Physiotherapy as an immunoactive therapy? A pilot study.

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Abstract

OBJECTIVES: The aim of this study was to confirm the immunoregulatory and anti-inflammatory changes in the immunologic profile after two months of the facilitation physiotherapy in patients with multiple sclerosis; and to determine whether the changes in the immunologic profile correlate with the changes in dehydroepiandrosterone, the brain microstructure and clinical functions.
DESIGN & SETTING: A group of 12 patients with multiple sclerosis was examined twice: at the beginning and 2 months later after the patients had undergone the facilitation therapy. Standardized tests evaluating chosen clinical functions (balance, righting, equilibrium and protective reactions, tremor, dysdiadochokinesis, dysmetry, fine hand function and walking), immune parameters (parameters of the humoral and cellular immunity), dehydroepiandrosterone and diffusion tensor imaging (the fractional anisotropy, mean diffusivity) were measured. The patients underwent the facilitation physiotherapy in two sessions lasting two hours each week for two months.
RESULTS: All clinical and diffusion tensor imaging parameters significantly improved following the therapy. Without the correction for multiple comparisons, there were significant changes in the IgG, IgG1 subclasses, in the numbers of Neutrophils and Lymphocytes, the T cells (CD3+) absolute number, the T cytotoxic subpopulation (CD3+CD8+) absolute number, B cells (CD19+) and the Natural

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killer cells. In addition, there was a significant correlation between the changes in the clinical functions and the changes in IgG1 (r=0.67), and between the changes in the mean diffusivity and the changes in CD3+CD8+ absolute (r=–0.61). The changes in the immune parameters and the mentioned correlations were not significant in view of the number of comparisons and thus necessitate further validation. No changes in the dehydroepiandrosterone concentration after the therapy were confirmed.

CONCLUSION: The study suggests new possibilities of physiotherapy to influence the psycho–neuro–endocrine–immune response in patients with multiple sclerosis.

Abbreviations:

BBS	- Berg Balance Scale
clinALL	- overall clinical change
clinOPT	- optimally weighted sum of clinical parameters
CNS	- central nervous system
CRP	- C-reactive protein
DD	- dysdiadochokinesis
DHEA	- dehydroepiandrosterone
DM	- dysmetry
DTI	- diffusion tensor imaging
DWI	- diffusion-weighted images
EDSS	- Expanded Disability Status Scale
FA	- fractional anisotropy
FLAIR	- fluid attenuated inversion recovery
fMRI	- functional magnetic resonance imaging
FOV	- field of view
HPA	- hypothalamus-pituitary-adrenal
IFN	- Interferon
IL	- interleukin
IQR	- interquartile range
IRI	- Immunoregulatory index (CD4+/CD8+)
LTD	- long-term depression
LTP	- long term potentiation
MD	- mean diffusivity
MPRAGE	- magnetization-prepared rapid acquisition gradient echo
MRI	- magnetic resonance imaging
MS	- multiple sclerosis
NHPT	- Nine Hole Peg Test
NK	- natural killers
NMDA	- N-methylD-apartate
PCA	- principal component analysis
REP	- righting, equilibrium and protective reactions
ROI	- regions of interest
SD	- standard deviation
SE EPI	- spin-echo echo-planar imaging
T	- tremor
T25 - FW	- Timed 25 – Foot Walk
TE	- echo time
TMB	- tetramethylbenzidine
TNF	- tumour necrosis factor
TR	- repetition time

INTRODUCTION

Multiple sclerosis is a chronic autoimmune disease, pathologically characterized by the presence of areas of demyelination and T-cell perivascular inflammation and axonal degeneration in the brain white matter (Lassmann 1999; Trapp *et al.* 1998). The combination of these pathological processes results in the impairment of the synaptic plasticity and the subsequent destabilization of the neuronal network and limitation of the plastic potential of the brain (Hemmer *et al.* 2002). MS causes acute or subacute neurological abnormalities that manifest themselves through a wide range of symptoms, e.g. muscular weakness, spasticity, ataxia or balance dysfunction. They entail a wide range of complications and related problems in normal life such as problems with walking or arm function (Thompson 2001; Henze *et al.* 2006).

