Complement activation kindles the transition of acute post-traumatic brain injury to a chronic inflammatory disease

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Traumatic brain injury (TBI) remains a major cause of disability among young adults in both civilian and military settings contributing to a high burden on healthcare systems (Badhiwala et al., 2019). Sequel of TBI, even mild injuries, include motor and sensory dysfunction, neurocognitive decline, neuropsychiatric complications, as well as increased risk of neurodegenerative and neurovascular events such as Alzheimer's disease and stroke (Breunig et al., 2013; Burke et al., 2013; Li et al., 2017). Despite the acute nature of the insult in TBI, pathological changes in the traumatized brain are better recognized as a chronic rather than an acute neurological disease, a phenomenon that remains under-investigated. Robust clinical data support the role of neuroinflammation in propagating neurodegenerative changes following TBI with a pivotal role of the complement system as an early trigger and chronic propagator of this response (Alawieh et al., 2018, 2021; Mallah et al., 2021). Hereby, we discuss how the role of complement pathways in different phases of injury after TBI was investigated using clinically relevant targeted complement inhibitors (Alawieh and Tomlinson, 2016; Alawieh et al., 2018, 2021; Mallah et al., 2021).

The complement system is a component of the innate and adaptive immune response that can be activated via one of three different pathways: the classical, lectin or alternative pathway. The classical pathway is usually initiated by the binding of C1q to antibody Fc domains. The lectin pathway is initiated by the binding of mannose-binding lectin to certain carbohydrates, including those present on natural antibodies. The alternative pathway can be spontaneously activated, and serves as an amplification loop for the other pathways. All pathways converge with the cleavage of C3 to produce C3a and C3b. C3a is a soluble inflammatory peptide and C3b is covalently bound to the activating surface. C3b is further cleaved to yield membrane-bound iC3b and C3d, opsonins that are recognized by receptors (such as C receptor 2 and 3, CR2 and CR3) on immune cells. C3 cleavage also leads to downstream C5 cleavage to yield C5a and C5b. C5a has inflammatory activities, whereas C5b initiates the terminal pathway and formation of the membrane attack complex that can cause direct cell lysis. Complement is regulated by various fluid phases and membrane-bound inhibitors. Of relevance, our group has developed injury-site targeted inhibitors of complement activation that home to the site of complement activation and inhibit different steps in the activation cascade. Several generations of these inhibitors have been reviewed elsewhere (Alawieh and Tomlinson, 2016), and they include Complement Receptor 2 (CR2)-targeted inhibitors. These are fusion proteins of CR2 that bind complement activation products C3b/iC3b/C3d which are covalently deposited on sites of active inflammation, and one of three complement inhibitors; namely, Crry (inhibits C3 cleavage by all pathways), factor H (fH, inhibits the alternative pathway) and CD59 (inhibits the membrane attack complex). These inhibitors constitute a clinical investigation toolbox to probe the role of different complement activation products in complement-dependent pathologies.

Our group among others has investigated the contribution of complement system activation to TBI pathology, and these studies have implicated all three pathways of complement activation (Ruseva et al., 2015; Alawieh et al., 2018, 2021; Mallah et al., 2021). Early work has focused on targeting the membrane attack complex demonstrating increased levels of the C5b-9 complex in the CSF of TBI patients and improved acute outcomes with inhibition of this pathway in preclinical models (Bellander et al., 2001; Stahel et al., 2001; Ruseva et al., 2015). However, subsequent study by our group took advantage of the repertoire of injury-site targeted inhibitors against the different complement pathways to probe the differential role of each pathway in TBI pathology (Alawieh et al., 2018). These inhibitors included CR2-Crry (inhibits C3 cleavage by all pathways), CR2-fH (inhibits the alternative pathway), and CR2-CD59 (inhibits the membrane attack complex). After acute administration of complement inhibitors in murine TBI, this work demonstrated that although acute outcomes were comparable and better than controls when either of the inhibitors was used, chronic outcomes were dependent specifically on suppression of C3 activation rather than membrane attack complex. Inhibition of the membrane attack complex alone (via CR2-CD59) reduced acute neuronal cell loss, but did not suppress the release of upstream complement activation products including C3b and C3a, both of which are potent activators of systemic and local inflammatory cells. This early activation of the C3 convertase resulted in an ongoing neuroinflammatory response characterized by astrogliosis and microgliosis up to 30 days after the initial insult. Only the inhibition of the C3 convertase or the alternative pathway (via CR2-Crry and CR2-fH respectively) was sufficient to suppress the ongoing neuroinflammatory response by 30 days and limit neurocognitive decline (Alawieh et al., 2018).

