Original Investigation

Transdermal Application of Myelin Peptides in Multiple Sclerosis Treatment

Agata Walczak, MD, PhD; Malgorzata Siger, MD, PhD; Agnieszka Ciach, MD, PhD; Marian Szczepanik, PhD; Krzysztof Selmaj, MD, PhD

IMPORTANCE Demonstration of efficacious antigen-specific therapy in multiple sclerosis.

OBJECTIVE To assess the safety and efficacy of transdermally applied myelin peptides in patients with relapsing-remitting multiple sclerosis.

DESIGN One-year double-blind, placebo-controlled cohort study.

SETTING Referral center.

PARTICIPANTS Thirty outpatients aged 18 to 55 years with relapsing-remitting multiple sclerosis.

INTERVENTION Skin patch with a mixture of 3 myelin peptides, MBP85-99, MOG35-55, and PLP139-155.

MAIN OUTCOMES AND MEASURES Cumulative number of active gadolinium-enhanced (Gd^+) lesions per patient per scan, mean volume of Gd^+ lesions, cumulative number of new T2 lesions, and T2 lesion and T1 lesion volume change from baseline to the end of the study. Total number of relapses during the year of the study per patient (annual relapse rate), proportion of relapse-free patients, and proportion of patients with 3 months of confirmed disability worsening on the Expanded Disability Status Scale at month 12.

RESULTS All patients completed the study. Compared with placebo, treatment with a myelin peptide skin patch (1 mg) showed a 66.5% reduction in the cumulative number of Gd^+ lesions ($P = .02$) during the 12 months of the study. The annual relapse rate in patients treated with a mixture of myelin peptides (1 mg) was significantly lower compared with the placebo group (0.43 vs 1.4; $P = .007$). Treatment with a myelin peptide skin patch was well tolerated and no serious adverse events were reported.

CONCLUSIONS AND RELEVANCE In patients with relapsing-remitting multiple sclerosis, treatment with a myelin peptide skin patch significantly reduced both magnetic resonance imaging and clinically defined measures of disease activity and was safe and well tolerated.

Editorial

JAMA Neurol. doi:10.1001/jamaneurol.2013.3022
Published online July 1, 2013.

Author Affiliations: Department of Neurology, Medical University of Lodz, Lodz, Poland (Walczak, Siger, Ciach, Selmaj); Department of Medical Biology, Jagiellonian University College of Medicine, Krakow, Poland (Szczepanik).

Corresponding Author: Krzysztof Selmaj, MD, PhD, Department of Neurology, Medical University of Lodz, 22 Kopcinskiego St, 90-153 Lodz, Poland (kselmaj@afazja.am.lodz.pl).

Induction of antigen-specific tolerance in multiple sclerosis (MS) remains the ultimate goal of therapy for this disease. The pathogenesis of MS is thought to result from antigen-specific autoimmunity[1,2] where autoreactive immune cells, T and B lymphocytes, directed at myelin-related peptides mediate the destruction of myelin within the central nervous system.[3-5] All currently available therapies in MS attenuate global function of the immune system without discrimination of antigen specificity.[6-8] This approach has led to some success for MS therapy but at the same time exposes patients to the risk of increased susceptibility to infectious agents,[9] the induction of opportunistic infections,[10] and increased risk for cancer.[11] In contrast, antigen-specific therapy aims to selectively target cells specific for a given antigen, thus disabling only a small part of the immune system responsible for an autoimmune response, which for MS includes antigens associated with the myelin sheath.[12]

In MS, several antigens have been linked with autoimmunity.[13-15] Among them, a prominent role has been proposed for peptides from 3 major myelin proteins, myelin basic protein (MBP), myelin oligodendrocyte glycoprotein (MOG), and proteolipid protein (PLP). Mapping and sequencing studies identified immunogenic peptide epitopes within these proteins as potentially responsible for the induction of an autoimmune response in MS.[16] However, immunogenic peptides, if applied in an altered form in an appropriate environment and at low concentrations, might not induce an immune response but instead might lead to immune tolerance.[17] The skin is a key first line of immune defense protecting us from pathogen invasion and the external environment. Thus, the immune system of the skin possesses large numbers of immune cells controlling both the induction of an immune response and immunotolerance. Transdermal application of myelin antigens in animals sensitized for experimental autoimmune encephalomyelitis showed an attenuating effect on disease expression.[18,19] Thus, the transdermal route of antigen application might prove to be effective in the development of antigen-induced immune tolerance in humans.

In this study, we conducted a double-blind, placebo-controlled trial with 3 myelin peptides, MBP85-99, PLP139-151, and MOG35-55, applied transdermally as a skin patch in 30 patients with relapsing-remitting MS over the course of 1 year. We tested the hypothesis that a mixture of these peptides would decrease disease activity in MS as assessed by an effect on magnetic resonance imaging (MRI) and clinical outcomes. We also assessed the safety and tolerability of transdermal application of myelin peptides.