Unfortunately, there is no curative treatment of MS available yet. However, it has been confirmed that the immunosuppressive and immunomodulatory pharmacologic treatment ameliorates the course of MS (Polman *et al.* 1995; 2006). Recent research has been looking for the development of a novel immune-centred/ immunoactive therapeutic approach enabling the regulation of the complex neuroimmune crosstalk occurring within the CNS (Di Filippo *et al.* 2008). MS requires a complex and life-long treatment, including physiotherapy (Khan *et al.* 2007). A number of pilot studies in physiotherapy/rehabilitation/physiology of load confirmed that a regular load of the submaximal intensity leads to the adaptation processes of the immune system (immunomodulation) (Heesen *et al.* 2003; Schulz *et al.* 2004; Castellano *et al.* 2008; White *et al.* 2006).

In this study, we were interested in determining whether the facilitation physiotherapy also possesses the immunoactive potential. We hypothesised that this kind of therapy can modulate the immune system through 1) an activation of the cerebellum and consequently via hypothalamus – paleocerebellum and the neocerebellum limbic system (Molitari *et al.* 2002); part of a limbic system is hypothalamus that owing to the hypothalamus-pituitary-adrenal axis (Kern & Ziemssen 2008) can influence immunomodulation, 2) a neuroimmune crosstalk during the LTP induction where the molecules that mediate the immune function parallel modulate the synaptic memory processes (Bains &Oliet 2007; Volterra & Meldolesi 2005; Di Filippo *et al.* 2008; Cotman & Berchtold 2002).

In literature, we did not find any research that evaluates the immunomodulation effect of any physiotherapeutic technique based on the neurophysiologic basis, that is, either facilitation (e.g. proprioceptive neuromuscular facilitation, Vojta reflex locomotion) or a task-oriented approach (e.g. the contemporary Bobath concept). To the best of our knowledge, this pilot study is the first one that attempts to evaluate this area.

The aim is to confirm the imunoregulatory and anti-inflammatory changes in the immunologic profile (parameters of the humoral and cellular immunity) after two months of facilitation physiotherapy in patients with multiple sclerosis; and to correlate the changes in the immunologic profile with the changes in dehydroepiandrosteron, the brain microstructure (diffusion tensor imaging) and clinical functions.

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MATERIAL AND METHODS

Study design

Standardized tests evaluating the clinical functions, the immune parameters, DHEA and DTI were examined twice: at the beginning and 2 months later after the patients had undergone the facilitation therapy.

Patients' characteristics

12 outpatients with a definite multiple sclerosis according to the revised McDonald criteria (Polman *et al.* 2005) were randomly selected from the MS Centre (Department of Neurology, The Third Medical Faculty and Faculty Hospital Královské Vinohrady in Prague) databases based on the criteria for inclusion in the study: both genders; the stability of their clinical status in the three preceding months; the minimum of two years on the imunomodulatory drugs (glatiramer acetate, interferon beta-1a, interferon beta-1b, mitoxantrone), the minimum of six months without physiotherapy, prevailing motor impairment; clinical manifestation as similar as possible; the ability to move independently and to walk for no less than 200 meters with a set of crutches (EDSS ≤5); the indication and ability to undergo ambulatory physiotherapy (e.g. motivation to cooperate actively, the ability to visit the centre regularly); and the typical multiple ovoid or confluent lesions in the periventricular and juxta-cortical white matter (confirmed on the MRI). All patients agreed to participate and signed an informed consent necessary for being enrolled in the study (the patients were informed about the objective and design of the study, the course of the examinations, their safety/risks; the patients were briefed about their rights – to be informed, not to participate in the study, to withdraw their consent at any point of time during the study, and the confidentiality of their data).

Physiotherapeutic program

Patients were receiving the facilitation physiotherapy in two two-hour sessions each week for two months from an experienced (ten years of a clinical experience with neurological patients) physiotherapist. In this therapy, somatosensory afferent (manual and verbal) stimuli were applied in the precisely given positions and motor functions (sitting, standing up, sitting down, standing, and walking). These activities were practiced repeatedly (3–7 times) within a practice session.

Examination

The basic characteristics of the patients such as the EDSS (Kurtzke 1983), duration of the disease, disease type, sex, age, and the pharmacologic treatment were determined by an independent board of certified neurologists at the start of the program.

