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Short communication

Myeloid-derived suppressor cells mediate immune suppression in spinal cord injury

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ABSTRACT

Spinal cord injury (SCI) is characterized by the loss of motor and sensory functions in areas below the level of the lesion and numerous accompanying deficits. Previous studies have suggested that myeloid-derived suppressor cell (MDSC)-induced immune depression may play a pivotal role in the course of SCI. However, the concrete mechanism of these changes regarding immune suppression remains unknown. Here, we created an SCI mouse model to gain further evidence regarding the relationship between MDSCs following SCI and T lymphocyte suppression. We showed that in the SCI mouse model, the expanding MDSCs have the capacity to suppress T cell proliferation, and this suppression could be reversed by blocking the arginase.

1. Introduction

Spinal cord injury (SCI) is a devastating condition of the central nervous system (CNS) that often causes damage to nerve roots or myelinated fiber tracts carrying signals to and from the brain (Ramer et al., 2014). In addition to the loss of sensation and motor function below the injury level, individuals affected with SCI will also often experience other complications, including bowel and bladder dysfunctions, urinary and respiratory system infections, and serious decubital ulcers (Taweel and Seyam, 2015; Oh and Eun, 2015; Sezer et al., 2015).

During the transition from an acute stage to a chronic stage of recovery after SCI, immune depression plays an important role that exacerbates the symptoms associated with the present neurologic deficits (Schwab et al., 2014). Especially in the acute stage of SCI, infections following immune depression are one of the leading causes of death (Soden et al., 2000). Animal studies have demonstrated that monocyte density increases at 24 h, while lymphocyte numbers significantly decrease (Stirling and Yong, 2008). Further research has indicated that both T cell mediated immunity and B cell mediated humoral immunity are disordered in SCI subjects (Campagnolo et al., 2008).

Recently, myeloid-derived suppressor cells (MDSCs) have become a popular topic in the research of immune depression (Schwab et al., 2014; Motz and Coukos, 2013). MDSCs are a heterogeneous population of cells that expand during the course of many diseases, such as carcinoma, inflammation, infection and sepsis (Gabrilovich and Nagaraj, 2009). In pathological conditions, MDSCs might affect immune depression by upregulating the expression of immune suppressive factors such as arginase and inducible nitric oxide synthase (iNOS) (Ostrand-Rosenberg and Sinha, 2009). MDSCs have a remarkable ability to suppress T cell responses. It has also been reported that MDSCs can regulate humoral immunity and innate immune responses by modulating the cytokine production of macrophages (Gabrilovich and Nagaraj, 2009). To date, however, no definite conclusion has been reached about whether MDSCs are involved in the course of immune suppression following SCI.

The purposes of our study were to first establish an SCI mouse model and to observe the changes in the lymphocyte subpopulation in the acute, sub-acute and chronic stages of SCI; to compare MDSCs invading the spleen and blood of the experimental and control groups; and, finally, to observe the inhibition of SCI-induced $CD11b^+$ $Gr\text{-}1^+$ cells on T cell proliferation in vitro and to thus analyze the important role of SCI-induced MDSCs in mediating immune suppression.

2. Methods

2.1. Animals

All procedures were performed according to the National Institutes of Health Guide for Care and Use of Laboratory Animals and were approved by the Institutional Animal Care and Use Committee at the Beijing Charity Hospital, China Rehabilitation Research Center (LLSC20110084).

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The subjects for the experiment were adult (8–10 weeks old, weighing 18–25 g), male C57BL/6 mice (n = 41), which were purchased from the Biophysics Institute of Chinese Academy of Sciences. The quality assessments of the selected animals were performed according to the ARRIVE guidelines (Kilkenny et al., 2012). All animals were provided with a fresh diet and tap water ad libitum throughout the experiment; environmental conditions were continuously regulated automatically. Every ten animals were housed in a cage within a well-ventilated room, and all animals were submitted to a daily rhythm of 12 h with artificial light and 12 h of darkness. The temperature of the room was maintained at 23 ± 1 °C.

2.2. Grouping and model creating

Animals were randomly assigned to the experimental group (n = 18), the control group (n = 18) and the normal group (n = 5). The experimental group mice were deeply anesthetized with an intraperitoneal injection of 10% chloral hydrate (5 mL/kg). An incision was made in the skin of these mice. The muscle and the tissue overlying the spinal column was blunt dissected away, revealing the laminae. Using the spinous process of T10 as a landmark, laminae 9 and 10 were carefully removed to expose the spinal cord. Extradural compression of the spinal cord at the vertebral level of T9/10 was achieved using a modified aneurysm clip with a closing force of 8 g, producing mechanical trauma. The clip was left in place for 1 min and then removed (Joshi and Fehlings, 2002; Wells et al., 2003). The muscle layers over the laminectomies and the skin on the back were aseptically closed with a surgical suture. Postoperatively, these animals were heated using overhead lamps until recovery from surgical anesthesia was complete. In our experiment, animals did not receive any prophylactic antibiotics or a solution to prevent dehydration.

