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Mast cells, glia and neuroinflammation: partners in crime?

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Abbreviations: AD, Alzheimer disease; ALS, amyotrophic lateral sclerosis; BBB, blood-brain barrier; CNS, central nervous system; EAE, experimental autoimmune encephalomyelitis; FAAH, fatty acid amide hydrolase; IL-1 β , interleukin-1 β ; MPL,

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monophosphoryl lipid A; MPTP, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine; MS, multiple sclerosis; NAAA, lysosomal NAE-hydrolyzing acid amidase; NAE, N-acylethanolamine; NGF, nerve growth factor; PAMP, pathogen-associated molecular patterns; PEA, palmitoylethanolamide; TLR, Toll-like receptor; TNF- α , tumour necrosis factor- α

Summary

Glia and microglia in particular elaborate pro-inflammatory molecules which play key roles in CNS disorders from neuropathic pain and epilepsy to neurodegenerative diseases.

Microglia respond also to pro-inflammatory signals released from other non-neuronal cells, mainly those of immune origin such as mast cells. The latter are found in most tissues, are CNS resident, and traverse the blood-spinal cord and blood-brain barriers when barrier compromise results from CNS pathology. Growing evidence of mast cell - glia communication opens new perspectives for development of therapies targeting neuroinflammation by differentially modulating activation of non-neuronal cells normally controlling neuronal sensitization – both peripherally and centrally. Mast cells and glia possess endogenous homeostatic mechanisms/molecules that can be up-regulated as a result of tissue damage or stimulation of inflammatory responses. Such molecules include the N-acylethanolamine family. One such member, N-palmitoylethanolamine is proposed to have a key role in maintenance of cellular homeostasis in the face of external stressors provoking, for example, inflammation. N-palmitoylethanolamine has proven efficacious in mast-cell mediated experimental models of acute and neurogenic inflammation. This review will provide an overview of recent progress relating to the pathobiology of neuroinflammation, the role of microglia, neuro-immune interactions involving mast cells and the possibility that mast cell–microglia cross talk contributes to the exacerbation of acute symptoms of chronic

neurodegenerative disease and accelerate disease progression, as well as promote pain transmission pathways. We will conclude by considering the therapeutic potential of treating systemic inflammation or blockade of signalling pathways from the periphery to the brain in such settings.

Introduction

Inflammation is fundamentally a protective cellular response aimed at removing injurious stimuli and initiating the healing process. However, when prolonged, inflammation overrides the bounds of physiological control and eventually becomes destructive. Inflammation increasingly surfaces as a key element in the pathobiology of chronic pain, neurodegenerative diseases, stroke, spinal cord injury, and perhaps even neuropsychiatric disorders.¹⁻⁵ A plethora of pro-inflammatory cytokines, eicosanoids, and other immune neurotoxins, have been found in cerebrospinal fluid and/or affected brain regions of patients with neurodegenerative disorders.⁶ Consider also that nuclear factor- κ B, a requisite transcription factor for most pro-inflammatory molecules, is activated in the substantia nigra pars compacta of Parkinson disease patients and 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-intoxicated mice and monkeys, and its selective inhibition of NF- κ B protects dopaminergic neurones from MPTP toxicity.⁷ It is intriguing to note that neuroinflammation may also raise the brain's sensitivity to stress.⁸ Indeed, a recently published study by Zhang et al.⁹ reports that inflammation-activated signalling pathways in the brain's hypothalamus control the production of ageing related hormones. This finding provides a link between inflammation, stress responses and ageing. Inflammation thus constitutes an important target for neuronal protection in neurodegenerative disorders and neuropathic pain, the latter resulting from damage or disease affecting the somatosensory system.¹⁰

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Recognition that an extensive communication exists between the immune system and the central nervous system (CNS) is, no doubt, one of the more fundamental advances in neuroscience in recent times. Inflammatory cytokines occupy a key niche in this network, regulating host responses to infection, inflammation, stress, and trauma. Glial cell activation has been implicated in the pathogenesis of Alzheimer disease (AD), Parkinson disease, cerebral ischemia, multiple sclerosis (MS)^{2,7,11} and motor neurone disease,¹² and possibly schizophrenia and depression.^{4,13} Microglia-mediated neuroinflammatory processes are proposed to compromise healthy brain aging, as well.¹⁴ Found to accumulate at sites of injury or plaques in neurodegenerative CNS diseases,^{11,15} microglia scavenge dead cells and secrete neurone survival factors; the latter may have beneficial effects in the recovery of injured CNS. However, inappropriate and prolonged activation of glia can cause autoimmune responses leading to brain injury and neuronal cell death.^{2,11,15} Glia provide a link also between neuroinflammation and neuropathic pain;¹⁶ microglia, in particular, show increased activity in multiple pain processing pathways in response to peripheral injury.¹⁷ Systemic inflammation gives rise to signals that communicate with the brain and leads to changes in metabolism and behaviour - including the expression of a pro-inflammatory phenotype by microglia.¹⁸ It has been proposed that in multiple chronic disease states, and in ageing, microglia are primed by prior pathology, or by genetic predisposition, to respond more vigorously to subsequent inflammatory stimulation, thus transforming an adaptive CNS inflammatory response to systemic inflammation, into one with deleterious consequences.¹⁹ It thus goes without saying that delineating the signalling pathways underlying glial cell activation is crucial in the design of agents capable of antagonising such signalling steps – which may translate into therapeutic benefit for neurodegenerative disorders and neuropathic pain.

