Inflammasome signalling in brain function and neurodegenerative disease

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Abstract | The mammalian CNS is an intricate and fragile structure, which on one hand is open to change in order to store information, but on the other hand is vulnerable to damage from injury, pathogen invasion or neurodegeneration. During senescence and neurodegeneration, activation of the innate immune system can occur. Inflammasomes are signalling complexes that regulate cells of the immune system, which in the brain mainly includes microglial cells. In microglia, the NLRP3 (NOD-, LRR- and pyrin domain-containing 3) inflammasome becomes activated when these cells sense proteins such as misfolded or aggregated amyloid-β, α-synuclein and prion protein or superoxide dismutase, ATP and members of the complement pathway. Several other inflammasomes have been described in microglia and the other cells of the brain, including astrocytes and neurons, where their activation and subsequent caspase 1 cleavage contribute to disease development and progression.

Innate immune system
Evolutionarily conserved arm of the immune system that recognizes pathogens and molecules arising in danger situations via germline-encoded signalling receptors and provides the first line of defence.

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All classes of animal or plant life require an efficient immune system that is able to detect and respond to damage to tissues or to the invasion of pathogenic microorganisms. Most multicellular species have only one immune system — the innate immune system — which provides rapid but nonspecific defence against infection. Jawed vertebrates (gnathostomes), however, evolved an additional, pathogen-specific immune response system, the adaptive immune system. This system is characterized by T lymphocytes and B lymphocytes, which express a large variety of specific receptors that arise through genetic recombination[1]. Innate immunity relies on several classes of germline-encoded signalling receptors that can directly sense classes of molecules with specific molecular patterns of foreign origin, allowing cells of the innate immune system to recognize and respond to a large variety of microorganisms with a limited set of signalling receptors[2]. Because these receptors can recognize molecular patterns, they are also called pattern recognition receptors (PRRs). Another important function of innate immune signalling receptors is their ability to sense altered host molecules or host molecules that appear in non-physiological subcellular localizations under conditions of tissue stress or after tissue damage. The recognition of these so-called danger signals[3,4] is highly relevant for efficient detection of sterile tissue damage, metabolic alterations and general stress in tissues.

Innate immune signalling receptors are expressed in a variety of specialized innate immune cells, such as macrophages, dendritic cells or neutrophils, where their activation leads to changes in cellular metabolism and signalling networks and that together alter transcriptional responses and cellular functions. Innate immune cell activation leads to changes in their antimicrobial activity, migration and output of inflammatory molecules. A variety of these innate immune receptors that are expressed in classical immune cells can also be found in non-immune cells such as stromal cells, epithelial cells or endothelial cells. In these cells, innate immune signalling receptors are part of the cell-autonomous immunity that protects these non-immune cells from infections and that can contribute to inflammatory tissue responses.

In addition to their function in infection control and the response to tissue damage, tissue-resident immune cells, such as macrophages, also support physiological tissue functions and are involved in tissue remodelling and adaptation processes. For example, in the brain, microglia are the principal innate immune cell that executes a number of physiological functions important for the maintenance of tissue homeostasis, synapse remodelling and neurotrophic factor secretion.

Innate immune functions are generally beneficial for the host, as they provide efficient protection from infectious organisms and lead to reconstitution of tissue homeostasis after injurious insults. However, innate immune mechanisms can also lead to non-resolving inflammatory reactions during chronic infections, repeated tissue damage or in response to overabundance of innate immune triggers present in tissues. A premier

example of the latter response is the ongoing inflammation found in a variety of neurodegenerative diseases that can be maintained by the key innate immune sensor for danger signals, the NLRP3 (NOD-, LRR- and pyrin domain-containing 3) inflammasome.

In this Review, we discuss the latest findings at the intersection between the fields of innate immunity and neurodegeneration. We focus our discussions on the latest developments in inflammasome biology, describe the current understanding of how inflammasomes can be triggered in the brain and summarize our knowledge on the relevance of inflammasome activation for the pathogenesis of various neurodegenerative diseases.

Principles of inflammasome activation

By gathering molecular clues that indicate either the invasion of tissues by microorganisms or events causing tissue damage or both, innate immune sensors integrate crucial information that is in turn translated into a signalling event aimed to fight off the invading microbial intruders and to repair damaged tissues. The main goal of innate immune activation by infectious or non-infectious insults is to set in motion mechanisms that aim to prevent further tissue or organ dysfunction and to regain tissue homeostasis[5].

Different modes of signal transduction have evolved that allow the cell to respond appropriately to a diverse nature of threats. Most innate immune signalling receptors, such as representatives of the Toll-like receptor (TLR) or RIG-I-like receptor (RLR) families and others, induce a complex network of signalling events that result in the de novo synthesis through transcriptional regulation of effector molecules, such as cytokines or chemokines[6,7] or non-proteinaceous signalling molecules such as bioactive lipid species. These factors act in a paracrine fashion, both locally and systemically, to alert other cell types of the imminent danger of infectious processes or to set in motion processes involved in the repair of damaged tissues. Innate immune signalling receptors can further mediate other important effector functions, such as phagocytosis and reactive oxygen production, leading to killing of microorganisms. Excitingly, even alterations in metabolic processes can be induced by activation of innate immune system signalling, a process that aids in the cellular adaptation to increased energy demands and gives rise to enhanced availability of building blocks for the de novo synthesis of the nucleic acids, proteins, lipids or metabolites required for the altered cellular functions during stress situations[8].

Members of another family of innate immune sensors, the NOD-like receptors (NLRs), include proteins that control post-transcriptional (rather than transcriptional signalling) events upon sensing of a particular trigger. After the activation of the cytosolic signalling receptor proteins, a process is set in motion that leads to the conversion of the inflammatory caspase 1 from an inactive precursor molecule into the cleaved and active enzyme. This activation process is triggered following the formation of a large, multi-molecular signalling complex that is called the inflammasome[9] (FIG. 1). Once the inflammasome sensor molecules are activated by a trigger, they undergo conformational changes leading to the loss of an autoinhibited state, which allows these molecules to interact with themselves, thus undergoing oligomerization, in which they can then trigger the helical fibrillar assembly of a downstream adaptor protein called apoptosis-associated speck-like protein containing a CARD (ASC; also known as PYCARD)[10]. The fibrillar ASC acts as a molecular platform that recruits pro-caspase 1, which is then activated by proximity-induced autocatalysis. Active caspase 1 then induces the post-transcriptional processing of pro-inflammatory cytokines of the interleukin-1β (IL-1β) family; in addition, caspase 1 can also mediate a pro-inflammatory form of cell death called pyroptosis[11].

Similar to other innate immune signalling receptors, a key function of inflammasomes is to detect and respond to microorganisms and to tissue damage. The inflammasome sensor molecules of the absent in melanoma 2 (AIM2)-like receptor sense various forms of nucleic acids (AIM2 and interferon-γ (IFNγ)-inducible protein 16 (IFI16)), whereas the NLR inflammasome sensors recognize a broad range of molecules signifying an infection or a breach of cellular integrity. For example, microbial-derived metabolites are sensed by NLRP6 (REFS[12,13]), and toxins that alter the cellular cytoskeleton indirectly stimulate the pyrin inflammasome[14]. The NLRP3 inflammasome has the ability to respond to a broad range of aggregated substances owing to recognition of damage to internal membranes[15], which appears to be important for numerous diseases in which crystal or proteinaceous aggregate deposition can be observed (FIG. 2). In addition, the NLRP3 inflammasome is activated by triggers that alter cellular ion homeostasis and that perturb certain metabolic pathways[16].

