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Neuroprotective Effect of Vaccination with Autoantigen-Pulsed Dendritic Cells After Spinal Cord Injury

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Background. **Studies have shown that the development of a properly controlled autoreactive T cell response can serve as a therapeutic approach for spinal cord injury (SCI). Thus, vaccination with mature dendritic cells (DCs) pulsed with central nervous system (CNS) antigens that can prime autoreactive T cells have the potential for treating SCI.**

Materials and Methods. **Mature DCs pulsed with spinal cord homogenate (SCH), nonpulsed mature DC or phosphate-buffer solution (PBS) were injected into spinal cord-injured mice peritoneally. The functional recovery of spinal cord was measured by Basso mouse scale and footprint analysis. Spinal cord specimen was preserved for immunohistochemical staining to detect T cell infiltration, differentiation of neural stem/progenitor cells, and tissue preservation. RT-PCR and enzyme linked immunosorbent assay (ELISA) was used to detect the expression of cytokines and neurotrophic factors.**

Results. **Vaccination with DCs pulsed with SCH promoted pronounced functional recovery from SCI. The neuroprotection induced by SCH-pulsed DCs (SCH-DC) correlated to the accumulation of $CD4^+$ T cells in the lesion site. SCH-DC markedly affected the production of interferon-γ, interleukin-12, and granulocyte-macrophage colony stimulating factor. SCH-DC also promoted expression of neurotrophic factors in the injured spinal cord and spleen cells. Furthermore, vaccination with SCH-DC enhanced neuronal differentiation of neural stem/progenitor cells, and it led to better tissue preservation.**

Conclusion. **The results of the present study suggest that DC-mediated immune regulation may be a potential therapeutic approach aimed at shifting the balance between immune and nerve cells in order to treat SCI.**

Key Words: **spinal cord injury; dendritic cell; T cell; immune microenvironment; cytokines; neurotrophic factor.**

INTRODUCTION

Following primary mechanical spinal cord injury (SCI), secondary pathology processes contribute to further damage. The local immune response has been identified as a key component of secondary damage following SCI [1, 2]. It is traditionally believed that the immune response in the damaged central nervous system (CNS) is harmful and should therefore be suppressed [3]. However, accumulating evidence indicates that the immune system can play both detrimental and beneficial roles in the nervous system [4]. Various immune cells including T cells, NK cells, macrophages, dendritic cells (DCs), and microglia participate in limiting damage to the nervous system during CNS trauma and in the process of repair after injury [4, 5].

Previous studies have shown that self-reactive T cells under certain conditions can be harmful to CNS because the transfer of myelin basic protein (MBP) peptide-specific T cells to naive recipients causes experimental autoimmune encephalitis (EAE) [6, 7]. However, recent studies have suggested that under some conditions, autoimmune T cells may convey

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"protective autoimmunity" to neurons and axons after mechanical nerve injury [5, 8–10]. In rodent models of optic nerve crush or SCI, passive or active immunization with T-cells specific to CNS-associated myelin antigens reduces neuronal loss and promotes immune neuroprotection [11–14]. Studies over the last few years have provided evidence indicating that a properly controlled T cell-mediated autoimmune response plays a key role in neurogenesis of the injured spinal cord and its protection from secondary degeneration [5]. Therefore, boosting the T cell response specific for CNS antigens could be considered a potential way of ameliorating SCI [8].

DCs have emerged as a key component of the immune balance and are critically involved in the T cell-mediated immune response. Current data indicate that DC functions are related to the stage of their maturation. After an antigenic stimulus, immature DCs become fully mature DCs, as shown by the up-regulation of major histocompatibility complex (MHC) and costimulatory molecules such as CD80 and CD86. These decisive signals from the DCs, concurrently with the release of proinflammatory cytokines, induce an antigen-specific T-cell immune response [15]. The capacity for the *in vitro* generation and manipulation of immunocompetent DCs promotes their use in immunotherapy approaches, particularly in cancer. Studies have shown that injection of DCs loaded with tumor-associated antigens leads to an anti-tumor immune response, and multiple clinical trials have been carried out using this method [16, 17]. Moreover, in animal models of different diseases, vaccination with specific antigen- or peptide-pulsed DCs has prevented the development of arthritis, diabetes, and EAE [18–20].

