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A Systematic Review of Directly Applied Biologic Therapies for Acute Spinal Cord Injury

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Abstract

An increasing number of therapies for spinal cord injury (SCI) are emerging from the laboratory and seeking translation into human clinical trials. Many of these are administered as soon as possible after injury with the hope of attenuating secondary damage and maximizing the extent of spared neurologic tissue. In this article, we systematically reviewed the available preclinical research on such neuroprotective therapies that are administered in a non-invasive manner for acute SCI. Specifically, we reviewed treatments that have a relatively high potential for translation due to the fact that they are already used in human clinical applications or are available in a form that could be administered to humans. These included: erythropoietin, NSAIDs, anti-CD11d antibodies, minocycline, progesterone, estrogen, magnesium, riluzole, polyethylene glycol, atorvastatin, inosine, and pioglitazone. The literature was systematically reviewed to examine studies in which an *in vivo* animal model was utilized to assess the efficacy of the therapy in a traumatic spinal cord injury paradigm. Using these criteria, 122 studies were identified and reviewed in detail. Wide variations exist in the animal species, injury models, and experimental designs reported in the preclinical literature on the therapies reviewed. The review highlights the extent of investigation that has occurred in these specific therapies, and points out gaps in our knowledge that would be potentially valuable prior to human translation.

Key words: anti-nogo antibodies; biologic therapies; cethrin; chondroitinase ABC; spinal cord injury

Introduction

A number of biological therapeutic interventions have been developed for traumatic spinal cord injury (SCI) in which the treatment reaches the injured spinal cord by direct application rather than by systemic distribution. While their mechanisms of action may share similarities with some pharmacologic agents drugs (reviewed in a separate article), their mode of direct application imposes practical considerations that are distinct from drugs, which can be administered orally or parenterally. The delivery of biological therapeutic interventions to the spinal cord can be achieved by any of three possible routes, namely:

1. Applying the agent to the overlying dura (and depending on penetration through the dura, diffusion through the cerebrospinal fluid [CSF] within the intrathecal space and into the cord)
2. Injecting the agent into the intrathecal space (and depending on its diffusion through the CSF into the cord)
3. Injecting the agent directly into the spinal cord

Within the enormous scope of pre-clinical investigation for SCI, there are numerous compounds that have been evaluated after some form of direct application to the spinal cord. Here, we limit the scope of this systematic review to three therapeutic strategies that have received considerable attention in the past two decades and are in various stages of clinical translation to promote endogenous neuroregenerative repair following SCI: (1) the degradation of inhibitory chondroitin sulfate proteoglycans with chondroitinase ABC; (2) the neutralization of myelin-mediated inhibition of neurite outgrowth with anti-Nogo (IN-1) antibodies or other Nogo-related approaches; and (3) the inhibition of Rho activation. The present systematic review provides an overview of the body of pre-clinical evidence that supports, or fails to support,

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the translation of the aforementioned neuroregenerative biological therapeutic strategies into human trials for SCI.

Methods

A PubMed search was conducted on "the therapy" and "spinal cord injury" (e.g., "chondroitinase ABC and spinal cord injury"). From the list of studies generated through this fairly indiscriminate search, we then applied the following inclusion criteria to systematically review the pre-clinical literature on biological therapies applicable to SCI:

1. Studies that include testing of the therapy in an *in vivo* animal model of spinal cord injury (i.e., exclusively *in vitro* studies were excluded)
2. Studies in which the spinal cord is *traumatically* injured with a contusion or compression device or is partially or completely transected (i.e., non-traumatic local or global ischemia, photochemical reaction, traumatic root avulsion, or dorsal root entry zone models were excluded)
3. At least *two peer-reviewed publications* available on the therapy (i.e., biological therapies supported by less than two peer-reviewed publications were excluded)

The data from the studies that fit the criteria were then tabulated to depict the animal model, injury model, treatment's dose and timing, experimental groups tested in the study, number of animals used (or "n" per group), and reported behavioral and non-behavioral outcomes (e.g., histologic, biochemical, or physiologic outcomes). A summary statement about the body of literature was then generated.

Results

Using this selection process, we identified the following therapies: chondroitinase ABC, anti-Nogo approaches, and Rho antagonists (Table 1). The PubMed searches on these therapies were initially conducted in the spring/summer of 2008 by SCI researchers across Canada and an updated search was conducted in June 2009. By applying the previously described criteria (essentially, *in vivo* animal studies utilizing a traumatic model of spinal cord injury), the following studies were generated, and the tables for each of these respective therapies are listed below.

Chondroitinase ABC

This systematic review revealed 23 studies, which utilized chondroitinase ABC (ChABC) in an *in vivo* model of SCI (Table 2). As would be expected, the majority of studies involved the use of the rat species, although one mouse model (Carter et al., 2008) and one cat model (Tester and Howland, 2008) were tested as well. The injury models typically were that of "sharp" or "crush" injuries, such as a hemisection, over-hemisection, transection, dorsal crush, or forceps compression, reflecting the mechanism of action of ChABC and the desire to measure axonal sprouting/growth in response to it. Two studies employed an NYU impactor for a thoracic contusion injury (Ikegami et al., 2005; Iseda et al., 2008). Notably, Iseda and colleagues (2008) actually compared ChABC in both a contusion and a hemisection model and reported that ChABC promoted sprouting only in the hemisection, but not the contusion, SCI model.

Table 1. Directly Applied Biologic Therapies

Therapy	*Mode of administration*	*Published studies meeting criteria (no.)*
Chondroitinase ABC	Direct injection into cord	25
Anti-Nogo approaches	Intrathecal application	24
Anti-Rho approaches	Direct injection into cord or extradural application (e.g., Cethrin)	9

The majority of injuries occurred in the thoracic spine, although eight of the 24 articles employed cervical injury models. The mode and timing of administration in this series of articles varied substantially. The ChABC was typically either injected directly into the cord at various depths and distances from the injury site or delivered via intrathecal injection/infusion. The dosing regimen ranged from immediate, single application (e.g., Tan et al., 2006; Yick et al., 2003, 2004) to continuous infusion over many weeks (e.g., Massey et al., 2008).

A number of researchers have described the use of ChABC as a supplement to a cell transplant therapy such as Schwann cells, olfactory-ensheathing glia, peripheral nerve transplants, fetal cells transplants, or neural precursor cells (Chau et al., 2004; Fouad et al., 2005, 2009; Houle et al., 2006; Ikegami et al., 2005; Vavrek et al., 2007). The inclusion of these studies in this table could be debated as the ChABC was utilized as an adjunct (and by itself these are less informative about the therapeutic applicability of ChABC as a stand-alone therapy). Nonetheless, we include them here as they fulfilled the basic criteria of being *in vivo* assessments within a traumatic SCI model.

In keeping with the interest in ChABC's putative mechanism of action, 13 of the 24 studies were solely focused on anatomical/histologic outcomes, with no measurement of behavioral outcome (Carter et al., 2008; Chau et al., 2004; Fouad et al., 2009; Iaci et al., 2007; Ikegami et al., 2005; Iseda et al., 2008; Massey et al., 2006, 2008; Shields et al., 2008; Tan et al., 2006; Tom and Houlé, 2008; Vavrek et al., 2007; Xia et al., 2008; Yick et al., 2003). In general, independent laboratories report ChABC promoting increased axonal sprouting (particularly serotonergic fibers) using either anterograde or retrograde tracing techniques. As an adjunct treatment to a transplantation therapy (e.g., Schwann cells, peripheral nerve graft), ChABC reportedly improved axonal growth and/or serotonergic sprouting (Chau et al., 2004; Fouad et al., 2005; Houle et al., 2006; Shields et al., 2008; Tom and Houlé, 2008). Xia and colleagues (2008) reported a reduced cystic cavity, suggestive of some neuroprotective effect. Carter and colleagues (2009) reported the prevention of neuron atrophy in the cortical regions that contained CSNs projecting to the thoracic spinal cord. In the same article, the effect on the intracellular signaling was demonstrated.