Clinical function

Balance by the Berg Balance Scale (Berg *et al.* 1995) and by the righting, equilibrium and protective reactions (Guarna *et al.* 1988, Davies 1993), tremor (Fahn *et al.* 1993), dysdiadochokinesis, and dysmetry (Alusi *et al.* 200), a fine hand function by the Nine Hole Peg Test and walking by the Timed 25 – Foot Walk (Morris 2000) were evaluated.

All the assessments were collected by the same examiner at the same time of the day, on the same day of the week. The measures were administered in the same order each time.

The MRI acquisition protocol

All subjects in this study underwent MRI examinations on a 3T MR scanner (Siemens Trio Tim, Erlangen, Germany) using a 12-channel phased-array head coil with the following protocol according to Ibrahim *et al.* 2011: 1) T1-weighted (T1W) 3D magnetization-prepared rapid acquisition gradient echo (MPRAGE) with a voxel size of $0.85 \times 0.85 \times 0.85$ mm^3 sagittal slices, 2) 3D T2-weighted fluid attenuated inversion recovery (FLAIR) with a voxel size of $1 \times 1 \times 1$ mm^3, 176 sagittal slices. 3) Diffusion-weighted images (DWI) using a spin-echo echo-planar imaging (SE EPI) sequence with the parameters: a voxel size of $2 \times 2 \times 2$ mm^3, 44 axial slices, 3 averages, the number of the diffusion directions 20, and two b values: 0, 1 000 s/mm^2.

The DTI data were corrected for distortions and eddy current effects using the FSL (www.fmrib.ox.ac.uk/fsl/index.html). The b=0 EPI images of the DTI image set were co-registered to a T1-weighted 3D MPRAGE. The EPI and the T1W 3D MPRAGE co-registered images were then normalized to a T1 template (SPM8, http://www.fil.ion.ucl.ac.uk/spm/software/spm8). The normalized DTI indices (fractional anisotropy FA and mean diffusivity MD) were then calculated using the MedINRIA (http://www-sop.inria.fr/asclepios/software/MedINRIA). The regions of interest (ROIs) of the corpus callosum for the FA and MD calculation were manually selected in the midsagittal slice of the T1W 3D MPRAGE normalized image. For the sake of accuracy, the same ROI of the CC was used for each subject at the beginning and the end of the study (the ROIs geometries of the CC were copied and pasted) (more details in Ibrahim *et al.* 2011).

The immune parameters

The cytokines detection

The colorimetric sandwich ELISAs for cytokines detection (IL-2, IL-6 and IL-10) was used (Quantikine Human IL immunoassay, R and D Systems, MN, USA). Briefly, a microplate pre-coated with a capture antibody was used, samples or standards were added and the analyte present was bound by the immobilized antibody. After the washing, an HRP-labeled detection antibody was added. After 15 minutes, a Tetramethylbenzidine (TMB) substrate solution was added to the wells and the absorbance at 450 nm was measured (Leung *et al.* 2003).

Immunoglobulins

The concentration of the immunoglobulins IgG, subclasses IgG 1–4, IgA, IgM, IgE was measured by nephelometry. Briefly, fresh blood was drawn, the plasma separated and frozen at –20 °C until the analysis took place. The precipitation of the antigen-antibody immunocomplexes (the specimen immunoglobulins and the appropriate animal polyclonal antibody) was measured by a nephelometer BNII (Siemens Medical Solutions, Dade Behring, Germany), according to the manufacturer's procedure (Nakamura *et al.* 1991).

Immune cells phenotype

For the determination of the T, B and NK populations the blood aliquots (100 µl) were incubated with the fluorescently labelled monoclonal antibodies (20 µl) for 20 minutes at room temperature. The monoclonal antibodies were PE- or FITC- conjugated anti-CD3, anti-CD4 or anti-CD8, anti-CD19, anti-CD (16+56) and an isotype control. After the incubation, the red blood cells were lysed, and the cells fixed until analysis. The cells were analyzed by a flow cytometry (FACSCalibur, Becton Dickinson, Belgium). The fluorescence data were collected for 2 x 10^4 cells and analyzed by the CELLQUEST software. The relative proportion and absolute numbers were determined from the white blood cell count performed from the same blood sample (Gratama *et al.* 1998, Lim *et al.* 1998).

Dehydroepiandrosterone

DHEA has been measured by a rutine radioimmunologic determination from the Immunotech (Czech Division, Marseille, France) (Bicikova *et al.* 2001) on the Institute of Endocrinology.