It is now generally accepted that antigen-pulsed DCs are efficient at priming the proliferative response of $CD4^+$ T-cells and T-cell clones [21, 22]. Immature DCs are poor antigen-presenting cells that are involved in the induction of peripheral T cell tolerance, while mature DCs are endowed with the capacity to initiate an antigen-specific T cell response [23, 24]. Previously performed studies have reported the neuroprotective effect of vaccination with immature DCs [25, 26], but a potential caveat to these studies is that once immature DCs are introduced into the host, immature DCs might cause tolerance instead of antigen-specific T cell proliferation. Therefore, to evoke T cell-mediated protective autoimmunity in the present study, we used mature DCs to investigate their therapeutic potentials for treating SCI. We show that vaccination with SCH-pulsed DCs (SCH-DC) leads to a protective effect from SCI in mice, and we explore the cellular basis and cytokine network in the injured spinal cord.

MATERIALS AND METHODS

Animals and Spinal Cord Injury

Adult BALB/c mice (weighing 24 to 26 g) were purchased from the Animal Breeding Center of Harbin Medical University (Harbin, PR China). All surgical procedures and postoperative animal care were carried out in accordance with the Guide for the Care and Use of Laboratory Animals (National Research Council, 1996, USA) and was approved by the Animal Use and Care Committee of School of Medicine, Harbin Medical University. In accordance with the clip compression model described previously [27], all mice were anesthetized by intraperitoneal injection of 10% chloral hydrate (5 mL/kg) and then positioned on a cork platform. The skin was incised along the midline of the back, and the paravertebral muscles of the thoracic-level (T8–T10) vertebrae were dissected out. Laminectomy was performed at the T9 level under visual guidance using an operating microscope. A 9-se-long extradural compression with a vascular clip (with 8 *g* force) was performed around the exposed spinal cord in order to cause an acute compression injury. Animals were left to recover on a warm pad until thermoregulation and an alert state were reestablished. Animals received manual bladder expression twice daily until the return of bladder function; they also received appropriate veterinary care when needed.

Antigens

A homogenate of the spinal cord (SCH) was harvested from BALB/c mice (aged 4–6 weeks, weighing 18–23 g). The process is described in brief as follows: BALB/c mice were euthanized by anesthesia overdose, and T 7–10 were sectioned. The spinal cord segments were ground and filtered (200 mm), then ground by an ultrasonic homogenizer and finally centrifuged at 15,000 rpm for 20 min. The supernatant was collected, and the protein concentration was measured using the bicinchoninic acid assay (BCA) method.

Preparation of Mouse DCs

DCs were obtained from bone marrow using a method described previously [28]. The brief process is described as follows: femurs and tibias were removed from the dead mature male BALB/c mice, the muscles and connective tissues were stripped, and bones were placed in 70% ethanol for 5 min for disinfection and then washed with phosphate-buffer solution (PBS). Both ends of the bones were cut with scissors, and the marrow was flushed out with calcium-free and magnesium-free PBS using a syringe with a 23-gauge needle. Cell aggregates were broken down by vigorous pipetting. Red blood cells were lysed with ACK buffer (150 mM NH_4Cl, 10 mM $KHCO_3$, 0.1 mM Na_2EDTA, pH 7.2–7.4). At d 0, bone marrow cells were counted and plated at a density of 0.5×10^6 cells/mL in a 2.5 mL flask (total 5 mL). The cells were cultured in RPMI-1640 medium (Gibco Invitrogen, Beijing, PR China) supplemented with 100 mg/mL penicillin and streptomycin, 2 mM L-glutamine, 50 mM b-mercaptoethanol, 1 mM pyruvate, 1:100 nonessential amino acids, and 10% heat-inactivated and filtered fetal calf serum (Gibco Invitrogen, Grand Island, NY) (referred to hereafter as DC medium). Cytokine recombinant murine granulocyte macrophage colony-stimulating factor (rmGM-CSF, PeproTech, Rocky Hill, NJ) at a concentration of 200 U/mL was added at d 0. On d 3, an additional 2.5 mL of RPMI-1640 medium containing 200 U/mL rmGM-CSF was added to the plates. On d 6 and 8, half of the culture supernatant was collected and centrifuged, and the cell pellet was resuspended in 2.5 mL of fresh RPMI-1640 containing 200 U/mL rmGM-CSF and returned to the original plate. On d 10, cells were ready for use. Some cells were resuspended in fresh DC medium (without additional cytokines; 2×10^6 cells/mL) containing SCH (100 mg/mL) or not. DCs that were pulsed with SCH were stimulated with lipopolysaccharide (LPS, 1 μg/mL) for 24 h to obtain SCH-pulsed mature DCs. Unpulsed mature DCs were stimulated