As for behavioral outcomes, the use of cervical injury models by a number of authors allowed for the assessment of forelimb functional recovery. In the cervical injury models, evidence for improvements in forelimb function with ChABC was modest. While Yick and colleagues (2004), Houle and

Table 2. Chondroitinase ABC

Study	*Animal model and injury model*	*Intervention and timing*	*Experimental group*	*Outcome: histologic/biochemical/physiological/behavioral*
Fouad et al., 2009	Model: adult female Fischer rats Injury: T8 transection, 1 mm spinal cord removed	ChABC • 2 μL of 10 μg/mL SCs + OEG, 30 μL • In the lesion site in Mg-filled channel at 0 h PI	SCI + • ChABC + SC + OEG in Mg channel ($n = 8$) • Mg channel alone ($n = 7$) No SCI + • Control ($n = 4$)	Histologic/biochemical/physiological: Cell implantation and ChABC delivery prevented thickening of the bladder; prevented disorganization of $\alpha\alpha$-smooth muscle bundles, collagen (type III) deposition (at 14 wk) and reduced viscoelasticity in the bladder walls. • Sparse and fairly short serotonergic fibers in both treated and untreated cords. Behavioral: Cell implantation and ChABC delivery resulted in improvements in bladder function.
Carter et al., 2008	Model: male and female YFP-H mice, 12 w, 20–25 g Injury: T12 crush with forceps to a depth of 0.5 mm	ChABC • 6 μL of 10 U/mL, given ICV or IT Penicillinase, ICV or IT • 6 μL of 10 U/mL • Slow bolus injection at 0 h PI then on 2, 4, 6, 8, and 10 d PI	SCI + • ChABC, ICV ($n = 8$) • ChABC, IT ($n = 8$) • Penicillinase, ICV ($n = 8$) • Penicillinase, IT ($n = 8$) • Control PI ($n = 7$) • Sham + ChABC ($n = 4$) • Sham + penicillinase ($n = 4$) • Sham ($n = 11$)	Histologic/biochemical/physiological: ChABC given ICV or IT similarly degraded CSPGs at the site of injury. • ChABC promoted neuroprotection of corticospinal projection neurons and prevented cell atrophy by >50% in the cortical regions that contained CSNs projecting to the thoracic spinal cord. • ChABC led to robust sprouting at the lesion site (with possible retrograde protection of neuronal cell bodies). • ChABC significantly increased phosphorylation of ERK1 (p44 MAPK, but not p42 MAPK) at lesion site. Only intrathecal ChABC (but not IVC) ChABC increased PKC $\beta\beta$II and Akt expression. • No significant differences in GSK-3$\beta\beta$ expression or phosphorylation Behavioral: not reported
Xia et al., 2008	Model: adult female SD rats, 250–300 g Injury: T9-10 lateral hemisection, 2 mm tissue removed	ChABC • 6 μL of 10 U/mL Vimentin anti-sense cDNA in retrovirus • 6 μL, titer 3.9×10^8 cfu/mL • Starting on day of injury, direct infusion to lesion site; repeated every 2nd day for total of 8 infusions	SCI + • ChABC • ChABC + vimentin anti-sense cDNA • Vimentin anti-sense cDNA • Saline or retrovisus without vimentin cDNA $N \geq 3$/group	Histologic/biochemical/physiological: ChABC and combined treatment (ChABC + vimentin anti-sense cDNA) greatly reduced CSPGs (CS-56) immunostaining in the scar at 2 wk PI, and reduced cystic cavity at 8 wk PI. • GFAP and vimentin expression was dramatically upregulated after SCI, especially in the scar tissue. Vimentin anti-sense, but not ChABC, reduced vimentin and GFAP expression at lesion site. Behavioral: not reported
Shields et al., 2008	Model: adult female SD rats, 200–225 g Injury: C3 dorsal hemisection (laceration with Vibraknife, 1.5 mm deep)	ChABC • 0.18 U in 6 μL (high dose) • 0.06 U in 6 μL (low dose) Penicillinase • 6 μL (30 μg/mL) 5-wk survival	SCI + • ChABC high dose ($n = 9$) • ChABC low dose ($n = 8$) • Penicillinase ($n = 5$)	Histologic/biochemical/physiological: ChABC significantly enhanced the sensory axonal regeneration (number and length of axons) in a dose-dependent manner. Axons extended across the lesion gap and into the distal spinal cord stump in 2 of 8 (low dose) and in 3 of 9 (high dose) rats compared with none in the control group.

(Continued)

Table 2. Chondroitinase ABC (Continued)

Study	Animal model and injury model	Intervention and timing	Experimental group	Outcome: histologic/biochemical/physiological/behavioral
		• Starting on day of injury, direct infusion to lesion site; repeated every 2nd day for total of 5 infusions		• ChABC decreased CSPG, NG-2, and CS56 expression in a dose-dependent manner. The presence of CSPG cleaved products (2B6 immunoreactivity) was apparent in both ChABC-treated groups. • ChABC infusion did not influence laminin and GFAP immunoreactivity in the cord. Behavioral: not reported
Iseda et al., 2008	Model: adult female SD rats, 10–12 wk old Injury: • T11 lateral hemisection • T11 contusion MASCIS impactor (10.0 g × 12.5 mm)	ChABC • 3 μL of 1.125 U/μL in aCSF • 1 μL injected at the lesion site; 1 μL injected 2 mm rostrally and 2 mm caudally (injections 1 mm deep in the midline of the cord) • Within 1 h PI–acute • 1, 2, or 4 wk PI–delayed	Hemisection SCI + • ChABC, acute • ChABC, 1, 2, or 4 wk delayed • aCSF Contusion SCI + • ChABC, 1 or 4 wk delayed • aCSF In total, 48 contused and 48 hemisected rats (but n/group not reported)	Histologic/biochemical/physiological: compared the spinal cord contusion and hemisection models • Intraspinal injection of 3.375 U ChABC acutely or 1, 2, or 4 wk PI effectively eliminated CSPG to nearly undetectable levels in both hemisected and contused spinal cords from 4 d to 3 wk after treatment, with an increase of 2B6 immunoreactivity at the injured site (digested product of CSPG). • Many CST axons grew around the lesion site in hemisected cords after both acute and chronic ChABC treatment, but not in contused spinal cords. • Significant increases in GAP43 staining in the injury site vs. controls at 4 d, but no differences detected by 3 wk. • High dose of ChABC did not produce detectable changes in staining for GFAP in contusion model. Behavioral: not reported
Tom and Houlé, 2008	Model: adult female SD rats, 225–250 g Injury: • Aspiration lesion of C5 right dorsal quadrant of ~1 mm^3	ChABC • 1 μL of 20 U/mL • Microinjection into the ventral spinal cord immediately rostral and caudal to C5 at 0 h PI, then on d 2 and 4 ChABC + PNG extending from C3 to C5	C5 SCI + • ChABC ($n = 3$) • Saline ($n = 5$) C5 SCI and PNG + • ChABC ($n = 4$) • Saline ($n = 8$)	Histologic/biochemical/physiological: ChABC digested CSPG (based on 2B6 immunoreactivity) • Microinjections of ChABC did not affect the inflammatory response (ED1 + macrophages/microglia) at 5 d PI. • In the PNG experiment to assess regeneration, ChABC promoted significantly more axons to grow out of the graft and re-enter the spinal cord, extending at least 500 μm beyond the PNG-spinal cord interface. Behavioral: not reported
Iaci et al., 2007	Model: adult female Long-Evan rats Injury: T9/10 forceps crush injury for 15 s (blade separation, 0.9 mm)	ChABC • 0.06 U/rat per dose in 4 μL of aCSF Penicillinase 228 μg/mL in 4 μL aCSF • Intrathecal infusion just caudal to T9/10 at 0 h PI, then every 2nd d for up to 2 wk	SCI + • ChABC • Penicillinase • aCSF Sacrificed on 1, 7, 14, 21 and 35 d, $n = 3$–5/time point	Histologic/biochemical/physiological: ChABC intensively cleaved the GAG chains rostral and caudal to injury site; ChABC did not affect the NG2, neurocan, or phosphacan protein content and had a little impact upon NG-2 and neurocan mRNA. Hence, the effects of ChABC are likely due to removal of GAG chains but not a decrease in CPSG content. Behavioral: not reported

Vavrek et al., 2007	Model: adult female Fisher rats Injury: T8 transection, 4 mm spinal cord removed	ChABC • 2 μL of 10 μg/mL at 0 h PI, then every 2nd day for 4 wk SCs, 5×10^6 cells at 0 h PI in Mg-filled channel OEG, 10^5 cells into each stump at 0 h PI	SCI + • ChABC + SCs in Mg channel + OEG ($n = 5$) • No treatment ($n = 4$)	Histologic/biochemical/physiological: Treated rats had retrogradely FlouroGold-labeled cells in the reticulospinal nuclei, vestibular nuclei, and the raphe nucleus, as well as in the spinal cord. Cell numbers were highest in the thoracic spinal cord (T7-T8) and the lateral vestibular nucleus. • The animal with the largest bridge diameter also presented the largest number of regenerated cells. • Positive correlation was found between the number of regenerated cells and amount of ChABC digestion. • No labeled cells were present within the red nucleus, nor were they found in the sensorimotor cortex. • No retrogradely labeled cell bodies were found in the untreated group. Behavioral: not reported
Garcia-Alias et al., 2008	Model: Lister hooded rats, 250–300 g Injury: C4 dorsal column crush (forceps compression $\times$ 20 s)	ChABC • 6 μL at 100 U/mL = 0.6 U, injection via cannula into the right lateral ventricle, given 0 h PI, or at 2, 4, or 7 d, then q48h for total of 7 applications	SCI + • ChABC • Control SCI $N = 6$/group	Histologic/biochemical/physiological: With CST anterograde tracing, all ChABC groups had less dieback and more axonal growth than did control group, but the statistics are not reported. Behavioral: Staircase pellet retrieval task (no. and %): effect only in the acute ChABC at 42 d PI ($p < 0.05$). Grip strength: no difference among groups. Placing response: all ChABC groups better than control ($p < 0.05$). Forepaw stride length: all ChABC groups better than control at 42 d PI ($p < 0.05$)
Massey et al., 2008	Model: male SD rats, 275–350 g Injury: T1 dorsal column crush (forceps compression $\times$ 10 s)	ChABC • 690 nL at 20 U/mL NT-3 lentivirus • Microinjection 0.3 mm deep to the lateral junction of the right fasciculus gracilus and the right nucleus gracilis • ChABC given 0 h PI, NT-3 given 12 d PI	SCI + • ChABC + NT-3 ($n = 6$) • ChABC ($n = 4$) • NT-3 ($n = 5$) • Vehicle $n = 6$)	Histologic/biochemical/physiological: Regeneration into gracile nucleus of GFP-labeled DRG implanted rostral to lesion site 14 d PI. Control, 336.95 μm +/− 140.96; ChABC, 1276.2 μm +/− 243.5, $p < 0.05$; NT-3, 1118.5 μm +/− 219.8, $p < 0.01$; ChABC + NT-3, 10,820.3 μm +/− 2583.5, $p < 0.001$. Behavioral: not reported
Tester and Howland, 2008	Model: adult female cat Injury: T10 hemisection	ChABC • 0.025 U, via gelfoam placed in lesion site at 0 h PI for 30 min, then q48h for 1 mo via port tubing sutured to muscle	SCI + • ChABC ($n = 3$) • Deactivated ChABC ($n = 2$) • Port placement + saline ($n = 1$) • Control SCI ($n = 3$)	Histologic/biochemical/physiological: Sprouting of 5-HT fibers into the rostral portion of the lesion showed a trend ($p = 0.08$) for greater numbers in the ChABC group. Behavioral: Plantar stepping: no difference of onset of recovery Ladder, pegboard, narrow beam: quicker rate of recovery in the ChABC group ($p < 0.05$). On pegboard at 16 and 20 wk PI, ChABC were better than controls.