While it is widely accepted that glial cell activation contributes to neuropathology, one must not forget that microglia and astrocytes also respond to pro-inflammatory signals released from other cells of immune origin. In this view, mast cells represent a potentially important (and underappreciated) peripheral immune signalling link to the brain in an inflammatory setting. Mast cells share similarities with basophil granulocytes in blood, although the former are likely generated by different precursor cells in the bone marrow.²⁰ In contrast to basophils, mast cells circulate in an immature form until choosing a tissue site to settle - which probably determines their precise characteristics. These effector cells of the innate immune system are present in most tissues in the vicinity of blood vessels, particularly near surfaces exposed to the environment.²¹ Mast cells participate in innate host defence reactions, occur in peripheral tissues innervated by small calibre sensory nerve fibres and within the endoneurial compartment of peripheral nerves, and in meninges and cerebral blood vessels. During development they enter the brain by way of penetrating blood vessels, with which they remain associated.²² Mast cells can move through normal brain blood-brain barrier (BBB),²³ but may also traverse the blood-spinal cord barrier and BBB when compromised by disease. Interestingly, they are capable of phagocytosis and antigen presentation, and can modulate the adaptive immune response.

Mast cells produce a vast array of mediators, which include biogenic amines, cytokines, enzymes, lipid metabolites, ATP, neuropeptides, growth factors and nitric oxide (Table 1).²⁴ Because of their heterogeneity, however, no single mast cell makes all of these. By nature of their immune regulatory role they participate in IgE switching by B cells,²⁵ and the release of chemoattractants that recruit eosinophils²⁶ and monocytes.²⁷ Certain disease states, like those involving autoimmune demyelination are accompanied by an increased absolute number of mast cells within the CNS,³⁷ as well as those undergoing degranulation.²⁸ Mast cell trypase is

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elevated in the cerebrospinal fluid of MS patients.²⁹ At the other end of the equation, activated mast cells can provoke demyelination³⁰ and induce apoptotic oligodendrocyte cell death.³¹ Brain mast cells might even 'bridge' the immune system and anxiety-like behaviour.³² Theoharides and colleagues³³ have suggested that perinatal mast cell activation by infectious, stress-related, environmental or allergic triggers can lead to release of pro-inflammatory and neurotoxic molecules, thus contributing to brain inflammation and autism spectrum disorders pathogenesis, at least in a subgroup of these patients.

Microglia, mast cells and nervous system disease

Neuropathic pain

Persistent pain represents a substantial and growing unmet medical need, affecting nearly half of people seeking medical care in the US alone. Among all types of chronic pain, neuropathic pain stands out: this is pain resulting from damage, degeneration or dysfunction of the sensory nervous system, and remains largely untreatable. Central neuropathic pain is found in spinal cord injury, MS, and some strokes, while the common causes of painful peripheral neuropathies are diabetes and other metabolic conditions. Neuropathic pain is common in cancer as a direct result of cancer on peripheral nerves or as a side effect of chemotherapy. The triggering and maintenance of neuropathic pain states depends very much on Schwann cells, spinal microglia and astrocytes, together with elements of the peripheral immune system.³⁴ Release of interleukin-1 β (IL-1 β) from spinal microglia as a consequence of inflammation/injury IL-1 β may, by engaging its receptor induce phosphorylation of the N-methyl-D-aspartate receptor NR1 subunit to strengthen painful signal transmission.³⁵ Under pathological conditions dorsal horn microglia become activated and show up-regulated expression of purinergic receptors,^{36,37} whose inhibition or deletion strongly attenuates neuropathic pain.³⁶⁻³⁸

Upon degranulation, mast cells release algogenic (from the Greek 'algos' for pain) substances which activate or sensitise nociceptors, thereby contributing directly to neuropathic pain.³⁹ Nerve-resident peripheral nerve mast cells represent the first line of activation at the point of damage and facilitate recruitment of neutrophils and macrophages.⁴⁰ Mast cell degranulation activates trigemino-cervical and lumbosacral pain pathways and elicits widespread tactile pain hypersensitivity,⁴¹ possibly mediated by a sensitising effect of histamine on nociceptors.⁵⁶ Rapid release of nerve growth factor (NGF) from mast cells also produces sensitization of nociceptors via the latter's high-affinity NGF-trkA receptors (and indirectly via other peripheral cell types).⁴² Interestingly, mast cells themselves respond to NGF, in a paracrine/autocrine fashion.⁴³ These events promote the recruitment of T-cells, which reinforce and maintain inflammatory reactions. The released mediators/factors can induce activity in axons and/or undergo retrograde transport to the cell body of dorsal root ganglion neurones, thereby affecting gene expression. Further, mast cells may enhance recruitment of other key immune cell types which, in turn, release pro-nociceptive mediators, such as IL-6.⁴⁴ Systemic glucocorticoid therapy reduces pain and the number of tumour necrosis factor- α (TNF- α)-positive mast cells in rats with chronic constrictive injury, strengthening a role for mast cells in chronic pain states.⁴⁵ Mast cells are important mediators of chronic visceral pain,⁴⁶ as well.

Ischemia and traumatic brain injury

Stroke and traumatic brain injury are characterized by an inflammatory response in which microglia activation and macrophage/neutrophil infiltration are important elements.⁴⁷ Left unchecked, this can ultimately lead to secondary injury. Yet, in certain instances attenuation of microglial activation can be beneficial.⁴⁸ Considerable efforts have been directed also to inhibiting the consequences of blood-borne neutrophil and phagocyte infiltration in ischemia.

