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The CD95- and the TNF-Ligand/Receptor systems in spinal cord injury of wild-type and mutant mice

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Shortly after injury of the spinal cord cells in the injured area undergo apoptosis (Crowe et al., 1997). Main inducers of the apoptotic program in other models of neurodegenerative diseases, such as stroke (Martin-Villalba et al., 1999; Martin-Villalba et al., 2001) are the CD95- and TNF-ligand/receptor (L/R) systems.

Here, we have examined the role of TNF and CD95L in SCI. Expression of these ligands increases upon injury of the spinal cord (Li et al., 2000a; Xu et al., 1998; Zurita et al., 2001), but no functional data have been shown so far. To address whether these death systems are involved in SCI-induced damage, we used a rodent model of SCI. Following transection of the dorsal two thirds of the spinal cord, therapeutic neutralization of CD95L alone, or both CD95L and TNF, dramatically reduced apoptotic cell death.

In addition, we took advantage of mouse mutants defective either in a functional CD95L (*gld*), mice defective in TNF (*tnf* $^{-/-}$), or hybrids of both (*tnf* $^{-/-}$ /*gld*). Surprisingly, we found that SCI-induced apoptotic cell death and inflammation decreased only in animals lacking both CD95L and TNF compared with *wt* animals. Therefore, in order to study the role of the CD95-

and the TNF-L/R systems on a long term basis following SCI, we decided to acutely neutralize CD95L and/or TNF in *wt* animals rather than using mice deficient in these ligands.

Importantly, mice treated with anti-CD95L or a mixture of anti-CD95L and anti-TNF antibodies, but not anti-TNF alone, were capable of initiating active movements several weeks post-injury. The locomotor performance of the animals was assessed in a double-blind manner before and at 1, 2, 3, and 4 weeks after injury. For this, the following tasks were tested: the overall locomotor performance was assessed using the BBB locomotor rating scale with slight modifications (Basso et al., 1995); deficits in descending motor control were examined by the grid-walk test (Metz et al., 2000); locomotor performance in the absence of cutaneous and proprioceptive input from the limbs to the spinal cord was checked in the swimming task; coordination and skill-learning was checked in the rotarod test; finally, the reaction of animals to otherwise non-nociceptive mechanical stimuli was tested by the “von Frey test”. For all the tasks tested, no statistically significant differences were found either between the two control groups, or between the two treated groups for any of the time points studied, respectively (Wilcoxon Rank Sum test).

The improvement in locomotor performance in animals lacking CD95L activity was mirrored by an increase in regenerating fibers at the site of the lesion and a parallel upregulation of the Growth associated protein-43 (GAP-43). Moreover, neutralization of CD95L led to an increase of expression of the neuronal marker, βIII-Tubulin and of Myelin basic protein (MBP), an indirect marker of oligodendrocyte viability.

Our work indicates that neutralization of CD95L may constitute a potent treatment for human spinal injuries. It would, on the one side keep neurons alive until a permissive environment is created and guidance cues arrive. On the other side, the resulting protection of oligodendrocytes would maintain the myelin insulation of the injured axons and allow a good transmission of electrical stimuli once connections are made. We demonstrated that following SCI neutralization of CD95L acts on three levels: protection of neurons, inhibition of demyelination, and promotion of regeneration.