Control mice experienced anesthesia and removal of the dorsal arch of the vertebrae without injuring the spinal cord. Next, the bladders of the experimental mice were manually voided twice per day for the duration of the study. Urine pH was monitored weekly throughout the study, and the animals were observed daily for signs of infection or abnormal wound healing at the site of surgery.

Normal mice were fed a standard diet without any vertebrae operation. There were no overt signs of infection in any mice. Meanwhile, the animals that were contused in a nonsymmetrical manner were excluded from the experimental analysis.

2.3. Collection of blood and spleen

After 3, 10 and 28 days of creating the animal models, we collected blood via the bulbus oculi. Then, we euthanized 6 mice by dislocating the vertebrae colli and harvested the spleen. Blood and tissue samples were then prepared for flow cytometry. Untreated or sham-injured animals served as controls.

2.4. Isolation of cells

The isolations of MDSCs and T cells were performed according to the manufacturer's instructions (Miltenyi Biotec). The purity of the cell separation ranged between 85 and 90% (Makarenkova et al., 2006).

2.5. Flow cytometry analysis

Harvested cells were washed in fluorescence activated cell sorter (FACS) medium (1 × PBS supplemented with 0.1% BSA and 0.1% NaN_3) and stained with the following Abs: FITC-labeled anti-mouse CD4, CD11b and B220 and PE-labeled GR1 and CD8 (BD Pharmingen). The fluorescence was measured using a FACScan flow cytometer (BD Biosciences), and the data analysis was performed using Cell Quest software (BD Biosciences). Cell sorting was performed on a MoFlo cell sorter (Dako Cytomation).

2.6. T cell proliferation assay

Purified MDSCs isolated from the experimental mice were added in 1×10^5 normal splenic T cells in flat bottom 96-well plates. These co-cultures were promptly stimulated with 1 μg/mL anti-CD3. Then, ^{3}H-thymidine was added 24 h later, and the cells were allowed to proliferate for another 18 h before the incorporated radioactivity was measured. T cells cultured alone served as the control. In addition, we used nor-NOHA (0.5 mM, Calbiochem, added from the beginning of the culture) to block the arginase.(Wells et al., 2003). The average level of ^{3}H-thymidine incorporation plus or minus the standard deviation (SD) is expressed as counts per minute (CPM) ± SD.

2.7. Statistical analysis

Data are expressed as the means with the standard deviation. Comparisons between two groups were assessed using Student's t-test. Data were analyzed using SPSS 13.0 for windows software. p values less than 0.05 were considered statistically significant.

3. Results

3.1. SCI mice model

After the mouse model was created, all 36 animals appeared to be lethargic and exhibited signs of poor grooming and weight loss. One mouse in the experimental group and 1 mouse in the control group died during the course of the experiment. The other mice were successfully used in the experiments.

3.2. Change in the T cell percentage after spinal cord injury

In the PBMCs of SCI mice, the $CD8^+$ and $CD4^+$ T lymphocytes remained constant in normal mice throughout the experimental period ($CD8^+$ 16.36% ± 4.21%, $CD4^+$ 21.68% ± 6.29%), while these cells dramatically decreased by approximately 50% at day 3 in the acute stage ($CD8^+$ 4.90% ± 2.35%, $CD4^+$ 8.27% ± 3.98%) after SCI ($p < 0.05$, compared to the normal group). Subsequently, the percentage of $CD8^+$ and $CD4^+$ T cells started to recover but remained at suppressive levels at day 10 in the subacute stage ($CD8^+$ 8.26% ± 2.67%, $CD4^+$ 16.48% ± 3.84%) until day 28 in the chronic stage ($CD8^+$ 13.52% ± 4.52%, $CD4^+$ 24.80% ± 5.23%), at which time, the $CD8^+$ and $CD4^+$ T cells recovered to almost normal levels (Fig. 1A, C). This percentage of T cells in the splenocytes similarly changed, with more limited ranges compared to those in the PBMCs (Fig. 1B, D).

3.3. Changes in the B cell percentage after spinal cord injury

Compared to the normal groups, the percentage of B cells in the PBMCs and splenocytes had no statistical changes at day 3, 10 or 28 (Fig. 2). Interestingly, it seemed that the percentage of B cells even increased in the acute stage at day 3 in the spleen, although there was no significant difference (39.56% ± 8.42% vs. 56.32% ± 17.13%, $p > 0.05$). This is inconsistent with previous studies and can be plausibly explained as being divergent in the strain of mice and method of measuring preferences from published evidence (Riegger et al., 2007, 2009).

3.4. Expression of MDSCs in the blood and spleen after spinal cord injury

First, we analyzed the changes in the MDSC percentage after SCI (Fig. 3A, C). In the PBMCs, compared to the normal group, the percentage of $CD11b^+$ $Gr\text{-}1^+$ cells (7.61% ± 2.84%) dramatically increased by approximately 2-fold at day 3 (19.13% ± 7.12%) after SCI ($p < 0.05$). Subsequently, at day 10, during the subacute stage, the $CD11b^+$ $Gr\text{-}1^+$ cell percentage started to decrease (12.43% ± 3.81%) but remained at high levels. Then, the $CD11b^+$ $Gr\text{-}1^+$ cell percentage continued to