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Neuroinflammation, Mast Cells and Glia: Dangerous Liaisons

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Abstract

The perspective of neuronflammation as an epiphenomenon following neuron damage is being replaced by the awareness of glia and their importance in neural functions and disorders. Systemic inflammation generates signals that communicate with the brain and leads to changes in metabolism and behavior, with microglia assuming a pro-inflammatory phenotype. Identification of potential peripheral-to-central cellular links is thus a critical step in designing effective therapeutics. Mast cells may fulfill such a role. These resident immune cells are found close to and within peripheral nerves and in brain parenchyma/meninges, where they exercise a key role in orchestrating the inflammatory process from initiation through chronic activation. Mast cells and glia engage in cross-talk that contributes to accelerate disease progression; such interactions become exaggerated with aging and increased cell sensitivity to stress. Emerging evidence for oligodendrocytes, independent of myelin and support of axonal integrity, points to their having strong immune functions, innate immune receptor expression, and production/response to chemokines and cytokines that modulate immune responses in the central nervous system while engaging in cross-talk with microglia and astrocytes. In this review we summarize the findings related to our understanding of the biology and cellular signalling mechanisms of neuroinflammation, with emphasis on mast cell-glia interactions.

Keywords

mast cells, microglia, astrocytes, oligodendrocytes, neuro-immune, neuroinflammation, neurodegeneration, neuropathic pain, immunosenescence

Introduction

Organisms are endowed by nature with various self-defense mechanisms to maintain homeostasis. As an example, the innate immune system is equipped to distinguish between friend and foe or self and non-self. One important component of such natural mechanisms is inflammation (Nathan and Ding 2010). Most of us would probably associate inflammation with pain, yet this complex biological response is a fundamental part of the body's response to injury and infection designed to eliminate the initial cause of cell injury and initiate tissue repair. In most cases the inflammatory event is innocuous and brief, as the swelling and itching of a mosquito bite. Inflammation is associated also with serious, chronic disorders such as inflammatory bowel disease and rheumatoid arthritis (Castellheim and others 2009). Manifestation of inflammation in the nervous system ('neuroinflammation') can be especially perilous. Although not a primary cause of disease, neuroinflammation contributes importantly to the pathogenesis of chronic pain and neuropathic pain (Ellis and Bennett 2014; Martini and Willison 2016; Myers and others 2006), chronic neurodegenerative diseases (Amor and others 2014; Freeman and Ting 2015; Iadecola and Anrather 2011; McGeer and McGeer 2013; Ransohoff 2016), neuropsychiatric illness (Castanon and others 2015; Najjar and others 2013; Theoharides and others 2015; Wohleb and others 2016), autism spectrum disorder (Noriega and Savelkoul, 2014; Theoharides and others 2016), and probably even temporal lobe epilepsy (Marchi and others 2014). Intriguingly, there appears to be close link between psychosocial stressors and neuroinflammation (Calcia and others 2016; Rivat and others 2010). A common theme in the above pathologies is the persistent nature of inflammation. As Nathan and Ding (2010) pointed out: "The problem with inflammation is not how often it starts, but how often it fails to subside". Without doubt, non-resolving inflammation is one of the principal contributors to the medical burden in today's society.

Our understanding of the immune system's role in neuroinflammation came to light with the revelation that extensive bi-directional lines of communication exist between the former and the central nervous system (CNS). Inflammatory cytokines and chemokines occupy a key niche in this

highway, regulating host responses to infection, inflammation, stress and trauma (Le Thuc and others 2015). Mast cells and microglia (the principal neuroimmune sentinels of the brain), two innate immune system cell types, together with astrocytes, represent key protagonists linking peripheral immune signalling to the brain in an inflammatory setting. These cell populations constitute important sources of inflammatory mediators and may have cardinal roles in conditions ranging from neuropathic pain (Miller 2005; Old and others 2015; Pekny and others 2016; Peng and others 2015; Sakamoto and others 2016; Thacker and others 2007) to neurodegenerative diseases (Amor and Woodruffe 2014; Appel and others 2011; Cunningham, 2013; Harcha and others 2015; Medeiros and LaFerla 2013; Ransohoff 2016; Silver and Curley 2013) and neuropsychiatric disorders (Prinz and Priller 2014; Theoharides and others 2015; Wohleb and others 2016). Such interactions take on added significance with aging, in which the process of ‘immunosenescence’ leads to increased sensitivity of microglia and mast cells to environmental stressors and may well be a factor in low-grade non-resolving inflammation in the elderly (Skaper 2015).

The realization that inflammation is an integral feature of numerous neurological conditions - for which in many cases there is no adequate therapy - leaves an enormous unmet medical need. However, progress in understanding the nature of inflammation’s origin and contribution to disease pathobiology is growing rapidly, as investigators explore nervous system-immune interactions. It bears mentioning that the above considerations have not addressed another key glial cell type, which is abundant in the CNS, namely, the oligodendrocyte. These myelin-producing cells also support axonal functions and long-term integrity, have strong immune functions, express a wide variety of innate immune receptors, and produce and respond to chemokines and cytokines that modulate immune responses in the CNS and engage in cross-talk with other glia cell types. The intent of this review will be to describe recent contributions in our understanding of the biology and cellular signaling mechanisms of neuroinflammation, with emphasis on a mast cell-glia (microglia, astrocyte, oligodendrocyte) interactions.

Mast Cells

A number of cell types participate in neuroinflammation, including glia and immune system-derived cells, both tissue-resident and blood-borne. Neuroinflammation-oriented reviews generally focus on the roles of microglia and other immune cell types such as T cells, along with astrocytes (Grace and others 2014; Hanisch and Kettenmann 2007; Le Thuc and others 2015; Old and others 2015). While glial cell activation represents an important contribution to neuropathology, these cells are responsive also to inflammatory signals originating from other cells of immune origin, such as mast cells. These granulated resident immune cells, first described by Paul Ehrlich in the late 19th century (Ehrlich 1878) are close relatives to basophil granulocytes in blood, both expressing CD34 and containing cytoplasmic granules loaded with histamine and heparin, the former released upon engagement of IgE. Tissue mast cells have a unique haematopoietic lineage development: unlike other myeloid-derived cells, immature lineage progenitors, once entering the circulation undergo transendothelial recruitment into peripheral tissues which regulate the appearance of secretory granules with a particular protease phenotype (**Box 1**) (Prussin and Metcalfe 2003). Apart from their participation in innate host defense reactions, mast cells display a widespread perivascular tissue distribution, and are found predominantly at the interface between the host and the external environment – as such they are strategically placed to act not only as first responders in harmful situations but also as environmental ‘sensors’ to communicate with other cellular elements involved in physiological and/or immune responses (da Dilva and others 2014). Mast cells occur in peripheral tissues innervated by small calibre sensory nerve fibres (**Fig. 1**) and within the endoneurial compartment of peripheral nerves, as well as in meninges and cerebral blood vessels. They are capable of phagocytosis and antigen presentation, and can modulate acquired immune responses (via the high-affinity IgE receptor, FcεRI) (Kalesnikoff and Galli 2008). Mast cells have long been known to participate in inflammatory processes, and are present and recruited to all inflammatory sites. Indeed, mast cells play an important role in orchestrating the whole inflammatory process from initiation events to chronic activation (**Box 1**).

