Inflammasomes in the CNS

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Abstract | Microglia and macrophages in the CNS contain multimolecular complexes termed inflammasomes. Inflammasomes function as intracellular sensors for infectious agents as well as for host-derived danger signals that are associated with neurological diseases, including meningitis, stroke and Alzheimer's disease. Assembly of an inflammasome activates caspase 1 and, subsequently, the proteolysis and release of the cytokines interleukin-1β and interleukin-18, as well as pyroptotic cell death. Since the discovery of inflammasomes in 2002, there has been burgeoning recognition of their complexities and functions. Here, we review the current understanding of the functions of different inflammasomes in the CNS and their roles in neurological diseases.

Mononuclear phagocytic cells
A family of non-lymphocyte immune cells, including monocytes, macrophages, microglia and dendritic cells.

Pathogen-associated molecular patterns
Specific elements that are produced by microorganisms and that can induce innate immune responses. These elements are recognized by specific receptors that are expressed at the surface of macrophages, dendritic cells and microglia.

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Over the past decade, there has been a surge in information regarding the composition and actions of the CNS's innate immune system, which includes microglia, trafficking macrophages and astrocytes[1]. The interactions between innate immune cells and infiltrating adaptive immune cells (T and B lymphocytes) in the CNS have also become better understood in recent years, and this has prompted the recognition that each of these cell types contributes to the development of inflammation in the CNS — a highly orchestrated response by the immune system to infections or to non-infectious (sterile) disorders such as stroke, multiple sclerosis and neurodegenerative diseases[2,3] (BOX 1). Thus, inflammation in the CNS has both pathogenic and protective effects depending on the biological circumstances.

The initiation of the inflammatory response involves the recently discovered multiprotein complexes termed 'inflammasomes'. Tschopp and colleagues initially described the inflammasome concept in the early 2000s when they revealed a pivotal link between tissue injury, innate immune processes and caspase 1-dependent responses[4]. An inflammasome is a cytosolic, multiprotein platform that enables the activation of pro-inflammatory caspases, chiefly caspase 1 (REF. 5) (FIG. 1). This leads to the cleavage and release of pro-inflammatory cytokines and, consequently, to a potent inflammatory response. Thus, inflammasomes are essential protein complexes that direct the innate immune system's responses to pathogenic stimuli. Inflammasome activation has since been implicated in the mechanisms underlying infectious, immune and inflammatory processes.

Mechanistic insights into the functions of inflammasomes have largely come from studies of rodent mononuclear phagocytic cells such as macrophages and microglia, although the relevance of inflammasome signalling to human health was demonstrated in early studies by the existence of cryopyrin-associated periodic syndromes[6].

The growing interest in inflammasomes has prompted a concurrent appreciation of their role in neurological diseases. In this Review, we first describe structures and functions of different inflammasomes, concentrating on four of the best-understood complexes, and then discuss the major consequences of inflammasome activation in the CNS, highlighting specific inflammasome complexes in the pathogenesis of acute and chronic neurological diseases.

The inflammasomes

The innate immune system acts at the frontline of the broader immune response through the sensing of pathogen-associated molecular patterns and danger-associated molecular patterns on infectious agents or disease-associated host molecules by pattern-recognition receptors. In the CNS, pattern-recognition receptors are primarily expressed by microglia, macrophages and astrocytes. These receptors are either membrane-bound and sense extracellular or endosomally located signals (in this case, they are known as Toll-like receptors) or are located within the cytoplasm and sense intracellular signals (in which case they are known as NOD-like receptors (NLRs)). Only cytosolic receptors are involved in the formation of inflammasomes.

Inflammasome complexes generally have three main components: a cytosolic pattern-recognition receptor, the enzyme caspase 1 and an adaptor protein that facilitates the interaction between the two. The receptor is either a member of the NLR family of proteins or a member of the pyrin and HIN domain-containing

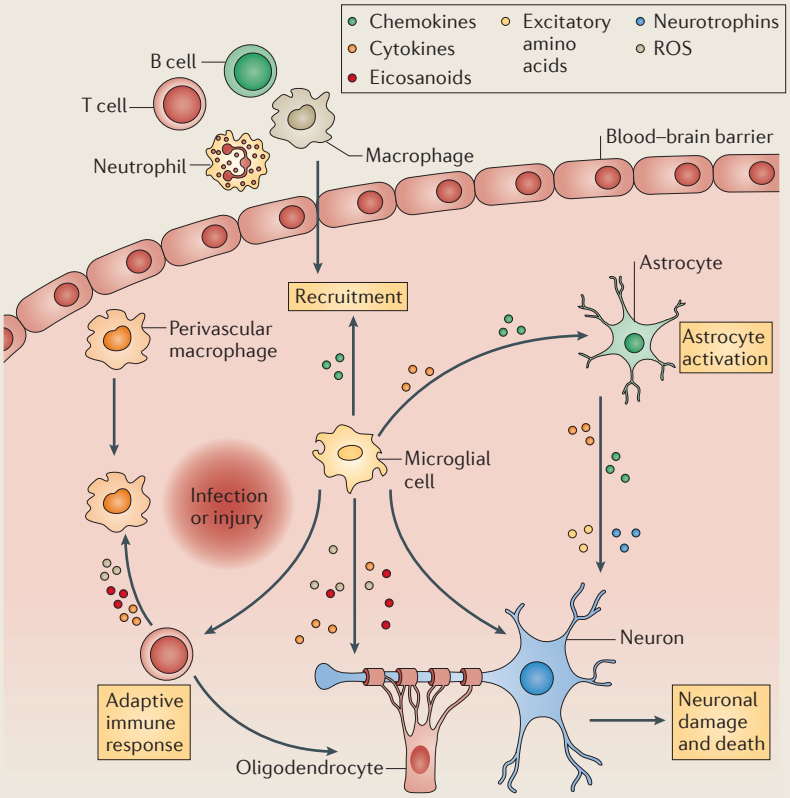
Danger-associated molecular patterns
(Also known as damage-associated molecular patterns.) Cellular molecules that are exposed after damage-induced necrosis. They are recognized by specialized receptors that are expressed at the surface of macrophages, dendritic cells and microglia.

Innate immunity
Immune responses based on the sensing of conserved pathogen or danger-associated molecular patterns (PAMPs or DAMPs, respectively) by germline-encoded pattern-recognition receptors without previous recognition or memory, unlike adaptive immunity.

Box 1 | Innate immune activation in the CNS

Innate immune activation in the CNS can be initiated locally and subsequently lead to alterations in the tissue microenvironment at almost every level of control, from gene expression and cellular differentiation to changes in cellular composition through the recruitment of blood-derived cells (see the figure). Microglia are the chief innate immune cells within the CNS, but they are complemented by CNS-derived macrophages that are located in the meninges, choroid plexus and perivascular space[139]. These cells constantly survey the proximal environment through the pattern-recognition receptors that they express (for example, Toll-like receptors and NOD-like receptors (NLRs)). When these cells sense tissue injury or a foreign (infectious) agent, a network of activation pathways is induced in microglia, resulting in an altered microglial morphology, intense respiratory metabolism and the expression and release of immune molecules (for example, cytokines, chemokines and reactive oxygen species (ROS))[2]. This response promotes the recruitment of peripheral innate immune cells (macrophages and neutrophils) and adaptive immune cells (T cells and B cells) to the site of CNS injury as well as further activation of nearby glial cells. At the site of injury, microglia with pathogenic phenotypes and microglia with reparative phenotypes are present[139]. Astrocytes are highly sensitive to the effects of proximal and remote immune cells. Indeed, astrogliosis (which is characterized by increased astrocyte proliferation and activation) is a hallmark of CNS inflammation. Activated astrocytes show many changes in their expression profile, including the upregulation of cytokines and chemokines[14]. The diversity of secreted factors from astrocytes — often with opposing functions — indicate that, as with microglial populations, a balanced response is required to achieve the most desired outcome (that is, a reduction of the inflammation and the initiation of tissue repair after elimination of the danger). Neurons can also contribute to innate immune responses by sensing stress or infections through intrinsic pattern-recognition receptors and subsequently releasing factors that recruit an inflammatory response[140].

