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Microglia-Müller Cell Interactions in the Retina

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Abstract

Microglia and Müller cells are cell types that feature prominently in responses to disease and injury in the retina. However, their mutual interactions have not been investigated in detail. Here, we review evidence that indicate that these two cell populations exchange functionally significant signals under uninjured conditions and during retinal inflammation. Under normal conditions, Müller cells constitute a potential source of extracellular ATP that mediates the activity-dependent regulation of microglial dynamic process motility. Following microglial activation in inflammation, microglia can signal to Müller cells, influencing their morphological, molecular, and functional responses. Microglia-Müller cell interactions appear to be a mode of bi-directional communications that help shape the overall injury response in the retina.

Keywords

Müller cells; microglia; inflammation; retina; gliosis

42.1 Introduction

Glia populations in the central nervous system (CNS) consist primarily of microglia, the main resident immune cells, and macroglia, which include astrocytes and oligodendrocytes. These non-neuronal cell populations are intimately integrated into healthy neuronal function, play important homeostatic roles in maintaining the CNS *milieu*, and participate prominently in tissue responses to diseases, inflammation and injury [1–4].

In the retina, microglia and macroglia are similarly represented. Retinal microglia are found distributed throughout the inner retina in a laminated fashion [5], and are involved in retinal responses to injury and disease [6]. Retinal macroglia, consisting of astrocytes and Müller cells, play key roles in supporting neuronal functions [7–10], and demonstrate gliotic changes in response to pathological insults [11]. However, how these two retinal cell populations interact and collaborate with each other is incompletely understood. As microglial and Müller cells responses to disease and injury have been ascribed both adaptive and maladaptive aspects, it is of interest to determine if and how microglia- Müller cell interactions help shape the features of the overall retinal injury response [6, 7, 12].

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42.2 Microglia-Müller Cell Interactions in the Healthy Retina

Live-cell imaging studies have revealed that “resting” microglial processes are highly dynamic and can occupy the surrounding extracellular milieu through these constant movements [13–17]. This “resting” phenotype has been linked to key constitutive microglial functions such as cleaning up apoptotic cells and cellular debris [18, 19], pruning excess or dysfunctional synapses [20, 21], providing trophic factors [22–24], and regulating synaptic functions and plasticity [25–27]. Recent studies have shown that microglia dynamically remodel synapses by engulfing pre-synaptic synapses in an activity-dependent manner [27]. Resting microglia can preferentially contact active neurons and down-regulate their activity, maintaining stability in overall activity levels [28]. These findings reveal that microglial process behavior and morphology are likely regulated by activity-dependent signaling involving neurons and macroglia.

As neurotransmission is the prominent mode of communication occurring between neurons and glia, it is a candidate factor for regulating microglial behavior. In live imaging experiments of *ex vivo* retinal explants, we found that excitatory glutamatergic neurotransmission occurring via AMPA and kainate channels exerted a positive effect on microglial morphology and process motility [15]. Blockade of glutamatergic neurotransmission using the antagonists, NBQX and GYKI, resulted in decreased microglial dendritic arbor size and decreased process motility, while application of glutamatergic agonists AMPA and kainate exerted opposite effects. Conversely, inhibitory GABAergic neurotransmission occurring via $GABA_A$ channels was found to negatively regulate microglial morphology and process motility; bicuculline blockade of $GABA_A$ receptors increased process motility while application of GABA decreased it.

Interestingly, these effects do not appear to be mediated by direct reception of glutamatergic or GABAergic signaling on microglial cells. We were unable to colocalize ionotropic glutamate receptors GluR2/3 on microglia using immunohistochemical studies. Electrophysiological studies demonstrate that microglia do not directly respond to the application of glutamatergic or GABAergic agonists, but respond only to application of ATP, which is likely mediated through P2 receptors expressed on microglia [15, 28]. Current evidence indicates that while microglial process motility is sensitive to overall levels of neuronal activity, which is determined by a balance between excitatory and inhibitory forms of neurotransmission, it is the activity-dependent release of extracellular ATP that constitutes the direct signal to microglia regulating their dynamic behavior [28].

The precise cellular source of ATP in the retina relevant to microglial regulation has not been definitively established, but Müller cells, a prominent source of extracellular ATP, are likely involved. Extracellular glutamate can induce Müller cells to release ATP [29] via several pathways including vesicle exocytosis [30], connexin hemichannels [31], and pannexin channels [32, 33]. In the retina, we found that probenecid, an inhibitor of pannexin channels, decreased microglial morphology and process motility, and was not rescued by the application of extracellular AMPA [15]. Taken together, these data reveal that ongoing excitatory and inhibitory neurotransmissions determine overall activity levels in the retina, which likely modulate ATP release from Müller cells via pannexin channels, thus

influencing “resting” microglial behavior. This scenario posits that in the uninjured healthy retina, Müller cell-microglia communication is a constitutive ongoing phenomenon - Müller cell signals inform retinal microglia on ongoing levels of neuronal activity, which are then integrated to drive a behavioral response in microglia that is commensurate with their functions of activity-regulation, synapse modification, and trophic factor production.