Activated mast cells secrete a plethora of vasoactive, pro-nociceptive and pro-inflammatory mediators, from biogenic amines, cytokines, enzymes, lipid metabolites and ATP, to neuropeptides, growth factors (i.e. tumor necrosis factor alpha (TNF- α) interleukin (IL)-1,2,3,4,6, and 13, nerve growth factor (NGF) and vascular endothelial growth factor (VEGF)) and nitric oxide (**Fig. 2**) (Kalesnikoff and Galli 2008; Silver and Curley 2013). Mast cell heterogeneity is a function of their tissue location and species, which is reflected in the repertoire of mediators elaborated. Within the context of their immune regulatory role they release chemoattractants that recruit eosinophils (Wardlaw and others 1986) and monocytes (Perry and others 1993). In addition to serving as effector cells, mast cells are capable of inducing T cell activation, recruitment, proliferation, and cytokine secretion in an antigen-dependent manner (Bulfone-Paus and Bahri 2015). Mast cell – T helper cell cooperation is expressed in terms of the immunological synapse (or immune synapse / supramolecular activation cluster (Monks and others 1998)), a structure which forms during recognition of peptide fragments (antigens) of pathogens bound to major histocompatibility complex II molecules on antigen-presenting cells (e.g. mast cells) by the T cell receptor (Grakoui and others 1999). We usually think of synapses as long-lasting structures formed between two neurons, or between neurons and a non-neuronal target cell such as muscle cells. Although immune synapses are more short-lived than neural synapses, they are nevertheless structured and essential for cell activation. Lymphocyte function-associated antigen 1 (LFA-1) is expressed by T cells, B cells, macrophages and neutrophils. It functions as an adhesion molecule by binding to intercellular adhesion molecule-1 (ICAM-1) on antigen-presenting cells (e.g. mast cells; **Fig. 2**) (Varma and others 2006). Further, the interaction between CD28 on T cells and its ligands CD86 and CD80 on mast cells is crucial for an optimal activation of antigen-specific T cells. T cell stimulation through CD28 in addition to the T-cell receptor can provide a potent signal for the production of various interleukins (Nakajima and others 1997). A complex relationship between actin dynamics and T cell activation appears to exist, with T cell polarization driven by actin cytoskeleton remodelling (Kwon and others 2008); the cytoskeleton of the antigen-presenting cell may also play an active role in T

cell activation (Comrie and Burkhardt 2016). In addition, mast cell secretion of TNF- α and direct cell-cell interactions between mast cell OX40L and T cell OX40 contribute to the ability of IgE- and antigen-stimulated mast cells to enhance T cell activation (Nakae and others 2006) and polarize the secretory machinery of the latter towards the mast cell (Gaudenzio and others 2009). Mast cells are involved in the development of various T cell-associated immune responses, including experimental autoimmune encephalomyelitis (EAE) (Gregory and others 2006), as will be discussed later in this review.

Blood-brain barrier

Healthy brain vessels possess a functional blood-brain barrier (BBB) which supports cells of the surrounding brain parenchyma, while protecting it from immune cells and harmful substances in the blood. Mast cells are found within the dura and meninges, and on the brain side of the BBB where they contact astrocytic end-feet (Silver and Curley 2013). Mast cells are able to traverse a healthy BBB (Silverman and others 2000), as well as the blood-spinal cord barrier (and BBB) in pathological conditions. Ischemia-triggered mast cell degranulation appears to play a role in initiating the early phase of ischemic damage (Lindsberg and others 2010). Increased mast cell degranulation has been reported after stroke in the immature brain (Biran and others 2008) and transient global ischemia in the adult rat (Hu and others 2004). Cerebral mast cells, through their release of proteases regulate acute microvascular gelatinase (matrix metalloproteinases-2 and -9) activation, thereby effecting BBB disruption following transient cerebral ischemia (Mattila et al., 2011). Another factor, not often considered, is the potential role of VEGF. Elevated VEGF levels contribute to early stroke pathology, including BBB breakdown, vascular leakage and edema. BBB disruption by VEGF increases extravasation of glutamate and albumin, in turn activating astrocytes and altering K⁺ homeostasis in brain parenchyma leading to hyperexcitation of neurons (Lapilover and others 2012) and entry of inflammatory cells. Indeed, VEGF administration shortly after stroke increases vascular leakage and brain infarction (Zhang and others 2000). Activated microglia

secrete prostaglandin E2, which induces VEGF expression in mast cells (Li and others 2015). Prolonged VEGF elevation increases angiogenic sprouting and causes breakdown of the BBB (Lange and others 2016). Indeed, pharmacological or genetic manipulation of mast cells in rat models reduces BBB permeability, brain edema and neutrophil recruitment after ischemia (Jin and others 2007; Mattila and others 2011), and diminishes brain edema and hematoma volume and improves outcome after experimental intracerebral hemorrhage (Strbian and others 2007). Using a mouse model of focal cerebral ischemia, McKittrick and others (2015) describe that mast cells act on the basal membrane, thus promoting BBB damage, brain edema, prolonged extravasation, neutrophil infiltration and hemorrhage. Moreover, it has been postulated that in EAE activation of meningeal mast cells elicits production of TNF- α and early neutrophil recruitment, thereby promoting breakdown of the local BBB and cerebrospinal fluid-blood barrier allowing initial immune cell (e.g. T cells) access to the CNS (Christy and others 2013; Sayad and others 2010). These findings suggest that meningeal inflammation anticipates CNS immune cell infiltration and point to mast cells as among the earliest participants in these disease-initiating events (Christy and others 2013).

