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Research paper

Exercise training attenuates experimental autoimmune encephalomyelitis by peripheral immunomodulation rather than direct neuroprotection

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ABSTRACT

Background: Conflicting results exist on the effects of exercise training (ET) on Experimental Autoimmune Encephalomyelitis (EAE), nor is it known how exercise impacts on disease progression.
Objective: We examined whether ET ameliorates the development of EAE by modulating the systemic immune system or exerting direct neuroprotective effects on the CNS.
Methods: Healthy mice were subjected to 6 weeks of motorized treadmill running. The Proteolipid protein (PLP)-induced transfer EAE model in mice was utilized. To assess effects of ET on systemic autoimmunity, lymph-node (LN)-T cells from trained- *vs.* sedentary donor mice were transferred to naïve recipients. To assess direct neuroprotective effects of ET, PLP-reactive LN-T cells were transferred into recipient mice that were trained prior to EAE transfer or to sedentary mice. EAE severity was assessed *in vivo* and the characteristics of encephalitogenic LN-T cells derived from PLP-immunized mice were evaluated *in vitro*.
Results: LN-T cells obtained from trained mice induced an attenuated clinical and pathological EAE in recipient mice *vs.* cells derived from sedentary animals. Training inhibited the activation, proliferation and cytokine gene expression of PLP-reactive T cells in response to CNS-derived autoantigen, but strongly enhanced their proliferation in response to Concanavalin A, a non-specific stimulus. However, there was no difference in EAE severity when autoreactive encephalitogenic T cells were transferred to trained *vs.* sedentary recipient mice.
Conclusion: ET inhibits immune system responses to an auto-antigen to attenuate EAE, rather than generally suppressing the immune system, but does not induce a direct neuro-protective effect against EAE.

1. Introduction

Multiple sclerosis (MS) is an immune-mediated disease of the central nervous system (CNS), leading to CNS demyelination and axonal damage (Frohman et al. 2006). During the course of MS, autoreactive T cells are activated in the peripheral lymphoid organs and migrate across the blood-brain barrier to induce inflammatory lesions within the CNS (McFarland and Martin 2007). MS causes chronic irreversible functional impairments and there is a requirement for therapeutic strategies that can reduce the deleterious impact of the disease on the quality of life of MS patients.

Various clinical trials demonstrated the safety of exercise training (ET) in MS patients and suggested it may induce various physiological and functional beneficial effects (Motl and Pilutti 2012; Pilutti et al. 2014). Furthermore, there is growing evidence linking between ET and the immune system (Gjevestad et al. 2015; Gleeson et al. 2011; Walsh et al. 2011). Regular physical activity reduces the risk of chronic diseases, partly owing to the anti-inflammatory effects of exercise (Baek 2016; Gleeson et al. 2011; Pruimboom et al. 2015; Spielman et al. 2016). Anti-inflammatory effects of ET are also documented in MS patients (Florindo 2014). These studies prompted additional research of ET for MS and specifically investigation of the beneficial effects and mechanisms of action of ET on Experimental Autoimmune Encephalomyelitis (EAE).

EAE is the most commonly used animal model to study MS therapies (Robinson et al. 2014). EAE is characterized by T-cell and monocyte infiltration in the CNS, targeting proteins that are expressed by myelin-producing oligodendrocytes, such as proteolipid protein (PLP), myelin basic protein (MBP) and myelin oligodendrocyte glycoprotein (MOG). This inflammatory process results in demyelination, axonal damage and subsequently progressive hind limb paralysis (Kuerten and Angelov 2008; Robinson et al. 2014; Wekerle 2008).

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Previous studies examined the effects of ET on EAE with conflicting results (Benson et al. 2015; Bernardes et al. 2016; Bernardes et al. 2013; Klaren et al. 2016; Patel and White 2013; Pryor et al. 2015; Rossi et al. 2009; Souza et al. 2016). It is not known whether ET mediates beneficial effects in EAE *via* modulation of the systemic immune system and/or by inducing direct CNS neuroprotective effects. Therefore, the aims of the current study were: (1) to examine the clinical and neuropathological effects of ET in a clinically relevant model of MS; and (2) to investigate whether ET ameliorates EAE by modulating the systemic immune system, or exerting direct neuroprotective effects on the CNS in EAE. We employed here a unique experimental paradigm using the transfer EAE model that enabled us to distinguish between the potential systemic and central effects of ET in EAE. The data demonstrate that ET modulates the peripheral immune system responses to the myelin antigen to attenuate EAE, rather than inducing a CNS neuroprotective effect.

2. Materials and methods

2.1. Experimental animals

Female SJL/JCrHsd mice (6–7 weeks of age) were obtained from Envigo Inc., Israel. Animals were housed in communal cages at 22 ± 1 °C under a 12-h light/dark cycle (lights on at 07:00 hours), with free access to food and water. Animal experimentation has received approval by the Institutional Animal Care and Use Committee, and the studies were conducted in accordance with the United States Public Health Service's Policy on Humane Care and Use of Laboratory Animals. Behavioral experiments were performed between 8:00 a.m. and 4:00 p.m.

