be defined.
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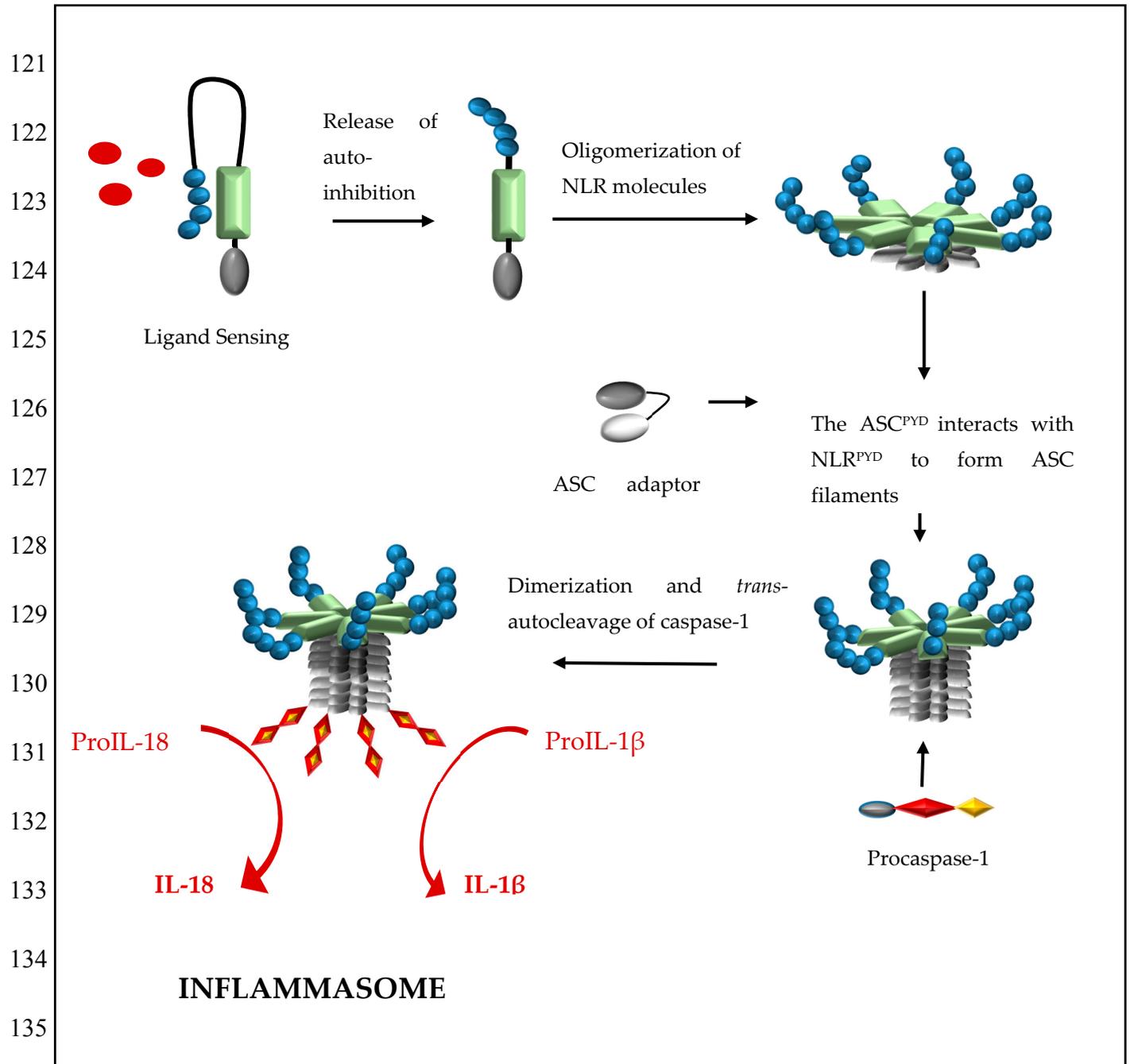
Figure 2: Formation of a NOD-like receptor protein inflammasome containing an N-terminal Pyrin domain

Formation of a nucleotide-binding oligomerization domain (NOD)-like receptor protein (NLR) inflammasome is initiated by ligand activation of the NLR protein. This causes the NLR proteins to oligomerize through their NACHT domains to create a platform of NLR[PYD] molecules at the N-terminal and through NLR[PYD]/ASC[PYD] interactions, nucleates helical ASC clusters to form a filament ASC structure. The aggregation of multiple ASC[CARD] molecules promotes ASC[CARD]/caspase-1[CARD] interactions which in turn brings caspase domains into close proximity for dimerization, *trans*-autocleavage, activation and the processing of pro-interleukin (IL)-1β and proIL-18 to their biologically active form, IL-1β and IL-18 respectively.
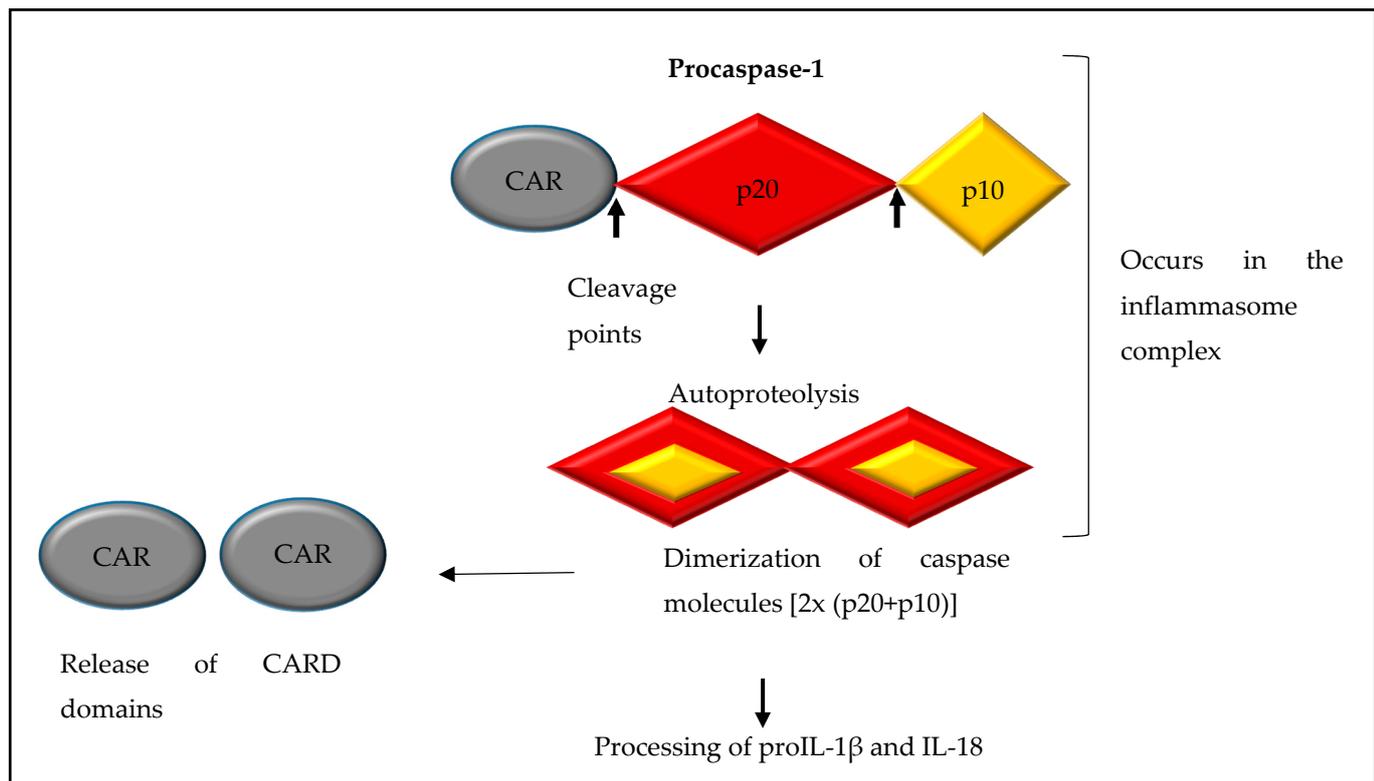
Figure 3: The mechanism for the inflammasome mediated catalytic conversion of procaspase-1 to caspase-1

Caspase-1 is initially synthesized as the inactive monomeric zymogen, procaspase-1. Binding of the procaspase-1[CARD] to ASC[CARD] filaments on the inflammasome complex results in the cleavage of procaspase-1 into a p35 fragment containing a CARD and a p10 fragment. Dimerization of the p10 and p20 and the removal of the procaspase-1[CARD] domain produces catalytically active caspase-1.

174   **3. Structure and formation of a pyrin and HIN domain (PYHIN) inflammasome complex**

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176   Two receptor in the pyrin and HIN domain (PYHIN) receptor family, absent in melanoma 2 (AIM2)

177   and interferon inducible protein 16 (IFI16) have shown the ability to form inflammasome complexes

178   (Figure 4).   Similar to NLRP inflammasomes, PYHIN inflammasomes, such as AIM2, upon ligand

179   activation oligomerize through their PYD domains to form a platform of AIM2^{PYD} molecules which

180   preferentially associates with ASC^{PYD} to form ASC filaments. The flexibly linked ASC^{CARD} clusters

181   along the ASC^{PYD} to form a platform for the binding of caspase-1^{CARD}. Similar to other NLRP

182   inflammasomes, the ASC filament structure forms the main body of the inflammasome. The

183   interaction of ASC^{CARD}/caspase-1^{CARD} brings caspase domains into close proximity for dimerization,

184   *trans*-autocleavage and activation, and the subsequent maturation of IL-1β and IL-18 [9].