Elderly patients often experience postoperative cognitive dysfunction following surgery and hospitalization (Terrando and others 2011). It has been suggested that extra-CNS surgical trauma, by provoking neuroinflammation, may be a critical contributor to surgery-induced cognitive dysfunction (Riedel and others 2014). Inflammatory mediators released by surgical stress may damage synapses and neurons, and ultimately lead to postoperative cognitive dysfunction (Eckenhoff and Laudansky 2013). Cerebral mast cells have been suggested to contribute to postoperative cognitive dysfunction by promoting BBB breakdown (Zhang and others 2016). It is intriguing also that acute stress (immobilization) may increase BBB permeability through activation of brain mast cells (Esposito and others 2001).

Blood-nerve barrier

Mast cells are present within the endoneurium, and their stimulation is capable of markedly altering blood-nerve barrier permeability, endoneurial fluid composition and nerve conduction (Harvey and others 1994). In an experimental model of diabetes produced by hyperglycemia, the authors observed a significant rise in permeability to [^{14}C]-labeled mannitol and water content, as well as significant increases in numbers of degranulating perivascular mast cells (Kalichman and others 1995).

Mast Cells and Glia in Inflammation-Associated Chronic Neuropathologies

Glial cells are activated in response to a number of different pathological states within the CNS, including chronic neurodegenerative disorders. Given the vast literature surrounding this facet of neuroinflammation, we will focus instead on evidence supporting their involvement along with mast cells in representative disease states. The reader is referred to several excellent recent reviews (Lull and Block 2010; Medeiros and LaFerla 2013; Smith and others 2011).

Alzheimer disease

An expanding body of evidence posits that Alzheimer disease (AD) pathogenesis is not restricted to the neuronal compartment, but encompasses interactions with immunological mechanisms in the brain. Indeed, microglia and astrocytes are key players in the pro-inflammatory environment of AD brain. Changes in microglia and astrocytes have been observed in the post-mortem brains of individuals with AD and in mouse transgenic models of AD over-expressing a mutant amyloid precursor protein and presenilin 1 bearing deposits of insoluble amyloid β -peptide ($\text{A}\beta$) (plaques) (Heneka and others 2015; Parvathenani and others 2003). Microglia surrounding $\text{A}\beta$ plaques in the frontal cortex of AD brain reportedly contain IL-1 β -positive microglia (Heneka and others 2015). Amyloid precursor protein reportedly also regulates microglial phenotype in a mouse model of AD (Manocha and others 2016). Triggering receptor expressed on myeloid cells 2 (TREM-2) is part of the immunoglobulin and lectin-like superfamily and functions as part of the innate immune system. TREM-2 is highly expressed by microglia (Hickman and others 2013) and mediates phagocytic

clearage of neuronal cell debris (Takahashi and others 2007). Mutations in this gene may be risk factors for AD (Guerreiro and others 2013). An endogenous ligand from TREM-2 has yet to be identified; however, TREM binding activity (a possible indication of TREM-2 ligand expression) has been observed on reactive astrocytes surrounding A β plaques and on damaged neurons and oligodendrocytes (Hsieh and others 2013; Heneka and others 2015).

Post-mortem studies of AD patients have evidenced mast cells close to amyloid plaque lesions in different brain regions (Maslinska and others 2007), although their involvement in disease onset and/or progression remains to be elucidated. Harcha and others (2015) have recently shed light on this question by showing that acute treatment of cultured mast cells with A β ₂₅₋₃₅ peptide activates Panx1 hemichannels, which mediate Ca²⁺ influx degranulation. Brain mast cells exhibited increased Panx1 and Cx43 hemichannel activity after acute A β ₂₅₋₃₅ treatment, which was accompanied by enhanced histamine release. In addition, mast cell numbers increased in cortex and hippocampus before onset of amyloid plaque formation and showed increased Panx1 and Cx43 hemichannel activity in a mouse model of AD. These authors suggest that mast cells are among the first brain cells to sense A β peptides and thus may play a critical role in the onset and progression of AD (Harcha and others 2015).

Traumatic brain injury (TBI) acts as an important epigenetic risk factor for AD (Sivanandam and Thakur 2012). Subjecting AD transgenic mice to TBI worsens behavioural deficits and aggravates A β deposition (Tajiri and others 2013), possibly due to immune cell dysregulation. Fluid percussion TBI resulted in cortical mast cell increase and changes in regulation of central histamine receptors (Lozada and others 2005). Mild TBI in the form of concussive head injury evoked persistent dural mast cell degranulation for at least 30 days, while blast trauma gave rise to a delayed mast cell degranulation response commencing at seven days that also persisted for at least 30 days (Levy and others 2015). Moreover, TBI leads also to microglial cell activation, which may be both rapid (Koshinaga and others 2000) and persistent following the injury event (Ertürk and others 2016; Ramlackhansingh and others 2011). Pro-inflammatory cytokines released by mast cells

and microglia can up-regulate expression of β -secretase (Sastre and others 2003), resulting in increased production of amyloidogenic A β species (Corrigan and others 2011). Mast cells are also early responders in the hypoxic-ischemic neonatal rat, their numbers increasing in ipsilateral hemisphere within the first several hours and prior to neuronal cell apoptosis and astrocyte/microglia activation.

Multiple sclerosis

MS is an inflammatory autoimmune disease characterized by the destruction of CNS myelin which insulates axons (Compston and Coles 2008), thereby allowing the rapid conduction of electrical impulses and delivery of the action potential to the target cell. MS is the most common cause of chronic neurological impairment in young people (Kamm and others 2014). Myelin destruction is initiated by the myelin-reactive T cells, and is further amplified by the inflammatory response of myeloid cells, including the brain-resident microglia and infiltrating inflammatory macrophages (Sospedra and Martin 2016). Microglia-mediated proteolysis of TREM-2 produces soluble TREM-2 (sTREM-2). Cerebrospinal fluid levels of sTREM-2 were reported increased in patients with relapsing-remitting MS, secondary progressive MS, and primary progressive MS, returning to control level with immunomodulatory drug (natalizumab) treatment (Öhrfelt and others 2016). Mast cells are also considered as playing an important role in the pathogenesis of MS (Conti and Kempuraj 2016; Kritas and others 2014; Theoharides and others 2008), where they mediate inflammation and demyelination by presenting myelin antigens to T cells and/or disrupting the BBB to permit entry of inflammatory cells and cytokines. Degranulating mast cells are seen in the brain of rats with EAE (Brenner and others 1994). Mast cells can be activated by myelin (Medic and others 2008), cause demyelination (Theoharides and others 1991) and induce oligodendrocyte death (Medic and others 2010). In mouse EAE models, however, some investigators argue for a lack of clear evidence whether mast cell effects on EAE development depend on mouse strain, immunization protocol, or type and severity of the disease (Nelissen and others 2013).

