HIGHLIGHTS

Neuroinflammation in glaucoma: soluble tumor necrosis factor alpha and the connection with excitotoxic damage

Inflammation is a complex and highly regulated response that occurs early after infection or injury. This process is initiated by cells of the immune system to re-establish tissue homeostasis. When the injury is persistent, however, chronic inflammation leads to overproduction of noxious mediators that contribute to cell dysfunction and death. The inflammatory response in the central nervous system (CNS), known as neuroinflammation, is achieved by activation of resident glia and monocyte-derived cells. Accumulating evidence indicates that this cellular response occurs in the early stages of numerous neurodegenerative diseases, triggering a cascade of events that converge to promote neuronal damage. Indeed, neuroinflammation has been reported in a host of CNS disorders including Alzheimer’s disease, Parkinson’s disease, amyotrophic lateral sclerosis, Huntington’s disease, multiple sclerosis, stroke, and glaucoma.

Glaucoma is a prevalent neurodegenerative disease and the leading cause of irreversible blindness worldwide affecting over 60 million people. Glaucoma is characterized by the progressive degeneration of retinal ganglion cells (RGC) and their axons in the optic nerve resulting in gradual vision loss. High intraocular pressure is the most significant known risk factor for developing the disease, but the mechanism by which elevated pressure promotes RGC damage is currently unknown. Current therapies are aimed at lowering intraocular pressure, but many patients continue to experience visual field loss even when pressure lowering treatments are implemented. A better understanding of the mechanisms causing glaucomatous neurodegeneration triggered by ocular hypertension injury is, therefore, essential to develop effective therapies.

Accumulating evidence indicates that neuroinflammation plays a key role in RGC damage in glaucoma. A number of studies have confirmed the presence of hallmark features of neuroinflammation in glaucoma animal models and human specimens including glial cell activation, upregulation of proinflammatory cytokines, induction of the complement cascade, and trans-endothelial cell migration of leukocytes (Soto and Howell, 2014). A critical modulator of the neuroinflammatory response in glaucoma is tumor necrosis factor alpha (TNFa). RGCs express the TNFa receptors 1 and 2 (TNFR1/2) and TNFa signaling has been linked to RGC death. For example, exogenous administration of TNFa promotes RGC loss and optic nerve degeneration, and genetic or pharmacological depletion of TNFa or its receptors stimulates RGC survival (Tezel et al., 2008). High-throughput characterization of the retinal proteome revealed significant upregulation of TNFa signaling in human glaucoma (Yang et al., 2011). TNFa levels have been shown to be elevated in aqueous humor samples from glaucoma patients (Sawada et al., 2010; Balaiya et al., 2011; Xin et al., 2013). Notably, TNFa gene polymorphisms are associated with primary open angle glaucoma (Fan et al., 2010; Bozkurt et al., 2012; Xin et al., 2013). A recent meta-analysis study (> 3,000 cases) showed that the TNFa 308G/A polymorphism is significantly linked with higher risk of developing primary open angle glaucoma, predominantly in the Asian population, but not with low tension or exfoliation glaucoma (Xin et al., 2013).

What is the source of TNFa in glaucoma? Chronically reactive glial cells are thought to become a sustained source of proinflammatory cytokines in the CNS. Traditionally, microglia are thought to be the primary source of TNFa after injury or in disease. Using a well-characterized rat model of ocular hypertension glaucoma (Morrison et al., 2015), our team recently demonstrated that high intraocular pressure stimulates production of TNFa by retinal glia (Cueva Vargas et al., 2015). Intriguingly, our results show that Müller cells, the most abundant glial cell type in the retina, rapidly upregulate TNFa in response to increased eye pressure. Müller cells are specialized radial glia that play critical structural, metabolic and support roles for retinal neurons. Consistent with their role as a source of TNFa, Müller cells exposed to selective blockers of the neurotrophin receptor p75NTR, an upstream activator of TNFa production in these cells, promoted RGC survival in models of traumatic axonal injury and excitotoxic damage (Lebrun-Julien et al., 2009a, b). In addition, we observed increased TNFa expression in retinal microglia with amoeboid shape, characteristic of a reactive state, rather than in quiescent cells with ramified morphology (Cueva Vargas et al., 2015). This finding is consistent with previous reports showing TNFa expression in microglia from human glaucomatous optic nerve head and rat retinas subjected to ocular hypertension (Roh et al., 2012). Of interest, high-dose irradiation leading to reduced microglial activation, and presumably decreased levels of proinflammatory mediators, attenuated RGC degeneration in a mouse model of inherited pigmented glaucoma (Howell et al., 2012). Collectively, these data suggest that both Müller cells and microglia respond rapidly to ocular hypertension by increasing TNFa production.

TNFa plays both homeostatic and pathophysiological roles in the CNS. TNFa is generated as a membrane-bound precursor that is cleaved by the cell surface protease TNFa-converting enzyme (TACE/ADAM17) to release the soluble 17-kDa protein. Both the transmembrane and secreted forms of TNFa are biologically active and play distinct roles in vivo. Soluble TNFa binds primarily to TNFR1 and regulates apoptosis and chronic inflammation, whereas membrane-bound TNFa displays a higher affinity for TNFR2 and mediates immunity against pathogens, resolution of inflammation and promotes myelination. Consistent with this, mice expressing only transmembrane TNFa suppress the onset and progression of autoimmune demyelination while maintaining host defenses against bacterial infection, septic shock and pulmonary fibrosis. Therefore, modulation of soluble versus transmembrane TNFa signaling might be a powerful strategy to achieve homeostasis in diseases with a neuroinflammatory component.

Which form of TNFa, soluble or transmembrane, is responsible for RGC death in glaucoma? To investigate this, we used an engineered dominant negative peptide, called XPro1595, that selectively inhibits soluble TNFa without interfering with transmembrane TNFa signalling (Zalevsky et al., 2007). XPro1595 binds only to soluble TNFa monomers and forms
in active heterotrimeric complexes. Our results demonstrate the key role of TNFα in the activation of RGCs, leading to RGC death in a TNFα-dependent manner. Using a cobalt (Co2+) permeability assay, we have shown that TNFα-exposed RGCs have increased Ca2+ permeability through AMPAR. This increased Ca2+ influx can lead to excitotoxicity, contributing to RGC death. Our findings suggest that targeting TNFα may be a potential therapeutic strategy for glaucoma.
to excitotoxic damage, can act on microglial metabotropic glutamate receptors through an autocrine loop to stimulate more TNFα synthesis. Collectively, these findings reveal a complex mode of action of TNFα: a direct effect on neurons to shift the balance between excitatory and inhibitory synaptic receptors, and an indirect effect on glial cells to regulate their ability to buffer glutamate and produce TNFα.

In conclusion, while endogenous TNFs play critical physiological roles in retinal homeostasis and neurotransmission, excess soluble TNFα results in CP-AMPAR upregulation, Ca²⁺ overload and neuronal death in glaucoma. These findings suggest that modulation of soluble TNFα signaling might be beneficial to counter the harmful effect of neuroinflammation and synaptic alterations in glaucomatous optic neuropathies.

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References