In contrast, less emphasis has been placed on brain-resident cell types able to mount an immediate host response in the cerebral parenchyma and meninges, for example, mast cells.⁴⁹ Analogous to peripheral nerve damage - and contrary to what was long believed⁵⁰ - mast cell activation is the "first responder" in this injury.⁵¹ Although CNS microglia/macrophages and endothelial cells produce TNF- α in response to stimuli, mast cells are "primed" to initiate acute inflammation with their stores of preformed TNF- α .⁵² Treatment with mast cell-stabilizing agents limits brain damage caused by perinatal hypoxia-ischemia and transient focal ischemia.^{51,53,54} Given their complement of vasoactive and matrix-degrading components like histamine, and proteases able to activate matrix metalloproteinases, mast cells are an early response element in the regulation of acute BBB changes after cerebral ischemia and hemorrhage.⁵⁵ By regulating acute microvascular gelatinase activation, cerebral mast cells can effect BBB disruption following transient cerebral ischemia.⁵⁶

Chronic neurodegenerative diseases

Microglia are activated in response to a number of different pathological states within the CNS including neurodegenerative disorders such as AD and Parkinson disease, MS, amyotrophic lateral sclerosis (ALS), and AIDS dementia complex. There is a vast literature on this facet of neuroinflammation which is beyond the scope of this article. The reader is referred to several excellent recent reviews.^{57,58} In the case of Parkinson disease, the oxidative stress response by microglial cells, most notably the activity of the enzyme NADPH oxidase, appears to play a central role in the ensuing pathology.⁶⁰

The case for mast cells, while somewhat analogous, depends to a greater extent on the specific disease. In MS patients, especially in chronic active plaques, mast cells have been noted generally clustered around venules and capillaries.⁶⁰ Mast cell-associated gene transcripts (e.g. tryptase) are also present in MS plaques, while mast cell mediators such as

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tryptase and histamine have been found in cerebrospinal fluid of MS patients, with levels rising during relapses.^{61,62} In mouse experimental autoimmune encephalomyelitis (EAE) models of MS, however, there is no clear evidence whether mast cell effects on EAE development depend on mouse strain, immunization protocol, or type and severity of the disease.⁶³ A role for mast cells in AD pathogenesis remains to be established, although one report claimed that fibrillar amyloid β -peptides trigger CD47-dependent mast cell secretory and phagocytic responses.⁶⁴ For Parkinson disease, there is no clear evidence to support a relationship between brain mast cells and Parkinson disease. As concerns mast cells and motor neurone disease, this point will be discussed separately below.

Mast cells and ALS: at the crossroads?

ALS, also known as Charcot's or Lou Gehrig's disease, is the most prevalent type of motor neurone disease, affecting around 4-6 per 100,000. ALS is characterized by a progressive dysfunction and degeneration of both upper motor neurones comprising the corticospinal tract, and lower motor neurones arising from the brainstem nuclei and ventral roots of the spinal cord. Neuroinflammation is now established as an important aspect of pathology in ALS.⁶⁵ There is a marked activation or proliferation of both microglia and astrocytes at specific disease stages in mouse models of ALS⁶⁶ and in humans *in vivo*.⁶⁷ There is also compelling evidence indicating impairment of all neurovascular unit components including the blood-brain and blood-spinal cord barriers in both patients and animal models of ALS.⁶⁸

As discussed earlier, mast cells participate in innate host defence reactions, and orchestrate neuroinflammatory processes. Meningeal mast cells contain preformed TNF- α (the only cell type to do so) and vasoactive mediators, which are able to regulate BBB⁶⁹ and blood-spinal cord barrier⁷⁰ function and facilitate the entry of lymphocytes, neutrophils and

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mast cells themselves⁷¹ when the barrier is compromised as a result of CNS pathology, e.g. in ischemic stroke and ALS, respectively. Indeed, degranulating⁷² and IL-17-expressing mast cells⁷⁰ have been observed in the spinal cord of ALS patients, and serum levels of this cytokine are reported to correlate with the duration and severity of the disease.⁷⁰ An interaction between mast cells and T regulatory lymphocytes can lead to increased IL-6 production by the former,⁷³ in turn promoting the activity of Th17 lymphocytes.⁷⁴ Moreover, IL-15 and IL-12 are elevated in the serum and cerebrospinal fluid of patients with ALS.⁷⁵ IL-15 is a mast cell chemoattractant⁷⁶ (potentially contributing to mast cell recruitment in inflammatory responses), while mast cells are an important source of IL-12.⁷⁷ Pathogen-associated molecular patterns (PAMPs) are molecules associated with groups of pathogens that are recognized by cells of the innate immune system. One important class of PAMP receptors is transmembrane Toll-like receptors (TLRs). IL-12 up-regulates expression of mast cell TLR2/TLR4⁷⁸ and proteinase-activated receptor-2,⁷⁹ emerging targets for neuroinflammation.⁸⁰

Functional TLR2 and TLR4 expressed by mast cells and microglia respond to molecules called damage associated molecular patterns, one being the high mobility group box 1 protein found elevated in spinal cord of ALS patients.⁸¹ Likewise, microglia-released IL-6 and CCL5 could, conceivably affect surface TLR2 and TLR4 expression on mast cells,⁸² resulting in the up-regulation of numerous chemokines to induce a pro-inflammatory profile in microglia.⁸³ Given that ALS patients frequently experience neuropathic pain, and that a transgenic mouse ALS model develops peripheral nerve inflammation,⁸⁴ one might ask whether or not mast cells play a role here, as well.

Microglia and mast cells: the other side of the coin

Activated microglia elaborate a potentially lethal cocktail of compounds capable of damaging neurones, oligodendrocytes, or extracellular matrix molecules, and depletion or blockade of microglia and macrophages prevents disease progression.⁸⁵ Microglia/macrophages, however, may deliver trophic factors,⁸⁶ and support myelin regeneration by phagocytic removal of obstructive myelin debris⁸⁷ or through activation and recruitment of endogenous oligodendrocyte precursor cells to the lesion site.⁸⁸ Microglia are found adjacent to amyloid deposits, and anti-inflammatory drugs that suppress the inflammatory response in microglia attenuate symptoms in a mouse model of AD.⁸⁹ Yet, in one study reducing or ablating resident microglia failed to alter amyloid plaque load in transgenic AD mouse models.⁹⁰ Further, deleting the microglial chemokine receptor CCR2 (which mediates the accumulation of mononuclear phagocytes at sites of inflammation) accelerated early disease progression and impaired microglial accumulation in an AD mouse model.⁹¹ Microglia activation via TLR4 signalling appears able to reduce, to some extent, A β deposits and preserve cognitive functions from A β -mediated neurotoxicity.⁹² However, prolonged activation of microglia is likely to result in a pro-inflammatory state. This idea is borne out in a newly published study in which the authors used monophosphoryl lipid A (MPL), a lipopolysaccharide-derived TLR4 agonist that exhibits unique immunomodulatory properties at doses that are non-pyrogenic.⁹³ Repeated, systemic injections of MPL, but not lipopolysaccharide, significantly improved AD-related pathology in a transgenic mouse model. MPL treatment led to a significant reduction in A β load in the brain of these mice, as well as enhanced cognitive function. MPL induced a potent phagocytic response by microglia while triggering only a moderate inflammatory reaction.¹¹⁷

As discussed earlier, acute CNS injuries are marked by a prolonged inflammatory response involving microglial activation and infiltration of macrophages and neutrophils.

