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Research report

Brain recruitment of dendritic cells following Li-pilocarpine induced status epilepticus in adult rats

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ABSTRACT

Activation of inflammatory and immune pathways may contribute to the development of epilepsy. Dendritic cells (DCs), a heterogeneous group of professional antigen presenting cells, can be detected in the brain under several inflammatory conditions. However, the spatiotemporal distribution and origin of seizure-induced DCs accumulation in the brain have not been explored yet. In the present study, we demonstrate the presence of CD11c-positive DCs in the hippocampus, thalamus and temporal cortex following Li-pilocarpine induced status epilepticus (SE) in rats. Recruitment of DCs occurs as early as 1 day following the induction of SE, reaching peak time at day 3, and still evident until day 5. The recruitment of DCs in the brain following lithium chloride administration was not detected. The observed DCs cannot be double-labeled with Iba-1 (an activated microglia marker) and whole-body radiation prevents seizure-induced DCs accumulation in brain parenchyma. Our data suggest that the recruitment of DCs in the epileptic brain may be derived from peripheral circulation and this population of immune cells may be involved in the immune processes after SE.

1. Introduction

Epileptogenesis refers to the process of developing spontaneous recurrent seizures (SRS) following an insult (Rakhade and Jensen, 2009). Animal models of epilepsy and human tissue studies suggest that epileptogenesis involves a cascade of molecular, cellular and neuronal network alterations, such as immediate early gene activation and changes in gene expression, alterations in neurotransmitter receptors and ion channels, necrotic and apoptotic neuronal death, mossy fiber sprouting, and aberrant neurogenesis (Jensen, 2009; Pitkanen and Lukasiuk, 2009). However, the details of the mechanisms underlying epileptogenesis remain largely unclear. Improved understanding of the mechanisms that are involved in the epileptogenic process should aid the development of disease-modifying treatment paradigms (Perucca et al., 2007; Pitkanen and Lukasiuk, 2009).

Recent experimental and clinical evidence highlights that the activation of inflammatory and immune pathways may contribute to the development of epilepsy (Aarli, 2000; Maroso et al., 2010; Vezzani et al., 2010). The temporal evolution of inflammatory processes after induction of status epilepticus (SE) was demonstrated in rodent brains. Pro-inflammatory cytokines are first expressed in the activated microglia and astrocytes, and an ensuing wave of inflammation is induced in neurons and endothelial cells of the blood–brain barrier (BBB) (Friedman, 2011; Kostulas et al., 2002). Pharmacological studies in experimental models of acute or chronic seizures demonstrated that targeting of specific pro-inflammatory pathways after SE shows antiepileptogenic effects (Ravizza et al., 2011). Furthermore, transient brain recruitment of peripheral immune cells was also reported (Fabene et al., 2008; Marchi et al., 2011). Together, these inflammatory and immune processes facilitate the excitability of the neural network, and are likely to be involved in the molecular, structural and synaptic changes characterizing epileptogenesis (Ravizza et al., 2011).

Dendritic cells (DCs) are a heterogeneous group of professional antigen presenting cells with a potent capacity to recognize and respond to danger signals. They participate in the initiation, maintenance and tolerance of the immune reactions in many neuroinflammatory diseases (Fischer and Reichmann, 2001; Hatterer et al., 2008; Stichel and Luebbert, 2007). CD11c, a pan DCs marker, is a type I transmembrane protein found at high levels on most DCs

Abbreviations: SRS, spontaneous recurrent seizures; SE, status epilepticus; BBB, blood–brain barrier; DCs, dendritic cells; MGG, May-Giemsa-Grunwald.

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(Reichmann et al., 2002). While normally residing in the meninges and choroid plexus, perivascular and intraparenchymal DCs can be detected in brain under several inflammatory conditions (Clarkson et al., 2012). Whether these DCs are derived from peripheral circulation (Hatterer et al., 2008; Steel et al., 2009) or from resident microglia (Chinnery et al., 2010; Prodinger et al., 2011) was still a matter of debate. Given the role of DCs in shaping of immune responses in the CNS, this population of immune cells may also contribute to the regulation of immunopathological cascade following seizures in the brain. However, the temporal-spatial distribution and the origin of DCs in epileptic brain have not been explored yet.