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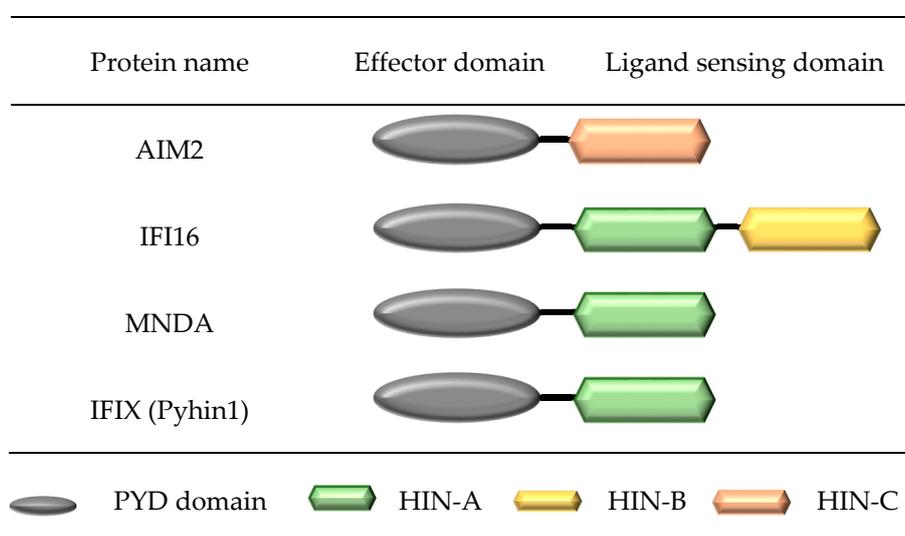
187

188   **Figure 4: Structure of the human pyrin and HIN domain (PYHIN) family**

189   The human pyrin and HIN (PYHIN) family of receptors comprises 4 members including, the

190   interferon inducible protein 16 (IFI16), absent in melanoma 2 (AIM2), myeloid nuclear differentiation

191   antigen (MNDA) and interferon inducible protein X [IFIX (Pyhin1)], while mouse contains 11

192   confirmed members [23]. All members consist of an N-terminal pyrin domain (PYD) domain attached

193   to one or more hemopoietic expression, interferon-inducibility, nuclear localisation (HIN-200)

194   domains at the C-terminal. Three distinct forms of HIN-200 have been characterised (HIN-A, -B and

195   -C) and are classified according to specific consensus motifs [24].

4. Ligand sensing of inflammasome complexes

Depending on the type of receptor protein in the complex, inflammasomes have the ability to respond to a wide array of pathogens and cellular danger signals. The LRR domains of the NLRP receptors and the HIN200 domains of the PYHIN receptors are thought to be involved in ligand interactions, however direct binding of an activating ligand to a receptor has only been demonstrated for the AIM2 and IFI16 inflammasomes.

5. The NLRP1 inflammasome

The NLRP1 inflammasome was one of the first inflammasomes to be described however efforts to unravel the processes that lead to activation have been hampered by species variations in the *NLRP1* gene. In humans the *NLRP1* gene is singular, while in mouse the gene encoding *Nlrp1* is polymorphic with three homologs, *Nlrp1a, Nlpr1b* and *Nlrp1c* [14]. Furthermore, the structure of mouse *Nlrp1* lacks the N-terminal PYD domain found in human NLRP1 and five different strain specific *Nlrp1b* alleles exist in inbred mice [25].

Nlrp1 is activated mainly by lethal toxin (LeTx) produced by *Bacillus anthracis* with variations in *Nlrp1b* providing sensitivity or resistance to the toxin [26]. LeTx is a bipartite toxin consisting of a protective antigen binding subunit and a catalytic lethal factor moiety. Binding of the protective antigen to anthrax binding sites translocates lethal factor into the host cytosol where it cleaves the N-termini of mitogen-activated protein kinase (MAPK) thereby disrupting cell signalling pathways. Initially lethal factor blocks cytokine production from numerous cell types, inhibits chemotaxis of neutrophils, induces apoptosis in activated macrophages and later induces cytokine-independent shock and death [27]. Caspase-1 and IL-1β deficient mice are more susceptible to *B.anthracis* infection indicating IL-1β production via the NLRP1b inflammasome is more important than ASC independent pyroptosis in the host protective response to *B.anthracis* [15, 27].

More recently NOD2 has been linked to NLRP1 dependent sensing of MDP and *B.anthracis* in activated cells where it produces a NOD2-NLRP1 inflammasome complex [28]. NOD2 is a known intracellular sensor of MDP and has the ability to contribute to the induction of NF-κB and MAPK transcription factors, however TLRs are much more effective in triggering these responses [29]. The absence of NOD2 prevents *B. anthracis* induced IL-1β secretion but has little effect on the transcription of proIL-1β indicating the importance of the NOD2-NLRP1 association in host defences against B. *anthracis* [28].

6. The NLRP3 inflammasome

The NLRP3 inflammasome has the ability to activate upon exposure to a wide range of whole pathogens, environmental irritants and structurally diverse DAMPs and PAMPs [2, 30, 31]. While the mechanisms are not yet fully understood it is thought that activation of NLRP3 occurs in response to host derived factors that are altered by these agents. While several models have been proposed for the activation of NLRP3 none have been found to be unified for all activating agents. The proposed mechanisms include;

1. K^+ efflux [32]
2. The generation of mitochondrial derived reactive oxygen species (mROS) [33]
3. Phagolysosomal destabilisation and the release of cathepsins [34]
4. The release of mitochondrial DNA or the mitochondrial phospholipid cardiolipin [35-37]

239   5.   Translocation to the mitochondria [33, 38, 39]

240   To add to the controversy, membrane permeation, phagolysosmal destabilisation, mitochondrial
241   damage and ROS production are all interrelated cellular events making the distinction between
242   bystander and causative activation events complicated.

243   In resting cells the basal expression of *NLRP3* is insufficient for inflammasome activation and
244   consequently two signals are required for the activation of the NLRP3 inflammasome [40, 41]. The
245   first signal is the NF-κB mediated transcription of *NLRP3* and *proIL-1β* from stimulation of TLR
246   antagonists or cytokines such as TNF-α and IL-1β. The second signal is the ligand activation step
247   which culminates in the activation of caspase-1 and the maturation of IL-1β and IL-18 [30, 42]. The
248   enhanced effect of guanylate binding protein (GBP5) on Nlrp3 inflammasome assembly in response
249   to bacteria and soluble but not crystalline inflammasome priming agents raises the possibility of
250   agent specific cofactors being required for inflammasome activation [43].

251   Particulate matter such as aluminium, silica, monosodium urate (MSU), calcium pyrophosphate
252   dehydrate crystals, cholesterol and amyloid β enters the cell by means of phagocytosis [34, 44-46].
253   The destabilisation of the phagolysosmal membrane and the release of the cysteine protease
254   cathepsins B into the cytosol is thought to also trigger NLRP3. Inhibitors of cathepsins B have been
255   shown to prevent caspase-1 activation induced by *N. gonorrhoeae [47]*. Interestingly, cathepsins
256   deficient mice show minimal defects in the activation of NLRP3 in response to particulate matter,
257   suggesting other off target effects may exist [48].

258   More recently, mitochondrial dysfunction and activation of the NLRP3 inflammasome has been an
259   area of intense research and much speculation. Mitochondrial reactive oxygen species (mROS) are
260   produced in response to cell stresses such as, hypoxia, starvation, pathogen infection, and growth
261   factor stimulation or membrane damage [49]. The release of mROS and oxidised mitochondrial DNA
262   have both been shown to activate the NLRP3 inflammasome [35, 50]. Interruption of ROS production
263   using inhibitors blocks NLRP3 activation suggesting ROS production upstream is necessary for
264   NLRP3 activation [50-52].

265   It has been proposed that NLRP3 associates with the mitochondria upon activation [38, 50] and when
266   exposed to non-crystalline activators, recruitment from the cytosol to the mitochondria is mediated
267   by the mitochondrial anti-viral signalling protein (MAVS) [39]. MAVS is also known as a
268   mitochondrial adaptor protein and plays a crucial role in RLR receptor signalling pathways leading
269   to type 1 IFN induction and NF-κB activation [53]. MAVS is thought to directly associate with the N-
270   terminus of NLRP3 to promote optimal inflammasome formation [39]. Consistent with a role for
271   MAVS in NLRP3 activation, MAVS deficient mice exposed to dextran sodium sulphate (DSS)
272   induced colitis fail to upregulate IL-1β [54].

273   Other work on mitochondrial dysfunction has demonstrated a ROS independent activation of *Nlrp3*
274   induced by the antibiotic linezolid whereby the mitochondrial specific lipid cardiolipin binds to *Nlrp3*
275   leading to the maturation of IL-1β [37]. Cardiolipin is a phospholipid exclusively found in the inner
276   mitochondrial membrane of eukaryotic cells. Cardiolipin plays a critical role in the activation of
277   caspase-8 and caspase-3 in the apoptotic cell death pathway which raises the possibility that the
278   inflammasome pathways are linked to the apoptosis pathways by processes that control
279   mitochondrial homeostasis.

