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REVIEW PAPER

The Role of Complement C3a Receptor in Stroke

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Abstract

The complement system is a key regulator of the innate immune response against diseased tissue that functions across multiple organ systems. Dysregulation of complement contributes to the pathogenesis of a number of neurological diseases including stroke. The C3a anaphylatoxin, via its cognate C3a receptor (C3aR), mediates inflammation by promoting breakdown of the blood–brain barrier and the massive infiltration of leukocytes into ischemic brain in experimental stroke models. Studies utilizing complement deficient mice as well as pharmacologic C3aR antagonists have shown a reduction in tissue injury and mortality in murine stroke models. The development of tissue-specific C3aR knockout mice and more specific C3aR antagonists is warranted to facilitate our understanding of the role of the C3aR in brain ischemia with the ultimate goal of clinical translation of therapies targeting C3aR in stroke patients.

Keywords Complement cascade · Central nervous system · Stroke · C3a receptor · C3a receptor antagonist

The complement system is a tightly-regulated cascade with a critical role in generating an immune response against diseased tissue. Excessive or inappropriate activation of complement contributes to the pathogenesis of various neuroinflammatory disorders including Alzheimer's disease (AD), Parkinson's disease (PD), Huntington's disease, and motor neuron disease (Heurich et al. 2011; Leblhuber et al. 1998; Morgan 2018; Wang et al. 2011). Components of the complement system are synthesized endogenously under conditions of homeostasis but can also cross the disrupted blood–brain barrier (BBB) and synthesized in the brain (Jacob and Alexander 2014; Noris and Remuzzi 2013). A detailed understanding of cerebral complement expression could provide important insight into the function of complement during normal development and may identify possible targets of intervention in various neurological diseases.

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Complement Cascade

The complement cascade is a critical component of innate immunity which functions to recognize and kill pathogens. The complement cascade is activated through three canonical pathways: the classical, lectin, and alternative pathways (Ducruet et al. 2009). The classical pathway is initiated by the interaction of C1q with antibodies, apoptotic cells, or serum amyloid P protein (Mollnes et al. 2002). The lectin pathway is initiated by the interaction of mannose binding lectin with surface carbohydrates on microbes with or protein cytokeratin present on ischemic endothelial cells (de Vries et al. 2004). The more recently discovered alternative pathway, which includes components such as factor B, factor D, and properdin, accounts for up to 80% of complement activation. This pathway can be spontaneously activated and serves as an amplification loop for the generation of C3b through an interaction with factors B and D (Blatt et al. 2016; Thurman and Holers 2006). All three pathways converge to assemble the C3 convertase which cleaves C3, producing C3a and C3b. C3b is then incorporated into the C5 convertase, leading to the generation of the chemotactic C5a fragment and formation of the membrane attack complex (C5b-9) which lyses the target cell (Merle et al. 2015; Ricklin et al. 2010). Interestingly, C3b can also be generated through extrinsic protease-mediated cleavage of C3 (Zhao et al.2017).

Complement Activation in the Central Nervous System

Complement component proteins are synthesized across various tissues (Noris and Remuzzi 2013). Circulating complement factors cannot migrate into the CNS under homeostatic conditions due to the presence of BBB. However, complement components extravasate into brain tissue under conditions of stress following the breakdown of the BBB in many neuropathologies (Dando et al. 2014; Daneman and Prat 2015). Additionally, complement is synthesized microglia, astrocytes, oligodendrocytes as well as neurons under both healthy and diseased conditions (Veerhuis et al. 2011; Woodruff et al. 2010). Complement in the brain is produced by several cell types. In particular, microglia expresses high levels of both C1q and CR3, a receptor that serves a crucial role in phagocytosis (Ehlers 2000; Schafer et al. 2000; Veerhuis et al. 1999). Additionally, astrocytes express C4 and C9 proteins (Walker et al. 1998). Furthermore, neuronal complement expression has been reported both in vivo and in vitro, with in situ hybridization experiments confirming the presence of C1q, C2, C3, C4, C5, C6, C7, C8, and C9 mRNA in postmortem Alzheimer disease (AD) and control brain tissue (Shen et al. 1997). Additionally, C3, factor H (FH), factor B (FB), C4, C1q, C5, C6, C7, and C9 expression has been reported in four human neuroblastoma cell lines including IMR32, SKNSH, SH-SY5Y, and KELLY (Thomas et al. 2000). Furthermore, hippocampal neurons, pyramidal cortical neurons, and cerebellar Purkinje neurons in normal human and murine brains constitutively express C5a and C3a anaphylatoxin receptors (Davoust et al. 1999; O'Barr et al. 2001). Due to the differential expression of complement in several neurological diseases, many in vivo studies targeting complement have been designed to achieve neuroprotection (O'Barr et al. 2001; Van Beek et al. 2000; Woodruff et al. 2010)