Granulocyte macrophage-colony stimulating factor (GM-CSF), a cytokine produced by T helper cells, plays a key role in orchestrating neuroinflammation in EAE. Meningeal mast cells appear to be an important contributor to myelin-specific T cell accumulation and GM-CSF expression, as T cells neither accumulate in meninges nor produce GM-CSF in the absence of mast cells (Russi and others 2016a,b). These authors also showed, by means of mast cell-T cell co-culture experiments and selective mast cell reconstitution of the meninges of mast cell-deficient mice that meningeal mast cells are an early source of caspase-1-(inflammasome)-dependent IL-1 β which, in turn, promotes GM-CSF expression by T cells and their encephalitogenicity. Further, acute MS patients appear to display mast cell-T cell co-localization in the meninges and CNS (Russi and others 2016b). Russi and colleagues (2016a) propose that mast cell-T cell crosstalk in the meninges is critical for EAE disease development and, possibly, also in human demyelinating disease (Russi and colleagues 2016b).

IL-17A is associated with many inflammatory and autoimmune diseases and may be involved in MS. Local production of IL-17A is not limited to cells involved in adaptive immune responses such as Th17 cells and cytotoxic T cells (Kolbinger and others 2016), but also innate immune cells such as mast cells (Kan and others 2016). IL-17A synergizes with other pro-inflammatory cytokines (e.g. those released by microglia and mast cells themselves), inducing the release of additional cytokines and chemokines that recruit new inflammatory cells. IL-17A adversely affects the functions of microglia, astrocytes, oligodendrocytes, neurons, neural precursor cells and endothelial cells (Kolbinger and others 2016).

IL-33, the newest member of the IL-1 cytokine family is implicated in a number of inflammatory and autoimmune diseases (Liew and others 2010). The IL-33 receptor, consisting of ST2 and IL-1 receptor accessory protein, is expressed particularly by T helper 2 cells and mast cells (Liew and others 2010). IL-33 is generally released as a danger signal ("alarmin") from damaged cells, and may also be processed and released from activated mast cells with subsequent autocrine and paracrine actions. IL-33 not only augments the stimulatory effects of IgE and substance P (SP)

on mast cells but can also trigger the release of cytokines from mast cells per se (Theoharides and others 2015). IL-33 inflammatory activity includes up-regulation of IL-6, IL-8, and IL-13. IL-33 is up-regulated in both the periphery and the CNS of MS patients (Christophi and others 2012), and administration of a blocking anti-IL-33 antibody in EAE mice during the induction phase significantly inhibited disease onset and severity (Li and others 2012). In human brain, IL-33 is expressed by various CNS resident cells including neurons, astrocytes, oligodendrocytes and microglia, while its receptor ST2 is mainly expressed by neurons. Expression levels and patterns of IL-33 and ST2 in acute and chronic MS brain lesion samples were enhanced compared with normal brain tissues (Allan and others 2016). These authors also demonstrated that IL-33 inhibits myelination in rat myelinating spinal cord co-cultures. Approximately 30-40% of patients with MS suffer from central neuropathic pain (Osterberg and others 2005). Conceivably, this could be linked to spinal cord oligodendrocyte-derived IL-33, and its reciprocal relationship with IL-33-induced production of TNF- α and IL-1 β in spinal cord (Zarpelon and others 2016).

Neuropathic pain

Acute pain is self-limiting and serves a protective biological function by acting as a warning of on-going tissue damage. It is a symptom of a disease process experienced in or around the injured or diseased tissue. Chronic pain, on the other hand, serves no protective biological function. Rather than being the symptom of a disease process, chronic pain is itself a disease process. Persistent pain represents a substantial and growing unmet medical need, affecting nearly half of people seeking medical care in the United States alone. Among all types of chronic pain, neuropathic pain stands out: as defined by the International Association for the Study of Pain, it results from damage or disease affecting the somatosensory system (Jensen and others 2011). Neuropathic pain may be either peripheral or central, depending on lesion location caused by diseases such as diabetes mellitus, herpes zoster, human immunodeficiency virus infection, medical interventions (chemotherapy, surgery), and injury, while the latter are most often caused by stroke, spinal cord

injury or multiple sclerosis (Kerstman and others 2013). In the case of peripheral neuropathic pain (painful neuropathy) chronic pain should be considered a brain disease in which alterations in neural networks affect multiple aspects of brain function, structure and chemistry (Borsook 2012). Neuropathic pain is associated with significant medical care costs and decreased worker productivity (Jay and Barkin 2014; Smith and Torrance 2012), with an estimated 3-4.5% of the global population affected by neuropathic pain - with the rate of incidence increasing as the global population ages (Smith and Torrance 2012). Indeed, a more recent systematic review of epidemiological studies estimates a population prevalence of pain with neuropathic characteristics to lie between 6.9% and 10% (van Hecke and others 2014).

Immune cells, in particular mast cells and microglia are co-protagonists of the somatosensory system, acting as the primary interlocutors for pain neurons, both in the periphery and at the spinal and supraspinal levels. Persistent alterations in mast cells and microglia lead to changes that promote persistent neuroinflammation which, in turn, impacts the functionality of neurons. Mast cell degranulation not only triggers the release of molecules (e.g. IL-6) which activate or sensitize nociceptors and directly contribute to neuropathic pain (Xanthos and others 2011) but also activates trigemino-cervical and lumbosacral pain pathways to elicit widespread tactile pain hypersensitivity (Levy and others 2012), the latter action possibly mediated by a sensitizing effect of histamine on nociceptors. By constituting the first line of activation at the site of damage, peripheral nerve-resident mast cells promote the recruitment of neutrophils and macrophages (Zuo and others 2003). Further, NGF, which can sensitize nociceptors by engaging their high-affinity TrkA receptors (and indirectly via other cell types) (Kelleher and others 2016) is rapidly released by mast cells (Leon and others 1994). Interestingly, mast cells respond to NGF in a paracrine/autocrine manner (Leon and others 1994). Working together, these events facilitate T-cell recruitment to reinforce and maintain inflammatory reactions. Mast cells may enhance recruitment of other key immune cell types which, in turn, release pro-nociceptive mediators. A role for mast cells in chronic pain states is further supported by recent studies showing that: glucocorticoid therapy reduces pain and TNF- α -

positive mast cell numbers in rats with chronic constrictive nerve injury (Hayashi and others 2011); mast cells are important mediators of chronic visceral pain (Done and others 2012). These concepts are schematically illustrated in more detail in **Fig. 3**.