Inflammasome activation in the brain

Historically, inflammasomes have been studied in cells of the myeloid lineage; therefore, most of the current brain-related literature refers to studies on microglia, the principal innate immune cell of the CNS. NLRP3, which was the first inflammasome to be studied in the brain, is predominantly located in microglia, and its function and binding components have been investigated in several cerebral pathologies, including Alzheimer disease (AD), Parkinson disease (PD), amyotrophic lateral sclerosis (ALS), traumatic brain injury (TBI) and CNS infection by viruses (see TABLE 1 and Supplementary Table 1 for details). Expression of NLRP3 can be found in other brain cells such as the oligodendrocytes of newborn mice after prenatal exposure to dexamethasone[17], a glucocorticoid receptor agonist, and in the astrocytes of mice[18] or rats[19] in disease models.

Although NLRP3 is the most commonly studied inflammasome within the CNS, a number of other inflammasomes have been implicated in the progression of neurodegenerative diseases, including NLRP1, NLRP2, NLRC4 (NLR family CARD domain-containing protein 4, also known as ice protease activating factor (IPAF)) and AIM2. These inflammasome complexes behave very similarly in that activation of their sensor leads to oligomerization, forming multiprotein complexes that serve as a platform to activate inflammatory caspases. Some differences remain, for example, NLRP1

Adaptive immune system
The vertebrates' immune subsystem that relies on clonal expansion of specialized immune cells in which highly specific receptors towards antigens are created through genetic recombination of antigen receptor gene segments to provide long-lasting acquired immunity.

T lymphocytes
A type of lymphocyte with cytotoxic, helper, regulatory and memory functions characterized by expression of the T cell receptor.

B lymphocytes
A type of lymphocyte that expresses the B cell receptor that recognizes specific antigens leading to the production of antibodies that function in providing humoral immunity.

Cell-autonomous immunity
The cell intrinsic immune defence that is provided by the function of innate immune signalling receptors expressed in the individual cell.

Inflammasome
Inflammasomes are multiprotein complexes, formed of an inflammasome sensor molecule with the adaptor ASC and the effector caspase 1, that mediate proteolytic activation of IL-1β family cytokines and pyroptotic cell death.

Pyroptosis
An inflammatory form of programmed cell death that is triggered by inflammatory caspases after activation of inflammasomes or cytoplasmic recognition of LPS and danger-associated molecules.

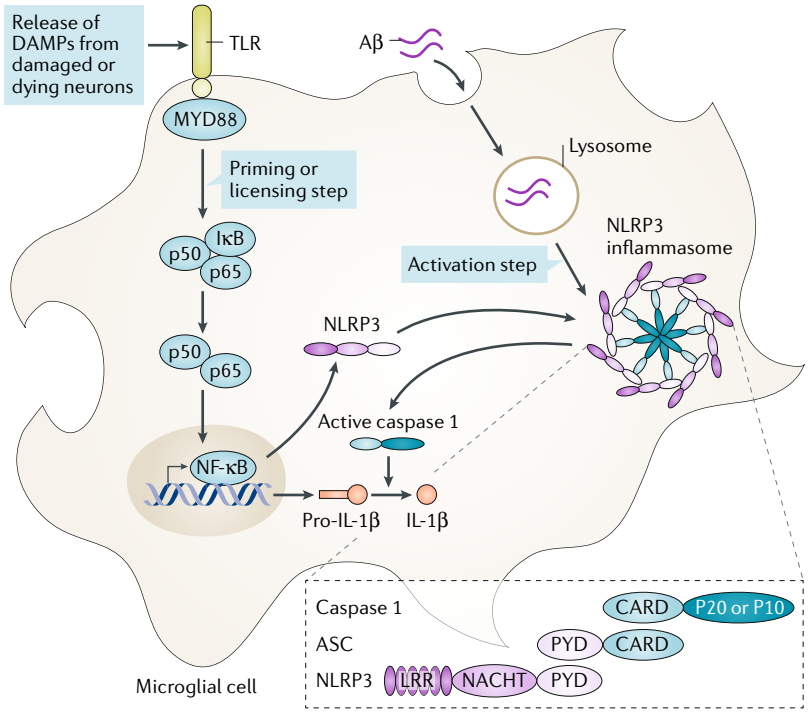

Fig. 1 | **Inflammasome activation in microglia.** Activation of the inflammasome and the production of interleukin-1β (IL-1β) is a tightly controlled process requiring two triggering steps: a priming step and an activation step. The binding of a pathogen-associated molecular pattern to a pattern recognition receptor activates the myeloid differentiation primary response protein MyD88 (MYD88)–nuclear factor-κB (NF-κB) pathway to produce pro-IL-1β. In Alzheimer disease, amyloid-β (Aβ) can induce lysosomal damage, which is the activation step required to induce assembly of the NLRP3 (NOD-, LRR- and pyrin domain-containing 3) inflammasome. Changes in potassium efflux or reactive oxygen species can also trigger inflammasome assembly. This process activates caspase 1, which in turn cleaves pro-IL-1β into IL-1β, allowing the microglial cell to release the bioactive pro-inflammatory cytokine. ASC, apoptosis-associated speck-like protein containing a CARD; CARD, caspase recruitment domain-containing protein; DAMP, danger-associated molecular pattern; IκB, inhibitor of NF-κB; LRR, leucine-rich repeat; NACHT, nucleotide-binding oligomerization domain; PYD, PYRIN domain; TLR, Toll-like receptor.

and NLRC4 can activate caspase 1 without ASC; however, ASC substantially improves the inflammasome complex formation and the production of IL-1β[9]. Several groups have shown that the NLRP1 inflammasome is upregulated in rat neurons after stimulation in vitro or in murine animal models in vivo[20,21], as well as in human neurons[22]. NLRP1 expression was further found in the microglia of monkeys[23] and humans[24] and in human pericytes[25].

The ability of NLRP2 to form an active inflammasome complex has been controversial; however, it is now known to associate with ASC and caspase 1 to form a functional inflammasome and was observed in human astrocytes, where it interacts with P2X purinoceptor 7 (P2X7) and the pannexin 1 channel[26]. The expression of NLRP2 has also been demonstrated in microglia[23] and human pericytes[25], whereas NLRC4 expression has been found in microglia and astrocytes in vitro[23,27], in human neurons[22] and in the adult cortex[27].

Although the previous inflammasomes have an NLR-sensing molecule, the AIM2 inflammasome has an AIM2 sensor that also connects to ASC (and subsequently caspase 1) via its pyrin domain, although AIM2 itself also contains a DNA-binding HIN (haematopoietic interferon-inducible nuclear proteins with a 200 amino acid repeat) domain[9]. AIM2 was found in most cells of the CNS, such as in rat and human neurons in vitro[22,28], in monkey and human microglia[23,24] and in human brain endothelial cells[29].