280   In addition, agents that induce NLRP3 activation, such as nigericin have demonstrated an ability to
281   disrupt mitochondrial homeostasis by reducing the intracellular concentration of the coenzyme

282   NAD^+. Low NAD^+ inactivates the α-tubulin deacetylase sirtuin 2 (SIRT2) and causes the accumulation
283   of acetylated α-tubulin. Excess acetylated α-tubulin mediates the microtubule transport of
284   mitochondria which drives the apposition of ASC on the mitochondria to NLRP3 on the endoplasmic
285   reticulum. Microtubule transport of organelles creates optimal sites for signal transduction between
286   ASC and NLRP3 and directs activation of NLRP3. Work using inhibitors of tubulin polymerisation
287   have demonstrated suppression of IL-1β [38].
288   Early work investigating caspase-1 activation by the NLRP3 inflammasome showed that K^+ efflux
289   accompanies NLRP3 activation [51, 55]   and a high extracellular concentration of K^+ blocks the
290   activation of not only the NLRP3 inflammasome but also the NLRP1, NLRC4 and AIM2
291   inflammasomes [56, 57]. ATP levels have been linked to K^+ efflux, such that high extracellular ATP
292   levels engage the ATP-gated purinergic $P2X_7$ receptor promoting the formation of the pannexin-1
293   pore, which induces K+ efflux [42, 58]. Previous work by Muñoz-Planillo [32] has shown that ROS
294   generation, opening of the pannexin-1 pore and K^+ efflux all occur upon stimulation with a variety of
295   bacterial pore-forming toxins, nigericin, ATP and particulate matter. However in contrast to other
296   work the permeation of the cell membrane to K^+ and Na^+ was found to be the only common step
297   induced by all NLRP3 antagonists and the primary activity that was necessary and sufficient for
298   caspase-1 activation. In addition, cytosolic K^+ efflux was found to be specific to NLRP3 activation and
299   was shown not to play a role in the activation of AIM2. These results await further clarification by
300   other independent researchers.
301
302   **7.   The NLRC4 inflammasome**
303   The NLRC4 inflammasome has been well characterised in the mouse system and plays an important
304   role in the detection of pathogenic bacteria [14]. The pathogenicity of a bacteria is reliant on functional
305   secretion systems including the type III and IV which act as needle-like structures delivering virulent
306   factors into the host's cytosol. NLRC4 is activated by two critical components of pathogenic bacteria,
307   a sequence motif found in the basal rod components of the type III (T3SS) and IV (T4SS) bacterial
308   secretion systems, and a similar sequence motif found in flagellin which is a component of their
309   flagellum apparatus [59, 60]. NLRC4 has been shown to detect basal rod components in *Salmonella*
310   *typhimurium*, *Legionella pneumophila*, *Burkholderia pseudomallei*, *Escherichia coli*, *Shingella flexneri*,
311   *Pseudomonas aeruginosa [61, 62]* and leaked cytosolic flagellin from *Listeria monocytogenes, Salmonella*
312   *typhimurium, Pseudomonas aeruginosa* and *Legionella pneumophila [60, 63, 64].*
313   Activation of the NLRC4 inflammasome involves the initial binding of a receptor protein from the
314   neuronal apoptosis inhibitory protein (NAIP) subfamily of NLRs to the activating ligand. NAIP
315   receptor proteins differ from other NLRs in that they contain multiple BIR domains at the N-terminus
316   instead of a CARD or PYD domain (Figure 1). In humans only one *NAIP* homolog is expressed,
317   whereas in mice the *NAIP* locus is polymorphic and seven paralogs of *Naip* (*Naip1-Naip7*) exist [62].
318   Human NAIP and its mouse ortholog, Naip1 recognise cytosolic T3SS needle proteins, Naip2 binds
319   T3SS rod components while Naip5 and Naip6 bind directly to bacterial flagellin [62, 64]. Binding of
320   a NAIP protein to a bacterial motif leads to the formation of the NAIP-NLRC4 inflammasome
321   complex and activation of caspase-1. Interestingly, in human U937 monocyte derived macrophages
322   NLRC4 activation does not occur in response to flagellin or T3SS rod protein but occurs in response
323   to the T3SS subunit Cprl from *Chromobacterium violaceum* which raises the possibility that other
324   accessory proteins may be involved in activation of the human NLRC4 inflammasome [64].

325

8. The AIM2 and IFI16 inflammasome

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Both the cytosolic AIM2 receptor and the nuclear IFI16 receptor directly bind their activating ligand double stranded DNA (dsDNA) via the C terminal HIN200 domain [65-67].   Non-sequence specific binding occurs at multiple sites along the dsDNA and is through electrostatic attractions between the positively charged HIN domain residues and the dsDNA sugar phosphate backbone [68].

The mechanisms that enable AIM2 and IFI16 to respond to viral, bacterial, mammalian and synthetic dsDNA while remaining unresponsive to self DNA are still unclear [14, 69].

Work using _Aim2_ deficient mice have demonstrated an essential role for AIM2 in the recognition of viruses and bacteria by the detection of cytosolic dsDNA. When compared to WT mice, _Aim2-/-_ mice experience higher mortality rates, higher bacterial loads and decrease production of caspase-1 generated cytokines after infection with _Fransicella tularenis_, suggesting AIM2 is necessary for detection of _Fransicella tularenis_ [70, 71].   Similarly, mouse macrophages deficient for _Aim2_ show an impaired ability to recognise not only _Fransicella tularenis_, but also vaccinia virus, murine cytomegalovirus (mCMV) with only partial recognition of _Listeria monocytogenes_ being demonstrated [65, 72].

341

9. The NLRP6, NLRP7 and NLRP12 inflammasomes

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In addition to the well-known NLRP1, NLRP3, NLRC4, AIM2 and IFI16 inflammasomes NLRP6, NLRP7 and NLRP12 have shown an ASC dependent activation of caspase-1. However, the signals that activate the NLRP6 and NLRP12 inflammasomes are yet to be determined [73].

Indeed, two independent studies have reported a lack of caspase-1 activation and IL-1β release in Nlpr6 deficient mouse macrophages in response to ATP and LPS which suggest the triggers that activate the NLRP6 inflammasome are different to those that activate the NLRP3 inflammasome [74, 75]. Recently NLRP7, which is not expressed in mice, was shown to form an ASC dependent inflammasome in human macrophages in response to microbial acylated lipopeptides [76].

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10. **Regulation of the inflammasome complex**

The potent inflammatory cytokines, IL-1β and IL-18 and the pyroptosis pathway all have the potential to cause tissue damage and disrupt an effective adaptive immune response. The mechanisms that lead to maturation of IL-1β and IL-18 are tightly controlled at several levels and multiple checkpoints along this process ensuring response appropriate levels.

In most cells the basal levels of many of the inflammasome constituents is insufficient for inflammasome formation. Consequently, the expression of the inflammasome components is regulated by NF-κB induced transcription and requires sensitization by a TLR or CLR ligand or stimulus from cytokine signalling pathways [30, 77]. In contrast to most cytokines IL-1β and IL-18 are produced as inactive zymogens requiring caspase-1 cleavage between Asp and Ala for maturation [3, 78]. The synthesis of precursor cytokines requiring activation prevents aberrant secretion of the leaderless IL-1β and IL-18 cytokines. Serine proteinases such as cathepsins G, elastase and in particular proteinase 3 found in neutrophils have also been shown to cleave proIL-1β to active IL-1β. While in monocytes autocrine production of ATP can activate caspase-1 and cleave proIL-1β, thereby releasing IL-1β by transcription only [79]. Worth noting is that during acute inflammatory conditions non-canonical maturation of IL-1β can also occur via caspase-11 and the NLRP3 inflammasome [6].

11. **Regulation by autoinhibition of the ligand sensing domain**

For most of the receptor proteins autoinhibition of the ligand sensing domain prevents unproductive intramolecular interactions by providing a tight on-site repression of the protein in the absence of a suitable activating ligand. For NLRP1, NLRP3, NLRP12 and NLRC4 receptors autoinhibition is achieved by the association of two chaperone proteins, ubiquitin ligase-associated protein (SGT1) and heat-shock protein 90 (HSP90) to the LRR domain. Upon ligand sensing SGT1 and HSP90 dissociate resulting in a conformation change within the protein which favours the recruitment of the ASC adaptor protein [80]. Whether autoinhibition of the sensing region occurs for the NLRP6 protein remains to be determined. For the PHYIN subfamily autoinhibition is provided by the molecular interactions between the PYD and HIN-200 domain and binding of DNA releases this autoinhibition [68].

12. **Priming events that regulate activation**

Specific priming events are known to regulate the activation of inflammasomes. The K-63 specific deubiquitinating enzyme BRCC3 mediates the deubiquitylation of NLRP3 which has recently been shown to occur in response to pattern recognition receptor stimulation [81]. Similarly, and as mentioned above, GBP5 enhances NLRP3 assembly in response to bacterial but not crystalline agents [43]. A priming event involving the phosphorylation of Ser533 by kinases like PKCδ is necessary before *Salmonella typhimurium* can activate the NLRC4 inflammasome. The phosphorylation of Ser533 is thought to result in a conformation change within the NLRC4 protein [82].

The anti-apoptotic proteins B-cell lymphoma 2 (Bcl-2) and B-cell lymphoma extra-large (Bcl-X$_L$) have been shown to regulate the NLRP1 inflammasome. By associating with NLRP1 via their loop domains Bcl-2 and Bcl-X$_L$ are able to suppress caspase-1 activation and IL-1β processing [83, 84]. Similar experiments for NLRP3 in Bcl-2 deficient macrophages have shown more caspase-1 processing while

395   Bcl-2 overexpressing macrophages demonstrated less caspase-1 processing suggesting NLRP3 may
396   also be regulated by Bcl-2 protein [35].
397   Activation of the NLRP3 inflammasomes is thought to be influenced by K^+ levels, indeed low
398   intracellular K+ level enhance caspase-1 activation [51, 55]. Similarly, high extracellular K^+ levels block
399   IL-1β release from the NLRC4 and AIM2 inflammasome suggesting the regulatory effect of K^+ may
400   be extended to other inflammasome complexes [70, 85]. Interestingly, the levels of extracellular K^+
401   need to block IL-1β release for the NLRP3 inflammasome is less than that needed for the NLRC4 or
402   AIM2 complex, while for the NLRP7 inflammasome high K^+ levels only slightly reduced IL-1β release
403   [76]. More work is needed to exclude off target effects and to determine the reasons for inflammasome
404   specific thresholds to K^+ levels.