Glia are important mediators of pain processes at the spinal level (Old and others 2015; Grace and others 2014). Microglia interact with spinal neurons at the site of injury, for example, by synthesizing and releasing IL-1 β that modulates neuronal cell activity (Watkins and others 2003), and can be activated through a number of cell surface molecules as well as respond to pro-inflammatory signals released from peripheral cells of immune origin, such as mast cells. Microglia express several subtypes of ionotropic P2X and metabotropic P2Y purinergic receptors that play a role in pain signalling in the spinal cord under pathological conditions (e.g. peripheral nerve injury) (Biber and others 2011; Kobayashi and others 2008; Skaper and others 2010). In such settings, dorsal horn microglia become activated with up-regulation of purinergic receptors which participate in neuropathic pain (Burnstock 2016; Tsuda 2016). Inhibiting the function or expression of these microglial receptors suppresses the aberrant excitability of dorsal horn neurons and neuropathic pain (Tsuda 2016), underlining spinal microglia as an important player in mechanisms for neuropathic pain and potential target for treating chronic pain states. In addition to the accepted roles for the P2X and P2Y receptors activated by ATP and ADP, respectively, recent evidence suggests a role also for the adenosine A2A receptor in microglial cytoskeletal rearrangements (Orr and others 2009; Luongo and others 2014) and the adenosine A1 receptor in moderating ATP-induced over-excitability of nociceptive neurons after microglia application to the spinal cord (Luongo and others 2014).

Cross-talk between mast cells and microglia, directly via cellular mediators and indirectly through somatosensory neurons, may contribute to amplification of peripheral pain signals at the spinal level (Bulanova and Paus 2010). Activated microglia contribute to pain states by releasing pro-inflammatory cytokines, chemokines, and proteases. Peng and others (2016) have suggested that microglia and monocytes may act synergistically to promote the transition from acute to

chronic pain after nerve injury. In addition, systemic inflammation can generate signals that communicate with the brain, leading to alterations in metabolism and behavior, including expression of a pro-inflammatory phenotype by microglia (Cunningham 2013). Astrocytes, the most abundant CNS glial cell type involved in neuroinflammation, also play a major role in pain facilitation and are a key contributor to neuropathic pain (Ji and others 2014; Milligan and Watkins 2009). The above findings encourage the notion that controlling mast cell-glia reactivity can provide an attractive therapeutic avenue for treating neuropathic pain (Gao and Ji 2010; Popiolek-Barczyk and Mika 2016; Skaper and Facci 2012). In this context it is interesting to point out that acute intracerebroventricular administration of N-palmitoylethanolamine, a congener of the endocannabinoid anandamide with analgesic and anti-inflammatory activities linked to mast cell / microglia modulation (Alhouayek and Muccioli 2014), reduced carrageenan-induced paw edema/hyperalgesia and prevented nuclear factor- κ B nuclear translocation in the spinal cord (D'Agostino and others 2007), supporting central involvement of these immune cells in peripheral inflammation.

To study the mechanisms of persistent pain, animal models of inflammatory hyperalgesia that mimic human clinical pain conditions have been developed based on the injection of inflammatory agents into the rat or mouse hind paw (Millan and others 1988). Neurogenic inflammation arises from the local release of inflammatory mediators from afferent neurons such as SP, calcitonin gene-related peptide, neurokinin A, and endothelin-3 (Geppetti and others 2008). Further, NGF (presumably mast cell-derived) can trigger nociception via transient receptor potential vanilloid 1 (TRPV1) and oxidative mechanisms (Eskander and others 2015). Once released, these neuropeptides provoke the egress of histamine from adjacent mast cells which, in turn, evokes the release of SP and calcitonin gene-related peptide - thus establishing a bidirectional link between histamine and neuropeptides in neurogenic inflammation (Rosa 2013). SP strongly induces VEGF in mast cells, and IL-33 contributes to the stimulation and release of VEGF in human mast cells in a dose-dependent manner and acts synergistically in combination with SP (Theoharides and others

2010) (**Fig. 4**). Within this context, incubation of rat peritoneal mast cells with NGF, together with specific antigen or SP to induce degranulation, leads to a significant increase in secretion of preloaded [³H]serotonin (**Table 1**); neither BDNF nor neurotrophin-3 was active.

The above considerations concerning chronic pain have not addressed another important glial cell type, which is abundant in the CNS, namely, oligodendrocytes. The latter cells are responsible for producing the myelin that ensheaths axons and forms the nodes of Ranvier that permit saltatory nerve conduction. More recently, additional roles for oligodendrocytes have come to light, including provision of trophic factors and metabolic support for neurons (Bankston and others 2013), as well as support of axonal functions and long-term integrity in a myelin-independent fashion (Nave 2010). For example, oligodendrocyte ablation in mice results in axonal pathology in the spinal dorsal horn and spinothalamic tract concurrent with the induction and maintenance of an exaggerated nociceptive sensitivity prior to frank demyelination independent of innate or adaptive immune responses (Gritsch and others 2014). Moreover, oligodendrocytes also have strong immune functions, express a wide variety of innate immune receptors, and produce and respond to chemokines and cytokines that modulate immune responses in the CNS (Peferoen and others 2014; Zeis and others 2016) and engage in cross-talk with microglia (Peferoen and others 2014). Inflammation triggers the production of N,N-dimethylsphingosine (DMS) in the dorsal horn, and its intrathecal administration is sufficient to induce neuropathic pain-like behavior in rats (Patti and others 2012). Interestingly, cultured human oligodendrocytes produce DMS, with DMS levels increasing when these cells are challenged with agents that damage white matter (Chen and others 2014), suggesting that damage to oligodendrocytes *in vivo* leads to increased DMS production which in turn drives inflammatory astrocyte responses involved in sensory neuron sensitization. In addition, a new study demonstrates that that spinal cord oligodendrocyte-derived IL-33 mediates neuropathic pain (Zarpelon and others 2016). An intriguing link between oligodendrocytes and NGF comes from a recent report by von Büdingen and others (2015), in which the authors demonstrate the direct binding of NGF to myelin oligodendrocyte glycoprotein, a minor component

of myelin and the only protein exclusively restricted to the outermost lamellae of compact CNS myelin (and which shares structural features with TrkA). Based on their findings, these authors posit that myelin oligodendrocyte glycoprotein may serve as a protective mechanism to deplete excess NGF and thereby prevent aberrant sprouting and neuropathic pain after peripheral nerve injury. Whether this mechanism links CNS demyelination with neuropathic pain as, for example, in MS (Solaro and others 2013) remains to be determined. Collectively, the above observations point to a heretofore unexpected role of oligodendrocytes in mediating neuropathic pain. The potential for crosstalk between the various glial cell types discussed will be explored further in the following section.

