Microglial NLRP3 inflammasome activation in multiple sclerosis

Melis Olcum[a], Bora Tastan[a,b], Cagla Kiser[a,b], Sermin Genc[a,b,c] and Kursad Genc[c,*]

[a]Izmir Biomedicine and Genome Center, Dokuz Eylul University Health Campus Balcova, Izmir, Turkey
[b]Izmir International Biomedicine and Genome Institute (iBG-Izmir), Dokuz Eylul University Health Campus, Balcova, Izmir, Turkey
[c]Department of Neuroscience, Institute of Health and Science, Dokuz Eylul University Health Campus, Balcova, Izmir, Turkey
*Corresponding author: E-mails: kkursadgenc@hotmail.com, kemal.genc@deu.edu.tr

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Abstract

Multiple sclerosis (MS) is a chronic, autoimmune and neuroinflammatory disease of the central nervous system (CNS) mediated by autoreactive T cells directed against myelin antigens. Although the crucial role of adaptive immunity is well established in MS, the contribution of innate immunity has only recently been appreciated. Microglia are the main innate immune cells of the CNS. Similar to other myeloid cells, microglia recognize both exogenous and host-derived endogenous danger signals through pattern recognition receptors (PRRs) localized on their cell surface such as Toll Like receptor 4, or in the cytosol such as NLRP3. The second one is the sensor protein of the multi-molecular NLRP3 inflammasome complex in activated microglia that promotes the maturation and secretion of proinflammatory cytokines, interleukin-1β and interleukin-18. Overactivation of microglia and aberrant activation of the NLRP3 inflammasome have been implicated in the pathogenesis of MS. Indeed, experimental data, together with post-mortem and clinical studies have revealed an increased expression of NLRP3 inflammasome complex elements in microglia and other immune cells. In this review, we focus on microglial NLRP3 inflammasome activation in MS. First, we overview the basic knowledge about MS, microglia and the NLRP3 inflammasome. Then, we summarize studies about microglial NLRP3 inflammasome activation in MS and its animal models. We also highlight experimental therapeutic approaches that target different steps of NLRP inflammasome activation. Finally, we discuss future research avenues and new methods in this rapidly evolving area.

List of Abbreviations

AD	Alzheimer's Disease
AIM	Absent in Melanoma
ALS	Amyotrophic Lateral Sclerosis
ASC	Apoptosis-associated Speck-like Protein
ATP	Adenosine Triphosphate
BBB	Blood Brain Barrier

BDNF	Brain Derived Neurotrophic Factor
CARD	Caspase Recruitment Domain
CNS	Central Nervous System
CSF	Cerebrospinal Fluid
DAMP	Damage-related Molecular Patterns
DC	Dendritic Cell
DMD	Disease Modifying Drug
DMF	Dimethyl Fumarate
EAE	Experimental Autoimmune Encephalomyelitis
EBV	Epstein Barr Virus
GM-CSF	Granulocyte-macrophage Colony Stimulating Factor
GSDMD	Gasdermin D
HLA	Human Leukocyte Antigen
IFN	Interferon
IGF	Insulin-like Growth Factor
IL	Interleukin
JNK	c-Jun N-terminal Kinase
LPS	Lipopolysaccharide
LRR	Leucine Rich Repeats
MBP	Myelin Basic Protein
MHC	Major Histocompatibility Complex
MMP	Matrix Metalloproteinase
MOG	Myelin Oligodendrocyte Glycoprotein
MRI	Magnetic Resonance Imaging
MS	Multiple Sclerosis
NF-KB	Nuclear Factor kappa-light-chain-enhancer of Activated B Cells
NIREG	Neuro-immune-regulator
NK	Natural Killer
NLRP3	Nucleotide-binding Domain and Leucine-rich Repeat Containing Protein 3
NOD	Nucleotide-binding Domain and Oligomerization Domain
OPC	Oligodendrocyte Precursor Cell
PAMP	Pathogen-associated Molecular Patterns
PBMC	Peripheral Blood Mononuclear Cell
PD	Parkinson's Disease
PLP	Proteolipid Protein
PNS	Peripheral Nervous System
PPMS	Primary Progressive Multiple Sclerosis
PRR	Pattern Recognition Receptors
PYD	Pyrin Domain
ROS	Reactive Oxygen Species
RRMS	Relapsing-remitting Multiple Sclerosis
SNP	Single Nucleotide Polymorphism
SPMS	Secondary Progressive Multiple Sclerosis
TBI	Traumatic Brain Injury
Th	T helper
TLR	Toll Like Receptor
Treg	T regulator

1. Multiple sclerosis

Multiple sclerosis (MS) is a chronic autoimmune disease of the central nervous system (CNS) characterized by demyelination and diffuse neurodegeneration that is observed both in the brain and spinal cord (Filippi et al., 2018). Autoreactive $CD4^+$ T cells have a central role in disease pathogenesis and they respond to myelin specific antigens, such as myelin basic protein (MBP), myelin oligodendrocyte glycoprotein (MOG) and proteolipid protein (PLP). Approximately 2.5 million people worldwide are affected by this disorder (Browne et al., 2014). MS is more common in young women and has a huge impact on quality of life and economic burden on society. Inflammatory attacks on myelinated axons may occur anywhere within the CNS, including the optic nerves, brainstem, periventricular white matter and cervical spinal cord.

Although the etiology of MS remains unclear, several genetic, environmental, and epigenetic factors are involved in disease pathogenesis (Olsson, Barcellos, & Alfredsson, 2017). Smoking, vitamin D deficiency, obesity, and infectious agents such as Epstein-Barr virus (EBV) are known environmental risk factors in the development of MS. Human leukocyte antigen (HLA) DRB1 *1501 has been associated with MS in many populations since the 1970s (Thompson, Baranzini, Geurts, Hemmer, & Ciccarelli, 2018). Recent genome-wide association studies in MS have identified several single nucleotide polymorphisms (SNPs) in both adaptive and innate immunity system genes (Parnell & Booth, 2017). Further functional studies are required to understand how these SNPs contribute to disease pathogenesis.

The clinical course of MS is classically categorized into four subtypes: Clinically isolated syndrome, relapsing-remitting multiple sclerosis (RRMS), secondary progressive multiple sclerosis (SPMS) and primary progressive multiple sclerosis (PPMS) (Filippi et al., 2018). About 85% of MS patients are initially diagnosed with RRMS that is characterized by recurrent attacks following total or partial recovery. Approximately 90% of untreated patients with RRMS evolve into SPMS within 20–25 years. 15% of patients can present PPMS which is characterized by the progressive worsening of symptoms from the onset of disease with no relapses (Klineova & Lublin, 2018).

The diagnosis of MS is based on clinical findings supported by laboratory evidences by neuroimaging and cerebrospinal fluid (CSF) analysis. Magnetic resonance imaging (MRI) evidence of demyelination is present of T2

hyperintense white matter lesions in the CNS. Spatial and temporal dissemination of MRI lesions is accepted as an indicator of disease progression. Gadolinium enhancement in T1-weighted MRI images is considered the hallmark of active plaques that represent the presence of inflammatory cells that penetrate the impaired blood-brain barrier (BBB) (Filippi et al., 2019). Presence of oligoclonal bands in CSF and brain imaging findings are included in the newly revised McDonald diagnostic criteria (2017) (Thompson, Banwell, et al., 2018).

A pathological hallmark of MS is focal demyelinating plaques which are located in both the white and gray matter of the brain, spinal cord, and optic nerves (Lassmann, 2018). Active demyelinating plaques are accompanied by densely populated phagocytic microglia and macrophages. Although the number of microglial cells in the center of plaques is reduced in chronic inactive lesions, a limited number of microglia are still present in periplaques area (Filippi et al., 2018). Additionally, chronic active plaques are sharply demarcated lesions and contain demyelination, partial axonal preservation, and reactive gliosis. Apart from these focal lesions, diffuse changes including perivascular inflammation, diffuse microglial activation, axonal injury, and astrogliosis in normal appearing white and gray matter are observed in patients with progressive MS (Lassmann, 2018). Cortical atrophy is a pathological hallmark of the progressive phase of disease that leads to cognitive dysfunction.

The underlying pathogenesis of the MS attack is demyelination caused by peripheral and central immune activation. Oligodendrocytes are the main glial cells that produce myelin in the CNS and exert metabolic support to neurons (Patel & Balabanov, 2012; Philips & Rothstein, 2017). Demyelination and tissue injury are partly mediated by the toxic products secreted by innate immune cells (Correale, Marrodan, & Ysrraelit, 2019; Patel & Balabanov, 2012) (Fig. 1). Besides, metabolic defects such as mitochondrial deficit, energy failure, reactive oxygen species (ROSs), excess of glutamate, ionic imbalance, and abnormality in iron metabolism contribute to oligodendrocyte apoptosis, subsequent neuronal damage and impaired remyelination (Adiele & Adiele, 2019; Correale et al., 2019). Reduced mitochondrial density in MS lesions and mitochondrial dysfunction are attributed as the main causes of neurodegeneration (Campbell & Mahad, 2018). Accumulation of excessive ROS is another cause of oligodendrocyte injury in MS (Ohl, Tenbrock, & Kipp, 2016). Impairment in the iron metabolism has a significant role in the pathogenesis of MS. Iron is mainly stored in microglia and it is essential trophic factor for myelin synthesis and

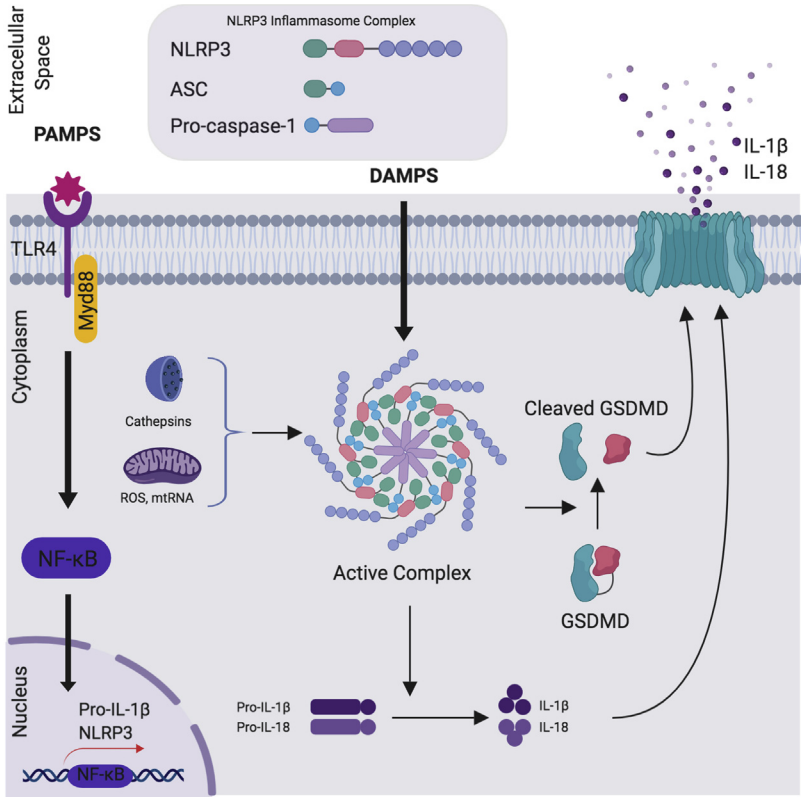

Fig. 1 Basic Mechanism of NLRP3 Inflammasome Activation.* DAMPs and/or PAMPs upregulates expression of NLRP3, pro-IL-1β and pro-IL-18 driven by nuclear translocation of NF-kB. Various stimuli such as ATP, K eflux, ROS and mtDNA activates formation of NLRP3 inflammasome complex. NLRP3 oligomerization in turn recruits ASC and pro-caspase-1 and causes caspase-1 autoactivation. Active caspase-1 cleaves pro-IL-1β and pro-IL-18 and induces pyroptosis through cleavege of Gasdermin D which promotes secretion of and IL-1β and IL-18. Simplified for clarity. See the text for details. *Created with BioRender.

oligodendrogenesis (Filippi et al., 2019). The reduced repair capacity of oligodendrocytes and irreversible axonal damage due to the lack of trophic factor support are other important factors that contribute to the pathogenesis of disease progression (Adiele & Adiele, 2019; Correale et al., 2019) (Fig. 1).

At the present time, there is no cure for MS. Interferon beta (IFN-β) was the first effective drug to be FDA approved for the treatment of RRMS in 1993 (Tintore, Vidal-Jordana, & Sastre-Garriga, 2019). Then, several disease-modifying drugs (DMDs) were introduced for MS treatment, including glatiramer acetate, teriflunomide, fingolimod, dimethyl fumarate,

natalizumab, alemtuzumab, rituximab, andocrelizumab and cladribine. All of these mentioned drugs target the immunologic etiology of the disease. They diminish the frequency and severity of acute attacks and improve patient's quality of life by reducing their disabilities. Due to their serious adverse effects, long-term usage of DMDs have been limited (Filippi et al., 2018). In addition, these drugs do not prevent the chronic progressive disability. Clinical trials that attenuate the progression of disease by inducing neurodegeneration with different stem cells are still ongoing (Genc, Bozan, Genc, & Genc, 2019). Therefore, there is still a need to find effective and safe treatments for MS.

2. Animal models

Even though MS has been known since the 18th century, access to biologic samples to study MS pathophysiology has been restricted to post-mortem tissues of MS patients (Praet, Guglielmetti, Berneman, Van Der Linden, & Ponsaerts, 2014). Animal models allow researchers to use different aspects to elucidate the possible mechanisms that underlie MS pathology including demyelination, inflammation and neurodegeneration. These models include experimental autoimmune encephalomyelitis (EAE), toxin-induced demyelination and virus-induced models (Praet et al., 2014). These models mimic certain aspects of the disease instead of its entire model due to the complex nature of MS (Kipp, Nyamoya, Hochstrasser, & Amor, 2017).

In EAE model, animals are immunized with myelin derived peptides including MOG, MBP and PLP (Tuusa, Raasakka, Ruskamo, & Kursula, 2017). These peptides are administrated with a bacterial adjuvant such as complete Freund's adjuvant or incomplete Freund's Adjuvant (Billiau & Matthys, 2001). For EAE induction, C57BL/6 and SJL/J mice strains and Lewis rats are frequently used to mimic the clinical and immunopathological aspects of human MS (Constantinescu, Farooqi, O'brien, & Gran, 2011). The EAE model is one of the most studied animal model as it has allowed researcher to develop treatments for MS when compared to other MS-mimicking models (Lassmann & Bradl, 2017). Ascending hind limb-tail paralysis and progressive loss of body weight are observed two weeks after immunization (Klaren, Motl, Woods, & Miller, 2014).

In the toxin-induced demyelinating models, administration of toxins such as cuprizone, lysophosphatidyl, lysolecithin and ethidium bromide

initiate demyelination and mimic MS in rodents (Lassmann & Bradl, 2017; Praet et al., 2014). In virus-induced MS models, usage of viral agents such as single-stranded RNA picornaviruses, Theiler's murine encephalomyelitis virus initiate demyelination and clinically mimic MS in animals (Gerhauser, Hansmann, Ciurkiewicz, Loscher, & Beineke, 2019).

3. Immunopathogenesis

3.1 Adaptive immunity

Both innate and adaptive immune systems are involved in the immunopathogenesis of MS. Exacerbation of disease is a result of the interaction between the peripheral immune cells including T cells, B cells, dendritic cells (DCs) and the immune cells of brain, especially microglia. There are two main hypotheses for initiation of disease: the outside-in and the inside-out (Gharagozloo et al., 2017) (Fig. 2). The outside-in hypothesis is the classical and most widely accepted one where peripheral immune cells are first activated out-side of the brain, then cross to the CNS via the damaged BBB and ultimately lead to immune mediated tissue injury (Thompson, Baranzini et al., 2018). T cells activation occurs in the presence of molecular mimicry between foreign peptides and self-peptides presented by DCs (Tai, Wang, Korner, Zhang, & Wei, 2018). In the inside-out hypothesis, disease starts within the CNS, results in oligodendroglial and axonal injury without the involvement of peripheral inflammatory cells and continues with an autoimmune peripheral reaction (Gharagozloo et al., 2017). Oligodendroglial and axonal injury can be caused by developmental abnormalities, mitochondrial dysfunction, ROS, infectious agents (viruses or bacteria) and environmental toxins (Filippi et al., 2019). Post-translationally modified myelin proteins formed after oligodendrocyte injury can enter peripheral circulation via the glymphatic system, and then activate the peripheral immune cells.

The role of adaptive immune responses in MS pathogenesis has been studied extensively. Basically, aberrantly activated myelin proteins-specific $CD4^+$ T helper (Th) cells and $CD8^+$ T cells to pass into the brain parenchyma and cause perivascular lesions (Gharagozloo et al., 2017). When $CD4^+$ Th cells are activated by antigen-presenting cells (APCs), they differentiate into various subtypes, such as Th1, Th2, Th17 and alter the immune response by secreting different cytokines (Baecher-Allan, Kaskow, & Weiner, 2018) (Fig. 2). While Th1 cells contribute to the clearance of

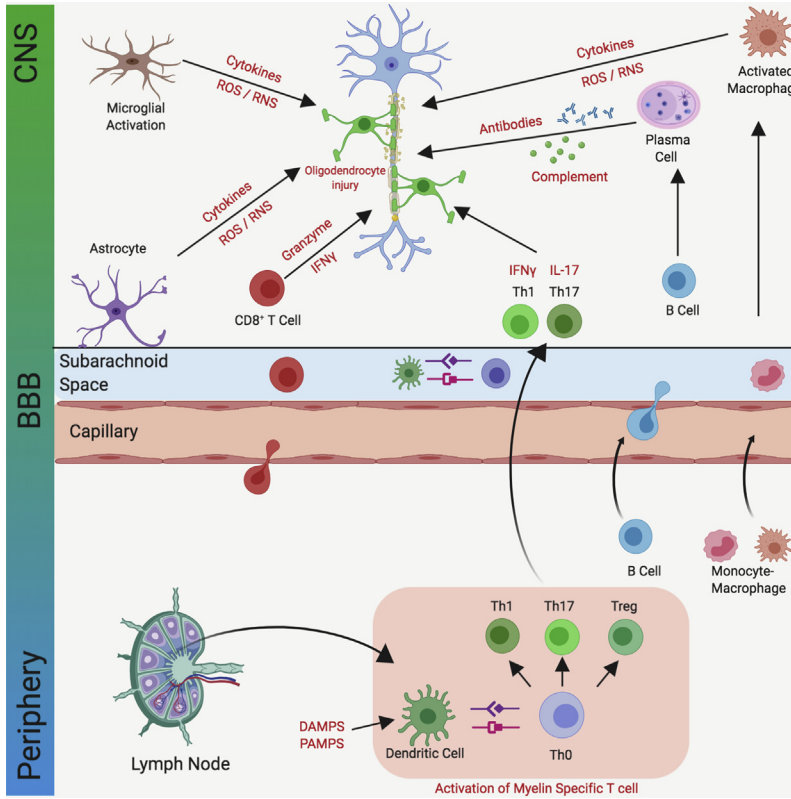

Fig. 2 Immunopathogenesis of Multiple Sclerosis.* Innate and adaptive immune responses lead demyelination and oligodendrocyte injury. *Created with BioRender. (*DAMPs*, Damage-associated molecular patterns; *PAMPs*, Pathogen-associated molecular pattern molecules; *Th*, T-helper cell; *IFN*, interferon; *IL*, interleukin; *TNF*, tumor necrosis factor; *NO*, nitric oxide). Simplified for clarity. See the text for details.

extracellular pathogens by releasing the proinflammatory cytokine, interferonγ (IFNγ), Th2 cells have an opposing effect on the response of Th1 cells. Naive $CD4^+$ T cells differentiate into interleukin-17 (IL-17) producing Th17 cells in the presence of interleukin-23 (IL-23). Both Th1 and Th17 cells are critically involved in the pathogenesis of MS, especially in the initial stages of lesion formation. $CD8^+$ cytotoxic T cells are the most common type of lymphocytes in MS lesions. IFN γ, perforin and granzyme secretion from these cells are responsible for the axonal and oligodendrocyte death.

