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Microglia dynamics and function in the CNS

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Summary of Recent Advances

Microglial cells constitute the resident immune cell population of the mammalian central nervous system. One striking feature of these cells is their highly dynamic nature under both normal and pathological brain conditions. The highly-branched processes of resting microglia display a constitutive mobility and undergo rapid directional movement towards sites of acute tissue disruption. Microglia can be converted by a large number of different stimuli to a chronically activated state by signaling through both purinergic and Toll-like receptor systems, among others. Recent work has uncovered some of the mechanisms underlying microglia dynamics and shed new light into the functional significance of this enigmatic member of the glial cell family.

Introduction

The mammalian central nervous system (CNS) is comprised of a number of cell types including both neurons and glial cells. Of the several types of glia present in the CNS, microglia represent a unique population with respect to both their developmental origins and their function. Like other myeloid populations, and distinct from all other CNS cells, which are derived from the neuroectoderm, microglia are a mesodermally derived population of cells. It has been shown that the precursors of adult microglia are bone marrow-derived cells that migrate to the CNS during embryonic development [1–2]. Perhaps owing to this origin, microglia share many commonalities with resident tissue macrophages in peripheral organ systems [••3].

In both mice and humans, microglia can be found postnatally in all regions of the CNS in a non-overlapping territorial fashion (fig 1), and comprise a large proportion of the total cellular makeup of the CNS- estimated to be as high as 12% [4]. Like their peripheral macrophage counterparts, microglia display a remarkable range in both morphology and activity, depending in part on the state of the surrounding tissue [3,5]. In this review we focus on research within the past two years regarding the dynamic nature and function of microglia. In the first section, we examine work on acute changes- the baseline motion of microglial processes, and their rapid response to CNS injury. In the second, we highlight research investigating the molecular mechanisms by which microglia become chronically activated, and discuss the functional implications of such activation in the CNS.

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Baseline microglial dynamics and rapid process extension

Recent findings have revealed that under physiological CNS conditions, microglia display a constant motility of their highly branched cellular processes within the intact mouse cerebral cortex and brain slices [6–8]. Similar observations have been made regarding microglial cells in both zebrafish [9] and leech [10]. *In vivo* studies on microglia have identified soluble ATP as well as connexin hemichannels to be important for baseline microglial motion [6]. More recently, the fractalkine receptor CX₃CR1 has been found to regulate the dynamics of retinal microglia processes as explants from animals null for this receptor demonstrate reduced basal motility [11]. In brain slices, the baseline motility of microglial processes is neither dependent on, nor altered by neuronal activity, including hippocampal LTP [12]. While the functional significance of this baseline motility remains unknown, experiments in mice have shown that microglial processes contact and pause on active neuronal synapses *in vivo*, suggesting a possible role of microglia motility in synaptic remodeling and/or function [13]. A recent electron microscopic study of brain microvasculature has found that the sole disruption in the continuous perivascular astroglial sheath surrounding the endothelium is contributed by the processes of microglia [14]. Thus, baseline microglial dynamics may also be involved in sensing and maintaining the integrity of the blood brain barrier.

Microglia processes are capable of rapid extension (~1.25 $\mu\text{m}/\text{min}$) towards sites of acute CNS damage [6,8]. Rapid process extension is dependent upon extracellular ATP sensed through microglial P2Y₁₂ receptors [6,15] and an associated outward potassium current [16]. Downstream intracellular signaling through PI3 kinase [17] and Akt phosphorylation [18], as well as integrin-beta-1 activation [19] has also been shown to be involved in P2Y₁₂ mediated chemoattraction. Recent work in both the mouse spinal cord [20] and the leech nerve cord [21] has demonstrated that nitric oxide (NO) is an additional guidance cue involved in directed movement of microglia. Furthermore, glutamate has been found to attract microglia in mouse spinal cord slices through an ATP-independent mechanism [22]. In a retinal injury model, the CX₃CR1 receptor has been implicated in the rapid response to injury as mice lacking this gene show reduced rates of process migration [11]. Interestingly, microglia activated by the bacterial product lipopolysaccharide (LPS) have been shown to retract their processes in response to ATP sensed through the adenosine 2A receptor [23]. This finding may explain in part a switch between ramified and amoeboid microglial morphology observed in some inflammatory states. Thus, studies in the past few years indicate that a variety of extrinsic and intrinsic factors are involved in rapid microglial process extension in response to injury. The rapid convergence of microglia processes at the site of injury suggests that microglia may provide a physical barrier to protect healthy tissue. In support of this idea, work using focal laser-damage in brain slice cultures showed that this action was able to contain the spread of damage to neighboring neuronal tissue [24].

