

## Review Article

# Physiological significance of astrogliosis after CNS injury

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In the injured central nervous system (CNS), reactive astrocytes form a glial scar and they are considered a physical barrier to prevent axonal regeneration by producing axonal growth inhibitors, such as chondroitin sulfate proteoglycans. However, the physiological role of reactive astrocytes remains to be elucidated. In this review, we showed that reactive astrocytes play a crucial role in wound healing and functional recovery. At the subacute phase of spinal cord injury (SCI), reactive astrocytes eventually migrated to the lesion epicenter and gradually compacted the infiltrated inflammatory cells and contracted the lesion area, and functional recovery was observed only in this repair phase. Selective deletion of the signal transducer and activator of transcription-3 (STAT3) in reactive astrocytes resulted in their limited migration associated with zinc signaling, markedly widespread damaged area and severe motor deficits. These results suggest that STAT3 is a key regulator of reactive astrocytes migration in the healing process after SCI, providing a beneficial aspect of reactive astrocytes after CNS injury.

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## Introduction

Since the regenerative capability of mammalian CNS is poor, SCI causes severe motor/sensory dysfunction and deficits can

often be permanent. It is now generally accepted that SCI is a two-step process involving the primary mechanical injury and a following cascade of auto-destructive injury. Mechanical trauma

rapidly lead to blood brain barrier disruption, neuronal cell death, axonal damage and demyelination, followed by a cascade of secondary injury that expands the additional inflammatory reaction at the lesion area<sup>1,2</sup>). This concept of “secondary” injury encompassing both necrotic and programmed cell death was postulated more than half a century ago, and has long been considered a major therapeutic target aimed at sparing tissue and function for anti-inflammatory and anti-apoptotic agents<sup>3,4</sup>), although primary mechanical injury is considered irreversible.

However, the actual clinical paralyzes of SCI patients as well as experimental SCI of rodents almost always exhibit severest state just after SCI, and gradual improvement to some extent with time course (except for complete paralysis), and there are very few patients with permanent deterioration<sup>5,6</sup>). If the secondary injury has a critical influence on the paralysis outcome, there would be a greater number of patients with deterioration after injury. Although the clinical time course of paralysis is suggesting that there is some sort of self-repair system after CNS injury even in rodents and primates, the mechanisms of gradual improvement in subacute phase is poorly understood and referred to as withdrawal of “spinal shock”. Understanding of the self-repair mechanism inherent in mammals is surely to lead to novel therapeutic strategy for the treatment of CNS injury.

## Astrogliosis and functional recovery

To interpret the process of paralysis improvement in the subacute phase, we examined serial histological sections of contused spinal cords and followed motor function in wild-type mice after produced contusion injury at thoracic 12 levels<sup>7</sup>). In this incomplete paralysis model, gradual functional recovery was observed until the subacute phase of injury (~ 2 weeks after injury), followed by limited recovery afterward.

Firstly, we tried to confirm the secondary injury process in the acute phase and found that the area of neural cell loss gradually enlarged in a rostral-caudal direction within a few days after SCI. Some portions of neurons were positive for cleaved caspase-3 indicated that the secondary injury process lasted for several days in this model during which we observed limited functional recovery. Meanwhile, astrocytes surrounding the lesion underwent a typical change of hypertrophy, process extension and increased expression of intermediate filaments such as GFAP and Nestin by 7 d after SCI, characteristic of “reactive astrocytes”.

Notably, these responsive astrocytes eventually migrated centripetally to the lesion epicenter and gradually compacted the CD11b-positive inflammatory cells, contracting the lesion area up until subacute phase after SCI as shown in Fig.1A. During

this process, we observed repair of injured tissue and gradual functional improvement, and reactive astrocytes formed a physical barrier against inflammatory cells, commonly referred to as glial scar (Fig.1B). After the migration of reactive astrocytes and completion of glial scar, functional improvement reached a plateau around 2 weeks after injury.