$CD4^+$ regulatory T (Treg) cells repress cell proliferation by secreting interleukin-10 (IL-10) (Baecher-Allan et al., 2018). Several defects in

suppressive effect of Treg cells have been linked to MS pathogenesis (Astier, Meiffren, Freeman, & Hafler, 2006; Danikowski, Jayaraman, & Prabhakar, 2017). Lack of inhibitory function of FoxP3$^+$ specific subtypes of Tregs has been associated with clinical exacerbation of MS.

γδ (gamma−delta) T cells constitute about 2% of the total blood lymphocytes (Baecher-Allan et al., 2018). They do not require antigen presentation via major histocompatibility complex (MHC) molecules for activation and secrete several cytokines, including IFN-γ, interleukin-4 (IL-4), and IL-17 after activation by non-peptide bacterial products. γδ T cells secrete IL-17 that enhance the generation of Th17 cells (Malik, Want, & Awasthi, 2016). The number of these cells has particularly been increased in around both active and chronic demyelinating lesions (Monteiro et al., 2018).

Recent anti-CD20 antibody treatment has revealed the importance of B cells in the immunopathogenesis of MS (Li, Patterson, & Bar-Or, 2018). Increased granulocyte-macrophage colony-stimulating factor (GM-CSF) and decreased IL-10 production have been detected in B cells of MS patients (Li, Rezk, et al., 2015). CD80$^+$ B cells were increased in PBMCs of MS patients, while IFN-β therapy was able to reduce this number (Genc, Dona, & Reder, 1997). B cells and plasma cells found in the brain parenchyma, meninges, and CSF of MS patients can produce anti-myelin antibodies that can be detected by the presence of oligoclonal bands (Michel et al., 2015).

Alteration in mature B cell count and activity exhibit a correlation between disease activity and treatment response (Arneth, 2019; Michel et al., 2015). Antibody-independent functions of B cells are to attract and activate T cells and myeloid cells for recruitment into the CNS and thus enhance cellular immune responses. Functional immunoglobins secreted from B cells and plasma cells contribute to myelin destruction through activating the classical complement system (Hemmer, Kerschensteiner, & Korn, 2015; Liu et al., 2017). Recent studies have confirmed that complement system is activated in MS (Plantone, Inglese, Salvetti, & Koudriavtseva, 2018).

3.2 Innate immunity

Innate immunity is a rapid responding system that is the first step of host defense mechanism (Heneka, Kummer, & Latz, 2014). Innate immunity response is not specific to the antigen. Innate immunity includes physical and chemical barriers and biological components. Biological components of innate immunity include several cell types and soluble inflammatory

mediators. Innate immune cells are neutrophils, monocytes, macrophages, dendritic cells, natural killer (NK) cells, innate lymphoid cells, mast cells and NK T cells in the PNS; and microglia, CNS-resident non-parenchymal macrophages (meningeal, perivascular and choroid plexus), and astrocytes in the CNS (Brown & Weinberg, 2018; Cui & Wan, 2019; Gross, Schulte-Mecklenbeck et al., 2016; Pierson, Wagner, & Goverman, 2018; Van Kaer, Postoak, Wang, Yang, & Wu, 2019). All of these cells have been implicated in MS immunopathogenesis (Mammana et al., 2018; Ponath, Park, & Pitt, 2018; Sie & Korn, 2017). Innate immune responses begin with recognition of potential external threats such as pathogen-associated molecular patterns (PAMPs) or internal threats such as damage-related molecular patterns (DAMPs) by pattern recognition receptors (PRRs) on myeloid cells.

Soluble mediators including cytokines, chemokines, proteins (cell adhesion molecules and complement proteins), microRNA (miRNA), enzymes (matrix metalloproteinases- MMPs, myeloperoxidase, inducible nitric oxide synthase, NADPH oxidases, and cyclo-oxygenase 2), and nucleosides such as adenosine triphosphate (ATP) enable cell-to-cell communication in innate immunity (Hannocks et al., 2019; Kothur, Wienholt, Brilot, & Dale, 2016; Wang et al., 2018). Cytokines, chemokines, nucleosides and cell adhesion molecules exert their function via their cognate receptors (Wang et al., 2018). ROS and reactive nitrogen species induce oxidative and nitrative stress, respectively (Ohl et al., 2016). Finally, pro-resolving mediators such as resolvins and maresines have been shown to promote resolution of inflammation (Schett & Neurath, 2018; Serhan & Levy, 2018). All of these soluble factors have been implicated in MS immunopathogenesis (Baecher-Allan et al., 2018; Filippi et al., 2018; Wang et al., 2018).

4. Microglia

Microglia are tissue-resident macrophages that comprise approximately 10% of the cells in the CNS (Wolf, Boddeke, & Kettenmann, 2017). They arise from yolk-sac primitive erythromyeloid progenitor cells which migrate into the brain at early prenatal embryogenic developmental stages (Guttenplan & Liddelow, 2019). Migration and colonization of microglia into the brain begin before the BBB formation between embryonic day 8 and 10 (E8-E10) in mice (Reu et al., 2017) and embryonic day 4,5 to 5 weeks in human (Sominsky, De Luca, & Spencer, 2018).

Microglia have distinct morphology in the adult brain. Prenatal and early postnatal microglia are mostly in the non-ramified state, indicating that microglia in the developing brain is continuously active (Wolf et al., 2017). At the end of the second week postnatally, microglia switch to a mature phenotype indicated by the expression of transmembrane protein 119 (Tmem119) and gain a ramified morphology (Lenz & Nelson, 2018).

4.1 Homeostatic microglia

Microglia play a crucial role in the development of the CNS. During the prenatal period, microglia contribute to synaptic elimination, neuronal cell death and also promote neural precursor cell proliferation and survival. Microglia regulate wiring of the forebrain circuits and modulate axonal outgrowth in prenatal mice (Tay, Savage, Hui, Bisht, & Tremblay, 2017). During development, microglia have an essential role in vasculogenesis and vascular sprouting by secreting soluble factors such as vascular endothelial growth factor (Kierdorf & Prinz, 2017).

During postnatal development, microglia eliminate the neurons that are not involved in functional circuits (Colonna & Butovsky, 2017; Ikegami, Haruwaka, & Wake, 2019). Microglia also shape neuronal synapses via synaptic pruning, which is the elimination of dendritic spines that are not receiving signals from synaptic contacts (Colonna & Butovsky, 2017). In early postnatal stage, microglia enhance cortical apoptosis which is essential for synaptic plasticity and behavioral adaptation (Bar & Barak, 2019; Nonaka & Nakanishi, 2019; Sominsky et al., 2018).

Together with their immune roles, microglia are essential modulators of processes including synaptogenesis, survival of synapses, neuronal maturation and activity of neurons in adults (Wolf et al., 2017). Microglia contribute to the maintenance of homeostasis of brain by continuously scanning the microenvironment with their ramified processes. Adult microglia express numerous surface molecules on their cell membrane to immediately respond to changes in their microenvironment by the release of several cytokines, chemokines, hormones, purines, neurotransmitters and neurotrophic factors. Microglial brain-derived neurotrophic factor (BDNF) is also a significant contributor to the regulation of learning-induced synapse formation in the healthy brain (Parkhurst et al., 2013). To maintain homeostasis, microglia express surface markers that include fractalkine receptor CX3C chemokine receptor 1 (CX3CR1), colony stimulating factor 1 receptor (CSF-1R), the integrin CD11b, surface glycoproteins F4/80 and CD68,

ionized calcium-binding adapter molecule 1 (IBA1), and pan-hematopoietic CD45 (Li & Barres, 2018; Tay et al., 2017).

4.2 Cell to cell interaction of microglia with other CNS cells

4.2.1 Interaction with neuron

Cell surface receptors and their ligands enable a bidirectional interaction between microglia with neurons. For instance, neurons express a ligand CX3L1 (also known as fractalkine) that is specific to the microglial receptor, CX3CR1 (Kabba et al., 2018). Neuro-immune-regulators (NIREGs) are proteins that regulate both the severity and duration of microglial responses (Bedoui, Neal, & Gasque, 2018). Constitutive expression of CD200, CD47 and chemokine (C-X-C motif) ligand 1 (CXCL1) on the neuronal surface are important in enhancing tissue resilience. Microglia remain in a steady state through interactions via their co-receptors CD200R, CD172a and CX3CR1 (Wolf et al., 2017). Neurons express numerous complement NIREGs, including CD55, CD46, CD59, and factor H. NIREGs are also involved in the regulation of cell stress, inflammatory responses and insurance of 'don't eat me' signal in order to prevent phagocytosis by microglia (Bedoui et al., 2018). Microglia can also modulate neuronal activity at the synaptic level (Marinelli, Basilico, Marrone, & Ragozzino, 2019).

4.2.2 Interaction with oligodendrocyte

There is a well-established balance between microglia and oligodendrocytes, which are responsible for myelin production. Microglia secrete growth factors that stimulate oligodendrocyte precursor cell (OPC) differentiation and myelination by promoting the expression of myelination-related proteins (Bar & Barak, 2019). Microglia can cross-talk with oligodendrocytes through CXCR1, CD200R, CSF-1R, cytokines, chemokines and neurotrophic factors during developmental stages. Additionally, oligodendrocytes can also activate microglia by releasing chemokines and cytokines (Kabba et al., 2018).

4.2.3 Interactions with astrocyte

Astrocytes account for almost 50% of all glial cell in the CNS (Kabba et al., 2018). They have key roles in the regulation of homeostasis, synaptic plasticity and neuroprotection (Li, Li, Zheng, & Qin, 2019). Astrocytes are critical players in the pathogenesis of EAE and MS (Brambilla, 2019; Ponath et al., 2018). Microglia-astrocyte cross-talk contributes to synaptic pruning during development (Thion, Ginhoux, & Garel, 2018). Astrocytes play a

central role in the synaptic maturation and ramification of microglia. They can also stimulate microglial proliferation by secreting soluble factors (Reemst, Noctor, Lucassen, & Hol, 2016). Activated microglia can stimulate reactive A1 astrocytes by secreting interleukin-1β (IL-1β), tumor necrosis factor-α (TNF-α), and complement component 1q both *in vitro* and *in vivo*. Activated A1 astrocytes then secrete unidentified neurotoxin(s) that can lead to neuronal and oligodendrocyte death (Hinkle, Dawson, & Dawson, 2019; Liddelow et al., 2017).

4.3 Immune functions of microglia

Microglia express variety of receptors related to the myeloid lineage, such as PPRs that recognize PAMPs and DAMPs (Wolf et al., 2017). In addition, to maintain brain homeostasis phagocytic microglia are known to express antigen presentation genes (Dubbelaar, Kracht, Eggen, & Boddeke, 2018). Resting microglia transform into an activated state and migrate to the lesion site once a signal is detected from an infection, trauma, neurodegenerative disease, loss of brain homeostasis, or programmed cell death (Eyo & Wu, 2019).

4.3.1 Phagocytosis

Phagocytic microglia are essential for normal development, injury and brain regeneration. In addition to removing complete or part of cells, microglia also have an important role in the clearance of misfolded proteins and myelin debris (Kettenmann, Hanisch, Noda, & Verkhratsky, 2011). Microglia respond to demyelination injury and facilitate the remyelination process by phagocytosis of myelin debris, remodeling the extracellular matrix and secreting trophic factors that is required for OPCs (Lloyd, Davies, & Miron, 2017). Microglial cells phagocyte pathogens, apoptotic cells and cellular debris through secreting cytokines and producing ROS (Tay et al., 2017).

4.3.2 Antigen presentation

Phagocytic and endocytic microglia have receptors such as TLRs that can effectively mediate antigen capture (Fiebich, Akter, & Akundi, 2014). Activated microglia also possess a lysosomal machinery in order to process antigens and express MHC Class II along with costimulatory molecules required for myelin peptide presentation to antigen specific $CD4^+$ T cells in the CNS (Schetters, Gomez-Nicola, Garcia-Vallejo, & Van Kooyk, 2017).

4.3.3 Microglial polarization

Microglia can polarize into distinct phenotypes depending on the nature of stimuli. Various conditions including age and CNS disorders, microenvironmental factors can influence microglial polarization. In the classical activation, M1 microglia comprise the first defense of innate immunity that accounts for the first few hours to days after stimulation. Microglia polarize into the M1 phenotype by lipopolysaccharide (LPS), interferon-γ and bacterial debris (Kabba et al., 2018), then secrete proinflammatory cytokines IL-1β, IL-6, and TNF-α that contribute to innate responses.

Microglia can also polarize into the M2 phenotype by IL-4 and interleukin-13 (IL-13) (alternative activation). M2 microglia play role in tissue repair once activation signals are received from damaged and dysfunctional neurons. To provide resolution of inflammation microglia then secrete several anti-inflammatory cytokines and neurotrophic factors that include IL-4, IL-10, insulin-like growth factor-1 (IGF-1), transforming growth factor-β and BDNF. However, microglial activation status is now considered to be a highly dynamic process that is tightly regulated. After single-cell transcriptomics and mass cytometry studies in both mice and human, the dichotomic view remains oversimplified.

5. Microglia in EAE and MS

Microglia play a fundamental role in all MS lesions (Zrzavy et al., 2017). After inflammation or CNS injury, microglia become activated (Wang et al., 2019). Activated microglia or disease associated microglia found in lesions and white matter in MS patients have an increased expression of pro-inflammatory genes (Wang et al., 2019). The relationship between microglial activation and its contribution to disease progression was demonstrated by a decrease in P2Y purinoceptor 12^{+} homeostatic microglia and an increase in $TMEM119^{+}$ microglia (Zrzavy et al., 2017). The role of activated microglia in the EAE model have also been studied. Centonze et al. demonstrated that activated microglia triggered excitation of post-synaptic currents by triggering TNF-α release and caused subsequent glutamate excitoxicity (Centonze et al., 2009, 2010).

Microglia and T cells are found in MS lesions (Luo et al., 2017). Activated microglia cause myelin sheath and oligodendrocyte damage via secreting ROS, reactive nitrogen species and glutamate (Luo et al., 2017; Voet, Prinz, & Van Loo, 2019). Pro-inflammatory cytokines secreted

from microglia exert a neurotoxic effect in MS (Surace & Block, 2012; Voet et al., 2019). Microglia also secrete chemokines for the recruitment of infiltrating cells (monocyte, macrophage and lymphocyte).

Microglia have also beneficial effects including phagocytosis, BDNF secretion and remyelination in MS (Yamasaki et al., 2014). Microglial triggering receptor expressed on myeloid cells 2, CR3 and signal regulatory protein-α receptors take place in the phagocytosis of myelin debris (Voet et al., 2019). Microglia contribute to remyelination by secreting IGF-1 and fibroblast growth factor (FGF-2) and induce proliferation of OPCs (Luo et al., 2017).

6. NLRP3 inflammasome

Inflammasomes are intracellular multiprotein complexes that mediate innate immune responses against PAMPs and DAMPs. The components and activation mechanisms of inflammasomes has been demonstrated by Tschopp and his group in 2002 (Martinon, Burns, & Tschopp, 2002). Activation of inflammasomes ultimately result in proteolytic cleavage and secretion of proinflammatory cytokines, pro-IL-1β and IL-18 that are crucial for the clearance of pathogens or injured cells (Hanamsagar, Torres, & Kielian, 2011). However, overactivation or dysregulation of inflammasomes are known to contribute to the pathogenesis of several disorders, including infections, autoimmune, and neurodegenerative diseases.

6.1 Sensors

Inflammasome sensor proteins are grouped based on their structural features. The nucleotide-binding, leucine-rich repeat containing proteins (NLR) represent a family of cytosolic sensors that are key components of the inflammasome complex (Delbridge & O'riordan, 2007). Each family member contains a C-terminal leucine-rich repeat region (LRR) required for ligand detection, a central nucleotide-binding and oligomerization domain (NOD or NACHT), and an N-terminal effector domain which differs in each NLR subfamily proteins, such as caspase recruitment domain (CARD), and pyrin domain (PYD) (Areschoug & Gordon, 2008). Various inflammasome sensors can recognize diverse molecules, including nucleic acids, bacterial proteins, toxins, metabolites and protein aggregates (Mangan et al., 2018).

6.2 NLRP3 triggers

The NLRP3 inflammasome is the most extensively studied inflammasome complex and prototype of NLRP proteins. Activation of the NLRP3 inflammasome triggered by LPS and ATP has been characterized by Mariathasan and his group in 2006. The NLRP3 inflammasome can be activated by a wide range of triggers including pathogen-derived ligands, such as cell wall components, nucleic acids, and toxins; environmental crystalline particles like silica, asbestos, and alum; and endogenous danger signals like ATP, uric acid crystals and aggregated proteins (Braga et al., 2017; Leemans, Cassel, & Sutterwala, 2011; Yeon, Yang, Lee, & Lee, 2017).

6.3 Components of NLRP3 complex and their domains

The NLRP3 inflammasome is formed by a sensor protein (NLRP3), an adaptor molecule (apoptosis-associated speck-like protein-ASC); and an effector enzyme (caspase-1) (Broz & Dixit, 2016) (Fig. 3). NLRP3 has three domains: a pyrin domain, a nucleotide binding NACHT domain and a LRR domain. The NACHT domain binds ATP and exerts ATPase activity. The pyrin domain is involved in homotypic interactions with the ASC adaptor protein. ASC contains two distinct domains: the N-terminal pyrin and the C-terminal CARD domains. ASC has a unique adaptor function that brings together PYD- and CARD-containing proteins to assemble an inflammasome. When ASC specks are generated one speck per cell, it is accepted as an evidence of inflammasome activation. Nucleation of ASC allows formation of CARD−CARD interactions with pro-caspase-1 (Kumar, Kawai, & Akira, 2011). Subsequently, caspase-1 undergoes auto cleavage and promotes the maturation and secretion of proinflammatory mediators IL1b and IL-18 (Mangan et al., 2018; Schroder & Tschopp, 2010) (Fig. 3).

6.4 Mechanisms of NLRP3 inflammasome activation

6.4.1 Priming

Canonical activation of the NLRP3 inflammasome occurs in two steps: priming and activation. In many resting cells, the basal expression of NLRP3 and IL-1β protein levels are not sufficient to activate the NLRP3 inflammasome. The priming signal leads to nuclear factor kappa B (NF-kB) translocation into nucleus and is subsequently accompanied with an increase in the messenger ribonucleic acid (mRNA) levels of both NLRP3 and pro-IL-1β (Sun, 2011; Sutterwala, Haasken, & Cassel, 2014; Green et al., 2018). The priming step can be triggered various stimuli such as LPS,

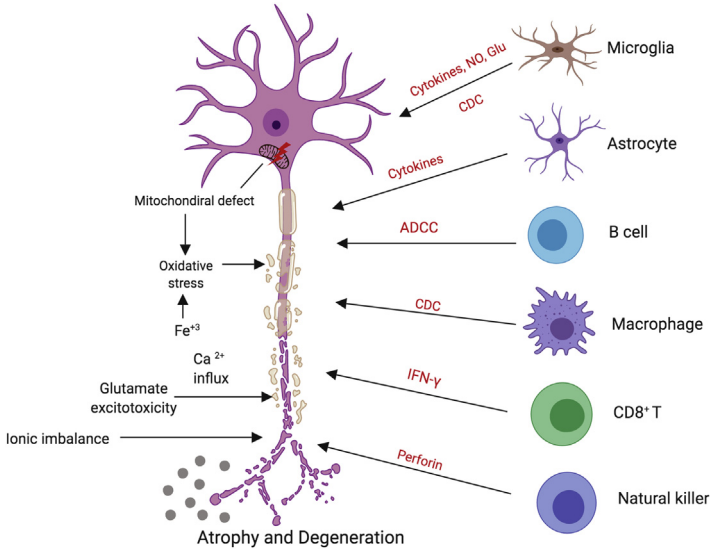

Fig. 3 Mechanisms of cell injury in MS.* Immune dependent and independent mechanisms induce demyelination and neuronal degeneration. * Created with BioRender. (*NO*, nitric oxide, *CDC*, complement dependent cytotoxicity, *ADCC*, antibody dependent cellular cytotoxicity). See the text for further detail.

cytokines, high mobility group box 1 and advanced glycation end products through toll Like Receptor 4 (TLR4); TNF-α and IL-1β via their cognate receptors (Mukherjee et al., 2009). In addition to the transcriptional priming step, recent studies suggest that non-transcriptional priming mechanisms can also effectively activate NLRP3 within minutes through post-translational modifications, such as phosphorylation of NLRP3 by c-Jun N-terminal kinase (JNK) or mitogen-activated protein kinase (Sun, 2011).