Although the evolutionary function of innate immunity is to protect against effects of injury, innate immune responses can also promote immunopathology when they are excessive and through off-target actions. In the CNS, this can cause neuronal and oligodendrocyte dysfunction and cell death in some circumstances. Indeed, cytokines and ROS can modulate glutamate receptor function, which can have excitotoxic effects; similarly, astrocytes that are activated by cytokines can promote excitotoxicity through altered regulation of extracellular glutamate levels[1,2].



(PYHIN) family of proteins (see below). The NLRs are encoded by a family of 22 genes in humans and contain a carboxy-terminal leucine-rich repeat (LRR) domain, a conserved central NACHT domain (which is essential for the nucleotide binding and protein oligomerization that are required to form multiprotein inflammasome complexes) and a variable amino-terminal domain that defines several NLR subfamilies (FIG. 2). Members of the NLRP subfamily carry an N-terminal pyrin domain (PYD). NLRP1 and NLRP3 are the best studied of this group, but NLRP2, NLRP6 and NLRP12 can also form caspase 1-activating inflammasomes[5,7]. Another well-studied inflammasome-forming NLR is NLRC4, which contains an N-terminal caspase activation and recruitment domain (CARD).

Following activation and oligomerization, NLRPs recruit, via homotypic protein interactions, the adaptor apoptosis-associated speck-like protein containing a CARD (ASC)[8]. This is the second component of most inflammasomes. ASC, which is composed of a PYD and a CARD, acts as an adaptor between the PYD of the respective NLRP protein and the CARD of procaspase 1,

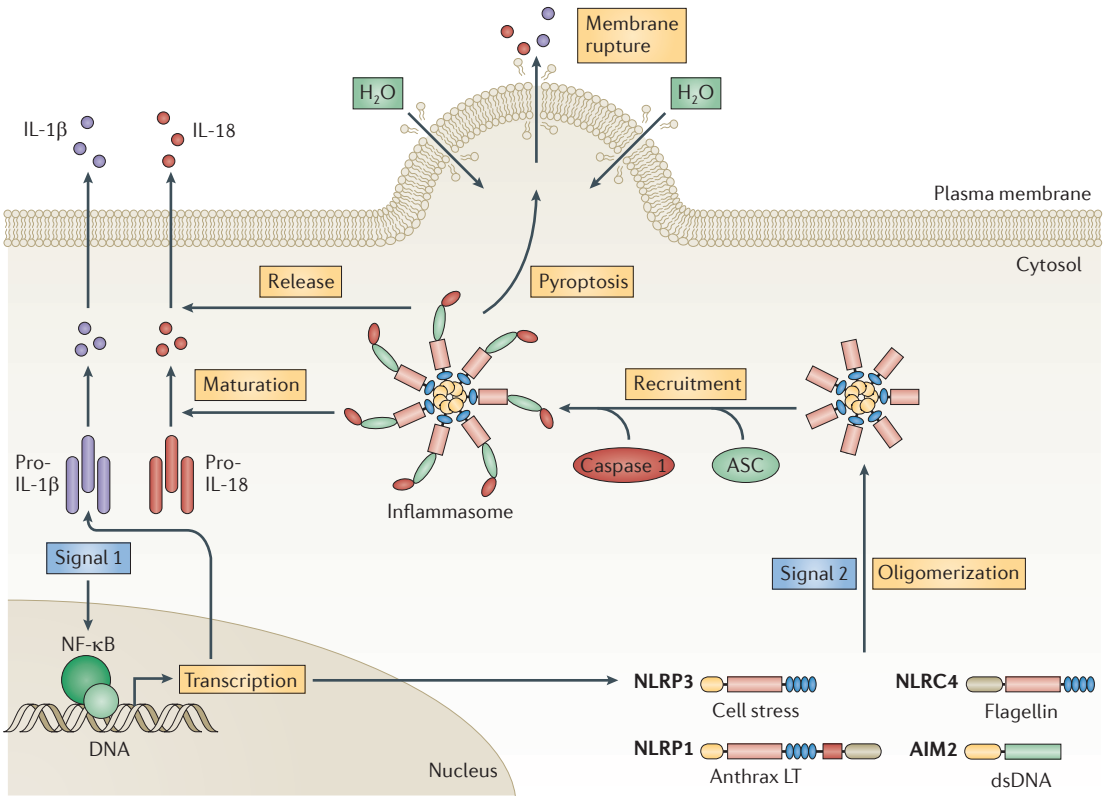


Figure 1 | **Inflammasome activation.** In response to individual pathogens or host-derived insults, a number of cytosolic sensors (that is, NOD-, LRR- and pyrin domain-containing 1 (NLRP1), NLRP3, NLRP5 (not shown), NOD-, LRR- and caspase activation and recruitment domain (CARD)-containing 4 (NLRC4) and absent in melanoma 2 (AIM2)) are capable of forming inflammasomes that mediate common downstream events. A priming stimulus (signal 1), acting through the nuclear factor-κB (NF-κB) pathway, often precedes assembly of the inflammasome complex in order to upregulate the expression of pro-interleukin-1β (pro-IL-1β) and NLRP3. Upon ligand sensing or enzymatic activation within the cytosol (signal 2), the cytosolic sensors oligomerize to form an activation platform for caspase 1. For some complexes, recruitment of caspase 1 also requires an additional adaptor protein, ASC (apoptosis-associated speck-like protein containing a CARD). Through its protease activity, caspase 1 regulates the maturation and release of IL-1β and IL-18 but also triggers pyroptotic cell death. For some inflammasome complexes, the direct activating stimulus is known, but for other complexes (that is, NLRP3) activation has been associated with a range of physiological stressors, including ion fluxes, endosomal rupture, reactive oxygen species or mitochondrial dysfunction. dsDNA, double-stranded DNA; LT, lethal toxin.

which is the third component of inflammasomes (FIG. 2). NLRC4 and NLRP1 are exceptions, as they can directly interact with procaspase 1 through their respective CARDs. However, ASC might still be required for optimal activation of these complexes[9,10].

The PYHIN-containing inflammasomes comprise absent in melanoma 2 (AIM2) and interferon-inducible protein 16 (IFI16). AIM2 and IFI16 are both composed of two major domains, a C-terminal DNA-binding HIN domain and an N-terminal PYD that mediates homotypic interactions with ASC and, subsequently, procaspase 1 (REF. 5).

The mechanisms underlying the activation of inflammasomes are an area of substantial discussion. The PYHIN inflammasomes are clearly activated by double-stranded DNA, but a clear ligand has not been identified for most of the NLR-forming inflammasomes. A number of recent reviews have been published on this subject[5,11].

It is thought that the recruitment of procaspase 1 into the inflammasome induces auto-proteolytic conversion of the pro-enzyme into active caspase 1. Activation of caspase 1 leads to the cleavage and subsequent release of interleukin-1β (IL-1β) and IL-18, primarily from innate immune cells. The CNS is particularly sensitive to IL-1β and IL-18 signalling because multiple neural cell types in the CNS express receptors for these cytokines[12,13]. The signalling cascades that are induced by cytokines have effects at a systemic level (that is, sickness behaviour and activation of the hypothalamus–pituitary–adrenal axis) as well as at the local site of infection or injury (that is, proliferation and activation of microglia and astrocytes)[12–14]. Caspase 1 activation and the subsequent cytokine cleavage and release promote immunopathogenic conditions that can lead to neuronal death. In addition to inducing cytokine release, caspase 1 activation can mediate, under some circumstances, a form of necrotic cell death known as pyroptosis, and there is evidence suggesting that caspase 1 has a direct role in initiating cell death pathways within neurons[15,16].

The focus of this Review is on the NLRC4, NLRP1, NLRP3 and AIM2 inflammasomes because they have garnered the most attention in neuroscience. The initial

Pyroptosis
A caspase 1-dependent form of programmed cell death that is characterized by necrosis-like swelling and rupture of the cell membrane.