Complement C3a Receptor

The complement C3a anaphylatoxin is physiologically regulated by serum carboxypeptidase N, which inactivates C3a by cleaving a single arginine residue (Noris and Remuzzi 2013). The pentapeptide sequence at the carboxyl terminus of C3a, Leu-Gly-Leu-Ala-Arg, is required for C3a activity (Caporale et al. 1980). C3a attracts and activates phagocytic cells at sites of inflammation. The effects of C3a are mediated through its receptor, C3aR. The C3a receptor is expressed on a wide range of cell types including monocyte/macrophages, platelets, polymorphonuclear leukocytes, mast cells, neutrophils, eosinophils, basophils, and adipocytes (Martin et al. 1997). In brain tissue, C3aR is expressed in microglia, neurons and astrocytes (Davoust et al. 1999). In the Human CNS, C3aR mRNA expression has been reported in the cerebral cortex, cerebellum, and spinal cord tissue, and has been localized to astrocytes and microglia under both normal and inflammatory conditions (Ames et al. 1996; Boos et al. 2005; Lacy et al. 1995; Reemst et al. 2016; Tornetta et al. 1997).

The human C3a receptor is a seven-transmembrane G protein-coupled receptor (Ames et al. 1996). Binding of C3a to the C3aR causes Ca^{2+} mobilization through a pertussis sensitive G_i-coupled activation which enhances mast cell degranulation (Beatty and Beatty 1975; Norgauer et al. 1993). Two site interactions with C3aR are necessary for functional activation. The extracellular *N*-terminal part, the second extracellular loops, and some residues of the transmembrane domains of the receptors bind C3a, resulting in a conformation change in the receptor (Chao et al. 1999). This in turn leads to G protein activation which, triggers intracellular kinases that activate diverse signals. The second extracellular loop of C3aR is approximately 172 residues and sequences adjacent to the transmembrane domains contain multiple aspartate residues which play an important role in C3a binding interaction of the receptor with the ligand (Chao et al. 1999). Studies utilizing mutant human C3aR suggest that the serine and threonine residues on the intracellular C terminal may undergo phosphorylation and influence ligand associated C3aR desensitization, internalization, and G protein-mediated signal transduction (Settmacher et al. 2003). On comparative analysis, the C3a receptor is most closely related to the C5a receptor, with 37% homology (Crass et al. 1999).

Stroke

Stroke is the fifth leading cause of death in the United States and is the leading cause of disability in the developed world (Benjamin et al. 2017). Stroke is extremely prevalent, with a lifetime risk between 8 and 10% (Woodruff et al. 2011). Although there have been significant improvements in stroke prevention strategies, treatment options for acute stroke remain limited. Currently, tissue-plasminogen activator (t-PA) is the only FDA approved pharmacologic treatment for ischemic stroke. However, the efficacy of t-PA is limited due in large part to a short therapeutic window and $< 10\%$ of stroke patients receive t-PA (Liu et al. 2018; Miller et al. 2011). t-PA also exacerbates brain edema and increase hemorrhagic transformation (Gravanis and Tsirka 2008; Zhao et al. 2017). Thus, the development of adjunctive or standalone neuroprotective therapies is desperately needed.

The pathogenesis of stroke is complex and involves multiphasic processes. Ischemic stroke is characterized by an abrupt disturbance of cerebral circulation which engenders neurological deficits. In stroke, cerebral blood flow is reduced which impairs normal cellular functions and produces necrotic cell death within the ischemic core. There is an overall loss of cellular homeostasis, resulting in increased intracellular calcium, excitotoxicity, free radical-mediated toxicity, cytokine production, complement activation, disruption of the BBB, and leukocyte infiltration (Chen et al. 2011). Reperfusion enhances inflammatory responses in the microcirculation which exacerbates injury tissue and impairs recovery and repair processes (Kalogeris et al. 2012). Intrinsic variability in the sensitivity of neuronal populations to ischemia also contributes to the extent of ischemic injury (Pfisterer and Khodosevich 2017).

Over the last several years complement has emerged as a critical mediator of post-stroke inflammation (Sochocka et al. 2017). Several studies have shown that complement activation in both brain tissue and the periphery correlates with severity correlate with severity of stroke and functional disability in both stroke patients and experimental models (Mocco et al. 2006). In particular, the C3a and C5a anaphylatoxins and their cognate receptors have emerged as important mediators of post-ischemic neurological injury (Arumugam et al. 2009). However, several studies have also suggested that complement is also involved in neuroregeneration after stroke (Stokowska et al. 2017; Yanamadala and Friedlander 2010). These dual functions of complement need to be thoroughly understood in order to facilitate the development of safe and efficient anti-complement therapeutics. In order to assess the role of C3aR as a potential therapeutic target we will review its function under both normal and pathological conditions across different experimental models of stroke.