Statistical analysis

The differences in the clinical parameters and DTI parameters after the therapy were analyzed by a paired t test. The differences in the immune parameters were analyzed by the Wilcoxon signed-rank test. Two outliers (DD and DM in the 4th patient in first measurement, the Grubbs test (Grubbs 1950) $p<0.001$, assumed to be missprints) were removed from the analysis. One-sided alternative (improvement) was tested in the clinical parameters and in the DTI parameters, a two-sided alternative was assumed in the immune parameters.

The differences were assumed to be significant and marked by an asterisk * in case the achieved p value was less than or equal to 0.05. With respect to multiple comparisons, such results need further validation. The results significant also after the Bonferroni correction for multiple comparisons (p-value less than or equal to 0.05/number of comparisons in a batch) were marked by two asterisks **.

To examine the overall clinical change (clinALL), we computed the average of the clinical parameters (the BBS, T, DD, DM, REP, the standardized logarithm of the NHPT, a standardized logarithm of the T25 – FW) standardized into interval [0, 1], where 0 represents the worst possible outcome and 1 represents the best possible outcome.

Also, we described the overall clinical change by the optimally weighted sum of the clinical parameters (clinOPT). These weights were computed by the principal component analysis used on the data of the first measurement. The resulting parameter (clinOPT=0.154 BBS + 0.080 REP + 0.004 T – 0.806 DD – 1.066 DM – 0.045 NHPT – 1.379 T25 – FW) is an optimal combination of the clinical parameters, so that its variance in the first measurement is maximalized. For the purpose of this analysis, five missing values in the first measurement (DD and DM in 4th patient, T25 – FW and NHPT in 5th patient, and T25 – FW in 11th patient) were replaced by the average values in an appropriate parameter in this measurement.

The correlations between the differences in the immune parameters and the differences in the clinical or the DTI parameters were computed by the Spearman correlation.

Statistical analyses were preformed by environment *R*, version 2.11.1 (R Development Core Team 2010) and its packages *FactoMineR* (Husson *et al.* 2010) and *outliers* (Komsta 2010).

Tab. 1. The changes in the clinical parameters after the therapy.

	difference			t test
	mean	SD	N	*p*-value
BBS	2.15	3.61	12	0.032*
REP	4.42	4.62	12	0.003**
T	–2.96	2.14	12	<0.001**
DD	–1.82	1.50	11	0.001**
DM	–1.27	1.03	11	0.001**
NHPT	–0.90	0.94	11	0.005**
T25 - FW	–0.60	0.57	10	0.004**
clinALL	0.06	0.03	9	<0.001**

clinALL was computed as an average of clinical parameters (BBS, REP, T, DD, DM, NHPT, T25 -FW) standardized into interval [0, 1], where 0 represents the worst possible outcome while 1 represents the best possible outcome. *significant change ($p\leq0.05$); **change significant also after the Bonferroni correction ($p\leq0.05/8$)

RESULTS

All tested clinical parameters significantly improved following the therapy. All changes were significant also after the correction for multiple comparisons, except for changes in the BBS (Table 1). In addition, the DTI parameters significantly improved after the therapy, the FA in the CC significantly increased ($p<0.001$) and the MD decreased ($p=0.014$).

There were no significant changes in the immune parameters when considering the number of comparisons. Without the correction for multiple com-

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Tab. 2. The changes in the immune parameters after the therapy.

	difference			Wilcoxon
	median	IQR	N	*p*-value
Cytokines				
IL-2	0.00	69.33	12	0.178
IL-6	–2.11	3.09	12	0.068
IL-10	0.78	4.25	12	0.689
Immunoglobulins				
IgG	0.64	0.61	12	0.005*
IgG1	0.26	0.37	12	0.012*
IgG2	0.12	0.47	12	0.455
IgG3	–0.01	0.04	12	1.000
IgG4	0.00	0.02	12	0.799
IgA	–0.16	0.20	12	0.116
IgM	–0.03	0.05	12	0.286
IgE	0.00	2.05	12	0.272
Immune cell phenotype				
Leukocytes	0.15	1.50	12	0.638
Neutrophils	–3.80	6.05	12	0.019*
Lymphocytes	3.50	5.95	12	0.027*
Monocytes	0.40	3.35	12	0.519
Eosinophils	0.25	1.43	12	0.610
CD3+ relative cells	–1.00	4.18	12	0.388
CD3+ absolute	0.24	0.29	12	0.016*
CD4+ relative	–0.45	5.23	12	0.308
CD4+ absolute	0.15	0.15	12	0.064
CD3+CD8+ relative	0.80	3.80	11	0.638
CD3+CD8+ absolute	0.09	0.10	12	0.009*
CD19+ relative	–1.25	1.75	12	0.005*
CD19+ absolute	0.00	0.05	12	0.380
IRICD4+CD8+	–0.10	0.48	12	0.265
NK, CD16+56+ relative	–0.25	2.65	12	0.409
NK, CD16+56+ absolute	0.02	0.02	12	0.031*