6.4.2 Activation

Diverse endogenous and exogenous signals trigger the assembly of the inflammasome components. However, the exact mechanism whether the same inflammasome sensor is activated by various danger signals remains unknown. One plausible explanation is that NLRP3 can sense these stimuli indirectly via intracellular upstream cellular stress signals, including ion flux, ROS, lysosomal disruption and Golgi disassembly (Groslambert & Py, 2018).

The most common ion flux that activates the NLRP3 inflammasome is potassium (K^+) efflux. Various stimuli like ATP, crystalline and nigericin

decrease the intracellular concentration of K^+ in cells. But, K^+ efflux is not specific for NLRP3 inflammasome activation (Munoz-Planillo et al., 2013). Increase in cytosolic calcium (Ca^{++}) leads to NLRP3 inflammasome activation through increasing mitochondrial ROS production (Csordas & Hajnoczky, 2009; Lemasters, Theruvath, Zhong, & Nieminen, 2009). Sodium (Na^+) influx and chloride (Cl^-) efflux are also involved in NLRP3 inflammasome activation (Yang, Wang, Kouadir, Song, & Shi, 2019).

ROS and especially mitochondrial ROS (mtROS) are well known endogenous triggers for NLRP3 inflammasome activation. Several NLRP3 inflammasome agonists increase ROS production, and ROS inhibition suppress NLRP3 inflammasome activity. Although these findings support the role of ROS in the activation of the NLRP3 inflammasome, the exact mechanism of how NLRP3 inflammasome sense ROS is still not known. It is possible to think that NEK7, a NLRP3 interacting protein, can either sense ROS production or K^+ efflux instead of NLRP3 itself (Gross, Mishra et al., 2016). Intracellular ROS can also indirectly activate the NLRP3 inflammasome by enhancing the interaction between thioredoxin-interacting protein and NLRP3 (Tschopp & Schroder, 2010).

Mitochondrial dysfunction also contributes to inflammasome activation. Anti-apoptotic mitochondrial proteins B-cell lymphoma 2 and B-cell lymphoma-extra-large inhibit the NLRP1 inflammasome (Faustin et al., 2009). Chemicals that induce mitochondrial membrane pore formation can activate the NLRP3 inflammasome through the release of mitochondrial deoxyribonucleic acid or by altering mitophagy (Allam et al., 2014). Mitochondrial localization of NLRP3 and its specific interactions with mitochondrial proteins like mitofusin2 and cardiolipin have been implicated to be an activation mechanism for inflammasomes (Iyer et al., 2013).

Lysosomal destabilization acts on both the priming and activation steps for inflammasomes. The inefficient clearance of large foreign particles such as silica may cause lysosomal ruptures and the release of the lysosomal enzyme Cathepsin B can then activate the NLRP3 inflammasome (Hornung et al., 2008). Lysosomes are involved in the regulation of intracellular calcium homeostasis. Lysosomal calcium signaling acts at the priming step by stabilizing pro-IL-1β mRNA (Weber & Schilling, 2014). Lysosomal membrane rupture activates the NLRP3 inflammasome due to the K^+ efflux after pore opening (Munoz-Planillo et al., 2013).

Recent studies have shown the role of the Golgi apparatus in inflammasome activation. NLRP3 first triggers the disassembly of the trans-golgi

network into vesicles, and then induces NLRP3 aggregation (Chen & Chen, 2018). NLRP3 is translocated from the endoplasmic reticulum to Golgi by cholesterol biosynthesis regulator proteins (sterol regulatory element-binding protein2 and sterol regulatory element-binding protein cleavage-activating protein), which has been proposed to be a requirement for inflammasome activation (Guo et al., 2018).

6.5 Other activation mechanisms of NLRP3 inflammasome

Caspase-4/5 in humans, and caspase-11 in mice are responsible for noncanonical inflammasome activation (Yi, 2018). Cytosolic LPS is the main inducer of the noncanonical pathway. Caspase-11 senses cytosolic LPS through binding to the lipid A portion of LPS and leads to pyroptosis without the involvement of caspase-1. Active caspases cleave gasdermin-D (GSDMD) and cause the release of the N-terminal fragment that drives pyroptosis. Consequent, K^+ efflux is responsible for the release of inflammatory cytokines. The N-terminal fragment of GSDMD can also activate the NLRP3 inflammasome via an undefined mechanism.

Alternative activation of the NLRP3 inflammasome is mediated by the TLR4 signaling pathway (Yang et al., 2019). Alternative activation exerts specific features including requirement of ASC and caspase-1, independency on K efflux and lack of ASC speck formation and pyroptosis. Signaling cascade continues TLR4- TIR-domain-containing adapter-inducing interferon-β - receptor-interacting serine/threonine-protein kinase 1-Fas-associated protein with death domain-caspase-8 to induce NLRP3 inflammasome activation and subsequently leads to IL-1β and IL-18 secretion (Gurung et al., 2014). The role of caspase-8 activation in the NLRP3 inflammasome by alternative pathway has not yet been completely elucidated. Caspase-8 activation may act as a priming signal that directly cleaves pro-IL-1β and contributes to the secretion of cytokines. In the absence of inhibitor of apoptosis protein (IAP), caspase-8 mediated cell death signal may act as an inflammasome signal (Gurung & Kanneganti, 2015).

6.6 Regulation

Inflammasome function is tightly regulated by several mechanisms (Rathinam, Vanaja, & Fitzgerald, 2012), as uncontrolled activation may lead to a variety of autoimmune diseases and metabolic disorders. Regulation of NLRP3 may occur at the transcriptional, post-transcriptional and post-translational levels (Song & Li, 2018).

6.6.1 Negative regulators of NLRP3 inflammasome

Negative regulators limit inflammasome activity and can interfere at different steps of inflammasome formation (Tozser & Benko, 2016). For example, the heat shock protein tripartite motif-containing protein 30, acts as a negative regulator through the inhibition of ROS production. Nitric oxide, carbon monoxide, small heterodimer partner protein, inhibitor of nuclear factor Kappa-B kinase subunit α, and G protein signaling modulator-3 have all been reported to act as negative regulators of the NLRP3 inflammasome. Autophagy also negatively regulates the NLRP3 inflammasome by several mechanisms, including the clearance of intracellular inflammasome inducer sources, suppression of IL-1β release, and degradation of inflammasome complex proteins (Rodgers, Bowman, Liang, & Jung, 2014).

6.6.2 Post-transcriptional regulation

Regulatory molecules like microRNAs (miRNAs) and RNA binding proteins can limit the amount and stability of mRNAs that encode essential proteins for the priming and/or activation steps, thus ultimately regulate NLRP3 inflammasome activity (Tezcan et al., 2019). miRNAs are 20–23 nt long noncoding RNAs that control gene expression via binding to the 3′UTR of coding mRNAs. Several miRNAs have been identified as post-transcriptional regulatory mechanisms for NLRP3 activity. miRa-146a is a negative regulator of the NLRP3 inflammasome at priming step and acts by targeting the NF-κB signaling molecules TNF receptor associated factor 6, and interleukin-1 receptor-associated kinase 1 (Singer et al., 2018). miR-223 can bind the 3′ UTR region of NLRP3 mRNA, and reduce NLRP3 expression. Due to their higher expression in myeloid cells, miR-223 plays a crucial role in innate immune responses (Zhao et al., 2018).

6.6.3 Post-translational regulation

Common post-translational modifications of proteins, such as phosphorylation, methylation, acetylation, glycosylation, ubiquitination, sumoylation and s-nitrosylation are involved in the regulation process. Ubiquitination type modifications are crucial for the regulation of the NLRP3 inflammasome. A20 is a NF-kB responsive deubiquitinase that can either activate or suppress inflammasome activation based on cellular signals (Das, Chen, Hendriks, & Kool, 2018; Rothschild, Mcdaniel, Ringel-Scaia, & Allen, 2018; Yue, Stone, & Lin, 2018). While the deubiquitinating enzyme BRCA1/BRCA2-containing complex subunit 3 induces NLRP3 activation, E3 ubiquitin ligase F-box L2 ubiquitinates the lysine-689 residue on

NLRP3 to promote its proteosomal degradation (Py, Kim, Vakifahmetoglu-Norberg, & Yuan, 2013). Phosphorylation of NLRP3 is another regulatory mechanism for the inflammasome activation. The protein tyrosine phosphatase non-receptor type 22 activates NLRP3 by dephosphorylating the tyrosine-861 residue (Spalinger et al., 2016). Phosphorylation of NLRP3 at the serine-295 residue prevents inflammasome assembly formation by inhibiting the ATPase activity (Mortimer, Moreau, Macdonald, & Chadee, 2016). Phosphorylation of NLRP3 can also act as a positive regulator of inflammasome activation. A recent study demonstrated that the serine-194 residue phosphorylated by JNK-1 can drive NLRP3 assembly formation (Song et al., 2017).

6.7 Consequences of NLRP3 inflammasome activation

6.7.1 Secretion of cytokines

IL-1β is a crucial player for proper inflammasome functioning in the CNS (Dinarello, 2018). By damaging endothelial cells, IL-1β disrupts the structural integrity of the BBB. When astrocytes secrete the chemokine, CCR2, peripheral immune cells start infiltrating into the CNS through the impaired BBB (Mccandless et al., 2009). Microglia and astrocytes are also activated by IL-1 β, and in turn can stimulate T cells and generate pro-inflammatory cytokines such as IL-6 and TNF-α (Ferrari et al., 2004). IL-1β mediates neuronal injury via glutamate excitotoxicity that is detected in the pathogenesis of MS (Gosselin & Rivest, 2007; Kostic, Zivkovic, & Stojanovic, 2013; Lin & Edelson, 2017; Rossi et al., 2012).

IL-18 is another cytokine processed by the NLRP3 inflammasome activation in MS (Sedimbi, Hagglof, & Karlsson, 2013). Recent studies have revealed the involvement of IL-18 in disease pathology in both *in vivo* animal models and in clinical studies (Dinarello, 2018). IL-18 has similar effects to IL-1β on T and B cells (Nakanishi, 2018). It also upregulates the expressions of caspase-1, MMPs, and pro-inflammatory cytokines by activating several signaling pathways in microglia (Bossu et al., 2009).

6.7.2 Pyroptosis

Caspase-1 activation by the inflammasome leads to programmed cell death mediated by GSDMD, namely pyroptosis. When GSDMD is cleaved by caspase-1, the C terminal fragment is removed, and its inhibition at the N terminal of gasdermin (GSDMD-NT) is abolished. Then, GSDMD-NT translocates and binds to phosphatidylinositol phosphates and phosphatidylserine in the inner leaflet of the membrane and oligomerizes to form

~15 nm pores (Rathkey et al., 2018). These pores let water into the cell, increase intracellular pressure causing membrane rupture, and finally induce the release of pro-inflammatory cytokines. Non-canonical inflammasome activation by caspases 4, 5 and 11 also activate the cleavage of GSDMD to induce pyroptosis (Liu, Zhang et al., 2016). Pyroptotic cell death attracts leukocytes to the site of inflammation, induces subsequent inflammatory responses, and leads to severe tissue damage (Bortolotti, Faure, & Kipnis, 2018).

6.8 NLRP3 inflammasome informs adaptive immunity

The IL-1 family cytokine signaling is important in adaptive immunity (Garlanda, Dinarello, & Mantovani, 2013). IL-1β provides survival signals for naive T cells and is crucial for the differentiation of T helper cell subtype Th17. This connection is involved in sterile autoimmune activity in MS (Wang, Ma, Wu, & Zhu, 2013).

Similar to IL-1β, IL-18 also plays role in the induction of adaptive immunity (Raupach, Peuschel, Monack, & Zychlinsky, 2006). IL-18 is described as IFN-γ inducing factor for T cells under pathogenic insults, and mediates differentiation of Th1 cell subtype and NK cells (Chaix et al., 2008; Harrison et al., 2015).

7. NLRP3 in CNS disorders

Microglia has been the focus of many NLRP3 inflammasome studies as they express a variety of inflammation sensing proteins. Microglial NLRP3 inflammasome activation has been extensively studied in acute and chronic CNS disorders, including Alzheimer's disease (AD), Parkinson's disease (PD), amyotrophic lateral sclerosis (ALS), frontotemporal dementia, stroke, traumatic brain and spinal cord injuries. *In vitro* studies revealed that aggregated ß-amyloid (Aβ) and hyperphosphorylated tau proteins induced the activation of the NLRP3 inflammasome in microglia (Stancu et al., 2019). A recent postmortem study has reported the increased expression of ASC, caspase-1, IL-1β in the cerebral cortex of AD patients (Li, Ismael et al., 2019).

Similar to Aβ, aggregated αSyn, a pathological protein accumulated in PD, can act as DAMPs to activate microglial NLRP3 inflammasome, thereby inducing mitochondrial impairment, ROS production and Cathepsin B activity (Bai Yang et al., 2018; Mouton-Liger et al., 2018;

Panicker et al., 2019; Sarkar et al., 2017). *In vivo* studies using either genetic (mutation in α-synuclein, parkin, PTEN-induced kinase 1) or toxin-based (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine, 6-hydroxydopamine) models have demonstrated the contribution of microglial NLRP3 inflammasome to PD pathogenesis (Lee et al., 2019; Mao et al., 2017; Mouton-Liger et al., 2018; Zhou et al., 2016). Microglial NLRP3 inflammasome activation has been observed in patients with ALS and frontotemporal dementia in which pathological proteins (mutant superoxide dismutase and TAR DNA-binding protein 43) have been accumulated (Johann et al., 2015; Leal-Lasarte, Franco, Labrador-Garrido, Pozo, & Roodveldt, 2017).

NLRP3 inflammasome activation is predominantly observed in microglia in animal models of traumatic brain (TBI) and spinal cord injury (Lin et al., 2017; Mortezaee, Khanlarkhani, Beyer, & Zendedel, 2018; Xu et al., 2018). NLRP3 inhibition in these models were able to rescue neurons from microglia mediated cell death (Mortezaee et al., 2018; Xu et al., 2018). In addition, the concentration of NLRP3 was elevated in the CSF of children with severe TBI, supporting the idea that the NLRP3 inflammasome is activated upon trauma (Wallisch et al., 2017). Post-ischemic inflammatory responses exert deleterious effects on stroke sequelae. Microglial NLRP3 inflammasome activation was also detected at the early stages in ischemic stroke animal models (Fann et al., 2013; Gong, Pan, Shen, Li, & Peng, 2018).

In addition to microglia, recent studies point out that NLRP3 inflammasome activation is also observed in other CNS cells, such as astrocytes, neurons, cerebral endothelial cells and pericytes in experimental models of CNS disorders. Immune cells (T cells and myeloid cells) express components of the NLRP3 inflammasome complex in disease conditions, such as MS. Further studies are required to gain insight and a comprehensive understanding of the role of NLRP3 inflammasome activation in the CNS and peripheral immune cells.

8. Microglial NLRP3 in EAE

The critical role of NLRP3 inflammasome activity in MS pathology has been demonstrated in several animal models. The NLRP3 inflammasome complex components NLRP3, ASC and pro-caspase-1 has been activated in EAE (Place & Kanneganti, 2017) (Table 1). NLRP3 inflammasome amplifies the neuroinflammation and contributes the progression of EAE (Inoue & Shinohara, 2013; Jafarzadeh & Nemati, 2018).

One of the hallmarks of NLRP3 inflammasome activation is the production and secretion of pro-inflammatory cytokines, IL-1β and IL-18 (Malik & Kanneganti, 2017). IL-1β has a fundamental role in MS (Lin & Edelson, 2017; Mendiola & Cardona, 2018). Many earlier studies have demonstrated an upregulation of these pro-inflammatory cytokines at both the mRNA and protein level (Lukens, Barr, Chaplin, Chi, & Kanneganti, 2012; Mckenzie et al., 2018; Zhang et al., 2018; Znalesniak et al., 2016). In a study by Lukens et al., IL-1β protein expression was significantly increased in EAE and this increase was shown to be specifically mediated by caspase-1 activity (Lukens et al., 2012). EAE induced mice demonstrated a significant increase in IL-1β and other inflammasome components protein levels in the cerebellum and spinal cord (Znalesniak et al., 2016). Similarly, another study showed that EAE induction significantly increased IL-1β mRNA levels in the hindbrain and spinal cord, whereas IL-1β protein expression was only upregulated in the spinal cord (Mckenzie et al., 2018). EAE induction in IL-1β $^{-/-}$ mice demonstrated that this deficiency led to a decrease in $CD4^{+}$ T cells together with a subsequent reduction of GM-CSF secreted by these cells (Lukens et al., 2012). Another study in EAE induced mice revealed that IL-1β deficient microglia secreted less inflammatory cytokines and chemokines including IL-6, IL-13, CXCL-1 and chemokine (C-X-C motif) ligand 2 and failed to enhance EAE progression (Zhang et al., 2018).

Activation of the NLRP3 inflammasome is required for GM-CSF production, which contributes to the development of EAE. It has been demonstrated that IL-1β produced by the NLRP3 inflammasome complex is required for the regulation of GM-CSF production along with the MYD88 adaptor protein. The experiments with IL-1R knock out mice revealed that IL-1R is required for the production GM-CSF and this deficiency reduced the clinical score in EAE induced mice. In addition, the number of MHC-II positive microglia was reduced in EAE induced IL-1R $^{-/-}$ mice (Lukens et al., 2012). IL-18 also plays a critical role in the pathogenesis of MS exacerbations (Karni, Koldzic, Bharanidharan, Khoury, & Weiner, 2002). In the EAE model, IL-18 protein expression was significantly increased and its deficiency led to a significant decrease in the clinical score of disease (Gris et al., 2010).

The alterations in the level of NLRP3 inflammasome complex proteins in EAE have been reported in several studies. The expression of NLRP3 was markedly increased both at the mRNA (Gris et al., 2010; Znalesniak et al., 2016; Mckenzie et al., 2018) and protein level (Bai, Wang et al., 2018b; Ke et al., 2017). Caspase-1 mRNA and protein levels together with the NLRP3

Table 1 NLRP3 in EAE models.

