

# Inflammasomes in neuroinflammatory and neurodegenerative diseases

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## Abstract

Neuroinflammation and neurodegeneration often result from the aberrant deposition of aggregated host proteins, including amyloid- $\beta$ ,  $\alpha$ -synuclein, and prions, that can activate inflammasomes. Inflammasomes function as intracellular sensors of both microbial pathogens and foreign as well as host-derived danger signals. Upon activation, they induce an innate immune response by secreting the inflammatory cytokines interleukin (IL)-1 $\beta$  and IL-18, and additionally by inducing pyroptosis, a lytic cell death mode that releases additional inflammatory mediators. Microglia are the prominent innate immune cells in the brain for inflammasome activation. However, additional CNS-resident cell types including astrocytes and neurons, as well as infiltrating myeloid cells from the periphery, express and activate inflammasomes. In this review, we will discuss current understanding of the role of inflammasomes in common degenerative diseases of the brain and highlight inflammasome-targeted strategies that may potentially treat these diseases.

**Keywords** disease; inflammasome; inflammation; microglia; neurodegeneration

**DOI** 10.15252/emmm.201810248 | Received 26 December 2018 | Revised 21 March 2019 | Accepted 3 April 2019 | Published online 23 April 2019

**EMBO Mol Med (2019) 11: e10248**

See the Glossary for abbreviations used in this article.

## Introduction

The innate immune system is a rapid and coordinated cellular defense response that aims to eliminate the threat posed by both sterile and infectious insults. Recognition of these pathogenic agents is mediated by pattern recognition receptors (PRRs) that sense pathogen-associated molecular patterns (PAMPs) and host- or environment-derived danger-associated molecular patterns (DAMPs). In the central nervous system (CNS), these PRRs are primarily expressed by microglia, astrocytes, and macrophages, but also

oligodendrocytes, neurons, and endothelial cells express a repertoire of PRRs (Lampron *et al*, 2013; Walsh *et al*, 2014). PRRs can either be membrane-bound, as is the case for the Toll-like receptors (TLRs), to sense signals in the extracellular environment or in the endosome, or they can be intracellular, as with the nucleotide-binding domain and leucine-rich repeat-containing receptors (NLRs) and AIM2-like receptors (ALRs). An important subgroup of cytosolic PRRs that includes members of the NLR and ALR families as well as the tripartite motif (TRIM) family member pyrin critically contributes to the innate immune response by assembling so-called “inflammasomes”.

## General concepts of inflammasome biology

Inflammasomes are cytosolic multiprotein complexes which upon assembly activate the pro-inflammatory caspase-1 (see Glossary) that is responsible for the maturation and secretion of the inflammatory cytokines IL-1 $\beta$  and IL-18, and additionally induce pyroptosis (Lamkanfi & Dixit, 2014; Broz & Dixit, 2016). Briefly, inflammasome-inducing stimuli trigger the oligomerization of PRR proteins and the recruitment of pre-existing procaspase-1 zymogens into the complex, leading to their proximity-induced autoactivation to generate active caspase-1. Consequently, caspase-1 will cleave the biologically inactive pro-peptides pro-IL-1 $\beta$  and pro-IL-18 into mature cytokines which are then secreted by the cell. Next to its role in the maturation of pro-IL-1 $\beta$  and pro-IL-18, caspase-1 can also induce a pro-inflammatory form of cell death, pyroptosis, that features early plasma membrane rupture, thereby releasing the soluble intracellular fraction that fuels the inflammatory response (Lamkanfi & Dixit, 2012, 2014). Central in this process is the executioner protein gasdermin D (GSDMD)—a substrate of murine caspase-1 and caspase-11, and human caspase-1, caspase-4, and caspase-5—the amino-terminal domain of which upon cleavage oligomerizes and perforates the plasma membrane to induce cell swelling and osmotic lysis (Shi *et al*, 2017) (Fig 1).

One way to classify inflammasome complexes is based on the receptor that initiates signaling (Lamkanfi & Dixit, 2012). The core inflammasome components consist of the cytosolic NLR, ALR, and

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## Glossary

### Blood–brain barrier (BBB)

a physiological barrier between the blood and the CNS parenchyma. The BBB is formed by endothelial cells that are joined by complex tight junctions and coated by a basement membrane and an additional membrane of astrocytic end-feet, known as the glia limitans.

### Caspases

a family of cysteine-dependent aspartate specific proteases that play a central role in inflammation and programmed cell death.

### Cerebrospinal fluid (CSF)

contained in the ventricles of the brain and the cranial and spinal subarachnoid spaces. It provides a mean for transporting different molecules, including cytokines, neurotransmitters, and hormones, but also resides innate immune cells.

### CNS macrophages

non-parenchymal macrophages of the CNS that are located in meninges, choroid plexus, and perivascular spaces.

### Creutzfeldt–Jakob disease

fatal degenerative prion disease that can be sporadic, hereditary, or acquired. The acquired form of the disease is caused by exposure to the misfolded scrapie form of the PrP protein.

### Cuprizone-induced demyelination

an experimental model to study local demyelination of the corpus callosum, which is induced by the administration of the copper chelator cuprizone (bis(cyclohexanone)oxalaldihydrazone) to the food of mice during 5 weeks.

### Dopaminergic neurons

the main dopamine-producing cells of the CNS and essential for controlling key functions of the brain, including voluntary movement, reward processing, mood, and working memory.

### Encephalomyelitis

general term to describe inflammation in the brain or spinal cord. Autoimmune encephalomyelitis is caused by an abnormal immune response to a self-antigen.

### Experimental autoimmune encephalomyelitis (EAE)

the main rodent model of MS. EAE is actively induced by peripheral immunization with myelin-specific proteins or peptides in combination with an adjuvant, or passively by transfer of encephalitogenic T cells.

### Familial Mediterranean Fever

the most common monogenic autoinflammatory disease and is characterized by periodic fevers with childhood onset, and frequently associated with serositis and joint pain. It predominantly affects Mediterranean populations.

### Grand-mal seizures

or tonic-clonic seizures: seizures that are characterized by the loss of consciousness and violent muscle contractions. This seizure comes in two phases: a tonic phase followed by a clonic phase. The brief tonic phase features loss of consciousness and muscle stiffening, while in the clonic phase the muscles go into rhythmic contractions.

### Induced pluripotent stem cells (iPSCs)

generated by genetic reprogramming of adult somatic cells. The advantage over other types of stem cells is that they are not derived from a human embryo.

### Ischemic infarct

an infarct caused by the interruption of the blood flow in the brain due to occlusion of a cerebral vessel.

### Middle cerebral artery occlusion

experimental stroke model that involves the permanent or transient occlusion of the middle cerebral artery. The middle cerebral artery is one of the three arteries that supply blood to the cerebrum. In the “filament model”, a suture filament is (transiently) introduced into the internal carotid artery and forwarded until the tip occludes the middle cerebral artery.

### Neonatal-onset multisystem inflammatory disease (NOMID)

or chronic infantile neurologic cutaneous and articular (CINCA): the most severe cryopyrin-associated periodic syndrome (CAPS). NOMID is characterized by neonatal-onset skin lesions, chronic aseptic meningitis, and recurrent fever along with joint symptoms.

### Sepsis

a life-threatening systemic inflammatory response syndrome (SIRS) caused by the body's response to an infection.

### Spatial memory

an important cognitive function that allows us to recall three-dimensional objects or places.

### Steatohepatitis

a type of fatty liver disease and is characterized by the accumulation of lipids and the infiltration of inflammatory cells in the hepatic parenchyma.