405   **13. Regulation by POPs and COPs**
406   In humans, pyrin only proteins (POPs) and CARD-only proteins (COPs) regulate the inflammasome
407   at the level of death fold interactions. With the exception of caspase-12, POPs and COPs are lacking
408   from the mouse genome which suggests humans have evolved more complex inflammasome
409   regulatory systems [86]. The POPs include, POP1 (also known as PYDC1) and POP2 (also known as
410   PYDC2) and both inhibit PYD interactions between the receptor protein and the ASC adaptor
411   molecule. POP1 shows a higher homology to ASC[PYD] than POP2 and therefore inhibits inflammasome
412   formation by sequestering ASC from other inflammasome forming NLRs [87]. POP2 is surprisingly
413   similar to the PYD domain of NLRP2 and NLRP7 and is thought to interact with other NLR[PYD]
414   proteins thereby preventing inflammasome formation [88]. Both POP1 and POP2 can prevent NF-κB
415   activation [87, 88].
416   The COPS proteins consists of several members including, CARD16 (also known as pseudo-ICE or
417   COP1), CARD17 (also known as INCA), CARD18 (also known as ICEBERG), caspase-12s and Nod2-
418   S.[89] COP proteins act as decoy inhibitors and sequester procaspase-1 via CARD-CARD interactions
419   thereby preventing its activation in the inflammasome. For example, CARD 17 is upregulated by IFN-
420   γ in the monocytic cell lines THP-1 and U937 and interacts with procaspase-1 to supress IL-1β
421   processing and release in LPS stimulated macrophages [90].
422

423   **14. Regulation by Type I interferons**
424   Type I interferons restrict IL-1β production by two distinct mechanisms. Depending on the cell type,
425   type I interferons through the STAT3 signalling pathway can induce autocrine and paracrine
426   production of the anti-inflammatory cytokine IL-10 which inhibits the synthesis of proIL-1β and
427   proIL-18. Additionally, type I interferons signalling through the STAT1 transcription factor can
428   repress the activity of the NLRP1 and NLRP3 inflammasome thereby subduing IL-1β production [91].
429   For the AIM2 inflammasome, *Irf3* deficient mouse macrophages, which are unable to secrete type I
430   interferons, have impaired AIM2 activation in response to *Francisella tularensis* infection indicating
431   that an intact type I interferon response is required for AIM2 activation [70]. Interestingly, activation
432   of the AIM2 inflammasome in response to mouse cytomegalovirus does not require an intact type I
433   interferon response [72]. The mechanisms pertaining to the selective requirement of type I interferons
434   for the clearance of certain infections remain unclear.
435   Evidence suggests that cells of the adaptive immune response can also dampen inflammasome
436   activation. In mouse macrophages and dendritic cells, effector CD4+ T cells and memory T cells
437   suppress activation of the NLRP1 and NLRP3 inflammasomes. For the NLRP3 inflammasome the

438 inhibitory effect requires the cell-to cell contact and could be mimicked by macrophage stimulation
439 with members of the TNF family such as, CD40L, OX40L and RANKL. Interestingly, the negative
440 feedback loop exerted by T cells is only evident for the NLRP1 and NLRP3 inflammasome and was
441 absent for the NLRC4 inflammasome [92].

442 **15. Regulation from inflammasome components**

443 Inflammasomes components can themselves indirectly impact on inflammasome formation and IL-
444 1β release. For example, NLRP12 acts as a negative regulator of the NF-κB pathway through its
445 interaction and regulation of NIK and TRAF3, and dysregulation of NF-κB is associated with colonic
446 inflammation and cancer [93]. NLRP10 interacts with ASC, even though it lacks a ligand sensing
447 LRR, and is thought to negatively regulate the inflammasome by sequestering ASC [94, 95]. The ASC
448 adaptor protein, in addition to the full length ASC also exists as three novel isoforms, ASC-b, ASC-c
449 and ASC-d. ASC-c exerted an inhibitory effect on NLRP3 inflammasome formation by only colocalise
450 with caspase-1 and not NLRP3. ASC-d failed to colocalise with either caspase-1 or NLRP3 suggesting
451 an undetermined function for this isoform [11].

452 Emerging evidence indicates NLRP7 is able to regulate inflammasomes, however conflict reports
453 argue the nature of the negative regulation. Reconstitution experiments in HEK293 cells have shown
454 that NLRP7 inhibits NLRP3 and caspase-1 mediated release of IL-1β and co-immunoprecipitation
455 studies indicated NLRP7 directly interacts with procaspase-1 and proIL-1β [96]. While other work
456 focusing on NLRP7 overexpression and gene specific mutations have indicated that NLRP7 inhibits
457 NF-κB activation by an unknown mechanism or inhibits release of IL-1β [97]. Positive regulation is
458 affirmed by the formation of the NLRP7 inflammasome in response to microbial acylated
459 lipopeptides [76].

460

461 **16. Inflammasome complexes and the intestinal environment**

462 Mouse models that stimulate colitis, such as DSS, have provide an accessible framework for
463 investigating the role of inflammasomes in diseases that affect the gastrointestinal tract. Differences
464 in the experimental conditions used for colitis induction and pathogen infection have resulted in
465 many discrepancies regarding the redundant or necessary role of individual inflammasome
466 complexes in protecting against colitis [16].

467 Mice deficient in *Nlrp3, Nlrc4, IL-1β, Casp1/11* and *Asc*, when challenged by DSS, have all shown
468 increased susceptibility to colitis, disease exacerbation, frequent mortality and increased tumor
469 formation when compared to DSS challenged wild type mice, suggesting these components aid in
470 colitis protection [98-104]. Disease exacerbation has also been a feature of DSS challenged *Nlrp6*
471 deficient mice [105]. Not reported in Nlrc4 and Nlrp3 deficient mice but associated with Nlrp6
472 deficiency is a reduction in the thickness of the mucus layer and the development of a transferable
473 colitis forming microbiota dominated by TM7 and *Prevotellaceae* species (*Bacteroidetes* phyla). The
474 reduction in mucus has been attributed to defects in mucin granule exocytosis and reduce autophagy
475 mechanisms in goblet cells which suggests that, unlike NLRP3 and NLRC4, NLRP6 orchestrates
476 downstream mechanisms involved in bacterial defences [105, 106].

477 Alterations in the composition of the gut microbiota have been reported for IBD patients [107, 108].
478 In general, UC patients exhibit higher overall bacterial counts while in CD the bacterial counts are
479 lower but associated with a higher proportion of unclassified *Bacteroidetes* spp. and a higher diversity
480 of TM7 phylotyes. Increases in *Enterobacteriaceae,* adherent-invasive strains of *Escherichia coli* and

481   *Ruminococcus gnavus* populations and a decrease in *Faecalibacterium* and *Roseburia* have been reported
482   for ileal CD [109-111]. Interestingly, disease remission in UC induces microbial populations
483   comparable to healthy patients while in CD the microbial population is reportedly not altered by
484   disease remission, remaining constant in active and quiescent disease states [109].
485   Human work investigating the role of individual inflammasomes on gastrointestinal diseases are
486   currently lacking. It has however been demonstrated that the expression of inflammasome
487   components such as *CASP1, IL-1β, IFI16* and *AIM2* increases in active disease [112-114].
488
489   **17. Future direction**
490   The dysregulation of inflammasomes and their importance in maintaining intestinal health has been
491   demonstrated by mice deficient in inflammasome components in DSS models of intestinal
492   inflammation. Population based studies have identified possible risk polymorphisms associated with
493   UC and CD. IL-1 neutralising agent have provided remarkable reduction in clinical symptoms for
494   CAPS patients. Taken altogether, it highlights the potential therapeutic benefits of targeting
495   individual inflammasome complexes to complement mainstream therapeutic options.
496   Currently, the focus of treatment for IBD patients is to induce and maintain clinical remission. This
497   is usually achieved by a combination of antibiotics, vitamin support, immunomodulators,
498   corticosteroids, 5-aminosalicylates, biologic therapies and surgery [1]. For many patients however
499   active disease persists despite treatment. Recently, the compound MCC950 was shown to inhibit
500   NLRP3 non-canonical and canonical IL-1β production in both mouse bone-marrow derived
501   macrophages and human monocyte-derived macrophages [115]. The ability of this compound to
502   consistently inhibit inflammasome mediated IL-1β production provides promise for clinical trials and
503   future therapeutic options.
504   While mice work has been pivotal to determining the regulatory role of inflammasomes in intestinal
505   health, questions still remain. Firstly, what is the exact role of inflammasomes, such as the NLRP3
506   complex, in intestinal immune responses? Secondly, how accurately do the finding in mice translate
507   to the mechanisms that induce colitis in humans? Future research now needs to focus on individual
508   inflammasomes complexes; how they present in active human disease, what mechanisms they
509   influence downstream and if blockading alleviates disease symptoms.
510
511
512
513

514   **Patent**

519

520   **Conflicts of interest:** The authors disclose no conflicts of interest.

521

522   **Author Contributions:** Nicole Ranson wrote the manuscript. All authors critically reviewed the

523   manuscript before submission.

524

References

525

526 1. Bickston S.J; Bloomfeld　R.S, *Handbook of Inflammatory Bowel Disease*. First Edition ed.;
527 Lippincott Williams and Wilkins, a Wolters Kluwer business: 2010; p 1-175.

528 2. Schroder, K.; Tschopp, J., The inflammasomes. *Cell* **2010,** 140, (6), 821-32.

529 3. Dinarello, C. A., Interleukin 1 and interleukin 18 as mediators of inflammation and the aging
530 process. *The American journal of clinical nutrition* **2006,** 83, (2), 447S-455S.