Flagellin
The primary component protein of bacterial flagella.

PrgJ
A protein component of the Gram-negative type III secretion system (T3SS). The T3SS is a needle-like structure found on the bacterial surface, which enables the injection of bacterial proteins into host cells in order to facilitate infection.

characterization of specific inflammasome complexes was carried out in non-CNS cell types, but it has been reported that the NLRC4, NLRP1 and NLRP3 complexes are activated in microglia (see below), and many of their actions have been shown to be similar to those in non-CNS cells. NLRP1 has been a particular focus of studies that have addressed inflammasome function within neurons[17–19]. The AIM2 inflammasome has not yet been studied in the nervous system, but it warrants mention here because it is well characterized and because it is a prominent focus of contemporary inflammasome research. Moreover, AIM2 has been implicated in the response to *Listeria monocytogenes*, a causative agent of meningitis[20].

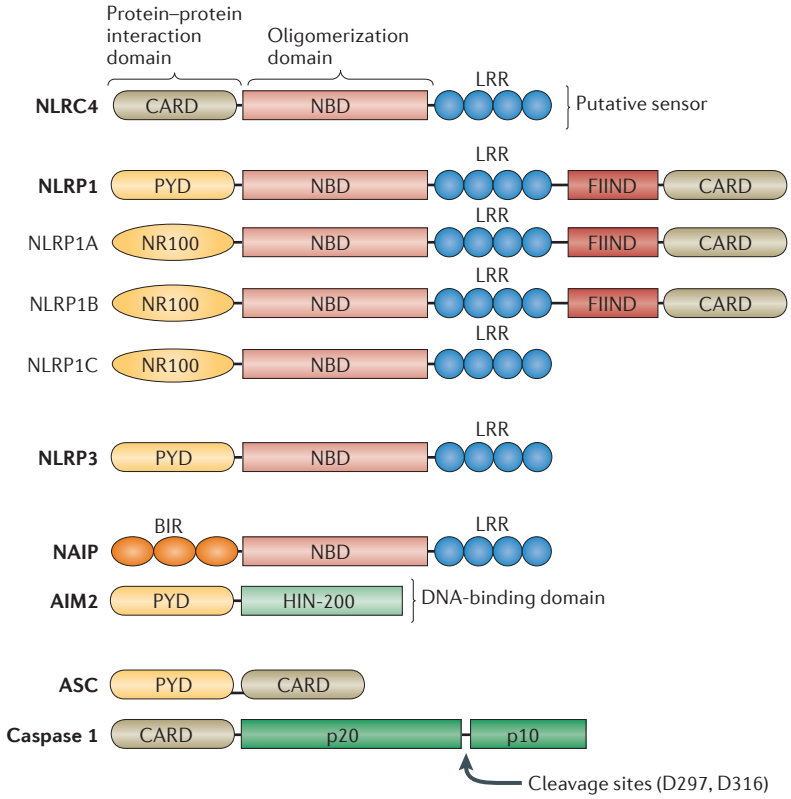


Figure 2 | **Inflammasome components and domain structure.** The activation and formation of inflammasome complexes is mediated through several protein domains. In NOD-like receptors (NLRs), the putative sensory component is formed by the carboxy-terminal leucine-rich repeat (LRR). Oligomerization of NLRs is mediated by the nucleotide-binding domain (NBD). The pyrin domain (PYD) mediates protein–protein interactions between the inflammasome sensor and the adaptor apoptosis-associated speck-like protein containing a caspase activation and recruitment domain (CARD) (ASC), which also contains a PYD. The CARD of ASC mediates protein–protein interactions with the CARD of procaspase 1. NOD-, LRR- and CARD-containing 4 (NLRC4) and NOD-, LRR- and PYD-containing 1 (NLRP1) can also directly interact with procaspase 1 through their respective CARDs. NLRP1 contains a unique function-to-find domain (FIIND), which is involved in inflammasome activation through auto-proteolysis. In the murine proteins NLRP1A, NLRP1B and NLRP1C, the amino-terminal PYD is replaced by an NR100 domain (amino-terminal domain of rodent NLRP1 of about 100 amino acids), which has no known homologue in humans. Neuronal apoptosis inhibitory proteins (NAIPs) are a subfamily of the NLRs and contain a baculovirus inhibitor of apoptosis repeat (BIR) domain. In absent in melanoma 2 (AIM2), the HIN-200 DNA- binding domain is the putative sensory component; the PYD in AIM2 mediates interactions with ASC.

The NLRC4 inflammasome. The NLRC4 inflammasome has been identified as a key sensor of bacteria that infect macrophages and related cells, including microglia[21,22]. Bacterial pathogens that are sensed by the NLRC4 inflammasome within microglia or macrophages and that are known to cause meningitis or encephalitis include *Legionella pneumophila* and *L. monocytogenes*[20,21]. The NLRC4 inflammasome senses both bacterial flagellin and PrgJ, a component of the bacterial type III secretion system[22,23]. Recent studies in mice have shown that neuronal apoptosis inhibitory proteins (NAIPs), a subfamily of the NLRs, are the direct sensors of flagellin or PrgJ; NAIP2 binds to PrgJ, and NAIP5 binds to flagellin[24,25]. These NAIPs can subsequently interact with NLRC4 and trigger inflammasome activation.

It should be noted that unlike in mice, only one NAIP-encoding gene exists in humans, and its role in NLRC4 activation is not fully understood[25,26]. Indeed, the human NAIP protein has been best characterized within the CNS as an inhibitor of apoptosis[27].

The NLRP1 inflammasome. NLRP1 was the first inflammasome to be characterized[4]; however, studies of this inflammasome have been hampered by issues such as the uncertainty as to the identity of a specific ligand, the complexity of its domain structure, possible enzymatic modification and the substantial divergence between human and mouse NLRP1. Human NLRP1 is unique among inflammasome-forming NLRs as it possesses two protein–protein interaction domains: an N-terminal PYD and a C-terminal CARD. Although ASC is not part of this inflammasome, it might enhance NLRP1 activation[9]. Mice have three *Nlrp1* genes (*Nlrp1a*, *Nlrp1b* and *Nlrp1c*). Instead of a PYD, murine NLRP1 proteins have a unique N-terminal domain for which there is no known homologue in humans[28,29]. A distinct feature of NLRP1 in both humans and mice is the presence of a function-to-find domain (FIIND) at the C-terminal end[4,30] (FIG. 2).

The bacterial metalloproteinase anthrax lethal toxin (LT) in *Bacillus anthracis* (which causes meningitis and sepsis in humans and in other species[31]) is an established activator of NLRP1 in rodents but not in humans[32]. Rat NLRP1 and mouse NLRP1B are activated by LT-mediated proteolysis of the N-terminal domain[33]. Activation of the NRLP1 inflammasome by LT triggers caspase 1-dependent cell death and cytokine release from mouse macrophages and bone marrow-derived (myeloid) dendritic cells[32]. However, this response is dependent on the mouse strain, and resistance or susceptibility to LT has been linked to allelic variants of the *Nlrp1b* gene[28].

The activation of human NLRP1 seems to require auto-proteolysis at the FIIND[30,34]. Interestingly, a naturally occurring splice variant of human NLRP1 fails to undergo auto-proteolysis at the FIIND region, which prevents subsequent maturation of IL-1β, whereas a single-nucleotide polymorphism (SNP) that alters this region enhances processing and activation of NLRP1 (REF. 30). This and other SNPs in the FIIND region have been linked to Crohn's disease and to increased susceptibility to bacterial meningitis[35], which suggests that the NLRP1 inflammasome has a role in these conditions.