Role of C3aR in Stroke Pathophysiology

Several studies have established the C3aR as a promising therapeutic target by demonstrating a deleterious role for C3 in stroke. A comparative analysis of the susceptibility of mice deficient in the complement proteins C1q, C3, C5 to transient focal cerebral ischemia (Mocco et al. 2006) showed that only $C3^{-/-}$ mice demonstrated reductions in infarct volume, neurological deficit score, decreased granulocyte infiltration, and reduced oxidative stress when assessed at 24 h post reperfusion. These authors then showed that administration of a pharmacologic C3aR antagonist achieved comparable neurological improvement and stroke volume reduction, suggesting that C3 associated cerebral injury is mediated via C3a receptor-mediated signaling (Jarlestedt et al. 2013; Mocco et al. 2006; Zhao et al. 2017).

Increased cerebral expression of C3aR has been observed in various murine models of focal cerebral ischemia including both transient and permanent middle cerebral artery occlusion (MCAO). The expression of C3aR reached its peak 2 days after MCAO and C3aR immunostaining was increased in macrophage-like cells and reactive astrocytes 7 days post-occlusion (Van Beek et al. 2000). Interestingly, C3aR mRNA expression was reduced to 25% of control animals within 3 h of MCAO but expression was highest at 24 h post-occlusion, suggesting a massive infiltration of leukocytes bearing C3aR as the source of this expression (Barnum et al. 2002). These findings were supported by us in transient focal cerebral ischemia where we reported increased C3aR expression on granulocytes and endothelial cells in the ischemic region at 24 h as well as on infiltrating T-lymphocytes at 7 days following reperfused stroke (Ducruet et al. 2008; Zhao et al. 2017). Taken together, these studies suggest that increased post-ischemic C3aR expression contributes to inflammation and oxidative stress post-stroke although the underlying mechanism remains unclear.

C3aR Inhibition and Stroke

Tissue-plasminogen activator (t-PA) remains the only FDA approved pharmacologic treatment for ischemic stroke patients, but its universal use is limited due to its short therapeutic window and potential adverse effects. A recent study (Zhao et al. 2017) shows that t-PA promotes C3 cleavage through plasmin-mediated mechanism, resulting in C3aR-associated enhanced endothelial cell permeability. This contributes to brain edema and hemorrhage in stroke, which is attenuated by a C3a receptor antagonist. It has been shown that genetic manipulation of C3 decreased post-ischemic granulocyte infiltration and reduced oxidative stress (Mocco et al. 2006). Pharmacological inhibition of C3a receptor by SB290157 inhibited ischemic reperfusion injury resulting in improved neurological function, reduced infarct volume, and attenuated oxidative stress (Ames et al. 2001; Ducruet et al. 2012; Mocco et al. 2006).

In spite of these studies suggesting a benefit of C3aR antagonism, a single study suggests that genetic C3 deletion is associated with greater neurological damage and reduced post-stroke neurogenesis in a permanent MCAO occlusion model (Rahpeymai et al. 2006). These authors also proposed that C3a regulates neural progenitor cell migration, differentiation, and increases the survival of astrocytes (Rahpeymai et al. 2006; Shinjyo et al. 2009; Stokowska et al. 2017). They also showed that neural progenitor cells and immature neurons express C3aR. Along these same lines, another report showed that C3aR deficiency in mice resulted in neonatal hypoxia–ischemia associated memory impairment which can be improved

with C3a treatment in wild-type controls (Jarlestedt et al. 2013). Another recent study suggested that C3a receptor deficiency decreased post-stroke axonal sprouting and plasticity in the peri-infarct cortex which can be recovered by intranasal C3a administration (Stokowska et al. 2017). These reports have suggested that C3a/C3aR signaling is associated with axonal regeneration and sprouting, neural plasticity, cell replacement, suggesting a role in regeneration of neuronal functions that can extend to the chronic phase of stroke recovery (Jauneau et al. 2006; Vasek et al. 2016). However, a study using C3a/GFAP transgenic overexpressing mice did not show any change in neurogenesis when compared to controls (Bogestal et al. 2007). Along the same lines, Ducruet et al. (2012) attributed these seemingly conflicting roles for C3aR in neurogenesis to a dose-dependent response of SB290157. A higher dose of C3aRA in a permanent model of ischemia contributed to the negative outcomes on neural progenitor proliferation in the SVZ whereas using a low dose produced a neuroprotective benefit (Rahpeymai et al. 2006). Although they were not able to find any evidence for new mature neurons either in the SVZ or in the peri-infarct region, an increase in DCX+ BrdU+ cells in the ischemic hemisphere was noted (Jin et al. 2001; Sun et al. 2003). Ducruet et al. (2012) also reported a delayed infiltration of ischemic C3aR expressing T-lymphocytes in the brain and delayed C3aRA administration (72 h post injury) was associated with significant reductions in subcortical injury. This suggests that activated T-cells may interfere with endogenous neurogenesis although the mechanism responsible for this effect is unknown.