Organism	EAE model	Inflammasome components identified	Site of detection	Method of detection	Clinical outcome	Outcome	References
Mouse (C57BL/6, *Nlrp3*$^{-/-}$, *Casp-1*$^{-/-}$, *Il-18*$^{-/-}$)	MOG_{35-55}	NLRP3, IL-1β, IL-18, GFAP	spinal cord	clinical score, RT-PCR, ELISA	Decreased clinical score in *Nlrp3*$^{-/-}$ and *Il-18*$^{-/-}$ mice	Increased NLRP3 mRNA expression in EAE induced mice Reduced demyelination and astrogliosis in *Nlrp3*$^{-/-}$ mice Reduced IL-18 cytokine expression, MOG-specific Th1 and Th17 T cell response and reduced inflammatory infiltrate in the CNS in *Nlrp3*$^{-/-}$ mice Reduced IFN-γ and IL-17 in *Il-18*$^{-/-}$ mice	(Gris et al., 2010)
Mouse (C57BL/6, *Nlrp3*$^{-/-}$, *Casp1*$^{-/-}$, *Il-1β*$^{-/-}$, *Il-18*$^{-/-}$)	MOG_{35-55}	N/A	spinal cord	clinical score	Decreased clinical score in *Nlrp3*$^{-/-}$mice	Reduced demyelination in *Nlrp3*$^{-/-}$ mice	(Jha et al., 2010)

Mouse (C57BL/6, *Nlrp3*$^{-/-}$, *Casp1*$^{-/-}$, *Il-1β*$^{-/-}$, *Il-18*$^{-/-}$)	8-10 week-old male mice, 0.2% cuprizone, 6 weeks	NLRP3, IL-1β, IBA1, GFAP	corpus callosum	RT-PCR, ELISA	N/A	Increased NLRP3 expression and IL-1β Decreased microgliosis and astrogliosis in *Nlrp3*$^{-/-}$ mice Delayed demyelination and decreased mature oligodendrocyte death in *Nlrp3*$^{-/-}$ mice Delayed demyelination and decreased astrogliosis, microgliosis and mature oligodendrocyte death in *Casp1*$^{-/-}$ mice	(Jha et al., 2010)
Mouse (C57BL/6, *Nlrp3*$^{-/-}$, *Casp1*$^{-/-}$, *Asc*$^{-/-}$)	MOG_{35-55}	N/A	spinal cord	clinical score	Decreased clinical score in *Asc*$^{-/-}$ and *Casp1*$^{-/-}$ mice	Decreased MOG-specific T cells	(Shaw et al., 2010)

(*Continued*)

Table 1 NLRP3 in EAE models.—cont'd

Organism	EAE model	Inflammasome components identified	Site of detection	Method of detection	Clinical outcome	Outcome	References
Mouse (C57BL/6, *Casp1*$^{-/-}$, *Il-1β*$^{-/-}$, *Il-1R*$^{-/-}$)	7-12 week-sex-matched mice, MOG_{35-55}	IL-1β	spinal cord	clinical score, Flow cytometry, ELISA	Decreased EAE clinical score in *Il-1R*$^{-/-}$	Reduced number of MHC-II positive microglia in EAE induced *Il-1R*$^{-/-}$ mice Decreased IL-1β expression in EAE induced *Casp1*$^{-/-}$	(Lukens et al., 2012)
Mouse C57BL/6	8-12 week-old male mice, MOG_{35-55}	NLRP3, IL-1β	spinal cord cerebellum	RT-PCR	N/A	Increased expression of NLRP3 and IL-1β mRNA	(Znalesniak et al., 2016)
Mouse (C57BL/6, *Nlrp3*$^{-/-}$, *Nlrc4*$^{-/-}$, *Asc*$^{-/-}$, *Casp1*$^{-/-}$, *Casp11*$^{-/-}$, *Il-1β*$^{-/-}$, *Il-18*$^{-/-}$)	8-10 week-old male mice, 0.2% cuprizone, 3—4 weeks	IL-1β, Caspase-1, IBA1, GFAP	BMDM	ELISA,WB, IF	N/A	Increased NLRC4 protein expression in IBA1 and GFAP positive cells in cuprizone induced mice Decreased microgliosis, astrogliosis and demyelination in *Nlrp3*$^{-/-}$ *Nlrc4*$^{-/-}$ DKO mice	(Freeman et al., 2017)

Mouse (C57BL/6)	10-12 week-old female mice, MOG_{35-55}	NLRP3, IL-1β, Caspase-1, NLRP1, Caspase-11, GSDMD, IBA1	hind brain spinal cord	IF, IHC, RT-PCR	N/A	Increased IL-1β, Caspase-1, NLRP3 and GSDMD mRNA expression in EAE induced mice Increased microgliosis and pyroptosis in EAE induced mice	(Mckenzie et al., 2018)
Mouse (C57BL/6, $CX3Cr1Cre^{EREYFP}$, $CX3Cr1^{GFP}$, *Nlrp3*$^{-/-}$, *Casp8*$^{fl/fl}$, *IRAKM*$^{fl/fl}$, *Casp8*$^{-/-}$, *Ripk3*$^{-/-}$, *Il-1β*$^{-/-}$	MOG_{35-55}, Adoptive immunization	IL-1β,ASC, Caspase-8, NLRP3, Caspase-1, IBA1	brain spinal cord	clinical score, ELISA, RT-PCR, Flow cytometry	Decreased clinical score in *Asc*$^{\Delta microglia}$, *Ripk3*$^{-/-}$, *Casp8*$^{\Delta microglia}$ mice	Decreased IL-1β mRNA expression in EAE induced *Asc*$^{\Delta microglia}$ mice Caspase-8 activity in NLRP3/ASC dependent manner in EAE induced mice Increased Caspase-8 activity in microglia in brain of EAE induced mice Microglia-intrinsic ASC increased Th1-and Th17 induced EAE in mice.	(Zhang et al., 2018)

Casp, Caspase; *NLRC*, NLR family CARD domain-containing protein 4; *Ripk*, Receptor interacting protein kinase; *IRAKM*, interleukin 1 receptor-associated kinase M.

expression was also reported to be increased in EAE induced mice (Bai, Wang et al., 2018; Ke et al., 2017; Mckenzie et al., 2018; Shao et al., 2014).

Knock out animal studies have confirmed the crucial role of NLRP3 inflammasome activation in the pathogenesis of EAE. Deficiency of these inflammasome complex proteins reduced disease progression and improved clinical scores (Gris et al., 2010; Jha et al., 2010; Mckenzie et al., 2018; Zhang et al., 2018; Znalesniak et al., 2016). EAE induction in *Nlrp3*$^{-/-}$ mice caused demyelination and astrogliosis that was accompanied with a reduced clinical score. Jha et al. demonstrated the critical role of NLRP3 inflammasome activation by using the cuprizone model to mimic MS. Deficiency in NLRP3 induced demyelination and caused mature oligodendrocyte death. *Nlrp3*$^{-/-}$ mice also displayed reduced microgliosis and astrogliosis following cuprizone induction. Furthermore, mice lacking caspase-1 were presented with delayed demyelination and reduced gliosis (Jha et al., 2010).

The role of ASC, another component of the NLRP3 inflammasome complex, was reported in EAE induced ASC KO mice with delayed disease progression, reduced MOG-specific T cells in the lymph node and improved clinical score (Shaw et al., 2010). The requirement of microglia specific ASC at the effector stage of EAE has been reported (Zhang et al., 2018). ASC deficiency in microglia led to a reduction in infiltration of immune cells, including $CD4^+$ T cells, B cells, neutrophils, and macrophages and attenuation of EAE. Furthermore, IL-1β is produced via IL-1 Receptor-Associated Kinase M (IRAKM)/ASC-NLRP3−caspase-8 axis and contributes to pathogenesis of EAE IRAKM-caspase-8 inflammasome activation is also necessary in microglia in EAE induced mice.

Another inflammasome complex, NLR family CARD domain-containing protein 4 (NLRC4) together with NLRP3 elicit disease progression in cuprizone-induced demyelination model. The expression of NLRC4 was increased in the corpus callosum of cuprizone-induced mice, especially in astrocytes and microglia. The use of *Nlrc4*$^{-/-}$, *Nlrp3*$^{-/-}$, and *Nlrp3*$^{-/-}$ *Nlrc4*$^{-/-}$ mice revealed that deficiency in NLRC4 and NLRP3 reduced microglia accumulation and astrocyte positive cells in the corpus callosum of cuprizone induced mice. Furthermore, reduced demyelination in these mice was reported (Freeman et al., 2017).

The noncanonical activation of the NLRP3 inflammasome and pyroptosis play role in the pathogenesis of EAE. The mRNA expression of NLRP3, IL-1β, caspase-1, GSDMD, caspase-11, NLRC4, and absent in melanoma 2 (AIM2) was increased in the hindbrain of EAE induced

mice. Additionally, GSDMD expression was elevated in spinal cord lesions and was colocalized with Iba-1 positive cells. GSDMD and caspase-1 positive microglia and oligodendrocytes suggest that these cells undergo pyroptosis (Mckenzie et al., 2018).

NLRP3 inflammasome activation also informs adaptive immunity (Deng, Yu, & Wang, 2019; Evavold & Kagan, 2018). NLRP3 deficiency diminished the Th1 and Th17 T cell response and reduced infiltration of inflammatory cells, including macrophages, $CD4^+$, and $CD8^+$ T cells in the spinal cord and brain (Gris et al., 2010; Inoue, Williams, Gunn, & Shinohara, 2012). NLRP3 inflammasome signaling may enhance the chemotactic ability of immune cells by increasing their chemotaxis-related protein expressions such as α4β1 integrin, chemokine (C-C motif) ligand 7, chemokine (C-C motif) ligand 8, and chemokine (C-X-C motif) ligand 16 (Inoue, Shinohara et al., 2012).

9. Experimental therapeutic implications

Although there is currently no cure for MS, several treatment options that provide symptom management are available. The drugs developed for this purpose target different molecular mechanisms that underlie MS. Inflammasome activation is one of the mechanisms that contribute greatly to MS progression. Several drugs that are currently in use have been shown to be effective against inflammasome activation in MS.

One of these drugs is IFN-β which has been widely used (Scheu et al., 2019) and is the first effective drug to be FDA approved for the treatment of MS (Tintore et al., 2019). The effect and mechanism of IFN-β on NLRP3 inflammasome activation has been shown in the EAE model (Inoue, Williams et al., 2012, 2016) (Table 2). The studies revealed that the efficacy of IFN-β treatment in EAE was remarkably dependent on the activation of the NLRP3 inflammasome. IFN-β treatment was effective as long as EAE progression relied on NLRP3 inflammasome activation (Inoue, William et al., 2012). Interestingly, the occurrence of NLRP3 inflammasome-independent EAE subtype called Type-B EAE showed resistance to IFN-β treatment. The high immunization in Type-B EAE caused strong stimulation of an innate immune response by subsequently bypassing the NLRP3 inflammasome in the EAE model (Inoue et al., 2016).

Another drug shown to be effective in suppressing NLRP3 inflammasome activation in MS is prednisolone (Yu et al., 2018) (Table 2).

Table 2 Experimental NLRP3 inflammasome inhibitors in EAE Models.

Name of therapeutic agent	Class of therapeutic agent	Organism	EAE model	Inflammasome components affected by treatment	Site of detection	Method of detection	Outcome	References
Ac-YVAD-cmk	Caspase-1 inhibitor	Mouse (C57BL/6)	MOG_{35-55}	IL-18		Flow cytometry, EAE clinical score	Reduced EAE clinical Score in caspase-1 inhibitor treated EAE induced mice Increased IL-18 expression in $CD4^+$ T cells from EAE induced mice	(Lalor et al., 2011)
IFNβ	Cytokine	Mouse (C57BL/6, *Asc*$^{-/-}$, *Nlrp3*$^{-/-}$, *Ifnar1*$^{-/-}$, 2D2 TCR Tg)	Active: MOG_{35-55} Passive: Transfer of $CD4^+$ T cells	IL-1β, Caspase-1	serum spleen	ELISA,WB	Decreased IL-1β and Caspase-1 protein expression in *Asc*$^{-/-}$ and *Nlrp3*$^{-/-}$ EAE-induced mice Decreased IL-1β and Caspase-1 protein expression in IFNβ treated EAE induced mice Increased IL-1β and Caspase-1 protein expression in EAE induced *Ifnar1*$^{-/-}$ mice	(Inoue et al., 2012b)

HU-308	Agonist for CB2 (a peripheral cannabinoid receptor)	Mouse (C57BL/6, CB2R-KO)	8-9 week-old female mice, MOG_{35-55}	NLRP3, IL-1β, Caspase-1	brain spinal cord	WB, RT-PCR	Decreased NLRP3 mRNA; IL-1β, Caspase-1 protein expression in HU-308 treated EAE-induced mice	(Shao et al., 2014)
DRα1-mMOG-35-55	Modified MOG	Mouse (C57BL/6, DR*1501-Tg and DR*1502-Tg mice)	8-12 week-old female mice, MOG_{35-55}	NLRP3, IL-1β	spinal cord	RT-PCR, Flow cytometry	Decreased NLRP3 and IL-1β in DRα1-mMOG-35-55 treated EAE-induced DR*1501-Tg mice Increased number of M2 polarized microglia	(Benedek et al., 2015)
MCC950	NLRP3 inflammasome inhibitor	Mouse (C57BL/6)	MOG_{35-55}			EAE clinical score	Attenuation of EAE in MCC950 treated EAE induced mice	(Coll et al., 2015)
IFNβ	Cytokine	Mouse (C57BL/6, *Asc*$^{-/-}$, *Nlrp3*$^{-/-}$, *Cxcr2*$^{fl/fl}$)	6-8 week-old female mice, MOG_{35-55}, Passive induction	IL-1β	spinal cord	ELISA	Increased EAE clinical Score in *Nlrp3*$^{-/-}$ and *Asc*$^{-/-}$ mice Decreased IFNβ efficiency in severe EAE induced mice	(Inoue et al., 2016)

(*Continued*)

Table 2 Experimental NLRP3 inflammasome inhibitors in EAE Models.—cont'd

Name of therapeutic agent	Class of therapeutic agent	Organism	EAE model	Inflammasome components affected by treatment	Site of detection	Method of detection	Outcome	References
Progesterone	Hormone	Mouse (C57BL/6)	6-8 week-old female, mice, 0.2% cuprizone, 5 weeks	NLRP3, IL-18, IBA1, GFAP	corpus callosum	RT-PCR, WB, IF	Decreased NLRP3 and IL-18 mRNA and protein expression in progesterone treated cuprizone induced mice Decreased IBA1 and GFAP positive cells in progesterone treated cuprizone induced mice	(Aryanpour et al., 2017)
JC-171	NLRP3 Inflammasome Inhibitor	Mouse (C57BL/6)	MOG_{35-55}	IL-1β	spinal cord	ELISA	Decreased IL-1β in spinal cord of JC-171 treated EAE induced mice	(Guo et al., 2017)
PNU282987	α7 nAChR Agonist	Mouse (C57BL/6, B6.129P2-$Cnr2^{tm1Dgen}$/J)	8-9 week-old female mice, MOG_{35-55}	NLRP3, Caspase-1, IL-1β, IL-18	spinal cord	WB	Decreased expression of NLRP3, IL-1β, IL-18, Caspase-1, protein expression in spinal cord of PNU282987 treated EAE induced mice	(Ke et al., 2017)

Name of therapeutic agent	Class of therapeutic agent	Organism	EAE model	Inflammasome components affected by treatment	Site of detection	Method of detection	Outcome	References
2ccPA	Natural Phospholipid Mediator	Mouse (C57BL/6)	10 week-old male mice, 0.2% cuprizone, 5-6-10 weeks	NLRP3, IL-1β, P2X7R, IBA1, GFAP	corpus callosum	RT-PCR, IF	Decreased NLRP3, IL-1β, P2X7R, IBA1 and GFAP mRNA expression in 2ccPA treated cuprizone induced mice Decreased IBA1 positive cells in 2cc PA treated cuprizone induced mice	(Yamamoto et al., 2017)
Tetramethylpyrazine	Alkaloid	Mouse (C57BL/6)	12-week-old female mice, MOG_{35-55}	NLRP3, Caspase-1, IL-18, IBA1, GFAP	spinal cord	WB, ELI SA, IF	Decreased NLRP3, Caspase-1 and IL-18 protein level in tetramethyl-pyrazine treated EAE-induced mice Decreased microgliosis and astrogliosis in tetramethyl-pyrazine treated EAE-induced mice	(Bai et al., 2018b)

(*Continued*)

Table 2 Experimental NLRP3 inflammasome inhibitors in EAE Models.—cont'd

Name of therapeutic agent	Class of therapeutic agent	Organism	EAE model	Inflammasome components affected by treatment	Site of detection	Method of detection	Outcome	References
VX-765	Caspase-1 inhibitor	Mouse (C57BL/6)	10-12 week-old female mice, MOG_{35-55}	NLRP3, IL-1β, Caspase-1, NLRP1, Caspase-11, GSDMD, IBA1	hind brain spinal cord	IF, IHC, RT-PCR	Decreased IL-1β, Caspase-1, GSDMD and subsequently microgliosis and pyroptosis in VX-765 treated EAE induced mice Decreased IL-1β, NLRP3, NLRP1, Caspase-1, Caspase-11, mRNA expression	(Mckenzie et al., 2018)
PMX205	Antagonist of C5a receptor	Mouse (Biozzi AB/H)	7-8 week-old, syngeneic Biozzi AB/H spinal cord homogenate (SCH)	NLRP3, IL-1β, ASC, IBA1, AIM2, NLRP1, NLRC4	spinal cord	IHC, RT-PCR, IF	Decreased IL-1β and IBA1 protein expression; NLRP3, IL-1β, ASC, IBA1, AIM2, NLRP1, NLRC4 mRNA expression in PMX205 treated EAE induced mice	(Michailidou et al., 2018)

							Decreased microglial inflammasome activation in C6 antisense treated EAE induced mice	
Prednisone	Corticosteroid	Mouse (C57BL/6)	8 week-old male, mice, 0.3%, cuprizone, 3 weeks	NLRP3, IL-1β, Caspase-1, IBA1, GFAP	corpus callosum	WB, IF	Decreased NLRP3, Caspase-1 and IL-1β protein expression in prednisone treated cuprizone induced mice Decreased IBA1 and GFAP positive cells in prednisone treated cuprizone induced mice	(Yu et al., 2018)
Oligodendrocyte secreted conditioned medium		Mouse (C57BL/6)	10-week-old female mice, MOG_{35-55}	NLRP3, IL-1β, IBA1, GFAP	spinal cord	WB, RT-PCR	Decreased expression of NLRP3, IL-1β, IBA1, GFAP protein levels and IL-1β and GFAP mRNA expression in spinal cord of 100xCM treated mice	(Jahanbazi Jahan-Abad et al., 2019)

(*Continued*)

Table 2 Experimental NLRP3 inflammasome inhibitors in EAE Models.—cont'd

Name of therapeutic agent	Class of therapeutic agent	Organism	EAE model	Inflammasome components affected by treatment	Site of detection	Method of detection	Outcome	References
Rapamycin & MCC950	mTORC1 complex inhibitor & NLRP3 inflammasome inhibitor	Mouse (C57BL/6)	8-10 week-old female, MOG_{35-55}	IL-1β	brain	WB	Decreased IL-1β protein expression in MCC950 treated EAE induced mice	(Xu et al., 2019)

Ac-YVAD-cmk, selective and irreversible caspase-1 inhibitor, *HU-308*, CB2 receptor agonist, *MCC950*, NLRP3 inhibitor, *JC-171*, Inhibitor of IL-1β release, *2ccPA*, 2-carba cyclic phosphatidic acid, *VX765*, Caspase-1 inhibitor, *PMX-205*, Suppressor of ROS-induced activation of the NLRP3 inflammasome.

Prednisolone or prednisone, a prodrug which is metabolized into prednisolone in the liver, is an immunosuppressive drug used in several autoimmune diseases, including asthma and rheumatic disorders (Becker, 2013). A recent study demonstrated the underlying mechanism of the effects of prednisone in MS by using the cuprizone-induced demyelination model (Yu et al., 2018). Prednisone treatment decreased disease scores, improved cognitive function, and reversed demyelination and gliosis. Furthermore, prednisone attenuated NLRP3 inflammasome activation and the subsequent inflammatory cytokine secretion, supporting the idea that NLRP3 inflammasome suppression could be a key mediator for prednisone activity.