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However, the fact that microglia accumulation at the lesion site and penumbra hints to a possible neuroprotective role. Indeed, genetic ablation of microglial cells results in a larger infarct after transient middle cerebral artery occlusion,⁹⁴ while microglia injected into the circulation of Mongolian gerbils home to an ischemic hippocampal lesion (facilitated by ischemia-induced BBB compromise) and improve neurone survival.⁹⁵ Further, microglia may protect hippocampal neurones from excitotoxicity.⁹⁶ Resting (ramified) microglia respond to, and repair, subclinical abnormalities of the brain without a complete activation transformation,⁴⁸ and are probably also key players in developmental synaptic pruning (discussed in more detail elsewhere⁹⁷).

Potential beneficial actions of mast cells should not be overlooked. Mast cells of human origin express and store angiogenin (a member of the ribonuclease A superfamily) within their granules, which is released upon stimulation by FcεRI-mediated signals, TLR ligands and NGF.⁹⁸ NGF stimulated human mast cells release greater amounts of angiogenin compared with FcεRI cross-linking. Human angiogenin is reported to be neuroprotective and to promote the survival and neuritogenesis of motor neurones,⁹⁹ and recent studies associating angiogenin gene mutations with ALS¹⁰⁰ suggest a possible disease link. Mast cells are a source of serotonin in the hippocampus, which can contribute to behavioural and physiological functions in the hippocampus.¹⁰¹ Outside the nervous system, mast cells may contribute to wound healing.¹⁰²

A mast cell – glia dialogue

Given their frequent proximity at sites of neuroinflammation, and the potential for peripheral (e.g. spinal) inflammation to influence supraspinal behaviours, it is conceivable that lines of communication exist between these two cell types. There is imounting evidence

for this, which will be briefly summarised here (see ref. 97 for a more detailed discussion). There is growing interest in TLR signalling pathways in neurodegenerative disorders and neuropathic pain, and especially TLR-2 and TLR-4. Engagement of TLR2/TLR4 on mast cells leads to release cytokines which recruit immune cells to the sites of injury. Likewise, microglial recruitment depends on signalling pathways involving TLR2/TLR4. Mast cell activation will up-regulate chemokine expression, including CCL5/RANTES, which induces a pro-inflammatory profile in microglia. Microglia-released IL-6 and CCL5 could, in turn affect mast cell expression of TLR2/TLR4. ATP, released from damaged cells/tissues, is a potent stimulus for microglia *in vitro*. ATP may act as an autocrine/paracrine factor for mast cells, and ATP released from one mast cell (e.g. FcεR1 cross-linking, stress) can diffuse several hundreds of micrometres to elicit a rise in Ca^{2+} in neighbouring cells.¹⁰³ ATP binding to P2 receptors may stimulate release of IL-33 from microglia pre-activated ('primed') with PAMPs via TLRs.¹⁰⁴ IL-33 can induce mast cells to secrete IL-6, IL-13 and CCL2 - in turn modulating microglia activity. Other examples include mast cell tryptase cleavage/activation of proteinase-activated receptor 2 on microglia (resulting in P2X₄ receptor up-regulation and brain-derived neurotrophic factor release),¹⁰⁵ while microglia-derived IL-6 and TNF-α up-regulate mast cell expression of proteinase-activated receptor 2, resulting in mast cell activation and TNF-α release.^{106,107} Elements of the complement system are also potential participants in this communication network, as the receptor for the chemoattractant C5a is markedly up-regulated on reactive astrocytes and microglia in inflamed CNS tissue,¹⁰⁸ C5a peptide is released in neuroinflammation,¹⁰⁹ and there is crosstalk between C5a and TLR4; mast cell C5a receptor is up-regulated upon activation, and C5a receptor is a strong mast cell chemoattractant signal towards C5a peptide. These last findings point to an additional element whereby microglia and mast cells may work in concert to promote neuroinflammation (Table 2).

Taping endogenous mechanisms as a therapeutic approach to neuroinflammation

Targeting microglial and mast cell activation is emerging as promising avenue for neuropathic pain,¹²⁹ as well as agents which inhibit neurotoxic glial cell activation.¹³⁰ Given the dangers that neuroinflammation poses to the organism, it would not be surprising if Nature has endowed the body with the capacity for self-defence. Indeed, we now recognize the existence of molecules involved in endogenous protective mechanisms, which are activated following different types of tissue damage or stimulation of inflammatory responses and nociceptive fibres. For example, chronic inflammatory processes such as those sustaining neuropathic pain may be counteracted by a program of resolution that includes the production of lipid mediators able to switch off inflammation.¹³¹ Chronic inflammatory conditions may lower the levels or actions of these molecules;¹³² conceivably, administration of such lipid mediators might provide an avenue *"to commandeer nature's own anti-inflammatory mechanisms and induce a "dominant" program of resolution"*.¹³³ One interesting class of such natural mediators comprises the N-acyl ethanolamines (NAEs), which are composed of a fatty acid and ethanolamine – the so-called fatty acid ethanolamines. Principal fatty acid ethanolamine family members include the endocannabinoid N-arachidonylethanolamine (anandamide) and its congeners N-stearoylethanolamine, N-oleoylethanolamine and N-palmitoylethanolamine (PEA, or palmitoylethanolamide).¹³⁴ PEA and its congeners are formed from N-acylated phosphatidylethanolamine (NAPE) by several enzymatic pathways,¹³⁵ the principal one involving a membrane-associated NAPE-phospholipase D which generates the respective NAE and phosphatidic acid (Fig. 2).¹³⁶ This enzyme converts N-palmitoyl-phosphatidyl-ethanolamine into PEA. In the mammalian brain, NAEs are hydrolysed by: (i) fatty acid amide hydrolase (FAAH) in the endoplasmic reticulum, which breaks down NAEs into the corresponding fatty acid and ethanolamine;¹³⁷ (ii) lysosomal NAE-hydrolysing acid amidase (NAAA) (Fig. 2).¹³⁸ NAAA is found mainly in

