

Title: Centripetal migration of infiltrating macrophage drives spontaneous regeneration after spinal cord injury

Kazu Kobayakawa¹, Seiji Okada², Hirokazu Saiwai¹, Kazuya Yokota¹, Kiyoshi Tarukado¹, Kennichi Kawaguchi¹, Gentaro Ono¹, Kazuki Kitade¹, V. Reggie Edgerton³, Takeshi Maeda⁴, Yasuharu Nakashima¹

¹Department of Orthopedic Surgery, Kyushu University, Fukuoka, Japan, ²Department of Orthopedic Surgery, Osaka University, Suita, Japan,

³Department of Integrative Biology and Physiology, University of California, Los Angeles, Los Angeles, CA,

⁴Department of Orthopedic Surgery, Spinal Injuries Center, Iizuka, Fukuoka, Japan

Email of Presenting Author: kobayakawa.kazu.000@m.kyushu-u.ac.jp

Disclosures:

K Kobayakawa (N), S Okada(N), H Saiwai(N), K Yokota(N), K Tarukado(N), K Kawaguchi(N)
G Ono(N), K Kitade(N), VR Edgerton(N), T Maeda(N) Y Nakashima(N)

INTRODUCTION: Traumatic Spinal cord injury (SCI) is a devastating disorder resulting in permanent motor/sensory dysfunction. Mechanical injury causes bleeding and disruption of the blood–spinal cord barrier, followed by the infiltration of circulating cells such as macrophages and neutrophils. In the acute phase of SCI, it is known that these cells secrete inflammatory cytokines, inducing a secondary injury cascade to the spinal cord, but after the acute phase of SCI, little is known about the behavior of these cells. In the post-acute phase, long-distance axonal retraction from the initial site of injury (called axonal dieback) occurs, and infiltrating macrophages touching axons exacerbate this axonal injury. However, in this phase, spontaneous tissue repair such as remyelination and axonal regeneration also occurs with partial motor recovery. To understand such complex pathophysiology, we performed comprehensive screenings such as RNA sequencing (RNA-seq) of injured spinal cord.

METHODS: Spinal cord injury. All study protocols involving mice were approved by the Committee of Ethics on Animal Experimentation of our institution and conducted in accordance with the National Institutes of Health guidelines for the care and use of animals. To generate *IRF8*^{−/−};EGFP⁺ mice, we crossed *IRF8*^{−/−} and CAG-EGFP mice. Microglial IRF8-deficient chimeric mice were generated by transferring the bone marrow cells (BMCs) of EGFP⁺ *IRF8*^{+/+} mice into *IRF8*^{−/−} recipient mice, and macrophagic IRF8-deficient chimeric mice were generated by transferring the BMCs of EGFP⁺ *IRF8*^{−/−} mice into WT recipient mice after irradiation. Spinal cord injury was induced at the 10th thoracic level using an Infinite Horizons Impactor. The motor function was evaluated according to a locomotor open-field rating scale, the BMS, a footprint analysis and a grip walk test. RNA sequencing. The mRNA sequencing library was prepared using the NEBNext Ultra Directional Library Prep Kit for Illumina, and the samples were sequenced on an Illumina HiSeq 1500 system. The gene expression levels (FPKM) were calculated using the TopHat and Cufflinks software programs with default parameters. An enrichment analysis for the biological processes was performed based on the GO database annotations with DAVID. Statistical analysis. Tests were two sided, and the level of significance was set at 0.05, using the JMP software program.

RESULTS SECTION: A time-course RNA-seq analysis revealed prominent IRF8 expression in the spinal cord during the recovery phase after SCI. IRF8 is expressed in CD68⁺ macrophages after SCI. Macrophages centripetally migrate toward the lesion epicenter after infiltrating into the wide range of spinal cord, depending on the gradient of chemoattractant C5a. However, macrophages lacking interferon regulatory factor 8 (IRF8) cannot migrate toward the epicenter and remain widely scattered in the injured cord with profound axonal loss and little remyelination, resulting in a poor functional outcome after SCI. Time-lapse imaging and P2X/YRs blockade revealed that macrophage migration via IRF8 was caused by purinergic receptors involved in the C5a-directed migration. Conversely, pharmacological promotion of IRF8 activation facilitated macrophage centripetal movement, thereby improving the SCI recovery.

DISCUSSION: Detrimental effects of macrophages such as promotion of forming scars, damaging axons, and decreasing spared myelin were also shown in several studies using clodronate liposomes for depletion of infiltrating macrophages after SCI. *IRF8*^{−/−} macrophages widely scattered in injured spinal cord during recovery phase might exert these detrimental effects on a larger range of spinal cord, resulting in poor functional recovery after SCI. Our results indicate negative correlations between the ranges of macrophages in injured cord and the LFB⁺ areas or GFAP-area. Given that the numbers of infiltrating macrophages in WT and *IRF8*^{−/−} mice after SCI are comparable, these results provide a new concept that not only the numbers but also the location of infiltrating macrophages affect remyelination and formation of glial scar. In conclusion, lack of epicenter-directed macrophage migration led to worse functional recovery after SCI. IRF8 drives macrophage migration toward C5a, regulating the purinergic receptor expression. Our findings provide deeper insight into the role of macrophage centripetal movement in spontaneous recovery, providing the therapeutic potential of promoting infiltrating cell migration toward the lesion epicenter after traumatic CNS injury.

SIGNIFICANCE/CLINICAL RELEVANCE: Migration of macrophages via IRF8 toward a core lesion is important for the spontaneous recovery after spinal cord injury. Our findings provide a novel therapeutic target for central nervous system injury.

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IMAGES AND TABLES:

