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INVASION OF LESION TERRITORY BY REGENERATING FIBERS AFTER SPINAL CORD INJURY IN ADULT MACAQUE MONKEYS

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Abstract—In adult macaque monkeys subjected to an incomplete spinal cord injury (SCI), corticospinal (CS) fibers are rarely observed to grow in the lesion territory. This situation is little affected by the application of an anti-Nogo-A antibody which otherwise fosters the growth of CS fibers rostrally and caudally to the lesion. However, when using the Sternberger monoclonal-incorporated antibody 32 (SMI-32), a marker detecting a non-phosphorylated neurofilament epitope, numerous SMI-32-positive (+) fibers were observed in the spinal lesion territory of 18 adult macaque monkeys; eight of these animals had received a control antibody infusion intrathecally for 1 month after the injury, five animals an anti-Nogo-A antibody, and five animals received an anti-Nogo-A antibody together with brain-derived neurotrophic factor (BDNF). These fibers occupied the whole dorso-ventral axis of the lesion site with a tendency to accumulate on the ventral side, and their trajectories were erratic. Most of these fibers (about 87%) were larger than 1.3 μm and densely SMI-32 (+) stained. In the undamaged spinal tissue, motoneurons form the only large population of SMI-32 (+) neurons which are densely stained and have large diameter axons. These data therefore suggest that a sizeable proportion of the fibers seen in the lesion territory originate from motoneurons, although fibers of other origins could also contribute. Neither the presence of the antibody neutralizing Nogo-A alone, nor the presence of the antibody neutralizing Nogo-A combined with BDNF influenced the number or the length of the SMI-32 (+) fibers in the spinal lesion area. In summary, our data show that after a spinal cord lesion in adult monkeys, the lesion site is colonized by fibers, a large portion of which presumably originate from motoneurons.

Key words: spinal cord lesion, scar tissue, primate, regeneration, BDNF, Nogo-A.

INTRODUCTION

In the adult central nervous system (CNS) of mammals, axotomized nerve fibers fail to regenerate over long distances. Among the factors contributing to this failure, the development of a scar tissue at the lesion site leads to the formation of an environment hostile to nerve fibers' regrowth and acting as a barrier against axonal regeneration into and across the lesion territory. Indeed, the scar tissue contains various neurite growth-inhibiting factors produced by cells such as microglial cells, meningeal cells, astrocytes or oligodendrocytes (Reier et al., 1983; Schwab and Bartholdi, 1996; Schwab, 2002; David and Lacroix, 2003). These last years, several growth-inhibiting molecules expressed in the scar tissue were identified such as the chondroitin sulfate proteoglycans (CSPGs), tenascin-c or semaphorins (Rudge and Silver, 1990; McKeon et al., 1991; Davies et al., 1997, 1999; Fawcett and Asher, 1999; Pasterkamp and Verhaagen, 2001). Nogo-A present in CNS myelin and oligodendrocytes inhibits the regeneration of spinal-lesioned axons (Schwab, 2004, 2010). In rodents, the application at the level of the spinal cord of an antibody neutralizing Nogo-A promotes both corticospinal (CS) fibers regeneration and functional recovery (Schnell and Schwab, 1990; Thallmair et al., 1998; Schwab, 2004; Liebscher et al., 2005; Maier et al., 2009). Experiments conducted on primates lead to similar results (Freund et al., 2006, 2007, 2009). In particular, these experiments have shown that in anti-Nogo-A antibody-treated monkeys, CS fibers sprout and regenerate rostrally, around and caudally to the spinal lesion (Fouad et al., 2004; Freund et al., 2006, 2007). However, in these animals, CS fibers were only exceptionally observed inside the lesion territory. Nevertheless, it remains possible that following spinal cord injury (SCI), neurons distinct from CS neurons show an ability to regenerate and to grow in or even through the scar tissue.

Neurofilaments constitute the main structural element of the neuronal cytoskeleton and the presence in neurons of non-phosphorylated forms of neurofilaments can be shown using the Sternberger monoclonal-incorporated

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Abbreviations: BDNF, brain-derived neurotrophic factor; CS, corticospinal; CSPGs, chondroitin sulfate proteoglycans; PBS, phosphate-buffered saline; SMI-32, Sternberger monoclonal-incorporated antibody 32.