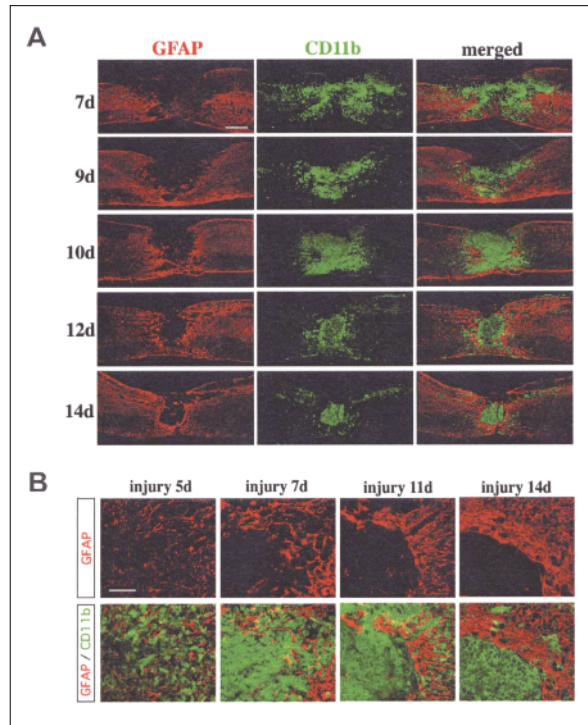
This glial scar contains extracellular matrix molecules that chemically inhibit axonal regeneration as well as physically, and has only been considered to definitely play a crucial part in CNS regeneration failure in the chronic phase of SCI<sup>8</sup>). However, the process observed at subacute phase strongly suggested that the emergence and migration of reactive astrocytes have a prominent role in the repair of injured tissue and the restoration of motor function before completion of the glial scar.

## The migration mechanism and Stat3 signaling

The regulatory mechanisms behind the reactive response of astrocytes remain elusive. We investigated the role of Stat3 signaling since Stat3 is a principal mediator in a variety of biological processes including wound healing and the movement of various types of cells<sup>9,10</sup>). In addition, several reports suggested Stat3 mediates certain aspects of astrogliosis downstream of the action of cytokines such as interleukin (IL)-6, leukemia inhibitory factor (LIF) and ciliary neurotrophic factor (CNTF) after CNS injury<sup>11-14</sup>).

In the injured spinal cord, phosphorylated Stat3 prominently increased at 12 h after injury, which remained detectable with western blotting for 2 weeks. We observed phosphorylation and nuclear translocation of Stat3 mainly in reactive astrocytes surrounding the lesion in immunohistochemistry, but not in distant areas for several days after injury.

To elucidate the role of Stat3 in reactive astrocytes, we performed experiments by using mice with a selective deletion of STAT3 under the control of Nestin gene promoter/enhancer<sup>15</sup>) (STAT3<sup>N<sup>-/-</sup></sup>), which are activated in reactive astrocytes after SCI. STAT3<sup>N<sup>-/-</sup></sup> mice showed no apparent abnormalities in motor function and development, although they showed signs of hyperphagia and leptin resistance<sup>15</sup>). At 2 weeks after injury, widespread tissue damage, demyelination and severe motor deficit were observed in this conditional STAT3 knockout mouse compared to wild-type mice (Fig.2A,C). Interestingly, although the comparable tissue damage and reactive gliosis was observed around the lesion at acute phase of injury in both type of mice, the configuration of these cells remained relatively unchanged for the chronic phase of injury owing to their limited migration. As a



**Fig.1** Compaction of inflammatory cells by reactive astrocytes and glial scar formation (Figure adapted from Okada et al.<sup>7)</sup>.

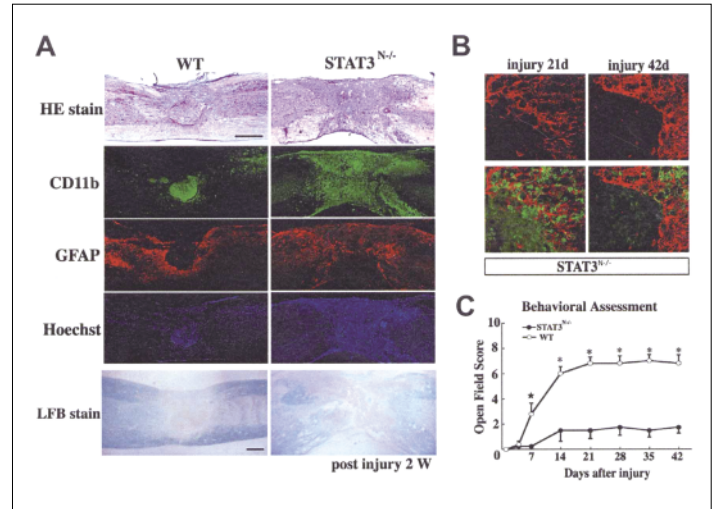
A) GFAP-positive reactive astrocytes gradually confine the area of CD11b-positive inflammatory cell infiltration. Scale bar, 500  $\mu$ m.

