Commentary

Targeted Modulation of the Neuroinflammatory Response after Spinal Cord Injury

The Ongoing Quest for the “Holy Grail”

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Healing of the injured spinal cord represents one of the remaining challenging frontiers in medicine. The post-traumatic neuroinflammatory response has been shown to contribute, at least in part, to the development of secondary neuronal cell death.¹ Research strategies to prevent these pathological sequelae after spinal cord injury (SCI) have largely failed in translation from the “bench to bedside.”² A classic example related to our lack of understanding the basic immunological mechanisms that lead to delayed neuronal cell death is the failure to administer high-dose methylprednisolone to improve neurological outcome after SCI.³ While considered a standard of care incorporated in clinical guidelines for many years, based on promising data from the NASCIS trials, high-dose steroids recently were recognized to be harmful, rather than beneficial, in the management of acute SCI, and their application in the field of neurotrauma is now considered obsolete.^{4–6}

Current research strategies in neurotrauma are therefore aimed at targeting more specific pathways of inflammation, such as the pharmacological inhibition of the complement cascade, which represents the major effector arm of the innate immune system.⁷ Interestingly, the first studies investigating complement activation in patients with SCI were published more than three decades ago. In 1980, Rebhuhn and Botvin⁸ showed that about two-thirds of all patients with SCI had elevated complement levels, and the authors postulated that complement activation may propagate a “self-feeding” immunological response responsible for the failure of regeneration of the injured spinal cord. The recent availability of higher quality complement reagents, including genetically engineered mice and tissue-targeted chimeric complement

inhibitors, currently allows for the more detailed elucidation of specific pathways of complement activation involved in the pathophysiology of neuroinflammation after SCI^{7,9} (Figure 1).

The recent study by Qiao and colleagues¹⁰ published in this issue of *The American Journal of Pathology* was designed to analyze the role of the alternative activation pathway (factor B) and terminal lytic pathway (C5b-9) in contributing to the secondary neuropathological sequelae after traumatic SCI in adult C57BL/6 mice.¹⁰ In the first part of the study, a traumatic spinal cord contusion was applied to mice with a genetic deficiency for factor B (fB^{-/-}). These mice therefore lacked a functional alternative pathway of complement activation.¹¹ Impressively, fB^{-/-} mice showed significantly improved locomotor function scores for up to 21 days after trauma, compared to wild-type controls. The neurological improvement was substantiated by significantly reduced neutrophil infiltration, complement deposition, and tissue damage in the injured spinal cord of fB^{-/-} mice, compared to that of wild-type littermates. These data imply a crucial role of complement activation, via the alternative pathway, in the development of posttraumatic neuroinflammation and propagation of delayed neuronal injury. In the second part of the study, these positive insights derived from gene knockout mice were translated to a pharmacological approach using a neutralizing monoclonal anti-factor B antibody (mAb1379), which is a potent inhibitor of the alternative complement activation pathway.^{12–13} Using a clinically relevant paradigm of systemic (intravenous) compound administration at 1 and 12 hours after trauma,

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Figure 1. Simplified depiction of the three traditional complement activation pathways and the potential pharmacological interventions designed to ameliorate the extent of neuroinflammation and neuropathology after spinal cord injury. See text for details.

the authors were able to replicate the neuroprotective effects seen in $fB^{-/-}$ mice, to the pharmacological mAb1379-treatment model in wild-type mice, using vehicle-injected animals as appropriate controls. Finally, the third part of the study was designed to determine the role of the membrane attack complex (C5b-9), as the terminal downstream event of complement activation, in the neuropathophysiology after SCI. For this purpose, mice lacking the gene for the membrane-bound complement regulatory molecule CD59a ($CD59a^{-/-}$) were subjected to traumatic spinal cord injury and analyzed by the same outcome parameters as for the $fB^{-/-}$ mice. One of the putative mechanisms of complement-mediated neuronal death after spinal cord injury is represented by posttraumatic activation of phosphatidyl inositol-specific phospholipase C (PI-PLC), which renders neurons vulnerable to membrane attack complex-mediated lysis by shedding of the glycosyl phosphatidyl inositol-anchored glycoprotein CD59a from neuronal membranes.^{14–15} In contrast with data from $fB^{-/-}$ mice and mAb1379-treated animals, $CD59a^{-/-}$ mice showed a significant deterioration of neurological scores from 11 to 21 days after SCI, compared to wild-type littermates. In addition, deposition

of complement C9, as a surrogate marker for the membrane attack complex (C5b-9), was significantly increased in the spinal cord of $CD59a^{-/-}$ mice and associated with exacerbated tissue damage and local neutrophil infiltration.¹⁰

The exact cellular and molecular mechanisms of complement-mediated neuropathology after spinal cord injury remain far from fully understood. The present study¹⁰ sheds further light toward our understanding of the immunological pathophysiology of SCI, as it offers novel insights into the impact of the different complement activation pathways (classical, alternative, lectin, and terminal pathway) and their involvement in posttraumatic neuroinflammation and neurodegeneration^{16–17} (Figure 1).

Recent experimental studies have provided initial evidence of involvement of the classical pathway of complement activation in the pathogenesis of neuronal tissue damage and adverse neurological outcome after SCI.¹⁸ In contrast, data from multiple studies on inflammatory conditions in and outside the CNS have revealed that the selective inhibition of the alternative complement pathway may represent a promising new immunomodulatory approach for multiple neuroinflammatory disor-

ders.^{7,11,13,19–21} The current experimental study¹⁰ implies that the therapeutic inhibition of the alternative pathway (factor B) during a clinically relevant time window within 1 to 12 hours after trauma may represent a promising new avenue in the search for a pharmacological remedy to host-mediated progressive inflammation and neurodegeneration within the injured spinal cord. In this regard, it is notable that the monoclonal anti-factor B antibody used in the present study has cross-reactivity across species and was originally designed as an anti-human antibody.^{12–13} Thus, this specific compound has potential for promising “bench-to-bedside” translation in the future. Similarly, the therapeutic blockade of membrane attack complex assembly, by the use of anti-C9 antibodies, or stimulation/up-regulation of CD59 expression (eg, through gene therapy), may represent a new strategy to avoid “innocent bystander” complement-mediated neuronal lysis after spinal cord injury.

Future experimental studies must be designed to titrate the optimal dosage, time window of administration, and time intervals for repeated injections of these new generation complement inhibitory and regulatory molecules. One shortcoming of these therapeutic approaches is the potential for a limited extent of complement inhibition related to the half-life of these compounds and peak concentration at the site of the injured tissue. In this regard, repeated injections at closer time intervals and the use of newly available chimeric compounds consisting of complement inhibitors fused to complement receptors that bind at the site of complement activation, such as CR2-factor H and CR2-Crry molecules,^{9,22} may represent the future “golden bullet” for amelioration of complement-mediated exacerbation of SCI.

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