(Continued)

Table 2. Chondroitinase ABC (Continued)

Study	*Animal model and injury model*	*Intervention and timing*	*Experimental group*	*Outcome: histologic/biochemical/physiological/behavioral*
Barritt et al., 2006	Model: male Wistar rats, 220–250 g Injury: C4 dorsal column crush	ChABC • 6 μL at 10 U/mL = 0.060 U, intrathecal bolus injections delivered via a flexible Silastic catheter inserted subdurally at the C4 level and pushed 8 mm, given at 0 h PI, then q48h for 10 d	SCI+ • ChABC • SCI control No SCI+ • ChABC • control $N = 16$/group	Histologic/biochemical/physiological: In anterograde CST tracing, ChABC group produced increased sprouting proximal to the lesion site, and distal to injury site at C5 ($p < 0.05$), but not C6. ChABC elicited increased 5HT sprouting caudal to the injury in the ventral horn ($p < 0.05$). Behavioral: No difference in thermal or mechanical stimuli among the groups. However, the injured animals did not develop thermal hyperalgesia or mechanical allodynia.
Houle et al., 2006	Model: female SD rats, 225–250 g Injury: C3 hemisection	ChABC • 0.005 U at 0 h PI, then 0.011 U/d for 7 d via an osmotic minipump and cannula	SCI+ • PNG transplant + ChABC ($n = 7$) • PNG transplant ($n = 5$)	Histologic/biochemical/physiological: In rubrospinal anterograde tracing, mean length of axons entering spinal cord from PNG: control PNG = 11.5 +/− 5.3 mm, PNG + ChABC = 1271.6 +/− 48.5 mm ($p < 0.01$) Behavioral: Rearing cylinder–no statistical effect. Rope ladder–no quantifiable difference. Grooming task: control, 1.6; ChABC, 2.86 ($p < 0.05$)
Huang et al., 2006	Model: Female SD Rats 250–300 g Injury: T8 Transection	ChABC • 6 μL at 1 U/mL = 0.006 U, or at 5 U/mL = 0.030 U, infusion via catheter placed in the epidural space from C1 down to T8 level, at 0 h PI, then q48h for 2 wk	SCI+ • ChABC • Tube • Saline • Control SCI $N = 4$/group	Histologic/biochemical/physiological: In anterograde CST tracing, only the 1 U/mL (0.006 U) ChABC group was reported to induce growth across lesion site (but not quantified). Large cysts were found in the 5 U/mL (0.030 U) ChABC group. Behavioral: BBB: ChABC at the dose of 1 U/mL (0.006 U) had higher scores compared to controls (peak BBB in control ~2.7, ChABC ~6). No effect with 5 U/mL (0.030 U) of ChABC
Kim et al., 2006	Model: female SD rats, 200–250 g Injury: C4 over hemisection	ChABC • 0.2 U delivered to injury site via gelfoam at 0 h PI, then at 3, 7, and 11 d via intrathecal injections	SCI+ • ChABC ($n = 7$) • Fetal spinal cord transplant + ChABC ($n = 18$) • Fetal spinal cord transplant ($n = 19$) • Control SCI ($n = 7$)	Histologic/biochemical/physiological: In anterograde CST tracing, no effect in any group. 5HT staining in the ventral gray matter distal (C6/7) to the injury site improved only in the transplant + ChABC group (45.2%) compared to control (21.7%, $p < 0.05$). ChABC (26.3%), transplant (30.4%). Behavioral: Gridwalk: no effect of ChABC, but there was an effect of transplant + ChABC ($p < 0.01$). With rearing cylinder, no effect in any group, but "functional touching" in the cylinder and tape removal were improved only in the transplant + ChABC group.
Massey et al., 2006	Model: male SD rats, 250–300 g Injury: C6/7 quadsection	ChABC • 1 μL at 50 U/mL = 0.050 U, right medulla microinjection at 0.3–0.5 mm below the dorsal pial surface and just lateral to the cuneate nucleus, given at 0 h PI	SCI+ • ChABC ($n = 6$) • Vehicle to cuneate nucleus of medulla ($n = 5$)	Histologic/biochemical/physiological: CTB-labeled sensory fibers in the brainstem. The ChABC group had significantly more labeling ($p < 0.01$) (control 165.41 +/− 67.73, ChABC 323.73 +/− 69.53). Microelectrode recording from cuneate nucleus: significantly more sites responding to forelimb stimulation in ChABC group ($p < 0.001$) (control = 46.3% +/− 3.3, ChABC 74.5% +/− 4.8) Behavioral: not reported

Tan et al., 2006	Model: female SD rats, 175–200 g Injury: T8 dorsal bilateral transaction, lesion extended ventrally to the depth of the central canal (~1.5 mm)	Anti-NG2 MAbs • 50 μL of neutralizing or non-neutralizing anti-NG2 MAbs, via gelfoam at injury site ChABC • 10 μL of 2.5 U/mL, via gelfoam at injury site, given at 0 h PI	SCI + • Anti-NG2 MAbs ($n = 7$) • Non-neutralizing anti-NG2 MAbs ($n = 5$) • Conditioning treatment + anti-NG2 MAbs ($n = 7$) • Conditioning treatment + non-neutralizing anti-NG2 MAbs ($n = 7$) • ChABC ($n = 6$) • Penicillinase control ($n = 3$)	Histologic/biochemical/physiological: In animals treated with the neutralizing anti-NG-2 antibodies, labeled axons penetrated the caudal border of the lesion and grew into and beyond the lesion center. Combining a conditioning treatment with NG-2 MAbs resulted in sensory axon regeneration past the glial scar and into the white matter rostral to the injury site. The combinatorial approach neutralized extrinsic inhibition and increased intrinsic growth results in anatomically correct axon regeneration, a prerequisite for functional recovery. Behavioral: not reported
Caggiano et al., 2005	Model: female Long-Evans rats Injury: T9/10 mild, moderate, and severe forcep compression injury	ChABC • 0.06 U, delivered intrathecally using a mechanical syringe, 10 min PI, then q48h for 2 wk	SCI + • Mild ChABC ($n = 13$) • Moderate ChABC ($n = 15$) • Severe ChABC ($n = 7$) • Mild control ($n = 14$) • Moderate control ($n = 15$) • Severe aCSF control ($n = 5$) • Severe control ($n = 6$)	Histologic/biochemical/physiological: not reported Behavioral: BBB: ChABC (peak, 9.1 +/− 0.64) improved scores in moderate injury (control peak BBB, 6.85 +/− 0.48), $p < 0.05$ from 21 d PI onward (except d 42 and 49) and severe injury control (peak BBB, 3.67–4.0 +/− 1.2–0.87), ChABC (7.5 +/− 1.43), $p < 0.05$ at 28, 49, and 63 d PI, (but not at 35, 42, 56, 70, or 77). No effect on mild injury (control, 9.7 +/− 0.63, ChABC, 9.9 +/− 0.5). Bladder: Reduced residual volume in the ChABC group in the moderate injury (but not in the other two injury models).
Fouad et al., 2005	Model: female Fischer rats 165−180 g Injury: T8 complete transection	ChABC • 2 μL at 10 U/mL = 0.02 U, + Mg bridge + SCs and rostrocaudal OEC grafts, delivery via a minipump, given at 0 h PI, then q48h for 4 wk	SCI + • Mg bridge + SC and rostrocaudal OEC grafts ($n = 6$) • Mg bridge + SCs and rostrocaudal OEC grafts + ChABC ($n = 5$) • Mg bridge (control, $n = 8$)	Histologic/biochemical/physiological: 5-HT fibers distal to lesion were higher in the transplant + ChABC than control, but no correlation between 5-HT and behavior. Corticospinal and medial reticular formation: Only a few axons entered the graft and none were observed caudal to graft–no difference between groups. Number of myelinated axons in the bridge was significantly higher in the transplant + ChABC. Myelinated axons in bridge correlated with BBB ($r = 0.63$). Behavioral: BBB: The transplant + ChABC had higher scores than the other two groups at 8 and 9 wk PI. Von Frey hair stimulation at base of tail: Both transplant and transplant + ChABC had lower thresholds.
Ikegami et al., 2005	Model: female SD rats, 230−250 g Injury: T10 NYU impactor (10 g × 25 mm)	ChABC • 12 mL at 200 U/mL = 2.4 U/d + NPSC + Infusion via minipump with the tip of the silicon tube placed just caudal to the level of injury (vertebral T11), continuously from 0 h PI for 2 wk, NPSC transplanted 1 wk after injury	SCI + • NPSC + ChABC ($n = 14$) • NPSC + inactivated ChABC ($n = 7$) • inactivated ChABC ($n = 5$)	Histologic/biochemical/physiological: NPSC migration: ChABC was reported (pictures displayed) to promote greater migration of NPSC into the host tissue (not quantified). Total GAP-43 positive fibers in the lesion center revealed differences among the groups ($p < 0.05$): control ~200; NPSC ~500; ChABC + NPSC ~1400. Behavioral: not reported