Methods

Patients

The study and all procedures were performed after approval from the ethics committee of the Medical University of Lodz and with informed consent from all participants. Thirty patients were enrolled in the study. Inclusion criteria included age from 18 to 55 years, a definite diagnosis of relapsing-remitting MS by the McDonald criteria,[20] screening disability score on the Expanded Disability Status Scale (EDSS) between 0 and 5.5, and 1 or more relapses within the previous year. Patients were excluded if they had been treated within 1 month before screening with intravenous steroids or within 3 months before screening with interferon-beta or glatiramer acetate or had ever been treated with mitoxantrone hydrochloride, cyclophosphamide, or natalizumab. Subjects participating in the trial included 22 women and 8 men with a mean (SD) age of 36.9 (8.0) years and a mean (SD) duration of MS of 8.3 (6.4) years.

Study Design

After providing informed consent, patients were randomized in 1 of 3 arms to receive placebo (n = 10 patients), a mixture of 1 mg of PLP139-151, 1 mg of MOG35-55, and 1 mg of MBP85-99 (n = 16), or a mixture of 10 mg of PLP139-151, 10 mg of MOG35-55, and 10 mg of MBP85-99 (n = 4). The 10-mg group was included primarily for safety reasons. Myelin antigens were dissolved in phosphate-buffered saline and were applied transdermally in an adhesive skin patch placed on the right upper arm that was changed once per week for 4 weeks and then once per month for 11 months. Phosphate-buffered saline served as the placebo treatment because it could not be differentiated visually from the myelin peptide preparations.

Magnetic Resonance Imagining

Magnetic resonance imaging of the brain with intravenous injection of gadolinium (Gd) was performed using a 1.5-T Siemens Avanto Plus magnet. Magnetic resonance imaging was performed at screening and repeated every 3 months until the end of the study. A fast spin echo (repetition time, 2200-3000 milliseconds; echo time, 15-50 milliseconds/80-120 milliseconds; echo train length, 4-6; 3-mm slice thickness; and 44 contiguous axial slices) sequence was used to obtain proton density- and T2-weighted images. Conventional spin echo T1-weighted images (repetition time, 600-650 milliseconds; echo time, 10-20 milliseconds) with the same scan geometry were obtained 5 minutes after injection of 0.1 mmol/kg of Gd. Slices were positioned to run parallel to a line joining the most inferioanterior and inferioposterior parts of the corpus callosum. Patients were carefully repositioned at follow-up according to published guidelines.

The identification of Gd-enhanced (Gd^+), T2-hyperintense, and T1-hypointense lesions was done by consensus of 1 experienced observer (M.S.). The number of total and new Gd^+ lesions, new T2-hyperintense lesions, and new T1-hypointense lesions were then counted. The Gd^+ and new T2 lesions were scored as measures of inflammatory activity between 2 consecutive scans. T2 lesion volume is a measure of overall lesion burden accumulating along the course of disease. The identified lesions were outlined by trained neuroradiologists using a semiautomated segmentation technique and lesion volumes were calculated automatically. Treating and examining neurologists were blinded to MRI results during the study.

Outcome Measures

The primary efficacy outcome measure was the cumulative number of active Gd^+ lesions per patient per scan during the year of the study. Secondary outcome measures included mean

Table 1. Demographic Data

Group	Mean (SD)		
		Myelin Peptide Skin Patch	
	Placebo (n = 10)	1 mg (n = 16)	10 mg (n = 4)
Male, No.	1	5	2
Female, No.	9	11	2
Age, y	38.25 (8.9)	35 (7.45)	36.5 (5.97)
Duration of multiple sclerosis, y	6.5 (4.79)	9.19 (7.54)	9.5 (4.25)
Baseline EDSS score	3.17 (1.17)	2.37 (1.62)	2.13 (1.44)
ARR prior to study	1.2 (1.1)	1.1 (0.12)	1.0

Abbreviations: ARR, annual relapse rate; EDSS, Expanded Disability Status Scale.

Table 2. MRI Outcome Measures[a]

MRI Measure	Mean (SD)			*P* Value[b]
		Myelin Peptide Skin Patch		
	Placebo	1 mg	10 mg	
cnGd$^+$ per patient per slide	0.0255 (0.01)	0.0085 (0.01)	0.0341 (0.03)	.02
vol cnGd$^+$ per patient, mm^3	13.38 (53.59)	8.4 (16.2)	23.98 (53.59)	.07
cn new T2 lesion per patient	2.4 (0.9)	0.75 (0.25)	1.25 (0.27)	.09
Δ T2 lesion volume per patient, mm^3	1925.72 (3142.6)	−845.4 (3734.4)	1265.1 (2055.2)	.01
Δ T1 lesion volume per patient, mm^3	1237.5 (1869.4)	−220.4 (936.0)	−63.2 (1544.8)	.01

Abbreviations: cn new T2 lesion, cumulative number of new T2 lesions; cnGd$^+$, cumulative number of Gd$^+$ lesions; Gd$^+$, gadolinium enhanced; MRI, magnetic resonance imaging; vol cnGd$^+$, volume of cumulative Gd$^+$ lesions; Δ, change from the beginning to the end of the study.