Dimethyl fumarate (DMF), another DMD used in MS has been shown to ameliorate NLRP3 inflammasome activation. DMF is an immunomodulatory drug approved by US Food and Drug Administration (FDA) for the treatment of the relapsing forms of multiple sclerosis (RRMS) (Liu, Zhou et al., 2016). The exact mechanism of action of DMF has not been elucidated, but studies showed that DMF modulates nuclear factor erythroid 2-related factor 2-dependent and independent signaling pathways (Yadav, Soin, Ito, & Dhib-Jalbut, 2019) *in vitro* inflammasome model in THP-1 monocytes (Miglio, Veglia, & Fantozzi, 2015) and *in vivo* colitis model (Liu, Zhou et al., 2016). Both studies demonstrated that DMF ameliorated NLRP3 inflammasome activation and decreased the expression of inflammasome markers. However, the effect of DMF on NLRP3 activation in microglial cells has yet to be investigated.

The effects of natural compounds in the amelioration of NLRP3 inflammasome activation have been extensively studied. A myriad of natural compounds have been shown to have anti-inflammatory effect and suppress NLRP3 inflammasome activity in the CNS, including resveratrol (Fu et al., 2013), sulforaphane (Greaney, Maier, Leppla, & Moayeri, 2016), quercetin (Wang, Pan, Zhang, Wang, & Kong, 2012), and curcumin (Li, Wang et al., 2015). These natural compounds possess anti-inflammatory and anti-oxidative effects that ameliorate NLRP3 inflammasome activation. For instance, tetramethylpyrazine found in the herbal medicine chuanxiong has been demonstrated to decrease the expression of inflammasome markers, NLRP3, caspase-1 and IL-18. Additionally, treatment improved the clinical score and reduced microgliosis and astrogliosis in the EAE-induced mice (Bai, Wang et al., 2018). Another natural compound 2-carba-cyclic phosphatidic acid, a phospholipid mediator, improved the clinical score, prevented axonal injury and reduced Iba + microglia in the corpus callosum in the cuprizone model, supporting the potential use of natural compounds

(Yamamoto et al., 2017). Although these tested compounds have different action mechanisms in the suppression of the NLRP3 inflammasome pathway, combinatorial therapies of these natural compounds could have a synergistic effect for MS treatment.

Inhibition of NLRP3 inflammasome complex proteins by small compounds could be a rationale therapeutic approach for autoimmune disorders. The use of caspase-1 inhibitors for MS treatment has been well studied (Lalor et al., 2011; Mckenzie et al., 2018). Caspase-1, the effector protein of the NLRP3 inflammasome complex, is responsible for the maturation of IL-1β and IL-18 that is known to contribute to disease progression. The use of Ac-YVAD-cmk, an inhibitor of caspase-1, led to the suppression of IL-17 and IFN-γ production by $CD4^+$ T cells and subsequently mitigated EAE (Lalor et al., 2011). Another caspase-1 inhibitor, VX-765 has also been shown to be a potent candidate molecule for the treatment of MS. In the study, inhibition of caspase-1 by VX-765 in a EAE induced mice model was sufficient to reduce neuroinflammation, inhibit axonal injury and improve neurobehavioral outcomes. Furthermore, EAE induced mice treated with VX-765 had reduced expressions of IL-1β, GSDMD, caspase-1 proteins and several inflammasome/inflammation related genes in their spinal cords. Finally, VX-765 also reduced NLRP3 inflammasome activation in nigericin induced human fetal primary microglial cells (Mckenzie et al., 2018).

NLRP3 inhibitors have demonstrated to be potential therapeutic targets in MS. A number of studies focused on the use of NLRP3 inhibitors such as MCC950 (Coll et al., 2015; Xu et al., 2019), JC-171 (Guo et al., 2017) for the treatment of MS. Studies revealed that a small molecule inhibitor, MCC950, decreased IL-17 and IFN-γ producing $CD3^+$ T cells and attenuated the severity of disease (Coll et al., 2015). MCC950 treated EAE induced mice had reduced nerve damage, astrogliosis and IL-1β protein expression. Another inhibitor, JC-171, had a similar effect on the EAE progression. Histopathological evaluation of the spinal cord revealed that JC-171 reduced myelin damage and slowed disease progression by decreasing IL-1β protein expression and the number of $IL17A^+$ $CD4^+$ Th17 cells in EAE induced mice (Guo et al., 2017).

A recent study with a novel therapeutic approach revealed the use of 3D-culture system in which neurotrophic factors in oligodendrocyte-derived conditioned media to be effective in reducing demyelination, and the infiltration of immune cells in EAE mice (Jahanbazi Jahan-Abad et al., 2019). Furthermore, conditioned media increased myelin marker expression,

suppressed microglial and astrocytic activation, and reduced inflammasome markers NLRP3 and Il-1β by ultimately reducing the severity of EAE. Another novel approach using modified MOG reversed EAE disease progression by reducing immune cell infiltration including monocytes, activated microglia ($CD11b^{+}CD45hi$) and $CD3^{+}$ T cells, expression of pro-inflammatory genes (NLRP3, IL-1β and CD74), altering the polarization state of macrophages and activating critical genes involved in neuronal survival and regeneration (Huwe1, MBP and histone deacetylase 5) (Benedek et al., 2015).

Several candidate molecules have been shown to be potent for the treatment of MS. For example, HU-308, cannabinoid receptor 2 (CB2R) agonist (Shao et al., 2014), progesterone, a hormone (Aryanpour et al., 2017), PNU282987, α7 nicotinic acetylcholine receptors agonist (Ke et al., 2017) and PMX205, C5a receptor antagonist (Michailidou et al., 2018) have all attenuated NLRP3 inflammasome activation and decreased the clinical score in EAE and cuprizone animal models.

HU-308, an agonist for CB2R, leads to autophagy and ameliorates NLRP3 inflammasome activity in the EAE model. By activating the autophagy pathway, HU-308 was able to reduce demyelination and inflammatory cell infiltration and the expression of inflammasome markers both in the brain and spinal cord (Shao et al., 2014). Progesterone, a natural anti-inflammatory hormone, decreases NLRP3 and IL-18 mRNA and protein expressions, protects oligodendrocytes against demyelination and improves behavioral score in EAE induced mice. Progesterone also alteres the polarization state of microglial cells from M1 into M2 phenotype (Aryanpour et al., 2017). A study with PNU282987, a selective α7nAChR agonist, revealed that PNU282987 modulates β-arrestin-1 expression in the spinal cord and decreases the expression of inflammasome markers NLRP3, caspase-1, IL-1β and IL-18 in a β-arrestin-1-mediated manner (Ke et al., 2017).

PMX205, a specific inhibitor of the C5a receptor 1, suppressed NLRP3 inflammasome activation in different cells (Laudisi et al., 2013; Triantafilou, Hughes, Triantafilou, & Morgan, 2013). As a result, inhibition of the complement system by PMX205 decreased genes that are key for inflammasome-related microglial activation: NLRP3, IL-1β, ASC, AIM2, NLR Family Pyrin Domain Containing 1 -NLRP1, NLRC4. PMX205 was also able to reduce IL-1β and Iba1 protein expressions that correlated with reduced demyelination, the extent of axonal injury and improve clinical scores. (Michailidou et al., 2018).

Taken together, there is still a need for potent treatment strategies for MS. The NLRP3 inflammasome pathway could be an ideal candidate for the development of new therapeutic strategies. The regulation of the NLRP3 inflammasome pathway can contribute greatly to the management and treatment of MS. A study using miR-223 mimic and miR-223 deficient mice in EAE could serve as good example for the modulation of the NLRP3 signaling pathway for potential treatment strategies (Cantoni et al., 2017).

10. NLRP3 in MS

Epidemiological studies suggest that the genetic susceptibility as one of the main causes of MS pathology aside from viral infections of EBV or environmental reasons. Gene association studies aim to explain the genetic susceptibility parameters critical in MS development. (Baranzini & Oksenberg, 2017). SNPs in the NLRP3 gene encoding region is of additional importance as inflammasome activity has been previously associated with autoimmune diseases (Table 3). There are about 60 SNPs in the NLRP3 gene which have been linked to several diseases such as cryopyrin-associated periodic syndromes, Muckle Well's syndrome, and AD (Aksentijevich et al., 2002; Compeyrot-Lacassagne, Tran, Guillaume-Czitrom, Marie, & Kone-Paut, 2009; Hoffman, Mueller, Broide, Wanderer, & Kolodner, 2001; Schuh et al., 2015; Zhang et al., 2011). A study in the Iranian population with MS revealed lower frequency of CG rs10754558, and higher frequency of CC rs3806265 in NLRP3 (Imani et al., 2018). In a Brazilian cohort, peripheral blood mononuclear cell (PBMC) analysis for 8 SNPs and inflammasome elements were evaluated. The NLRP3 gene rs35829419 C > A SNP was found to be related to MS severity (Soares, Oliveira, & Pontillo, 2019). This study supports experimental models regarding a constitutive activation of inflammasome in disease pathology that in turn triggers the release of pro-inflammatory cytokines, IL-1β and IL1-18. Elevated IL-18 is reported to be relevant to the clinical progression of MS. In a previous study, same polymorphism exhibited a non-significant trend for the association between IFN- β responders (Malhotra et al., 2015). However, in a larger cohort, the same group reported that the results could not be replicated (Malhotra et al., 2018). The most recent study relates several increased rare variants of NLRP3 and CASP1 genes to MS patients (Vidmar et al., 2019). These mutations reportedly play a key role in the inflammasome signaling pathway and inactivate the inflammasome via autophagy/mitophagy and type-1 interferon response.

Table 3 NLRP3 in multiple sclerosis.

Subjects/(population)	Inflammasome components identified	Method	Main findings	References
Genotype: 403 responders and 386 non-responders (European) Expression: 97 MS patients (28 IFN-β responders, 69 non responder), 14 healthy controls	NLRP3	Genotyping qPCR	A non-significant trend for association between rs35829419 and IFN- β response Baseline mRNA expression levels of NLRP3 increased in PBMC samples of non-responders NLRP3 mRNA expression increased in responders after IFN- β treatment	(Malhotra et al., 2015)
421 IFN-β responders, 198 non responders (European)	14 NLRP3 polymorphisms	TaqMan genotyping	No significant associations between the response to IFN-β and NLRP3 polymorphisms	(Malhotra et al., 2018)
Genotyping: 209 MS, 36 NMO, 233 healthy controls (Brazil)	8 SNPs in NLRP1, NLRP3, NLRC4,	Genotyping qPCR	C > A at rs35829419 in NLRP3 gene related to MS severity	(Soares et al., 2019)

(*Continued*)

Table 3 NLRP3 in multiple sclerosis.—cont'd

Subjects/(population)	Inflammasome components identified	Method	Main findings	References
Expression: after LPS + ATP treatment: 9 MS and 10 healthy controls			The expression of inflammasome genes up-regulated in LPS-treated MS monocytes	
Genotyping: 187 RRMS, 122 healthy controls (Iranian)	NLRP3 (rs10754558, rs3806265, rs4612666 and rs35829419)	TaqMan genotyping qPCR	C > T at rs3806265 NLRP3 are associated with MS risk	(Imani et al., 2018)
Expression: 37 MS (18 relapse, 19 remission), 22 healthy controls			Lower CG genotype at rs10754558 in NLRP3 gene in MS patients NLRP3 expression decreased with IFN- β treatment	
4 MS patients and 9 healthy control brain tissue samples	NLRP3, ASC, Caspase-1	immunohistochemistry	NLRP3, ASC and Caspase-1 and IL-1β expressed in active lesions of MS, reduced in chronic inactive lesions	(Kawana et al., 2013)

15 MS patients and 12 other disease controls, brain tissue samples	Caspase-1, NLRP1, NLRP3, AIM2	qPCR immunohistochemistry	Upregulation of Caspase-1, NLRP1, NLRP3, AIM2 at the transcript level in white matter of MS patients, IL-1β immunoreactivity detected in brain monocytic cells.	(Mckenzie et al., 2014)
7 MS patients, 4 other disease control brain tissue samples and CSF	NLRP3, Caspase-1	qPCR Bioplex ELISA	NLRP3 and IL-1β gene expression levels increased in MS plaques. IL-18 cytokine secretion is also increased in CSF	(Voet et al., 2018)
32 MS patients and 120 age-matched controls serum	Caspase-1, ASC	Protein expression Simple plex assay	Caspase-1, ASC, and IL-18 are elevated in the serum of MS patients	(Keane et al., 2018)
47 MS patients and 46 healthy controls PBMC	Caspase-1	Competitive RT-PCR Western blot	Caspase-1 and IL-18 expression increased in PBMC of MS	(Huang et al., 2004)
MS and control patients after IFN- β treatment PBMC culture	Caspase-1	WB	IFN-β Inhibits Caspase-1 expression in activated monocytes from healthy person (*in vitro*) Lower IL-1β were seen in serum of IL-1 β treated MS patients.	(Guarda et al., 2011)

(*Continued*)

Table 3 NLRP3 in multiple sclerosis.—cont'd

Subjects/(population)	Inflammasome components identified	Method	Main findings	References
32 MS patients and 37 healthy controls	NLRP3 and Caspase-1	qPCR	NLRP3 and Caspase-1 mRNA levels were significantly higher in RRMS	(Peelen et al., 2015)
PBMC in ex vivo assays: 18 MS patients and 21 age-matched healthy controls			Culture supernatant derived from LPS/ATP stimulated PBMC promotes IL-17 and suppresses IL-10 in $CD4^+$ T cells	
30 MS patients (22 women and 8 men) PBMC	NLRP3, ASC, Caspase-1	NLRP3 and ASC: qPCR	mRNA expressions of NLRP3 decreased with IFN-β 1a	(Noroozi et al., 2017)
		Caspase-1: WB	Plasma levels of IL-1β decreased with IFN-β 1a	

NMO, Neuromyelitis optica.

In earlier studies, IL-1β secretion from MHC II^+ microglia in brain lesions draw attention to the role of inflammasome activation in MS in the CNS (Burm et al., 2016). Inflammasome components have also been evaluated and perivascular macrophages and astrocytes were found to be predominantly located in active MS lesions (Kawana et al., 2013). In addition to former immunohistochemical analysis, significant up-regulation of inflammasome-associated genes (caspase-1, NLRP1, NLRP3, AIM2) and IL-1β in the white matter of MS patients were reported by quantitative PCR (qPCR) (Mckenzie, Reinke, Branton, Lu, & Power, 2014; Voet et al., 2018). Additionally, NLRP3 inflammasome activation end product IL-18 secretion was increased in the CSF of MS patients (Voet et al., 2018).

Peripheral activation of the NLRP3 inflammasomes was detected in serum samples collected from 120 normal donors and 32 MS patients. The protein levels of caspase-1, ASC, and IL-18 in the serum of MS patients were higher when compared to the control group (Keane, Dietrich, & De Rivero Vaccari, 2018). This study suggests ASC and caspase-1 to be biomarkers for MS with high sensitivities at 90% and 80%, respectively.

PBMC derived from MS patients have also been frequently used in studies. IFN- treatment suppress the level of IL1b in serum (Guarda et al., 2011). Elevated caspase-1 mRNA levels were reported in PBMCs derived from MS patients when compared to controls (Huang, Huang, & Hillert, 2004; Peelen et al., 2015). IFN-β modulates the expression of inflammasomes at the mRNA level, thereby decreasing IL-1β secretion and improving the clinical presentation of MS (Noroozi, Meimand, Arababadi, Nakhaee, & Asadikaram, 2017). Women are more sensitive to IFN-β treatment, and a decrease in NLRP3 expression has been reported after 6 months of therapy in females patients but not in males. IFN-β pre-activation enables activated memory T cells to reduce IL-1β release from monocytes. Preactivated human memory T cells can also downregulate the P2X purinoceptor 7 mRNA expression and extracellular calcium following ATP stimulation (Beynon, Quintana, & Weiner, 2012).

There is a clear consensus in the literature that NLRP3 plays a central role in response to IFN-β. Even after IFN-β treatment, responders maintain a decreased level of NLRP3 and IL-1β. Clinical studies have common limitations such as: treatment naïve patients are hard to find, groups are mostly heterogeneous and low in patient number, and studies fail to include ethnicities or sex differences.

11. Future perspective

Since the contribution of microglia to the pathophysiology of MS is well established, they have been the subject of MS research for decades. Up to this point, researchers have been focused on the origin and roles of microglia in disorders of the CNS. The advancement in life science technologies have enabled researchers to identify specific microglial phenotypes, which revealed remarkable differences among healthy or disease associated microglia. However, there are likely sub-microglial phenotypes present in the CNS, and the identification of these sub groups certainly will provide a better understanding of MS (Ajami et al., 2018; Yeh & Ikezu, 2019). Single cell technologies such as mass cytometry will further assist in the identification of new disease associated sub-microglial and oligodendroglial phenotypes in MS (Clark, Lee, Smallwood, Kelsey, & Reik, 2016; Mrdjen et al., 2018; Van Bruggen, Agirre, & Castelo-Branco, 2017). Furthermore, omics studies including genomics, transcriptomics, and epigenomics will play an important role in the characterization of sub-microglial populations in healthy and disease-associated states (Cuevas-Diaz Duran, Wei, & Wu, 2017; Shema, Bernstein, & Buenrostro, 2019). Single-cell high-throughput analysis of the CSF and PBMC samples from MS patients will increase our understanding about MS pathogenesis (Cuevas-Diaz Duran et al., 2017).

The interactions between microglia and other cells in the CNS, including astrocytes, oligodendrocytes, neurons, CNS-resident macrophages and T cells direct the course of disease in MS and its animal models. The characterization of these cell-to-cell interactions using organoids, co-cultures, 3D culture systems, embryonic stem cell or induced pluripotent stem cell technologies will boost our scientific knowledge in MS pathophysiology (Cuascut & Hutton, 2019; Di Ruscio, Patti, Welner, Tenen, & Amabile, 2015; Ormel et al., 2018; Pocock & Piers, 2018; Song, Yan, Marzano, & Li, 2019; Watson, Kavanagh, Allenby, & Vassey, 2017). Future studies using microglial cell type-specific deletion of NLRP3 inflammasome genes and *in vivo* molecular imaging techniques are needed in new knockout and transgenic animal models (Guttenplan & Liddelow, 2019; Voet et al., 2019).

Animal models will continue to contribute to MS research for the development of new treatments and better understanding of the underlying disease mechanism. However, existing animal models could not completely fulfill the complexity and heterogeneity of MS, so novel animal models

will be required to recapitulate all aspects of this disorder (Lassmann & Bradl, 2017).

The improvements in basic life science should be translated to the clinical research. New imaging techniques like MRI, optical coherence tomography and positron emission tomography may be of great help in demonstrating the microglial states more effectively in patients (Filippi et al., 2019). Establishment of post-mortem brain tissue banks from healthy and MS patients will immensely contribute to MS research and be an invaluable source for human studies.

In conclusion, these newly developed methods and approaches will certainly elucidate our understanding of the complete role of microglia in MS and give rise to the development of novel, more specific and effective strategies for MS treatment.

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References

Adiele, R. C., & Adiele, C. A. (2019). Metabolic defects in multiple sclerosis. *Mitochondrion, 44*, 7–14.

Ajami, B., Samusik, N., Wieghofer, P., Ho, P. P., Crotti, A., Bjornson, Z., et al. (2018). Single-cell mass cytometry reveals distinct populations of brain myeloid cells in mouse neuroinflammation and neurodegeneration models. *Nature Neuroscience, 21*, 541–551.

Aksentijevich, I., Nowak, M., Mallah, M., Chae, J. J., Watford, W. T., Hofmann, S. R., et al. (2002). De novo CIAS1 mutations, cytokine activation, and evidence for genetic heterogeneity in patients with neonatal-onset multisystem inflammatory disease (NOMID): A new member of the expanding family of pyrin-associated autoinflammatory diseases. *Arthritis & Rheumatism, 46*, 3340–3348.

