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Taking Off the SOCS: Cytokine Signaling Spurs Regeneration

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Strategies to improve function after CNS injuries must contend with the failure of axons to regrow after transection in adult mammals. In this issue of *Neuron*, Smith et al. provide an important advance by demonstrating that SOCS3 acts as a key negative regulator of adult optic nerve regeneration.

A fundamental characteristic of the mature mammalian CNS is the failure of neurons to accomplish long-range axonal regeneration after injury. Regeneration failure is explained in part by the presence of numerous molecules that act as barriers for central axon growth (Benowitz and Yin, 2008). The recent identification of a receptor for chondroitin sulfate proteoglycans, known inhibitors of axon regeneration, provides an outstanding example of the ongoing progress in understanding inhibitory mechanisms (Shen et al., 2009). Nevertheless, blocking negative extracellular influences alone appears to be insufficient to enable extensive axon elongation after CNS injury.

In striking contrast to PNS neurons, CNS neurons undergo a permanent downregulation of axon growth potential during development (Goldberg et al., 2002). Exciting recent work has defined some of the mechanisms underlying this reduced intrinsic growth potential (Moore et al., 2009). Particularly important is the demonstration that adult CNS neurons possess a diminished capacity to activate the mTOR-regulated protein synthesis machinery after injury (Park et al., 2008). Elimination of phosphatase and tensin homolog (PTEN) activates mTOR and allows some CNS neurons to regenerate axons. In this edition of *Neuron*, Smith et al. report that suppressor of cytokine signaling 3 (SOCS3) is another critical negative regulator of CNS regeneration (Smith et al., 2009).

The SOCS family of proteins is comprised of eight family members that function as intracellular inhibitors of cytokine signaling. SOCS proteins primarily inhibit JAK-STAT signaling through binding to JAK and/or specific phospho-tyrosine residues on cytokine receptors. Many physiological functions are regulated by SOCS proteins, including inflammatory, immune, endocrine, and oncogenic responses (Croker et al., 2008). SOCS1, -2, and -3 are perhaps the best characterized of the family, and each is expressed in the nervous system (Miao et al., 2006; Park et al., 2009). Injury-induced cytokine signaling is critical for triggering adult regenerative responses, especially the successful regrowth that occurs in the PNS. In the CNS, lens injury activates cytokines and other processes that improve the regeneration of retinal ganglion cells (RGCs) after nerve crush and is akin to the conditioning lesion effect in the PNS (Leon et al., 2000; Leibinger et al., 2009). It is perhaps surprising then that little is known about the role of SOCS family members in regeneration.

A major impediment to CNS regeneration studies has been the technical difficulty of the assays employed. Spinal cord injury has been the most commonly utilized paradigm, but this model can be difficult to standardize. Tracing regenerating axons over many spinal cord segments is complex, and axon elongation can be difficult to distinguish from compensatory sprouting. Lesioning the optic nerve has become a favored model since these issues are more easily controlled. After a careful and full optic nerve crush in control animals, virtually no axons regenerate. Further, the favorable geometry of the model allows for a straightforward and quantitative assessment of strategies that promote regeneration. Park et al. demonstrated the power of this model in floxed allele mice using intravitreal injections of adenoviruses encoding Cre (AAV-Cre), which infect greater than 90% of RGCs (Park et al., 2008). In theory, the influence of almost any intracellular signaling molecule that suppresses the growth of mature RGC neurons can be assessed in this manner.

Here, the authors tested the role of SOCS3 by injecting AAV-Cre into the eye of SOCS3$^{fl/fl}$ mice. The optic nerve was crushed, and RGC regrowth and survival were evaluated. Strikingly, RGC axons regenerated up to 1.5 mm, and survival was significantly increased. Much of the regrowth occurred 3–7 days postinjury and was correlated with an increased level of phospho-S6 labeling, an indicator of mTOR activity. The extent of axon elongation following SOCS deletion was, however, less than that observed in PTEN-deleted mice (~3 mm), raising the possibility that additional growth-suppressing pathways may converge on mTOR (Park et al., 2008).

