Published in "Neurodegenerative Diseases 4(1): 51-56, 2007"
which should be cited to refer to this work.

Repair of the Injured Spinal Cord

A Joint Approach of Basic and Clinical Research

Anita D. Buchli[a] Eric Rouiller[c] Roland Mueller[b] Volker Dietz[b]
Martin E. Schwab[a]

[a]Brain Research Institute, University and ETH Zurich, and [b]Spinal Cord Injury Center, Balgrist University Hospital, Zurich, and [c]Department of Medicine, Unit of Physiology, University of Fribourg, Fribourg, Switzerland

Key Words
Spinal cord injury · Nogo-A · Axonal regeneration · Functional recovery · Neural plasticity · Animal models · Clinical trials

Abstract
The myelin protein Nogo-A is a potent inhibitor of neurite outgrowth in the central nervous system, thus contributing to the incapacity of fiber tracts in the adult spinal cord to regenerate after injury. In this review we report on a joint approach of different research groups to develop a therapy applying anti-Nogo-A antibodies to the injured spinal cord. While basic researchers took the initiative to provide means of neutralizing the inhibitory effect of Nogo-A and demonstrated enhanced fiber growth, regeneration and functional recovery both in rodent and primate models, clinical groups and rehabilitation engineers have sought to translate this novel strategy into a clinical setting.

Introduction

The aim of the project 'Spinal Cord Repair' of the NCCR 'Neural Plasticity and Repair' initiated by the Swiss National Science Foundation in 2001 is to develop novel treatments for spinal cord injury and implement these into a clinical setting. The challenge to bring together basic researchers, clinicians and engineers from different academic institutions (Federal Institute of Technology, University of Zurich and Fribourg, Balgrist University Hospital Zurich) and industry to collaborate on distinct aspects of this project resulted in an extremely fruitful exchange of ideas and has greatly accelerated the progress of the project.

One of the basic science groups within this project has developed regeneration-enhancing treatments for increased restoration of function after spinal cord injury. Their validation has now been successful in a preclinical setting in mouse, rat and monkey models – a key prerequisite to enter clinical trials. A network of paraplegic centers in Europe and North America has been created where clinical studies can be pursued ensuring comparable diagnostic methods, treatment schedules and follow-ups. Phase I clinical trials with an anti-Nogo-A antibody treatment of acute para- and tetraplegic patients have recently been initiated.

Basic Research and Proof of Principle of Regeneration-Enhancing Anti-Nogo Antibody Treatment in Rodent Models

Several lines of evidence suggested the presence of specific inhibitory factors responsible for the nonconducive properties of central nervous system (CNS) tissue for axonal regeneration in adult vertebrates. CNS white matter