Chronic microglial activation

In addition to the acute activities of microglia, these cells also demonstrate progressive changes often termed "activation." Depending on the nature of the initiating stimulus, these changes may include altered expression of cell surface markers and inflammation related genes, increased phagocytic ability, process retraction and acquisition of an amoeboid morphology, migration of cell bodies through CNS tissue, and/or increased proliferative rates [5]. Interestingly and perhaps not surprisingly, many of these alterations can be observed in monocytes and macrophages of the peripheral immune system during different inflammatory states.

Time course of chronic microglial activation

In contrast to the rapid pace of microglial process movement which takes place on a scale of seconds to minutes, the time course of chronic microglial activation is more prolonged. Although variable, these changes often take place over hours to weeks. For example, measurable changes in gene expression occur within six hours as evidenced by increased TLR2 mRNA levels in response to systemic LPS challenge [25]. Migration of microglial cell bodies toward damaged tissue begins as early as 3 hours post-injury in neonatal brain slices [26]. Significant accumulation of migrating microglia can be seen in hypoxic striatal tissue within 24 hours [27]. Proliferation of microglia has been observed two days after induction of non-invasive neural degeneration in mouse thalamus [28], or three days after sciatic nerve ligation in the spinal cord [29]. Microglial proliferation within the subventricular zone of hypoxic mouse brain peaks at two weeks after insult [30]. The extended and divergent time course of microglial activation suggests that the activation process is regulated by complex mechanisms that may differ significantly depending on the initiating stimulus.

Mechanisms and effects of activation

The switch from a ramified state to one of activation is triggered by a diverse array of stimuli, sensed by a large assortment of receptors. The downstream output, and overall effects of microglial activation are similarly complex [3]. Because the vast number of studies on these topics prohibits a complete discussion of all work here, in this review we will limit our discussion to a select group of well-characterized signaling pathways and molecules that demonstrate clear involvement in chronic microglial activation.

Purinoreceptors—Purines are released into the extracellular space constantly by neurotransmission, as well as from damaged or dying cells. Many lines of evidence have indicated that purinergic receptors of the P1, P2X, and P2Y receptor families are associated with chronic microglial activation. Signaling through the P1 adenosine receptor A2A drives proliferation of spinal microglia after nerve injury [31], and intrathecal blockade of this receptor abolishes neuropathic pain in the same model [32]. The P2X7 receptor (P2X7R) can drive morphological microglial activation and proliferation in the absence of other stimuli when over-expressed *in vitro* [33]. It appears that activation of this receptor decreases microglial potential for glutamate transport which may be important for excitotoxic injury [34]. Blockade of P2X7R impairs microglial release of the major proinflammatory cytokine IL-1 β in response to LPS [35] or amyloid- β (A β) [36]. Inhibition of microglial activation with the P2X7R antagonist Brilliant Blue G leads to improved clinical outcome in a model of spinal cord injury [37]. Furthermore, increased expression of P2Y12R has been reported in spinal microglia following peripheral nerve injury, and pharmacological P2Y12R blockade reverses tactile allodynia in lesioned mice [38–39]. In addition, mRNA levels of, and ion conductance through both P2X7 and P2Y12 are increased in microglia after experimentally induced status epilepticus [40]. A similar transcriptional up-regulation of these receptors was found in microglia from mice carrying a human mutant form of superoxide dismutase 1 (mSOD1), leading to increased production of tumor necrosis factor alpha (TNF- α) and NO, and increased toxicity toward cultured primary neurons [41]. Interestingly the expression levels of multiple microglial P2 receptors vary with age in mice, which may have implications for aging-related functional changes in these cells [42].

Toll-like receptors—Toll-like receptors (TLRs) are expressed by a variety of cells of the innate immune system and are part of a larger family of so-called pattern recognition receptors that are able to ligate and sense danger signals in the form of pathogen associated molecular patterns [43]. These ligands are typically the products of invading bacteria, fungi,