(Continued)

Table 2. Chondroitinase ABC (Continued)

Study	*Animal model and injury model*	*Intervention and timing*	*Experimental group*	*Outcome: histologic/biochemical/physiological/behavioral*
Chau et al., 2004	Model: female Fischer rats Injury: T8 hemisection	ChABC • 0.06 U/d with SC-seeded guidance channels, intrathecal infusion via minipump from 0 h PI to ~d 16	SCI + • 3 mm SC/Mg mini-channel + ChABC • 3 mm SC/Mg mini-channel $N = 12$/group	Histologic/biochemical/physiological: In anterograde tracing, control animals displayed no growth caudal to mini-channel. ChABC group: 7/12 animals displayed growth caudal to mini-channel with the mean axonal growth in the 7 animals being 3.18 mm +/− 0.98 (other 5 not included). Number of myelinated axons in the channel was higher in the ChABC group ($p < 0.002$, $n = 5$; control ~450, ChABC ~1550). Cavitation was reduced in the ChABC group ($p < 0.02$, $n = 3$). Behavioral: not reported
Yick et al., 2004	Model: SD rat 200−250 g Injury: C7 right hemisection	ChABC • 10 μL of 2.5 U/mL = 0.025 U via soaked gelfoam placed on top of lesion site, at 0 h PI Lithium chloride IP • 85 mg/kg (correct dose in erratum), daily	SCI + • ChABC • Lithium • Lithium + ChABC • PBS $N = 4$/group for behavioral analysis, Western blotting, and immunohistochemistry $N = 5$/group for axonal tracing	Histologic/biochemical/physiological: Retrograde tracing (Fluoro-gold) of rubrospinal neurons. Lithium–no effect, ChABC (22.1 % +/− 4.6) better than control (2.3% +/− 0.9, $p < 0.001$); lithium + ChABC (41.5% +/− 4.6) better than ChABC and control ($p < 0.001$). Behavioral: Forelimb use in cylinder: lithium (18.1%, +/− 3.7)–no effect; ChABC group (29.1% +/− 1.9) better than control (7.7% +/− 3.0, $p < 0.01$). Lithium + ChABC (38.7% +/− 5.2) better than control ($p < 0.01$) and ChABC ($p < 0.05$).
Yick et al., 2003	Model: female SD rats, 9 wk old Injury: T11 hemisection	ChABC • 10 μL of 2.5 U/mL = 0.025 U via soaked gelfoam placed on top of lesion site, at 0 h PI	SCI + • ChABC Sham $N = 6$/group	Histologic/biochemical/physiological: C7 retrograde labeling of L1 Clarke's neurons at 4 wk PI: ChABC, 12.3% +/− ~1.5; control 0. Behavioral: not reported

Bradbury, *Nature*, 2002	Model: Adult Male Wister rat Injury: C4 dorsal column crush	ChABC • 6 μl at 10U/ml = 0.06 U Bolus injections through an intrathecal silastic catheter with the tip just rostral to the SCI. • injections began @ Oh PI, then every 2nd day for 10 days	1. SCI + ChABC (n = 12 + 5) 2. SCI control (n = 12 + 9) 3. Sham (n = 12 + 8) (anatomical/e-phys + behavioral analysis)	Histologic / Biochemical / Physiological: ChABC Improved CST growth/sprouting 2 mm proximal thru 1 mm distal to lesion (but not 2 - 5 mm distal) $p < 0.05$. Behavioral: ChABC group improved; Beam walk 2-6 weeks; Grid walk 2-6 weeks, Stride length, Stride width. No effect on the sensory test of tape removal. Electrophysiology postsynaptic potentials were better in the ChABC group 1 - 7 mm distal to lesion ($p < 0.001$)
Yick, *NeuroReport*, 2000	Model: Female SD Rats 200 - 250 g; Injury: T11 hemisection	ChABC • 10 μl of ChABC at 1.0 U/ml = 0.010 U 2.5 U/ml = 0.025 U 5.0 U/ml = 0.050 U Soaked gelfoam placed on top of implanation site @ Oh PI	1. SCI + Peripheral nerve graft (PNG) + 1.0 U/ml ChABC (n = 6) 2. SCI + PNG + 2.5 U/ml ChABC (n = 8) 3. SCI + PNG + 5.0 U/ml ChABC (n = 6) 4. SCI + PNG + BDNF (n = 3) 5. SCI + PNG + vehicle (n = 6) 6. Control SCI + PNG (n = 6)	Histologic / Biochemical / Physiological: counting of Fluoro-Gold retrogradely labeled Clarke neurons reveals 5.8% with 1.0 U/ml ChABC, 2.0% with 2.5 U/ml ChABC, and 12.8% with 5.0 U/ml ChABC. No retrogradely labeled cells with BDNF, Vehicle, or PNG alone. Behavioral: Not tested

aCSF, artificial cerebrospinal fluid; BBB, Basso, Beattie and Bresnahan locomotor test; BMS, Basso Mouse Scale; C5, cervical vertebra 5; ChABC, chondroitinase ABC; CSPG, chondroitin sulfate proteoglycan; CST, corticospinal tract; CTB labeled, cholera toxin subunit B; DREZ, dorsal root entry zone; GAG, glycosaminoglycan; GAP, growth-associated protein; GSK-3$\beta\beta$, glycogen synthase kinase-3$\beta\beta$; GFAP, glial fibrillary acidic protein; ICV, intracerebroventricularly; IT, intrathecally; MAb, monoclonal antibody; Mg, Matrigel; NT-3, Neurotrophin-3; NPSC, neural precursor cells; OEG, olfactory-ensheathing glia; PI, post-injury; PNG, peripheral nerve graft; q48h, interval 48 hours; SC, Schwann cell; SCI: spinal cord injury; SD rats, Sprague-Dawley rats; T8, thoracic vertebra 8; YFP-H, yellow fluorescent protein.

colleagues (2006), and Garcia-Alias and colleagues (2008) reported some improved forelimb performance with ChABC treatment (the latter in a number of metrics), significant improvements with ChABC alone were not observed by others (Kim et al., 2006). In thoracic injury models, behavioral recovery with ChABC treatment alone was found to be modest in some studies (Caggiano et al., 2005; Huang et al., 2006; Tester and Howland, 2008), while others described no improvements (Barritt et al.). Fouad and colleagues (2009) reported the positive effects of ChABC on bladder function and morphology. In combination with a cell transplant therapy, behavioral recovery was greater when the transplant was combined with ChABC than without (Fouad et al., 2006; Kim et al., 2006), suggesting that the ChABC may play an important role as an adjunct therapy for such transplants.

Anti-Nogo approaches

The approach of targeting myelin inhibition, and specifically what is now known as Nogo and its downstream pathways, is one of the longest-studied therapeutic strategies in SCI, with the first description of the effect of anti-Nogo (IN-1) monoclonal antibody on corticospinal tract sprouting emerging in 1990, nearly 20 years ago. Everything published over the subsequent decade on this strategy came from Dr. Martin Schwab and his colleagues in Zurich as they sought to characterize the effects of this treatment. These studies employed rat and primate species and a sharp partial transection injury (e.g., dorsal hemisection, over-hemisection, unilateral pyramidotomy) of the thoracic or upper cervical spine, with an IN-1 secreting hybridoma implanted into the dorsal frontro-parietal cortex typically at the time of spinal cord injury. Many of these studies examined histologic outcomes exclusively, with a focus on whether the IN-1 antibody was stimulating axonal regeneration/sprouting (which typically it was). Behavioral outcomes were also improved in some studies. Certainly, there was compelling evidence from this single laboratory to support the promise of the IN-1 antibody therapy, providing a rationale to pursue the translation of this approach.

With the cloning of Nogo in 2000, the strategy surrounding this aspect of myelin inhibition has evolved into different approaches. The IN-1 monoclonal antibody approach (which involved the transplant of a hybridoma secreting IgM antibody) has been supplanted by an intrathecally applied anti-Nogo IgG antibody approach, which has now entered clinical trials. This intrathecally applied anti-Nogo IgG treatment has been tried in both rat and primate SCI models, with immediate post-injury administration leading to both anatomic and functional improvements (Freund et al., 2006, 2007, 2009; Liebscher et al., 2005; Wannier-Morino et al., 2008). This anti-Nogo IgG intrathecal approach has been translated into human clinical trials, with a European and Canadian trial being initiated in 2007.