2.2. Experimental design

The transfer-EAE experimental set-up was designed to enable the differentiation of the effects of ET on systemic autoimmunity, specifically on induction of lymph node (LN)-derived T cell encephalitogenicity (part 1, Fig. 1A), *vs.* direct neuro-protective effects of ET on the CNS (part 2, Fig. 1B).

Part 1 (Fig. 1A): To assess the modulatory effects of ET on systemic autoimmunity, we examined *in vivo* and *in vitro* the amount, potency and encephalitogenicity of LN-derived T cells from mice that underwent the ET program prior to PLP immunization (trained mice), compared with T cells from sedentary mice. To that end, healthy mice were subjected to a defined treadmill running program. This was followed by their immunization with a PLP peptide. Then, their LN-T cells were removed, stimulated in culture with PLP peptide and injected to naïve recipient mice, which developed EAE. Another group of recipient mice were injected with PLP-reactive LN T cells from sedentary mice and served as controls. Here we examined whether treadmill running of the donor mice modulated the systemic autoimmune process. Encephalitogenicity was examined (1) *in vivo* by examination of the clinical and pathological severity of EAE induced in recipient naïve SJL mice, following transfer of LN-T cells from trained- *vs.* sedentary donor mice; and (2) *in vitro*, at the day of LNCs removal or following secondary activation *in vitro* by the PLP auto-antigen, using activation and proliferation assays, as well as by T cell surface markers analysis and cytokine gene expression.

Part 2 (Fig. 1B): Healthy mice were subjected to a defined treadmill running program (trained mice), followed by injection of PLP-reactive, encephalitogenic LN-T cells from donor mice. Sedentary mice were injected with the same PLP-reactive LN-T cells and served as controls. Here we examined whether treadmill running program of the recipient mice prior to transfer of encephalitogenic T cells attenuated the severity of EAE *via* direct neuro-protective effects on the CNS.

EAE mice from both experimental protocols started to develop EAE clinical signs at 7–10 days post LN-T cell transfer, and were scored daily for neurological symptoms up to 50 days post transfer.

2.3. Forced treadmill exercise training (ET)

Physical training and performance tests were performed on a 5-lane treadmill designed for mice (Panlab Harvard Apparatus, USA). The back of each treadmill lane contained an electrified grid, which delivered a shock stimulus to stationary mice (0.1 mA). Before commencement of exercise performance tests and training program, mice were familiarized with treadmill running for 10 min on three consecutive days (first day at 8 cm/s with no electrical shock; second and third days at 8 cm/s with 0.1 mA electrical shocks). Performance tests were performed prior to the training and at the end of a 6-week training protocol. There was a 72 h interval between the two types of performance tests.

2.3.1. Exhaustion speed performance test

Maximal running speed for each mouse was assessed first by running the mice for 8 cm/s and then increasing the speed by 2 cm/s per minute until exhaustion (modified from (Qi et al. 2011)). Exhaustion was defined by an inability/refusal to continue when encouraged with a bottlebrush or a small puff of air.

2.3.2. Exercise tolerance performance test

Exercise tolerance was determined by running each mouse individually to exhaustion at 30 cm/s on a rodent-specific treadmill (modified from (Ritchie et al. 2014)). Exhaustion was defined as above.

2.3.3. Exercise training protocol

Mice were subjected to a 6-week treadmill running, 5 days per week, 1 session per day at 23 cm/s. According to the baseline exercise speed performance tests, this training speed corresponds to an exercise intensity of 55–60% of maximal speed (Table 1). The trained mice were subjected to an incremental exercise training protocol. Each training session consisted of a 5-min warm-up at 8 cm/s. For the first week, warm-up was followed by 10 min of training, and for the second week by 20 min of training. During the following 4 weeks, warm-up was followed by 30 min training at 23 s/min. To minimize potential confounding factors such as differences in stress, sound and light exposure, sedentary control mice were left on the treadmill without running for the same duration as the exercise groups. No animal was excluded from the experiments.

2.4. Muscle enzyme activities

At the end of the 6-week training period, trained and sedentary mice were sacrificed by decapitation 24 h after the last session (to minimize any potential residual effects of the last exercise bout). Soleus (type I, oxidative) and extensor digitorum longus (EDL; type II, glycolytic) muscles from trained and sedentary mice were dissected free, frozen in liquid nitrogen and analyzed for mitochondrial enzyme activities to assess the efficacy of the exercise protocol. Frozen muscles were freeze-dried, cleaned of non-muscle constituents, homogenized with ground glass homogenizers in ice-cold buffer (80 μl/mg dry wt) consisting of (in mM): 50 Tris-HCl, 1 EDTA, 0.05% (*v*/v) Triton X-100, pH 7.5. The extracts were centrifuged at 1400 × *g* (4 °C) for 1 min and the supernatant was assayed for citrate synthase (CS) and β-hydroxyacyl-CoA dehydrogenase (HAD) with standard spectrophotometric techniques, as described elsewhere (Zhang et al. 2007), as well as for protein (Biorad assay). Assays were conducted at 25 °C under conditions that yielded linearity with respect to time and extract volume.

2.5. Transfer experimental autoimmune encephalomyelitis (EAE)

Proteolipid protein $(PLP)_{139–151}$ transfer EAE model in 6–7- or 12–13 week-old female SJL/JCrHsd mice was utilized as previously