### Striata

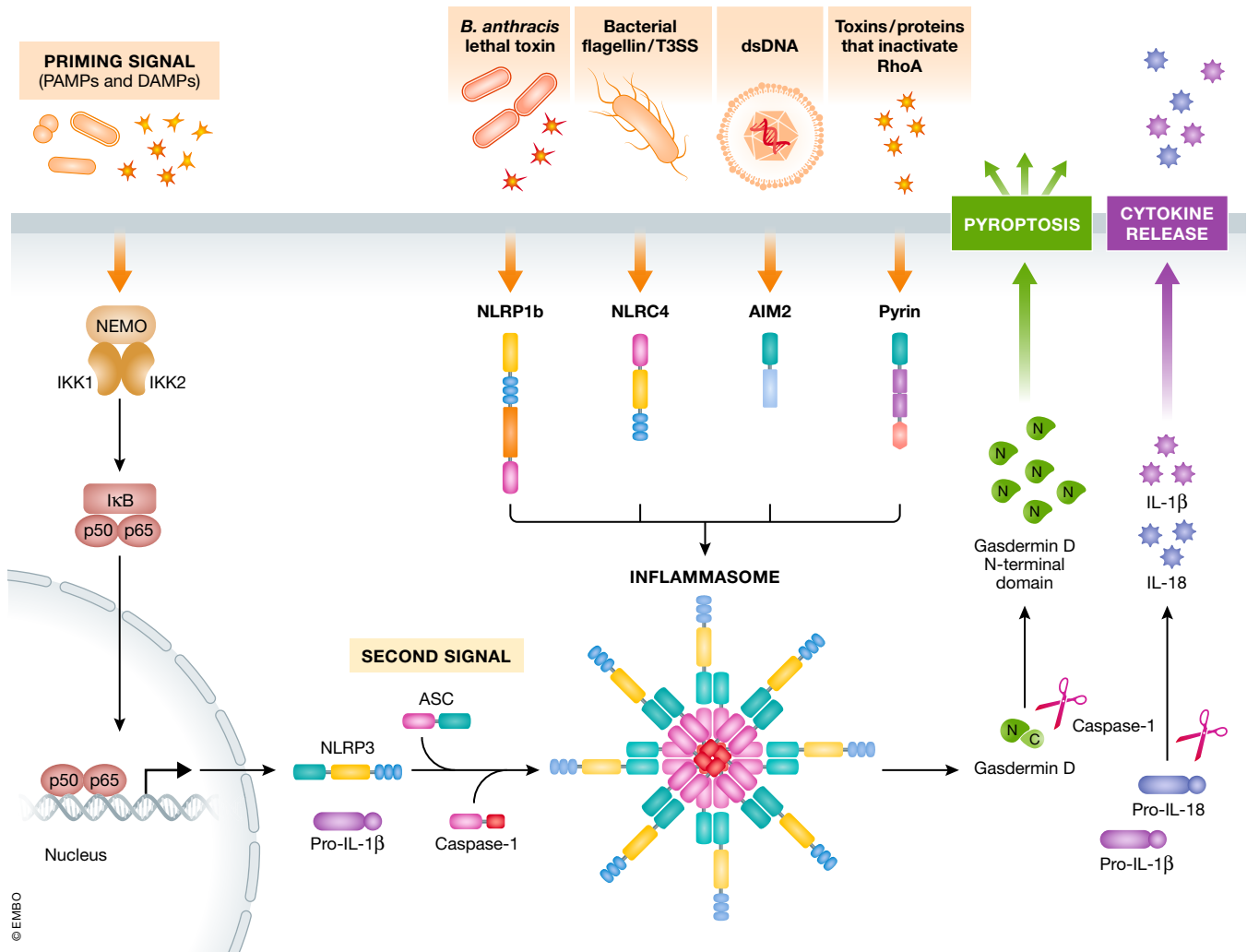
dopamine neurons from the substantia nigra pars compacta project to the caudate and the putamen of the basal ganglia, together referred to as the striatum.

### Substantia nigra pars compacta

structure in the brainstem that contains most of the dopamine-producing neurons.

pyrin receptors, the adaptor apoptosis-associated speck-like protein containing a caspase recruitment domain (ASC) and procaspase-1. ASC is composed of a pyrin domain (PYD) and a caspase recruitment domain (CARD) and functions as an adaptor that links the PYD of the NLR or pyrin and the CARD of procaspase-1 (Fig 2). Some inflammasomes, such as NLRC4 and NLRP1b, do not require ASC and may directly recruit procaspase-1 through their respective CARDS, although under wild-type conditions ASC does contribute to optimal caspase-1 activation and efficient secretion of IL-1 $\beta$  and IL-18 (Broz *et al*, 2010; Guey *et al*, 2014; Van Opdenbosch *et al*, 2014). Assembly and activation of inflammasomes requires detection of specific signals: Murine Nlrp1b responds to *Bacillus anthracis* lethal toxin (LeTx) (Boyden & Dietrich, 2006); NLRC4 detects intracellular flagellin and components of type III secretion systems (T3SS) of bacterial pathogens (Franchi *et al*, 2006; Miao *et al*, 2006, 2010); AIM2 physically binds cytosolic double-stranded DNA (dsDNA) (Hornung *et al*, 2009); and pyrin indirectly

responds to toxins that covalently inactivate the small GTPase RhoA (Xu *et al*, 2014). NLRP3, the most widely studied inflammasome, responds to a broad spectrum of activating stimuli that includes a suite of bacterial, fungal, and viral PAMPs, DAMPs such as ATP and uric acid crystals, and crystalline and aggregated substances such as asbestos, silica, and amyloid- $\beta$  fibrils (Lamkanfi & Dixit, 2012). NLRP3 activation is unique in the sense that it involves a two-step process. A first signal, or priming signal, results in the NF- $\kappa$ B-dependent transcriptional upregulation of NLRP3 and pro-IL-1 $\beta$ , but also controls post-translational modifications of NLRP3 as highlighted by the mapped ubiquitin and phosphorylation sites on NLRP3 (Yang *et al*, 2017). This is followed by a second “activation” signal that induces the oligomerization and activation of the NLRP3 inflammasome (Fig 1). Besides canonical NLRP3 inflammasome signaling, the non-canonical NLRP3 inflammasome pathway involves activation of caspase-11 (or its human orthologs caspase-4 and caspase-5) by



**Figure 1. Inflammasome activation and signaling.**

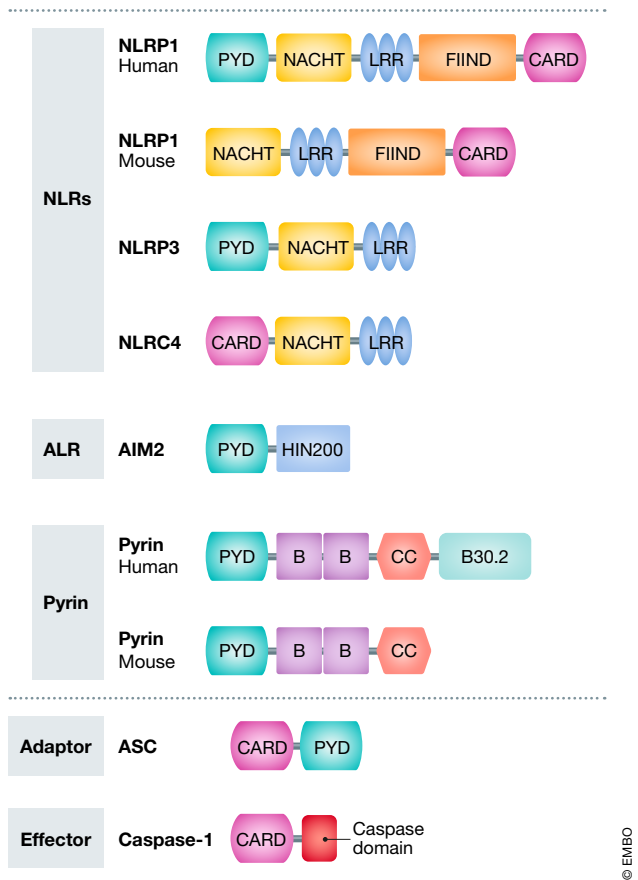
Inflammasomes assemble in a stimulus-specific manner. Different DAMPs and PAMPs are able to induce NLRP3, while NLRP1b responds to *Bacillus anthracis* lethal toxin, NLRC4 recognizes bacterial flagellin and/or the type III secretion system of bacterial pathogens, AIM2 is specifically activated by dsDNA, and pyrin recognizes the inactivation of RhoA by toxins and effector proteins. Activation of the NLRP3 inflammasome involves a two-step mechanism. The priming signal is detected by membrane-bound PRRs, including TLRs and C-type lectin receptors (CLRs) and induces NF- $\kappa$ B-dependent transcription of NLRP3 and pro-IL-1 $\beta$  precursor protein, and controls post-translational modifications that license NLRP3 activation. The second activation signal is necessary for inflammasome formation, depending on the oligomerization and subsequent activation of procaspase-1. Active caspase-1 then cleaves pro-IL-1 $\beta$  and pro-IL-18 to their mature forms IL-1 $\beta$  and IL-18 which get secreted. In addition, caspase-1 can cleave gasdermin D, releasing its N-terminal fragment which translocates to the plasma membrane inducing pore formation and pyroptotic cell death. In contrast to NLRP3, other inflammasome receptors do not need this initial priming signal to induce inflammasome activation and cytokine release.