IQR interquartile range; *significant change (p≤0.05)

parisons, there were significant changes in IgG, IgG1 subclasses, the number of Neutrophils and Lymphocytes, the T cells (CD3+) absolute number, the T cytotoxic subpopulaltion (CD3+CD8+) absolute number, B cells (CD19+) and Natural killer cells (NK absolute number), see Table 2. Males had significantly higher values of the DHEA than females, but there was no significant change in the DHEA after the therapy.

The correlations of the immune parameters that significantly changed after the therapy (IgG, IgG1, Neutrophils, Lymphocytes, CD3+ absolute, CD3+CD8+ absolute, CD19+ relative and NK absolute) with the overall clinical parameter (clinALL), optimal clinical parameter (clinOPT) and DTI parameters (FA and MD) are shown in Table 3. Significantly, the nonzero correlations between the changes in the clinOPT and IgG1 (0.67), and between the changes in the MD and CD3+CD8+ absolute (–0.61) were found. Since both the clinOPT and IgG1 significantly increased after the therapy and the correlation between the changes is positive (0.67), the greater is the change in the clinical parameters, the greater it is in IgG1. Since the MD significantly decreases after the therapy, CD3+CD8+ absolute increases, and the correlation between the changes is negative (–0.61), the greater the change in the MD is, the greater it is in the CD3+CD8+ absolute.

Tab. 3. The Correlation of the changes after the therapy in the immune parameters (significantly changed after the therapy) and in the clinical and DTI parameters.

	clinALL	clinOPT	FA	MD
IgG	0.12	0.19	-0.24	-0.02
IgG1	0.33	0.67*	-0.01	-0.42
Neutrophiles	-0.15	0.10	-0.17	0.42
Lymphocytes	0.18	-0.08	0.05	-0.36
CD3+ absolute	0.07	-0.23	-0.04	-0.15
CD3+CD8+ absolute	0.40	0.34	0.30	-0.61*
CD19+ relative	-0.23	0.13	-0.06	0.37
NK, CD16+56+ absolute	0.42	0.15	-0.44	0.05

clinALL was computed as an average of clinical parameters (BBS, REP, T, DD, DM, NHPT, T25 - FW) standardized into interval [0, 1], where 0 represents the worst possible outcome while 1 represents the best possible outcome
clinOPT was computed as a weighted average of the clinical parameters (0.154 BBS + 0.080 REP + 0.004 T – 0.806 DD – 1.066 DM – 0.045 NHPT – 1.379 T25 - FW). Weights were established by the principal component analysis (PCA) to maximize the variability in the 1st measurement
*significantly nonzero correlations ($p \leq 0.05$, $|r| \geq 0.58$)

DISCUSSION

The effect of the physiotherapy (e.g. massage, cooling and heating, electroacupuncture) on the immune system has already been evaluated in numerous studies and in respect of several diseases, e.g. for the chronic fatigue syndrome or breast cancer (Wang *et al.* 2009, Billhult *et al.* 2009; Hernandez-Reif *et al.* 2004; Dong *et al.* 2009; Kim *et al.* 2005). In multiple sclerosis, the effect of aerobic training (Heesen *et al.* 2003; Castellano *et al.* 2008; Schulz *et al.* 2004) and progressive resistance training (White *et al.* 2006) has been evaluated. Heesen *et al.* (2003) highlighted a trend towards a lower induction of TNF-alpha and IL-10 in the sedentary MS as compared with a trained healthy control directly after, and 30 min-

utes after, the completion of the aerobic training. Castellano *et al.* (2008) described that the MS subjects showed elevated TNF-alpha and plasma IFN-gamma at rest following eight-week aerobic training in comparison with healthy controls. By contrast, Schulz *et al.* (2004) did not find any changes in the immune parameters (plasma IL-6 and soluble IL-6) in people with MS after eight-week aerobic training. White *et al.* (2006) found that eight weeks of progressive resistance training were associated with decreased resting plasma concentration of IL-4, IL-10, CRP, IFNγ and a decreasing tendency for TNF while IL-2 and IL-6 remained unchanged. These kinds of training probably modulate the immune function through the local and systemic cytokine production (White & Castellano 2008; White *et al.* 2006).