531 4. Latz, E.; Xiao, T. S.; Stutz, A., Activation and regulation of the inflammasomes. *Nat Rev*
532 *Immunol* **2013,** 13, (6), 397-411.

533 5. Lamkanfi, M.; Walle, L. V.; Kanneganti, T. D., Deregulated inflammasome signaling in
534 disease. *Immunological reviews* **2011,** 243, (1), 163-73.

535 6. Lamkanfi, M.; Dixit, V. M., Mechanisms and functions of inflammasomes. *Cell* **2014,** 157, (5),
536 1013-22.

537 7. Aachoui, Y.; Leaf, I. A.; Hagar, J. A.; Fontana, M. F.; Campos, C. G.; Zak, D. E.; Tan, M. H.;
538 Cotter, P. A.; Vance, R. E.; Aderem, A.; Miao, E. A., Caspase-11 protects against bacteria that
539 escape the vacuole. *Science (New York, N.Y.)* **2013,** 339, (6122), 975-8.

540 8. Yazdi, A. S.; Guarda, G.; D'Ombrain, M. C.; Drexler, S. K., Inflammatory caspases in innate
541 immunity and inflammation. *J Innate Immun* **2010,** 2, (3), 228-37.

542 9. Lu, A.; Magupalli, V. G.; Ruan, J.; Yin, Q.; Atianand, M. K.; Vos, M. R.; Schroder, G. F.;
543 Fitzgerald, K. A.; Wu, H.; Egelman, E. H., Unified polymerization mechanism for the
544 assembly of ASC-dependent inflammasomes. *Cell* **2014,** 156, (6), 1193-206.

545 10. de Alba, E., Structure and interdomain dynamics of apoptosis-associated speck-like protein
546 containing a CARD (ASC). *The Journal of biological chemistry* **2009,** 284, (47), 32932-41.

547 11. Bryan, N. B.; Dorfleutner, A.; Kramer, S. J.; Yun, C.; Rojanasakul, Y.; Stehlik, C., Differential
548 splicing of the apoptosis-associated speck like protein containing a caspase recruitment
549 domain (ASC) regulates inflammasomes. *Journal of inflammation* **2010,** 7, 23.

550 12. Becker, C. E.; O'Neill, L. A., Inflammasomes in inflammatory disorders: the role of TLRs and
551 their interactions with NLRs. *Seminars in immunopathology* **2007,** 29, (3), 239-48.

552 13. Smith, P. D.; Mcdonald, T. T.; Blumberg, R. S.; editors, *Principles of Mucosal Immunology*.
553 Garland Science,Taylor and Francis Group, LLC: 2013.

554 14. Rathinam, V. A.; Vanaja, S. K.; Fitzgerald, K. A., Regulation of inflammasome signaling.
555 *Nature immunology* **2012,** 13, (4), 333-2.

556 15. Broz, P.; von Moltke, J.; Jones, J. W.; Vance, R. E.; Monack, D. M., Differential requirement for
557 Caspase-1 autoproteolysis in pathogen-induced cell death and cytokine processing. *Cell host*
558 *& microbe* **2010,** 8, (6), 471-83.

559 16. Franchi, L.; Munoz-Planillo, R.; Nunez, G., Sensing and reacting to microbes through the
560 inflammasomes. *Nature immunology* **2012,** 13, (4), 325-32.

561 17. Faustin, B.; Lartigue, L.; Bruey, J. M.; Luciano, F.; Sergienko, E.; Bailly-Maitre, B.; Volkmann,
562 N.; Hanein, D.; Rouiller, I.; Reed, J. C., Reconstituted NALP1 inflammasome reveals two-step
563 mechanism of caspase-1 activation. *Molecular cell* **2007,** 25, (5), 713-24.

564 18. D'Osualdo, A.; Weichenberger, C. X.; Wagner, R. N.; Godzik, A.; Wooley, J.; Reed, J. C.,
565 CARD8 and NLRP1 undergo autoproteolytic processing through a ZU5-like domain. *PloS*
566 *one* **2011,** 6, (11), e27396.

567 19. Pathan, N.; Marusawa, H.; Krajewska, M.; Matsuzawa, S.; Kim, H.; Okada, K.; Torii, S.;
568 Kitada, S.; Krajewski, S.; Welsh, K.; Pio, F.; Godzik, A.; Reed, J. C., TUCAN, an antiapoptotic

569　　　　　caspase-associated recruitment domain family protein overexpressed in cancer. *The Journal*
570　　　　　*of biological chemistry* **2001,** 276, (34), 32220-9.

571　　20.　Stilo, R.; Leonardi, A.; Formisano, L.; Di Jeso, B.; Vito, P.; Liguoro, D., TUCAN/CARDINAL
572　　　　　and DRAL participate in a common pathway for modulation of NF-kappaB activation. *FEBS*
573　　　　　*letters* **2002,** 521, (1-3), 165-9.

574　　21.　Tschopp, J.; Martinon, F.; Burns, K., NALPs: a novel protein family involved in inflammation.
575　　　　　*Nature reviews. Molecular cell biology* **2003,** 4, (2), 95-104.

576　　22.　Finger, J. N.; Lich, J. D.; Dare, L. C.; Cook, M. N.; Brown, K. K.; Duraiswami, C.; Bertin, J.;
577　　　　　Gough, P. J., Autolytic proteolysis within the function to find domain (FIIND) is required for
578　　　　　NLRP1 inflammasome activity. *The Journal of biological chemistry* **2012,** 287, (30), 25030-7.

579　　23.　Schattgen, S. A.; Fitzgerald, K. A., The PYHIN protein family as mediators of host defenses.
580　　　　　*Immunological reviews* **2011,** 243, (1), 109-18.

581　　24.　Cridland, J. A.; Curley, E. Z.; Wykes, M. N.; Schroder, K.; Sweet, M. J.; Roberts, T. L.; Ragan,
582　　　　　M. A.; Kassahn, K. S.; Stacey, K. J., The mammalian PYHIN gene family: phylogeny,
583　　　　　evolution and expression. *BMC evolutionary biology* **2012,** 12, 140.

584　　25.　Boyden, E. D.; Dietrich, W. F., Nalp1b controls mouse macrophage susceptibility to anthrax
585　　　　　lethal toxin. *Nat Genet* **2006,** 38, (2), 240-4.

586　　26.　Newman, Z. L.; Printz, M. P.; Liu, S.; Crown, D.; Breen, L.; Miller-Randolph, S.; Flodman, P.;
587　　　　　Leppla, S. H.; Moayeri, M., Susceptibility to anthrax lethal toxin-induced rat death is
588　　　　　controlled by a single chromosome 10 locus that includes rNlrp1. *PLoS pathogens* **2010,** 6, (5),
589　　　　　e1000906.

590　　27.　Terra, J. K.; Cote, C. K.; France, B.; Jenkins, A. L.; Bozue, J. A.; Welkos, S. L.; LeVine, S. M.;
591　　　　　Bradley, K. A., Cutting edge: resistance to Bacillus anthracis infection mediated by a lethal
592　　　　　toxin sensitive allele of Nalp1b/Nlrp1b. *Journal of immunology* **2010,** 184, (1), 17-20.

593　　28.　Hsu, L. C.; Ali, S. R.; McGillivray, S.; Tseng, P. H.; Mariathasan, S.; Humke, E. W.; Eckmann,
594　　　　　L.; Powell, J. J.; Nizet, V.; Dixit, V. M.; Karin, M., A NOD2-NALP1 complex mediates caspase-
595　　　　　1-dependent IL-1beta secretion in response to Bacillus anthracis infection and muramyl
596　　　　　dipeptide. *Proceedings of the National Academy of Sciences of the United States of America* **2008,**
597　　　　　105, (22), 7803-8.

598　　29.　Maeda, S.; Hsu, L. C.; Liu, H.; Bankston, L. A.; Iimura, M.; Kagnoff, M. F.; Eckmann, L.; Karin,
599　　　　　M., Nod2 mutation in Crohn's disease potentiates NF-kappaB activity and IL-1beta
600　　　　　processing. *Science (New York, N.Y.)* **2005,** 307, (5710), 734-8.

601　　30.　Martinon, F.; Mayor, A.; Tschopp, J., The inflammasomes: guardians of the body. *Annual*
602　　　　　*review of immunology* **2009,** 27, 229-65.

603　　31.　Anand, P. K.; Malireddi, R. K.; Kanneganti, T. D., Role of the nlrp3 inflammasome in
604　　　　　microbial infection. *Frontiers in microbiology* **2011,** 2, 12.

605　　32.　Munoz-Planillo, R.; Kuffa, P.; Martinez-Colon, G.; Smith, B. L.; Rajendiran, T. M.; Nunez, G.,
606　　　　　K(+) efflux is the common trigger of NLRP3 inflammasome activation by bacterial toxins and
607　　　　　particulate matter. *Immunity* **2013,** 38, (6), 1142-53.

608　　33.　Zhou, R.; Tardivel, A.; Thorens, B.; Choi, I.; Tschopp, J., Thioredoxin-interacting protein links
609　　　　　oxidative stress to inflammasome activation. *Nature immunology* **2010,** 11, (2), 136-40.

34. Hornung, V.; Bauernfeind, F.; Halle, A.; Samstad, E. O.; Kono, H.; Rock, K. L.; Fitzgerald, K. A.; Latz, E., Silica crystals and aluminum salts activate the NALP3 inflammasome through phagosomal destabilization. *Nature immunology* **2008,** 9, (8), 847-56.

35. Shimada, K.; Crother, T. R.; Karlin, J.; Dagvadorj, J.; Chiba, N.; Chen, S.; Ramanujan, V. K.; Wolf, A. J.; Vergnes, L.; Ojcius, D. M.; Rentsendorj, A.; Vargas, M.; Guerrero, C.; Wang, Y.; Fitzgerald, K. A.; Underhill, D. M.; Town, T.; Arditi, M., Oxidized mitochondrial DNA activates the NLRP3 inflammasome during apoptosis. *Immunity* **2012,** 36, (3), 401-14.