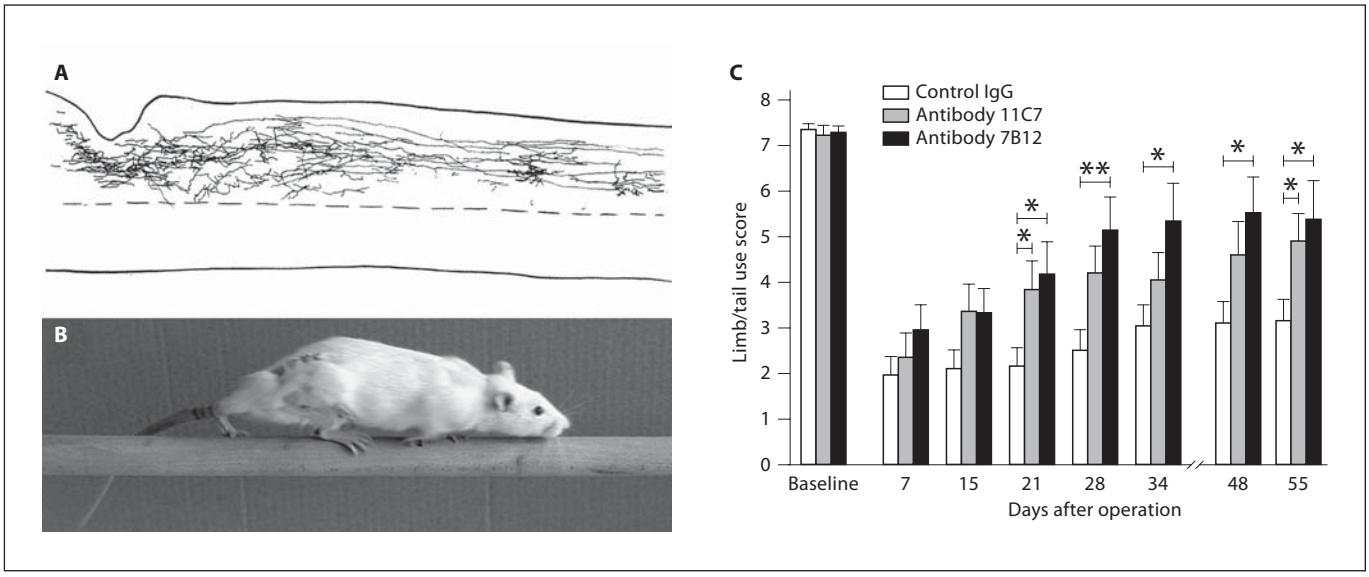

Fig. 1. Long-distance and functional regeneration after spinal cord injury in rats (**A**), and functional recovery in anti-Nogo-A antibody-treated rats after spinal cord injury (**B**, **C**). **A** Regeneration in the spinal cord: camera lucida drawing of sagittal sections of the lower thoracic spinal cord including the lesion site (left). Neutralization of Nogo-A leads to enhanced sprouting rostral to the lesion (left) and to fibers crossing the lesion on remaining tissue bridges into the caudal spinal cord and growing down the spinal cord over long distances (right to the lesion site). **B** Rat balancing over a narrow beam. **C** Anti-Nogo-A-treated animals (antibodies 11C7, 7B12) show improved performance in the swim test compared with the control IgG antibody-treated SCI rats. With permission from *Annals of Neurology*. * $p < 0.01$; ** $p < 0.001$.

contains myelin-associated neurite growth inhibitors such as Nogo-A that seem to play a crucial role in preventing regeneration of lesioned axons in adult animals [1, 2]. The same inhibitory myelin proteins probably also inhibit structural plasticity in higher vertebrates [2, 3]. Different antibodies that neutralize the inhibitory activity of Nogo-A have been developed and their regeneration-enhancing effect for CNS axons was tested both in vitro and in vivo.

In vivo studies were performed with adult rats lesioned at spinal cord thoracic level T8, which received anti-Nogo-A antibodies through the cerebrospinal fluid. In Nogo-A antibody-treated animals but not in control antibody-infused animals, regenerating corticospinal (CS) tract fibers were seen growing down the spinal cord (fig. 1A). These fibers exceeded a length of 8 mm and arborized profusely into the gray matter. In addition to regenerative growth of lesioned axons, sprouting and compensatory fiber growth was observed from spared, unlesioned fiber systems [3–6]. This suggested that both these phenomena – regeneration and structural plasticity – probably contribute to the often high degree of functional recovery observed in these animals. The behavioral results summarized below also suggest that the fibers growing and regenerating in the adult CNS tissue seem to be able to recognize functionally meaningful targets and to form new functional circuits.

In spinal cord-injured (SCI) adult rats, intrathecal infusion of anti-Nogo-A antibodies resulted in impressive improvements of functional recovery in particular of locomotor functions like swimming, running, or crossing horizontal ladders or beams (fig. 1B, C) [4, 6, 7]. In these studies malfunctions like pain or spasticity – both possible indicators for misled axons or erroneous connections – were consistently absent, suggesting that the new connections and circuits are formed with high specificity. Functional magnetic resonance imaging (functional MRI) was applied to study the cortical representation of forelimbs and hind limbs and their responsiveness to peripheral sensory stimulation after SCI. In contrast to control antibody-treated animals, SCI rats treated with anti-Nogo-A antibodies revealed significant cortical responses in functional MRI after hind paw stimulation, suggesting restitution of afferent spinocerebral pathways [6].