Another approach being developed by Dr. Stephen Strittmatter and his colleagues at Yale University is the competitive antagonism of the Nogo receptor with a synthetic Nogo-66 (1-40) peptide (Nogo extracellular peptide, residues 1-40), otherwise known as "NEP1-40" or "Nogo-66 receptor antagonist peptide." This has been applied both intrathecally and systemically (via subcutaneous injection) in mouse and rat studies utilizing both cervical and thoracic partial transection spinal cord injury models. In this acute treatment paradigm, NEP1-40 has been reported to promote both histologic improvements (i.e., enhanced sprouting/regeneration), and modest behavioral improvements (Atalay et al., 2007; Cao et al., 2008; Grandpre et al., 2002; Li and Strittmatter, 2003). It has also been reported by Li and Strittmatter (2003) to be effective in a subacute treatment paradigm with a 7-day delay post-injury prior to intervention. Steward and colleagues (2008) attempted to reproduce these findings with NEP1-40 in an NIH-funded replication study, and while there was a weak suggestion that NEP1-40 "created a situation that was slightly more conducive to axon regeneration," the robust sprouting/regeneration and improved behavioral recovery reported by Li and Strittmatter were not observed.

An additional approach, also pioneered by Strittmatter and colleagues, is the use of a soluble Nogo receptor ectodomain, which has been administered intrathecally at the time of injury in rodent thoracic contusion and partial transection models (Li et al., 2004; Wang et al., 2006). In both cases, there was improved behavioral recovery. In the study by Wang and colleagues (2006), a 3-day delay in treatment did not nullify the behavioral improvements.

Measured within the context of the SCI research community, the pre-clinical body of work behind the anti-Nogo approaches is considerable (Table 3). There are good examples of treatments that have been extensively studied, but the majority of investigation has resided within single laboratories. The significant benefits of NEP1-40 (Li and Strittmatter, 2003) were not observed in a formal, NIH-funded replication study (Steward et al., 2008), suggesting the need for further investigation to characterize the robustness of this intervention. The anti-Nogo antibody treatment has not undergone a similar replication study, although commercial intellectual property issues will likely preclude such an investigation. A recent study that evaluated the corticospinal tract after a dorsal hemisection injury in two different Nogo-deficient mutant mouse lines found enhanced regeneration in neither (Lee et al., 2009). Interpreting the implications of such negative findings in this loss-of-function experiment with the positive findings from Dr. Schwab's laboratory with the administration of anti-Nogo antibodies is difficult given the obvious differences in experimental paradigm.

Rho antagonists

The Rho pathway is recognized as an important biochemical signaling pathway in growth cone dynamics and neuronal apoptosis. Inhibition of Rho activation may therefore influence axonal sprouting/regeneration and secondary damage at the injury site. The strategy of inhibiting Rho within *in vivo* models of SCI has been explored by a number of investigators (Table 4). Dr. Lisa McKerracher and colleagues were the first research group to demonstrate that the application of Rho-kinase inhibitors, Y27632 and C3 transferase, directly to the SCI epicenter immediately after injury could significantly improve behavioral recovery within mice that had received a dorsal over-hemisection experimental lesion (Dergham et al., 2002). This C3 transferase approach was found to reduce RhoA activation after contusive thoracic SCI (Dubreuil et al., 2003).

Interestingly, the experience of others with C3 transferase early on was not similarly positive. Sung and colleagues

Table 3. Anti-Nogo Approaches

Study	*Animal model and injury model*	*Intervention and timing*	*Experimental group*	*Outcome: histologic/biochemical/physiological/behavioral*
Maier et al., 2009	Model: adult female SD rats, 200−250 g Injury: T8 T-shaped lesion (bilateral dorsal hemisection and complete midline transection)	Anti(amino)Nogo-A Ab (11C7) • Intrathecal infusion at T10 started at 0 h PI, then × 14 d (Ab solution 3 mg/mL) minipump delivered 5 μL/h, 3.1 μg IgG/mL Treadmill training (bipedal & quadrupedal)	SCI + • 11C7, non-trained • 11C7, trained • Control IgG, non-trained • Control IgG, trained $N = 17$/group for behavior and $n = 7$/group for morphology	Histologic/biochemical/physiological: 11C7 treatment increased the number of CST fibers with a significant increase after training and led to significant serotonergic innervation of motoneurons. • No difference in nociceptive primary afferent fibers (CGRP+) Behavioral: at 9 wk PI, 11C7-treated rats displayed consistent step cycles with good coordination and a very low level of paw drag, approaching the values of intact rats. • 11C7 treatment significantly improved performance in inclined grid walk. • Training alone improved locomotor function. • After combined treatment (11C7 + training), animals displayed worse locomotor function than 11C7 alone, suggesting interference of both movement strategies.
Freund et al., 2009	Model: male or female macaque monkeys, 3–5 kg, 3.5−6.9 yr old Injury: C7-C8 unilateral transection	Anti(amino)Nogo-A Ab (11C7 or MAb hNogo-A) • Ab concentrations of 3.7−10 mg/mL in PBS; delivered intrathecally 3−5 mm rostral to the lesion via osmotic minipump × 4 wk (started at 0 h PI)	SCI + • Anti-Nogo-A ($n = 7$; 3 hNogo-A, 4 11C7) • Control antibodies ($n = 6$)	Histologic/biochemical/physiological: This re-examines Freund results from 2006. Statistical analysis was conducted for recovery as a function of the size of the lesion. • The normalized number of axonal swellings caudal to the lesion and normalized number of CS axons crossing midline at C5 as a function of the lesion extent are significantly different in the groups. Behavioral: With modified Brinkman board test, anti-Nogo-A antibody-treated monkeys demonstrated significantly better recovery of their manual dexterity than the control antibody-treated monkeys in the total number of pellets and the contact time. • Animals receiving anti-Nogo-A antibody 1 wk PI exhibited complete recovery of function.
Wannier-Morino et al., 2008	Model: male or female macaque monkeys, 3–5 kg, 3.5−6.9 yr old Injury: C7-C8 unilateral transection	Anti(amino)Nogo-A Ab (11C7 or MAb hNogo-A) • Ab concentrations of 3.7−10 mg/mL in PBS; delivered intrathecally 3−5 mm rostral to the lesion via osmotic minipump × 4 wk (started at 0 h PI)	SCI + • Anti-Nogo-A (n = 7; 4hNogo-A, 3 11C7) • Control antibodies ($n = 4$) Uninjured controls ($n = 4$)	Histologic/biochemical/physiological: Anti-Nogo-A antibody treatment at the site of the lesion did not prevent reduction of the number of rubrospinal neurons in the contralesional red nucleus nor did it prevent shrinkage. Behavioral: not reported

(Continued)

Table 3. Anti-Nogo Approaches (Continued)

Study	*Animal model and injury model*	*Intervention and timing*	*Experimental group*	*Outcome: histologic/biochemical/physiological/behavioral*
Beaud et al., 2008	Model: adult macaque monkeys Injury: variable lesions at C7-8 targeting a hemisection	Anti(amino)Nogo-A Ab (11C7 or MAb hNogo-A) • Ab concentrations of 3.7−10 mg/mL in PBS; delivered intrathecally 3−5 mm rostral to the lesion via osmotic minipump × 4 wk (started at 0 h PI)	SCI + • Anti Nogo-A ($n = 5$; 4 hNogo-A and 1 11C7) • Control IgG ($n = 4$) Uninjured controls ($n = 5$)	Histologic/biochemical/physiological: no effect on atrophy of lesioned cell bodies Behavioral: not reported
Steward et al., 2008	Model: adult female C57Bl/6 mice Injury: T8 dorsal hemisection	NEP1-40 (Nogo 66) subcutaneous injection • Given at 0 and 4 h PI, or given at 7 d PI (delayed)	SCI + • NEP1-40 • Reverse peptide • Control $N = 10−11$/group for each	Histologic/biochemical/physiological: modest effect on axonal sprouting/regeneration Behavioral: no locomotor improvement (*Note:* This is an NIH-funded replication study of Li and Strittmatter, 2003 [see below].)
Cao et al., 2008	Model: adult female SD rats Injury: C4 right lateral funiculus section	NEP1-40 (Nogo 66) • 500 mM NEP1-40 via intrathecal infusion at 0.3 mL/h for 28 d • Started at 0 h PI	SCI + • NEP1-40 ($n = 8$) • Control ($n = 7$)	Histologic/biochemical/physiological: Rubrospinal tract and 5-HT sprouting increased. Behavioral: Gait and forelimb use recovered (but not to the end of experiment).
Atalay et al., 2007	Model: adult male SD rats Injury: T10 hemisection	NEP1-40 (Nogo 66) • Given at 0 h PI, directly applied to injury site	SCI + • NEP1-40 ($n = 23$) • Control ($n = 21$)	Histologic/biochemical/physiological: improved preservation of injured axons, enhanced axonal sprouting Behavioral: significantly enhanced motor recovery at 8 and 21 d PI
Freund et al., 2007	Model: adult macaque monkeys Injury: variable lesions at C7-8 targeting a hemisection	Anti(amino)Nogo A Ab (11C7 or MAb hNogo A) • Ab concentrations of 3−10 mg/mL in PBS; delivered intrathecally 3–5 mm rostral to the lesion via osmotic minipump × 4 wk (started at 0 h PI)	SCI + • Anti Nogo (11C7, $n = 7$) • Anti Nogo (hNogo-A, $n = 6$) Uninjured controls ($n = 3$)	Histologic/biochemical/physiological: fewer retraction bulbs, increased axon arbor length, increased CST sprouting rostral to injury site Behavioral: not assessed (some animals were assessed in Freund et al., 2006)
Freund et al., 2006	Model: adult macaque monkeys, 2.5−5.5 kg, 3−4 yr old Injury: variable lesions at C7-8 targeting a hemisection	Anti(amino)Nogo A Ab (11C7 or MAb hNogo A) • Ab concentrations of 3−10 mg/mL in PBS; delivered intrathecally 3–5 mm rostral to the lesion via osmotic minipump × 4 wk (started at 0 h PI)	SCI + • Anti Nogo (11C7, $n = 4$) • Anti Nogo (hNogo-A, n = 2) • Control IgG ($n = 6$)	Histologic/biochemical/physiological: enhanced CST sprouting distal to injury site (SCI lesion variable) Behavioral: improvement in reaching performance