macrophages, where it hydrolyses NAEs having less than 18 carbon atoms, i.e., PEA, but not N-oleoylethanolamine and N-stearoylethanolamine. In contrast, FAAH hydrolyses all three NAEs.

Molecules like PEA may, conceivably, function to maintain cellular homeostasis when faced with external stressors provoking, for example, inflammation.¹³⁹ In fact, PEA is produced/hydrolysed by microglia and mast cells,^{140,141} it down-modulates mast cell activation,¹⁴² and controls microglial cell behaviours.^{143,144} Tissue levels of PEA are elevated in brain areas involved in nociception and in spinal cord following neuropathic pain induction,¹⁴⁵ as well as conditions associated with pain development^{143,146} and in the interstitium of the trapezius muscle of women with chronic widespread pain and chronic neck-shoulder pain.¹⁴⁷ The above observations thus suggest that PEA maintains cellular homeostasis by acting as mediator of resolution of inflammatory processes (Table 3). A corollary to this idea is that pathological situations may arise in which endogenous PEA levels are inadequate to deal with the ensuing insult. In these cases, exogenous administration to effectively "top up" the body's own supply may be a therapeutically viable approach. Indeed, a growing number of studies demonstrate the validity of such; these are briefly summarized in Table 4. A more detailed discussion is outside the scope of the review, and will be covered elsewhere (Skaper S.D., submitted).

As NAAA preferentially hydrolyzes PEA, inhibition of its degradation represents a complementary therapeutic approach to treat inflammation. A number of selective NAAA inhibitors have been described to date;¹⁶⁸⁻¹⁷⁰ these dampen responses induced by inflammatory stimuli *in vivo* and *in vitro*, and elevate PEA levels *in vitro* as well.¹⁶⁸ The most recently identified compound, 1-(2-biphenyl-4-yl)ethyl-carbonyl pyrrolidine, is a reversible and competitive NAAA inhibitor: it reduces mRNA expression levels of inducible nitric

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oxide synthase and IL-6 and increases intracellular PEA levels in mouse macrophages upon lipopolysaccharide-induced inflammation.¹⁷¹

The numerous preclinical studies have encouraged clinical trials of PEA, mainly in the area of chronic and neuropathic pain. PEA reportedly improved myelinated-fibre function in patients with chemotherapy-induced painful neuropathy,¹⁷² and reduced neuropathic pain in a patient with MS.¹⁷³ A recently presented case series described the application and potential efficacy and safety of micronized and ultra-micronized PEA (formulations designed to improve bioavailability) in the treatment of various syndromes associated with chronic pain that is poorly responsive to standard therapies.¹⁷⁴ Some 40 or so clinical trials have been conducted to date, with a patient base exceeding 2000.¹⁷⁵ There has also been a case study reporting on the effects of ultramicronized PEA in sporadic ALS, in which treatment led to an improved clinical picture, as evidenced by electromyographic analysis and pulmonary function.¹⁷⁶ A more detailed description of PEA clinical trials will be discussed elsewhere (Skaper S.D., submitted). Importantly, PEA has no adverse effects at pharmacologically relevant doses.

What is the molecular basis underlying the effects of PEA? As suggested in a number of studies, PEA may be a ligand for peroxisome proliferator activated receptor (PPAR) α , one of a group of nuclear receptor proteins that function as transcription factors regulating the expression of genes. PPAR α - and γ -isoforms in particular are associated with pro-inflammatory events. PEA actions (anti-inflammatory,¹⁷⁷ anti-nociceptive/anti-neuropathic^{160,166,178} and neuroprotective^{155,159,179}) were either absent in PPAR α null mice or blocked by PPAR α antagonists. An 'entourage effect' has also been hypothesized to explain the pharmacological actions of PEA, whereby PEA enhances the anti-inflammatory and anti-nociceptive activity of other endogenous compounds by potentiating their affinity for a

receptor or by inhibiting their metabolic degradation.¹⁸⁰ Anandamide is a candidate molecule, as it possesses anti-inflammatory and anti-nociceptive effects. Anandamide and its congeners like PEA have in common the transient receptor potential vanilloid type 1 (TRPV1) receptor. The TRPV1 receptor, a non-selective cation channel expressed in small diameter sensory neurones, is activated by noxious heat, low pH and capsaicin. Anandamide itself is a TRPV1 receptor agonist, and PEA enhances anandamide stimulation of the human TRPV1 receptor¹⁸¹ in a cannabinoid CB2 receptor antagonist-sensitive fashion (although PEA shows no appreciable affinity for either CB1 or CB2 receptors) - which could be interpreted as PEA acting indirectly by potentiating anandamide actions.¹⁶² Mast cells¹⁸² and microglia¹⁸³ reportedly express TRPV1 receptors.