In the future, the impact of rehabilitative training on regeneration and plasticity in the spinal cord and brain (including the neocortex), and on functional recovery will be studied in adult rats. For this, a combination of specific lesion models with anti-Nogo-A antibody treatment, different tracing techniques and physical training are envisaged. In addition to behavioral analysis, these experiments allow us to study effects on fiber growth and anatomical plasticity.

Very little information is available on the distribution of therapeutic antibodies infused into the cerebrospinal fluid. We therefore studied the distribution and tissue penetration of antibodies after 7–14 days of intrathecal infusion in adult rats and monkeys [8]. Anti-Nogo-A antibodies reached the brain and whole spinal cord and penetrated deep into the parenchyma where they bound to oligodendrocytes and nerve cells. They were subsequently internalized together with the endogenous cell surface Nogo-A, leading to a down-regulation of CNS Nogo-A levels.

Nogo-A knockout mice, which can serve as a useful proof of principle for the inhibitory effect of Nogo-A on regeneration, were successfully generated. CS tract regeneration was enhanced following injury in the absence of Nogo-A [9]. To exclude a role of background genes, these Nogo-A knockout mice were successfully backcrossed into two different commonly used mouse strains (unpublished data). After spinal cord lesion, Nogo-A-deficient mice of both strains showed enhanced regenerative sprouting and long-distance regeneration as compared to wild-type mice of the same background. Surprisingly, however, one of the strains (SV129) displayed significantly higher numbers of regenerating fibers than the Nogo-A knockouts of the other strain (C57Bl/6). These results confirm that Nogo-A is an important endogenous inhibitor of axonal regeneration in the adult spinal cord; they also demonstrate that the effects of Nogo-A deletion can be modified by mouse strain-specific genes.

The reaction of neurons and CNS tissue to anti-Nogo-A antibody treatment on the one hand and the consequences of loss of Nogo-A expression in knockout animals on the other hand are being studied at the molecular level by a functional genomics approach. The aim of these experiments is to identify molecules associated with axon (re-)growth, to gain insight into signaling mechanisms involved in regeneration processes, and to find cues involved in neuronal circuit formation in the adult CNS.

Enhancement of Functional Recovery and Axonal Regrowth in Monkeys Treated with Anti-Nogo-A Antibodies after Lesion of the Cervical Spinal Cord

In order to translate the anti-Nogo-A antibody strategy from rodents to subhuman primates, monkeys have been subjected to SCI. Since the general organization of the CS system is considerably different between rodents and primates, a proof of principle in monkeys represents a crucial step before clinical application to human patients. Furthermore, the monkey model is more appropriate than rodents to test whether anti-Nogo-A antibody treatment does not generate undesired secondary effects, such as chronic pain.

Twelve adult Macaque monkeys (*Macaca fascicularis* or *Macaca mulatta*; 3.5–5 kg; 3–4 years old) were trained to perform a manual dexterity task (fig. 2B). The monkeys retrieved food pellets from 50 wells using the opposition of thumb and index, i.e. the precision grip. The number of pellets retrieved within 30 s was determined (fig. 2A). After about 2 months a subhemisection was performed unilaterally at the C7/C8 level [10–12]. The lesion completely interrupted the main CS component in the dorsolateral funiculus (fig. 2C, arrow). Immediately after the lesion, 6 monkeys were treated with an anti-Nogo-A antibody delivered intrathecally near the lesion site for 4 weeks from an osmotic pump. An inactive control antibody was infused in the other 6 monkeys. Behavioral testing was continued for 2–3 months postlesion in order to compare the extent and time course of functional recovery between the two groups of monkeys. Finally, an anterograde neuroanatomical tracer (biotinylated dextran amine, BDA) was injected in the primary motor cortex contralateral to the cervical lesion, in order to trace the CS tract and its regenerated fibers.

