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**“Effects of a Toll-like Receptor 9 Antagonist on Spinal Cord Astrocytes and the Glial Scar Following Spinal Cord Injury”**

by

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**ABSTRACT**

Traumatic spinal cord injury (SCI) leads to permanent neurological deficits. Following the primary mechanical insult, several endogenous mechanisms are triggered and either expand the damage or confer neuroprotection. Among those, is the formation of a glial scar at the injury epicenter which is a cellular process that plays both beneficial and detrimental roles. The glial scar physically isolates necrotic tissue from the nearby healthy tissue to prevent the uncontrollable spread of the damage. Paradoxically, the increased expression of chondroitin sulfate proteoglycans (CSPGs) at the glial scar is one of the determinants underlying inhibition of axonal regrowth which, in turn, limits functional recovery. Reactive astrocytes are one of the principal cell types that constitute the glial scar and are the main producers of CSPGs.

Astrocytes express toll-like receptors (TLRs), which are the first sensors of danger. Binding of endogenous ligands released by stressed or damaged cells activate TLRs and initiate neuroinflammation following SCI. The contribution of TLRs to SCI remains inadequately defined. Earlier studies have shown that a TLR9 antagonist, oligodeoxynucleotide 2088 (ODN 2088), administered intrathecally, reduces neuroinflammation, enhances white matter sparing, and improves the functional outcomes of SCI. Even though cells intrinsic to the SC, including neurons and astrocytes express TLR9, little is known about the effects of the antagonist on these cells in the context of SCI. In particular, the response of astrocytes to ODN 2088 is poorly defined. The current investigations were undertaken to unravel the effects of TLR9 antagonism on astrocyte function at the glial scarand *in vitro.*

Our studies indicated that intrathecal ODN 2088 treatment significantly decreases CSPG immunoreactivity and astrocyte proliferation at the glial scar in female C57Bl/6 mice sustaining a severe mid-thoracic (T8) SC contusion injury as compared to injured controls treated with vehicle. In addition, intrathecal administration of ODN 2088 preserved proximal CST axons. Addition of ODN 2088 to pure SC astrocyte cultures, significantly decreased proliferation through inhibition of ERK1/2/MAPK activation and reduced astrocyte migration in an *in vitro* scratch wound assay. In contrast, ODN 2088 enhanced the chemotaxis of peritoneal macrophages in macrophage-astrocyte co-cultures. Conditioned medium of astrocytes treated with the antagonist also promoted macrophage chemotaxis in a trans-well migration assay *in vitro*. The effects of the antagonist on macrophage chemotactic migration were partly mediated by increased C-C motif chemokine ligand 1 (CCL1) release by astrocytes and were inhibited by addition of an anti-CCL1 blocking antibody to the cultures. ODN 2088-mediated modulation of astrocyte proliferation, migration and chemotaxis were dependent on TLR9 expression since they were not observed in TLR9-/- astrocytes. Genetic deletion of TLR9 mimicked some of the effects of the TLR9 antagonist since proliferation and migration of TLR9-/- astrocytes were lower than that observed with TLR9+/+ astrocytes.

Taken together, these findings suggest that the TLR9 antagonist can modify the glial scar by limiting astrocyte proliferation, CSPG production and presumably, migration. These changes are paralleled by improved preservation of proximal axons supporting the idea that ODN 2088 treatment creates a favorable milieu that fosters axonal protection at the injury site.