cytosolic LPS, and the induction of pyroptosis through the cleavage of GSDMD and the release of high mobility group box 1 protein (HMGB1) and IL-1 $\alpha$  (Lamkanfi & Dixit, 2014; Shi *et al*, 2017). Apart from AIM2 and the NAIP/NLRC4 complexes, the common secondary messengers or physical ligands that bind and trigger inflammasome assembly await discovery.

### Inflammasomes and the central nervous system

IL-1 $\beta$  and IL-18 have important functions in the CNS, and many cell types in the brain express their cognate receptors that initiate inflammatory signaling cascades that may contribute to neuronal

injury and cell death (Allan *et al*, 2005; Alboni *et al*, 2010). Hence, increased levels of IL-1 $\beta$  and IL-18 are often observed upon CNS infection, brain injury, and neurodegenerative diseases (Heneka *et al*, 2014, 2018). IL-1 $\beta$  and IL-18 are also important for physiological functions in the CNS and have been shown to participate to processes of cognition, learning, and memory (Tsai, 2017). Pyroptosis additionally contributes to inflammasome-driven pathology through the release of other inflammatory mediators and DAMPs. Apoptosis and necroptosis are additional cell death modes that have been shown to promote neuroinflammation and neuronal degeneration in several neurodegenerative pathologies, including multiple sclerosis, amyotrophic lateral sclerosis, Parkinson's disease, and Alzheimer's disease (Zhang *et al*, 2017; Yuan *et al*, 2019).



**Figure 2. Domain structure of inflammasomes.**

A subset of NLRs and ALRs can trigger the formation of inflammasomes. NLR family members have a nucleotide-binding and oligomerization domain (NACHT/NBD), as well as leucine-rich repeat (LRR) motifs, typically located in the center and carboxy terminus of the NLR proteins, respectively. The NACHT motif is usually flanked by an additional amino-terminal domain, either CARD or PYD, and these domains are used for further sub-classification of inflammasomes. These domains allow the recruitment of adaptor and effector proteins to the inflammasome signaling complex. The NLR gene family consists of 22 human members and 34 murine members, many of which the function is not always clear (Lamkanfi & Dixit, 2012; Broz & Dixit, 2016). In addition to the NLR-containing inflammasomes, the ALR family member AIM2 can also assemble an inflammasome complex. AIM2 is characterized by an amino-terminal PYD domain and one or two DNA-binding HIN200 domains (Hornung *et al.*, 2009). Pyrin, also known as TRIM20, features a PYD domain, two B-boxes, and a coiled-coil domain, whereas the human pyrin also has an additional C-terminal B30.2 domain. ASC is the critical adaptor protein for many inflammasome complexes and is composed of CARD and PYD domains, the latter being necessary for homotypic interaction with a PYD-containing inflammasome sensor (NLRP3, AIM2). Pro-caspase-1 features a CARD domain, in addition to its caspase domain, and homotypic CARD interactions result in direct or indirect (via ASC) recruitment of pro-caspase-1 to the inflammasome complex. Inflammasome activation involves ASC and pro-caspase-1 recruitment, resulting in ASC oligomerization into a macromolecular aggregate, known as an ASC speck (Broz & Dixit, 2016).

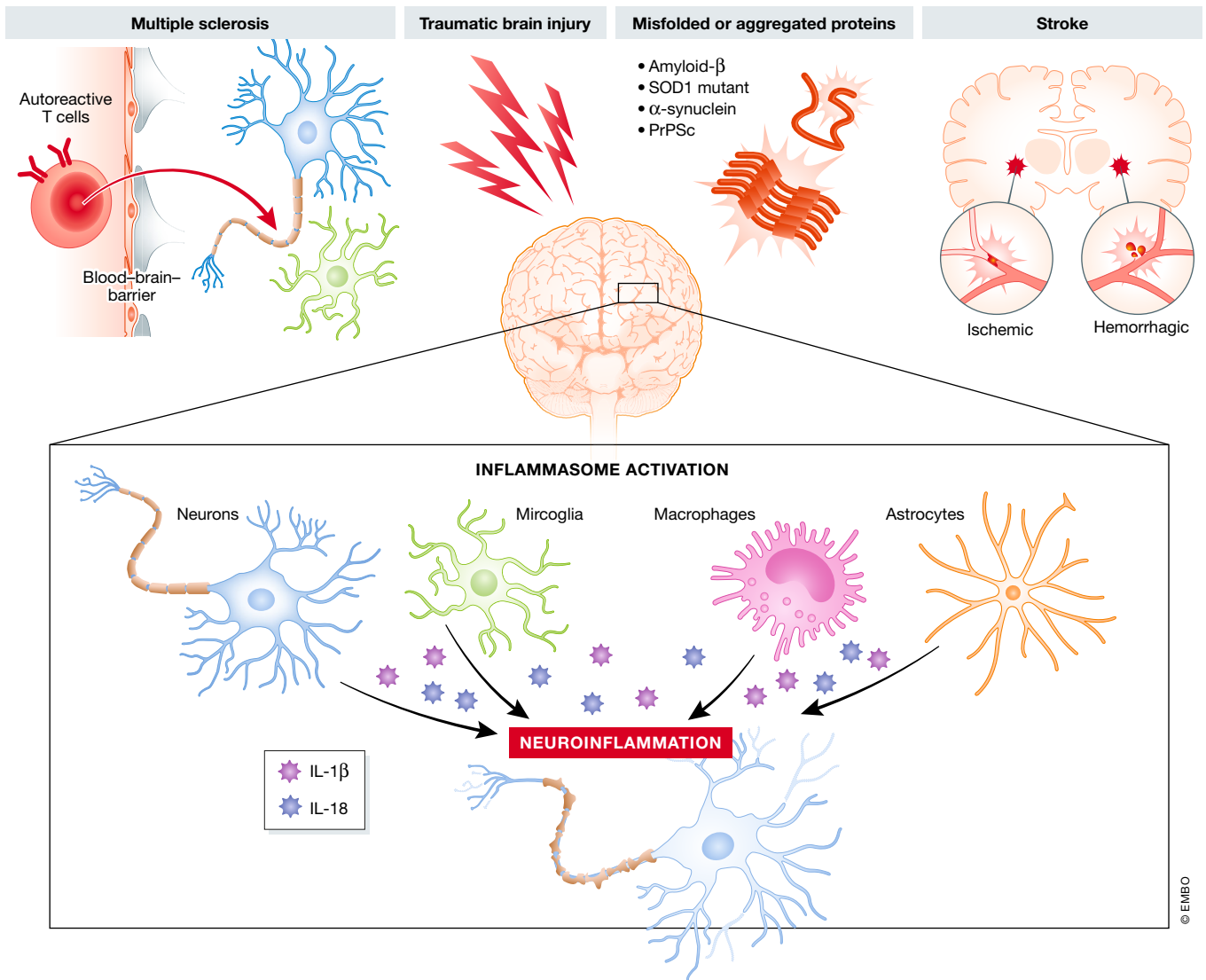
Although inflammasome signaling in the CNS is mainly attributed to microglia, the key innate immune cells of the brain, expression of inflammasome components has also been reported in other cell types of the CNS, including neurons (Kaushal *et al.*, 2015), astrocytes (Freeman *et al.*, 2017), perivascular CNS macrophages