In the brain, similar to the peripheral immune system, NLRP3 inflammasome activation requires a priming step provided by an innate immune sensor or cytokine receptor activation, followed by a second signal, which then causes the assembly and full activation of the NLRP3 inflammasome (FIG. 2), although it is important to note that the amplitude of IL-1β released from microglia is reduced in comparison with that observed from bone-marrow-derived macrophages (BMDMs) when studied in vitro[23,30,31]. In addition to the PRR activators described above, several other immune stimulatory molecular patterns may be present in the brain, including, but not restricted to, oligomeric and fibrillar forms of amyloid-β (Aβ)[32], glutamate and pannexin 1, which are able to induce microglial inflammasome activation. ATP is also known to stimulate inflammasome activation via triggering of the P2X7 receptor[22,33], as can α-synuclein (α-Syn)[34,35], transactive response (TAR) DNA-binding protein 43 (TDP43)[36], changes in the extracellular concentration of potassium[21] or mitochondrial dysfunction[37].

Sleep and inflammasome activation. Inflammasome activation may, in a time-restricted and space-restricted manner, also be involved in physiological functions of the brain. Thus, NLRP3-deficient mice have been shown to exhibit reduced non-rapid eye movement (NREM) sleep during the light period under normal housing conditions[38]. When challenged by sleep deprivation, wild-type mice but not NLRP3-deficient mice exhibited enhanced recovery sleep during NREM and rapid eye movement (REM) sleep states. These sleep patterns were accompanied by increased levels of caspase 1 activity and IL-1β levels in the somatosensory cortex. Together, these data suggest that NLRP3 inflammasome activity is involved in spontaneous sleep and sleep responses after sleep deprivation. Given the increased risk of sleep deprivation for neurodegenerative disease and in particular AD[39], one may speculate whether NLRP3-mediated neuroinflammation represents a mechanistic link.

Inflammasomes and senescence

Cellular senescence is a phenomenon in which normal cells cease to divide. Senescence can be triggered by telomere shortening, which ultimately leads to DNA damage. However, senescence can also be induced by telomere-independent pathways that occur after DNA damage resulting from increased reactive oxygen species (ROS) or the activation of oncogenes. Cellular senescence is associated with a number of morphological and phenotypic changes, such as alterations in the chromatin, resistance to apoptosis and, importantly, the presence of a unique secretory profile termed the senescence-associated secretory profile (SASP).

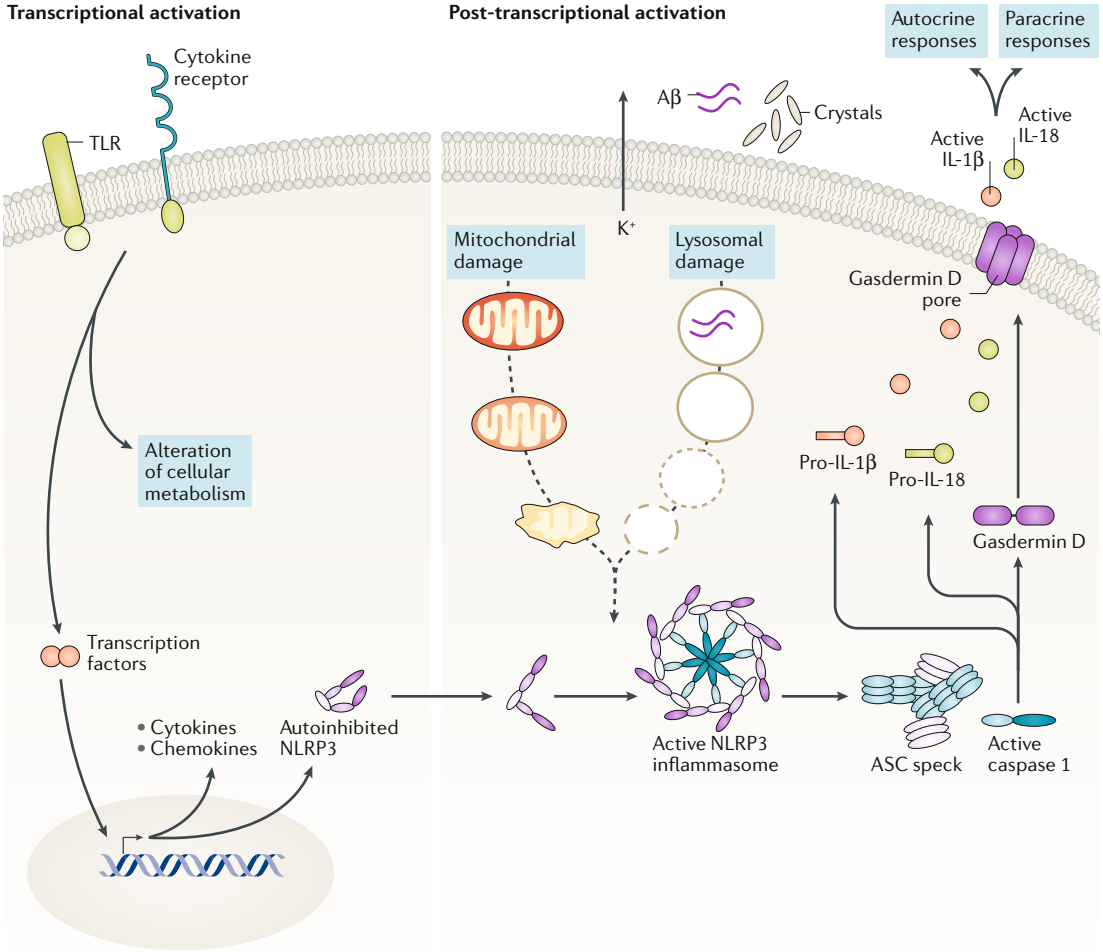

Fig. 2 | **NLRP3 transcriptional and post-transcriptional activation.** NLRP3 (NOD-, LRR- and pyrin domain-containing 3) is expressed at low levels in myeloid cells and requires a priming step for upregulation. Once transcriptionally induced, NLRP3 is produced in an autoinhibited state in the cytosol of the cell. A second signal is required to release the autoinhibited state of NLRP3. NLRP3 activators, such as aggregated amyloid-β (Aβ) or crystalline substances, induce lysosomal damage and cell stress, leading to mitochondrial disruption. NLRP3 responds to these signals and forms an active inflammasome, which nucleates the helical fibrillar assembly of apoptosis-associated speck-like protein containing a CARD (ASC) into ASC specks. ASC specks represent a molecular platform that recruits and activates pro-caspase 1 through autocatalysis. Active caspase 1 cleaves and activates pro-forms of the pro-inflammatory cytokines interleukin-1β (IL-1β), IL-18 and gasdermin D. Cleaved gasdermin D translocates to the plasma membrane and forms a pore through which the cleaved IL-1β and IL-18 molecules can be released into the extracellular space. In addition, gasdermin D can induce an inflammatory form of cell death, termed pyroptosis. The cleaved cytokines have both autocrine and paracrine effects as they can prime the inflammasome and act on many types of cells. TLR, Toll-like receptor.

