

Macrophages attract astrocytes to the center of the injured spinal cord via ADP-P2Y1R axis

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INTRODUCTION: Although spinal cord injury (SCI) causes severe disability, effective treatments have not been established. One of the reasons for this disability is the scar tissue that forms after SCI. After SCI, inflammatory cells such as macrophages infiltrate the injured area, and astrocytes migrate, forming a glial scar around macrophages. The glial scar inhibits axonal regeneration, resulting in significant permanent disability. However, the mechanism through which glial scar-forming astrocytes migrate to the injury site has not been clarified. Here we show that migrating macrophages attract reactive astrocytes toward the center of the lesion after SCI.

METHODS: All study protocols involving mice were approved by the Committee of Ethics on Animal Experimentation of our institution and were conducted in accordance with the Act on Welfare and Management of Animals. First, to clarify the interaction between astrocytes and macrophages, we generated macrophage interferon regulatory factor 8 (IRF8), which regulates macrophage migration after spinal cord injury, deficient chimeric mice by transferring the bone marrow cells of *IRF8*^{-/-} mice into *Nes-EGFP*⁺ mice, in which reactive astrocyte express EGFP, after irradiation. These mice were subjected to a contusion injury at the 10th thoracic level. After the injury, pathological changes were evaluated. Next, to investigate the mechanism in more detail, a transwell assay was performed using primary cultured astrocytes and macrophages. Transwell assay was performed in 5 groups: (1) the control group: astrocytes in the inserts, with the only medium in the well; (2) the macrophage group: astrocytes in the inserts, with macrophages in the well; (3) the ADP group: astrocytes in the inserts, with ADP in the well; (4) the apyrase group: astrocytes in the inserts, with macrophages and apyrase in the well; and (5) the MRS-2179 group: astrocytes treated with MRS-2179 in the inserts, with macrophages in the well. In each group, the percentage increase in migrating cells compared to the control group was evaluated. Finally, to determine whether the macrophage-derived ADP-astrocyte P2Y1R pathway, which we have identified, is also functional in vivo, we performed additional animal experiments: *IRF8* knockout mice were subjected to spinal cord injury, followed by continuous ADP administration. Wild-type mice were also subjected to spinal cord injury and treated with MRS-2179. The pathological evaluation was performed on the spinal cords of these mice. All statistical analyses were performed using the GraphPad Prism software program, version 9.1.2. Specifically, pathological assessments were tested by unpaired t-test, and transwell assays were tested by one-way ANOVA.

RESULTS SECTION: In chimeric mice lacking IRF8, macrophages failed to migrate to the center of injury and were widely scattered around the injured spinal cord, forming a huge glial scar around it (Fig. 1a, b). Furthermore, the presence or absence of IRF8 in macrophages did not alter astrocyte cell body size or proliferative ability (Fig. 1c, d). Next, we investigated the mechanism by which macrophages attract astrocytes by in vitro trans-well assay. The cell number of migrating astrocytes increased in the macrophage group and the ADP group, but not in the apyrase group and the MRS-2179 group (Fig. 2a, b). These results revealed that macrophage-derived ADP attracts astrocytes via P2Y1R. Finally, we evaluated whether this ADP-P2Y1R pathway could regulate astrocyte migration after spinal cord injury: continuous intraspinal infusion of ADP into *IRF8* knockout mice resulted in reduced glial scarring (Fig. 3a, b). On the other hand, Intraspinal injection of MRS-2179 into wild-type mice increased glial scarring. These results indicate that the ADP-P2Y1R pathway affects astrocyte migration, resulting in the glial scar formation after SCI.

DISCUSSION: We previously reported that STAT3 is involved in astrocyte migration, but the mechanism that controls the direction of astrocyte migration was not clear. We hypothesized that macrophages attract astrocytes because knockout of IRF8 changes the location of glial scars composed of astrocytes in accordance with changes in the distribution of macrophages. In this study, we show that macrophage-derived ADP attracts astrocytes via the astrocyte P2Y1R. However, there are still some unknowns regarding the mechanism of glial scar formation. For example, it is not yet clear as to what factors stop astrocyte migration. Since astrocytes are arranged in a row at the outer edge of the scar and are rarely present inside the glial scar, astrocyte migration is not regulated only by the concentration gradient of ADP; some factors inside the scar should stop astrocyte migration. However, the mechanism that stops the migration of astrocytes at the glial scar has not been elucidated. For example, PARP1 may be a candidate for the negative regulation of astrocyte migration. Stat3 is known to be essential for astrocyte migration, and PARP1 has been reported to inhibit Stat3 phosphorylation. This study did not provide any insights into such factors that inhibit astrocyte migration, and further studies are needed to elucidate the mechanism by which astrocytes form glial scars.

SIGNIFICANCE/CLINICAL RELEVANCE: We demonstrated that macrophages attract astrocytes via the ADP-P2Y1R pathway. Our findings provide deeper insight into the interaction between astrocytes and macrophages and suggest a potential therapeutic target for SCI.