Concluding remarks

Neuroinflammatory disorders are conditions where immune responses damage components of the nervous system (Karolinska Institute, 2013, www.ki.se). Inflammatory effectors derive from the innate and adaptive immune systems, as well as glia within the CNS. Microglia, in particular, act as sensors for disturbed brain tissue homeostasis and accumulate locally in response to neuronal cell injury or entry of foreign material in the brain.¹⁸⁴ Few studies until now have been directed to resident brain cell types capable of mounting immediate host responses in the brain and meninges. Mast cells are effector cells of the innate immune system, and represent the "first responders" to injury rather than microglia.⁵¹ Mast degranulation/mediator release is very rapid, while longer lasting activation elaborates *de novo* formed mediators. Mast cell degranulation does not result in cell demise; rather, mast cells are stable, multiple-use cells capable of surviving and delivering several consecutive hits.¹⁸⁵

Research to-date has largely focused on detrimental effects of neuroinflammation in association with psychiatric and neurodegenerative diseases, as well as neuropathic pain. Yet, we know little of glial and mast cell changes in human chronic pain - unequivocal demonstration that glial and mast cell activation occurs in hypersensitised patients remains an important gap. We lack also systematic studies which provide a correlation between the magnitude of glial and/or mast cell markers in cerebrospinal fluid or spinal tissue and the intensity of pain in patients.

Today's armamentarium to combat neuropathic pain (antidepressants, anticonvulsants, sodium channel blockers, glutamate receptor antagonists, opioids) treats the symptoms but not the underlying pathophysiology. Further, they provide at best transient relief in only a fraction of neuropathic pain patients and can produce serious CNS side effects. Agents like cromolyn, which stabilize mast cells, suppress development of hyperalgesia but do not affect microglia. Microglial inhibitors (e.g. minocycline) commonly utilized in pain models rely on their anti-inflammatory properties but may suffer from issues such as non-selectivity in targeting one cell population, as well as the risk of acute or cumulative toxicity. Incomplete understanding of mechanisms underlying the induction and maintenance of neuropathic pain has hindered more effective treatments, including elucidating the role of mast cells and how they might interact with microglia.

Clearly, much remains to be learned about signalling mechanisms that regulate neuroinflammation. One may envision the CNS neurone being subjected to 'assault' by a microglia-astrocyte-mast cell network, as a result of inadequate regulation of these non-neuronal cells because of excessive and/or persistent endogenous and/or endogenous stimuli, and/or an inadequate cellular inhibitory capacity (Fig. 4). Targeting endogenous regulators of

neuroinflammation may thus prove to be a viable strategy for affecting a diverse array of nervous system disorders. Within this context, the capacity of PEA to modulate the protective responses of animals during inflammation and pain led to the hypothesis that endogenous PEA may be a component of the complex homeostatic system controlling the basal threshold of both inflammation and pain. The production of PEA during inflammatory conditions supports this role, and emerging data that selective inhibition of PEA degradation is anti-inflammatory provides more direct evidence for the involvement of PEA in the control of pain and inflammation. It also leads one to ponder if we are not missing important therapeutic avenues by studying glia and mast cells in isolation from each other. Future studies should investigate the role of mast cells in inflammatory diseases as a network, which requires a critical examination of specific tissue localization, function, and dynamic interaction with endogenous cells.

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BOX 1

Mast cell ID chart

Origin and classification:

- First described by Paul Ehrlich in 1878 on the basis of their unique staining characteristics and large cytoplasmic granules
- Very close to basophil granulocytes in blood; current evidence suggests that mast cells are generated by different precursor cells in the bone marrow
- Thought to originate from bone marrow precursors expressing CD34; a distinct subset of mast cells can also be induced upon host responses to inflammation
- The hematopoietic lineage development of tissue mast cells is unique compared to other myeloid-derived cells because it is early lineage progenitors, undetectable by histochemistry, that leave the bone marrow to enter the circulation. These immature lineage mast cells immediately undergo transendothelial recruitment into peripheral tissues wherein the appearance of secretory granules with a particular protease phenotype is regulated by the peripheral tissue.
- Two types of mast cells are recognised, those from connective tissue and a distinct set of mucosal mast cells. The activities of the latter are dependent on T-cells
- Present in most tissues in the vicinity of blood vessels, and are especially prominent near the boundaries between the body's external environment and the internal milieu, such as the skin, mucosa of the lungs and digestive tract, as well as in the mouth, conjunctiva and nose
- Mast cells are also found within the nervous system, including meninges, brain parenchyma and nerve

Physiology:

- Play a key role in the inflammatory process
- Upon activation rapidly release granules into the interstitium
- Degranulation is caused by direct injury (e.g. physical or chemical), cross-linking of IgE receptors or by activated complement proteins
- Capable of elaborating a vast array of important cytokines and other inflammatory mediators
- Express multiple "pattern recognition receptors" thought to be involved in recognizing broad classes of pathogens
- Granules carry a variety of bioactive chemicals, proteoglycans, serine proteases, neuropeptides; can be transferred to adjacent cells of the immune system and neurones via transgranulation and their pseudopodia

Role in disease:

- Allergic reactions
- Anaphylactic shock
- Neuropathic pain
- Acute and chronic neurodegenerative disorders

Table 1. Mast cell mediators

- *Biogenic amines*
 - biogenic amines (histamine (2-5 pg/cell), serotonin)
- *Cytokines*
 - interleukins 1-6
 - leukemia inhibitory factor
 - tumour necrosis factor- α
 - interferon- γ
 - transforming growth factor- β
 - granulocyte-macrophage colony-stimulating factor
- *Enzymes*
 - acid hydrolases
 - chymase
 - phospholipases
 - rat mast-cell protease I and II
 - trypase
- *Lipid metabolites*
 - prostaglandin D2
 - leukotriene C4
 - platelet-activating factor
- *Other bioactive molecules*
 - neuropeptides (e.g. vasoactive intestinal peptide, substance P)
 - proteoglycans, mainly heparin (active as an anticoagulant)
 - nerve growth factor
 - ATP
 - nitric oxide

Table 2. Potential avenues of mast cell – glia communication

<i>Effector</i>	Biological actions		<i>Ref.</i>
	<i>Microglia/Astrocytes</i>	<i>Mast cells</i>	
TLR2, TLR4	Microglial release of IL-6 and CCL5 affects surface expression of TLR2/TLR4 on mast cells	Up-regulation of cytokine/chemokine release; CCL5/RANTES induces pro-inflammatory profile in microglia; recruitment of immune cells to site of injury	110-117
ATP/P2 receptors	ATP stimulates IL-33 release from microglia pre-activated with PAMPs via TLRs	IL-33 binds to its receptor on mast cells and induces secretion of IL-6, IL-13 and monocyte chemoattractant protein 1 which, in turn could modulate microglia activity	104,118,119
Proteinase-activated receptor 2 (PAR2)	Mast cell tryptase cleaves/activates PAR2 on microglia, resulting in P2X ₄ up-regulation and BDNF release	IL-6 and TNF- α from microglia can up-regulate mast cell expression of PAR2, which can result in mast cell activation and TNF- α release	106,107, 119,120
CXCR4/CXCL12	Promotes microglia migration and activation; CXCR4/CXCL12 are both up-regulated in hypoxia/ischemia	CXCR4 acts as mast cell chemotaxin	121-124
C5a receptor (C5aR)	C5aR up-regulated upon microglia activation; C5a peptide released in neuroinflammation; crosstalk between C5a and TLR4	C5aR up-regulated upon activation; a strong mast cell chemoattractant signal towards C5a peptide; crosstalk between C5a and TLR4	108,125,126
CD40/CD40L	Astrocytes display enhanced expression of CD40 - cross-talk with CD40L leads to production of inflammatory cytokines; astrocyte-derived cytokines/chemokines trigger mast cell degranulation	Display enhanced surface expression of CD40L respectively - cross-talk with CD40 leads to production of inflammatory cytokines	127,128

BDNF, brain-derived neurotrophic factor; IL, interleukin; PAMPs, pathogen-associate molecular patterns; PAR, proteinase-activated receptor; TLR, Toll-like receptor; TNF- α , tumour necrosis factor- α

[Modified from *The FASEB Journal*, 26(8), S.D. Skaper, P. Giusti, L. Facci, Microglia and mast cells: two tracks on the road to neuroinflammation, 3103-3117 (Table 1), Copyright

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Table 3. Changes in palmitoylethanolamide levels during neuroinflammation

Disease; tissue or body fluid	Change	Main finding	Ref.
Chronic relapsing experimental allergic encephalomyelitis; spinal cord	↑	~2-fold increase	148
Experimental acute stroke; striatal and cortical infarcted hemisphere	↑	~ 25-fold increase compared to controlateral (non-infarcted) areas	149
Experimental focal cerebral ischemia; ischemic cerebral cortex	↑	25-fold increase compared with sham-operated animals, at 24 hrs post-focal cerebral ischemia	143
Cerebral ischemia in man; penumbral tissue surrounding the primary ischemic lesion (microdialysis study)	↑	Significantly increased levels within the first day after ischemia	150
Chronic migraine or probable chronic migraine and probable analgesic-overuse headache; cerebrospinal fluid	↑	Significantly higher levels in the two patient groups (without significant difference between them) compared to control subjects	151
Chronic widespread pain and chronic neck-shoulder pain in women; microdialysis dialysate of the trapezius	↑	Significantly higher levels compared to healthy subjects, and correlation with pain intensity	147

Table 4. Preclinical studies showing anti-neuroinflammatory and/or neuroprotective effects of palmitoylethanolamide

Model	Action	Ref.
Compression model of spinal cord trauma in mice	Reduces spinal inflammation/tissue injury, ameliorates recovery of motor limb function Limits mast cell infiltration and activation; reduces activation of microglia and astrocytes	152,153
Traumatic brain injury in mice	Reduces edema and infarct size Improves neurobehavioural functions	154
MPTP mouse model of Parkinson disease	Protects against MPTP-induced neurotoxicity, microglial and astrocyte activation, and functional deficits	155
Stroke (middle cerebral artery occlusion in rats)	Reduces edema and infarct size Improves neurobehavioural functions	156
β -Amyloid peptide injection in rat brain	Counteracts reactive gliosis Reduces behaviour impairments	157
Chronic constriction injury in sciatic nerve	Anti-allodynic and anti-hyperalgesic effects Reduces mast cell activation Preservation of nerve structural integrity	158-160
Acute inflammation (formalin, dextran, carrageenan injection in rat hindpaw)	Reduces mast cell activation, tissue edema, inflammatory/mechanical hyperalgesia	161-166
WAG/Rij rat model of absence epilepsy	Anti-epileptic action	167

MPTP, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine

Figure Legends

Fig. 1. Electron microscopic image of an isolated tissue mast cell. Note the prominent appearance of numerous cytoplasmic granules.

Fig. 2. Palmitoylethanolamide synthesis and catabolism. A plasma membrane-associated N-acylated phosphatidylethanolamine-phospholipase D (PLD) converts N-palmitoyl-

phosphatidyl-ethanolamine (N-APE) into palmitoylethanolamide and phosphatidic acid. Palmitoylethanolamide is metabolized to palmitic acid and ethanolamine by both fatty acid amide hydrolase (FAAH, which also breaks down other fatty acid amides) and the more selective N-acyl ethanolamine-hydrolyzing acid amidase (NAAA). Tissue levels of palmitoylethanolamide increase in stressful settings such as peripheral tissue inflammation, neuroinflammation, and pain. See text for further details.