36. Nakahira, K.; Haspel, J. A.; Rathinam, V. A.; Lee, S. J.; Dolinay, T.; Lam, H. C.; Englert, J. A.; Rabinovitch, M.; Cernadas, M.; Kim, H. P.; Fitzgerald, K. A.; Ryter, S. W.; Choi, A. M., Autophagy proteins regulate innate immune responses by inhibiting the release of mitochondrial DNA mediated by the NALP3 inflammasome. *Nature immunology* **2011,** 12, (3), 222-30.

37. Iyer, S. S.; He, Q.; Janczy, J. R.; Elliott, E. I.; Zhong, Z.; Olivier, A. K.; Sadler, J. J.; Knepper-Adrian, V.; Han, R.; Qiao, L.; Eisenbarth, S. C.; Nauseef, W. M.; Cassel, S. L.; Sutterwala, F. S., Mitochondrial cardiolipin is required for Nlrp3 inflammasome activation. *Immunity* **2013,** 39, (2), 311-23.

38. Misawa, T.; Takahama, M.; Kozaki, T.; Lee, H.; Zou, J.; Saitoh, T.; Akira, S., Microtubule-driven spatial arrangement of mitochondria promotes activation of the NLRP3 inflammasome. *Nature immunology* **2013,** 14, (5), 454-60.

39. Subramanian, N.; Natarajan, K.; Clatworthy, M. R.; Wang, Z.; Germain, R. N., The adaptor MAVS promotes NLRP3 mitochondrial localization and inflammasome activation. *Cell* **2013,** 153, (2), 348-61.

40. Bauernfeind, F. G.; Horvath, G.; Stutz, A.; Alnemri, E. S.; MacDonald, K.; Speert, D.; Fernandes-Alnemri, T.; Wu, J.; Monks, B. G.; Fitzgerald, K. A.; Hornung, V.; Latz, E., Cutting edge: NF-kappaB activating pattern recognition and cytokine receptors license NLRP3 inflammasome activation by regulating NLRP3 expression. *Journal of immunology* **2009,** 183, (2), 787-91.

41. Franchi, L.; Eigenbrod, T.; Nunez, G., Cutting edge: TNF-alpha mediates sensitization to ATP and silica via the NLRP3 inflammasome in the absence of microbial stimulation. *Journal of immunology* **2009,** 183, (2), 792-6.

42. Gombault, A.; Baron, L.; Couillin, I., ATP release and purinergic signaling in NLRP3 inflammasome activation. *Frontiers in immunology* **2012,** 3, 414.

43. Shenoy, A. R.; Wellington, D. A.; Kumar, P.; Kassa, H.; Booth, C. J.; Cresswell, P.; MacMicking, J. D., GBP5 promotes NLRP3 inflammasome assembly and immunity in mammals. *Science (New York, N.Y.)* **2012,** 336, (6080), 481-5.

44. Martinon, F.; Petrilli, V.; Mayor, A.; Tardivel, A.; Tschopp, J., Gout-associated uric acid crystals activate the NALP3 inflammasome. *Nature* **2006,** 440, (7081), 237-41.

45. Rajamaki, K.; Lappalainen, J.; Oorni, K.; Valimaki, E.; Matikainen, S.; Kovanen, P. T.; Eklund, K. K., Cholesterol crystals activate the NLRP3 inflammasome in human macrophages: a novel link between cholesterol metabolism and inflammation. *PloS one* **2010,** 5, (7), e11765.

46. Halle, A.; Hornung, V.; Petzold, G. C.; Stewart, C. R.; Monks, B. G.; Reinheckel, T.; Fitzgerald, K. A.; Latz, E.; Moore, K. J.; Golenbock, D. T., The NALP3 inflammasome is involved in the innate immune response to amyloid-beta. *Nature immunology* **2008,** 9, (8), 857-65.

653   47.   Duncan, J. A.; Gao, X.; Huang, M. T.; O'Connor, B. P.; Thomas, C. E.; Willingham, S. B.;
654   Bergstralh, D. T.; Jarvis, G. A.; Sparling, P. F.; Ting, J. P., Neisseria gonorrhoeae activates the
655   proteinase cathepsin B to mediate the signaling activities of the NLRP3 and ASC-containing
656   inflammasome. *Journal of immunology* **2009,** 182, (10), 6460-9.

657   48.   Dostert, C.; Guarda, G.; Romero, J. F.; Menu, P.; Gross, O.; Tardivel, A.; Suva, M. L.; Stehle, J.
658   C.; Kopf, M.; Stamenkovic, I.; Corradin, G.; Tschopp, J., Malarial hemozoin is a Nalp3
659   inflammasome activating danger signal. *PloS one* **2009,** 4, (8), e6510.

660   49.   Sena, L. A.; Chandel, N. S., Physiological roles of mitochondrial reactive oxygen species.
661   *Molecular cell* **2012,** 48, (2), 158-67.

662   50.   Zhou, R.; Yazdi, A. S.; Menu, P.; Tschopp, J., A role for mitochondria in NLRP3
663   inflammasome activation. *Nature* **2011,** 469, (7329), 221-5.

664   51.   Petrilli, V.; Papin, S.; Dostert, C.; Mayor, A.; Martinon, F.; Tschopp, J., Activation of the
665   NALP3 inflammasome is triggered by low intracellular potassium concentration. *Cell death*
666   *and differentiation* **2007,** 14, (9), 1583-9.

667   52.   Dostert, C.; Petrilli, V.; Van Bruggen, R.; Steele, C.; Mossman, B. T.; Tschopp, J., Innate
668   immune activation through Nalp3 inflammasome sensing of asbestos and silica. *Science (New*
669   *York, N.Y.)* **2008,** 320, (5876), 674-7.

670   53.   Kawai, T.; Takahashi, K.; Sato, S.; Coban, C.; Kumar, H.; Kato, H.; Ishii, K. J.; Takeuchi, O.;
671   Akira, S., IPS-1, an adaptor triggering RIG-I- and Mda5-mediated type I interferon induction.
672   *Nature immunology* **2005,** 6, (10), 981-8.

673   54.   Li, X. D.; Chiu, Y. H.; Ismail, A. S.; Behrendt, C. L.; Wight-Carter, M.; Hooper, L. V.; Chen, Z.
674   J., Mitochondrial antiviral signaling protein (MAVS) monitors commensal bacteria and
675   induces an immune response that prevents experimental colitis. *Proceedings of the National*
676   *Academy of Sciences of the United States of America* **2011,** 108, (42), 17390-5.

677   55.   Lamkanfi, M.; Mueller, J. L.; Vitari, A. C.; Misaghi, S.; Fedorova, A.; Deshayes, K.; Lee, W. P.;
678   Hoffman, H. M.; Dixit, V. M., Glyburide inhibits the Cryopyrin/Nalp3 inflammasome. *The*
679   *Journal of cell biology* **2009,** 187, (1), 61-70.

680   56.   Bauernfeind, F.; Ablasser, A.; Bartok, E.; Kim, S.; Schmid-Burgk, J.; Cavlar, T.; Hornung, V.,
681   Inflammasomes: current understanding and open questions. *Cellular and molecular life sciences*
682   *: CMLS* **2011,** 68, (5), 765-83.

683   57.   Franchi, L.; Kanneganti, T. D.; Dubyak, G. R.; Nunez, G., Differential requirement of P2X7
684   receptor and intracellular K+ for caspase-1 activation induced by intracellular and
685   extracellular bacteria. *The Journal of biological chemistry* **2007,** 282, (26), 18810-8.

686   58.   Harder, J.; Franchi, L.; Munoz-Planillo, R.; Park, J. H.; Reimer, T.; Nunez, G., Activation of
687   the Nlrp3 inflammasome by Streptococcus pyogenes requires streptolysin O and NF-kappa
688   B activation but proceeds independently of TLR signaling and P2X7 receptor. *Journal of*
689   *immunology* **2009,** 183, (9), 5823-9.

690   59.   Franchi, L.; Amer, A.; Body-Malapel, M.; Kanneganti, T. D.; Ozoren, N.; Jagirdar, R.; Inohara,
691   N.; Vandenabeele, P.; Bertin, J.; Coyle, A.; Grant, E. P.; Nunez, G., Cytosolic flagellin requires
692   Ipaf for activation of caspase-1 and interleukin 1beta in salmonella-infected macrophages.
693   *Nature immunology* **2006,** 7, (6), 576-82.

694   60.   Miao, E. A.; Alpuche-Aranda, C. M.; Dors, M.; Clark, A. E.; Bader, M. W.; Miller, S. I.;
695   Aderem, A., Cytoplasmic flagellin activates caspase-1 and secretion of interleukin 1beta via
696   Ipaf. *Nature immunology* **2006,** 7, (6), 569-75.

697   61.   Miao, E. A.; Mao, D. P.; Yudkovsky, N.; Bonneau, R.; Lorang, C. G.; Warren, S. E.; Leaf, I. A.;
698   Aderem, A., Innate immune detection of the type III secretion apparatus through the NLRC4
699   inflammasome. *Proceedings of the National Academy of Sciences of the United States of America*
700   **2010,** 107, (7), 3076-80.

701   62.   Yang, J.; Zhao, Y.; Shi, J.; Shao, F., Human NAIP and mouse NAIP1 recognize bacterial type
702   III secretion needle protein for inflammasome activation. *Proceedings of the National Academy*
703   *of Sciences of the United States of America* **2013,** 110, (35), 14408-13.