When the NLRP1 inflammasome was first characterized, it was reported to be composed of NLRP1, ASC, caspase 1 and caspase 5 (REF. 4). Subsequent studies showed that the anti-apoptotic proteins B cell lymphoma-2 (BCL-2) and BCL-X_L could bind to and suppress the activation of NLRP1, suggesting that the NLRP1 inflammasome might be regulated by additional interactions outside those that are considered to make up the core complex[36]. This suggestion was reinforced by studies of the NLRP1 inflammasome in neurons, in which X-linked inhibitor of apoptosis protein (XIAP), P2X purinoreceptor 7 (P2X7) and the membrane channel pannexin 1 directly interacted with NLRP1 (REFS 17–19). In fact, the NLRP1 inflammasome in neurons seems to exist in a partially pre-assembled state; neuronal stress can promote both further association of its components and further proteolytic processing of caspase 1 (REFS 17–19).

The interaction of XIAP with the NLRP1 inflammasome complex in neurons is not fully understood, although the processing of XIAP would be expected to reduce its capacity to inhibit caspases[37]. Two recent studies have also implicated the IAPs more broadly (cIAP1, cIAP2 and XIAP) in the regulation of inflammasome activation in macrophages[38,39]. However, for reasons that are unclear, one of these studies reported that IAPs promote inflammasome activation[38], whereas the other reported that IAPs suppress it[39].

Extracellular ATP may be involved in the activation of inflammasomes; it binds to the P2X7 and thereby triggers a cation-channel-dependent K^+ efflux in macrophages[40]. This event has been particularly associated with NLRP3 activation (discussed below). In addition to triggering a K^+ efflux, extracellular ATP leads to the opening of a large membrane channel that is permeable to molecules that are up to 1 kD in size[40]. Studies using channel blockers have suggested that this large pore is formed by pannexin 1 and that the formation of the pannexin 1 channel is required for inflammasome activation, including the NLRP1 inflammasome in neurons[40,41]. Although the requirement for pannexin 1 in the activation of inflammasomes has not been supported by studies using pannexin-knockout cells or animals[42,43], recently identified problems with pannexin 1 knockouts will require these studies to be re-assessed[44]. It is also possible that pannexin 1 indirectly influences inflammasome function by amplifying the activating signal in some settings. Specifically, pannexin 1 channels can be activated by high extracellular K^+ levels and are known to mediate the release of intracellular ATP[45], which raises the possibility that pannexin 1 might be involved in sensing K^+ efflux or may amplify the external signals (that is, ATP) that are associated with inflammasome activation. Indeed, high extracellular K^+ levels have been reported to activate the NLRP1 inflammasome in neurons and to increase the expression of caspase 1 in astrocytes[19].

The existence of multiple mouse *Nlrp1* genes and the non-conservation of the response to anthrax LT leaves the relevance of mouse models of NLRP1 function to human diseases open to question. However, gene-association studies in humans have suggested that variations in the *NLRP1* sequence can underlie susceptibility to diseases such as bacterial meningitis and Alzheimer's disease[35,46]. In addition, studies in rats and in humans suggest that NLRP1 activation can be detrimental to the outcome of traumatic brain injury (TBI) or spinal cord injury[17,18].

The NLRP3 inflammasome. The NLRP3 inflammasome is the best-recognized and most widely implicated regulator of caspase 1 activation. Its N-terminal protein–protein interaction domain is a PYD, and it thus requires the adaptor ASC[6]. This complex is also noted for its broad array of activating stimuli, which include bacterial, fungal and viral components[47,48], endogenous danger signals such as extracellular ATP, amyloid-β and uric acid crystals[49–51], and environmental microparticles such as silica crystals[52].

NLRP3 activation is often described in terms of a two-step process requiring two signals. For example, many microbial Toll-like receptor ligands, such as lipopolysaccharide (LPS), have been shown to prime cells (signal 1) by inducing the transcription and translation of IL-1β and, in some cases, NLRP3 expression[49,50,53]. A secondary signal such as ATP (signal 2) is then required to trigger the formation of the inflammasome complex that leads to the activation of caspase 1 and the eventual cleavage and release of IL-1β (FIG. 1). This two-signal system is particularly robust in mice that receive LPS as the priming stimulus (that is, signal 1) and inflammasome activators as signal 2 (REFS 49,50). In human cells, this signal distinction is often less clear, perhaps because the priming stimulus might itself lead to the release of activators such as ATP[54,55]. The fact that various stimuli lead to NLRP3 activation suggests that the NLRP3 inflammasome acts as a general sensor of cellular damage or stress. Indeed, certain physiological events including ion fluxes, endosomal rupture, production of reactive oxygen species (ROS) and mitochondrial dysfunction have been repeatedly (although not universally) shown to trigger the activation of the NLRP3 inflammasome.

Ion fluxes, particularly K^+ efflux, occur in response to a number of NLRP3 activators. These include extracellular ATP and bacterial toxins that are capable of forming membrane pores[49,56]. Inflammasome activation can be inhibited by high extracellular cation concentrations[56], and recent evidence suggests that K^+ efflux is required to trigger NLRP3 activation in response to all known activators of the NLRP3 inflammasome[57]. In addition, two recent studies have reported that large water influxes into macrophages can activate NLRP3, although the studies drew separate conclusions about whether the lowering of intracellular K^+ was specifically required for this effect[58,59].

Endosomal rupture can mediate NLRP3 activation in response to large particulate matter such as silica, uric acid crystals and amyloid-β, as well as to viruses or bacterial toxins that disrupt endosomes[47,50–52,60,61]. The protease cathepsin B, which is released from damaged lysosomes, has been implicated in NLRP3 inflammasome activation, although it is important to keep in mind that the studies that suggest a role for cathepsin B in inflammasome activation primarily used cathepsin B

Crohn's disease
A chronic inflammatory disease of the intestines (especially the colon and ileum) that is associated with ulcers and fistulae.

Two-signal system
In reference to the two-step model of inflammasome activation: following an initial priming signal (signal 1) to induce expression of pro-interleukin-1β, a second signal (signal 2) is required to trigger formation of the inflammasome complex and activate caspase 1, which can then cleave interleukin-1β into its mature form before its eventual secretion.

inhibitors that might have off-target effects that could underlie the activation[47,50,52,60,62,63]. Endosomal rupture may also be an upstream trigger of K^+ efflux[57].

ROS production is consistently observed in the context of NLRP3 activation; indeed, several studies have used inhibitors of ROS production to block activation of this inflammasome[63–67]. ROS-sensitive thioredoxin-interacting protein (TXNIP) may bind and activate NLRP3 (REF. 66), but this proposal has been challenged by a subsequent study that found no evidence that NLRP3 activation requires TXNIP[68].

Damage to mitochondria leads to increased ROS production, and the accumulation of damaged mitochondria resulting from inhibition of autophagy has been reported to sensitize macrophages to ATP-dependent NLRP3 activation. Conversely, specifically blocking mitochondrial sources of ROS inhibited NLRP3 activation[69,70]. Two studies have also shown that NLRP3 activation requires the release of mitochondrial DNA into the cytoplasm[69,71]. In fact, the authors of one of these studies proposed that NLRP3 directly binds and senses oxidized mitochondrial DNA (in a way that may depend on ROS)[71]. These findings are of considerable interest because they imply a unified model of NLRP3 activation and link inflammasome activation to apoptosis, which also involves mitochondrial permeabilization. Investigations into the interactions between NLRP3 and mitochondria are continuing at a rapid pace; in a new twist, it was recently reported that when activated by non-crystalline stimuli, NLRP3 might physically associate with mitochondrial membranes to oligomerize into an inflammasome complex[72]. However, indicative of the ongoing debate within this area, another recent study has challenged the idea that mitochondrial dysfunction is required for NLRP3 activation[57].

The NLRP3 inflammasome is by far the most widely studied complex in neurological diseases (as well as outside the CNS). Most studies have focused on NLRP3 function in microglia or CNS macrophages, although NLRP3 has also been proposed to function in neurons[59,73]. Not surprisingly, most investigations of activation mechanisms of NLRP3 in CNS cells were modelled after studies performed in peripheral myeloid cells, and these investigations showed that many of the activation mechanisms described above —including ROS production, K^+ efflux and endosomal rupture — also apply to NLRP3 activation within microglia[50,62,67]. However, some aspects of inflammasome activation might differ between macrophages and microglia. For example, unlike in macrophages, LPS priming of IL-1β expression in mouse microglia might require caspase 8 activation[74].