(Kawana *et al.*, 2013), oligodendrocytes (Mckenzie *et al.*, 2018), and endothelial cells (Gong *et al.*, 2018) (Fig 3). However, current understanding of inflammasome activation in microglia and its role in CNS inflammation and disease is still fragmentary and primarily based on *in vitro* studies with primary microglia and microglial cell lines, and *in vivo* studies with transgenic knockout mice that lack expression of specific inflammasome components throughout the body. More appropriate research tools that allow CNS-specific inflammasome targeting are needed to investigate the relative contribution of local inflammasome signaling in specific brain cell types to overall CNS pathology. Furthermore, evidence for inflammasome activation in patients with neurodegenerative disease often relies on detection of higher IL-1 $\beta$  transcript levels and/or increased gene and protein expression of inflammasome components. However, increased expression levels of these NF- $\kappa$ B responsive genes are largely indicative of an ongoing inflammatory response rather than direct support for inflammasome engagement. We here review the current knowledge on the relevance of inflammasome activation for the most common neurodegenerative pathologies (Fig 3 and Table 1) and highlight emerging strategies for the treatment of inflammasome-driven diseases. Although inflammasome activation is also central to the pathology of many CNS infectious diseases, such as Zika, HIV, and West Nile virus, and bacterial infections that induce meningitis (Walsh *et al.*, 2014; Mamik & Power, 2017; Heneka *et al.*, 2018), these conditions are outside the scope of this review.

## Inflammasome activation in multiple sclerosis and experimental autoimmune encephalomyelitis

Multiple sclerosis (MS) is the most common chronic inflammatory disease of the CNS. MS is characterized by a compromised blood–brain barrier (BBB) that leads to immune cell infiltration from the periphery and local microglia and astrocyte activation, which together promote inflammation, demyelination, and neurodegeneration (Baecher-Allan *et al.*, 2018). Experimental autoimmune encephalomyelitis (EAE) is a widely used rodent model of MS (Ransohoff, 2012), and both IL-1 $\beta$  and IL-18 were shown to critically contribute to EAE (Sutton *et al.*, 2006; Gris *et al.*, 2010; Lévesque *et al.*, 2016). Most studies on the involvement of inflammasomes in MS have focused on the peripheral immune response that is shaped by lymphocytes and macrophages that enter the CNS during MS pathology. Caspase-1, IL-18, and IL-1 $\beta$  are upregulated in peripheral blood mononuclear cells (PBMCs) and cerebrospinal fluid (CSF) of MS patients, as are levels of the NLRP3 inflammasome-activating DAMPs ATP and uric acid (Inoue & Shinohara, 2013; Mamik & Power, 2017). Moreover, caspase-1 expression was shown to be elevated in acute and chronic demyelinating lesions (Voet *et al.*, 2018), and caspase-1 and ASC have recently been proposed as candidate biomarkers for MS onset (Keane *et al.*, 2018).

The role of the NLRP3 inflammasome in EAE-associated T-cell priming and trafficking into the CNS is supported by the analysis of NLRP3, caspase-1, and ASC knockout mice (Furlan *et al.*, 1999; Gris *et al.*, 2010; Inoue *et al.*, 2012a). Moreover, the pharmacological NLRP3 inflammasome inhibitor MCC950/CRID3 was shown to suppress IL-1 $\beta$  production and attenuate EAE severity in wild-type mice (Coll *et al.*, 2015). Similarly, pharmacological blockade of



**Figure 3. Inflammasome activation in neurodegenerative disease.**

Inflammasomes can be activated in the CNS in response to acute injury (traumatic brain injury and stroke), autoimmune-mediated injury (multiple sclerosis), and accumulation of misfolded or aggregated proteins in the brain (Alzheimer's disease, amyotrophic lateral sclerosis, Parkinson's disease, and prion disease). Inflammasome activation has been demonstrated in CNS-resident cell types, including microglia, astrocytes, and neurons, but also in CNS-infiltrating cells, such as in infiltrating macrophages. Although most research on neurodegenerative diseases has focused on the importance of the NLRP3 inflammasome, also other inflammasome types can be activated in the brain and have been demonstrated in neurodegenerative disorders. Overall, inflammasome activation results in caspase-1-mediated cleavage of pro-IL-1 $\beta$  and pro-IL-18, and the subsequent release of the mature cytokines. High levels of IL-1 $\beta$  and IL-18 can be detected in many neurodegenerative conditions and are considered to be crucial for the establishment of a chronic inflammatory environment, leading to neuronal dysfunction and eventually neurodegeneration.

caspase protease activity with Z-Val-Ala-DL-Asp-fluoromethylketone or the inflammatory caspase prodrug VX-765 reduced EAE symptoms in mice (Furlan *et al*, 1999; McKenzie *et al*, 2018). Interestingly, IFN $\beta$ , the first line treatment for MS, is only effective in an NLRP3 inflammasome-dependent EAE subtype, whereas NLRP3-independent EAE could not be reversed by IFN- $\beta$  therapy (Inoue *et al*, 2012b, 2016). We recently provided direct genetic evidence for the relevance of inflammasome signaling in microglia and border-associated macrophages during EAE (Voet *et al*, 2018). The anti-inflammatory protein A20 has been shown to negatively regulate NLRP3 inflammasome activation (Vande Walle *et al*,

2014), and deletion of A20 in microglia and CNS macrophages exacerbated EAE in mice due to NLRP3 hyperactivation that resulted in increased IL-1 $\beta$  secretion and CNS inflammation (Voet *et al*, 2018). CNS-intrinsic inflammasome activation was further reported in another study that showed caspase-1- and GSDMD-mediated pyroptosis in microglia, as well as in myelin-forming oligodendrocytes in the CNS of MS patients and EAE mice (McKenzie *et al*, 2018).

The relevance of the NLRP3 inflammasome to MS has also been demonstrated in the model of cuprizone-induced demyelination, in which NLRP3-deficient mice presented delayed demyelination,

**Table 1. Overview of the described neurodegenerative disease models.**

Disease model	Description
<b>Multiple sclerosis</b>	
EAE	EAE is actively induced by peripheral immunization with myelin-specific proteins or peptides in combination with an adjuvant, or passively by transfer of encephalitogenic T cells
Cuprizone	Administration of the copper chelator cuprizone will induce local demyelination of the corpus callosum
<b>Stroke</b>	
Permanent MCAO	Permanent occlusion of the middle cerebral artery is obtained using an intraluminal suture
Transient MCAO	Intraluminal suture MCAO utilizes a suture inserted into the middle cerebral artery to interrupt the blood flow for a specific duration and is afterward removed. Embolic stroke MCAO uses an autologous blood clot injected into the MCA to occlude the vessel
Intracerebral hemorrhage (ICH)	Stroke condition provoked by injection of autologous arterial blood into the basal ganglia
<b>TBI and SCI</b>	
Controlled cortical impact (CCI)	A mechanical model of traumatic brain injury. Following craniotomy, the CCI device mechanically transfers energy onto the dura mater damaging the cortex, and sometimes the subcortical structures
Impact acceleration model	The exposed skull is covered with a steel disk and a weight is dropped onto the steel disk
Contusion model of spinal cord injury	A transient force is applied by either an electromagnetic device or a weight-drop to displace and damage the spinal cord
<b>Alzheimer disease</b>	
APP/PS1	Mouse model expressing human APP695 with the Swedish double mutation (K670N/M671L) and a mutant human presenilin 1 (PS1-dE9)
3xTgAD	Mouse model expressing human APP695 with the Swedish double mutation (K670N/M671L), human PS1 with the M146V mutation, and human Tau with the P301L mutation
Tg2576	Mouse model expressing human APP695 with the Swedish double mutation (K670N/M671L) at the $\beta$ -secretase cleavage site
5xFAD	Mouse model expressing human APP and PSEN1 transgenes with a total of five AD-linked mutations: the Swedish (K670N/M671L), Florida (I716V), and London (V717I) mutations in APP, and M146L and L286V mutations in PSEN1
<b>Amotrophic lateral sclerosis</b>	
TgSOD1-G93A	Mouse model expressing a G93A mutant form of human SOD1
hSOD1 G37R	Mouse model expressing a G37R mutant form of human SOD1
hSOD1 G85R	Mouse model expressing a G85R mutant form of human SOD1
<b>Parkinson disease</b>	
A53T	Mouse model expressing the mutant human A53T alpha-synuclein.
LPS-induced PD	Injection of LPS in the left substantia nigra pars compacta
6-hydroxydopamine-induced PD	Injection of 6-hydroxydopamine in the medial forebrain bundle
MPTP-induced PD	Intraperitoneal injection of MPTP five times at 2-h intervals
<b>Prion disease</b>	
Scrapie-infected	Intracerebral infections with brain homogenate of scrapie strain 139A-infected mice or intracerebral injection with RML6 (passage 6 of Rocky Mountain Laboratory strain mouse-adapted scrapie prions)
Tg(CJD)	Mouse model expressing a misfolded mutant PrP (D177N/V128)
<b>Huntington disease</b>	
R6/2	Transgenic mice expressing exon 1 of human huntingtin with expanded CAG/polyglutamine repeat