The SASP includes a number of secreted proteins, and IL-1β is a key defining cytokine of this condition, along with other pro-inflammatory cytokines such as IL-6 and IL-8. Indeed, it has recently been demonstrated that the inflammasome and IL-1 signalling, with transforming growth factor-β (TGFβ) family ligands, can mediate the ability of senescent cells to induce senescence in neighbouring cells through paracrine mechanisms[40]. Many factors can induce senescence, such as ageing, the build-up of biological changes within the cell, cellular stress or sterile tissue inflammation. Importantly, microglia, astrocytes and cells of the neurovascular unit can become senescent, and these senescent cells are typically found in ageing and in many neurodegenerative diseases, including AD and PD[41]. In addition, SASP-related cytokines, including IL-1β, are also increased in the CNS and cerebrospinal fluid (CSF) of patients with neurodegenerative diseases[41,42].

Sterile tissue inflammation
Inflammation induced by a variety of insults such as molecules released from dying cells that may be injured owing to trauma or crystal deposition or in chronic conditions.

Ageing-related senescence in the CNS. Senescence within the CNS has been examined in vivo, using a senescence-accelerated mouse (SAM) model. Tha and colleagues demonstrated increased IL-1β in the brains of mice in the SAMP8 model of this condition, which was accompanied by changes in learning and memory[43]. Interestingly, ablation of *Nlrp3* protects the murine CNS from an age-related increase in the signalling pathways of two SASP pro-inflammatory cytokines — IL-1β and IL-8 (REF.[44]). Attenuated microglial and astrocytic activation was also observed, with a reduction in cell death and inflammatory pathways as determined by transcriptome analysis. Importantly, Youm and colleagues found that

Table 1 | **Inflammasome activation is also observed in other neurological and neurodegenerative conditions**

Inflammasome	Inflammasome components identified	Site of detection	Level and/or method of detection	Inflammasome inhibition	Species	Stimulants	Disease model	Citation
NLRP3 and NLRC4	IL-1β and caspase 1	BMDMs and astrocytes	ELISA, western blot and histology	*Nlrp3*$^{-/-}$, *Nlrc4*$^{-/-}$, *Il1b*$^{-/-}$, *Asc*$^{-/-}$ and *Casp1*$^{-/-}$	Mouse	Cuprizone	Demyelination	Freeman et al., 2017 (REF.[31])
NLRP3	IL-1β, IL-18 and caspase 1	Brain and microglia	ELISA	YVAD (caspase 1 inhibitor) and NLRP3 siRNA	Mouse	Japanese encephalitis virus	Encephalitis	Kaushik et al., 2012 (REF.[95])
NLRP1	Caspase 1	Neurons and hippocampus	Western blot and histology	–	Human	Patients with intractable mesial temporal lobe epilepsy	Epilepsy	Tan et al., 2015 (REF.[96])
–	Caspase 1 and IL-1β	Hippocampus	Western blot and ELISA	Caspase 1 siRNA and NRLP1 siRNA	Rat	Seizure induction after electrode implantation	Epilepsy	Tan et al., 2015 (REF.[96])
NLRP3	IL-1β, IL-18 and caspase 1	Hippocampus	Western blot, histology and ELISA	NLRP3 siRNA	Rat	Status epilepticus induced by electrode implantation	Epilepsy	Meng et al., 2014 (REF.[97])
NLRP3	IL-1β, caspase 1, ASC, NLRP3 and AIM2	Human foreskin fibroblast cells	Western blot and histology	–	Human	Infection with herpes simplex virus 1	Herpes simplex virus 1	Johnson et al., 2013 (REF.[98])
NLRC4	–	Astrocytes and microglia	Histology	–	Human	Patients with multiple sclerosis	Multiple sclerosis	Freeman et al., 2017 (REF.[31])
NLRP1 and NLRP3	ASC, IL-1β and IL-18	Brain	Histology	–	Human	Stroke	Stroke	Fann et al., 2013 (REF.[99])
NLRP1 and NLRP3	ASC, caspase 1, IL-1β and IL-18	Brain and neurons	Western blot and histology	YVAD	Mouse	Middle cerebral artery occlusion	Stroke	Fann et al., 2013 (REF.[99])
NLRP3	Caspase 1 and IL-1β	Brain, microglia and endothelial cells	Western blot, histology and ELISA	*Nlrp3*$^{-/-}$	Mouse	Middle cerebral artery occlusion	Stroke	Yang et al., 2014 (REF.[100])
–	ASC, IL-1β and caspase 1	Brain and sera	Western blot and mRNA	*Asc*$^{-/-}$	Mouse	Infection with the West Nile virus	West Nile virus	Kumar et al., 2013 (REF.[101])
NLRP3	IL-1β (human)	Plasma	ELISA and histology	*IL1R*$^{-/-}$, *Casp1*$^{-/-}$, *Nlrp3*$^{-/-}$ and *Myd88*$^{-/-}$	Human and mouse	Infection with the West Nile virus	West Nile virus	Ramos et al., 2012 (REF.[102])
	IL-1β (mouse)	Brain						

AIM2, absent in melanoma 2; ASC, apoptosis-associated speck-like protein containing a CARD; BMDM, bone-marrow-derived macrophage; ELISA, enzyme-linked immunosorbent assay; IL, interleukin; NLRC4, NOD-, LRR- and CARD-containing 4; NLRP3, NOD-, LRR- and pyrin domain-containing 3; siRNA, small interfering RNA.

depletion of *Nlrp3* improved the cognitive function of aged mice, which was associated with increased expression of learning and memory genes, and this effect was in part mediated by IL-1, as depletion of *Il1r* was similarly neuroprotective[44].

Within the aged brain, microglia are often described as immunosenescent owing to an age-related change in their response to various stimuli, with a key role for inflammasome-related signalling in these senescent cells. IL-1β is increased in the CNS of mice and rats with age[44,45], and fibrillar Aβ induces significantly more IL-1β in microglia prepared from aged versus young mice[46]. A similar age-related effect on microglial IL-1β production has also been observed by many groups using lipopolysaccharide (LPS)[47–49], which was associated with impaired murine behaviour[50]. Importantly, administration of IL-1β into the brains of mice induces potent neuroinflammation and is also associated with memory impairment and neurotoxicity[51]. Together, this altered inflammatory response in immunosenescent microglia may predispose the brain to neurodegenerative conditions such as AD and PD, in which IL-1β and inflammasome signalling are characteristically observed (FIG. 3).