Fig. 3. Palmitoylethanolamide (PEA) treatment reduces activation of astrocytes induced by 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) injection. Western blot analysis showed a significant expression of glial fibrillary acidic protein (GFAP) in the substantia nigra. PEA treatment significantly reduced the activation of astrocytes after MPTP injection. The relative expression of the protein band (~47 kDa) was standardized for densitometric analysis to β -actin levels, and reported as mean \pm s.e.m. from $n=5/6$ brains for each group. *** $p<0.001$ versus sham, ### $p<0.001$ versus MPTP + vehicle. [Modified from PLoS One 7(8):e41880. doi: 10.1371/journal.pone.0041880), E. Esposito, D. Impellizzeri, E. Mazzon, I. Paterniti, S. Cuzzocrea, Neuroprotective activities of palmitoylethanolamide in an animal model of Parkinson's disease. (Figure 10). (Ref. 155) © Esposito et al. This is an open-access article distributed under the terms of the Creative Commons Attribution License].

Fig. 4. Neuroinflammation may result from CNS neurones 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. Bidirectional communication may take place between microglia, mast cells and astrocytes, which can act to reinforce the deleterious signals acting on the neuron.

Fig. 1

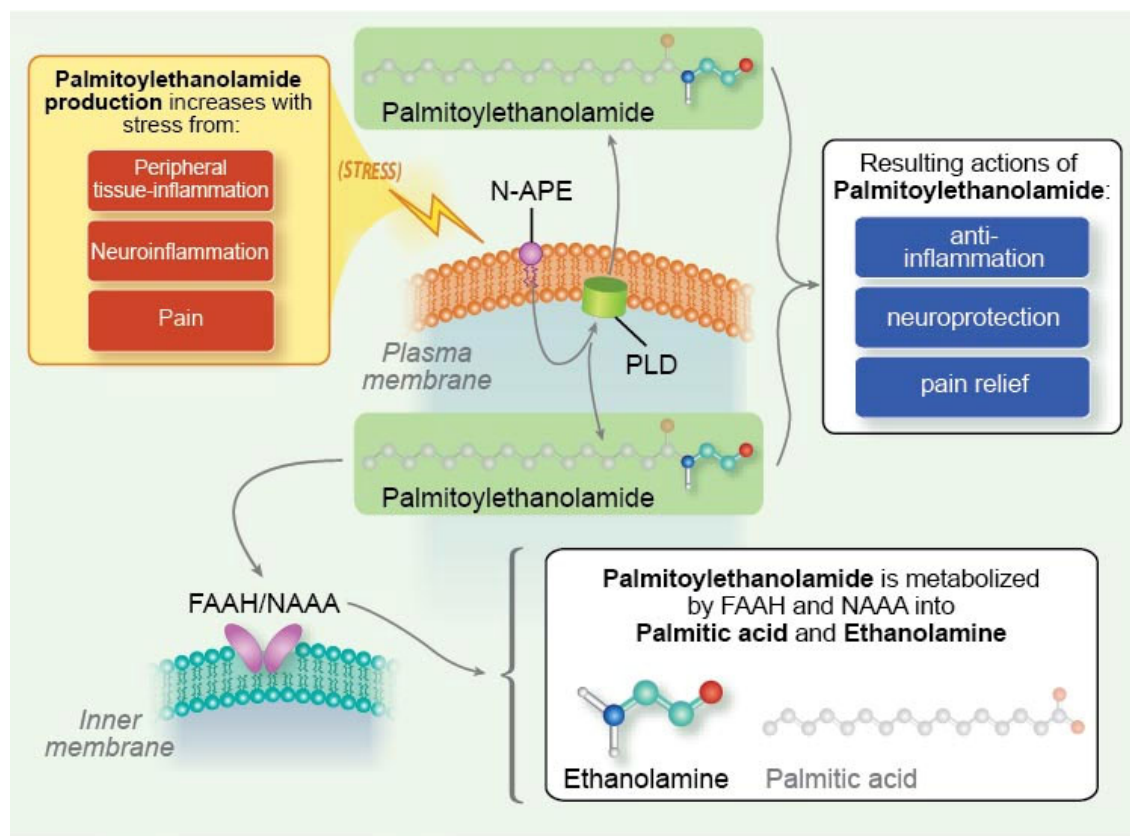
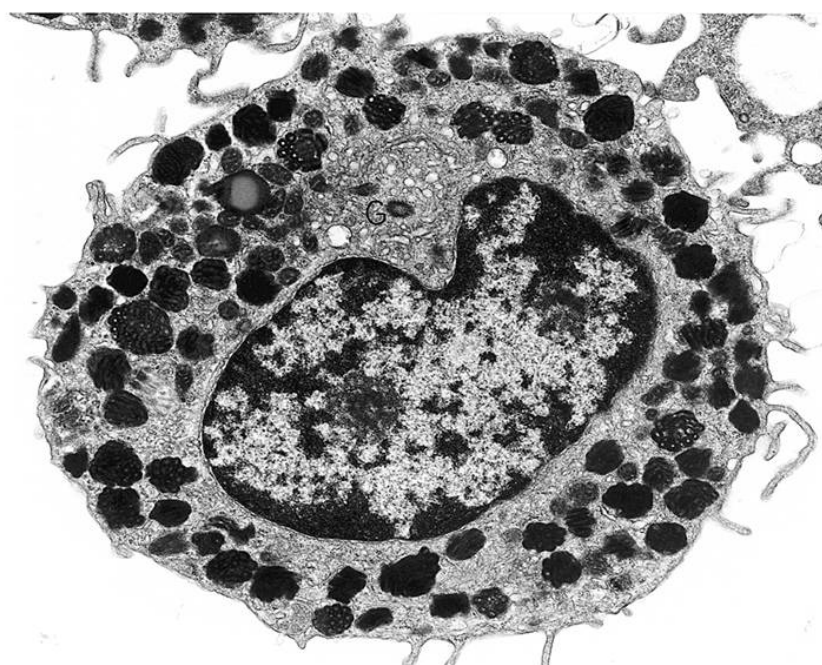


Fig. 3