The authors next reasoned that endogenous SOCS3 functions to dampen the response to injury-induced cytokines that act through gp130. Coexpression of gp130 and SOCS3 transcripts in adult RGCs was demonstrated. Further, injection of AAV-Cre into SOCS3$^{fl/fl}$ gp130$^{fl/fl}$ double-mutant retina failed to induce significant regrowth after optic nerve crush. Presumably, SOCS3 is reducing the intrinsic growth potential of central neurons by inhibiting the efficacy of JAK-STAT signaling, although STAT activation was not rigorously assessed. These experiments suggest that RGC regrowth following SOCS3 deletion is dependent upon signaling through a gp130-dependent cytokine signaling cascade.

To explore the identity of injury-induced signals linked to gp130, the expression of

CNTF, IL-6, and CT-1 was assessed. Of the three cytokines studied, only CNTF was found to show increased expression in the ganglion cell layer, which peaked 6 hr postinjury. These data suggest that CNTF may mediate an injury-induced response via gp130. Important in this regard are the recent results of Leibinger et al., which show that the effect of lens injury on RGC regeneration is reduced in *CNTF*$^{-/-}$ mice and totally blocked in *CNTF*$^{-/-}$ *LIF*$^{-/-}$ double-mutant mice (Leibinger et al., 2009). Together these results suggest that CNTF and LIF are functionally relevant injury-induced factors.

Having shown that SOCS3 blocks endogenous injury-induced signaling, the authors asked if regeneration in response to exogenously applied cytokines can be enhanced by deleting SOCS3. Consistent with previous reports, the injection of exogenous CNTF induced modest regeneration after nerve injury. Importantly, the combination of exogenous CNTF and SOCS3 deletion resulted in nearly a 2-fold increase in the number and length (~2.5 mm) of axons projecting past the crush site when compared to SOCS3 deletion alone. Many previous attempts at augmenting CNS regrowth with exogenous CNTF or other trophic factors have met with limited success. It is interesting to consider the possibility that SOCS3 (and other negative regulators) may have been acting as constitutive brakes, frustrating these prior efforts.

The findings of Smith et al. highlight the critical role of cytokine signaling in the successful regeneration of central axons. As the authors point out, simply activating the mTOR pathway is not enough to elicit growth in the absence of an injury-induced signal. It is clear that SOCS3 represents an outstanding target for further enhancing the effects of cytokines. Identification of the transcriptional targets of cytokine signaling will be an important focus for future research.

Much remains to be learned about the interactions among the intracellular pathways that have been associated with successful regeneration. For example, the growth-promoting effects of cyclic AMP appear to be mediated in part by downregulation of SOCS3 (Park et al., 2009). On the other hand, another regeneration-promoting factor, oncomodulin, promotes RGC regrowth in a JAK/STAT-independent manner (Yin et al., 2006). Regeneration-induced signals acting through receptor tyrosine kinases and activating the MAPK pathway (Lorber et al., 2009) may be similarly independent of SOCS3. Importantly, as the authors have shown, loss of SOCS3 restores activation of the protein synthesis machinery, which is presumably required for the effects of all growth-enhancing signals.

Are the current approaches being developed in rodent models capable of triggering clinically relevant responses in the human CNS? Importantly, few of these interventions have been tested in a primate model where the anatomy and distances of axon growth that must be achieved would better approximate the situation in humans. Further, the complexity of human injuries that are associated with large scars and cavity formation in the spinal cord are not really addressed in these optic nerve models. Leaving the enormous translational obstacles aside, there appears to be reason for cautious optimism over the long term. Combinatorial therapies show progressive enhancements of axon regrowth in rodent models. Additionally, appropriate combinatorial therapies can result in functional improvement even after long-standing injury (Kadoya et al., 2009). Smith et al. provide compelling data suggesting that SOCS3 silencing may add to the beneficial effects of current therapeutic approaches.

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