704   63.   Wu, J.; Fernandes-Alnemri, T.; Alnemri, E. S., Involvement of the AIM2, NLRC4, and NLRP3
705   inflammasomes in caspase-1 activation by Listeria monocytogenes. *Journal of clinical*
706   *immunology* **2010,** 30, (5), 693-702.

707   64.   Zhao, Y.; Yang, J.; Shi, J.; Gong, Y. N.; Lu, Q.; Xu, H.; Liu, L.; Shao, F., The NLRC4
708   inflammasome receptors for bacterial flagellin and type III secretion apparatus. *Nature* **2011,**
709   477, (7366), 596-600.

710   65.   Fernandes-Alnemri, T.; Yu, J. W.; Datta, P.; Wu, J.; Alnemri, E. S., AIM2 activates the
711   inflammasome and cell death in response to cytoplasmic DNA. *Nature* **2009,** 458, (7237), 509-
712   13.

713   66.   Hornung, V.; Ablasser, A.; Charrel-Dennis, M.; Bauernfeind, F.; Horvath, G.; Caffrey, D. R.;
714   Latz, E.; Fitzgerald, K. A., AIM2 recognizes cytosolic dsDNA and forms a caspase-1-
715   activating inflammasome with ASC. *Nature* **2009,** 458, (7237), 514-8.

716   67.   Chu, X.; Chen, W.; Li, N.; Hu, X. Z.; Du, C. T.; Yu, S. X.; Zhou, M.; Zhang, X. J.; Jiang, G. M.;
717   Han, W. Y.; Deng, X. M.; Yang, Y. J., Cytosolic double-stranded DNA induces nonnecroptotic
718   programmed cell death in trophoblasts via IFI16. *The Journal of infectious diseases* **2014,** 210,
719   (9), 1476-86.

720   68.   Jin, T.; Perry, A.; Jiang, J.; Smith, P.; Curry, J. A.; Unterholzner, L.; Jiang, Z.; Horvath, G.;
721   Rathinam, V. A.; Johnstone, R. W.; Hornung, V.; Latz, E.; Bowie, A. G.; Fitzgerald, K. A.; Xiao,
722   T. S., Structures of the HIN domain:DNA complexes reveal ligand binding and activation
723   mechanisms of the AIM2 inflammasome and IFI16 receptor. *Immunity* **2012,** 36, (4), 561-71.

724   69.   Muruve, D. A.; Petrilli, V.; Zaiss, A. K.; White, L. R.; Clark, S. A.; Ross, P. J.; Parks, R. J.;
725   Tschopp, J., The inflammasome recognizes cytosolic microbial and host DNA and triggers an
726   innate immune response. *Nature* **2008,** 452, (7183), 103-7.

727   70.   Fernandes-Alnemri, T.; Yu, J. W.; Juliana, C.; Solorzano, L.; Kang, S.; Wu, J.; Datta, P.;
728   McCormick, M.; Huang, L.; McDermott, E.; Eisenlohr, L.; Landel, C. P.; Alnemri, E. S., The
729   AIM2 inflammasome is critical for innate immunity to Francisella tularensis. *Nature*
730   *immunology* **2010,** 11, (5), 385-93.

731   71.   Jones, J. W.; Kayagaki, N.; Broz, P.; Henry, T.; Newton, K.; O'Rourke, K.; Chan, S.; Dong, J.;
732   Qu, Y.; Roose-Girma, M.; Dixit, V. M.; Monack, D. M., Absent in melanoma 2 is required for
733   innate immune recognition of Francisella tularensis. *Proceedings of the National Academy of*
734   *Sciences of the United States of America* **2010,** 107, (21), 9771-6.

735   72.   Rathinam, V. A.; Jiang, Z.; Waggoner, S. N.; Sharma, S.; Cole, L. E.; Waggoner, L.; Vanaja, S.
736   K.; Monks, B. G.; Ganesan, S.; Latz, E.; Hornung, V.; Vogel, S. N.; Szomolanyi-Tsuda, E.;

Fitzgerald, K. A., The AIM2 inflammasome is essential for host defense against cytosolic bacteria and DNA viruses. *Nature immunology* **2010,** 11, (5), 395-402.

73. Chen, G. Y., Role of Nlrp6 and Nlrp12 in the maintenance of intestinal homeostasis. *European journal of immunology* **2014,** 44, (2), 321-7.

74. Chen, G. Y.; Liu, M.; Wang, F.; Bertin, J.; Nunez, G., A functional role for Nlrp6 in intestinal inflammation and tumorigenesis. *Journal of immunology* **2011,** 186, (12), 7187-94.

75. Anand, P. K.; Malireddi, R. K.; Lukens, J. R.; Vogel, P.; Bertin, J.; Lamkanfi, M.; Kanneganti, T. D., NLRP6 negatively regulates innate immunity and host defence against bacterial pathogens. *Nature* **2012,** 488, (7411), 389-93.

76. Khare, S.; Dorfleutner, A.; Bryan, N. B.; Yun, C.; Radian, A. D.; de Almeida, L.; Rojanasakul, Y.; Stehlik, C., An NLRP7-containing inflammasome mediates recognition of microbial lipopeptides in human macrophages. *Immunity* **2012,** 36, (3), 464-76.

77. Guarda, G.; So, A., Regulation of inflammasome activity. *Immunology* **2010,** 130, (3), 329-36.

78. Martinon, F.; Tschopp, J., Inflammatory caspases and inflammasomes: master switches of inflammation. *Cell death and differentiation* **2007,** 14, (1), 10-22.

79. van de Veerdonk, F. L.; Netea, M. G.; Dinarello, C. A.; Joosten, L. A., Inflammasome activation and IL-1beta and IL-18 processing during infection. *Trends in immunology* **2011,** 32, (3), 110-6.

80. Mayor, A.; Martinon, F.; De Smedt, T.; Petrilli, V.; Tschopp, J., A crucial function of SGT1 and HSP90 in inflammasome activity links mammalian and plant innate immune responses. *Nature immunology* **2007,** 8, (5), 497-503.

81. Py, B. F.; Kim, M. S.; Vakifahmetoglu-Norberg, H.; Yuan, J., Deubiquitination of NLRP3 by BRCC3 critically regulates inflammasome activity. *Molecular cell* **2013,** 49, (2), 331-8.

82. Qu, Y.; Misaghi, S.; Izrael-Tomasevic, A.; Newton, K.; Gilmour, L. L.; Lamkanfi, M.; Louie, S.; Kayagaki, N.; Liu, J.; Komuves, L.; Cupp, J. E.; Arnott, D.; Monack, D.; Dixit, V. M., Phosphorylation of NLRC4 is critical for inflammasome activation. *Nature* **2012,** 490, (7421), 539-42.

83. Bruey, J. M.; Bruey-Sedano, N.; Luciano, F.; Zhai, D.; Balpai, R.; Xu, C.; Kress, C. L.; Bailly-Maitre, B.; Li, X.; Osterman, A.; Matsuzawa, S.; Terskikh, A. V.; Faustin, B.; Reed, J. C., Bcl-2 and Bcl-XL regulate proinflammatory caspase-1 activation by interaction with NALP1. *Cell* **2007,** 129, (1), 45-56.

84. Faustin, B.; Chen, Y.; Zhai, D.; Le Negrate, G.; Lartigue, L.; Satterthwait, A.; Reed, J. C., Mechanism of Bcl-2 and Bcl-X(L) inhibition of NLRP1 inflammasome: loop domain-dependent suppression of ATP binding and oligomerization. *Proceedings of the National Academy of Sciences of the United States of America* **2009,** 106, (10), 3935-40.

85. Arlehamn, C. S.; Petrilli, V.; Gross, O.; Tschopp, J.; Evans, T. J., The role of potassium in inflammasome activation by bacteria. *The Journal of biological chemistry* **2010,** 285, (14), 10508-18.

86. da Cunha, J. P.; Galante, P. A.; de Souza, S. J., Different evolutionary strategies for the origin of caspase-1 inhibitors. *Journal of molecular evolution* **2008,** 66, (6), 591-7.

87. Stehlik, C.; Krajewska, M.; Welsh, K.; Krajewski, S.; Godzik, A.; Reed, J. C., The PAAD/PYRIN-only protein POP1/ASC2 is a modulator of ASC-mediated nuclear-factor-kappa B and pro-caspase-1 regulation. *The Biochemical journal* **2003,** 373, (Pt 1), 101-13.

780 88. Bedoya, F.; Sandler, L. L.; Harton, J. A., Pyrin-only protein 2 modulates NF-kappaB and
781   disrupts ASC:CLR interactions. *Journal of immunology* **2007,** 178, (6), 3837-45.

782 89. Stehlik, C.; Dorfleutner, A., COPs and POPs: modulators of inflammasome activity. *Journal*
783   *of immunology* **2007,** 179, (12), 7993-8.

784 90. Lamkanfi, M.; Denecker, G.; Kalai, M.; D'Hondt, K.; Meeus, A.; Declercq, W.; Saelens, X.;
785   Vandenabeele, P., INCA, a novel human caspase recruitment domain protein that inhibits
786   interleukin-1beta generation. *The Journal of biological chemistry* **2004,** 279, (50), 51729-38.

787 91. Guarda, G.; Braun, M.; Staehli, F.; Tardivel, A.; Mattmann, C.; Forster, I.; Farlik, M.; Decker,
788   T.; Du Pasquier, R. A.; Romero, P.; Tschopp, J., Type I interferon inhibits interleukin-1
789   production and inflammasome activation. *Immunity* **2011,** 34, (2), 213-23.

790 92. Guarda, G.; Dostert, C.; Staehli, F.; Cabalzar, K.; Castillo, R.; Tardivel, A.; Schneider, P.;
791   Tschopp, J., T cells dampen innate immune responses through inhibition of NLRP1 and
792   NLRP3 inflammasomes. *Nature* **2009,** 460, (7252), 269-73.