oligodendrocyte loss, and neuroinflammation (Jha *et al*, 2010). Additional support was provided by similar observations in caspase-1 and IL-18 knockout mice, which unlike IL-1 $\beta$  knockout mice showed protection (Jha *et al*, 2010). Moreover, IL-1 $\beta$  and IL-18 had a differential effect on the process of remyelination after cuprizone treatment, demonstrating a delay in remyelination in IL-1 $\beta$  knockout mice, whereas accelerated remyelination was seen in IL-18 knockouts (Jha *et al*, 2010).

### Inflammasome activation in stroke

Neuroinflammation plays a crucial pathological role in stroke, and IL-1 $\beta$  has been identified as a key cytokine in stroke pathology. So far, four distinct inflammasomes have been implicated in stroke, *viz.* NLRP1, NLRP3, NLRC4, and AIM2 (Barrington *et al*, 2017). Additionally, early studies implicated caspase-11 in middle cerebral artery occlusion (MCAO), a mouse model of stroke (Kang *et al*,

2000). Expression levels of NLRP3 and NLRP1, as well as cleavage products of caspase-1, IL-1 $\beta$ , and IL-18, were shown to be elevated in postmortem brain tissue of stroke patients (Fann *et al*, 2013). Studies with pharmacological inhibitors of inflammatory caspases and analysis of caspase-1-deficient mice—that were later shown to also lack caspase-11 expression (Kayagaki *et al*, 2011)—showed protection in experimental models of stroke (Friedlander *et al*, 1997; Hara *et al*, 1997; Schielke *et al*, 1998; Rabuffetti *et al*, 2000; Ross *et al*, 2007). In agreement, mice deficient in both IL-1 $\alpha$  and IL-1 $\beta$  exhibited dramatically reduced ischemic infarct volumes in transient MCAO (Boutin *et al*, 2001), whereas IL-18-deficient mice did not show protection in this model (Wheeler *et al*, 2003). NLRP3 deficiency in mice, treatment with the NLRP3 inflammasome inhibitor MCC950/CRID3, and intracerebroventricular injection of NLRP3 siRNAs all ameliorated clinical outcomes in experimental models of stroke (Ma *et al*, 2014; Yang *et al*, 2014; Yuan *et al*, 2015; Ismael *et al*, 2018b). Also, NLRC4- and AIM2-deficient mice were shown to have significantly smaller infarct volumes compared to wild-type mice subjected to tMCAO, with was associated with strongly reduced microglia cell activation and leukocyte recruitment to the infarct site (Denes *et al*, 2015). Finally, an expression analysis in the peri-infarct zone of transient MCAO rats revealed elevated levels of the inflammasome components NLRP1, NLRP3, AIM2, NLRC4, and ASC (Lammerding *et al*, 2016), consistent with the potential activation of multiple inflammasomes in stroke.

### Inflammasome activation in traumatic brain and spinal cord injury

Patients with traumatic brain injury (TBI) have significantly higher levels of inflammasome markers in their CSF, including ASC, caspase-1, NLRP1, and NLRP3 (Adamczak *et al*, 2012; Wallisch *et al*, 2017). However, NLRP1b and ASC knockout mice did not show any improvement in motor recovery or lesion volume in conditions of TBI, compared to control mice, although their levels of IL-1 $\beta$  were reduced (Brickler *et al*, 2016). NLRP3 expression localizing to neurons, microglia, and astrocytes was also detected in experimental TBI (Liu *et al*, 2013a), and NLRP3-deficient mice as well as pharmacological blockade of NLRP3 activation were shown to improve recovery from TBI (Irrera *et al*, 2017). Treatment with IL-1 $\beta$  neutralizing antibodies also was shown to improve the cognitive outcome following TBI (Clausen *et al*, 2009). In contrast to IL-1 $\beta$  levels, which rapidly increase after TBI, IL-18 levels gradually increase over several days, suggesting that IL-1 $\beta$  may be involved in the early phase of TBI, while IL-18 may contribute to pathogenesis at later phases of the disease. In agreement, IL-18 inhibition resulted in improved neurologic recovery after 7 days with little effect on the early response being observed immediately after injury (Yatsiv *et al*, 2002).

Similar to TBI, spinal cord injury (SCI) induced higher levels of NLRP1, ASC, caspase-1, IL-1 $\beta$ , and IL-18 in mice, and therapeutic neutralization of ASC was proposed to result in tissue sparing and functional improvement due to reduced inflammasome activation in neurons (de Rivero Vaccari *et al*, 2008). Furthermore, expression of heme oxygenase-1 was shown to suppress NLRP1 inflammasome activation and neuronal cell death, and lead to improved functional recovery after SCI (Lin *et al*, 2016). Also, enhanced NLRP3

expression can be demonstrated in SCI, predominantly in neurons, but also in microglia and astrocytes (Zendedel *et al*, 2016).

### Inflammasome activation in Alzheimer's disease

Alzheimer's disease (AD) is the most common age-related neurodegenerative disorder and is characterized by progressive memory deficits and cognitive impairment (Sala Frigerio & De Strooper, 2016). AD pathology is dominantly explained by the amyloid hypothesis, which suggests that the increasing extracellular deposition of misfolded amyloid-beta (A $\beta$ ) is the primary cause of the disease. Although there is overwhelming evidence for a pathogenic role for A $\beta$  in AD, neuroinflammation is increasingly regarded as another key component that actively contributes to AD pathology (De Strooper & Karran, 2016; Sala Frigerio & De Strooper, 2016). Neuroinflammation in AD is primarily driven by the CNS-resident microglia population (Sarlus & Heneka, 2017; Shi & Holtzman, 2018). Although it is recognized that microglia may exert benign and reparative activities in AD through the phagocytic removal of A $\beta$  deposits, the accumulation of A $\beta$  may also prime microglial cells and promote their activation and production of inflammatory mediators. Moreover, upon A $\beta$  accumulation, microglial cells may become progressively impaired in their ability to phagocytose A $\beta$  (Sarlus & Heneka, 2017; Shi & Holtzman, 2018). Furthermore, a microglial inflammatory state would also impact on other CNS-resident cells and result in synaptic dysfunction and neuronal damage.

Many recent studies have implicated inflammasomes in the development of AD. Elevated expression of IL-1 $\beta$  has been reported in microglia that surround A $\beta$  plaques of AD patients (Griffin *et al*, 1989; Simard *et al*, 2006). Additionally, gene expression analysis of cultured PBMCs from AD patients revealed higher expression of NLRP3, ASC, caspase-1, and caspase-5 as well as the cytokines IL-1 $\beta$  and IL-18 (Saresella *et al*, 2016). The expression levels of NLRC4 and ASC were also found significantly elevated in brain samples of a subgroup of sporadic AD patients (Liu & Chan, 2014). Finally, genetic variants of NLRP1 have been associated with AD risk (Pontillo *et al*, 2012), and the amount of NLRP1 immunopositive neurons was strongly increased in AD brain (Kaushal *et al*, 2015).

