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NEURODEGENERATION AND INFLAMMATION IN HIPPOCAMPUS IN EXPERIMENTAL AUTOIMMUNE ENCEPHALOMYELITIS INDUCED IN RATS BY ONE – TIME ADMINISTRATION OF ENCEPHALITOGENIC T CELLS

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Abstract—Cognitive dysfunction is relatively frequent in multiple sclerosis (MS) and it happens from the early stages of the disease. There is increasing evidence that the grey matter may be involved in autoimmune inflammation during relapses of MS. The purpose of this study was to evaluate if a single transfer of encephalitogenic T cells, mimicking a relapse of MS, may cause hippocampal damage and memory disturbances in rats. Lewis rats were injected with anti-MBP CD4+ T cells, that induced one-phase autoimmune encephalomyelitis (EAE) with full recovery from motor impairments at 10–15 days. The spatial learning and memory were tested by the Morris water maze test in control and EAE animals, 30 and 90 days post-induction (dpi). The neural injury and inflammation was investigated in the hippocampus by immunohistochemistry and quantitative analyses. There was a marked decrease in the number of CA1 and CA4 pyramidal neurons 5 dpi. The loss of neurons then aggravated till the 90 dpi. An increase in microglial and astroglial activation and in pro-inflammatory cytokines mRNA expression in the hippocampus, were present 30 and 90 dpi. Nerve growth factor and brain-derived neurotrophic factor mRNA levels were also significantly elevated. The water maze test, however, did not reveal memory deficits. The present data indicate that a single transfer of autoimmune T cells results in preserved inflammation and probable on-going neuronal injury in the hippocampus, long after recovery from motor disturbances. These findings suggest that any relapse of the MS may start the neurodegenerative process in the hippocampus, which is not necessarily connected with memory deficits.

Key words: autoimmune encephalomyelitis, hippocampus, multiple sclerosis, memory, neurodegeneration, neuroinflammation.

INTRODUCTION

Cognitive impairment is detected in about half of multiple sclerosis (MS) patients. The most common impairments are disturbances in memory, attention and concentration, information processing and visuospatial abilities (Amato et al., 2006). In the early stages of MS, it is postulated that cognitive deficits may depend on subtle alterations in synaptic transmission that lead to altered neuronal connectivity (Mandolesi et al., 2010). In the later stages, axonal pathology, the leading hallmark of MS, is associated with impairment of the neuronal network, which manifests as disconnection syndrome or subcortical cognitive impairment (Rovaris et al., 2006; Lund et al., 2012). In addition, there are grey matter lesions in the cerebral cortex and in the brain stem nuclei of MS patients (Kidd et al., 1999; Kutzelnigg et al., 2005; Seewann et al., 2011). Kutzelnigg and Lassmann (2006) report cortical band-like subpial demyelination and destruction, especially in patients with the progressive form of the disease. A new neuroimaging technique, double inversion recovery (DIR) magnetic resonance sequence and high-field magnetic resonance, recently revealed cortical and hippocampal inflammatory lesions that are undetectable using routine MRI (Rinaldi et al., 2010; Seewann et al., 2012). Grey matter lesions have been shown to correlate with cognitive decline, particularly, visuospatial memory disturbances correlated with hippocampal injury detected by DIR (Schmierer et al., 2010). However, hippocampal atrophy has also been shown in MS patients without any memory deficits (Roosendaal et al., 2010).

In spite of aforementioned frequent cases of cognitive impairments in MS patients, the mechanisms responsible for their occurrence are not clear. To better elucidate these mechanisms studies with the use of various experimental models of MS are carried on.

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Abbreviations: BDNF, brain-derived neurotrophic factor; DIR, double inversion recovery; DNF, brain-derived neurotrophic factor; dpi, days post EAE induction; EAE, autoimmune encephalomyelitis; GAPDH, glyceraldehyde 3-phosphate dehydrogenase; GFAP, glial fibrillary acidic protein; Iba1, ionized calcium-binding adaptor molecule 1; IL, interleukin; MLV, Moloney murine leukaemia virus; MOG, myelin oligodendrocyte glycoprotein; MRI, magnetic resonance imaging; NeuN, nuclear neuron protein; NGF, nerve growth factor; NGF, nerve growth factor; PBS, phosphate-buffered saline; TNF, tumour necrosis factor.

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In MS experimental model, autoimmune encephalomyelitis (EAE), the atrophy of CA1 hippocampal region as well as inflammatory lesions in hippocampus, cortex and cerebellum, along with some memory disturbances, have been recently described (Ziehn et al., 2010; Mangiardi et al., 2011). The site and extension of the observed brain lesions depended on animal species and type of EAE induction.