Mast Cells and Glia: a Complex Communication Network

Recruitment and activation of different immune cell populations in a defined temporal pattern requires reciprocal communication amongst these cells. Consider, for example, brain-derived neurotrophic factor (BDNF), which plays a key role in eliciting pain hypersensitivity. Peripheral nerve injury up-regulates the purinergic P2X₄ receptor in spinal microglia to mediate BDNF release and neuropathic pain (Ulmann and others 2008), and depends on mast cell tryptase cleavage of proteinase-activated receptor 2 (PAR2) to activate PAR2-bearing microglia (Yuan and others 2010). Microglia-released IL-6 and TNF- α , up-regulating PAR2 expression on nearby mast cells (Zhang and others 2010) could, as the authors suggest, result in a BDNF-driven feedback loop between these cells.

Extracellular ATP is a ubiquitous danger signal released from infected or damaged cells, acting via purinoceptors on target cells, and is an important signaling pathway in the development of neuropathic pain (Burnstock 2016). ATP is a potent stimulus for microglia. Expression of different P2 receptor subtypes can vary as a function of the species and source from which mast cells are derived (Bulanova and Bulfone-Paus 2010). ATP from one mast cell can diffuse several hundred micrometers trigger a rise in Ca²⁺ in neighboring cells (Osipchuk and Cahalan 1992). Microglia

whose Toll-like receptors have been activated by pathogen-associated molecular patterns respond to ATP with the release of IL-33 which can subsequently bind to its cognate mast cell receptor and induce the secretion of inflammatory molecules to modulate glial cell activity.

There is evidence to suggest that elements of the complement system also participate in this crosstalk. The chemoattractant C5a receptor is up-regulated on reactive astrocytes and microglia and C5a is released in neuroinflammation (Griffin and others 2007). The latter acts as a strong chemoattractant signal for mast cells whose C5a receptor is up-regulated upon activation of these cells.

Cluster of differentiation 40 (CD40) is a co-stimulatory protein found on antigen-presenting cells (including mast cells; **Fig. 2**) and is required for their activation. The binding of CD154 (CD40L) on T helper cells to CD40 activates antigen presenting cells and induces a variety of downstream effects. Studies by Kim and others (2011) point to the existence of cross-talk between astrocytes and mast cells through CD40-CD40L interaction which induces cytokine and chemokine production via Rho-family GTPases/ Ca^{2+} -dependent protein kinase C isoforms, mitogen-activated protein kinases, nuclear factor- κB and signal transducer and activator of transcription 1. These cytokines subsequently re-activate astrocytes, and enhance the production of a variety of cytokines.

Toll-like receptors (TLRs) represent a major class of pathogen-associated molecular patterns, molecules associated with groups of pathogens recognized by innate immune system cells (e.g. microglia and mast cells). Activation of mast cell TLR2/TLR4 triggers cytokine release which recruits immune cells to the sites of injury; microglia recruitment depends on signaling pathways involving TLR2/TLR4 (Aguirre and others 2013; Pietrzak and others 2011). Mast cell activation up-regulates also chemokine expression (e.g. CCL5/RANTES), which can induce a pro-inflammatory response in microglia, as well. Further, microglia-derived IL-6 and CCL5 may, in turn, affect mast cell expression of TLR2/TLR4. **Table 2** summarizes the above-discussed points.

Conceivably, glia – glia interactions could comprise an additional element whereby glia and mast cells work in concert to promote neuroinflammation. For example, Wang and others (2014)

have demonstrated microglia – astrocyte interaction at the level of translocator protein, a marker of gliosis in neurodegeneration. Microglia and astrocytes are also capable of exercising a reciprocal interaction through their release of pro-inflammatory cytokines/chemokines (Le Thuc and others 2015). Moreover, a crosstalk between oligodendrocytes and microglia has been proposed, involving exosomes - small vesicles containing proteins, lipids and regulatory RNAs (Peferoen and others 2014) (**Box 2**).

Immunosenescence

Non-resolving inflammation takes on added significance in the context of aging, a phenomenon associated with elevated levels of circulating cytokines and pro-inflammatory markers, and age-related changes in the immune system (so-called 'immunosenescence') (Mate and others 2014; Michaud and others 2013). The changes that mast cells and microglia undergo as the organism ages represent an important element of immunosenescence. Mast cells have been observed to display various alterations in the aging animal, such as: reprogramming of degranulation (expressed as heightened sensitivity to prostaglandin E2) (Nguyen and others 2005); increased cell numbers in the dermis (role in tissue damage and aging changes in skin?) (Gunin and others 2011); decline in cell development due to senescent stromal cell impairment (Tsuboi and others 2011). Dysregulation of microglia is likely to play a prominent role in aging, as well. Aging models are characterized by an increased inflammatory state of microglia, 'primed' to be activated and resistant to regulation (Eggen and other 2013; Norden and Godbout 2013; Rawji and others 2016). Priming renders microglia susceptible to a secondary inflammatory stimulus, which can then trigger an exaggerated inflammatory response (Perry and Holmes 2014) (**Fig. 5**). The age-related decline of myelin proteins was found to be highly correlated with activation of astrocytes and microglia in the rat CNS (Xie and others 2013). Age-related myelin degradation (and possibly also oligodendrocyte turnover) (Young and others 2013) burdens the clearance function of microglia during aging, thereby leading to lysosomal storage and possibly contributing to microglial senescence and

immune dysfunction in aging (Safaiyan and others 2016). It has also been suggested that the alarmin high mobility group box 1 (HMGB1), which can be released under chronic pathological conditions and initiate inflammatory cascades, mediates neuroinflammatory priming in the aged brain (Fonken and others 2016). Moreover, priming of spinal microglia following activation by diverse forms of peripheral trauma/inflammation can lead to enhanced pain intensity / duration (Hains and others 2010). When the aged brain's immune system is challenged, microglial activation is amplified and prolonged. Left unchecked, this primed state of microglia may ultimately manifest itself in deleterious behavioral and cognitive consequences. Interestingly, dystrophic (senescent) rather than activated microglial cells are associated with tau pathology in human brain and have been suggested to precede neurodegeneration in AD (Streit and others 2009). The appearance of dystrophic microglia has been noted also in mouse brain with aging (Cristino and others 2015; Punzo and others 2016).