B) GFAP-positive reactive astrocytes gradually form glial scar and seclude inflammatory cells. Scale bar, 100  $\mu$ m.

result, the impaired contraction of inflammatory cells by reactive astrocytes brought about widespread damage and limited recovery in only STAT3<sup>N/-</sup> mice (Fig.2B,C).

To further investigate the relationship between STAT3 signaling and function of reactive astrocytes, analysis of SCI in SOCS3<sup>N/-</sup> mice<sup>16)</sup> was conducted. SOCS3 is the negative feedback molecule of STAT3 and the “bipolar” relationship between STAT3 and SOCS3 has been noted in several selective deletion experiments<sup>15,16)</sup>. In the injured spinal cord in SOCS3<sup>N/-</sup> mice, rapid migration of reactive astrocytes to seclude inflammatory cells, enhanced contraction of lesion area and dramatic improvement in functional recovery were observed. These results suggest that STAT3 signaling associated with the migration of reactive astrocytes is key regulator in the healing process after SCI.

Regarding the downstream of Stat3, several reports indicated a possible molecular link between Stat3-zinc signaling and cell movement<sup>17)</sup>. The zinc transporter LIV1 was found to be the tran-



**Fig.2** Compaction of inflammatory cells by reactive astrocytes and functional recovery were limited in STAT3<sup>N/-</sup> mice (Figure adapted from Okada et al.<sup>7)</sup>.

A) Compared to WT mice, the infiltration of CD11b-positive cells, GFAP negative area and demyelination were greater in STAT3<sup>N/-</sup> mice at 2 weeks after injury. Scale bar, 500  $\mu$ m and 300  $\mu$ m.

B) Glial scar formation and seclusion of inflammatory cells were insufficient in STAT3<sup>N/-</sup> mice even at 3 weeks after injury.

C) Time course of functional recovery of lower limbs (open filed score). While gradual recovery was observed in the subacute phase in WT mice, little improvement was observed in STAT3<sup>N/-</sup> mice. Data are mean  $\pm$  s.e.m. \*  $p < 0.01$ , and \*  $p < 0.05$ .

scriptional downstream target of Stat3 and essential for the nuclear localization of Snail, a transcriptional repressor of the Cdh1 gene which encodes E-cadherin. The absence of Stat3 therefore causes dysregulation of cell adhesion and impairs cell movement. In this model, selective deletion of Stat3 in reactive astrocytes brought about their limited migration and impaired healing process after SCI. In addition, another study reported that zinc deficiency impaired compaction of inflammatory cells by reactive astrocytes after CNS injury similar to STAT3<sup>N/-</sup> mice<sup>18,19)</sup>. On the other hand, astrocyte-targeted IL-6-expressing transgenic mice showed prompt migration of reactive astrocytes and compaction of inflammatory cells after CNS injury similar to SOCS3<sup>N/-</sup> mice<sup>20)</sup>. It stands to reason that enhanced phosphorylation of Stat3 in reactive astrocytes brought about the similar phenotype to SOCS3<sup>N/-</sup> mice after SCI in this transgenic mice. We also confirmed the robust expression of LIV1 mRNA in reactive astrocytes of wild-type mice but limited expression in STAT3<sup>N/-</sup> mice in this model<sup>7)</sup>. Thus, Stat3-Zinc signaling could

be a radically new therapeutic target for the treatment of CNS injury.

## The pleiotropic role for astrogliosis

Astrogliosis is intrinsically loosely defined term. After CSN injury, the astrocytes around the lesion response to injury and undergo a typical change of hypertrophy, process extension. These reactive astrocytes are gradually integrated and form a physical barrier, commonly referred to as glial scar<sup>21)</sup> as shown in Fig.1B. This process after CNS injury is roughly described as “reactive gliosis” or “astrogliosis”. As mentioned above, astrogliosis is considered to be detrimental for regeneration of CNS since they secrete chondroitin sulphate proteoglycans (CSPGs), which inhibit axonal outgrowth<sup>8)</sup>. Owing to this inhibitory molecule, severed axons within long myelinated tracts cannot regenerate past the lesion. In fact, treatment with chondroitinase after SCI resulted in degradation of CSPGs at the lesion site, and allowed axonal regeneration and recovery of locomotor and proprioceptive functions<sup>22)</sup>. In mice lacking both GFAP and vimentin, reduced astroglial reactivity resulted in improved sprouting of axons and functional restoration after SCI<sup>23)</sup>.