Allam, R., Lawlor, K. E., Yu, E. C., Mildenhall, A. L., Moujalled, D. M., Lewis, R. S., et al. (2014). Mitochondrial apoptosis is dispensable for NLRP3 inflammasome activation but non-apoptotic caspase-8 is required for inflammasome priming. *EMBO Reports, 15*, 982–990.

Areschoug, T., & Gordon, S. (2008). Pattern recognition receptors and their role in innate immunity: Focus on microbial protein ligands. *Contributions to Microbiology, 15*, 45–60.

Arneth, B. M. (2019). Impact of B cells to the pathophysiology of multiple sclerosis. *Journal of Neuroinflammation, 16*, 128.

Aryanpour, R., Pasbakhsh, P., Zibara, K., Namjoo, Z., Beigi Boroujeni, F., Shahbeigi, S., et al. (2017). Progesterone therapy induces an M1 to M2 switch in microglia phenotype and suppresses NLRP3 inflammasome in a cuprizone-induced demyelination mouse model. *International Immunopharmacology, 51*, 131–139.

Astier, A. L., Meiffren, G., Freeman, S., & Hafler, D. A. (2006). Alterations in CD46-mediated Tr1 regulatory T cells in patients with multiple sclerosis. *Journal of Clinical Investigation, 116*, 3252–3257.

Baecher-Allan, C., Kaskow, B. J., & Weiner, H. L. (2018). Multiple sclerosis: Mechanisms and immunotherapy. *Neuron, 97*, 742–768.

Bai, H., Yang, B., Yu, W., Xiao, Y., Yu, D., & Zhang, Q. (2018a). Cathepsin B links oxidative stress to the activation of NLRP3 inflammasome. *Experimental Cell Research, 362*, 180–187.

Bai, X. Y., Wang, X. F., Zhang, L. S., Du, P. C., Cao, Z., & Hou, Y. (2018b). Tetramethylpyrazine ameliorates experimental autoimmune encephalomyelitis by modulating the inflammatory response. *Biochemical and Biophysical Research Communications, 503*, 1968–1972.

Bar, E., & Barak, B. (2019). Microglia roles in synaptic plasticity and myelination in homeostatic conditions and neurodevelopmental disorders. *Glia*. http://dx.doi.org/10.1002/glia.23637. PMID: 31058364. [Epub ahead of print] Review.

Baranzini, S. E., & Oksenberg, J. R. (2017). The genetics of multiple sclerosis: From 0 to 200 in 50 years. *Trends in genetics, 33*, 960–970.

Becker, D. E. (2013). Basic and clinical pharmacology of glucocorticosteroids. *Anesthesia Progress, 60*, 25–31. quiz 32.

Bedoui, Y., Neal, J. W., & Gasque, P. (2018). The neuro-immune-regulators (NIREGs) promote tissue resilience; a vital component of the host's defense strategy against neuroinflammation. *Journal of Neuroimmune Pharmacology, 13*, 309–329.

Benedek, G., Meza-Romero, R., Jordan, K., Keenlyside, L., Offner, H., & Vandenbark, A. A. (2015). HLA-DRalpha1-mMOG-35-55 treatment of experimental autoimmune encephalomyelitis reduces CNS inflammation, enhances M2 macrophage frequency, and promotes neuroprotection. *Journal of Neuroinflammation, 12*, 123.

Beynon, V., Quintana, F. J., & Weiner, H. L. (2012). Activated human CD4+CD45RO+ memory T-cells indirectly inhibit NLRP3 inflammasome activation through downregulation of P2X7R signalling. *PLoS One, 7*, e39576.

Billiau, A., & Matthys, P. (2001). Modes of action of Freund's adjuvants in experimental models of autoimmune diseases. *Journal of Leukocyte Biology, 70*, 849–860.

Bortolotti, P., Faure, E., & Kipnis, E. (2018). Inflammasomes in tissue damages and immune disorders after trauma. *Frontiers in Immunology, 9*, 1900.

Bossu, P., Salani, F., Cacciari, C., Picchetto, L., Cao, M., Bizzoni, F., et al. (2009). Disease outcome, alexithymia and depression are differently associated with serum IL-18 levels in acute stroke. *Current Neurovascular Research, 6*, 163–170.

Braga, T. T., Forni, M. F., Correa-Costa, M., Ramos, R. N., Barbuto, J. A., Branco, P., et al. (2017). Soluble uric acid activates the NLRP3 inflammasome. *Scientific Reports, 7*, 39884.

Brambilla, R. (2019). The contribution of astrocytes to the neuroinflammatory response in multiple sclerosis and experimental autoimmune encephalomyelitis. *Acta Neuropathologica, 137*, 757–783.

Brown, M. A., & Weinberg, R. B. (2018). Mast cells and innate lymphoid cells: Underappreciated players in CNS autoimmune demyelinating disease. *Frontiers in Immunology, 9*, 514.

Browne, P., Chandraratna, D., Angood, C., Tremlett, H., Baker, C., Taylor, B. V., et al. (2014). Atlas of multiple sclerosis 2013: A growing global problem with widespread inequity. *Neurology, 83*, 1022–1024.

Broz, P., & Dixit, V. M. (2016). Inflammasomes: Mechanism of assembly, regulation and signalling. *Nature Reviews Immunology, 16*, 407–420.

Burm, S. M., Peferoen, L. A., Zuiderwijk-Sick, E. A., Haanstra, K. G., T Hart, B. A., Van Der Valk, P., et al. (2016). Expression of IL-1beta in rhesus EAE and MS lesions is mainly induced in the CNS itself. *Journal of Neuroinflammation, 13*, 138.

Campbell, G., & Mahad, D. J. (2018). Mitochondrial dysfunction and axon degeneration in progressive multiple sclerosis. *FEBS Letters, 592*, 1113–1121.

Cantoni, C., Cignarella, F., Ghezzi, L., Mikesell, B., Bollman, B., Berrien-Elliott, M. M., et al. (2017). Mir-223 regulates the number and function of myeloid-derived suppressor

cells in multiple sclerosis and experimental autoimmune encephalomyelitis. *Acta Neuropathologica, 133*, 61–77.
Centonze, D., Muzio, L., Rossi, S., Cavasinni, F., De Chiara, V., Bergami, A., et al. (2009). Inflammation triggers synaptic alteration and degeneration in experimental autoimmune encephalomyelitis. *Journal of Neuroscience, 29*, 3442–3452.
Centonze, D., Muzio, L., Rossi, S., Furlan, R., Bernardi, G., & Martino, G. (2010). The link between inflammation, synaptic transmission and neurodegeneration in multiple sclerosis. *Cell Death & Differentiation, 17*, 1083–1091.
Chaix, J., Tessmer, M. S., Hoebe, K., Fuseri, N., Ryffel, B., Dalod, M., et al. (2008). Cutting edge: Priming of NK cells by IL-18. *The Journal of Immunology, 181*, 1627–1631.
Chen, J., & Chen, Z. J. (2018). PtdIns4P on dispersed trans-Golgi network mediates NLRP3 inflammasome activation. *Nature, 564*, 71–76.
Clark, S. J., Lee, H. J., Smallwood, S. A., Kelsey, G., & Reik, W. (2016). Single-cell epigenomics: Powerful new methods for understanding gene regulation and cell identity. *Genome Biology, 17*, 72.
Coll, R. C., Robertson, A. A., Chae, J. J., Higgins, S. C., Munoz-Planillo, R., Inserra, M. C., et al. (2015). A small-molecule inhibitor of the NLRP3 inflammasome for the treatment of inflammatory diseases. *Nature Medicine, 21*, 248–255.
Colonna, M., & Butovsky, O. (2017). Microglia function in the central nervous system during Health and neurodegeneration. *Annual Review of Immunology, 35*, 441–468.
Compeyrot-Lacassagne, S., Tran, T. A., Guillaume-Czitrom, S., Marie, I., & Kone-Paut, I. (2009). Brain multiple sclerosis-like lesions in a patient with Muckle-Wells syndrome. *Rheumatology, 48*, 1618–1619.
Constantinescu, C. S., Farooqi, N., O'brien, K., & Gran, B. (2011). Experimental autoimmune encephalomyelitis (EAE) as a model for multiple sclerosis (MS). *British Journal of Pharmacology, 164*, 1079–1106.
Correale, J., Marrodan, M., & Ysrraelit, M. C. (2019). Mechanisms of neurodegeneration and axonal dysfunction in progressive multiple sclerosis. *Biomedicines, 7*.
Csordas, G., & Hajnoczky, G. (2009). SR/ER-mitochondrial local communication: Calcium and ROS. *Biochimica et Biophysica Acta, 1787*, 1352–1362.
Cuascut, F. X., & Hutton, G. J. (2019). Stem cell-based therapies for multiple sclerosis: Current perspectives. *Biomedicines, 7*.
Cuevas-Diaz Duran, R., Wei, H., & Wu, J. Q. (2017). Single-cell RNA-sequencing of the brain. *Clinical and Translational Medicine, 6*, 20.
Cui, Y., & Wan, Q. (2019). NKT cells in neurological diseases. *Frontiers in Cellular Neuroscience, 13*, 245.
Danikowski, K. M., Jayaraman, S., & Prabhakar, B. S. (2017). Regulatory T cells in multiple sclerosis and myasthenia gravis. *Journal of Neuroinflammation, 14*, 117.
Das, T., Chen, Z., Hendriks, R. W., & Kool, M. (2018). A20/Tumor necrosis factor alpha-induced protein 3 in immune cells controls development of autoinflammation and autoimmunity: Lessons from mouse models. *Frontiers in Immunology, 9*, 104.
Delbridge, L. M., & O'riordan, M. X. (2007). Innate recognition of intracellular bacteria. *Current Opinion in Immunology, 19*, 10–16.
Deng, J., Yu, X. Q., & Wang, P. H. (2019). Inflammasome activation and Th17 responses. *Molecular Immunology, 107*, 142–164.
Di Ruscio, A., Patti, F., Welner, R. S., Tenen, D. G., & Amabile, G. (2015). Multiple sclerosis: Getting personal with induced pluripotent stem cells. *Cell Death & Disease, 6*, e1806.
Dinarello, C. A. (2018). Overview of the IL-1 family in innate inflammation and acquired immunity. *Immunological Reviews, 281*, 8–27.
Dubbelaar, M. L., Kracht, L., Eggen, B. J. L., & Boddeke, E. (2018). The kaleidoscope of microglial phenotypes. *Frontiers in Immunology, 9*, 1753.

Evavold, C. L., & Kagan, J. C. (2018). How inflammasomes inform adaptive immunity. *Journal of Molecular Biology, 430*, 217–237.
Eyo, U. B., & Wu, L. J. (2019). Microglia: Lifelong patrolling immune cells of the brain. *Progress in Neurobiology, 179*, 101614.
Fann, D. Y., Lee, S. Y., Manzanero, S., Tang, S. C., Gelderblom, M., Chunduri, P., et al. (2013). Intravenous immunoglobulin suppresses NLRP1 and NLRP3 inflammasome-mediated neuronal death in ischemic stroke. *Cell Death & Disease, 4*, e790.
Faustin, B., Chen, Y., Zhai, D., Le Negrate, G., Lartigue, L., Satterthwait, A., et al. (2009). Mechanism of Bcl-2 and Bcl-X(L) inhibition of NLRP1 inflammasome: Loop domain-dependent suppression of ATP binding and oligomerization. *Proceedings of the National Academy of Sciences of the U S A, 106*, 3935–3940.
Ferrari, C. C., Depino, A. M., Prada, F., Muraro, N., Campbell, S., Podhajcer, O., et al. (2004). Reversible demyelination, blood-brain barrier breakdown, and pronounced neutrophil recruitment induced by chronic IL-1 expression in the brain. *American Journal Of Pathology, 165*, 1827–1837.
Fiebich, B. L., Akter, S., & Akundi, R. S. (2014). The two-hit hypothesis for neuroinflammation: Role of exogenous ATP in modulating inflammation in the brain. *Frontiers in Cellular Neuroscience, 8*, 260.
Filippi, M., Bar-Or, A., Piehl, F., Preziosa, P., Solari, A., Vukusic, S., et al. (2018). Multiple sclerosis. *Nat Rev Dis Primers, 4*, 43.
Filippi, M., Bruck, W., Chard, D., Fazekas, F., Geurts, J. J. G., Enzinger, C., et al.Attendees of the Correlation Between, P., and Workshop, M.R.I.F.I.M. (2019). Association between pathological and MRI findings in multiple sclerosis. *The Lancet Neurology, 18*, 198–210.
Freeman, L., Guo, H., David, C. N., Brickey, W. J., Jha, S., & Ting, J. P. (2017). NLR members NLRC4 and NLRP3 mediate sterile inflammasome activation in microglia and astrocytes. *Journal of Experimental Medicine, 214*, 1351–1370.
Fu, Y., Wang, Y., Du, L., Xu, C., Cao, J., Fan, T., et al. (2013). Resveratrol inhibits ionising irradiation-induced inflammation in MSCs by activating SIRT1 and limiting NLRP-3 inflammasome activation. *International Journal of Molecular Sciences, 14*, 14105–14118.
Garlanda, C., Dinarello, C. A., & Mantovani, A. (2013). The interleukin-1 family: Back to the future. *Immunity, 39*, 1003–1018.
Genc, B., Bozan, H. R., Genc, S., & Genc, K. (2019). Stem cell therapy for multiple sclerosis. *Advances in Experimental Medicine & Biology, 1084*, 145–174. http://dx.doi.org/10.1007/5584_2018_247. PMID: 30039439.
Genc, K., Dona, D. L., & Reder, A. T. (1997). Increased CD80(+) B cells in active multiple sclerosis and reversal by interferon beta-1b therapy. *Journal of Clinical Investigation, 99*, 2664–2671.
Gerhauser, I., Hansmann, F., Ciurkiewicz, M., Loscher, W., & Beineke, A. (2019). Facets of Theiler's murine encephalomyelitis virus-induced diseases: An update. *International Journal of Molecular Sciences, 20*.
Gharagozloo, M., Gris, K. V., Mahvelati, T., Amrani, A., Lukens, J. R., & Gris, D. (2017). NLR-dependent regulation of inflammation in multiple sclerosis. *Frontiers in Immunology, 8*, 2012.
Gong, Z., Pan, J., Shen, Q., Li, M., & Peng, Y. (2018). Mitochondrial dysfunction induces NLRP3 inflammasome activation during cerebral ischemia/reperfusion injury. *Journal of Neuroinflammation, 15*, 242.
Gosselin, D., & Rivest, S. (2007). Role of IL-1 and TNF in the brain: Twenty years of progress on a Dr. Jekyll/Mr. Hyde duality of the innate immune system. *Brain, Behavior, and Immunity, 21*, 281–289.

Greaney, A. J., Maier, N. K., Leppla, S. H., & Moayeri, M. (2016). Sulforaphane inhibits multiple inflammasomes through an Nrf2-independent mechanism. *Journal of Leukocyte Biology, 99*, 189–199.

Green, J. P., Yu, S., Martin-Sanchez, F., Pelegrin, P., Lopez-Castejon, G., Lawrence, C. B., et al. (2018). Chloride regulates dynamic NLRP3-dependent ASC oligomerization and inflammasome priming. *Proceedings of the National Academy of Sciences of the U S A, 115*, E9371–E9380.

Gris, D., Ye, Z., Iocca, H. A., Wen, H., Craven, R. R., Gris, P., et al. (2010). NLRP3 plays a critical role in the development of experimental autoimmune encephalomyelitis by mediating Th1 and Th17 responses. *The Journal of Immunology, 185*, 974–981.

Groslambert, M., & Py, B. F. (2018). Spotlight on the NLRP3 inflammasome pathway. *Journal of Inflammation Research, 11*, 359–374.

Gross, C. C., Schulte-Mecklenbeck, A., Wiendl, H., Marcenaro, E., Kerlero De Rosbo, N., Uccelli, A., et al. (2016a). Regulatory functions of natural killer cells in multiple sclerosis. *Frontiers in Immunology, 7*, 606.

Gross, C. J., Mishra, R., Schneider, K. S., Medard, G., Wettmarshausen, J., Dittlein, D. C., et al. (2016b). K(+) efflux-independent NLRP3 inflammasome activation by small molecules targeting Mitochondria. *Immunity, 45*, 761–773.

Guarda, G., Braun, M., Staehli, F., Tardivel, A., Mattmann, C., Forster, I., et al. (2011). Type I interferon inhibits interleukin-1 production and inflammasome activation. *Immunity, 34*, 213–223.

Guo, C., Chi, Z., Jiang, D., Xu, T., Yu, W., Wang, Z., et al. (2018). Cholesterol homeostatic regulator SCAP-SREBP2 integrates NLRP3 inflammasome activation and cholesterol Biosynthetic signaling in macrophages. *Immunity, 49*, 842–856 e847.

Guo, C., Fulp, J. W., Jiang, Y., Li, X., Chojnacki, J. E., Wu, J., et al. (2017). Development and characterization of a hydroxyl-sulfonamide analogue, 5-chloro-N-[2-(4-hydroxy-sulfamoyl-phenyl)-ethyl]-2-methoxy-benzamide, as a novel NLRP3 inflammasome inhibitor for potential treatment of multiple sclerosis. *ACS Chemical Neuroscience, 8*, 2194–2201.

Gurung, P., Anand, P. K., Malireddi, R. K., Vande Walle, L., Van Opdenbosch, N., Dillon, C. P., et al. (2014). FADD and caspase-8 mediate priming and activation of the canonical and noncanonical Nlrp3 inflammasomes. *The Journal of Immunology, 192*, 1835–1846.

Gurung, P., & Kanneganti, T. D. (2015). Novel roles for caspase-8 in IL-1beta and inflammasome regulation. *American Journal Of Pathology, 185*, 17–25.

Guttenplan, K. A., & Liddelow, S. A. (2019). Astrocytes and microglia: Models and tools. *Journal of Experimental Medicine, 216*, 71–83.

Hanamsagar, R., Torres, V., & Kielian, T. (2011). Inflammasome activation and IL-1beta/IL-18 processing are influenced by distinct pathways in microglia. *Journal of Neurochemistry, 119*, 736–748.

Hannocks, M. J., Zhang, X., Gerwien, H., Chashchina, A., Burmeister, M., Korpos, E., et al. (2019). The gelatinases, MMP-2 and MMP-9, as fine tuners of neuroinflammatory processes. *Matrix Biology, 75–76*, 102–113.

Harrison, O. J., Srinivasan, N., Pott, J., Schiering, C., Krausgruber, T., Ilott, N. E., et al. (2015). Epithelial-derived IL-18 regulates Th17 cell differentiation and Foxp3(+) Treg cell function in the intestine. *Mucosal Immunology, 8*, 1226–1236.

Hemmer, B., Kerschensteiner, M., & Korn, T. (2015). Role of the innate and adaptive immune responses in the course of multiple sclerosis. *The Lancet Neurology, 14*, 406–419.

Heneka, M. T., Kummer, M. P., & Latz, E. (2014). Innate immune activation in neurodegenerative disease. *Nature Reviews Immunology, 14*, 463–477.

Hinkle, J. T., Dawson, V. L., & Dawson, T. M. (2019). The A1 astrocyte paradigm: New avenues for pharmacological intervention in neurodegeneration. *Movement Disorders, 34*, 959–969.

Hoffman, H. M., Mueller, J. L., Broide, D. H., Wanderer, A. A., & Kolodner, R. D. (2001). Mutation of a new gene encoding a putative pyrin-like protein causes familial cold autoinflammatory syndrome and Muckle-Wells syndrome. *Nature Genetics, 29*, 301–305.

Hornung, V., Bauernfeind, F., Halle, A., Samstad, E. O., Kono, H., Rock, K. L., et al. (2008). Silica crystals and aluminum salts activate the NALP3 inflammasome through phagosomal destabilization. *Nature Immunology, 9*, 847–856.