The triggers of NLRP3 inflammasome activation within the CNS are in many cases unknown or are assumed based on studies of peripheral responses (for example, to a given infection). Molecules of specific relevance to neurological diseases that have been reported to activate this inflammasome in microglia and macrophages include amyloid-β, prion protein (PrP) and α-synuclein[50,63,75]. As a danger signal that is often released in the context of neurological damage, ATP is also thought to be a likely trigger for inflammasome activation in the CNS[62,76,77].

The importance of NLRP3 for human health is clearly demonstrated by the existence of cryopyrin-associated periodic syndromes, which arise from gain-of-function mutations in the *NLRP3* gene[6]. These syndromes are characterized by repeated episodes of systemic inflammation and also include neurological signs and symptoms such as fever, chronic (aseptic) meningitis and headache. One of the immediate benefits of linking these disorders to *NLRP3* genotypes has been the finding that many of the signs and symptoms can be treated with inhibitors of IL-1β signalling[78].

The AIM2 inflammasome. AIM2 is a cytosolic receptor that is essential for the sensing of double-stranded DNA[79]. It consists of two major domains, a C-terminal DNA-binding HIN domain and an N-terminal PYD that mediates homotypic interactions with ASC. Through its HIN domain, AIM2 binds and senses double-stranded DNA (largely virus- and bacterially derived) that is present in the cytoplasm[8]. Upon DNA binding, an auto-inhibitory interaction between the HIN domain and PYD is lifted, leading to the formation of a complex of AIM2 molecules binding along the DNA strand, and this results in the recruitment of ASC and caspase 1 (REF. 80). Such auto-inhibitory interactions that prevent oligomerization in the absence of stimulus sensing are thought to be a shared mechanism for many inflammasome-forming cytosolic sensors. The AIM2 inflammasome thus exemplifies the receptor–ligand-mediated model of inflammasome activation[8].

CNS expression of inflammasome components

The highest expression of inflammasome components — NLRs and PYHINs, the adaptor protein ASC and caspase 1 — is found in myeloid cells and/or in tissues that are rich in innate immune cells[81–83]. Tissue expression profiles suggest that the CNS is fully equipped with the components of the NLRP1 and NLRP3 inflammasomes[82].

Not surprisingly, mouse microglia express components of the NLRP3 and NLRC4 inflammasomes, which are activated by relevant stimuli[21,50,67,76]. However, it is not known whether CNS mononuclear phagocytic cells — including resident microglia, perivascular macrophages and meningeal macrophages — exhibit differential expression and activation of inflammasomes nor whether there is differential expression of inflammasomes in different anatomical CNS sites.

Increasing evidence suggests that inflammasomes exist in non-myeloid cell types in the CNS. Interestingly, several studies have reported caspase 1 activation, IL-1β cleavage and the expression of inflammasome-forming NLRs in neurons[16,41,59,73,81]. In addition, recent studies have shown NLRP3 inflammasome expression in retinal-pigmented epithelium and NLRP2 inflammasome expression within astrocytes[7,84].

The functional consequences of inflammasome activation are ultimately determined by the substrate specificity of caspase 1. Over 100 caspase 1 substrates have now been reported through single-protein studies and large protein screens[85,86]. However, for most of these substrates, the physiological relevance of their cleavage

Non-myeloid cell types
Cells of the CNS other than microglia and macrophages, including astrocytes, oligodendrocytes and neurons.

is not known. Hence, the well-established caspase 1 substrates IL-1β and IL-18 still dominate current research into inflammasome activation.

IL-1β and IL-18 signal through their respective receptors, IL-1R1 and IL-18R, triggering nuclear factor-κB-dependent transcriptional events[12,13]. IL-1β is a key initiator of inflammation; it makes important contributions to cellular activation and cytokine production. IL-18 is considered to be an important regulator of interferon-γ responses in T cells and natural killer cells. In the CNS, microglia, astrocytes and neurons express receptors for these cytokines, which thereby participate in systemic responses such as fever as well as in local inflammation within neural tissue[12–14].

Because inflammasome engagement is a common upstream regulatory mechanism, IL-1β and IL-18 are often released in the same experimental pathological circumstances. Indeed, increased levels of each cytokine are observed in several neurological diseases, including viral encephalitis, stroke, Alzheimer's disease and multiple sclerosis[73,87–89]. Although these observations suggest that inflammasomes may be involved in these conditions, there is currently little direct evidence that any particular inflammasome is involved in a given CNS pathology, and the limited available evidence comes from studies in transgenic mouse strains that lack a specific inflammasome.

Inflammasome-dependent cell death

Caspase proteases (BOX 2) are functionally classified as either apoptotic or inflammatory; inflammatory caspases, including caspase 1, are not thought to initiate apoptotic cell death pathways[90]. However, another form of programmed cell death, termed pyroptosis, does involve inflammatory caspases, specifically caspase 1. In mice, pyroptosis is most often observed during bacterial infections of macrophages[15]. During pyroptosis, cells rapidly swell as the cell membrane becomes more permeable, leading to the cell's eventual rupture and the release of cellular contents[15,32]. By contrast, in apoptosis, caspases cleave hundreds of cellular substrates to dismantle the cell internally and membrane integrity is maintained[90]. It is assumed that pyroptosis limits bacterial growth and enables the rapid release of IL-1β and other danger signals into the environment surrounding the cell and, in this manner, facilitates resolution of infection.

Pyroptosis has not been studied in CNS cell types other than microglia. Mouse microglia can undergo pyroptosis in response to *L. pneumophila*[21], a response that is similar to that of peripheral macrophages[91]. Other species of bacteria that are known to infect the CNS and cause meningitis, such as *L. monocytogenes*, *Staphylococcus aureus* and *Mycobacterium tuberculosis*, can induce pyroptosis in peripheral myeloid cells[20,92,93]. In addition, pyroptosis has been implicated in the death of microglia during cytomegalovirus retinitis[94].

Although caspase 1-dependent cell death of microglia involves pyroptosis[21], the link between inflammasome activation and neuron death is still unclear. Multiple mechanisms are possible, including inflammasome activation in neurons that promotes neurodegeneration through cytokine release[84,95], a non-canonical mechanism by which caspase 1 activates apoptosis[16], and the caspase 1-dependent activation of pyroptosis within neurons.

Disentangling inflammasome-dependent cytokine release from neuronal degeneration is complicated if the same cell is both the source and the target of cytokine signalling. Neurons express activated caspase 1 under various conditions of stress and are capable of releasing IL-1β[16,41,59,73]. In some conditions, neurons might promote immunopathogenesis through inflammasome-dependent release of cytokines without caspase 1 directly activating cell death pathways within these cells. Indeed, a study in a mouse model of macular degeneration showed that aberrant accumulation of RNA transcripts (caused by deficient Dicer activity within the retinal-pigmented epithelium) activated the NLRP3 inflammasome and triggered the subsequent release of IL-18, which promoted apoptotic cell death in an autocrine manner[84]. A similar autocrine mechanism has been suggested to promote neuronal apoptosis in response to downregulation of superoxide dismutase 1 (SOD1)[95].