In all monkeys, the hand homolateral to the lesion was dramatically impaired immediately after the lesion and then, over a period of a few weeks, progressive partial recovery of manual dexterity took place. The 2 monkeys shown in figure 2A exhibited a comparable cervical cord lesion, but the anti-Nogo-A antibody-treated monkey (red) recovered faster and much more completely than the control antibody-treated animal (blue). Overall, control antibody-treated monkeys exhibited a recovery of manual dexterity that was inversely correlated to the lesion extent (fig. 2D, blue circles), whereas anti-Nogo-A antibody-treated monkeys recovered faster and almost completely, irrespective of lesion size (fig. 2D, red squares). In addition to the above-mentioned ability to grasp pieces of food with the precision grip, a better re-

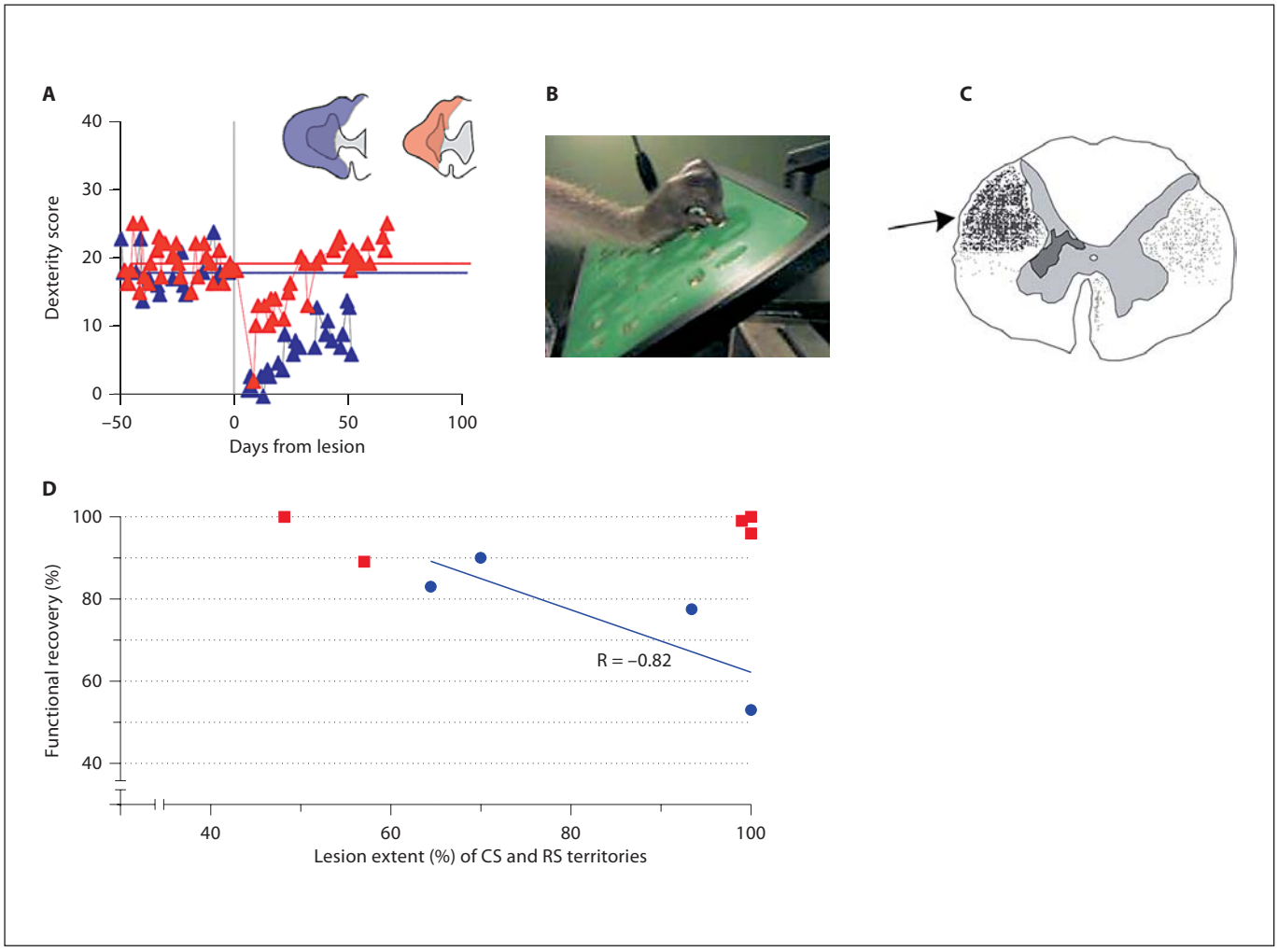

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Fig. 2. A Typical manual dexterity score (successful food pellet retrieval) derived from daily sessions of precision grip task (Modified Brinkman Board) for 2 monkeys: control antibody-treated (blue) and anti-Nogo-A antibody-treated (red). The dexterity score is high before the lesion, falls to a very low level after the spinal cord injury, and recovers to about 30–40% in the control, but to 100% of the original performance in the anti-Nogo-A antibody-treated animal. The insets (top) show the extent of the lesion on a cross section of the cervical spinal cord, for the control antibody- (blue) and anti-Nogo-A antibody-treated monkeys (red). The lesion completely interrupted the dorsolateral funiculus and covered a comparable extent of the hemicord in the 2 monkeys. **B** View of the left hand of a monkey while performing the grasping of a pellet from a well in the Brinkman Board test. **C** Frontal section of the cervical cord in an intact monkey showing the distribution of the corticospinal (CS) axons as a result of BDA injection in the right motor cortex. Each labeled CS axon is represented by a small dot. The arrow points to the main CS component, the crossed dorsolateral funiculus, which was destroyed by

covery in anti-Nogo-A antibody-treated monkeys was also observed for behavioral tasks testing other motor parameters, such as force and ballistic grasping or catching of moving objects.