*In vitro* studies have shown that fibrillar A $\beta$  activates the NLRP3 inflammasome when phagocytosed by microglia, leading to activation of caspase-1 and release of IL-1 $\beta$  (Halle *et al*, 2008). NLRP3 inflammasome activation has also been documented *in vivo* in the transgenic APP/PS1 mouse model of AD, and deficiency in NLRP3 significantly ameliorated spatial memory deficits and hyperactive behavior in these mice, which was associated with reduced hippocampal and cortical A $\beta$  deposition, smaller plaque volumes, decreased levels of pro-inflammatory cytokines, and improved microglial phagocytic ability (Heneka *et al*, 2013). Similar results were obtained in APP/PS1 mice that were deficient in caspase-1 (Heneka *et al*, 2013). Inhibition of the NLRP3 inflammasome by MCC950/CRID3 also promoted amyloid- $\beta$  clearance and ameliorated cognitive function in APP/PS1 mice (Dempsey *et al*, 2017). Indirect inhibition of NLRP3 inflammasome activation by clinically approved fenamate non-steroidal anti-inflammatory drugs that target cyclooxygenase enzymes and volume-regulated anion channels (VRAC) also was shown to suppress microglia-mediated neuroinflammation and memory loss in 3 $\times$ TgAD mice (Daniels

et al, 2016). Finally, pharmacological inhibition of caspase-1 activity by VX-765 reduced amyloid- $\beta$  accumulation, brain inflammation, and cognitive impairment (Flores et al, 2018). Collectively, these findings suggest that misfolded A $\beta$  activates the microglial NLRP3 inflammasome, which triggers the release of pro-inflammatory factors that perpetrate a chronic neuroinflammatory environment and promote AD pathology. In addition, NLRP3 inflammasome activity also results in the extracellular release of micrometer-sized ASC particles that may function as danger signals and alert surrounding macrophages (Baroja-Mazo et al, 2014). These ASC specks were shown to physically bind to A $\beta$  to seed and spread A $\beta$  pathology in a prion-like manner by promoting misfolded protein aggregation and plaque formation in the APP/PS1 AD model (Venegas et al, 2017).

A pathogenic role for IL-1 $\beta$  in AD has been demonstrated in mice with a deficiency in IL-1 receptor antagonist (IL-1ra), which resulted in increased vulnerability to intracerebroventricular injection with human oligomeric A $\beta$ 1–42 (Craft et al, 2005). Another study showed that IL-1 $\beta$  injection in the cerebral hemisphere increases A $\beta$ -APP protein levels in wild-type mice (Sheng et al, 1996). In contrast, sustained hippocampal IL-1 $\beta$  overexpression in APP/PS1 mice was shown to reduce plaque pathology, an observation that might be explained by the increased phagocytic activity of microglia and macrophages (Shaftel et al, 2007). Similar observations were done in 3xTgAD mice, but tau phosphorylation was also increased in these mice, demonstrating the complex role of IL-1 signaling in AD (Ghosh et al, 2013). IL-1R1 deficiency in mice had no effect on A $\beta$  deposition in Tg2567 AD mice (Das et al, 2006). Similarly, IL-18 deletion did not confer a protective effect in APP/PS1 mice (Tzeng et al, 2018). However, these mice were shown to be highly susceptible to grand-mal seizures and exhibited increased excitatory synaptic proteins and dendritic spine density, an imbalance which leads to a higher basal excitatory synaptic transmission (Tzeng et al, 2018). Therefore, IL-18 may have an unexpected role in controlling neuronal activity.

Although the focus has been on the role of NLRP3 in AD, the role of other inflammasomes has also been characterized in the context of AD. The AIM2 inflammasome was shown to increase A $\beta$  deposition, microglia activation, and cytokine production, but does not affect behavior or memory in transgenic 5xFAD mice (Wu et al, 2017). NLRP1 knockdown in APP/PS1 mice was shown to result in significantly reduced neuronal pyroptosis and to rescue cognitive impairments (Tan et al, 2014). Finally, the NLRC4 inflammasome was shown to enhance neuronal A $\beta$  levels through activated pro-inflammatory astrocytes (Liu & Chan, 2014).

### Inflammasome activation in amyotrophic lateral sclerosis

Amyotrophic lateral sclerosis (ALS) is a fatal neurodegenerative disease that is caused by the progressive loss of upper and lower motor neurons and leads to muscle weakness and wasting (Taylor et al, 2016). Twenty percent of familial ALS cases are caused by mutations in the gene that encodes superoxide dismutase 1 (SOD1), and many experimental models of ALS rely on expression of mutant SOD1. Deficiency in caspase-1 and IL-1 $\beta$ , as well as treatment with the recombinant IL-1 receptor antagonist anakinra, all significantly extended the lifespan of mutant SOD1 transgenic animals (Meissner

et al, 2010). Consistently, the expression of NLRP3, NLRC4, AIM2, and caspase-1 activation has been demonstrated in neural tissue of mutant SOD1 transgenic animals (Pasinelli et al, 1998; Johann et al, 2015; Gugliandolo et al, 2018), and mutant SOD1 was shown to activate caspase-1 and IL-1 $\beta$  in microglia (Meissner et al, 2010; Lehmann et al, 2018). Increased caspase-1 levels have also been detected in serum of ALS patients (H $\check{z}$ becka et al, 2001), and analysis of postmortem spinal cord tissue showed increased NLRP3, ASC, caspase-1, and IL-18 expression levels, with spinal cord astrocytes identified as the main NLRP3 inflammasome-expressing cell type (Johann et al, 2015). However, clinical studies with anakinra in ALS patients have not shown a significant reduction in disease progression (Maier et al, 2015), suggesting that inflammasome activation does not play a major role in ALS, or that the pathology is driven by IL-18 or by DAMPs via pyroptosis.

### Inflammasome activation in Parkinson's disease

Parkinson's disease (PD) is a progressive neurodegenerative disorder caused by the loss of dopaminergic neurons in the substantia nigra pars compacta. Lewy bodies are pathological hallmarks of the disease that predominantly consist of intra-neuronal aggregates of fibrillar  $\alpha$ -synuclein (Przedborski, 2017). Analysis of the serum of PD patients revealed increased levels of IL-1 $\beta$  and caspase-1 (Zhou et al, 2016), and elevated IL-1 $\beta$  levels were observed in the striata of PD patients (Mogi et al, 1994). The midbrain of A53T transgenic mice, which model PD based on the overexpression of mutant human A53T  $\alpha$ -synuclein, also contained increased IL-1 $\beta$  concentrations (Zhou et al, 2016). Chronic expression of IL-1 $\beta$  in the substantia nigra of rats by a recombinant adenovirus expressing IL-1 $\beta$  was shown to induce progressive death of dopaminergic neurons and resulted in motor impairments (Ferrari et al, 2006). However, most of the evidence linking PD to inflammasome signaling comes from *in vitro* studies, and the importance of inflammasomes for the disease is not completely understood.  $\alpha$ -Synuclein was shown to trigger activation of the NLRP3 inflammasome in human monocytes and BV2 microglial cells (Codolo et al, 2013; Gustot et al, 2015; Zhou et al, 2016), but not in primary microglia (Gustin et al, 2015). *In vivo*, microinjection of the caspase-1 inhibitor Ac-YVAD-CMK was shown to reduce the expression of NLRP3 inflammasome signaling proteins and improve the number of dopaminergic neurons in LPS-induced and 6-hydroxydopamine-induced PD in rats (Mao et al, 2017). In agreement, NLRP3 knockout mice were resistant to the loss of nigral dopaminergic neurons induced by treatment with the neurotoxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), which was associated with a reduction in caspase-1 activation and IL-1 $\beta$  and IL-18 secretion (Yan et al, 2015). Recently, caspase-1 was shown to cleave  $\alpha$ -synuclein *in vitro*, generating an aggregation-prone protein that was toxic to cultured neurons (Wang et al, 2016). Finally, mutations in *Parkin*, *PARK2*, *PARK6*, and *PINK1* have been identified in patients with autosomal recessive early-onset PD, and microglia and macrophages from *PARK2* and *PINK1* knockout mice and patients with *PARK2* mutations have been shown to display an exacerbated NLRP3 inflammasome response, possibly due to impaired expression of the anti-inflammatory protein A20 that negatively regulates NLRP3 inflammasome activation (Mouton-Liger et al, 2018).