Huang, W. X., Huang, P., & Hillert, J. (2004). Increased expression of caspase-1 and interleukin-18 in peripheral blood mononuclear cells in patients with multiple sclerosis. *Multiple Sclerosis, 10*, 482–487.

Ikegami, A., Haruwaka, K., & Wake, H. (2019). Microglia: Lifelong modulator of neural circuits. *Neuropathology, 39*, 173–180.

Imani, D., Azimi, A., Salehi, Z., Rezaei, N., Emamnejad, R., Sadr, M., et al. (2018). Association of nod-like receptor protein-3 single nucleotide gene polymorphisms and expression with the susceptibility to relapsing-remitting multiple sclerosis. *International Journal of Immunogenetics, 45*, 329–336.

Inoue, M., Chen, P. H., Siecinski, S., Li, Q. J., Liu, C., Steinman, L., et al. (2016). An interferon-beta-resistant and NLRP3 inflammasome-independent subtype of EAE with neuronal damage. *Nature Neuroscience, 19*, 1599–1609.

Inoue, M., & Shinohara, M. L. (2013). NLRP3 inflammasome and MS/EAE. *Autoimmune Diseases, 2013*, 859145.

Inoue, M., Williams, K. L., Gunn, M. D., & Shinohara, M. L. (2012a). NLRP3 inflammasome induces chemotactic immune cell migration to the CNS in experimental autoimmune encephalomyelitis. *Proceedings of the National Academy of Sciences of the U S A, 109*, 10480–10485.

Inoue, M., Williams, K. L., Oliver, T., Vandenabeele, P., Rajan, J. V., Miao, E. A., et al. (2012b). Interferon-beta therapy against EAE is effective only when development of the disease depends on the NLRP3 inflammasome. *Science Signaling, 5*, ra38.

Iyer, S. S., He, Q., Janczy, J. R., Elliott, E. I., Zhong, Z., Olivier, A. K., et al. (2013). Mitochondrial cardiolipin is required for Nlrp3 inflammasome activation. *Immunity, 39*, 311–323.

Jafarzadeh, A., & Nemati, M. (2018). Therapeutic potentials of ginger for treatment of multiple sclerosis: A review with emphasis on its immunomodulatory, anti-inflammatory and anti-oxidative properties. *Journal of Neuroimmunology, 324*, 54–75.

Jahanbazi Jahan-Abad, A., Karima, S., Sahab Negah, S., Noorbakhsh, F., Borhani-Haghighi, M., & Gorji, A. (2019). Therapeutic potential of conditioned medium derived from oligodendrocytes cultured in a self-assembling peptide nanoscaffold in experimental autoimmune encephalomyelitis. *Brain Research, 1711*, 226–235.

Jha, S., Srivastava, S. Y., Brickey, W. J., Iocca, H., Toews, A., Morrison, J. P., et al. (2010). The inflammasome sensor, NLRP3, regulates CNS inflammation and demyelination via caspase-1 and interleukin-18. *Journal of Neuroscience, 30*, 15811–15820.

Johann, S., Heitzer, M., Kanagaratnam, M., Goswami, A., Rizo, T., Weis, J., et al. (2015). NLRP3 inflammasome is expressed by astrocytes in the SOD1 mouse model of ALS and in human sporadic ALS patients. *Glia, 63*, 2260–2273.

Kabba, J. A., Xu, Y., Christian, H., Ruan, W., Chenai, K., Xiang, Y., et al. (2018). Microglia: Housekeeper of the central nervous system. *Cellular and Molecular Neurobiology, 38*, 53–71.

Karni, A., Koldzic, D. N., Bharanidharan, P., Khoury, S. J., & Weiner, H. L. (2002). IL-18 is linked to raised IFN-gamma in multiple sclerosis and is induced by activated CD4(+) T cells via CD40-CD40 ligand interactions. *Journal of Neuroimmunology, 125*, 134–140.

Kawana, N., Yamamoto, Y., Ishida, T., Saito, Y., Konno, H., Arima, K., et al. (2013). Reactive astrocytes and perivascular macrophages express NLRP3 inflammasome in active demyelinating lesions of multiple sclerosis and necrotic lesions of neuromyelitis optica and cerebral infarction. *Clinical and Experimental Neuroimmunology, 4*, 296–304.

Ke, P., Shao, B. Z., Xu, Z. Q., Chen, X. W., Wei, W., & Liu, C. (2017). Activating alpha7 nicotinic acetylcholine receptor inhibits NLRP3 inflammasome through regulation of beta-arrestin-1. *CNS Neuroscience and Therapeutics, 23*, 875–884.

Keane, R. W., Dietrich, W. D., & De Rivero Vaccari, J. P. (2018). Inflammasome proteins as biomarkers of multiple sclerosis. *Frontiers in Neurology, 9*, 135.

Kettenmann, H., Hanisch, U. K., Noda, M., & Verkhratsky, A. (2011). Physiology of microglia. *Physiological Reviews, 91*, 461–553.

Kierdorf, K., & Prinz, M. (2017). Microglia in steady state. *Journal of Clinical Investigation, 127*, 3201–3209.

Kipp, M., Nyamoya, S., Hochstrasser, T., & Amor, S. (2017). Multiple sclerosis animal models: A clinical and histopathological perspective. *Brain Pathology, 27*, 123–137.

Klaren, R. E., Motl, R. W., Woods, J. A., & Miller, S. D. (2014). Effects of exercise in experimental autoimmune encephalomyelitis (an animal model of multiple sclerosis). *Journal of Neuroimmunology, 274*, 14–19.

Klineova, S., & Lublin, F. D. (2018). Clinical course of multiple sclerosis. *Cold Spring Harb Perspect Med, 8*.

Kostic, M., Zivkovic, N., & Stojanovic, I. (2013). Multiple sclerosis and glutamate excitotoxicity. *Reviews in the Neurosciences, 24*, 71–88.

Kothur, K., Wienholt, L., Brilot, F., & Dale, R. C. (2016). CSF cytokines/chemokines as biomarkers in neuroinflammatory CNS disorders: A systematic review. *Cytokine, 77*, 227–237.

Kumar, H., Kawai, T., & Akira, S. (2011). Pathogen recognition by the innate immune system. *International Reviews of Immunology, 30*, 16–34.

Lalor, S. J., Dungan, L. S., Sutton, C. E., Basdeo, S. A., Fletcher, J. M., & Mills, K. H. (2011). Caspase-1-processed cytokines IL-1beta and IL-18 promote IL-17 production by gammadelta and CD4 T cells that mediate autoimmunity. *The Journal of Immunology, 186*, 5738–5748.

Lassmann, H. (2018). Multiple sclerosis pathology. *Cold Spring Harb Perspect Med, 8*.

Lassmann, H., & Bradl, M. (2017). Multiple sclerosis: Experimental models and reality. *Acta Neuropathologica, 133*, 223–244.

Laudisi, F., Spreafico, R., Evrard, M., Hughes, T. R., Mandriani, B., Kandasamy, M., et al. (2013). Cutting edge: The NLRP3 inflammasome links complement-mediated inflammation and IL-1beta release. *The Journal of Immunology, 191*, 1006–1010.

Leal-Lasarte, M. M., Franco, J. M., Labrador-Garrido, A., Pozo, D., & Roodveldt, C. (2017). Extracellular TDP-43 aggregates target MAPK/MAK/MRK overlapping kinase (MOK) and trigger caspase-3/IL-18 signaling in microglia. *The FASEB Journal, 31*, 2797–2816.

Lee, E., Hwang, I., Park, S., Hong, S., Hwang, B., Cho, Y., et al. (2019). MPTP-driven NLRP3 inflammasome activation in microglia plays a central role in dopaminergic neurodegeneration. *Cell Death & Differentiation, 26*, 213–228.

Leemans, J. C., Cassel, S. L., & Sutterwala, F. S. (2011). Sensing damage by the NLRP3 inflammasome. *Immunological Reviews, 243*, 152–162.

Lemasters, J. J., Theruvath, T. P., Zhong, Z., & Nieminen, A. L. (2009). Mitochondrial calcium and the permeability transition in cell death. *Biochimica et Biophysica Acta, 1787*, 1395–1401.

Lenz, K. M., & Nelson, L. H. (2018). Microglia and beyond: Innate immune cells as regulators of brain development and behavioral function. *Frontiers in Immunology, 9*, 698.

Li, Q., & Barres, B. A. (2018). Microglia and macrophages in brain homeostasis and disease. *Nature Reviews Immunology, 18*, 225–242.

Li, L., Ismael, S., Nasoohi, S., Sakata, K., Liao, F. F., Mcdonald, M. P., et al. (2019b). Thioredoxin-interacting protein (TXNIP) associated NLRP3 inflammasome activation in human alzheimer's disease brain. *Journal of Alzheimer's Disease, 68*, 255–265.

Li, Y., Li, J., Li, S., Li, Y., Wang, X., Liu, B., et al. (2015b). Curcumin attenuates glutamate neurotoxicity in the hippocampus by suppression of ER stress-associated TXNIP/NLRP3 inflammasome activation in a manner dependent on AMPK. *Toxicology and Applied Pharmacology, 286*, 53–63.

Li, K., Li, J., Zheng, J., & Qin, S. (2019a). Reactive astrocytes in neurodegenerative diseases. *Aging Dis, 10*, 664–675.

Li, R., Patterson, K. R., & Bar-Or, A. (2018). Reassessing B cell contributions in multiple sclerosis. *Nature Immunology, 19*, 696–707.

Li, R., Rezk, A., Miyazaki, Y., Hilgenberg, E., Touil, H., Shen, P., et al. (2015a). Proinflammatory GM-CSF-producing B cells in multiple sclerosis and B cell depletion therapy. *Science Translational Medicine, 7*, 310ra166.

Liddelow, S. A., Guttenplan, K. A., Clarke, L. E., Bennett, F. C., Bohlen, C. J., Schirmer, L., et al. (2017). Neurotoxic reactive astrocytes are induced by activated microglia. *Nature, 541*, 481–487.

Lin, C. C., & Edelson, B. T. (2017). New insights into the role of IL-1beta in experimental autoimmune encephalomyelitis and multiple sclerosis. *The Journal of Immunology, 198*, 4553–4560.

Lin, Z. H., Wang, S. Y., Chen, L. L., Zhuang, J. Y., Ke, Q. F., Xiao, D. R., et al. (2017). Methylene Blue Mitigates acute neuroinflammation after spinal cord injury through inhibiting NLRP3 inflammasome activation in microglia. *Frontiers in Cellular Neuroscience, 11*, 391.

Liu, X., Zhang, Z., Ruan, J., Pan, Y., Magupalli, V. G., Wu, H., et al. (2016a). Inflammasome-activated gasdermin D causes pyroptosis by forming membrane pores. *Nature, 535*, 153–158.

Liu, X., Zhou, W., Zhang, X., Lu, P., Du, Q., Tao, L., et al. (2016b). Dimethyl fumarate ameliorates dextran sulfate sodium-induced murine experimental colitis by activating Nrf2 and suppressing NLRP3 inflammasome activation. *Biochemical Pharmacology, 112*, 37–49.

Liu, Y., Given, K. S., Harlow, D. E., Matschulat, A. M., Macklin, W. B., Bennett, J. L., et al. (2017). Myelin-specific multiple sclerosis antibodies cause complement-dependent oligodendrocyte loss and demyelination. *Acta Neuropathol Commun, 5*, 25.

Lloyd, A. F., Davies, C. L., & Miron, V. E. (2017). Microglia: Origins, homeostasis, and roles in myelin repair. *Current Opinion in Neurobiology, 47*, 113–120.

Lukens, J. R., Barr, M. J., Chaplin, D. D., Chi, H., & Kanneganti, T. D. (2012). Inflammasome-derived IL-1beta regulates the production of GM-CSF by CD4(+) T cells and gammadelta T cells. *The Journal of Immunology, 188*, 3107–3115.

Luo, C., Jian, C., Liao, Y., Huang, Q., Wu, Y., Liu, X., et al. (2017). The role of microglia in multiple sclerosis. *Neuropsychiatric Disease and Treatment, 13*, 1661–1667.

Malhotra, S., Rio, J., Urcelay, E., Nurtdinov, R., Bustamante, M. F., Fernandez, O., et al. (2015). NLRP3 inflammasome is associated with the response to IFN-beta in patients with multiple sclerosis. *Brain, 138*, 644–652.

Malhotra, S., Sorosina, M., Rio, J., Peroni, S., Midaglia, L., Villar, L. M., et al. (2018). NLRP3 polymorphisms and response to interferon-beta in multiple sclerosis patients. *Multiple Sclerosis, 24*, 1507–1510.

Malik, A., & Kanneganti, T. D. (2017). Inflammasome activation and assembly at a glance. *Journal of Cell Science, 130*, 3955–3963.

Malik, S., Want, M. Y., & Awasthi, A. (2016). The emerging roles of gamma-delta T cells in tissue inflammation in experimental autoimmune encephalomyelitis. *Frontiers in Immunology, 7*, 14.

Mammana, S., Fagone, P., Cavalli, E., Basile, M. S., Petralia, M. C., Nicoletti, F., et al. (2018). The role of macrophages in neuroinflammatory and neurodegenerative pathways of alzheimer's disease, amyotrophic lateral sclerosis, and multiple sclerosis: Pathogenetic cellular effectors and potential therapeutic targets. *International Journal of Molecular Sciences, 19*.

Mangan, M. S. J., Olhava, E. J., Roush, W. R., Seidel, H. M., Glick, G. D., & Latz, E. (2018). Targeting the NLRP3 inflammasome in inflammatory diseases. *Nature Reviews Drug Discovery, 17*, 588–606.

Mao, Z., Liu, C., Ji, S., Yang, Q., Ye, H., Han, H., et al. (2017). The NLRP3 inflammasome is involved in the pathogenesis of Parkinson's disease in rats. *Neurochemical Research, 42*, 1104–1115.

Marinelli, S., Basilico, B., Marrone, M. C., & Ragozzino, D. (2019). Microglia-neuron cross-talk: Signaling mechanism and control of synaptic transmission. *Seminars in Cell & Developmental Biology, 94*. pii: S1084-9521(18)30170-8. https://doi.org/10.1016/j.semcdb.2019.05.017. PMID: 31112798, [Epubahead of print] Review

Martinon, F., Burns, K., & Tschopp, J. (2002). The inflammasome: A molecular platform triggering activation of inflammatory caspases and processing of proIL-beta. *Molecular Cell, 10*, 417–426.

Mccandless, E. E., Budde, M., Lees, J. R., Dorsey, D., Lyng, E., & Klein, R. S. (2009). IL-1R signaling within the central nervous system regulates CXCL12 expression at the blood-brain barrier and disease severity during experimental autoimmune encephalomyelitis. *The Journal of Immunology, 183*, 613–620.

Mckenzie, B., Reinke, S., Branton, W., Lu, J.-Q., & Power, C. (2014). Activation of multiple inflammasomes within the central nervous system during experimental autoimmune encephalomyelitis and multiple sclerosis. *Journal of Neuroimmunology, 275*, 163.

Mckenzie, B. A., Mamik, M. K., Saito, L. B., Boghozian, R., Monaco, M. C., Major, E. O., et al. (2018). Caspase-1 inhibition prevents glial inflammasome activation and pyroptosis in models of multiple sclerosis. *Proceedings of the National Academy of Sciences of the U S A, 115*, E6065–E6074.

Mendiola, A. S., & Cardona, A. E. (2018). The IL-1beta phenomena in neuroinflammatory diseases. *Journal of Neural Transmission, 125*, 781–795.

Michailidou, I., Jongejan, A., Vreijling, J. P., Georgakopoulou, T., De Wissel, M. B., Wolterman, R. A., et al. (2018). Systemic inhibition of the membrane attack complex impedes neuroinflammation in chronic relapsing experimental autoimmune encephalomyelitis. *Acta Neuropathol Commun, 6*, 36.

Michel, L., Touil, H., Pikor, N. B., Gommerman, J. L., Prat, A., & Bar-Or, A. (2015). B cells in the multiple sclerosis central nervous system: Trafficking and contribution to CNS-compartmentalized inflammation. *Frontiers in Immunology, 6*, 636.

Miglio, G., Veglia, E., & Fantozzi, R. (2015). Fumaric acid esters prevent the NLRP3 inflammasome-mediated and ATP-triggered pyroptosis of differentiated THP-1 cells. *International Immunopharmacology, 28*, 215–219.

Monteiro, A., Cruto, C., Rosado, P., Martinho, A., Rosado, L., Fonseca, M., et al. (2018). Characterization of circulating gamma-delta T cells in relapsing vs remission multiple sclerosis. *Journal of Neuroimmunology, 318*, 65–71.

Mortezaee, K., Khanlarkhani, N., Beyer, C., & Zendedel, A. (2018). Inflammasome: Its role in traumatic brain and spinal cord injury. *Journal of Cellular Physiology, 233*, 5160–5169.

Mortimer, L., Moreau, F., Macdonald, J. A., & Chadee, K. (2016). NLRP3 inflammasome inhibition is disrupted in a group of auto-inflammatory disease CAPS mutations. *Nature Immunology, 17*, 1176–1186.

Mouton-Liger, F., Rosazza, T., Sepulveda-Diaz, J., Ieang, A., Hassoun, S. M., Claire, E., et al. (2018). Parkin deficiency modulates NLRP3 inflammasome activation by attenuating an A20-dependent negative feedback loop. *Glia, 66*, 1736–1751.

Mrdjen, D., Pavlovic, A., Hartmann, F. J., Schreiner, B., Utz, S. G., Leung, B. P., et al. (2018). High-dimensional single-cell Mapping of central nervous system immune cells reveals distinct myeloid subsets in Health, aging, and disease. *Immunity, 48*, 380–395 e386.

Mukherjee, S., Chen, L. Y., Papadimos, T. J., Huang, S., Zuraw, B. L., & Pan, Z. K. (2009). Lipopolysaccharide-driven Th2 cytokine production in macrophages is regulated by both MyD88 and TRAM. *Journal of Biological Chemistry, 284*, 29391–29398.

Munoz-Planillo, R., Kuffa, P., Martinez-Colon, G., Smith, B. L., Rajendiran, T. M., & Nunez, G. (2013). K(+) efflux is the common trigger of NLRP3 inflammasome activation by bacterial toxins and particulate matter. *Immunity, 38*, 1142–1153.

Nakanishi, K. (2018). Unique action of interleukin-18 on T cells and other immune cells. *Frontiers in Immunology, 9*, 763.

Nonaka, S., & Nakanishi, H. (2019). Microglial clearance of focal apoptotic synapses. *Neuroscience Letters, 707*, 134317.

Noroozi, S., Meimand, H.a. E., Arababadi, M. K., Nakhaee, N., & Asadikaram, G. (2017). The effects of IFN-beta 1a on the expression of inflammasomes and apoptosis-associated speck-like proteins in multiple sclerosis patients. *Molecular Neurobiology, 54*, 3031–3037.

Ohl, K., Tenbrock, K., & Kipp, M. (2016). Oxidative stress in multiple sclerosis: Central and peripheral mode of action. *Experimental Neurology, 277*, 58–67.

Olsson, T., Barcellos, L. F., & Alfredsson, L. (2017). Interactions between genetic, lifestyle and environmental risk factors for multiple sclerosis. *Nature Reviews Neurology, 13*, 25–36.

Ormel, P. R., Vieira De Sa, R., Van Bodegraven, E. J., Karst, H., Harschnitz, O., Sneeboer, M.a. M., et al. (2018). Microglia innately develop within cerebral organoids. *Nature Communications, 9*, 4167.

Panicker, N., Sarkar, S., Harischandra, D. S., Neal, M., Kam, T. I., Jin, H., et al. (2019). Fyn kinase regulates misfolded alpha-synuclein uptake and NLRP3 inflammasome activation in microglia. *Journal of Experimental Medicine, 216*, 1411–1430.