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Table 1. Individual characteristics and summary data for all animals included in the study

Name	Mk-CB	Mk-CBo	Mk-CC	Mk-CGa	Mk-CG	Mk-CH	Mk-CP	MK-CS		
	1,2,3,4	–	1,3	–	1,2,3,4	1,2,3,4	1,2,3	1,3,4		
Species	Fasc.	Fasc.	Mul.	Fasc.	Fasc.	Fasc.	Fasc.	Mul.		
Sex	♀	♂	♂	♂	♂	♂	♀	♂		
mAB received	Control	Control	Control	Control	Control	Control	Control	Control		
Amount mAB (mg)	80	14	14.8	14	36	36	14.8	14.8		
BDNF treatment	No	No	No	No	No	No	No	No		
Amount BDNF (mg)	–	–	–	–	–	–	–	–		
BDA transport time (days)	78	99	21	87	70	62	78	35		
Lesion to BDA injection delay (days)	147	93	86	69	70	76	81	167		
Hemisection extent (%)	75	93	38	73	51	90	45	63		
Functional recovery (%)	78	99	83	100	90	53	83	22		
Age of the animal at the sacrifice (years)	5	∼4	∼4	∼4	∼4	∼4	6.9	4.5		
Nb. fibers with Ø < 1.3 μm	266	190	64	83	34	232	143	24		
Nb. fibers with Ø > 1.3 μm	780	884	305	989	404	2307	657	158		
Nb. fibers in the lesion territory	1046	1074	369	1072	438	2539	800	182		
Cumulative length for fibers with Ø < 1.3 μm	13.6	21.6	4.5	6.0	3.1	21.9	20.5	2.7		
Cumulative length for fibers with Ø > 1.3 μm	63.0	94.0	27.6	72.6	31.2	178.9	86.5	18.9		
Cumulative length for all fibers in the lesion	76.5	115.6	32.0	78.6	34.3	200.8	107.0	21.6		
Volume of BDA injection (in μl)	20			34.5	24	20	24			
Nb. of BDA injection sites	10			21	12	10	12			
Name	Mk-AC	Mk-AF	Mk-AG	Mk-AK	Mk-AM	Mk-ABB	Mk-ABMa	Mk-ABMx	Mk-ABP	Mk-ABS
	1,2,3,4	1,3,4	2,4	3	1,2,3,4	–	–	–	–	–
Species	Fasc.	Mul.	Fasc.	Fasc.	Fasc.	Fasc.	Fasc.	Fasc.	Fasc.	Fasc.
Sex	♂	♂	♂	♂	♂	♂	♂	♂	♂	♂
mAB received	hNogoA	11C7	hNogoA	hNogoA	hNogoA	hNogoA	hNogoA	hNogoA	hNogoA	hNogoA
Amount mAB (mg)	36	14.8	14.6	36	36	36	36	36	36	36
BDNF treatment	No	No	No	No	No	Yes	Yes	Yes	Yes	Yes
Amount BDNF (mg)	–	–	–	–	–	1.4	1.4	1.4	1.4	1.4
BDA transport time (days)	64	71	70	62	69	76	65	79	69	62
Lesion to BDA injection delay (days)	71	187	42	90	69	91	84	179	111	111
Hemisection extent (%)	85	56	78	86	80	83	94	95	77	93
Functional recovery (%)	100	57	100	100	96	93	68	78	70	66
Age of the animal at the sacrifice (years)	∼4	6.25	3.5	3.5	∼4	5	∼4	5	4.5	4.5
Nb. fibers with Ø < 1.3 μm	73	47	46	301	145	32	49	124	705	328
Nb. fibers with Ø > 1.3 μm	821	82	94	505	1607	526	918	1017	1199	953
Nb. fibers in the lesion territory	894	129	140	806	1752	558	967	1141	1904	1281
Cumulative length for fibers with Ø < 1.3 μm	6.6	4.7	3.2	26.2	17.5	4.6	3.1	12.3	41.2	23.6
Cumulative length for fibers with Ø > 1.3 μm	59.9	4.3	8.3	75.9	131.3	56.3	77.2	93.4	88.0	67.9
Cumulative length for all fibers in the lesion	66.5	9.0	11.5	102.1	148.8	60.9	80.4	105.7	129.2	91.5
Volume of BDA injection (in μl)	20		28		19	26	28.5	34.5		
Nb. of injection sites	10		15		10	12	19	27		