and viruses. In the periphery, activation of TLRs leads to robust inflammatory responses that are important for host defense, but are also implicated in autoimmune and chronic inflammatory diseases [43]. Of the TLRs thus far characterized in mice, TLRs 1–9 are expressed in microglia. Interestingly, although microglial TLRs recognize their canonical ligands—such as LPS from CNS-invading *Citrobacter koseri* by TLR4 [44], they have also been shown to bind unique CNS-derived signals. For example, TLR2 expressed by microglia is activated by the Alzheimer's Disease (AD) related protein amyloid- β (A β) [45–46], as well as by ischemic injury [47]. A unique heterodimer composed of TLRs 4 and 6 along with its co-receptor CD36 has also recently been implicated in A β -induced, microglia-mediated CNS inflammation [48]. Microglial TLRs 2 and 4 have been implicated in the sensing of long-chain fatty acids and subsequent inflammation in the hypothalamus. Genetic or pharmacological blockade of these receptors leads to changes in feeding behaviors and a decrease in pro-inflammatory cytokine production within this brain region—a finding which may have relevance for diet induced obesity [49]. TLR signaling may also play a role in neurotoxicity induced by cell injury or death. HSP60 protein released from dying neurons activates microglial TLR4 signaling, leading to increased nitric oxide (NO) production, and further neuron death [50]. TLR4 ligation has even been implicated in ethanol induced activation of microglia and subsequent production of neurotoxic substances [51].

TNF- α signaling—TNF- α is the archetypal pro-inflammatory cytokine. Its release by cells of the peripheral immune system is not only required for host defense but also associated with deleterious effects to the surrounding tissues [52]. TNF- α secreted by microglia may also play a similarly divergent role. For example, microglial TNF- α inhibits the growth and division of oligodendrocyte precursor cells in culture [53]—a finding which may prove relevant for demyelinating conditions such as multiple sclerosis. TNF- α has also been implicated in synaptic alterations and degeneration during experimental autoimmune encephalomyelitis (EAE) [54]. In contrast to the potentially toxic effects of microglial-derived TNF- α , several recent studies have shown its release to be protective. TNF- α signaling initiated by microglial release is protective for neurons after induction of focal cerebral ischemia [55]. Additionally, TNF- α may be neuroprotective against A β induced toxicity as TNF- α pre-conditioning reduced neuronal apoptosis in response to intracerebral A β injection [56].

Together, the aforementioned studies indicate that signaling through purinergic and toll-like receptor systems plays an important role in chronic microglial activation with significant functional consequences. Furthermore, TNF- α released from chronically activated microglia may play both protective and toxic roles within the CNS, possibly depending on the context in which it is released.

Conclusions

Microglia are a numerous and highly dynamic population of CNS cells with multiple potential functions in both the normal and perturbed brain (Fig. 2). The initial reports describing the rapid and constitutive motion of microglial processes have raised the possibilities that microglia monitor the brain's microenvironment for damage or danger signals, sequester neurotransmitters or other soluble factors, sense the state of neuronal synapses, or possibly even function in antigen capture reminiscent of peripheral dendritic cells. However caution is warranted considering that these interpretations have limited or no experimental support. At this time the function of the baseline motility of microglial processes, or their rapid extension towards sites of CNS damage remains unclear.

Regarding the function of activated microglia in pathological brain states, many inroads have recently been made into understanding both the mechanisms by which microglial

activation occurs, and the effects of this activation on the CNS. The outcomes of chronic microglial activation are often sorted into the categories of "beneficial" or "deleterious" with regard to the surrounding CNS tissue. However, it should be kept in mind that the outcome of microglial activation is complex and likely context dependent. Thus, a given facet of microglial activation may prove to be required for one aspect of CNS function, while simultaneously proving detrimental to another. While much progress has been made in understanding microglia activation and its effects, the continued development of tools for the study of microglia in the intact CNS will be of great importance in unraveling their complex and multiple functions.

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Figure 1. Resting microglia in the mouse cerebral cortex. Microglia are labeled by EGFP expressed from the CX₃CR1 promoter. Note the highly ramified appearance of individual microglial cells, as well as the "tiled" or non-overlapping processes of microglia in the cortex. Scale bar= 100 μ m. Figure courtesy of Dr. Hui-tai Xu.

Figure 2.

The multiple activities of microglia. Microglia exist in a highly ramified state within normal brain tissue (center). (a) The processes of microglia display constitutive motility which is dependent on ATP signaling. The functional significance of this baseline motility remains unclear. (b) Microglial processes are rapidly recruited to sites of CNS tissue damage. The signals responsible for this recruitment include ATP and NO, etc. (c) Signaling through many pathways including those utilizing purinergic (P1, P2X, and P2Y) and toll-like receptors (TLRs) leads to a state of chronic microglial activation. As a result, microglia may release soluble factors that act in a trophic, protective, or anti-inflammatory manner on surrounding CNS cells.