## Inflammasome activation in prion disease

Prion diseases are a group of rapidly progressive neurodegenerative disorders caused by misfolded aggregated infectious prion proteins (PrP) (Sigurdson *et al*, 2018). IL-1 $\beta$  levels are increased in the CSF of patients with Creutzfeldt–Jakob disease (CJD) (Van Everbroeck *et al*, 2002), and IL-1 $\beta$  and cleaved caspase-1 levels were reported to be upregulated in the brains of scrapie-infected mice (Schultz *et al*, 2004). Stimulation of microglia with an amyloidogenic PrP peptide induced NLRP3 activation and IL-1 $\beta$  secretion *in vitro* (Shi *et al*, 2012). Stimulation of microglia with PrP fibrils was also shown to induce toxicity in neurons (Hafner-Bratkovič *et al*, 2012). Mechanistically, NLRP3 inflammasome activation was suggested to negatively regulate autophagy in microglia, thereby contributing to neurodegeneration (Lai *et al*, 2018). Inhibition of IL-1R signaling with anakinra was shown to reduce seizure susceptibility in a CJD mouse model (Bertani *et al*, 2017). However, genetic ablation of NLRP3 or ASC in mice did not affect prion pathogenesis, suggesting that the NLRP3 inflammasome does not play a significant role in prion disease (Nuvolone *et al*, 2015).

## Inflammasome activation in Huntington's disease

Huntington's disease (HD) is an autosomal dominant progressive neurodegenerative disease that is caused by the expansion of a trinucleotide CAG repeat in the 5' coding region of the huntingtin gene, leading to the expression of an abnormal protein that gradually damages cells in the brain (Caron *et al*, 2018). Caspase-1 activation can be detected in the brains of HD patients and in mouse models of HD, and inhibition of caspase-1 was shown to slow down disease progression in the R6/2 mouse model of HD (Ona *et al*, 1999). Mechanistically, caspase-1 was shown to cleave mutant and wild-type huntingtin *in vitro* (Wellington *et al*, 1998) and *in vivo* (Ona *et al*, 1999), potentially contributing to the neurodegeneration seen in HD. Similarly, treatment with the tetracycline derivative minocycline was shown to delay disease progression by inhibition of caspase-1 and caspase-3 expression (Chen *et al*, 2000).

## Pharmacological targeting of inflammasomes

The central role of inflammasomes in neuroinflammatory responses, in particular NLRP3, makes it an attractive drug target for neurodegenerative diseases. Currently approved therapies in the clinic target the downstream effector cytokines released by inflammasome activation, such as anakinra (IL-1 receptor antagonist), canakinumab (IL-1 $\beta$  neutralizing antibody), and riloncept (soluble decoy receptor for IL-1 $\beta$  and IL-1 $\alpha$ ) (Dinarello *et al*, 2012). While valuable in treating autoinflammatory diseases (Van Gorp *et al*, 2019), these IL-1 inhibitors do not prevent pyroptosis and IL-18-driven immune activation. The need for subcutaneous administration of these biological agents represents another drawback of these biological agents (Moran *et al*, 2013; Rossi-Semerano *et al*, 2015), especially in the case of anakinra, which requires daily injections due to a short half-life of around 4–6 h (Granowitz *et al*, 1992). Moreover, constitutive neutralization of IL-1 $\beta$ -driven signaling increases the risk for infections, as observed in the recent CANTOS trial (Ridker

*et al*, 2017). Finally, the concentration of anakinra in CSF of healthy non-human primates did not surpass 0.2–0.3% of the peripheral serum concentration, demonstrating poor penetration of the BBB (Fox *et al*, 2010). These observations urge for the development of small molecule inflammasome antagonists with improved pharmacokinetic properties and efficacy, and that are able to cross the BBB.

Several small molecule inhibitors that target the inflammasome components caspase-1, NLRP3, and GSDMD have been reported (Table 2). Since caspase-1 activation is common to all inflammasome complexes, caspase-1 inhibition would serve as a pan-inflammasome inhibitory strategy, while compounds targeting NLRP3 have a narrower selectivity profile. Targeting the inflammasome adaptor ASC would have an intermediate selectivity profile; however, compounds that target ASC have not been reported to date (Mangan *et al*, 2018). A number of NLRP3 inhibitory compounds have been identified and validated in a suite of disease models (Table 2). However, for most agents it is not known whether they directly target NLRP3 or indirectly act on the pathway via other mechanisms, hampering clinical development of these molecules (Mangan *et al*, 2018). To date, only very few compounds targeting NLRP3 or caspase-1 have entered clinical trials. RP-1127, an intravenous formulation of the NLRP3 inflammasome inhibitor glyburide (Lamkanfi *et al*, 2009), is being tested in a clinical trial for stroke (EudraCT 2017-004854-41) after a positively evaluated pilot study (ClinicalTrials.gov: NCT01268683) (Sheth *et al*, 2014). RP-1127 is also being tested in a clinical trial for TBI (ClinicalTrials.gov: NCT01454154). In addition, the caspase-1 inhibitor VX-765 entered a phase II clinical trial for use in epilepsy (ClinicalTrials.gov: NCT01048255) and psoriasis (ClinicalTrials.gov: NCT00205465), but further development has been discontinued (Mackenzie *et al*, 2010). The encouraging activity of small molecule NLRP3 inflammasome antagonists in preclinical studies warrants the development of inflammasome-targeting therapies for clinical use.

Pyroptosis is emerging as another key mechanism that contributes to inflammasome-driven pathology in metabolic, autoimmune, and neurodegenerative diseases. Cleavage of GSDMD by the inflammatory caspase-1 and caspase-11 (mouse), or caspase-1, caspase-4, and caspase-5 (human) releases an amino-terminal pore-forming GSDMD domain that oligomerizes and inserts in the plasma membrane, inducing membrane rupture and leakage of the cytosolic contents (Vande Walle & Lamkanfi, 2016). Hence, GSDMD inhibition has been proposed as a novel therapeutic strategy to prevent inflammasome-driven pathology in different diseases. Pyroptosis has recently been shown to be the critical mechanism of IL-1 $\beta$ -mediated systemic pathology in the autoinflammatory disease Familial Mediterranean Fever (FMF), which is caused by missense mutations in *Mefv* that activates the pyrin inflammasome (Kanneganti *et al*, 2018). Similarly, GSDMD was shown to control inflammasome-driven pathology in neonatal-onset multisystem inflammatory disease (NOMID), which is caused by activating mutations in the inflammasome sensor *NLRP3* (Xiao *et al*, 2018). Indeed, FMF knockin mice and NOMID mice were shown to be fully protected from developing the respective autoinflammatory syndromes when crossed into a *Gsdmd*-deficient background (Kanneganti *et al*, 2018; Xiao *et al*, 2018). GSDMD was also demonstrated to play a key role in the pathogenesis of steatohepatitis by controlling cytokine secretion, and *Gsdmd* knockout mice exhibit decreased severity of steatosis and inflammation in a high-fat