This study is the first one evaluating the immunomodulation effect of the physiotherapeutic methods based on the neurophysiologic basis which predominantly uses the facilitation principles. There are two possible explanations of the effect mechanisms. Firstly, following the facilitation physiotherapy, the afferent inputs to the cerebellum are increased through the somatosensory afferent stimuli. The cerebellum is connected with the limbic system by a multiple tract (via motor cortex, hypothalamus and via hypothalamus – paleocerebellum and neocerebellum) (Molitary *et al.* 2002; Ito 2006; Onat & Cavdar 2003). The hypothalamus is part of the limbic system that can influence the immune system due to the HPA axis, the hierarchically organized endocrine system. The HPA axis responds to the physical and psychological challenge so as to adapt to the challenging internal and external circumstances by the release of hormones including a glucocorticoid cortisol hormone. This hormone has both the enhancing and inhibiting influence on the immune function depending on the exact domain (acute versus chronic, systemic versus local), thereby helping establish a fine-tuned balance prerequisite for health and well-being (Kern & Ziemssen 2008; McEwen *et al.* 1997; del Rey & Besedovsky 2000). Secondly, the facilitation physiotherapy has an immunoactive potential because it modulates plastic changes. Authors (Di Filippo *et al.* 2008) refer to recent evidence regarding the glial cells which a) actively take part in the immune responses in the CNS and b), as the third element of the synapse, actively contribute to the neurotransmission, neuronal excitability, and several forms of the synaptic plasticity, such as the LTP and LTD (Bains & Oliet 2007; Volterra & Meldolesi 2005) – due to these mechanisms, the information is stored in the CNS (Bear & Malenka 1994; Buonomano & Merzenich 1998). In this study, the positive effect of the facilitation physiotherapy on the clinical functions under control of the cerebellum and brain microstructure in patients with MS has been confirmed. These results confirm the above described hypotheses concerning the effect mechanisms.

The facilitation therapy uses the principles of sensory-motor learning (Rasova *et al.* 2010; Horak 1991; Faissner *et al.* 1996) and this is probably the way it influences the interconnection of the neural networks (Squire 1986; Dobkin 2003) in the context of the general molecular, cellular and behavioural mechanisms for recovery, such as repair, compensation and adaptation (Matthews *et al.* 2004). The adaptive changes involve unmasking the existing but latent connections, the experience-dependent increases in the dendritic spines and synaptogenesis, and the modulation of the synaptic efficacy such as LTP or LTD (Bütefisch 2006). Several imaging studies using the fMRI (Morgen *et al.* 2004; Rasova *et al.* 2005; 2009) and a pilot DTI study (Ibrahim *et al.* 2011) confirmed that the training of the motor functions in MS patients leads to adaptive and plastic changes of the CNS on a system level. In this study, we were looking for a correlation between the changes in the DTI and the changes of the immune parameters after the therapy. The facilitation therapy through restoring the LTP can activate both brain plastic changes and immunomodulation. That is to say that an active neuroimmune crosstalk occurs during the LTP induction. Neuronal and immunological functions are simultaneously influenced by the modulation of the neurotransmitter signalling pathways (the molecules that primarily mediate the immune function, e.g. cytokines modulate actively the synaptic memory processes) and due to the activation of the ionotropic and metabotropic glutamate receptors (they are expressed by both neurons and immune cells and, in this way, they can modulate either the LTP induction or the viability and functionality of lymphocytes) (Boldyrev *et al.* 2005).