The inflammatory model of EAE, mimicking MS relapse, may be evoked by anti-MBP CD4+ T cells transfer to Lewis rats. The passive transfer of T cells produce monophasic disease with full recovery in about 10–15 days (Flügel et al., 2001). The pathological changes in the spinal cord and the brain include inflammatory infiltrations and minimal demyelination. This model is a major experimental tool for investigating T-cell function in MS (Wekerle, 2008).

In our earlier studies, we observed that encephalitogenic T cell transfer in rats induced early hippocampal injury (Kurkowska-Jastrzebska et al., 2007, 2010a). The current study was undertaken to evaluate whether encephalitogenic anti-MBP CD4+ T cells caused hippocampal inflammation and neuronal injury in a late phase of the disease and whether these phenomena were correlated with memory impairment. In contrast to the early stages when the severity of motor impairment makes it difficult to provide cognitive tests correctly studies of memory can be conducted in the late phases of EAE.

EXPERIMENTAL PROCEDURES

Animals

The study involved four groups (n = 12 rats/group) of 10-week-old female Lewis rats: two control groups and two EAE groups. One control and one EAE group was used for tests at 30 and 90 days post-induction (dpi), respectively. The additional group of rats (six animals per group) was injected with T cells to evaluate early neurodegeneration in the hippocampus. Animals were sacrificed 5 days after T cells administration when the aggravation of EAE signs was the biggest. Animals were housed in standard controlled conditions with free access to food and water. The study was conducted in accordance with the National Institute of Health (NIH) guidelines for the care and use of laboratory animals and was approved by the local ethics committee.

The EAE model

EAE was induced by intravenous injection of re-stimulated anti-MBP CD4+ T cells (4 million T cells per rat) suspended in the 0.9% NaCl. The control rats received intravenous injection of 0.9% NaCl. All rats that received anti-MBP T cells developed monophasic EAE 3–4 days after administration and recovered completely after 10–15 days as described previously (Kurkowska-Jastrzebska et al., 2007, 2010a). No animals died of EAE. Animals were weighted every day and examined for the aggravation of motor symptoms using the common clinical scores. Clinical scores (0–5) were assigned as follows: 0 – no signs; 1 – flaccid tail; 2 – hind limb weakness or abnormal gait; 3 – complete hind limb paralysis; 4 – complete hind limb paralysis with forelimb weakness; 5 – moribund or deceased. The course of the clinical symptoms of EAE is shown at Fig. 1.

Morris water maze test

The Morris water maze test was performed twice, at 30 and 90 dpi. Animals were sacrificed 24 h after the memory test. A modified version of the Morris water maze was used (Widy-Tyszkiewicz et al., 1993). The circular pool (140-cm in diameter) was divided into four equal quadrants (designated north-east NE, north-west NW, south-east SE, and south-west SW). The animals were trained to locate a hidden platform in the pool (placed in SE quadrant) using distal visual cues on the walls around the pool. Animals from the EAE and control groups were given one six-trial session daily for four consecutive days. The memory test was performed with the platform removed from the pool after four days of training. The number of times the animal crossed over the previous platform position and the time spent in the goal quadrant (SE) were recorded and analysed. The visible platform test was carried out on the same day, with the time to locate the platform (latency) and the distance travelled recorded. Data from the tests were recorded and analysed using a VHS image analysis system (Chromotrack, San Diego Instruments, San Diego, CA, USA).

Tissue preparation

For immunohistochemistry, the animals were sacrificed by transcardial perfusion (under sodium pentobarbital anaesthesia) with 0.1 M phosphate-buffered saline (PBS) with heparin (5 units/ml) followed by 4% paraformaldehyde. Brains were removed, postfixed in the same fixative and cryoprotected in 30% sucrose in 0.1 M PBS for at least 48 h at 4 °C. Frozen 20-μm and 40-μm consecutive sections were cut in coronal plane and collected in PBS with 0.1% sodium azide. Free floating adjacent sections were processed as described below.

For immunoblotting and real-time polymerase chain reaction (RT-PCR), animals were sacrificed by decapitation, brains were rapidly removed and hippocampi surgically dissected. The anterior parts of the hippocampi, relevant to the position of −2 mm and −3.5 mm posterior to the bregma, were separated and used for the studies.

Immunohistochemistry

Immunohistochemistry was used to detect the neuronal nuclear antigen (NeuN), microglial OX4 antigen and astroglial glial acidic fibrillary protein (GFAP) as described previously. (Kurkowska-Jastrzebska et al., 2007, 2010a) After blocking of unspecified bindings, consecutive brain sections were incubated with polyclonal rabbit anti-NeuN antibody (1:300, Millipore, Temecula, CA, USA), polyclonal rabbit anti-GFAP antibody (1:300, Millipore, USA, Temecula, CA, USA) or