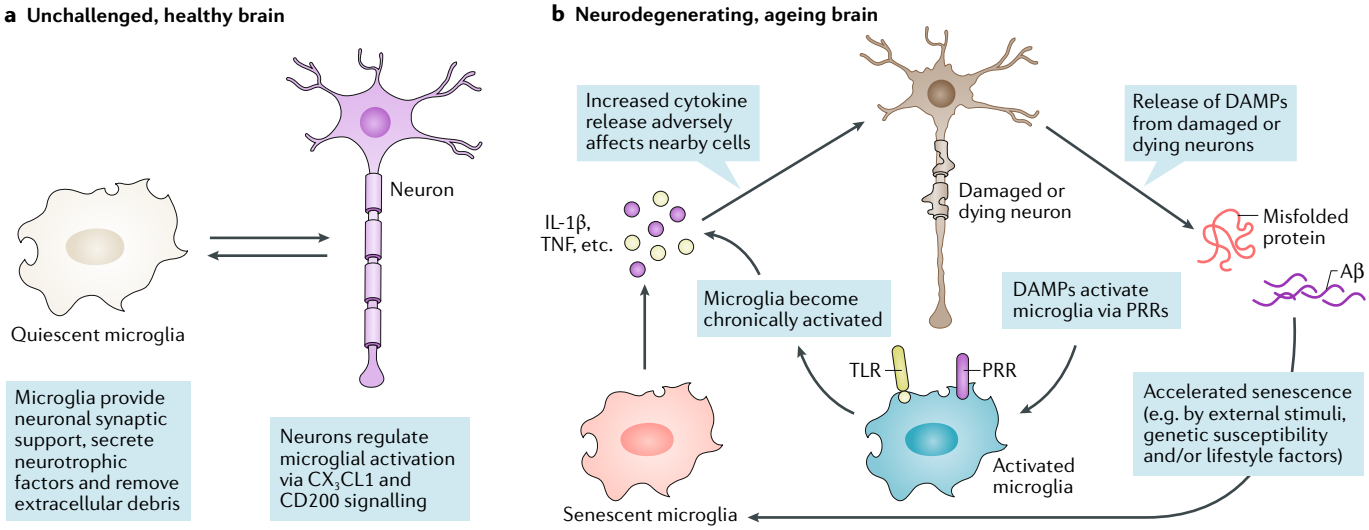

Fig. 3 | **Microglia in the healthy and ageing brain. a** | Microglia and neurons exist in controlled homeostasis within the CNS. Microglia remain in close contact with neurons, where they release a range of neurotrophic factors to support neuronal function while also modulating synapses. In addition, microglia remove extracellular debris via phagocytosis, thus maintaining stability within the brain. In turn, neurons also affect microglial activity. There are a number of receptor–ligand interactions between these cells, including CX_3C-chemokine ligand 1 (CX_3CL1) and CD200 that maintain microglia in a quiescent state. **b** | In the ageing or neurodegenerative brain, damaged or dying neurons release danger-associated molecular patterns (DAMPs) such as amyloid-β (Aβ), which accumulate and activate microglia via pattern recognition receptors (PRRs) and Toll-like receptors (TLRs). Over time, this results in chronically activated, senescent microglia that produce increased levels of pro-inflammatory cytokines (including interleukin-1β (IL-1β)) that negatively affect neighbouring cells and neurons, thus accelerating this inflammatory cascade.

Inflammasome activation in AD

AD is the most prevalent neurodegenerative disorder and the most studied with respect to inflammasome activation (see Supplementary Table 1 for details); indeed, a growing number of studies have found a clear association between the microglia-mediated immune response and AD progression[52,53]. AD is characterized by the accumulation and deposition of Aβ and by the intraneuronal formation of neurofibrillary tangles (NFTs) consisting of hyperphosphorylated tau proteins, both of which are danger signals and are known to activate microglia cells[32,54,55]. Aβ is generated by sequential processing of two proteases and is usually removed from the brain by transport into the CSF, the blood vessels and local degradation by microglia[56]. If the Aβ concentration rises above a critical threshold, oligomers and fibrils form and deposit in so-called senile plaques. At this stage, Aβ, and in particular its oligomeric or fibrillar forms, acts as a danger-associated molecular pattern (DAMP) and is able to cause inflammasome activation. In human AD brains, caspase activity was strongly increased when compared with non-demented and age-matched controls[57]. Interestingly, this phenomenon was also detected in brain samples from patients suffering from mild cognitive impairment due to AD and from early-onset AD patients, suggesting that NLRP3 inflammasome activation represents an early rather than a late pathogenic event. Many of these findings were replicated in monocytes derived from these patients suffering from AD once primed with LPS and stimulated with Aβ1–42 preparations. In these experiments, peripheral monocytes from patients with AD were found to co-express NLRP3, ASC and caspase 1 and showed increased release of IL-1β and IL-18 (REF.[58]).

Aβ induces inflammasome activation. Microglial NLRP3 inflammasome activation upon exposure to fibrillar Aβ was first described in vitro by Halle and colleagues in 2008. In these experiments, fibrillar Aβ was able to induce microglial IL-1β release in an NLRP3-dependent and ASC-dependent manner[32]. Incubation with z-YVAD, a caspase 1 inhibitor, was equally effective at inhibiting IL-1β production. Similarly, genetic caspase 1 deficiency blocked CC-chemokine ligand 2 (CCL2), CCL4 and CXC-chemokine ligand 2 (CXCL2) release. In addition to fibrillar Aβ, soluble Aβ peptides can induce NLRP3 inflammasome activity mediated by CD36 (REF.[59]). Of note, soluble Aβ was equally potent at inducing IL-1β when compared to fibrillar Aβ in these experiments. Similarly, oligomeric preparations of Aβ can induce caspase-1-mediated IL-1β production, which can be blocked by the ROS scavenger *N*-acetyl-L-cysteine but is independent from Aβ uptake or cathepsin B[60]. Although these experiments yielded interesting and novel insights, they should be interpreted with caution as it seems unclear whether synthetic peptide-derived soluble and oligomeric Aβ preparations remain stable or convert into fibrils before or during stimulation of microglia under cell culture conditions. However, another study suggests that Aβ seems to require the fully assembled inflammasome to induce IL-1β release, whereas other immune stimulatory triggers relevant for neurodegeneration such as chromogranin A stimulate IL-1β production through different pathways[46]. Activation of NLRP3 in response to Aβ is further regulated by autophagy-related protein 7 (ATG7)-mediated autophagy as cellular ATG7 deficiency led to increased caspase 1 cleavage, ASC speck formation and IL-1β release in microglia and BV2 cells[61]. Interestingly, cell culture media from these

hyperstimulated ATG7-deficient microglia induced a greater loss of neuronal dendrites, suggesting that microglial inflammasome activation needs to be tightly controlled in order to limit neuronal destruction. It is important to note that synapse loss can occur in areas distant from Aβ deposits; however, it is clear that there is significantly greater synapse loss in the vicinity of Aβ-containing plaques[62,63].

It was recently shown that astrocytes have a functional NLRP3 inflammasome[31], and astrocytes can generate IL-1β upon uptake of Aβ in an ASC-dependent fashion[64]. However, the activation of other inflammasomes also occurs in astrocytes. Palmitate has been described to induce the activation of the NLRC4 inflammasome in rat astrocytes, causing IL-1β maturation in an ASC-dependent manner[27]. Furthermore, human astrocytes have been found to express the NLRP2 inflammasome, which was bound to ASC, caspase 1, pannexin and P2X7. In these experiments, the ATP-induced and NLRP2-mediated IL-1β production was abolished by pannexin inhibition[26]. Alternatively, astrocytes may incorporate NLRP3-containing ASC specks, which can be released by immunostimulated pyroptotic microglia, a mechanism that has already been described for macrophages[65]. In this case, astrocytes would obtain a ready-to-use inflammasome and thereby would be capable of generating NLRP3-inflammasome-dependent cytokines, including IL-1β.