Several studies have also linked the activation of caspase 1 (an inflammatory caspase) to neuronal death by apoptosis[16,96,97]. These findings are contradictory to the consensus view that inflammatory caspases and apoptotic caspases are functionally distinct; indeed, many of the early studies suggesting caspase 1-dependent apoptosis were based on the use of caspase 1 inhibitors with limited specificity[96,97]. The most compelling evidence for caspase 1-dependent apoptosis comes from a study showing that caspase 1-deficient cortical neurons were resistant to oxygen- and glucose deprivation-induced apoptosis[16]. The authors proposed that caspase 1-dependent activation

Box 2 | **The expanding role of caspases in the CNS**

Caspases are broadly classified as being either pro-inflammatory or pro-apoptotic. In humans, the inflammatory caspases include caspases 1, 4 and 5, whereas in mice they include caspases 1 and 11. The primary function of these enzymes is to regulate the maturation and release of cytokines. The apoptotic caspases (caspases 3, 6, 7, 8 and 9) execute apoptotic cell death through the extrinsic or intrinsic pathways[94]. In neurological disease, inflammatory caspases promote immune activation, whereas apoptotic caspases are activated in neurons in response to immune molecule-mediated cytotoxicity, diminished growth factor signalling and excitotoxicity[98,141]. Caspases also regulate non-apoptotic forms of programmed cell death. For example, pyroptosis requires caspase 1 activation, and necroptosis — a receptor-interacting protein kinase 1 (RIPK1)- and RIPK3-dependent form of cell death — is negatively regulated by caspase 8. Both pyroptosis and necroptosis occur in myeloid cells[15,142], and, unlike in apoptosis, cells undergoing these two forms of cell death display necrosis-like features, which include the rapid swelling and subsequent rupture of cells.

Neuronal necrosis caused by ischaemia is often considered to be an uncontrolled process, but there is increasing evidence that regulated pathways, particularly the necroptosis pathway, might be involved[143]. This raises the questions of whether caspase 1 or caspase 8 regulates necrosis within the CNS and whether necrosis-regulating pathways can be targeted therapeutically. Caspases 3, 7 and 9, which are primarily known for mediating apoptosis, might also mediate synaptic remodelling of neurons and activation of microglia in response to lipopolysaccharide[75,144]. In addition, caspase 6 might contribute to the pathogenesis of Huntington's disease and Alzheimer's disease by processing amyloid precursor protein, tau or mutant huntingtin[145].

of the pro-apoptotic molecule BH3-interacting domain (BID) triggered apoptosis through the mitochondria-based intrinsic pathway[16]. Although the identification of caspase 1 in neurons concurrent with the release of IL-1β points to inflammasome activation in these cells[16], the proposed mechanism that caspase 1 might trigger apoptosis through the mitochondrial pathway remains to be confirmed. A recent study in myeloid cells reported that mitochondrial membrane disruption triggered by conventional inducers of apoptosis could lead to the downstream activation of the NLRP3 inflammasome[71]. This observation raises the question of whether activation of caspase 1 in cells subject to oxygen- and glucose deprivation-induced apoptosis occurs before or after mitochondrial disruption.

Inflammasome activation in neurons might also have a role in cell death through the triggering of pyroptosis, which has the appearance of necrosis but is dependent on caspase 1 activation[15]. Necrotic cell death contributes to neurodegeneration, particularly in the context of excitotoxicity[98]. It is interesting to note that inflammasome activation within neurons has been reported in an *in vivo* kainic acid model of excitotoxicity[59] and has also been implicated in enteric neuron death due to stimulation of P2X7 during colitis[77]. However, it has yet to be demonstrated whether pyroptosis occurs in neurons.

Inflammasomes in neurological diseases

Inflammasome activation is under current investigation across a broad spectrum of neurological diseases, including infections, acute sterile brain injury and chronic neurodegenerative diseases (FIG. 3; TABLE 1).

Inflammasome activation in acute brain infections. As innate immune sensing underlies the activation of inflammasome complexes, acute infections that lead to meningitis or encephalitis have the potential to contribute to inflammasome activation within the CNS.

Importantly, the consequences of inflammasome activation in response to a given pathogen can be different inside versus outside the CNS, as has been shown in studies of the bacterium *Streptococcus pneumoniae*, a common cause of both bacterial meningitis and pneumonia. In a mouse model of pneumonia, NLRP3 inflammasome sensing of this bacterium has a protective effect, as mice deficient in NLRP3 exhibited a more severe disease

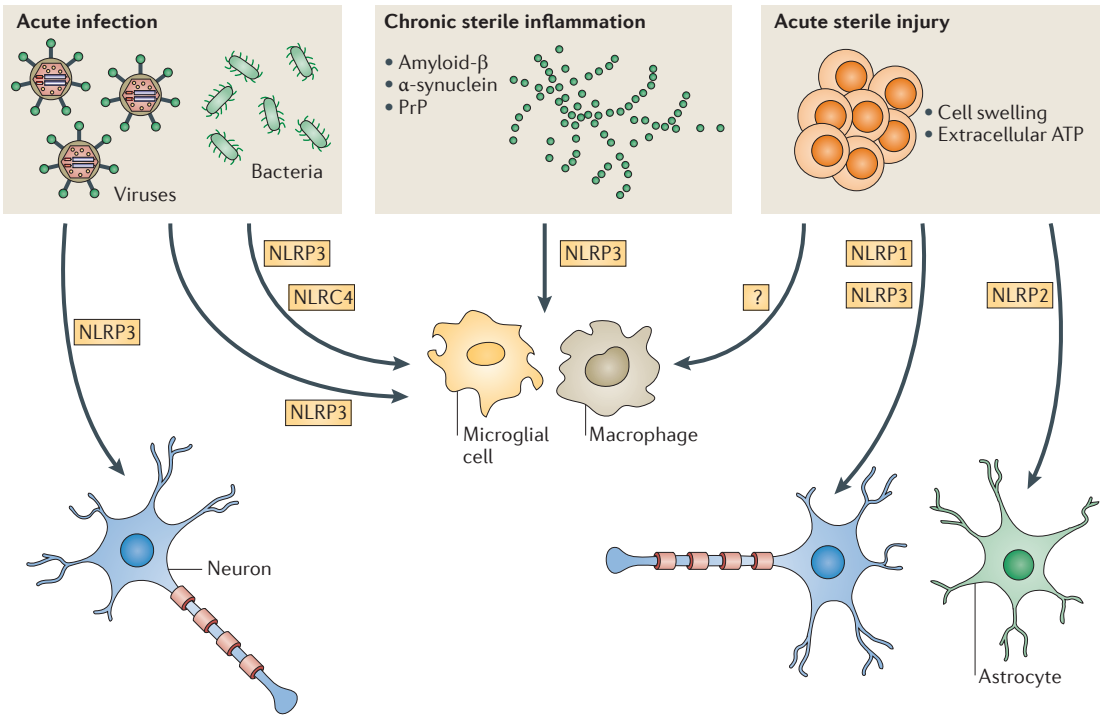


Figure 3 | **Location and identity of inflammasome complexes implicated in neurological conditions.** The inflammasome can be activated in the CNS under diverse conditions that trigger inflammation, including acute infection, chronic sterile inflammation and acute sterile injury. In the case of acute infections, pathogen-associated molecular patterns on viruses and bacteria are thought to activate either the NOD-, LRR- and pyrin domain-containing 3 (NLRP3) or NOD-, LRR- and caspase activation and recruitment domain-containing 4 (NLRC4) inflammasomes. Inflammasome activation in response to acute infection primarily occurs in microglia and macrophages, although it has also been reported to occur in neurons infected by West Nile virus. In the case of chronic sterile inflammation, misfolded proteins such as amyloid-β, α-synuclein and prion protein (PrP) activate the NLRP3 inflammasome within microglia and macrophages. In acute sterile injury such as stroke, traumatic brain injury or spinal cord injury, the NLRP1 and NLRP3 inflammasomes assemble within neurons. The proximal triggers of inflammasome formation in this context may include extracellular ATP and/or cell swelling. Extracellular ATP has also been reported to trigger the NLRP2 inflammasome in astrocytes. It is currently not known how acute sterile injury triggers inflammasome activation in microglia or macrophages.