However, the basic phenomena of reactive gliosis appear conserved throughout vertebrate evolution. Thus, reactive gliosis has advantages for functional restoration or survival. Actually, glial scar provide several important beneficial functions for stabilizing fragile CNS tissue and repair of the blood-brain barrier after injury. Their primary role is to seclude the injury site from healthy tissue, preventing a cascading wave of uncontrolled tissue damage<sup>8)</sup>. The selective ablation of dividing astrocytes using ganciclovir and GFAP-TK transgenic mice resulted in severe leukocyte infiltration, tissue disruption, demyelination and neuronal death<sup>24)</sup>. Here, we showed that Stat3 signaling in reactive astrocytes have a considerable role in the repair of injured tissue and the recovery of motor function.

Although these results seem to conflict with one another, consideration of the timeframe in which these events were observed suggests a possible phase-dependent role of reactive astrocytes. In mice lacking both GFAP and vimentin, functional recovery was observed later than 2 weeks after injury<sup>23)</sup>, whereas substantial recovery was completed within 2 weeks after injury in Nes-Stat3<sup>-/-</sup> and Nes-Socs3<sup>-/-</sup> mice, suggesting that reactive astrocytes in the subacute phase repair tissue and restore function, whereas in the chronic phase of injury they impair axonal regeneration as a physical and chemical barrier. These reports also indicate that different potential effects of reactive gliosis are likely to be context dependent and regulated by different intracellular

signaling pathways.

## Concluding remarks

In this review, we have shown that Stat3 is a key regulator of reactive astrocytes migration and beneficial aspects of reactive astrocytes after CNS injury. Stimulation of reactive astrocytes migration might thus represent a potential target for intervention in the treatment of CNS trauma. However, the precise mechanism of reactive response in astrocytes as well as the functional recovery by reactive astrocytes remains elusive. For functional restoration, reorganization of interactions between descending inputs and the lumbosacral locomotor circuits is required. In addition, developed glial scar actually inhibit the regeneration and reorganization of spinal circuits in the chronic phase of injury. Future elucidation of both intrinsic and extrinsic astrocytes response mechanisms might contribute to achieve a better understanding of the pathophysiology of CNS injury.

## References

- 1) Beattie MS: Inflammation and apoptosis: linked therapeutic targets in spinal cord injury. *Trends Mol Med*, 10: 580-583, 2004.
- 2) Crowe MJ, Bresnahan JC, Shuman SL, Masters JN, and Beattie MS: Apoptosis and delayed degeneration after spinal cord injury in rats and monkeys. *Nat Med*, 3: 73-76, 1997.
- 3) Demjen D, Klusmann S, Kleber S, Zuliani C, Stieltjes B, Metzger C, Hirt UA, Walczak H, Falk W, Essig M, Edler L, Krammer PH, Martin-Villalba A: Neutralization of CD95 ligand promotes regeneration and functional recovery after spinal cord injury. *Nat Med*, 10: 389-395, 2004.
- 4) Okada S, Nakamura M, Mikami Y, Shimazaki T, Mihara M, Ohsugi Y, Iwamoto Y, Yoshizaki K, Kishimoto T, Toyama Y, Okano H: Blockade of interleukin-6 receptor suppresses reactive astrogliosis and ameliorates functional recovery in experimental spinal cord injury. *J Neurosci Res*, 15: 265-276, 2004.
- 5) Frankel HL, Hancock DO, Hyslop G, Melzak J, Michaelis LS, Ungar GH, Vernon JD, Walsh JJ: The value of postural reduction in the initial management of closed injuries of the spine with paraplegia and tetraplegia. *I Paraplegia*, 7: 179-192, 1969.
- 6) Ueta T, Shiba K: Cervical Cord Injuries; Comprehensive Treatment in the Acute Stage and Neurological Prognosis. *The Journal of the Japan Spine Research Society*, 12: 389-417, 2001.