A detailed anatomical investigation of the transected CS tract showed that application of antibodies neutralizing Nogo-A led to a significant increase in BDA-labeled CS axonal arbors and CS axonal swellings caudal to the lesion, as compared to control antibody-treated monkeys. Rostral to the lesion, the anti-Nogo-A antibody treatment induced a reduction of the incidence of axonal retraction bulbs, attenuation of CS axonal dieback and increased sprouting of CS fibers. Very few CS axons truly regenerated by recrossing the lesion; instead, anti-Nogo-A antibody treatment enhanced collateral sprouting, which gave rise to BDA-labeled axons seen growing around the lesion medially in the gray matter. The fibers then elongated into the denervated gray matter territory caudal to the lesion. The presence of CS axonal arbors caudal to the lesion correlated with the level of functional recovery, suggesting that the regenerative sprouting of the CS tract contributed to the recovery of manual dexterity. Very importantly, the monkeys treated for 4 weeks with the anti-Nogo-A antibody did not show any sign of chronic pain or change of general behavior, neither with their mates within the colony in the animal room nor with respect to the experimenters. The monkeys were in good health as indicated by a stable body weight postlesion during the infusion of the antibody. This study in monkeys extended the findings with anti-Nogo-A antibody therapy in rodents to primates, paving the way for clinical application to human SCI patients.

the lesion in the present study. **D** For 9 monkeys, the plot shows the relationship between the extent of the spinal cord lesion (abscissa) and the degree of functional recovery (ordinate). A functional recovery of 100% means that the postlesion score came back to the level of the prelesion score. The extent of the lesion was expressed in percent of the zone in the white matter of the hemicord including the dorsolateral funiculus (main CS tract component) and the rubrospinal (RS) tract. In control antibody-treated monkeys (●), the functional recovery was inversely correlated to the lesion extent ($r = -0.82$); in contrast, all the anti-Nogo-A antibody-treated animals (■) reached 90–100% of recovery.