Wang et al., 2006	Model: adult female SD rats Injury: Moderate T8 contusion	NgR(310)ecto-Fc, intrathecally • Via minipump at 0.29 mg/kg/day × 28 d; started at time of injury or 3 d PI	SCI + • NgR(310) ecto-Fc ($n = 8$) • Control ($n = 8$)	Histologic/biochemical/physiological: increased 5-HT fiber sprouting Behavioral: enhanced locomotor recovery (BBB) in both acute and delayed group. Delayed therapy was as efficacious as acute therapy.
Liebscher et al., 2005	Model: female Lewis rats, 160–190 g Injury: T8 T-shaped lesion (bilateral dorsal hemisection and complete midline transection)	Anti (amino) Nogo-A Ab (11C7 or 7B12) • Intrathecal infusion at T10 started at 0 h PI, then × 14 d (Ab solution 3 mg/mL) minipump delivered 5 mL/h, 3.1 mg IgG/mL	SCI + • Anti Nogo A Ab (7B12) • Anti Nogo A Ab (11C7) • Control Ab Total $n = 69$; n/group not reported	Histologic/biochemical/physiological: Anti–Nogo-A: CST sprouting was significantly higher in both treated groups than in control. Enhanced CST regeneration Behavioral: improvement on horizontal ladder, swimming, but not in BBB score
Fouad et al., 2004	Model: monkey *Callitrix jacchus* (marmoset) Injury: T8 dorsolateral quadrant	MAb IN-1 • Ab-secreting hybridoma implanted into CSF at 0 h PI	SCI + • IN-1 ($n = 4$) • Control Ab ($n = 2$)	Histologic/biochemical/physiological: growth and regeneration of CST up to 5 mm caudal of lesion Behavioral: not reported
Li et al., 2004	Model: adult female SD rats Injury: T6/7, dorsal hemisection	NgR(310) ecto-Fc, intrathecal • Given at 0 h PI	SCI + • NgR(310) ecto-Fc ($n = 6$) • Control ($n = 6$)	Histologic/biochemical/physiological: Intrathecal NgR(310) ecto-Fc ecto stimulates dCST sprouting in rats rostral to an over-hemisection, induces sprouting and synaptic connection of CST fibers and serotonergic axons, and improves electrophysiology measures (motor-evoked potentials). Behavioral: improved locomotor recovery
Li and Strittmatter, 2003	Model: adult Bl/6 mice Injury: T6 dorsal over-hemisection	NEP1-40 (Nogo 66) • Given at 0 and 4 h PI or given at 7 d PI (delayed) Subcutaneous infusion with minipump × 14 d, or daily intraperitoneal injections × 14 d	• NEP1-40 ($n = 57$) • Control ($n = 34$)	Histologic/biochemical/physiological: extensive growth of 5-HT and CST fibers Behavioral: positive effect of delayed treatment on locomotor recovery, dose-dependent effects following systemic application
GrandPré et al., 2002	Model: female SD rats, 175–250 g Injury: T6/7, dorsal hemisection	NEP1-40 (Nogo 66) • 75 μg/kg, given intrathecally at 0 h PI and then for 4 wk	SCI + • NEP1-40 ($n = 16$) • Control ($n = 18$)	Histologic/biochemical/physiological: CST sprouting rostral to lesion. NEP1-40 treatment prevented decrease in the density of 5-HT-positive fibers below injury site. Behavioral: substantially improved recovery in open field locomotion measured in a subgroup of animals ($n = 6$ and 7)
Raineteau et al., 2002	Model: adult Lewis rats Injury: bilateral pyramidotomy	MAb IN-1 • Ab-secreting hybridoma implanted at 0 h PI	SCI + • IN-1 ($n = 31$) • Control Ab ($n = 24$) • No treatment ($n = 11$) No SCI ($n = 25$)	Histologic/biochemical/physiological: reorganization of rubrospinal tract. Numerous RST fibers entered the ventral horn after bPT and IN-1 Ab treatment, targeted specially to the ventral regions of the spinal grey matter. RST fibers invading the ventral horn made close appositions with motoneurons. • Electrophysiology: Reduced latency and threshold for muscle stimulation in IN-1 Ab treated animals confirmed the innervation of the proximal motoneuron pool. Behavioral: not reported

(Continued)

Table 3. Anti-Nogo Approaches (Continued)

Study	*Animal model and injury model*	*Intervention and timing*	*Experimental group*	*Outcome: histologic/biochemical/physiological/behavioral*
Merkler et al., 2001	Model: Lewis Rats, 200–250 g Injury: T8, large over-hemisection (2/3 of cord)	MAb IN-1 • Ab-secreting hybridoma implanted at 0 h PI	SCI + • IN-1 • Control Ab $N = 10$/group	Histologic/biochemical/physiological: not reported Behavioral: recovery in locomotion (electromyographic recordings, kinematics, and horizontal ladder)
Raineteau et al., 2001	Model: Lewis rats, 2.5 mo Injury: bilateral pyramidotomy	MAb IN-1 • Ab-secreting hybridoma implanted at 0 h PI	SCI + • IN-1 ($n = 23$) • Control Ab ($n = 19$) • Lesion only ($n = 7$) No SCI ($n = 12$)	Histologic/biochemical/physiological: 2-fold increase in the number of collaterals emerging from rubrospinal tract and brain stimulation Behavioral: recovery in grasping
Brosamle et al., 2000	Model: female Lewis rats, 6–8 wk old Injury: T8 bilateral dorsal hemisection	IN-1 Fab • 5 mg/mL for 0.5 μL/h $\times$ 14 d delivery via minipump, started at 0 h PI	SCI + • IN-1 Fab ($n = 18$) • Neutralized Fab ($n = 7$) • BSA (control, $n = 5$)	Histologic/biochemical/physiological: rIN-1 Fab. Regenerating fibers grew >9 mm beyond the lesion. Behavioral: not reported
Raineteau et al., 1999	Model: adult Lewis rats, 2.5 mo old Injury: unilateral pyramidotomy	MAb IN-1 • Ab-secreting hybridoma implanted at 0 h PI	SCI + • IN-1 ($n = 6$) • Control ($n = 6$) • Lesion only ($n = 6$)	Histologic/biochemical/physiological: regenerative sprouting enhanced Behavioral: reported in Z'Graggen et al., 1998
Thallmair et al., 1998	Model: Lewis rats, 2–3 mo old Injury: unilateral pyramidotomy	MAb IN-1 • Ab-secreting hybridoma implanted at 0 h PI	SCI + • IN-1 • Control Ab $N = 25$/group	Histologic/biochemical/physiological: increase in sprouting, crossing the midline, branching, bouton-like structures, increased number of cortico-bulbar projections from parvocellular red nucleus and pons Behavioral: enhanced recovery in grasping, rope climbing, and sticky paper test
Von Meyenburg et al., 1998	Model: Lewis rats, 90–120 g, 5–6 wk old Injury: T8 bilateral dorsal hemisection	MAb IN-1 • Hybridoma implanted at 2 or 8 wk PI (with NT-3)	SCI + • IN-1 + NT-3 ($n = 9$, 2 wk) • Control Ab + NT-3 ($n = 8$, 2 wk) • IN-1 + NT-3 ($n = 20$, 8 wk) • Control Ab + NT-3 ($n = 11$, 8 wk) • No treatment	Histologic/biochemical/physiological: In 2 wk group, CST fibers regenerated for 2–11.4 mm; 8 wk group, CST fibers regenerated 2 mm into caudal spinal cord. Behavioral: Locomotor recovery in both IN-1/NT-3 groups was non-significant (was increased in 2 wk group for both AB treatment, but not significant, so behavioral recovery was mostly due to NT-3).