In the present study, we employed a Li-Pilocarpine induced SE model to investigate the presence, time course and spatial distribution of CD11c-positive DCs in the rat brain. Then we sought to examine the origin of these DCs by using whole-body irradiation and double-staining with CD11c and Iba-1, a marker of activated microglia.

2. Materials and methods

2.1. Animals

Adult male Sprague-Dawley rats weighing between 200 and 250 g were used in this study. The rats were housed under controlled temperature and light conditions (12 h light/dark cycle with lights on at 8:00 AM), with ad libitum access to food and water. All procedures used were in strict accordance with the guidelines established by the U.S. NIH and were approved by the Fourth Military Medical University Animal Care Committee.

2.2. Induction of status epilepticus

SE was induced by Li-pilocarpine intraperitoneal (i.p.) injection as described previously (Yang et al., 2010). An aqueous solution of lithium chloride (3 eEq/kg, i.p.; Sigma–Aldrich, St. Louis, MO) was injected 18–20 h prior to the administration of pilocarpine (30 mg/kg, i.p., dissolved in saline; Sigma–Aldrich). Rats were pretreated with scopolamine methyl bromide (1 mg/kg, i.p.; Sigma–Aldrich) 20 min prior to pilocarpine injection to reduce its peripheral effects. Development of seizures were evaluated by behavioral (Racine's scale) assessment. Seizures were terminated with diazepam (10 mg/kg, i.p.) when rats experienced stage 4 or greater seizures for 90 min. For immunohistochemistry, the rats were sacrificed at 12 h, 1, 3, 5, 7 and 30 days following SE (n = 6, 6, 6, 6, 5 and 5 in each group, respectively). An equal volume of physiological saline instead of lithium chloride and pilocarpine were given to the control rats (n = 6). Another group of rats were treated with lithium chloride as described above, whereas pilocarpine administration was omitted. These rats were sacrificed at 12 h, 1, 3, 5 and 7 days following lithium chloride treatment as well (n = 3 in each group).

2.3. Tissue fixation and immunohistochemistry

Under anesthesia with sodium pentobarbital (50 mg/kg, i.p.), rats were perfused transcardially with 0.9% saline, followed by 4% paraformaldehyde in 0.1 M PBS (pH 7.4). The brains were immediately removed, postfixed for 2 h in the same fixative, and placed in 20% sucrose at 4 °C until they sank. Coronal sections (40 μm thickness) were cut on a freezing microtome, stored in PBS. For immunohistochemical detection of CD11c-positive DCs, sections were washed free-floating in 0.01 M PBS, then incubated overnight at 4 °C with mouse-derived monoclonal antibody against CD11c (1:500; Abcam Inc, American). After incubation, they were washed with PBS and incubated in biotinylated goat anti-mouse secondary antibodies (1:500; Sigma–Aldrich) for 2 h at room temperature, followed by rinsing in PBS and incubation in avidin–biotin–peroxidase complex (1:500; Sigma–Aldrich) for 2 h. After a final wash, sections were colorized by the 3,3-diaminobenzidine (DAB)-nickel solution through peroxidase reaction.

For double-labeling, cryostat sections were incubated with a cocktail solution containing the primary antibodies against CD11c and Iba-1 (rabbit-derived polyclonal, 1:500; Abcam Inc, American). Following three rinses in PBS, sections were labeled with secondary Alexa Fluor 594 conjugated goat anti-rabbit IgG and Alexa Fluor 488 conjugated goat anti-mouse IgG (1:500, Molecular Probes, Eugene, OR, USA) for 2 h. Omission of the primary or secondary antibody served as negative controls. The specificity of immunolabeling was also verified on sections of peripheral lymphoid organs (Supplementary Figure 1). The labeled sections were rinsed and cover-slipped. Double immunofluorescence staining was examined under a laser scanning confocal microscope (FluoView300, Olympus, Tokyo, Japan).

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.brainresbull.2012.11.007.