**Table 2. Overview of pharmacological compounds targeting inflammasome signaling.**

Compounds	Benefit (+)/detriment (–)	Neurodegenerative disease model
<b>Caspase-1 inhibitors</b>		
VX-765 and VX-740	(–) No further development after phase II clinical trial for use in epilepsy and psoriasis (Mangan et al, 2018)	Alzheimer disease (Flores et al, 2018) EAE (Mckenzie et al, 2018)
<b>NLRP3 inflammasome inhibitors</b>		
<i>Sulfonylurea-based compounds</i>		
Glyburide	(+) Specific for NLRP3 inflammasomes; significantly delays LPS-induced mortality (Lamkanfi et al, 2009) (–) High dosage required (Marchetti et al, 2014); cardiovascular side effects (Riddle, 2003)	TBI (Simard et al, 2012) Ischemic stroke (Simard et al, 2012)
CP-412,245 and CP-424,174	(+) Oral administration of CP-424,174 selectively blocks IL-1 production in mice (Perregaux et al, 2001)	
CRID1 and CRID2	(–) No <i>in vivo</i> evidence	
MCC950 (also known as CRID3 or CP-456,773)	(+) NLRP3 inflammasome specific (Coll et al, 2015); inhibits NLRP3 activation by all known stimuli (Mangan et al, 2018) (–) Precise molecular target unknown	Alzheimer disease (Dempsey et al, 2017) EAE (Coll et al, 2015) TBI (Ismael et al, 2018a; Xu et al, 2018) Stroke (Ismael et al, 2018b; Ren et al, 2018)
16673-34-0	(+) Lacks cyclohexylurea group responsible for hypoglycemic activity; prevents NLRP-mediated myocardial injury (Marchetti et al, 2014)	
Hybrid molecules (combining MCC950 and glyburide)	(–) Moderately effective at inhibiting NLRP3 compared to MCC950 (Hill et al, 2017)	
<i>Fenamate classes of NSAIDs</i>		
Flufenamic and mefenamic acid	(+) Inhibits NLRP3 by blocking VRACs (Daniels et al, 2016) (–) Lack of specificity, inhibits multiple inflammatory nodes (Daniels et al, 2016)	Alzheimer disease (Daniels et al, 2016)
<i>Michael acceptors</i>		
Parthenolide	(–) No suitable pharmacological properties (Baldwin et al, 2016); inhibits NF- $\kappa$ B-dependent signaling (Saadane et al, 2007)	Stroke (Dong et al, 2013)
BAY 11-7082	(–) Not specific; inhibits NF- $\kappa$ B-dependent signaling (Lee et al, 2012)	TBI (Irrera et al, 2017)
3,4-methylenedioxy- $\beta$ -nitrostyrene (MNS)	(–) Modest potency (Baldwin et al, 2016)	
Acrylate and acrylamide derivatives (ex. IFN58, IFN39)	(+) Oral administration of IFN39 alleviates DNBS-induced colitis in rats (Cocco et al, 2017)	
<i>Novel boron compound series</i>		
NBC13	(+) Significantly decreases LPS-induced IL-1 $\beta$ production <i>in vivo</i> (Baldwin et al, 2017)	
<i>Other NLRP3 inhibitors</i>		
Fc11a-2	(+) <i>In vivo</i> efficacy in DSS-induced colitis (Liu et al, 2013b)	
CY-09	(+) Therapeutic effect in mouse models of CAPS and type 2 diabetes (Jiang et al, 2017)	
JC-171	(+) Treatment effective in EAE in both prophylactic and therapeutic settings (Guo et al, 2017)	EAE (Guo et al, 2017)
OLT-177	(+) No adverse effects in preliminary clinical testing of healthy humans (Marchetti et al, 2018)	
$\beta$ -hydroxybutyrate (BHB)	(–) Not specific for NLRP3; can inhibit HDACs (Youm et al, 2015)	
<b>GSDMD inhibitors</b>		
Antabuse	(+) Efficacious in sepsis models (preprint: Hu et al, 2018)	
Necrosulfonamide	(+) Efficacious in sepsis models (Rathkey et al, 2018)	

diet-induced model of non-alcoholic steatohepatitis (Xu *et al*, 2017). Pharmacologic inhibition of GSDMD by necrosulfonamide also was efficacious in sepsis models (Rathkey *et al*, 2018). Finally, antabuse (disulfiram), a drug used to treat alcohol addiction, was shown to inhibit GSDMD pore formation and IL-1 $\beta$  secretion in human and mouse cells, and LPS-induced septic death and IL-1 $\beta$  secretion in mice (preprint: Hu *et al*, 2018). Together, these observations suggest that inhibition of GSDMD-dependent pyroptosis may also hold promise for the treatment of inflammasome-mediated neuroinflammatory pathology.

## Concluding remarks and future perspectives

Although only discovered in 2002 (Martinon *et al*, 2002), inflammasomes are now widely regarded as central regulators of innate immunity playing an indispensable role in the host defense against stress conditions and pathogens that might be harmful to the host. However, inflammasome activation leading to excessive or prolonged inflammation can also contribute to a damaging and destructive environment resulting in the development of inflammatory diseases, including neurodegenerative diseases (Lamkanfi & Dixit, 2012).

Most research in the field has focused on the role of the NLRP3 inflammasome, largely neglecting the potential importance of other inflammasome complexes in CNS disorders. Future research will have to investigate and characterize less well-known inflammasomes and expand the understanding of inflammasome signaling and its importance for neurodegeneration. The generation of new mouse models and reagents that allow selective targeting of the inflammatory mechanisms initiated by these inflammasomes will be crucial to this end. Also, although the importance of inflammasome activation has been demonstrated in many neurodegenerative diseases and experimental model systems, their specific role in the different CNS cell types is not always clear. Most research has focused on microglia, but other brain cell types also have functional inflammasomes which may contribute to disease pathology (Walsh *et al*, 2014). Since most studies published so far have investigated inflammasome activation using conventional “full body” knockout mice which do not allow to specify the relevant cell types involved, cell type-specific inflammasome mutant mice will be needed to address the importance of inflammasome signaling in CNS-specific cell types, assessing their importance for cytokine production, pyroptosis, and CNS inflammation. Also, new techniques including single-cell RNA sequencing and mass cytometry, in combination with high-resolution microscopy and *in vivo* cell imaging, should help to get a better view on the spectrum of inflammasomes that is expressed in the different CNS-resident cell types in different (pathological) conditions.

Major progress needs to be made in translating findings from animal studies to humans. However, key differences exist between human and mouse inflammasomes. Humans have only a single *NLRP1* gene, while mice have three isoforms of *Nlrp1*. Similarly, humans have two orthologues for caspase-11, *viz.* caspase-4 and caspase-5 (Lamkanfi & Dixit, 2012). More research using human cell types, eventually based on iPS technology and patient-derived material, will be needed to translate findings more effectively from rodents to the human conditions they model.

Finally, since inflammasome signaling is central to many neurodegenerative diseases, targeting their activation is regarded as a potential therapy to treat these diseases. Targeting the downstream IL-1 $\beta$  and IL-18 cytokines is probably not the best approach since these cytokines are needed for optimal immunity in the host. Moreover, cytokine-targeting biologics have limited capacity to penetrate the blood–brain barrier. Therefore, modulating the activity of specific inflammasome sensors and cell type-specific inflammasome targeting with small molecules will be vital in this context and may have better potential as therapies to treat patients that suffer from neurodegenerative diseases (Guo *et al*, 2015). Also, pyroptosis is now being identified as a critical mechanism driving inflammatory pathology, suggesting GSDMD inhibition as a potential anti-inflammatory strategy (Kanneganti *et al*, 2018; Mckenzie *et al*, 2018; Xiao *et al*, 2018).

## Acknowledgements

S.V. is a predoctoral fellow with the Institute for the Promotion of Innovation by Science and Technology (IWT) and is supported by grants from the Charcot Foundation and the Queen Elisabeth Medical Foundation. S.S. is a predoctoral fellow with the FWO and is supported by a “Hope in Head” Rotary grant. Work in the G.v.L. laboratory is supported by research grants from the FWO, the Queen Elisabeth Medical Foundation, the CBC Banque Prize, the Charcot Foundation, the “Belgian Foundation Against Cancer”, and the “Concerted Research Actions” (GOA) of the Ghent University. M.L. is supported by European Research Council Grant 683144 (PyroPop).

## Conflict of interest

The authors declare that they have no conflict of interest.

### Pending issues

- (i) The cell type-specific contribution of inflammasome signaling to neurodegenerative diseases, both peripheral and CNS resident.
- (ii) Direct evidence for inflammasome activation and signaling in patients with neurodegenerative disease.
- (iii) Characterization of the central activation mechanism and high-resolution structure of inflammasome proteins.
- (iv) Development of compounds able to cross the BBB and that selectively inhibits those inflammasomes that contribute to the pathogenicity.

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