PERSPECTIVE

Complement pathway in Alzheimer's pathology and retinal neurodegenerative disorders – the road ahead

Chronic inflammation has increasingly been acknowledged as a hallmark feature of several progressive neurodegenerative disorders. Accruing evidence indicates that sustained inflammation compromises the core neuroprotective mechanisms underlying neural injury in Alzheimer's disease (AD) and retinal neurodegenerative disorders. Innate immunity and activation of the classical complement pathways are suggested to play important roles in normal central nervous system physiology and complex tissue remodeling during the disease process (Gasque et al., 2000). The pathway is implicated in normal brain development and is also involved in the inflammatory response in a wide range of neurodegenerative conditions either directly or indirectly through recruitment and activation of immune cells. The classical, alternative and lectin complement pathways, together encompass about 30 plasma and membrane-bound proteins. The classical pathway is comprised of about 20 proteins including several serine proteinases and proteinase inhibitors connected as part of an amplifying cascade. Activation of the classical pathway is triggered once C1q is attached to the immune complexes containing IgG or IgM leading to production of the C3 convertase (Merle et al., 2015).

Although complement constitutes the core of innate immune defence mechanisms in blood, several complement proteins are also expressed in the brain and in the retina. Complement activation has, interestingly, been reported in a wide range of retinal and brain disorders including AD, depression, seizures, multiple sclerosis, age related macular degeneration, diabetic retinopathy, uveoretinitis and glaucoma. More recently, involvement of this pathway in AD pathophysiology (Winston et al., 2019) and retinal disorders (Kassa et al., 2019) has gained significant attention. C1q, C3 and C4 are well expressed in the brain and have been shown to exhibit several fold elevated expression in AD (Zhou et al., 2008). Increased C1q expression was also reported in glaucoma conditions. Whether the increased expression in the brain and the retina is due to augmentation in synthesis of these proteins in the neuronal and glial cells or is sourced from the blood due to compromised blood-brain and blood-retinal barriers is not well established. The complement pathway, however, likely continues to perform its well-known functions of tissue homeostasis, and debris and protein aggregate clearance in the brain and retina, or at least works in that direction. In the long term, chronic activation of complement proteins may promote neurotoxicity and induce synapse and neuronal cell degeneration.

It is still unclear as to how the complement pathway is activated in the brain and retina, but abnormal protein aggregation in neurodegenerative conditions seems to be the most likely stimulus for complement activation. For instance, amyloid β and tau aggregation and protein deposition may trigger complement activation in AD (Mirzaei et al., 2019). Interestingly, both these proteins are also elevated in the ganglion cell layer in glaucoma. Amyloid aggregates in drusen deposits are also a key feature of age-related macular degeneration (AMD) pathology. Amyloid deposition in the brain and retina promotes microglial and astrocytic activation that in turn may stimulate complement activation. Synaptic degenerative changes in AD mouse models has been shown to be associated with complement activation and C1q levels were shown to correspond to increase tau levels in the brain. More recently, Litvinchuk et al. (2018) demonstrated C3a receptor inhibition to play a role in reducing tau pathology in AD mice. Complement pathway repressor complement factor H, in contrast, was shown to be reduced in the retina of AD mouse model. An *in vitro* study demonstrated the activation of alternative and complement pathway as a result of both fibrillar amyloid β1–40 and β1–42 peptide binding to C3 and the globular heads of C1q proteins. C1q plays a key role in intensifying the toxicity of soluble amyloid β oligomers on synapses as well as memory forming biochemical processes in the hippocampus (Kolev et al., 2009). Multiple reports highlighting involvement of complement pathway proteins in animal models of AD and human subjects has resulted in growing interest in manipulating the complement pathway, in particular, the classical pathway in the brain to improve function or restore homeostasis.