Following these studies, the question remains whether the role of complement in driving neuroinflammation is restricted only to the acute phase of injury following TBI. Histological data show that, even up to 90 days post-TBI, complement activation products were still deposited in the injured brain and associated with ongoing microglial proliferation and neuronal loss (Alawieh et al., 2018, 2021). This led to subsequent long-term studies in mice where complement C3 activation was inhibited via the targeted inhibitor CR2-Crry starting at 2 months after the onset of TBI. At the time of initiation of treatment, animals of the treatment and control cohorts have already sustained significant cognitive deficits as compared to their naïve controls and showed similar patterns of complement activation. However, when CR2-Crry was administered as three doses over 1 week starting 8 weeks after the initial insult, these cognitive

deficits were reversed showing that, even in a delayed fashion, inhibition of pathologic C3 activation can allow for reversal of cognitive decline incurred after TBI. At the same time, the dynamic nature of this response at the delayed timepoint further supports the nature of TBI pathology as a chronic rather than an acute neuroinflammatory disease (Alawieh et al., 2021). In the same work, the introduction of rehabilitation resulted in an additive effect to complement inhibition, a mechanism based on the neuroplastic response that could still be exploited in chronically injured brains. In fact, using super-resolution microscopy, our group demonstrated that this chronic complement activity in the injured brain was responsible for complement-dependent phagocytosis of synaptic connections in the hippocampus, a process that is directly correlated with cognitive performance in tested animals (Alawieh et al., 2021). Loss of synaptic density is an early process in neurodegeneration and results in limited trophic supports for recovering neurons, and reduces the neuronal substrate that is able to engage in neuroplastic and neuro-regenerative mechanisms. These studies show that complement is not only a trigger for the neuroinflammatory response after acute TBI but also continues to kindle the neuroinflammatory response later in the phases of injury limiting neurocognitive recovery. Complement-driven neuroinflammation poses a significant brake on the neurodegenerative response in the recovering brain as characterized by limited neurogenesis in the subventricular zone, decrease dendritic and synaptic arborization, and chronic loss of neurons in the brain with robust complement activity following injury (Alawieh et al., 2018, 2021; Mallah et al., 2021).

Supporting this hypothesis, follow-up work from our group assessed whether continuous suppression of complement activity in murine TBI was required to prevent the reinstatement of cognitive deficits over 6 months of recovery compared to transient short-term inhibition. In mice treated with complement inhibitor (CR2-Crry) over a single week 2 months after injury, cognitive deficits recurred by 6 months of recovery whereas mice receiving weekly dosing of complement inhibitor maintained cognitive performance comparable to naïve mice on spatial learning and memory tasks.

Collectively, these studies take a unique advantage of the clinically relevant targeted complement inhibitors that allow for local suppression of complement activation at different stages of TBI pathology. Compared to genetic knockout models that do not allow for a temporal investigation of this role, the use of these inhibitors has demonstrated that the C3 activation step is a pivotal event in triggering the neuroinflammatory response after TBI, and that this trigger is an ongoing phenomenon that keeps propagating the inflammatory response in the chronic phases of injury unless therapeutically inhibited. In addition to providing new insight into TBI pathology, these targeted approaches for complement modulation open a new avenue for potential new therapeutics for the treatment of the large population of TBI patients. Notably, these inhibitors, among other targeted complement inhibitors, are currently in different stages of clinical development, and the humanized version of CR2-fH (namely TT30) has been found to be safe in a phase 1 clinical trial (Risitano et al., 2015).

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