- 7) Okada S, Nakamura M, Katoh H, Miyao T, Shimazaki T, Ishii K, Yamane J, Yoshimura A, Iwamoto Y, Toyama Y, Okano H. Conditional ablation of Stat3 or Socs3 discloses a dual role for reactive astrocytes after spinal cord injury. *Nat Med*, 12:829-34, 2006.
- 8) Silver J, Miller JH: Regeneration beyond the glial scar. *Nat Rev Neurosci*, 5: 146-156, 2004.
- 9) Sano S, Itami S, Takeda K, Tarutani M, Yamaguchi Y, Miura H, Yoshikawa K, Akira S, Takeda J: Keratinocyte-specific ablation of Stat3 exhibits impaired skin remodeling, but does not affect skin morphogenesis. *EMBO J*, 18: 4657-68, 1999.
- 10) Yamashita S, Miyagi C, Carmany-Rampey A, Shimizu T, Fujii R, Schier AF, Hirano T. Stat3 Controls Cell Movements during Zebrafish Gastrulation. *Dev Cell*, 2: 363-375, 2002.
- 11) Sriram K, Benkovic SA, Hebert MA, Miller DB, O'Callaghan JP: Induction of gp130-related cytokines and activation of JAK2/STAT3 pathway in astrocytes precedes up-regulation of glial fibrillary acidic protein in the 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine model of neurodegeneration: key signaling pathway for astrogliosis in vivo? *J Biol Chem*, 279: 19936-19947, 2004.
- 12) Kerr BJ, Patterson PH: Potent pro-inflammatory actions of leukemia inhibitory factor in the spinal cord of the adult mouse. *Exp Neurol*, 188: 391-407, 2004.
- 13) Xia XG, Hofmann HD, Deller T, Kirsch M: Induction of STAT3 signaling inactivated astrocytes and sprouting septal neurons following entorhinal cortex lesion in adult rats. *Mol Cell Neurosci*, 21: 379-392, 2002.
- 14) Klein MA, Möller JC, Jones LL, Bluethmann H, Kreutzberg GW, Raivich G: Impaired neuroglial activation in interleukin-6 deficient mice. *Glia*, 19, 227-233, 1997.
- 15) Gao Q, Wolfgang MJ, Neschen S, Morino K, Horvath TL, Shulman GI, Fu XY: Disruption of neural signal transducer and activator of transcription 3 causes obesity, diabetes, infertility, and thermal dysregulation. *Proc Natl Acad Sci USA*, 101: 4661-4666, 2004.
- 16) Mori H, Hanada R, Hanada T, Aki D, Mashima R, Nishinakamura H, Torisu T, Chien KR, Yasukawa H, Yoshimura A: Socs3 deficiency in the brain elevates leptin sensitivity and confers resistance to diet-induced obesity. *Nat Med*, 10: 739-743, 2004.
- 17) Yamashita S, Miyagi C, Fukada T, Kagara N, Che YS, Hirano T: Zinc transporter LIV1 controls epithelial-mesenchymal transition in zebrafish gastrula organizer. *Nature*, 429: 298-302, 2004.
- 18) Penkowa M, Giral M, Thomsen PS, Carrasco J, Hidalgo J: Zinc or copper deficiency-induced impaired inflammatory response to brain trauma may be caused by the concomitant metallothionein changes. *J Neurotrauma*, 18: 447-463, 2001.
- 19) Penkowa M, Carrasco J, Giral M, Moos T, Hidalgo J: CNS wound healing is severely depressed in metallothionein I- and II-deficient mice. *J Neurosci*, 19: 2535-2545, 1999.
- 20) Penkowa M, Giral M, Lago N, Camats J, Carrasco J, Hernández J, Molinero A, Campbell IL, Hidalgo J: Astrocyte-targeted expression of IL-6 protects the CNS against a focal brain injury. *Exp Neurol*, 181: 130-148, 2003.
- 21) Frisén J, Johansson CB, Török C, Risling M, Lendahl U: Rapid, widespread, and longlasting induction of nestin contributes to the generation of glial scar tissue after CNS injury. *J Cell Biol*, 131: 453-464, 1995.
- 22) Bradbury EJ, Moon LD, Popat RJ, King VR, Bennett GS, Patel PN, Fawcett JW, McMahon SB: Chondroitinase ABC promotes functional recovery after spinal cord injury. *Nature*, 416: 636-640, 2002.
- 23) Menet V, Prieto M, Privat A, Giménez y Ribotta M: Axonal plasticity and functional recovery after spinal cord injury in mice deficient in both glial fibrillary acidic protein and vimentin genes. *Proc Natl Acad Sci USA*, 100: 8999-9004, 2003.
- 24) Faulkner JR, Herrmann JE, Woo MJ, Tansey KE, Doan NB, Sofroniew MV: Reactive astrocytes protect tissue and preserve function after spinal cord injury. *J Neurosci*, 24: 2143-2155, 2004.