Z'Graggen et al., 1998	Model: female Lewis rats, 45–120 d old Injury: unilateral pyramidotomy	MAb IN-1 • Ab-secreting hybridoma implanted at 0 h PI	SCI + • IN-1 ($n = 16$) • Control Ab ($n = 16$) • Lesion only ($n = 16$) Sham + • IN-1 ($n = 6$) • No treatment ($n = 6$)	Histologic/biochemical/physiological: enhanced CST sprouting of the (corticopontine) and midline crossings Behavioral: enhanced recovery in grasping
Bregman et al., 1995	Model: Lewis Rats, 6–8 wk old Injury: T7 over-hemisection	MAb IN-1 • Ab-secreting hybridoma implanted at 0 h PI HRP	SCI + • IN-1 ($n = 23$) • Control Ab (HRP, $n = 22$) • Lesion only ($n = 16$) • Unlesioned control ($n = 16$)	Histologic/biochemical/physiological: Sprouting of CST fibers at lesion site, regeneration up to 11 mm within 3 wk, and these axons were maintained up to 12 wk later. IN-1 greatly increased serotonergic and noradrenergic projections. Behavioral: recovery in stride length; recovery of specific reflex (contact-placing response) and locomotor functions after spinal cord injury in these adult rats
Schnell and Schwab, 1990	Model: Lewis rats, 2–6 wk old Injury: T5-7 dorsal hemisection	MAb IN-1 • Ab-secreting hybridoma implanted at 0 h PI into dorsal fronto-parietal cortex with cyclosporin A, given 7–10 d pre-injury and continuous for a few weeks HRP • Via implanting antibody-secreting hybridoma cell injected into dorsal fronto-pariental cortex with cyclosporin A, given 7–10 d pre-injury and continuous for a few weeks	SCI + • IN-1 ($n = 9$) • HRP hybridoma cells ($n = 2$) • Non-treated control ($n = 3$)	Histologic/biochemical/physiological: sprouting of CST fibers at lesion site; regeneration up to 11 mm within 3 wk Behavioral: not reported

Ab, antibody; BBB, Basso, Beattie and Bresnahan locomotor test; C5, cervical vertebra 5; CGRP, calcitonin gene related protein; CSF, cerebrospinal fluid; CST, cortico-spinal tract; HRP, horseradish peroxidase; KO, knockout; MEP, motor-evoked potentials; PI, post-injury; SCI, spinal cord injury; SD rats, Sprague-Dawley rats; T8, thoracic vertebra 8.

Table 4. Rho Antagonists

Study	*Animal model and injury model*	*Intervention and timing*	*Experimental group*	*Outcome: histologic/biochemical/physiological/behavioral*
Lord-Fontaine et al., 2008	Model: female SD rats or female Balb-c mice Injury: T9 NYU impactor (rat), 10 g × 25 mm or T7 dorsal over-hemisection (mouse)	BA-210 (Cethrin) • 15 μg in fibrin sealant applied extradurally (rat) at time of injury • 1 mg in fibrin sealant applied onto exposed cord (mouse) immediately or at 24 or 72 h PI	Rat SCI+ • BA-210 in fibrin sealant (n = 11) • Vehicle in fibrin sealant (n = 12) Mouse SCI+ • BA-210 at 0 h PI (n = 4) • BA-210 at 24 h PI (n = 6) • BA-210 at 72 h PI (n = 6) • Fibrin sealant controls	Histologic/biochemical/physiological: BA-210 reduced lesion extent and increased spared white matter in rat SCI model (with immediate treatment). No description of histologic outcomes in mouse SCI model. Behavioral: BA-210 significantly improved BBB scores in the rat SCI model at 6, 7, and 8 wk (~9 vs. just under 8). There was no evidence of the induction of mechanical allodynia in BA-210 treated animals. BA-210 also significantly improved BBB scores in the mouse SCI model at 16 d PI when administered immediately or 24 h PI. No significant difference in locomotor function in the BA-210 vs. control animals with the 72 h PI treatment.
Nishio et al., 2006	Model: male Wistar rats 205–245 g Injury: T9 NYU impactor, 10 g × 25 mm	Fasudil, Rho-kinase inhibitor • Via an osmotic minipump, 15 μg/μL at rate of 12 μL/d, resulting in the infusion of 180 μg/day started 0 h PI or started 4 wk PI Saline • Started immediately or delayed	SCI+ • Fasudil (n = 5) • Saline (immediate, n = 5) • Saline (delayed, n = 7)	Histologic/biochemical/physiological: CST sprouting by immediate, but not delayed, Fasudil treatment Behavioral: Immediate, but not delayed Fasudil treatment results in improved BBB hindlimb function.
Chan et al., 2005	Model: adult male SD rats Injury: C4/C5 dorsal column transection	Y-27632, Rho-kinase inhibitor • Via osmotic minipumps implanted into the subcutaneous space at the back of the neck, 2 mM, 160 μg (low dose), or 20 mM, 1600 μg (high dose) per animal over 2 wk, started 0 h PI	SCI+ • Low-dose Y27632 (n = 15) • High-dose Y-27632 (n = 20) • PBS (n = 18)	Histologic/biochemical/physiological: High-dose Y27632 significantly increased the distance of the longest axon compared to controls. Low-dose Y27632-treated animals had decreased sprouting in the distal grey matter compared to controls, while high-dose-treated animals showed more sprouting. Behavioral: Low-dose Y27632 treatment appeared to be detrimental to neurological recovery. For the forepaw outward rotation angles, only the high-dose group showed significant recovery. Footslip and food pellet reaching tests indicated that high-dose Y27632 accelerated recovery, while low dose impeded it.
Yamagishi et al., 2005	Model: female Wistar rats, 200–250 g Injury: T9/T10 transection	Y-27632 • Via gel foam, 10 μL of 30 mM at 0 h PI	SCI+ • Y-27632 • PBS N = 6/group	Histologic/biochemical/physiological: Immunostaining 3 d post-axotomy showed that Y-27632 significantly abolished breakdown of microtubules and neurofilaments as well as axolemma in transected spinal cords. Results suggest that Rho-kinase inhibition delays Wallerian degeneration *in vivo*. Rho-kinase inihibition also promotes increased integrity of the dorsal CST in rats with transection cord injuries, as demonstrated with increased anterograde labeling of the CST. Behavioral: not reported

Tanaka et al., 2004	Model: male Wistar rats, 200–250 g Injury: T10 dorsal-hemisection, depth of 1.8 mm	Y-27632 • 3 mg per animal, at 0 h PI, delivered via osmotic minipump over 2 wk Fasudil • 3 mg per animal, delivered via osmotic minipump over 2 wk Cytoplasmic p21Cip1/WAF1 fusion protein GST-ΔNLS-p21-PTD-myc • 20 nmol/kg/d, delivered via osmotic minipump over 2 wk	SCI + • Y-27632 ($n = 26$) • HA-1077 ($n = 26$) • p21Cip1/WAF1 ($n = 29$) • GST-PTD-myc control ($n = 28$)	Histologic/biochemical/physiological: HA-1077 or Y-27632 treatments led to significantly enhanced sprouting, and GST-ΔNLS-p21-PTD-myc protein was even more effective. Immunoreactivity for Tuj1 and GFAP revealed that HA-1077 and Y-27632 were as effective as GST-ΔNLS-p21-PTD-myc protein in reducing the cavity area. EMG recordings demonstrated that GST-ΔNLS-p21-PTD-myc protein, HA-1077, and Y-27632 decrease ratios of irregular contractions between the TA and the VL muscles in comparison to GST-PTD-myc-treated control rats. Behavioral: BBB scores of rats with HA-1077, Y-27632, and GST-ΔNLS-p21-PTD-myc protein were significantly improved over the GST-PTD-myc-treated control rats.
Dubreuil et al., 2003	Model: female Long-Evans rats, 200–250 g and female Balb/c mice, 20–22 g Injury: • Rats: T10 dorsal over-hemisections, depth of 1.6 mm or NYU impactor, 25 g × cm • Mice: T8 dorsal over-hemisections	C3 transferase • *Clostridium botulium* exozyme (C3-05) in a fibrin matrix, injection into transected spinal cord • 50 μg at 0 h PI (rats) • 10 μg at 0 h PI (mice) • 1 μg in fibrin at 0 h PI (for TUNEL experiment)	SCI + • Rats, C3 treatment ($n = 13$) • Rats, untreated ($n = 11$) • Mice, C3 treatment ($n = 5$) • Mice, untreated ($n = 5$) Sham • Rats ($n = 11$) • Mice ($n = 5$)	Histologic/biochemical/physiological: To determine if endogenous cells in the spinal cord were able to take up and retain C3–05 after treatment, rat spinal cord double-labeled with an antibody specific for C3 and with cell type-specific markers showed intracellular C3 immunoreaction in neurons, astrocytes, and oligodendrocytes. Treatment with the Rho antagonist C3–05 after contusion or transection of the spinal cord reverses RhoA activation after injury. In both mice and rats treated with C3–05, the number of TUNEL-labeled cells was significantly reduced by ~50% after SCI. Behavioral: not reported
Fournier et al., 2003	Model: female SD rats 250−300 g Injury: T3/T4 dorsal hemisection, depth of 1.5 mm	C3 transferase • 300 μg per animal over 2 wk at a rate of 0.5 μL/h, delivered via intrathecal pump started at 0 h PI • Rho-kinase inhibitor, 340 μg per animal over 2 wk started at 0 h PI GST • 340 μg per animal over 2 wk started at 0 h PI	SCI + • C3 transferase ($n = 11$) • Y-27632 ($n = 12$) • GST control ($n = 10$) • PBS ($n = 15$)	Histologic/biochemical/physiological: C3 does not promote sprouting or long-distance regeneration of injured CST fibers after spinal cord lesions in the adult rat. The amount of scar tissue in C3-treated animals is significantly reduced in comparison to vehicle controls. Y-27632 enhances sprouting of rat CST fibers after dorsal hemisections. Behavioral: Rats were evaluated using the BBB score 2, 7, 14, 21, and 28 d postoperatively. C3 treatment resulted in delayed BBB locomotor recovery in comparison to GST treatment. Y-27632 treatment yields improved BBB locomotor recovery in comparison to PBS controls by 3 points in BBB scale in 2 wk.