To add to the controversy, SB290157, a small molecule antagonist of C3a receptor, has been reported to act as an full agonist in a variety of cell systems that express high levels of C3aR (Mathieu et al. 2005). It is reported that SB 290157 acts as an antagonist in a cellular system where receptor density is low and acts as an partial agonist in cell lines that express higher levels of C3aR (Mathieu et al. 2005; Therien 2005). Thus, there is a need for development of more specific antagonists of the C3aR. Recently, BR111, a specific antagonist of C3a receptor, was utilized to attenuate C3a-induced inflammation in rat paw edema model (Lohman et al. 2017). Future studies using this antagonist, or mice with tissue-specific deletion of C3aR, may provide a better insight into the role of C3aR modulation in stroke. Overall, the effect of complement inhibition on recovery mechanisms may function primarily through attenuation of inflammation which promotes recovery and growth-promoting processes. However, the role of the complement system in ischemic brain injury is complex and depends on the type of ischemic injury, the developmental stage of the brain, and the stage of post-ischemic recovery.

Clinical Translation of Strategies Targeting the C3a/C3aR Axis

Complement activation plays a prominent role in acute and chronic neurological disorders, including traumatic brain and spinal cord injury as well as Alzheimer's disease (Carpanini et al. 2019). The complex role of complement in the brain is highlighted manifested by the fact that C3a not only promotes inflammation but also stimulates neural plasticity following brain ischemia (Stokowska et al. 2017). Furthermore, the C3a/C3aR axis has been shown to promote post-operative cognitive decline, and C3aR inhibition improves post-operative memory function following orthopedic surgery (Xiong et al. 2018). Much of this complexity likely stems from cell-specific role of C3aR expression. Given the complexity of the role of C3a/C3aR axis in stroke and other neurological diseases as well as the concerns raised above with the lack of specificity of SB290157, further development of genetic and pharmacologic tools to specifically antagonize endothelial C3aR will be necessary prior to clinical translation in stroke.

Conclusion

In vitro and in vivo studies evaluating complement inhibitory strategies in the setting of ischemic stroke has engendered a field of novel neuroprotective candidates. SB20197 ameliorates post-ischemic neurological outcomes both with and without t-PA by targeting post-ischemic inflammation. Existing studies have suggested that the C3a/C3aR is a major contributor to post-stroke inflammation/injury, and that targeting C3aR can improve outcome after stroke beyond the acute phase (Fig. 1). However, additional preclinical studies are required utilizing more specific antagonists for C3aR in different models of stroke to provide better insight into the mechanisms involved in post-stroke injury, recovery, neurogenesis, and synaptic plasticity.

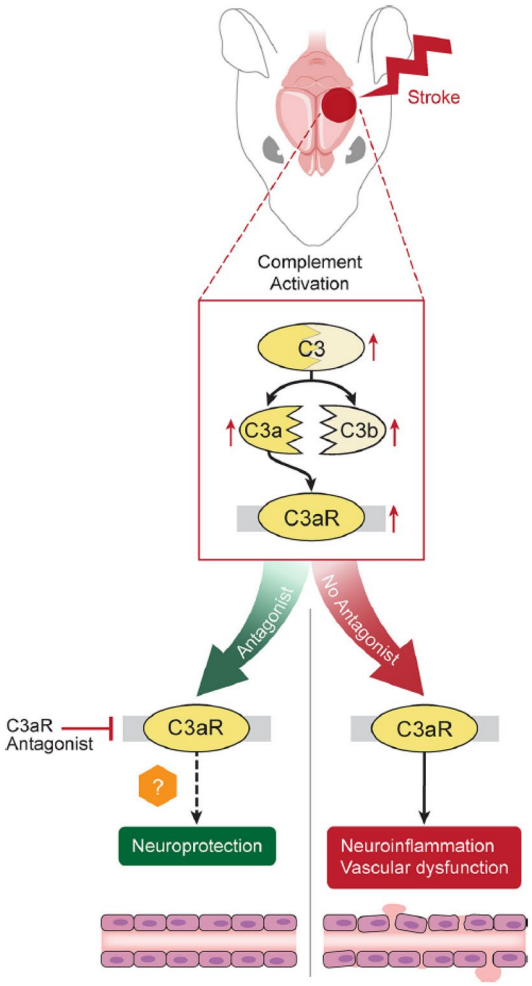

Fig. 1 Schematic diagram is showing hypothetical mechanisms of C3aR function in stroke. Reproduced with permission from Barrow Neurological Institute, Phoenix AZ

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Compliance with Ethical Standards

Conflict of interest The authors declare that they have no conflict of interest.

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