Multiple sclerosis is a chronic inflammatory disorder and was suggested to be a T cell-driven autoimmune disease. From our very brief approach to testing a basic immune cell subpopulation and its products, we can speculate only about the theoretical and clinical significance. Which immune cells do then have relevance for disease pathology? While the CD8+ T cells are dominant in the MS brain lesion edge and in the perivascular regions, most of the data show that the dominant population are the CD4+ T cells. These cells mediate the damage to the myelin either directly by a secretion of a variety of proinflammatory cytokines (ex. TNF alpha) or indirectly by attracting other immune cells such as B cells (an autoantibody formation), macrophages (aphagocytosis and antigen presentation), mast cells (degranulation) and the NK cells (cytotoxicity). Compelling evidence of this scenario involves providing it from an animal model (Martin *et al.* 1992). The recent point of view is reinterpreted in the light of the recent discovery of a Th17 cell (involved in the progression of the CNS autoimmunity), and the protective effect of the naturally occurring CD4+CD25+Foxp3 regulatory cells (Tregs) (Venken *et al.* 2010). Finally, the recent exciting results with the B-cell depleting agents highlight the pathogenic roles for key players and also for B cells (Meirer *et al.* 2011). To make this issue more difficult, most of the activity of the involved immune

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cells is performed locally at the CNS lesions, therefore it is very difficult to interpret our finding by analysing a peripheral blood. Definitely, we observe the decrease of the CD19+ B cells after the period of treatment and the increase of all T CD3+cells, which correlates with the hypothesis of the pathogenic role of the B cells.

Tregs are a very small proportion of all T cells, and we did not analyse this specific immunoregulatory subpopulation, we can only speculate that the possible changes of Tregs can be responsible for the increase of a total number of the T cells. The changes of an immunoglobulin IgG concentration and the major subclass IgG1 are very difficult to interpret. Up to now, we have not been fully knowledgeable about the nature of the oligoclonal bands, i.e. whether they derive from a small but random sample of the peripheral B cells or some pathogenic response. Our interpretation is that the facilitation physiotherapy has no effect on the antibody production in this cohort of patients.

A summary of the recent data indicates a striking beneficial role of DHEA and its derivatives on many autoimmune inflammatory diseases. In addition to the adrenal cortex, the DHEA is also produced in the central and peripheral nervous system and it ranks among the most active neurosteroids (Morfin *et al.* 2000). It is of interest that the DHEA (as other neurosteroids) is synthesized by glial cells (Baulieu & Schumacher 2000) and acts as a positive modulator of the NMDA receptors. One can speculate that the changed levels of the DHEA and other neuroactive steroids can play a serious role in myelinisation. Its neuroprotective role has already been documented (Friess *et al.* 2000; Allolio & Arlt 2002; Schumacher *et al.* 2003; Morfin & Starka 2001; Trincal *et al.* 2002; Bicikova *et al.* 2004).

In this study, the changes of the DHEA levels after the facilitation physiotherapy have been expected to occur, but not confirmed. Our hypothesis was based on the initial findings that showed the effect of the physiotherapy on the endocrine parameters. Jandova *et al.* (2008) described a significant decrease in the unconjugated DHEA after the balneotherapy that included the physiotherapy in thyroidectomized women. Hessen *et al.* (2003) confirmed that aerobic training significantly increases the epinephrine, norepinephrine, adrenocorticotropic hormone, and beta-endorphin levels in MS patients. We did not discover any significant changes of the DHEA level following the therapy. Nevertheless, we noted correlations with the changes of certain immune parameters. The trend towards the positive correlation of DHEA and IL-10 (r=0.50), and the trend towards the negative correlation of DHEA with IL-6 (r=–0.50) and a number of B cells (r=–0.56) after the treatment period support the idea of DHEA's anti-inflammatory and immunoregulating role. This role has already been confirmed in the 7-hydroxylated derivatives of DHEA (Dillon 2005) and some synthetic androstene derivatives that can limit the production of the autoimmune Th1 associated cytokines (Offner 2002).

In summary, this pilot study confirmed the imunoregulatory and anti-inflammatory changes in the immunologic profile after two months of the facilitation physiotherapy in patients with multiple sclerosis. The changes in the immunologic profile correlate with the changes in the dehydroepiandrosterone, brain microstructure and clinical functions. Given the number of tests in the batch, these results require further validation.

The endocrine, nervous and immune systems constitute a mutually dependent and complementary commonly linked functional network. There are new possibilities for physiotherapy to influence the psycho-neuro-endocrine-immune response.

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