Parkhurst, C. N., Yang, G., Ninan, I., Savas, J. N., Yates, J. R., 3rd, Lafaille, J. J., et al. (2013). Microglia promote learning-dependent synapse formation through brain-derived neurotrophic factor. *Cell, 155*, 1596–1609.

Parnell, G. P., & Booth, D. R. (2017). The multiple sclerosis (MS) genetic risk factors indicate both acquired and innate immune cell subsets contribute to MS pathogenesis and identify novel therapeutic opportunities. *Frontiers in Immunology, 8*, 425.

Patel, J., & Balabanov, R. (2012). Molecular mechanisms of oligodendrocyte injury in multiple sclerosis and experimental autoimmune encephalomyelitis. *International Journal of Molecular Sciences, 13*, 10647–10659.

Peelen, E., Damoiseaux, J., Muris, A. H., Knippenberg, S., Smolders, J., Hupperts, R., et al. (2015). Increased inflammasome related gene expression profile in PBMC may facilitate T helper 17 cell induction in multiple sclerosis. *Molecular Immunology, 63*, 521–529.

Philips, T., & Rothstein, J. D. (2017). Oligodendroglia: Metabolic supporters of neurons. *Journal of Clinical Investigation, 127*, 3271–3280.

Pierson, E. R., Wagner, C. A., & Goverman, J. M. (2018). The contribution of neutrophils to CNS autoimmunity. *Clinical Immunology, 189*, 23–28.

Place, D. E., & Kanneganti, T. D. (2017). Recent advances in inflammasome biology. *Current Opinion in Immunology, 50*, 32–38.

Plantone, D., Inglese, M., Salvetti, M., & Koudriavtseva, T. (2018). A perspective of coagulation dysfunction in multiple sclerosis and in experimental allergic encephalomyelitis. *Frontiers in Neurology, 9*, 1175.

Pocock, J. M., & Piers, T. M. (2018). Modelling microglial function with induced pluripotent stem cells: An update. *Nature Reviews Neuroscience, 19*, 445–452.

Ponath, G., Park, C., & Pitt, D. (2018). The role of astrocytes in multiple sclerosis. *Frontiers in Immunology, 9*, 217.

Praet, J., Guglielmetti, C., Berneman, Z., Van Der Linden, A., & Ponsaerts, P. (2014). Cellular and molecular neuropathology of the cuprizone mouse model: Clinical relevance for multiple sclerosis. *Neuroscience & Biobehavioral Reviews, 47*, 485–505.

Py, B. F., Kim, M. S., Vakifahmetoglu-Norberg, H., & Yuan, J. (2013). Deubiquitination of NLRP3 by BRCC3 critically regulates inflammasome activity. *Molecular Cell, 49*, 331–338.

Rathinam, V. A., Vanaja, S. K., & Fitzgerald, K. A. (2012). Regulation of inflammasome signaling. *Nature Immunology, 13*, 333–342.

Rathkey, J. K., Zhao, J., Liu, Z., Chen, Y., Yang, J., Kondolf, H. C., et al. (2018). Chemical disruption of the pyroptotic pore-forming protein gasdermin D inhibits inflammatory cell death and sepsis. *Sci Immunol, 3*.

Raupach, B., Peuschel, S. K., Monack, D. M., & Zychlinsky, A. (2006). Caspase-1-mediated activation of interleukin-1beta (IL-1beta) and IL-18 contributes to innate immune defenses against *Salmonella enterica* serovar Typhimurium infection. *Infection and Immunity, 74*, 4922–4926.

Reemst, K., Noctor, S. C., Lucassen, P. J., & Hol, E. M. (2016). The indispensable roles of microglia and astrocytes during brain development. *Frontiers in Human Neuroscience, 10*, 566.

Reu, P., Khosravi, A., Bernard, S., Mold, J. E., Salehpour, M., Alkass, K., et al. (2017). The lifespan and turnover of microglia in the human brain. *Cell Reports, 20*, 779–784.

Rodgers, M. A., Bowman, J. W., Liang, Q., & Jung, J. U. (2014). Regulation where autophagy intersects the inflammasome. *Antioxidants and Redox Signaling, 20*, 495–506.

Rossi, S., Furlan, R., De Chiara, V., Motta, C., Studer, V., Mori, F., et al. (2012). Interleukin-1beta causes synaptic hyperexcitability in multiple sclerosis. *Annals of Neurology, 71*, 76–83.

Rothschild, D. E., Mcdaniel, D. K., Ringel-Scaia, V. M., & Allen, I. C. (2018). Modulating inflammation through the negative regulation of NF-kappaB signaling. *Journal of Leukocyte Biology*. http://dx.doi.org/10.1002/JLB.3MIR0817-346RRR. PMID:29389019. [Epub ahead of print] Review.

Sarkar, S., Malovic, E., Harishchandra, D. S., Ghaisas, S., Panicker, N., Charli, A., et al. (2017). Mitochondrial impairment in microglia amplifies NLRP3 inflammasome proinflammatory signaling in cell culture and animal models of Parkinson's disease. *NPJ Parkinsons Dis, 3*, 30.

Schetters, S. T. T., Gomez-Nicola, D., Garcia-Vallejo, J. J., & Van Kooyk, Y. (2017). Neuroinflammation: Microglia and T Cells get ready to tango. *Frontiers in Immunology, 8*, 1905.

Schett, G., & Neurath, M. F. (2018). Resolution of chronic inflammatory disease: Universal and tissue-specific concepts. *Nature Communications, 9*, 3261.

Scheu, S., Ali, S., Mann-Nuttel, R., Richter, L., Arolt, V., Dannlowski, U., et al. (2019). Interferon beta-mediated protective functions of microglia in central nervous system Autoimmunity. *International Journal of Molecular Sciences, 20*.

Schroder, K., & Tschopp, J. (2010). The inflammasomes. *Cell, 140*, 821–832.

Schuh, E., Lohse, P., Ertl-Wagner, B., Witt, M., Krumbholz, M., Frankenberger, M., et al. (2015). Expanding spectrum of neurologic manifestations in patients with NLRP3 low-penetrance mutations. *Neurol Neuroimmunol Neuroinflamm, 2*, e109.

Sedimbi, S. K., Hagglof, T., & Karlsson, M. C. (2013). IL-18 in inflammatory and autoimmune disease. *Cellular and Molecular Life Sciences, 70*, 4795–4808.

Serhan, C. N., & Levy, B. D. (2018). Resolvins in inflammation: Emergence of the pro-resolving superfamily of mediators. *Journal of Clinical Investigation, 128*, 2657–2669.

Shao, B. Z., Wei, W., Ke, P., Xu, Z. Q., Zhou, J. X., & Liu, C. (2014). Activating cannabinoid receptor 2 alleviates pathogenesis of experimental autoimmune encephalomyelitis via activation of autophagy and inhibiting NLRP3 inflammasome. *CNS Neuroscience and Therapeutics, 20*, 1021–1028.

Shaw, P. J., Lukens, J. R., Burns, S., Chi, H., Mcgargill, M. A., & Kanneganti, T. D. (2010). Cutting edge: Critical role for PYCARD/ASC in the development of experimental autoimmune encephalomyelitis. *The Journal of Immunology, 184*, 4610–4614.

Shema, E., Bernstein, B. E., & Buenrostro, J. D. (2019). Single-cell and single-molecule epigenomics to uncover genome regulation at unprecedented resolution. *Nature Genetics, 51*, 19–25.

Sie, C., & Korn, T. (2017). Dendritic cells in central nervous system autoimmunity. *Seminars in Immunopathology, 39*, 99–111.

Singer, J. W., Fleischman, A., Al-Fayoumi, S., Mascarenhas, J. O., Yu, Q., & Agarwal, A. (2018). Inhibition of interleukin-1 receptor-associated kinase 1 (IRAK1) as a therapeutic strategy. *Oncotarget, 9*, 33416–33439.

Soares, J. L., Oliveira, E. M., & Pontillo, A. (2019). Variants in NLRP3 and NLRC4 inflammasome associate with susceptibility and severity of multiple sclerosis. *Mult Scler Relat Disord, 29*, 26–34.

Sominsky, L., De Luca, S., & Spencer, S. J. (2018). Microglia: Key players in neurodevelopment and neuronal plasticity. *The International Journal of Biochemistry & Cell Biology, 94*, 56–60.

Song, L., Yan, Y., Marzano, M., & Li, Y. (2019). Studying heterotypic Cell(-)Cell interactions in the human brain using pluripotent stem cell models for neurodegeneration. *Cells, 8*.

Song, N., & Li, T. (2018). Regulation of NLRP3 inflammasome by phosphorylation. *Frontiers in Immunology, 9*, 2305.

Song, N., Liu, Z. S., Xue, W., Bai, Z. F., Wang, Q. Y., Dai, J., et al. (2017). NLRP3 phosphorylation is an essential priming event for inflammasome activation. *Molecular Cell, 68*, 185–197 e186.

Spalinger, M. R., Kasper, S., Gottier, C., Lang, S., Atrott, K., Vavricka, S. R., et al. (2016). NLRP3 tyrosine phosphorylation is controlled by protein tyrosine phosphatase PTPN22. *Journal of Clinical Investigation, 126*, 1783–1800.

Stancu, I. C., Cremers, N., Vanrusselt, H., Couturier, J., Vanoosthuyse, A., Kessels, S., et al. (2019). Aggregated Tau activates NLRP3-ASC inflammasome exacerbating exogenously seeded and non-exogenously seeded Tau pathology in vivo. *Acta Neuropathologica, 137*, 599–617.

Sun, S. C. (2011). Non-canonical NF-kappaB signaling pathway. *Cell Research, 21*, 71–85.

Surace, M. J., & Block, M. L. (2012). Targeting microglia-mediated neurotoxicity: The potential of NOX2 inhibitors. *Cellular and Molecular Life Sciences, 69*, 2409–2427.

Sutterwala, F. S., Haasken, S., & Cassel, S. L. (2014). Mechanism of NLRP3 inflammasome activation. *Annals of the New York Academy of Sciences, 1319*, 82–95.

Tai, Y., Wang, Q., Korner, H., Zhang, L., & Wei, W. (2018). Molecular mechanisms of T cells activation by dendritic cells in autoimmune diseases. *Frontiers in Pharmacology, 9*, 642.

Tay, T. L., Savage, J. C., Hui, C. W., Bisht, K., & Tremblay, M.-È. (2017). Microglia across the lifespan: From origin to function in brain development, plasticity and cognition. *The Journal of Physiology, 595*, 1929–1945.

Tezcan, G., Martynova, E. V., Gilazieva, Z. E., Mcintyre, A., Rizvanov, A. A., & Khaiboullina, S. F. (2019). MicroRNA post-transcriptional regulation of the NLRP3 inflammasome in immunopathologies. *Frontiers in Pharmacology, 10*, 451.

Thion, M. S., Ginhoux, F., & Garel, S. (2018). Microglia and early brain development: An intimate journey. *Science, 362*, 185–189.

Thompson, A. J., Banwell, B. L., Barkhof, F., Carroll, W. M., Coetzee, T., Comi, G., et al. (2018a). Diagnosis of multiple sclerosis: 2017 revisions of the McDonald criteria. *The Lancet Neurology, 17*, 162–173.

Thompson, A. J., Baranzini, S. E., Geurts, J., Hemmer, B., & Ciccarelli, O. (2018b). Multiple sclerosis. *Lancet, 391*, 1622–1636.

Tintore, M., Vidal-Jordana, A., & Sastre-Garriga, J. (2019). Treatment of multiple sclerosis - success from bench to bedside. *Nature Reviews Neurology, 15*, 53–58.

Tozser, J., & Benko, S. (2016). Natural compounds as regulators of NLRP3 inflammasome-mediated IL-1beta production. *Mediators of Inflammation, 2016*, 5460302.

Triantafilou, K., Hughes, T. R., Triantafilou, M., & Morgan, B. P. (2013). The complement membrane attack complex triggers intracellular Ca2+ fluxes leading to NLRP3 inflammasome activation. *Journal of Cell Science, 126*, 2903–2913.

Tschopp, J., & Schroder, K. (2010). NLRP3 inflammasome activation: The convergence of multiple signalling pathways on ROS production? *Nature Reviews Immunology, 10*, 210–215.

Tuusa, J., Raasakka, A., Ruskamo, S., & Kursula, P. (2017). Myelin-derived and putative molecular mimic peptides share structural properties in aqueous and membrane-like environments. *Multiple Sclerosis and Demyelinating Disorders, 2*.

Van Bruggen, D., Agirre, E., & Castelo-Branco, G. (2017). Single-cell transcriptomic analysis of oligodendrocyte lineage cells. *Current Opinion in Neurobiology, 47*, 168–175.

Van Kaer, L., Postoak, J. L., Wang, C., Yang, G., & Wu, L. (2019). Innate, innate-like and adaptive lymphocytes in the pathogenesis of MS and EAE. *Cellular and Molecular Immunology, 16*, 531–539.

Vidmar, L., Maver, A., Drulovic, J., Sepcic, J., Novakovic, I., Ristic, S., et al. (2019). Multiple Sclerosis patients carry an increased burden of exceedingly rare genetic variants in the inflammasome regulatory genes. *Scientific Reports, 9*, 9171.

Voet, S., Mc Guire, C., Hagemeyer, N., Martens, A., Schroeder, A., Wieghofer, P., et al. (2018). A20 critically controls microglia activation and inhibits inflammasome-dependent neuroinflammation. *Nature Communications, 9*, 2036.

Voet, S., Prinz, M., & Van Loo, G. (2019). Microglia in central nervous system inflammation and multiple sclerosis pathology. *Trends in Molecular Medicine, 25*, 112–123.

Wallisch, J. S., Simon, D. W., Bayir, H., Bell, M. J., Kochanek, P. M., & Clark, R. S. B. (2017). Cerebrospinal fluid NLRP3 is increased after severe traumatic brain injury in infants and children. *Neurocritical Care, 27*, 44–50.

Wang, C., Pan, Y., Zhang, Q. Y., Wang, F. M., & Kong, L. D. (2012). Quercetin and allopurinol ameliorate kidney injury in STZ-treated rats with regulation of renal NLRP3 inflammasome activation and lipid accumulation. *PLoS One, 7*, e38285.

Wang, K., Song, F., Fernandez-Escobar, A., Luo, G., Wang, J. H., & Sun, Y. (2018). The properties of cytokines in multiple sclerosis: Pros and cons. *The American Journal of the Medical Sciences, 356*, 552–560.

Wang, J., Wang, J., Wang, J., Yang, B., Weng, Q., & He, Q. (2019). Targeting microglia and macrophages: A potential treatment strategy for multiple sclerosis. *Frontiers in Pharmacology, 10*, 286.

Wang, X., Ma, C., Wu, J., & Zhu, J. (2013). Roles of T helper 17 cells and interleukin-17 in neuroautoimmune diseases with emphasis on multiple sclerosis and Guillain-Barre syndrome as well as their animal models. *Journal of Neuroscience Research, 91*, 871–881.

Watson, P. M. D., Kavanagh, E., Allenby, G., & Vassey, M. (2017). Bioengineered 3D glial cell culture systems and applications for neurodegeneration and neuroinflammation. *SLAS Discov, 22*, 583–601.

Weber, K., & Schilling, J. D. (2014). Lysosomes integrate metabolic-inflammatory cross-talk in primary macrophage inflammasome activation. *Journal of Biological Chemistry, 289*, 9158–9171.

Wolf, S. A., Boddeke, H. W., & Kettenmann, H. (2017). Microglia in physiology and disease. *Annual Review of Physiology, 79*, 619–643.

Xu, L., Zhang, C., Jiang, N., He, D., Bai, Y., & Xin, Y. (2019). Rapamycin combined with MCC950 to treat multiple sclerosis in experimental autoimmune encephalomyelitis. *Journal of Cellular Biochemistry, 120*, 5160–5168.

Xu, X., Yin, D., Ren, H., Gao, W., Li, F., Sun, D., et al. (2018). Selective NLRP3 inflammasome inhibitor reduces neuroinflammation and improves long-term neurological outcomes in a murine model of traumatic brain injury. *Neurobiology of Disease, 117*, 15–27.

Yadav, S. K., Soin, D., Ito, K., & Dhib-Jalbut, S. (2019). Insight into the mechanism of action of dimethyl fumarate in multiple sclerosis. *Journal of Molecular Medicine (Berlin), 97*, 463–472.

Yamamoto, S., Yamashina, K., Ishikawa, M., Gotoh, M., Yagishita, S., Iwasa, K., et al. (2017). Protective and therapeutic role of 2-carba-cyclic phosphatidic acid in demyelinating disease. *Journal of Neuroinflammation, 14*, 142.

Yamasaki, R., Lu, H., Butovsky, O., Ohno, N., Rietsch, A. M., Cialic, R., et al. (2014). Differential roles of microglia and monocytes in the inflamed central nervous system. *Journal of Experimental Medicine, 211*, 1533–1549.

Yang, Y., Wang, H., Kouadir, M., Song, H., & Shi, F. (2019). Recent advances in the mechanisms of NLRP3 inflammasome activation and its inhibitors. *Cell Death & Disease, 10*, 128.

Yeh, H., & Ikezu, T. (2019). Transcriptional and epigenetic regulation of microglia in Health and disease. *Trends in Molecular Medicine, 25*, 96–111.

Yeon, S. H., Yang, G., Lee, H. E., & Lee, J. Y. (2017). Oxidized phosphatidylcholine induces the activation of NLRP3 inflammasome in macrophages. *Journal of Leukocyte Biology, 101*, 205–215.

Yi, Y. S. (2018). Regulatory roles of the caspase-11 non-canonical inflammasome in inflammatory diseases. *Immune Netw, 18*, e41.

Yu, H., Wu, M., Lu, G., Cao, T., Chen, N., Zhang, Y., et al. (2018). Prednisone alleviates demyelination through regulation of the NLRP3 inflammasome in a C57BL/6 mouse model of cuprizone-induced demyelination. *Brain Research, 1678*, 75–84.

Yue, Y., Stone, S., & Lin, W. (2018). Role of nuclear factor kappaB in multiple sclerosis and experimental autoimmune encephalomyelitis. *Neural Regen Res, 13*, 1507–1515.

Zhang, A. Q., Zeng, L., Gu, W., Zhang, L. Y., Zhou, J., Jiang, D. P., et al. (2011). Clinical relevance of single nucleotide polymorphisms within the entire NLRP3 gene in patients with major blunt trauma. *Critical Care, 15*, R280.

Zhang, C. J., Jiang, M., Zhou, H., Liu, W., Wang, C., Kang, Z., et al. (2018). TLR-stimulated IRAKM activates caspase-8 inflammasome in microglia and promotes neuroinflammation. *Journal of Clinical Investigation, 128*, 5399–5412.

Zhao, G., Jiang, K., Yang, Y., Zhang, T., Wu, H., Shaukat, A., et al. (2018). The potential therapeutic role of mir-223 in Bovine endometritis by targeting the NLRP3 inflammasome. *Frontiers in Immunology, 9*, 1916.

Zhou, Y., Lu, M., Du, R. H., Qiao, C., Jiang, C. Y., Zhang, K. Z., et al. (2016). MicroRNA-7 targets Nod-like receptor protein 3 inflammasome to modulate neuroinflammation in the pathogenesis of Parkinson's disease. *Molecular Neurodegeneration, 11*, 28.

Znalesniak, E. B., Fu, T., Guttek, K., Handel, U., Reinhold, D., & Hoffmann, W. (2016). Increased cerebral Tff1 expression in two murine models of neuroinflammation. *Cellular Physiology and Biochemistry, 39*, 2287–2296.

Zrzavy, T., Hametner, S., Wimmer, I., Butovsky, O., Weiner, H. L., & Lassmann, H. (2017). Loss of 'homeostatic' microglia and patterns of their activation in active multiple sclerosis. *Brain, 140*, 1900–1913.