In vivo, Aβ injection into the murine striatum caused an increase of cell surface glycoprotein F4/80 (also known as ADGRE1)-positive microglia and macrophages[32] and this phenomenon was inhibited in ASC- and caspase-1-knockout mice. Likewise, IL-1 receptor and myeloid differentiation primary response protein MyD88 (MYD88)-knockout failed to show microglial activation, suggesting that Aβ initiates a signalling cascade that involves MYD88 activation, NLRP3 inflammasome formation and ultimately caspase 1 cleavage.

NLRP3 inflammasome and AD pathology. To model AD more closely, mice expressing amyloid precursor protein (APP) and presenilin 1 (PS1) containing an exon 9 deletion (APP/PS1 mice; genes and mutations are associated with familial, early-onset AD) were crossed with NLRP3-knockout animals and subsequently analysed for neuropathological changes, learning and memory function as well as microglial phagocytosis and inflammatory gene expression. Aged APP/PS1 transgenic mice had an inflammatory phenotype in the neocortex and hippocampus, which was characterized by Aβ plaque-associated activated microglia displaying process retraction and volume increases of soma near branches and the cell soma itself, respectively[57]. Brains from APP/PS1 mice showed increased levels of pro-inflammatory mediators and microglia were found at Aβ plaques without any overt signs of phagocytic uptake. By contrast, APP/PS1/NLRP3-knockout animals showed immunohistological signs of Aβ phagocytosis and revealed a twofold increase in phagocytic clearance capacity in a quantitative flow cytometric-based in vivo uptake assay, in which the proportion of phagocytic cells is reliably quantified. In addition, brain lysates of these animals showed a twofold increase in insulin-degrading enzyme (IDE) expression. This finding is of particular interest, as IDE is able to degrade extracellular Aβ. The observed increase may bear functional relevance, as a paper by Leissring and colleagues previously demonstrated that increased IDE levels limit cerebral Aβ accumulation in a single APP transgenic mouse model[66]. The combined effect of the increased IDE production and phagocytic Aβ clearance reduced the cerebral Aβ load substantially in aged APP/PS1 animals[57]. NLRP3 expression was confined to microglia; therefore, it has been concluded that the observed changes were entirely due to NLRP3 modulation in these cells. In addition to Aβ removal, inhibition of this particular innate immune signalling pathway protected APP/PS1 animals from neuronal spine loss in the neocortex. Moreover, hippocampal long-term potentiation (LTP), a measure for the formation and consolidation of memory, was equally protected, suggesting that innate immune factors are responsible for LTP suppression instead of Aβ alone. One possible factor downstream of NLRP3 inflammasome activation is IL-1β itself, because this cytokine has previously been shown to suppress LTP in the dentate gyrus of wild-type rats[67]. Functional protection, however, was not restricted to electrophysiological measurements but was also found when animals were tested in several learning and memory paradigms, including spatial navigation memory and object recognition[57]. Importantly, NLRP3-knockout animals did not show any deficit in LTP or spatial memory performance, suggesting that the NLRP3 inflammasome is not involved in physiological microglial functions. Another feature of inflammasome activation is the formation of ASC specks, which represent multimer NLRP3 complexes. Those ASC specks can be found in activated microglial cells and in the extracellular space, when microglial cells undergo pyroptosis. Once released, ASC specks bind rapidly to Aβ peptides, thereby increasing their propensity to aggregate in a time-dependent and concentration-dependent manner[68]. ASC-bound Aβ was identified in brain samples from APP/PS1 mice and human AD cases. Employing an APP/PS1 mouse seeding model, it has been shown that ASC specks are able to seed Aβ deposition. In keeping with this, ASC-positive material was found in the core of Aβ plaques in the human AD brain. Together, these data suggest that innate immune cell pyroptosis and ASC speck release are involved in the earliest stage of Aβ deposition and progression of disease. ASC specks might have potential as a therapeutic target, which could reduce Aβ deposition and spreading of disease pathology.

In addition to the above-described intracellular inflammasome control mechanism by autophagy, NLRP3 activation in microglia seems to be counterbalanced in vivo by IL-33, another IL-1 cytokine family member[69]. Administration of IL-33 reduced NLRP3 inflammasome levels in APP/PS1 mice by increasing the proportion of microglia in an alternative type of activation. This phenotype was associated with an increased uptake of Aβ by microglia and improved memory performance, thus supporting the data obtained from

APP/PS1/NLRP3-knockout animals, although at present it is unclear whether IL-33 affects the NLRP3 inflammasome directly or indirectly via its ability to induce type 2 immune responses.

A role for inflammasome activation in causing cognitive dysfunction was strengthened by recent experiments demonstrating that *N*,*N*′-dimethyl-4,4′-bipyridinium dichloride (paraquat), a herbicide and mitochondrial toxin known to induce oxidative stress, increased caspase 1 and IL-1β levels associated with spatial memory dysfunction and increased Aβ deposition in wild-type and APP/PS1 brains[70]. These data were supported by experiments testing the effects of pharmacological NLRP3 inhibition. Thus, treatment of two different murine AD models with NLRP3 inhibitors by intraperitoneal injection reduced the cerebral Aβ load[71,72], improved memory performance[71] and was associated with reduced microglial cell activation, although the authors did not confirm target engagement in the brain. Likewise, inhibition of NLRP3 by the fenamate class of nonsteroidal anti-inflammatory drug (NSAIDs) inhibited the NLRP3 inflammasome via reversible blockade of volume-regulated anion channels in BMDMs and attenuated microglial activation and cognitive deficits in two rodent models of AD in vivo[73].

AIM2 and NLRP1 inflammasomes and AD. Inflammasome inhibition and subsequent Aβ removal may not always be linked to improved memory function, as shown in experiments analysing 5XFAD mice carrying a knockout of the AIM2 inflammasome[74]. In these experiments, AIM2 knockout was accompanied by reduced Aβ levels along with attenuated microglial activation. Nevertheless, neither open-field behaviour nor spatial memory performance improved in these animals. Of note, AIM2-knockout animals had increased levels of IL-6 and IL-18, which may account for memory dysfunction because IL-6 has been shown to suppress hippocampal LTP[75]. Alternatively, AIM2 products may have an as yet unknown role for memory formation.

In addition to NLRP3 and AIM2, neuronal expression of NLRP1 may contribute to AD pathogenesis. Rat primary cortical neurons had an upregulation of NLRP1 levels upon exposure to Aβ, and this finding was paralleled by an age-dependent increase of neuronal NLRP1 expression in APP/PS1 mice[20]. Neuronal Aβ stimulation causes increased caspase 1 activity and IL-1β release, which were attenuated by NLRP1 small interfering RNA (siRNA). NLRP1 did not affect the overall deposition of Aβ but reduced the number of TdT-mediated dUTP nick end labelling (TUNEL)-positive neurons, which indicates apoptotic cells, and improved spatial memory performance. However, it remains unclear from this study whether IL-1β was actively contributing to neuronal cell death or whether this occurred as a consequence of caspase 1 cleavage of further substrates. In line with this hypothesis, NLRP1-dependent caspase 1 activation was found to cause caspase 6 activation in serum-deprived and ATP-stimulated fetal human primary neurons[22]. Importantly, these authors also demonstrated increased expression of NLRP1 in the brains of patients with AD, with a 20–25-fold increase in NLRP1-immunopositive neurons in AD relative to non-cognitively impaired brains. As caspase 6 activity can cause axonal degeneration, it seems feasible to suggest that the NLRP1–caspase 1–caspase 6 signalling pathway may be involved in axonal damage in AD.