Fig. 3. Assisted treadmill training within a driven gait orthosis (Lokomat). Subjects are suspended in a harness. Body weight support as well as treadmill speed can be adapted to the patient's ability. The orthosis is equipped with sensors to provide feedback of the subject's performance to the patient and the therapist. Different patient-cooperative control strategies are implemented to enhance training success (picture courtesy of Hocoma AG, Volketswil, Switzerland).

Clinical Issues

To document the changes at the neurological and functional level after human spinal cord injury and therefore provide a historical control group for future clinical trials, a European multicenter study (EM-SCI) was initiated in 2001 [13]. In this study, standardized assessments covering neurological, functional and electrophysiological aspects are done at defined time points after the injury. Additional protocols monitoring pain, bladder function and acute care treatment will be included in the near future. New tests for voluntary function of the legs covering the whole rehabilitation period and neurophysiological methods for the assessment of impaired function of specific spinal pathways are underway. Analysis of the currently more than 700 patient data allows a better stratification of patient subgroups and a more precise prediction of outcome. This will help recognize even small improvements in the recovery of functions and to monitor any significant effect of a new treatment.

If such a new treatment could produce axonal regeneration in the lesioned human spinal cord the spinal circuits below the lesion should be preserved. This is especially true for clinically complete SCI patients where only regeneration approaches may succeed. Recently, signs of decreased neuronal function in the lower spinal cord were found in chronic complete SCI patients; they were restricted to the specific motor behaviors affected by the injury, e.g. locomotion [14]. The affected spinal neuronal circuits seem to be at least partially different from spinal reflex circuits as the latter do not show such decreased function [15]. Possible preventive effects of early motor training combined with pharmacological interventions (e.g. L-dopa) are being evaluated. Earlier results have already shown that loading and hip position afferents are essential to stimulate the spinal neuronal circuits involved in locomotion [16].

Long-term training studies with chronic incomplete SCI patients showed that these patients profit from an assisted locomotor training for their mobility [17]. Robotic devices for gait (Lokomat®, fig. 3) and arm function (ARMin) with novel training and feedback strategies have been introduced and are expected to enhance the positive output of assisted training in patients with SCI [18, 19].

New insights into the integrity of spinal circuits will be provided by studies applying high-resolution MRI techniques (functional MRI, diffusion tensor imaging) to the injured spinal cord [20]. Although very challenging, this approach holds great promise for the assessment of spared tract anatomy, residual spinal cord function, and the recovery of spinal pathways in a noninvasive manner.

Electrophysiological recordings at the level of the lesion have been shown to serve as an objective diagnostic tool to assess the integrity of autonomic afferent nerve pathways and to distinguish between central and peripheral lesions [21]. In SCI patients, synchronized activation and inactivation of the autonomic and somatic pathways was shown to be necessary for appropriate urine storage and coordinated bladder voiding. The chronology of bladder dysfunction has been assessed, providing the basis for conditional neuromodulation in patients with SCI [22]. This is a promising treatment for bladder dysfunction, which is a more confining issue for a majority of SCI patients than the walking impairment.

In conclusion, the concerted efforts of basic scientists working with rat and monkey models of SCI as well as cell biological and molecular biological tools on the one hand, and clinical researchers including neurorehabilitation specialists and engineers on the other hand in the NCCR project 'Spinal Cord Repair' have led to the full translation of basic science findings into a clinical trial: in close collaboration with Novartis Pharma in Basel, a phase I clinical trial in humans with anti-Nogo-A antibodies was started in the spring of 2006.

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