Table 1 | **Inflammasome activation in neurological diseases**

Immune process	Disease context	Inflammasome involvement	Cellular location	Refs
Acute encephalitis or meningitis	*S. pneumoniae* meningitis	The NLRP3 inflammasome contributes to brain injury by driving the IL-1β, IL-18 and subsequent IFNγ response	Macrophages	62,100
	S. aureus brain abscesses	Infection induces NLRP3 inflammasome-dependent IL-1β release from microglia	Microglia	76
	Cerebral malaria	Malarial haemozoin activates the NLRP3 inflammasome but activation of NLRP3 and caspase 1 seems to be unrelated to cerebral malaria	Macrophages	146,147
	JEV	JEV infection of microglia activates the NLRP3 inflammasome	Microglia	67
	WNV	*Nlrp3*- or *Asc*-knockout mice are more susceptible to CNS virulence mediated by WNV infection. Activation of inflammasomes has been reported in microglia and neurons	Microglia and neurons	73,103
Acute sterile inflammation	Stroke and/or ischaemic injury	NLRP1 inflammasome formation occurs in neurons after stroke in rodents. An NLRP1-specific antibody is protective	Neurons	113
	TBI	The NLRP1 inflammasome is detected in neurons after TBI in rats. An ASC-specific antibody is protective	Neurons	18
	Spinal cord injury	The NLRP1 inflammasome is detected in neurons after spinal cord injury in rats. An ASC-specific antibody is protective	Neurons	17
Chronic sterile inflammation	CNS demyelination	The NLRP3 inflammasome promotes disease in both experimental autoimmune encephalitis and cuprizone models of CNS demyelination	Microglia and macrophages	116,117,119
	Amyotrophic lateral sclerosis	Mutant SOD1 induces caspase 1-dependent IL-1β release from microglia in an ASC-dependent and NLRP3-independent manner. This release is protective *in vivo*	Microglia and macrophages	121
	Parkinson's disease	α-synuclein activates the NLRP3 inflammasome in monocytes	Macrophages	63
	AD	Amyloid-β activates the NLRP3 inflammasome in microglia. *Nlrp3*-knockout mice are protected in a mouse model of AD	Microglia and macrophages	50,124
	Prion disease	PrP fibrils or neurotoxic fragments activate the NLRP3 inflammasome in microglia	Microglia and macrophages	75,148

AD, Alzheimer's disease; ASC, apoptosis-associated speck-like protein containing a caspase activation and recruitment domain; IFNγ, interferon-γ; IL, interleukin; JEV, Japanese encephalitis virus; NLRP, NOD-, LRR- and pyrin domain-containing; PrP, prion protein; *S. aureus*, *Staphylococcus aureus*; *S. pneumoniae*, *Streptococcus pneumoniae*; SOD1, superoxide dismutase 1; TBI, traumatic brain injury; WNV, West Nile virus.

course[99]. By contrast, in mouse models of *S. pneumoniae* meningitis, NLRP3 inflammasome induction and the subsequent cytokine responses exacerbated brain pathology[62,100]. IL-1β or IL-18 signalling had minimal impact on bacterial growth within the brain but promoted pathogenic inflammatory responses in the brain[62,100,101]. Such observations suggest that inflammasome activation in this context does more harm than good.

Other bacterial pathogens that have been reported to activate specific inflammasomes (NLRP3 or NLRC4) in microglia include *S. aureus*, *M. tuberculosis* and *L. pneumophila*[21,76,102]. However, only *in vitro* responses to these bacteria have been examined, and the effects on *in vivo* survival remain unknown.

IL-1β and IL-18 signalling are neuroprotective in mouse models of viral encephalitis caused by West Nile virus (WNV)[73,103], influenza A[104] and herpes simplex virus[105]. In these disorders, cytokine signalling seems to control levels of viraemia and enhance neuronal survival. Inflammasomes have been specifically studied in the context of two related flaviviruses, Japanese encephalitis virus (JEV) and WNV, both of which can cause viral encephalitis. JEV infection of microglia activates the NLRP3 inflammasome, leading to the release of IL-1β and IL-18. One study in mice showed that both cytokines were components of the immune response, although the effect of these signals on survival was not determined[67]. WNV infection in humans is associated with increased plasma levels of IL-1β[73], and, in mice, deletion of either NLRP3 or the adaptor ASC reduced survival after WNV infection; these findings indicate a protective role for inflammasomes in WNV infection[103]. The consequences of ASC deficiency were different outside versus inside the CNS: the peripheral immune response was restricted, permitting increased WNV replication in ASC-knockout mice, whereas in the CNS ASC deficiency led to an exaggerated immune response, thereby promoting cell damage[103]. Interestingly, WNV infection unexpectedly induced IL-1β-release from neurons, and IL-1β signalling was required for effective control of WNV replication in cortical neurons and for survival[73].

Unlike inflammasome activation in pneumococcal pneumonia, which might be damaging, inflammasome activation in response to those acute viral infections that have been examined (chiefly WNV) seems to be part of a protective response. Whether this will be true for other viral infections of the brain remains to be seen. The ability of inflammasome-dependent responses to limit pathogen growth was clearly a major difference

between the response to bacteria or viruses. Therefore, the consequences of inflammasome activation in response to viruses that establish chronic infections in the brain, such has HIV, may be very different.

Acute sterile brain injuries. Acute brain injury such as that caused by stroke or TBI results in focal innate immune responses that lead to neural tissue damage[3]. Mice deficient in caspase 1 are protected from neural injury caused by ischaemic stroke[106]; this finding suggests that inflammasome activation has a pathogenic role in this process. *Il-18*-knockout mice are not protected from ischaemic injury in the CNS[107], but administration of an IL-1R antagonist protected rats from brain injury induced by ischaemia, even if it was administered after the ischaemic event[108]. This effect depended not only on IL-1β but also on IL-1α, as mice lacking both cytokines are resistant to the pathogenic effects of ischaemia[109]. Although IL-1α is not a direct substrate of caspase 1 (REFS 110,111), caspase 1 (and therefore inflammasome activation) has indirect effects on IL-1α by degrading the bound and inhibitory cytosolic IL-1R2 (REF. 111) or through caspase 1-dependent regulation of non-classical secretion systems[112].

Administration of antibodies directed against ASC or against NLRP1 was neuroprotective in rodent models of TBI or stroke, respectively[18,113]. Middle cerebral artery occlusion has been reported to induce the expression of NLRP1 and NLRC5 in rat neurons[114], and increased NLRP1 levels in the cerebrospinal fluid of patients with TBI are associated with a worse prognosis[115].

The regulation of inflammasome activation in response to acute sterile injury may involve the pannexin 1 channel. As discussed above, NLRP1 activation in neurons requires pannexin 1 (REFS 18,19). Recent studies of cortical spreading depression associated with headache and studies of colitis-associated enteric neuron death have also implicated pannexin 1 in the activation of inflammasomes[41,77]. However, in a mouse model of stroke, genetic ablation of the pannexin 1 channel improved outcomes (specifically, it reduced infarct size and led to fewer neurological deficits) but had no effect on IL-1β-release, suggesting that the effect of pannexin 1 activation on stroke might not involve inflammasome activation[43]. Because of the substantial existing literature on IL-1β signalling, targeting the consequences of inflammasome activation as therapy is a more immediate possibility for acute sterile brain injuries than for other CNS diseases. However, a greater understanding of how the inflammasome itself becomes activated in such injuries, particularly within neurons, is required to move the field forward.

Chronic sterile CNS inflammation. Chronic inflammation within the CNS can have detrimental consequences for brain function and structure[2]. Multiple sclerosis is the prototypic example of a chronic CNS inflammatory disorder. Investigations into a role for inflammasomes in multiple sclerosis have focused on the peripheral immune response in which activated T cells and macrophages infiltrate the CNS during multiple sclerosis relapses. These events can be modelled in mice by experimental autoimmune encephalitis (EAE). Several studies using *Nlrp3*- knockout mice and *Asc*-knockout mice have shown that the induction of EAE is dependent on the NLRP3 inflammasome[116,117]. Loss of NLRP3 and the subsequent loss of IL-1β and IL-18 signalling ameliorated the EAE disease course by reducing T cell priming and subsequent T cell trafficking into the CNS[116–118]. Inflammasome induction within the CNS has not been investigated in EAE models.