42.3 Microglia-Müller Cell Interactions in Retinal Inflammation Help Shape the Overall Injury Response

Upon the onset of inflammation, injury, or disease, microglia react rapidly by transitioning to an activated status within minutes [13, 14, 16], initiating the first steps of the inflammatory response that precede macroglial responses [34–36]. Like astrocytes in brain, Müller cells demonstrate activation and reactive gliosis under pathological conditions. Typical features of Müller cell gliosis involve cellular hypertrophy, up-regulation of intermediate filament expression (such as GFAP and vimentin), increased rates of proliferation, and down-regulation of glutamine synthetase (GS) expression [8]. We wanted to investigate whether signals from activated microglia in the acute aftermath of injury influence Müller cells in the overall injury response.

We explored the cellular signaling between microglia and Müller cells by using a simple *in vitro* co-culture system in which Müller cells were cultured alone, or co-cultured with microglia with or without lipopolysaccharide (LPS) pre-treatment. We found that Müller cells which were either cultured alone or co-cultured with unactivated microglia demonstrated a symmetrical, flat cell shape with prominent lamellipodia. However, those co-cultured with activated microglia transitioned to elongated, spindle/multipolar shapes, which was confirmed by quantitative image analysis [37]. These cells also interestingly decreased their mRNA expression of gliosis markers, such as glutamate aspartate transporter (GLAST) and vimentin. Their proliferation was also significantly decreased, without any increase in cellular apoptosis. In addition, Müller cells co-cultured with activated microglia expressed higher mRNA and protein levels of trophic factors, such as GDNF and LIF. Indeed, the conditioned media from Müller cells which had been previously co-cultured with activated microglia demonstrated greater neuroprotective effects in an *in vitro* assay of oxidatively-stressed 661W photoreceptors. These results indicate that Müller cells are highly responsive to the activation state of microglia, and that activated microglia can acutely induce Müller cell responses that are associated with adaptive and neuroprotective effects and do not involve expression of typical markers of gliosis.

In further experiments, we found that Müller cells co-cultured with activated microglia significantly increased their mRNA and protein expression of pro-inflammatory factors such as IL1β, IL6, and iNOS [37]. Nitrite production was increased, consistent with increased iNOS expression. When Müller cell conditioned media following activated microglia co-culture was collected and added to fresh, non-activated microglia, they were capable of inducing microglia activation as evidenced by increased microglial proliferation and pro-inflammatory cytokine production. These results suggest in response to situations of inflammation or injury, Müller cells and microglia can conduct mutual and reciprocal signaling that amplifies local inflammation in a positive feedback cycle.

We further examined whether physical interactions between microglia and Müller cells were altered in microglia-Müller cell signaling. We found that Müller cells co-cultured with activated microglia increased mRNA expression of adhesion molecules (VCAM-1 and ICAM-1). Müller cells exposed to activated microglia were also capable of retaining the largest number of microglia to their surfaces in a cell adhesion assay. Additionally, activated microglia induced Müller cells to express higher mRNA and protein levels of chemotactic cytokines (CCL2, CCL3), and the conditioned media from Müller cells exposed to activated microglia induced higher levels of microglial chemotaxis relative to controls. These results suggest that under the influence of activated microglia, Müller cells undergo changes related to their expression of cell-adhesion and chemotactic molecules, which serve to present a more conducive surface for microglial adhesion and migration. These features were recapitulated *in vivo* when retinal microglia activated by intravitreal injection of LPS demonstrated changes in physical contacts with Müller cells. Microglia in the unactivated, "resting" state have horizontally oriented processes that interdigitate with the orthogonally-oriented, radial Müller cell processes. Acutely following LPS injection, microglia transitioned to a more vertically-orientation where their processes fasciculated closely in an adherent manner with Müller cell processes which may serve as a scaffold for the radial migration of microglia.

Taken together, these data demonstrated the ability of microglia to induce changes in Müller cells as a function of their activation. Bi-directional microglia-Müller cell signaling appears to help shape an acute injury response that is characterized by (1) an amplification of activation, (2) adaptive neuroprotection, and (3) increased physical interaction between the two cell types that may help mobilize migratory microglia within the retina.

42.4 Perspectives

Given that reciprocal microglia- Müller cell interactions can shape acute retinal responses to injury and disease, future studies will examine their role in long-term responses and in chronic inflammation. The transition between acute, adaptive responses to chronic, maladaptive gliosis may be a result altered interactions changes in this particular locus. Discovery of relevant molecular signals will be instructive in understanding these multifaceted interactions. Microglia- Müller cell signaling may constitute a target for therapeutic interventions that can direct overall retinal injury responses towards beneficial, and away from detrimental, ends.

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