The ability of microglia and mast cells to communicate with each other may very well exacerbate the effects of aging on these cells' pro-inflammatory traits. Obesity (Kim and Do 2015), along with diabetes (Donath 2014) and cancer (Diakos and others 2014) are now recognized as chronic low-grade inflammatory states. Not surprisingly, incidence of the latter conditions generally increases with age, placing the elderly at greater risk for a state of *low-grade, non-resolving inflammation*. This concept has an underlying experimental basis, whereby Morris and others (2015) demonstrated that an endotoxin-induced, persistent state of low-grade inflammation is associated with innate immune programming. On a side note, there is mounting evidence that acute and chronic inflammatory pain states, including neuropathic pain (which is associated with low-grade chronic inflammation), markedly alter BBB permeability (Dos Santos and others 2014), perhaps as a consequence of changes in tight junction proteins (Brooks and others 2005). Loss of BBB integrity has been linked, as well, to cognitive dysfunction in aging (Chan-Ling and others 2007; Pelisch and others 2013; Rapp and others 2008).

Conclusions and Perspectives

Inflammation is a protective reaction initiated following injury or infection, and is intended to promote tissue repair and healing. When dysregulated, however, inflammation is transformed into a key pathological process in diverse disease states. Neuroinflammatory disorders are conditions where immune responses damage components of the nervous system, and can be especially devastating. Microglia represent well-described sensors for disturbed brain tissue homeostasis and accumulate locally in response to neuronal cell injury or entry of foreign material into the brain. Yet, we still have much to learn about resident brain cell types capable of mounting immediate host responses in the brain and meninges, such as mast cells. These innate immune effector cells have the capacity to evoke both a rapid and longer-term delayed responses. As discussed in this review, a highly complex interplay exists between a number of non-neuronal cells in the nervous system, including microglia, astrocytes, mast cells and oligodendrocytes (**Fig. 6**). Inclusion of the last cell type is a relatively recent addition to the equation, yet perhaps not all that surprising given the fact that they express a wide range of innate immune receptors, and produce and respond to chemokines and cytokines that modulate immune responses across the CNS.

These cellular behaviors, with their interconnected lines of communication can present a challenge when designing strategies to deal with the resolution of inflammation-associated neurological disorders (Fullerton and Gilroy, 2016). This issue is especially relevant when confronting chronic and neuropathic pain where, for the majority of patients, treatment options are woefully insufficient. For instance, opioids, the treatment of choice for acute severe pain, are contraindicated for chronic usage due to many side effects and their abuse potential, and newer anticonvulsants prescribed for pain are only effective for about one-third of patients. These two classes of drugs act primarily on neuronal circuits of the brain and spinal cord and treat the symptoms but not the underlying pathophysiology. Mast cell-stabilizing agents like cromolyn suppress development of hyperalgesia but do not affect microglia. Microglial inhibitors like minocycline, which are often used in pain models rely on their anti-inflammatory properties but

may be limited due to non-selectivity in targeting one cell population and the risk of acute or cumulative toxicity. An agent which is capable of modulating activation across glia as well as mast cells, while at the same time devoid of immunosuppressive activity, might prove efficacious in the resolution of inflammation and even restoration of tissue homeostasis.

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Conflict of Interest

The authors declare that there is no conflict of interest.

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Box 1

The basics of mast cell biology

Mast cells were first described by Paul Ehrlich in 1878 based on their unique staining characteristics and large cytoplasmic granules – a feature unique to these cells. They share certain features with another blood-borne immune cell, the basophil, although mast cells appear to be generated by different precursor cells in the bone marrow expressing CD34. The hematopoietic lineage development of tissue mast cells is unique compared to other myeloid-derived cells because it is early lineage progenitors, histochemically undetectable, that leave the bone marrow to enter the circulation. Immature lineage mast cells undergo transendothelial recruitment into peripheral tissues where they differentiate; appearance of secretory granules with a particular protease phenotype is regulated by the peripheral tissue. We now recognise two mast cell types, namely, those from connective tissue and a distinct set of mucosal mast cells – the latter's activities being dependent on T-cells. Mast cells found throughout all vascularised tissues, often close to blood vessels. Their prominence near boundaries between the body's external environment and the internal milieu (e.g. skin, lung mucosa and digestive tract), as well as in mouth, conjunctiva and nasal passages leaves them well-placed to function as sentinel cells in host defense. Mast cells are found also within the nervous system, including meninges, the brain parenchyma and peripheral nerves.

Mast cells play a key role in the inflammatory process, and their activation leads to rapid release of granules into the interstitium. Degranulation can be caused by a number of factors, including direct injury (e.g. physical or chemical), cross-linking of IgE receptors or by activated complement proteins. Mast cells have the capacity to release a wide array of bioactive agents, including cytokines, nociceptive peptides, chemoattractants and growth factors. These molecules can be transferred to adjacent cells of the immune system and neurons via transgranulation and their pseudopodia. In addition, they express a number of "pattern recognition receptors" which may allow them to recognize broad classes of pathogens.

Mast cells play important roles in diseases both outside and within the nervous system, including allergic reactions and anaphylactic shock, chronic and neuropathic pain, degenerative and traumatic neurological pathologies, mood disorders and autism.

Box 2

Exosomes

Exosomes are cell-derived vesicles widely distributed in virtually all eukaryotic fluids, including medium from cultured cells. They were initially observed in the maturing mammalian reticulocyte, and shown to participate in selective removal of many plasma membrane proteins. In most mammalian cells the plasma membrane is being continually recycled and portions internalized as endosomes, after which membrane parts of some endosomes are internalized as smaller vesicles called multivesicular bodies. Exosomes may follow two routes: release from the cell when multivesicular bodies fuse with the plasma membrane; direct release from the plasma membrane. Exosomes have specialized functions and appear to play key roles in coagulation, intercellular signalling, and scavenging. These vesicles contain a variety of molecular constituents of their cell of origin, including proteins and RNA. The protein composition of exosomes can vary with cell and tissue of origin, but the majority of exosomes contain an evolutionarily-conserved common set of proteins.