793 93. Allen, I. C.; Wilson, J. E.; Schneider, M.; Lich, J. D.; Roberts, R. A.; Arthur, J. C.; Woodford, R.
794   M.; Davis, B. K.; Uronis, J. M.; Herfarth, H. H.; Jobin, C.; Rogers, A. B.; Ting, J. P., NLRP12
795   suppresses colon inflammation and tumorigenesis through the negative regulation of
796   noncanonical NF-kappaB signaling. *Immunity* **2012,** 36, (5), 742-54.

797 94. Imamura, R.; Wang, Y.; Kinoshita, T.; Suzuki, M.; Noda, T.; Sagara, J.; Taniguchi, S.;
798   Okamoto, H.; Suda, T., Anti-inflammatory activity of PYNOD and its mechanism in humans
799   and mice. *Journal of immunology* **2010,** 184, (10), 5874-84.

800 95. Wang, Y.; Hasegawa, M.; Imamura, R.; Kinoshita, T.; Kondo, C.; Konaka, K.; Suda, T.,
801   PYNOD, a novel Apaf-1/CED4-like protein is an inhibitor of ASC and caspase-1. *International*
802   *immunology* **2004,** 16, (6), 777-86.

803 96. Kinoshita, T.; Wang, Y.; Hasegawa, M.; Imamura, R.; Suda, T., PYPAF3, a PYRIN-containing
804   APAF-1-like protein, is a feedback regulator of caspase-1-dependent interleukin-1beta
805   secretion. *The Journal of biological chemistry* **2005,** 280, (23), 21720-5.

806 97. Messaed, C.; Akoury, E.; Djuric, U.; Zeng, J.; Saleh, M.; Gilbert, L.; Seoud, M.; Qureshi, S.;
807   Slim, R., NLRP7, a nucleotide oligomerization domain-like receptor protein, is required for
808   normal cytokine secretion and co-localizes with Golgi and the microtubule-organizing
809   center. *The Journal of biological chemistry* **2011,** 286, (50), 43313-23.

810 98. Dupaul-Chicoine, J.; Yeretssian, G.; Doiron, K.; Bergstrom, K. S.; McIntire, C. R.; LeBlanc, P.
811   M.; Meunier, C.; Turbide, C.; Gros, P.; Beauchemin, N.; Vallance, B. A.; Saleh, M., Control of
812   intestinal homeostasis, colitis, and colitis-associated colorectal cancer by the inflammatory
813   caspases. *Immunity* **2010,** 32, (3), 367-78.

814 99. Li, P.; Allen, H.; Banerjee, S.; Franklin, S.; Herzog, L.; Johnston, C.; McDowell, J.; Paskind, M.;
815   Rodman, L.; Salfeld, J.; et al., Mice deficient in IL-1 beta-converting enzyme are defective in
816   production of mature IL-1 beta and resistant to endotoxic shock. *Cell* **1995,** 80, (3), 401-11.

817 100. Bauer, C.; Duewell, P.; Mayer, C.; Lehr, H. A.; Fitzgerald, K. A.; Dauer, M.; Tschopp, J.;
818   Endres, S.; Latz, E.; Schnurr, M., Colitis induced in mice with dextran sulfate sodium (DSS)
819   is mediated by the NLRP3 inflammasome. *Gut* **2010,** 59, (9), 1192-9.

820 101. Kuida, K.; Lippke, J. A.; Ku, G.; Harding, M. W.; Livingston, D. J.; Su, M. S.; Flavell, R. A.,
821   Altered cytokine export and apoptosis in mice deficient in interleukin-1 beta converting
822   enzyme. *Science (New York, N.Y.)* **1995,** 267, (5206), 2000-3.

823   102.   Zaki, M. H.; Boyd, K. L.; Vogel, P.; Kastan, M. B.; Lamkanfi, M.; Kanneganti, T. D., The NLRP3
824   inflammasome protects against loss of epithelial integrity and mortality during experimental
825   colitis. *Immunity* **2010,** 32, (3), 379-91.

826   103.   Hirota, S. A.; Ng, J.; Lueng, A.; Khajah, M.; Parhar, K.; Li, Y.; Lam, V.; Potentier, M. S.; Ng,
827   K.; Bawa, M.; McCafferty, D. M.; Rioux, K. P.; Ghosh, S.; Xavier, R. J.; Colgan, S. P.; Tschopp,
828   J.; Muruve, D.; MacDonald, J. A.; Beck, P. L., NLRP3 inflammasome plays a key role in the
829   regulation of intestinal homeostasis. *Inflammatory bowel diseases* **2011,** 17, (6), 1359-72.

830   104.   Allen, I. C.; TeKippe, E. M.; Woodford, R. M.; Uronis, J. M.; Holl, E. K.; Rogers, A. B.; Herfarth,
831   H. H.; Jobin, C.; Ting, J. P., The NLRP3 inflammasome functions as a negative regulator of
832   tumorigenesis during colitis-associated cancer. *The Journal of experimental medicine* **2010,** 207,
833   (5), 1045-56.

834   105.   Elinav, E.; Strowig, T.; Kau, A. L.; Henao-Mejia, J.; Thaiss, C. A.; Booth, C. J.; Peaper, D. R.;
835   Bertin, J.; Eisenbarth, S. C.; Gordon, J. I.; Flavell, R. A., NLRP6 inflammasome regulates
836   colonic microbial ecology and risk for colitis. *Cell* **2011,** 145, (5), 745-57.

837   106.   Wlodarska, M.; Thaiss, C. A.; Nowarski, R.; Henao-Mejia, J.; Zhang, J. P.; Brown, E. M.;
838   Frankel, G.; Levy, M.; Katz, M. N.; Philbrick, W. M.; Elinav, E.; Finlay, B. B.; Flavell, R. A.,
839   NLRP6 inflammasome orchestrates the colonic host-microbial interface by regulating goblet
840   cell mucus secretion. *Cell* **2014,** 156, (5), 1045-59.

841   107.   Swidsinski, A.; Loening-Baucke, V.; Vaneechoutte, M.; Doerffel, Y., Active Crohn's disease
842   and ulcerative colitis can be specifically diagnosed and monitored based on the biostructure
843   of the fecal flora. *Inflammatory bowel diseases* **2008,** 14, (2), 147-61.

844   108.   Frank, D. N.; St Amand, A. L.; Feldman, R. A.; Boedeker, E. C.; Harpaz, N.; Pace, N. R.,
845   Molecular-phylogenetic characterization of microbial community imbalances in human
846   inflammatory bowel diseases. *Proceedings of the National Academy of Sciences of the United States
847   of America* **2007,** 104, (34), 13780-5.

848   109.   Willing, B. P.; Dicksved, J.; Halfvarson, J.; Andersson, A. F.; Lucio, M.; Zheng, Z.; Jarnerot,
849   G.; Tysk, C.; Jansson, J. K.; Engstrand, L., A pyrosequencing study in twins shows that
850   gastrointestinal microbial profiles vary with inflammatory bowel disease phenotypes.
851   *Gastroenterology* **2010,** 139, (6), 1844-1854 e1.

852   110.   Martin, H. M.; Campbell, B. J.; Hart, C. A.; Mpofu, C.; Nayar, M.; Singh, R.; Englyst, H.;
853   Williams, H. F.; Rhodes, J. M., Enhanced Escherichia coli adherence and invasion in Crohn's
854   disease and colon cancer. *Gastroenterology* **2004,** 127, (1), 80-93.

855   111.   Neut, C.; Bulois, P.; Desreumaux, P.; Membre, J. M.; Lederman, E.; Gambiez, L.; Cortot, A.;
856   Quandalle, P.; van Kruiningen, H.; Colombel, J. F., Changes in the bacterial flora of the
857   neoterminal ileum after ileocolonic resection for Crohn's disease. *The American journal of
858   gastroenterology* **2002,** 97, (4), 939-46.

859   112.   Christophi, G. P.; Rong, R.; Holtzapple, P. G.; Massa, P. T.; Landas, S. K., Immune markers
860   and differential signaling networks in ulcerative colitis and Crohn's disease. *Inflammatory
861   bowel diseases* **2012,** 18, (12), 2342-56.

862   113.   McAlindon, M. E.; Hawkey, C. J.; Mahida, Y. R., Expression of interleukin 1 beta and
863   interleukin 1 beta converting enzyme by intestinal macrophages in health and inflammatory
864   bowel disease. *Gut* **1998,** 42, (2), 214-9.

865   114.   Vanhove, W.; Peeters, P. M.; Staelens, D.; Schraenen, A.; Van der Goten, J.; Cleynen, I.; De
866   Schepper, S.; Van Lommel, L.; Reynaert, N. L.; Schuit, F.; Van Assche, G.; Ferrante, M.; De
867   Hertogh, G.; Wouters, E. F.; Rutgeerts, P.; Vermeire, S.; Nys, K.; Arijs, I., Strong Upregulation
868   of AIM2 and IFI16 Inflammasomes in the Mucosa of Patients with Active Inflammatory
869   Bowel Disease. *Inflammatory bowel diseases* **2015**.
870   115.   Coll, R. C.; Robertson, A. A.; Chae, J. J.; Higgins, S. C.; Munoz-Planillo, R.; Inserra, M. C.;
871   Vetter, I.; Dungan, L. S.; Monks, B. G.; Stutz, A.; Croker, D. E.; Butler, M. S.; Haneklaus, M.;
872   Sutton, C. E.; Nunez, G.; Latz, E.; Kastner, D. L.; Mills, K. H.; Masters, S. L.; Schroder, K.;
873   Cooper, M. A.; O'Neill, L. A., A small-molecule inhibitor of the NLRP3 inflammasome for the
874   treatment of inflammatory diseases. *Nature medicine* **2015,** 21, (3), 248-55.
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877