Parkinson disease

α-Syn induces activation of the inflammasome. Similar to Aβ, fibrillar forms of α-Syn increased monocytic and microglial IL-1β release in a caspase-1-mediated fashion (see Supplementary Table 1 for details). This process requires the phagocytic uptake of α-Syn, ROS production, cathepsin B activity and ultimately NLRP3 inflammasome activation[34,35,76]. Interestingly, in human monocytes, this effect was blocked by an anti-TLR2 antibody, suggesting that binding of α-Syn to TLR2 is required for NLRP3 inflammasome activation and IL-1β production[34]. Similar to Aβ, α-Syn may also utilize a TLR–MYD88-mediated signalling pathway for microglial inflammasome activation[77]. Crossing caspase-1-knockout animals into the α-SynA53T mutant mouse model of PD, a mutation that is associated with familial PD as it induces overexpression of α-Syn, resulted in a strong reduction of microglial immunoreactivity. Of note, microRNA-7 reduced caspase 1 cleavage and IL-1β generation in the 1,2,3,6-methyl-phenyl-tetrahydropyridine/probenecid (MPTP) mouse model of PD, and this phenomenon was associated with neuroprotection of dopaminergic neurons in the substantia nigra[35]. In keeping with this, serum derived from patients with PD had reduced microRNA-7 but increased caspase 1 and IL-1β levels, although these findings must be interpreted with caution as the source of these inflammatory mediators remains elusive[35]. Additionally, α-Syn-induced inflammasome activation may require as yet unidentified signalling steps, as the ketone body hydroxybutyrate, known to strongly inhibit inflammasome activation by classical stimulants in macrophages, was not able to reduce α-Syn-induced inflammasome activation in microglia[33].

Inflammasome activation and α-Syn cleavage. α-Syn inflammasome activation may be only one axis of a mutual interaction. Recently, it has been shown that inflammasome activation through classical stimuli, including nigericin, paraquat, aluminium crystals, LPS or menadione, directly leads to α-Syn truncation through caspase-1-mediated cleavage. This truncation increased the propensity of α-Syn to aggregate and increased neuronal toxicity. In turn, neuronal loss was reduced by caspase 1 inhibition. The α-Syn-induced inflammasome activation and inflammasome-mediated α-Syn truncation and aggregation together may establish a self-propagating 'vicious cycle', which ultimately contributes to neuronal death through high cytokine levels and increased aggregated α-Syn[78]. Mitochondrial failure and increased ROS production have been linked to PD pathogenesis before, including several mechanisms that may contribute to neuronal dysfunction and demise. BMDMs were treated with rotenone, an inhibitor of the mitochondrial respiratory chain, to demonstrate priming of the NLRP3 inflammasome, which

was then fully activated in the presence of ATP[37]. The activation depended on ROS production and mitochondrial depolarization. If such a mechanism would also be in place in dopaminergic neurons, one may speculate whether one dimension of neuronal loss is mediated by mitochondrial deficits, ROS production and then subsequent α-Syn truncation and aggregation. In support of a neuroprotective effect of inflammasome inhibition in PD, the phenolic flavonoid baicalein was found to reduce inflammasome activation and apoptosis in the rat substantia nigra dopaminergic system[79].

Amyotrophic lateral sclerosis

Adaptive and innate immune mechanisms have both been implicated in the pathogenesis of ALS on the basis of data from human brain and spinal cord tissue as well as cell culture and transgenic mouse models[80]. Human superoxide dismutase 1 (SOD1) mutations such as SOD1G93A cause familial forms of dominantly inherited ALS and have been used to establish cell culture and animal models of ALS in order to study disease-related processes (for details, see Supplementary Table 1). In microglia, mutant SOD1G93A can activate caspase 1 cleavage in an ASC-dependent and NLRP3-dependent manner, suggesting that mutant SOD1 exposure activates the NLRP3 inflammasome and subsequent IL-1β release in an age-dependent manner[18,81]. ROS and peroxynitrite generation most likely contribute to this signalling cascade[82]. This mutant SOD1-triggered inflammasome activation depends on the formation of its amyloid conformation and its cellular uptake by endocytosis. When crossed into the murine SOD1G93A ALS model, genetic ablation of caspase 1 and IL-1β prolonged the survival of transgenic animals and reduced the astrocyte and microglial activation while also reducing the loss of choline acetyltransferase (ChAT)-immunopositive motor neurons in the ventral horn of the spinal cord. In the same in vivo model, treatment of animals with an anti-IL-1β antibody was equally protective. Linking these data to human disease, analysing several IL-1 family members in the CSF of sporadic ALS cases demonstrated significantly increased levels of total IL-18, its inhibitor IL-18 binding protein (IL-18BP) and free IL-18, suggesting an activation of an IL-1-cleaving inflammasome[83]. These data are in line with the detection of increased levels of NLRP3, ASC, cleaved caspase 1 and mature IL-18 in the spinal cord tissue of sporadic ALS cases[18].

Inflammasome activity regulates TDP43 expression. TDP43, which physiologically acts as a transcriptional repressor binding to RNA and DNA, is involved in several mechanisms of transcriptional repression, mRNA splicing and translational protein regulation. Its hyperphosphorylated and ubiquitylated form has been found to aggregate and accumulate intraneuronally in brains of patients with ALS or frontotemporal dementia[84]. In addition, elevated levels of TDP43 have also been described in chronic traumatic encephalopathy[85]. TDP43 caused motor neuron cell death in vitro, which depended on the activation of microglia and was mediated through a CD14, nuclear factor-κB (NF-κB) and NLRP3 signalling cascade[36]. In another model of murine neurotoxicity using 2,2′,4,4′-tetrabromodiphenyl ether (BDE-47), NLRP3 inflammasome activation was engaged in the upregulation of nuclear TDP43 through the downregulation of parkin[86]. TDP43 itself was causative for neuronal apoptosis in the hippocampus. Inhibition of NLRP3 or caspase 1 activity led to increased parkin levels and blocked TDP43-induced neurotoxicity, suggesting that under these circumstances, interference with NLRP3 could be a novel therapeutic strategy.

Traumatic brain injury

In contrast to the classical neurodegenerative diseases, where pathology begins within the brain and body and local processes primarily cause neuronal dysfunction and death, TBI arises from an external trauma and physical destruction of either the brain or the spinal cord. This type of cerebral damage involves innate immune activation (see Supplementary Table 1 for details) and the release of various inflammatory mediators, which account for secondary neuronal damage and functional deficits. The defined time of onset, however, enables specific analysis of the dynamics and time-dependent development of the immune response in the lesioned tissue.