(Continued)

Table 4. Rho Antagonists (Continued)

Study	*Animal model and injury model*	*Intervention and timing*	*Experimental group*	*Outcome: histologic/biochemical/physiological/behavioral*
Sung et al., 2003	Model: female SD rats, 250–300 g Injury: T9/T10 NYU impactor, 10 g × 1.25 cm	C3 transferase • Soaked in the gelfoam, directly to contused spinal cord, 10 μg dissolved in 0.1 cc PBS at 0 h PI Y-27632 oral • 10 mg/kg/day • At 0 h PI then × 10 d • At 5 d PI then × 10 d Fasudil IP, • 10 mg/kg in 1 cc saline at 0 h, then × 5 d	SCI + • C3 ($n = 5$) • Y-27632 ($n = 19$) • Fasudil ($n = 16$) • Vehicle (n = 8) Sham ($n = 5$)	Histologic/biochemical/physiological: Histological hematoxylin and eosin analysis of spinal cord 5 wk after a single dose of Fasudil showed less tissue damage when compared to control. Behavioral: At 1 wk post-SCI, 3 rats were severely emaciated and C3 was ended due to poor response. Motor function of rats receiving Fasudil improved significantly for both single dose and multiple dose regimens. Rats receiving Y-27632 immediately after spinal cord injury exhibited a reduction in BBB.
Dergham et al., 2002	Model: female BALB-c mice, 20 g Injury: T7 dorsal over-hemisection	C3 transferase • Via a fibrin adhesive delivery system (Tisseel VH kit), 50 μL of 1 mg/mL C3 in 25 μL of thrombin solution at 0 h PI or via collagen gels at 0 h PI Y27632 • In 25 μL of the thrombin solution at 0 h PI	SCI + • Fibrin + C3 ($n = 13$) • Collagen + C3 ($n = 12$) • Fibrin + Y27632 ($n = 5$) • Fibrin ($n = 10$) • Collagen ($n = 7$) • No treatment ($n = 13$) SCI + (for behavioral testing) • C3 ($n = 6$) • Untreated ($n = 6$)	Histologic/biochemical/physiological: After thoracic spinal cord lesion, only axons that regenerate long distances show upregulation of GAP-43 mRNA expression. *In situ* hybridization using 35S-labeled riboprobes on coronal brain sections revealed high levels of GAP-43 mRNA expression in neurons of the motor cortex of C3-treated animals whereas untreated animals showed GAP-43 signal similar to background. Behavioral: BBB hindlimb motor function testing revealed that mice treated with C3 or Y27632 had remarkable recovery within 24 h, already walking with weight support, which may be caused by increased neuroprotection in the lesioned spinal cord.

BBB, Basso, Beattie and Bresnahan locomotor test; C5, cervical vertebra 5; GAP, growth-associated protein; GST, glutathione S-transferase; PI, post-injury; SCI, spinal cord injury; SD rats, Sprague-Dawley rats; T8, thoracic vertebra 8.

(2003) reported that their C3-treated animals were severely emaciated, and many were terminated. Fournier and colleagues (2003) observed no axonal regeneration/sprouting and delayed hindlimb locomotor recovery, despite significantly reduced scar formation with C3-treated animals compared to fusion protein control-treated animals. Nonetheless, design improvements to create a C3-fusion protein consisting of the C3 transferase coupled to a transport sequence (i.e., BA-210) facilitate the ability for this biological compound to cross plasma membranes and gain increased distribution throughout the injured spinal cord. This more permeable form of C3 transferase has been translated into human clinical trials under the name Cethrin® (BioAxone Therapeutique, Montreal, Quebec, Canada). Rights to Cethrin have since been licensed to Alseres Pharmaceuticals (Hopkinton, MA). A recent report by McKerracher's group studied BA-210 in a rodent contusion injury model and examined different time windows of intervention within a mouse hemisection model (Lord-Fontaine et al., 2008). The authors reported improved behavioral outcomes with the BA-210 in both injury models, with an effective time window extending 24-h post-injury.

The other approach that has been studied is that of Y27632, a selective Rho-kinase inhibitor (downstream of Rho). This has been investigated both as an oral drug and as a directly applied agent. In mouse and rat contusion and partial transection models of SCI, this was found to promote behavioral recovery (Dergham et al., 2002; Fournier et al., 2003; Sung et al., 2003; Tanaka et al., 2004). Chan and colleagues (2005) demonstrated that low doses of Y27632 were detrimental to the rodent cord after dorsal hemisection, while higher doses tended to accelerate recovery.

Discussion

This article reviews three therapeutic approaches, which entail the direct application of a biological therapy to the injured spinal cord or overlying dura; two have already been commercialized and have moved forward into clinical trials (the anti-Nogo antibody trial [Novartis] and BA-210 [Cethrin]). The anti-Nogo antibody approach that evolved from the IN-1 antibody work has been fairly extensively studied, albeit almost exclusively by Dr. Schwab and Novartis. The C3 approach went into clinical trial as BA-210 after the studies of Dergham and colleagues (2002) and Dubreil and colleagues (2003) were reported. At this stage, replication studies of these patented technologies are unlikely to occur, although the NIH-funded replication of the initial NEP1-40 results has demonstrated how difficult it can be to demonstrate the robustness of the effect.

The fact that these therapies involve some form of direct application to the spinal cord influences their clinical translation in a number of ways. For one, the main target of these agents is to promote axonal growth/sprouting/plasticity by altering the inhibitory SCI environment or the response of axons to this environment. Yet most of the agents are tested in an acute injury paradigm with immediate application at the time of injury, and hence, sorting out the behavioral responses attributable to axonal growth versus those attributable to some form of neuroprotection is difficult. This was, for example, quite evident in the study by Dergham and colleagues (2002), where improved behavioral recovery was seen almost immediately after injury and would therefore be difficult to attribute to the promotion of axonal regeneration. The subsequent study by Dubreuil and colleagues (2003) pointed out that RhoA activation occurred locally at the site of injury after acute SCI and suggested a mechanism by which immediately applied C3 transferase (and also BA-210, or Cethrin) might have a neuroprotective effect. Conceivably, the anti-Nogo and ChABC approaches may be expected to have some additional neuroprotective effect in the rodent spinal cord as well, given that they are administered very early after injury in these pre-clinical studies.

From the patients' perspective, it matters little whether improved function is related to some form of neuroprotection or some effect on axonal growth. However, at a translational and operational level, the distinction between neuroprotection versus neuroregenerative mechanisms has substantial relevance, given that the need to directly apply these treatments to the exposed spinal cord or dura imposes significant practical challenges to the translation of these therapies. The time delay may be significant before such treatments can be then applied to an acutely injured patient, owing to transport time and availability of imaging and operating room facilities. So if it is indeed a neuroprotective mechanism of action that is being sought, the direct application to the spinal cord clearly has some limitations with respect to how quickly the therapy can be instituted. Incorporating that inherent delay in clinical treatment into experimental pre-clinical studies is simple enough (one just waits before applying the therapy); more difficult is taking the time window of intervention in human studies and extrapolating that to rodent studies (and vice versa). How similar or different the temporal pattern of relevant biological processes is between rodents and humans is unclear, although work by Dr. Lynne Weaver and colleagues would suggest that the time course of cellular invasion into the injured cord is reasonably similar between the two (Fleming et al., 2006). Irrespective of this, when reviewing the studies included in these tables, one cannot help but feel that more pre-clinical studies with delays of intervention are needed. In 21 of 23 ChABC studies, 22 of 24 anti-Nogo studies, and 8 of 9 anti-Rho studies (i.e., 91% of all studies), the intervention was applied immediately after the injury was induced.

The other important consideration in the translation of these directly applied biologic therapies is their biodistribution within the injured spinal cord. It is difficult to ascertain the degree and kinetics of the agents' distribution into the spinal cord, either via diffusion through the cerebrospinal fluid within the intrathecal space (e.g., anti-Nogo antibodies) or diffusion through the dura and then across the CSF (e.g., BA-210). For example, in most ChABC studies, a sharp partial transection model is employed (hence opening up the dura), and then the ChABC is directly injected into and/or infused onto the cord. How to translate the findings of such studies into a clinically relevant therapeutic paradigm is not clear. Similarly, partial transection injuries have been used for most anti-Nogo studies, so determining what the biodistribution of the antibody within a contused spinal cord is unclear. Finally, for an extradural application of a protein such as Cethrin, while the biodistribution in a rodent model of cord injury may be acceptable (where the CSF space is very small), how such a protein distributes to the injured human spinal cord when it must diffuse through a substantial amount of CSF is unknown. These are all important considerations for such directly applied therapies.

In summary, in this article we systematically reviewed the preclinical animal model data on ChABC, anti-Nogo approaches, and anti-Rho approaches. The question of whether any one of these approaches is ready for human translation has already been answered to some extent, as both the anti-Nogo antibody and the Rho antagonist Cethrin are well into clinical trials. Despite this, there remain questions about the time window of applicability and the biodistribution of these agents within the injured cord. Further pre-clinical work on these therapies is warranted in order to refine and optimize the treatment paradigms for human study.

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Discussion

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