Inflammasome activation is also implicated in an *in vivo* model of cuprizone-induced innate immune activation (in the CNS) and demyelination; however, the relevance of inflammasome activation is not completely understood, as IL-1β and IL-18 had different effects on remyelination after cuprizone treatment[119,120]. In this model of demyelinating disease, *Il-1β*-knockout mice had a disease phenotype that was similar to that of wild-type mice, but remyelination was delayed in these mice, suggesting that IL-1β might promote the repair process[120]. By contrast, *Il-18*-knockout mice had a less severe phenotype and remyelination was faster[119]. *Nlrp3*-knockout mice had a delayed onset of cuprizone-mediated pathology compared with wild-type mice, but the extent of remyelination was similar to that of wild-type mice[119]. These observations indicate that, on balance, inflammasome activation promotes pathology in this model and that IL-1β and IL-18 have opposing effects on the subsequent repair process.

In summary, although the causes of autoimmune demyelinating diseases such as multiple sclerosis remain unknown, inflammasome activation is potentially important both inside and outside the CNS.

Although neurodegenerative diseases that are characterized by the presence of misfolded protein aggregates — including amyotrophic lateral sclerosis (ALS), Alzheimer's disease, Parkinson's disease and Huntington's disease — are not usually considered to be prototypic inflammatory diseases, there is increasing evidence that innate immune responses contribute to their pathogenesis[2]. The sensitivity of innate immune cells to endogenous danger signals has prompted investigations into inflammasome (particularly the NLRP3 inflammasome) responses to misfolded proteins or aberrant protein deposits and into how they may contribute to the disease process.

Amyloid-β is the chief constituent of senile plaques in Alzheimer's disease and was the first molecule associated with a neurodegenerative disorder to be shown to activate an inflammasome[50]. Specifically, exposing LPS-primed macrophages to fibrillar amyloid-β activated caspase 1 and triggered IL-1β release; this response was dependent on NLRP3 and involved both endosomal rupture and cathepsin B release[50]. Similar findings, including endosomal rupture in the activation of the NLRP3 inflammasome, have been reported for α-synuclein (an aggregated protein found in Parkinson's disease) and PrP[63,75].

Mutations in *SOD1* that result in the formation of toxic misfolded protein aggregates are thought to be a major contributor to the pathogenesis of ALS. Stimulating microglia *in vitro* with mutant SOD1 induced caspase 1 activation in the microglia, and the

T cell priming
The process by which naive T cells are presented with an antigen in an immunogenic form, leading to their maturation into effector cells.

level of subsequent IL-1β secretion correlated with the degree of SOD1 misfolding[121]. Although inflammasome activation by SOD1 required endosomal rupture and was dependent on ASC[50], it did not require NLRP3 (REF. 121), suggesting that other inflammasomes can sense misfolded proteins such as mutant SOD1. Caspase 1 deficiency or IL-1β deficiency improved survival in mice expressing the mutant form of SOD1 (REF. 121), indicating that inflammasome activation in response to SOD1 promotes pathogenesis.

It remains a topic of debate whether the presence of immune cells or cytokines at sites such as amyloid-β plaques mediates pathogenic or reparative processes[2]. For example, studies into the role of IL-1β signalling in the progression of Alzheimer's disease have produced contradictory results. In one study, genetic deletion of IL-1RA (also known as IL-1RN) in mice increased microglia activation and reduced neuronal survival in response to intracerebroventricular infusion of human amyloid-β[122]. Although this finding suggests a pathogenic role for IL-1β, a subsequent study showed that hippocampal overexpression of IL-1β reduced plaque size in a mouse model of Alzheimer's disease because greater numbers of mononuclear phagocytic cells were recruited to the sites of plaque formation[123]. In diseases or disease models in which the same class of immune cell (that is, microglia and macrophages) has both protective or pathogenic functions, studies that manipulate the level of activity of a general modulator of this class of immune cell, such as IL-1β, could yield divergent results depending on the experimental design. The targeting of upstream regulatory processes of cytokine release, such as inflammasome activation, within macrophages or microglia might provide a better differentiation between cells that exert pathogenic versus reparative effects. This approach has recently been applied to another model of Alzheimer's disease[124]. Specifically, a follow-up study to the initial finding that amyloid-β activates the NLRP3 inflammasome suggested that this pathway can also promote disease progression. In this study, offspring of *Nlrp3*-knockout or caspase 1-knockout mice crossed with APP/PS1 transgenic mice (which develop Alzheimer's-like disease through the expression of mutant forms of amyloid-β precursor protein and presenilin 1) did not develop neurobehavioural defects or amyloid deposition[124]. These inflammasome-deficient mice had increased numbers of microglia with anti-inflammatory phenotypes, which presumably enabled efficient clearance of amyloid-β[124]. These observations imply that innate immune responses — and specifically inflammasomes — influence both susceptibility to neurodegenerative disease onset and disease severity.

Conclusions and future perspectives

The role of inflammasomes in the healthy CNS and CNS diseases has achieved substantial recognition in multiple animal models of neurological conditions, and new avenues of research are continually emerging. Investigations into the contributions of inflammasome induction to mood disorders, epilepsy and neuroAIDS are likely to follow.

Much of what may be considered in the future is still greatly informed by studies of inflammasome function outside the CNS. For example, the list of new inflammasome complexes being discovered and characterized — such as NLRP6 (REF. 125) and NLRP12 (REF. 126) — continues to grow and the roles of inflammatory caspases such as caspases 4, 5 and 11 are being scrutinized[127–129]. In addition, inflammasomes have recently been found to mediate the release of immune factors other than IL-1β and IL-18, including prostaglandins and leukotrienes[130], which could influence CNS function. For the most part, how these discoveries relate to the nervous system and CNS diseases remains to be investigated.

However, it is insufficient to rely on studies of isolated peripheral immune cells to direct future investigations into inflammasome function within the CNS. Microglia, astrocytes and neurons have all been reported to express inflammasomes, but little is known about how this diversity affects the regulation of IL-1β signalling at the tissue level. The conditions under which inflammasome activation occurs within neurons and astrocytes in particular deserve more attention. A major step forward would be to map inflammasome activation to specific brain regions and cell types directly during disease. This may involve the development of novel transgenic animals (that is, conditional knockouts), *in vivo* cell imaging and real-time imaging of inflammasome formation within cells.

Major progress is also required in the application of findings from animal models to humans. One of the most exciting findings of the early inflammasome field was the recognition that gain-of-function mutations in the *NLRP3* gene underlie cryopyrin-associated periodic syndromes in humans[6]. Genetic variants within individual inflammasomes have been linked to bacterial meningitis[35] and Alzheimer's disease[46], but few studies have examined inflammasome activation using human CNS cell types. The importance of doing so is underlined by the differences that are known to exist between mouse and human inflammasomes: for example, the functional diversity of different variants of the *Nlrp1* gene in mice as opposed to the single *NLRP1* gene identified in humans. Although obtaining human primary CNS cells has obvious challenges, sources of such cells are available.

Because the NLRP3 inflammasome has been identified as a sensor of misfolded proteins in several mouse models of neurodegenerative diseases, an important question is whether this inflammasome also senses misfolded proteins in different human patients. In addition, as fascinating recent work on amyloid-β fibrils from patients with Alzheimer's disease has shown that patients with divergent clinical histories have structurally distinct fibrils within the brain[131], an important development with regard to understanding inflammasome activation in Alzheimer's disease would be to identify whether distinct forms of amyloid-β fibrils derived from human patients have a greater or lesser capacity to activate NLRP3 and subsequent immune responses.

The ultimate goal is to develop therapies that target inflammasome activation, but most are in the early stages of development. Importantly, our understanding

of inflammasome activation (or suppression) in specific diseases in humans is currently complicated by the wide use of therapeutics that can activate inflammasomes: for example, statins[132,133]. IL-1β-targeting therapies, such as IL-1β-specific monoclonal antibodies and IL-1R antagonists, have been successfully used to treat cryopyrin-associated syndromes and are now being investigated for the treatment of arthritis and even stroke[78,108,134]. Similarly, the development of new drugs such as cytokine release inhibitory drug CRID3 and milk fat globule-EGF 8 (MFGE8), omega-3 fatty acids and the repurposing of existing drugs — such as glyburide — to target inflammasome functions are promising possibilities for future therapeutic interventions in neurological diseases[135–138].

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