Sustained elevation of C1q expression in the retina was observed in ischemia/reperfusion injury in mice and interestingly C1q deficient mice demonstrated significant functional and structural protection against the inner retinal damage (Kolev et al., 2009). Similar changes in C1q expression have been identified in the retina and optic nerve head of DBA/2J congenital mouse glaucoma model. C3 levels were also observed in the retinal ganglion cells in experimental glaucoma models and post-mortem human eyes and enhanced expression of C3 was evident in the retinal ganglion cells in culture under stress induced by serum deprivation of cells. C3a receptor was also shown to play a role in ocular morphogenesis and mice lacking C3a and C5a receptors exhibited caspase activation and progressive retinal degenerative changes (Yu et al., 2012). Remarkably, genetic modulation of C3 was shown recently to impart significant protection against onset and progression of inner retinal injury in DBA/2J chronic mouse glaucoma model. Our proteomics studies carried out in human post-mortem glaucoma retinas revealed that immune and inflammatory pathways associated with complement system such as C1q, C1s, C1r, C4a, C4b, C3, C5, C6, C7, C8a, C8b, C8g and C9 were over-represented in the vitreous and retinal samples (Mirzaei et al., 2017). Apart from its roles in mediating inflammation, the complement system has also been shown to play a role in synaptic development and pruning, processes that are negatively affected in AD and in retinal degeneration.

Several complement pathway linked genetic variants such as CFI, CFH, C3 and C9 have been shown to exhibit association with AMD. A number of clinical trials targeting different components of complement pathway such as C3, C5, factor D and properdin are underway in AMD. AMD is associated with increased drusen deposition and complement inhibitors such as clusterin, CD46 and vitronectin have been identified in drusen (Kawa et al., 2014). The significance of these observations in AMD pathophysiology is not clear but amyloid β colocalization with C3 products in the AMD retinas suggests that amyloid deposition might be playing a role in complement activation in the retina, similar to its effects in the brain in AD. Complement activation products C5a and C3a are constituents of drusen and elevated locally that might promote choroidal neo-vascularisation in the retina. C3a, C3d, Ba, C5a, and membrane attack complex C5b-9 have been shown to be increased

in blood of AMD patients. Transgenic mice lacking intact C3 and C3aR/C5aR demonstrated reduced vessel formation in laser induced neovascularisation model of AMD and this vascularisation was also suppressed in response to pharmacological blockade of these receptors (Kawa et al., 2014). Increased levels of C3a, CFB and C3c have been identified in the aqueous humour of uveitis patients suggesting its role in the intraocular inflammatory disease process. Inhibition of complement pathway by neutralising factor B was shown to suppress uveitis injury by modulating T cell responses in a rat model.

Research conducted in last decade or so has significantly increased our understanding of the involvement of complement pathway proteins in neurodegenerative and pro-inflammatory disease processes in AD and in retinal disorders. Apart from significant efforts in AMD however, where several clinical trials are underway targeting different complement components, we have not seen a significant advancement on complement pathway regulating therapeutic approaches in ocular disorders. Success in gene therapy to modulate C3 in a glaucoma model and concomitant neuroprotection suggests its promising role in glaucoma (Bosco et al., 2018). Until, we identify consistent beneficial effects of complement inhibition in pre-clinical and clinical trials, it might be difficult to establish whether complement activation is indeed detrimental for neural tissues or if it plays a protective role at least during initial stages of the disease process. It is possible that the process starts a protective phenomenon until it snowballs into a destructive process in chronic disease. The crosstalk between complement activation and effects of other inflammatory processes such as cytokine activation, microglial, dendritic cell and astrocyte function too needs to be resolved clearly for better understanding of complement pathway mediated disease processes. Greater understanding of the relative contribution of these different inflammatory components will facilitate combination therapies to resist cell-injury and improve neuroprotection. Another challenge in complement targeting perhaps is to design drugs that can cross blood brain and blood retinal barrier and can act specifically at the site of disease. Nevertheless, complement targeting may be a promising therapeutic approach to improve neural cell function and restore tissue homeostasis in the brain and retinal disorders.

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