Exosomes are thought to provide a means of intercellular communication and of transmission of macromolecules between cells via membrane vesicle trafficking, thereby influencing the immune system. In recent years, exosomes have been considered as having roles in the spread of proteins, lipids, mRNA, microRNA and DNA and as contributing factors in the development of several diseases. Given these properties of exosomes, there is now much interest in clinical applications of exosomes, with potential applications in prognosis, therapy, and as biomarkers.

Figure legends

Fig. 1. Safranin staining highlights cytoplasmic granules (red) in a tissue mast cell, located in close proximity to a small blood vessel and myelinated nerve fiber (note node of Ranvier). Degranulating mast cells have a wide ‘footprint’, their granules being able to travel up to 50 μm . This strategic location can influence the function of vascular structures as well as axons. (Image courtesy of Lucia Petrelli, Department of Neuroscience, University of Padua)

Fig. 2. The mast cell: a multifaceted immune cell. Schematic representation of mast cell activators and molecules. Mast cell activation triggers the rapid release of preformed effectors (tumor necrosis factor (TNF), interleukin-4 (IL-4) and granulocyte macrophage-colony stimulating factor (GM-CSF), among others) as well as de novo synthesis of cytokines (TNF), chemokines, and growth factors (e.g. nerve growth factor). Cell adhesion molecule 1 (CADM1) on mast cells promotes interaction with peripheral nociceptive neuron terminals via heterophilic binding to nectin-3 (Hagiyama and others 2013; Ito and Oonuma 2006). Intercellular contacts between T helper cells and antigen-presenting cells like mast cells initiate T-cell signaling, whereby T-cell surface receptors recognize antigens bound to major histocompatibility complex molecules on the antigen-presenting cell (Suurmond and others 2013). This process (which also engages adhesion receptors) creates a specialized junction between the two cell types – the so-called immunological synapse (Choudhuri and others 2014), which mediates delivery of effector molecules (via microvesicles) and intercellular signals across this cleft – in effect polarizing the secretory machinery of the T cell towards the mast cell. Cluster of differentiation 86 (CD86) is a protein expressed on antigen-presenting cells providing co-stimulatory signals necessary for T cell activation and survival. CD28 Engagement between CD28 on T cells and CD86 on mast cells is crucial for an optimal activation of antigen-specific T cells. TCR, T cell receptor.

Fig. 3. In physiological conditions, pain information begins at the nerve endings, which form a functional pain unit with the nearby tissue capillaries. Peripheral mast cells are a key element in this functional unit, given their proximity to sensory nerve endings and vasculature (Forsythe and Bienenstock 2012). Following injury or inflammatory stimuli, mast cell mediators (histamine, bradykinin, prostaglandins, some microRNAs) stimulate nociceptive afferents (Kajihara and others 2010; Park and others 2014). Neuropeptides (e.g. substance P) may also provoke mast cell degranulation, leading to a bidirectional positive feedback-loop (Matsuda and others 1989) to up-regulate local inflammation and increase pain. Mast cell recruitment of other immune cells, which release pro-nociceptive mediators, can affect not only injured zones but also adjacent territories, creating a secondary, widespread hyperalgesia (Zuo and others 2003). At the spinal level, mediators released from *dural* mast cells may reach the superficial laminae to modulate synaptic transmission and nociception (Michaloudi and others 2008). CNS mast cells are believed to have a role in central integration of pain, being especially concentrated in the thalamus - an essential nociceptive relay whose release of mediators such as histamine or serotonin might interact with third-order neurons targeting the cortex (Héron and Dubayle 2013; Silver and Curley 2013). Peripheral and brain mast cells *cooperate* with microglia and other immune cells to orchestrate onset of central sensitization. In fact, in the absence of a tight physiological control, mast cell-nerve terminal activity results in nociceptor sensitization, reduced pain threshold at the site of inflammation and, ultimately, dysfunctional pain signaling and hyperalgesia (Demir and others 2013; Zuo and others 2003). Persistent increased responsiveness of nociceptors can also sensitize spinal cord neurons, leading to central sensitization (Dirckx and others 2013).

Fig. 4. Peripheral sensitization and neurogenic inflammation. The interaction between nociceptive terminals and tissue mast cells conditions the peripheral threshold to pain. A lack of proper mast cell control, e.g. in response chemical, dysmetabolic or infective noxae leads to the abnormal release of growth factors like vascular endothelial growth factor (VEGF) and nerve growth factor

(NGF) which can cause neoinnervation and angiogenesis, as well as vasoactive amines (histamine, HIST) to provoke vasodilatation, edema and inflammatory cell infiltration. In neurogenic inflammation mediators like substance P (SP) are directly released from sensory nerves (slow velocity C-fibers) to produce vasodilatation, edema, and other manifestations of inflammation. Additionally, NGF can trigger nociception via transient receptor potential vanilloid 1 (TRPV1). SP induces the release of histamine from mast cells which, in turn, evokes the release of SP and calcitonin gene-related peptide from nerve terminals - thus establishing a bidirectional link between histamine and neuropeptides in neurogenic inflammation. SP strongly induces VEGF in mast cells, and IL-33 contributes to the stimulation and release of VEGF in human mast cells in a dose-dependent manner and acts synergistically in combination with SP.

Fig. 5. Schematic illustration of the effects of aging on microglial cell responsiveness to stress or injury. Age-related changes in the immune system (immunosenescence) are characterized by an increased inflammatory state of microglia, 'primed' to be activated and resistant to regulation. Priming renders microglia susceptible to a secondary inflammatory stimulus, which can then trigger an exaggerated inflammatory response (represented by the extended, red curve).

Fig. 6. Neuroinflammation may result from CNS neurons being impinged upon by a microglia-astrocyte-mast cell network, as a result of inadequate regulation of these non-neuronal cells due to excessive and/or persistent endogenous and/or endogenous stimuli, and/or an inadequate cellular inhibitory capacity. Two-way communication between microglia, mast cells and astrocytes may act to reinforce deleterious signals acting on the neuron. The oligodendrocyte adds another level of complexity to this interacting network of non-neuronal cells, being both a target and source of signals directed to the latter cell populations (Kiray and others 2016). Oligodendrocytes have strong immune functions, express a wide variety of innate immune receptors, and produce and respond to chemokines and cytokines that modulate immune responses in the CNS.