2.4. Western blotting

Additional epileptic rats were sacrificed at 1, 3, and 5 days following SE (n = 5, 5 and 4 in each group, respectively). Rats that treated with lithium chloride only (n = 3, 3 and 3 in each group, respectively) were sacrificed simultaneously. Rats that treated with physiological saline only (n = 3) were sacrificed three days later. The brains were removed, frozen in liquid nitrogen and stored at −80 °C. Tissues of hippocampus, thalamus and temporal cortex were gently dissected and placed in cold buffer containing 250 mM sucrose, 18 mM Tris–Hepes, pH 7.4, 1 mM EDTA, and complete protease inhibitor (Roche Applied Science, Indianapolis, IN). Tissues were disrupted using a PRO 200 homogenizer (PRO Scientific Inc., Monroe, CT). Samples of the protein were loaded on 12% SDS-polyacrylamide gels and transferred to nitrocellulose membranes. The samples were blocked with 5% non-fat milk in TBST for 1 h and incubated for 2 h at room temperature with anti-CD11c antibody (Santa Cruz, USA, 1:500). As a protein loading control, the samples were also incubated with anti-tubulin antibody (Sigma, USA, 1:3000). After washed with PBS, the samples were incubated for 1 h with HRP-conjugated goat anti-mouse IgG (Bio-Rad, USA) and HRP-conjugated goat anti-goat IgG (Bio-Rad, USA) antibodies. The density of western blotting bands was measured with the Image Quant 5.2 software (GE Healthcare Life Sciences).

2.5. Irradiation of animals

To examine whether brain DCs were derived from the peripheral circulation, depletion of circulating mononuclear cells was achieved in a separate group of rats subjected to whole-body irradiation before SE induction. These rats were irradiated at a dose of 9 Gy for a total time of 10.5 min (Anthony et al., 1997). The skull was shielded by a lead box. All irradiated animals were maintained with 100 mg/l of oxytetracycline in their drinking water. Three days after depletion, animals received treatment of Li-pilocarpine as detailed above. The rats were sacrificed at 1, 3 and 7 days after SE (n = 4, 4, and 3 in each group, respectively) and used for immunohistochemistry. In order to assess the level of mononuclear cell depletion, blood smears and May-Giemsa-Grunwald (MGG) staining were made at the time of perfusion as described previously (Amer et al., 2001). In brief, after the smears were fixed in methanol for 15 min, they were stained in May-Grunwald solution for 5 min and then in Giemsa solution for 10 min. The smears were dried and mounted after rinsed in running water (pH = 6.8) for 10 min. Age-matched normal rats were used as controls (n = 3, 3 and 3 in each group, respectively).

2.6. Statistical analysis

All data are expressed as mean ± SEM. The significance of differences between groups was calculated by analysis of variance (ANOVA), followed by post hoc testing for individual differences by Dunnett's tests. Student's T test was used for comparison of a single factor between two groups. Data management and statistical analyses were performed in SPSS v16.0. Statistical significance was set at $P < 0.05$.

3. Results

3.1. Recruitment of CD11c-positive DCs in brain parenchyma after SE

Sixty rats injected with Li-pilocarpine developed SE and twelve rats out of these died from SE. Seizures could be detected behaviorally within 15 min after Li-pilocarpine injection and the duration of SE was controlled within 90 min. Rats with lithium chloride treatment or control rats with saline-injection did not exhibit similar behavioral changes. Tissue sections were processed by immunohistochemistry to examine the presence of DCs in rats 12 h, 1, 3, 5, 7 and 30 days following SE. In the brain parenchyma of control animals (Fig. 1A–C), Li-treated rats (Fig. 1D–F) and epileptic rats 12 h after SE (data not shown), virtually no CD11c-positive cells were observed. In contrast, immunohistochemically detected DCs recruitment occurred at 1 day following the induction of SE in the hippocampus, thalamus and temporal cortex (Fig. 1G–I). The expression of CD11c reached a maximum at day 3 (Fig. 1J–L) and slightly declined until day 5 (Fig. 1M–O). However, there were no detectable DCs at 7 or 30 days after SE (data not shown). In agreement with the results of immunohistochemistry, immunoblot analysis demonstrated that the level of CD11c protein was higher at 3 days after SE than that at 1 day or 7 days after SE. In addition, there was no detectable expression of CD11c in control group and Li-treated groups (Fig. 2, photographs of CD11c expression in rats 12 h, 1 day and 7 days after