Inflammasome activation in traumatic brain injury. In spinal cord injury (SCI), increased caspase 1 and caspase 11 cleavage and subsequent generation of IL-1β and IL-18 were detected as early as 15 min post-SCI[87]. This phenomenon was due to activation of the NLRP1 inflammasome in rat spinal cord motor neurons and linked to cleavage of the X-linked inhibitor of apoptosis protein. Importantly, reducing activity of ASC, a critical component for any inflammasome assembly, by anti-ASC neutralizing antibodies blocked the NLRP1 activation and subsequent release of IL-1β and IL-18. This reduction was accompanied by reduced lesion volume of the spinal cord lesion and an improved functional outcome in several motor tests, including grip strength and sticker removal[87]. It appears that NLRP1 is not the only inflammasome that can be expressed in neurons following TBI. Experiments using the AIM2 activator poly(deoxyadenylic-deoxythymidylic) acid sodium salt (poly(dA:dT)) induced ASC speck formation, caspase 1 cleavage and release of IL-1β in embryonic cortical neurons[28]. AIM2 activation caused neuronal pyroptosis, which was prevented by pretreatment of cells with Ac-YVAD-CMK, a cell-permeable irreversible inhibitor of caspase 1 (REF.[28]). Of note, CSF derived from patients with TBI was able to induce AIM2 activation, caspase 1 cleavage and neuronal pyroptosis in vitro, and this was blocked by a pannexin 1 inhibitor, suggesting that pannexin 1, an ATP release channel, acts as a neuronal cell death signal in neuronal pyroptosis[28]. Similar to spinal cord trauma, NLRP3 components and its activation have been described in the cerebral cortex of a rat model of TBI. In this study, increased levels of ASC, NLRP3 and caspase 1 became detectable at 6 hours post-TBI, and the levels of increased cleaved caspase 1 were evident at 24 hours. Interestingly, pericontusional NLRP3

Pericontusional
The area of tissue surrounding a contusion or an injury in the brain, often caused by trauma or an impact to the head.

immunoreactivity was colocalized to ionized calcium-binding adaptor molecule 1 (IBA1; also known as AIF1, which identifies myeloid cells) but also to glial fibrillary acidic protein (GFAP) and the neuronal nuclear protein (neuN), thus identifying astrocytes and neurons, respectively. Unfortunately, this study did not fully confirm that NLRP3 was inside the cells (by imaging throughout the layers of the cell and demonstrating the internal presence of NLRP3 by sideward scatter), leaving the question of whether NLRP3 was truly found in neurons and astrocytes unanswered[19]. The role and impact of inflammasome activation may, however, depend on the precise model investigated, because a study using a controlled cortical impact model in mice did not find any neuroprotective effect of either NLRP1 or ASC knockout, despite increased IL-1β levels[88]. As this study used a very small number of animals per group, tendencies towards neuroprotection (for example, by NLRP1 deficiency) may have been missed.

CNS disease with neurodegenerative component

HIV-1-associated neurocognitive disorders. HIV-1-associated neurocognitive disorders (HANDs) represent an increasing clinical problem in long-term AIDS survivors, as the introduction of effective combined antiviral therapy regimens substantially lengthens the lifespan of these patients[89]. Clinically, HANDs affect up to 50% of HIV-infected patients and often manifest with learning and memory dysfunction and executive deficits. Neurobiologically, HANDs are characterized by changes in the cerebral energy metabolism, neuroinflammation, neuronal dysfunction and death. Various inflammasome components are increased in the HIV-infected brain, including increased mRNA levels of IL-1β and IL-18, suggesting functional activation[24]. Infection of human primary microglial cells with the HIV_{SF162} strain induced IL-1β release and microglial ASC speck formation that were dependent on the NLRP3 inflammasome and caspase 1 (REF.[24]). These data have been replicated in a model of feline immunodeficiency virus infection of cats, showing that IL-1β induction and microglial activation are associated with the occurrence of neurobehavioural deficits. Further defining the pathological pathogen-associated molecular pattern (PAMP), which accounts for microglial inflammasome activation, Chivero, Guo and colleagues found that the HIV-1 transactivator of transcription (Tat) protein is instrumental for the upregulation of NLRP3 and ASC levels themselves and for the increase in caspase 1 cleavage and subsequent IL-1β release[90]. Similarly, the HIV-1 viral protein R (Vpr) is able to induce NLRP3-dependent caspase 1 cleavage and IL-1β release. Furthermore, treatment of Vpr transgenic animals with the caspase 1 inhibitor VX-765 improved neurobehavioural deficits[91].

Zika viral infection. Similarly, Zika virus infection has been related to several neurological disorders such as congenital microcephaly, Guillain–Barré syndrome, myelopathy and encephalitis. Zika virus stimulation of a human astrocytoma cell line caused both an increase in IL-1β levels and decreased cell viability, although the study did not further define which Zika virus epitope or inflammasome is involved in this immune reaction[92]. This finding leaves a number of questions that need to be addressed before a role of inflammasome activation in any of the named Zika-evoked neurological disorders can be defined.

X-linked adrenoleukodystrophy. X-linked adrenoleukodystrophy (X-ALD; a genetic disease that affects the nervous system and the adrenal cortex) is caused by a loss-of-function mutation of the gene encoding for the ATP-binding cassette subfamily D member 1 (ABCD1). ABCD1 is involved in the transport of very-long-chain fatty acids from the cytosol to the peroxisome, a critical step for their degradation and disposal. In the brain, dysfunctional ABCD1 causes axonopathy of the spinal cord and inflammatory demyelination[93]. 25-Hydrocholesterol (25-HC) is a downstream product of cholesterol 25-hydroxylase, and this enzyme is increased in X-ALD patient-derived induced pluripotent stem cells. Indeed, 25-HC activates microglia and increases cerebral IL-1β levels when injected into the murine corpus callosum[94]. In LPS-primed mouse microglia, 25-HC causes NLRP3-dependent caspase 1 cleavage, leading to the release of IL-1β. This activation also requires 25-HC-induced potassium efflux and mitochondrial ROS production. Of note, NLRP3 deficiency or treatment with IL-1 receptor antagonist protein (IL-1ra) attenuated microglial activation upon cerebral 25-HC injection and prevented oligodendrocyte cell death.

Conclusions

In autoimmune diseases of the CNS, immunological mechanisms have always been considered to be of key importance. By contrast, this has not been the case for neurodegenerative disorders. For decades, inflammation in those diseases has been viewed purely as a bystander phenomenon, which was believed to not interfere with disease pathogenesis. Only recently have experimental, epidemiological, genetic and epigenetic data provided evidence for a role of innate immune mechanisms for neurodegenerative disease (see TABLE 1 and Supplementary Table 1 for details). At the centre of these mechanisms is the activation of the inflammasomes, which orchestrate the initiation, persistence and chronicity of innate immunity. Modulation of inflammasome activity in models of AD, PD and other neurodegenerative disease suggests a potential therapeutic role for substances that can effectively inhibit this immune signalling mechanism. Although most of the studies have addressed the function of the NLRP3 inflammasome, there is much less information available concerning the role of other inflammasomes, which could also influence disease phenotypes and progression. Before any inflammasome-targeted interventions may be tested in humans, further information is needed regarding when and where such interference with the brain's innate immune system will be beneficial. A further challenge is the development of drugs that cross the blood–brain barrier and selectively inhibit only those inflammasomes that are involved in the particular pathogenetic process.

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