[a] Statistical analysis was performed by the Mann-Whitney *U* test.

[b] *P* value for comparison between the 1-mg myelin peptide skin patch and placebo groups.

volume of Gd$^+$ lesions; cumulative number of new T2 lesions at months 3, 6, 9, and 12; and T2 lesion and T1 lesion volume change from baseline to 12 months. Clinical secondary outcomes involved the total number of relapses during the year of the study per patient (annual relapse rate [ARR]), the proportion of relapse-free patients, and the proportion of patients with 3 months of confirmed disability worsening on the EDSS at month 12. The worsening was defined as a decrease of EDSS score by 0.5 point confirmed after 3 months.

Statistical Analysis

The analysis of primary end points was based on the outcome of data from 2 groups (1-mg myelin peptide skin patch vs placebo or combined 1-mg and 10-mg myelin peptide skin patch vs placebo). Statistical analysis was performed with an SPSS analysis package (version 14.0; IBM SPSS). Normal distribution of every parameter was evaluated using the Kolmogorov-Smirnov test. The MRI data were analyzed using the Mann-Whitney *U* test. The significance level was set at $P = .05$.

Results

Demographics

Of 30 patients who entered the study, 10 were randomly allocated to receive placebo; 16, to receive a myelin peptide skin patch with 1 mg of each peptide; and 4, to receive a myelin peptide skin patch with 10 mg of each peptide. The demographic, clinical, and MRI characteristics at baseline are shown in **Table 1**. All 30 patients completed the study and there was no early termination or withdrawal from the study.

Safety and Tolerability

The most common adverse effect was local reaction in the area of the skin patch. Redness and itching of modest intensity were observed in 20% (4 of 20) of patients receiving myelin peptides (both doses 1 mg and 10 mg). No differences in the distribution of other adverse events, infection of upper respiratory tracts (n = 2) and lacrimation (n = 1), was observed among all 3 treatment groups. No serious adverse events were reported. Routine clinical laboratory testing (blood chemistry, hematology, and urinalysis) did not identify any significant abnormalities during the study period.

Magnetic Resonance Imaging

The results of MRI efficacy are summarized in **Table 2**. Treatment with the 1-mg myelin peptide skin patch showed a 66.5% reduction compared with placebo (0.0085 vs 0.0255) in the cumulative number of Gd$^+$ lesions per patient per scan ($P = .02$), the primary end point of the study (Table 2). Significant differences in favor of the 1-mg myelin peptide skin patch were also seen for all other examined secondary MRI outcomes. The cumulative change in volume of Gd$^+$ lesions per patient was lower in the 1-mg myelin peptide group vs the placebo group (8.4 mm^3 vs 13.38 mm^3; $P = .07$). The cumulative number of new T2 lesions was reduced by 3-fold (68.8%) in the 1-mg myelin peptide skin patch group vs the placebo group (0.75 vs 2.4; $P = .09$). The mean T2 lesion volume at 12 months in 1-mg myelin peptide group was lower by 19.7% compared with base-

Table 3. Clinical Outcomes

Clinical Outcomes	Placebo (n = 10)	Myelin Peptide Skin Patch 1 mg (n = 16)	Myelin Peptide Skin Patch 10 mg (n = 4)	*P* Value[a]
ARR, mean	1.4	0.43	0.25	.007
Relapse-free patients, No./total No.	1/10	10/16	3/4	.049
Disability-progression patients, No./total No.	7/10	3/16	1/4	.13
EDSS score change during the study, mean	0.75	0.08	0.00	.09

Abbreviations: ARR, annual relapse rate; EDSS, Expanded Disability Status Scale.

[a] *P* value for comparison between the 1-mg myelin peptide skin patch and placebo groups.

line (3450.4 vs 4295.8 mm^3), whereas in the placebo group, T2 lesion volume increased by 25.4% (7580.5 mm^3 vs 9506.2 mm^3). Thus, change in T2 lesion volume during the study was significantly different in the 1-mg myelin peptide group vs the placebo group ($P = .01$). Moreover, during the year of the study, T1 lesion volume in the 1-mg myelin peptide group decreased by 14.1% whereas it increased in the placebo group by 61.2% ($P = .01$). The MRI data from the 10-mg myelin peptide group were less efficacious than the 1-mg group. However, the low number of patients treated with the 10-mg dose did not allow for statistical assessment.

Clinical Outcomes

Clinical outcomes are summarized in **Table 3**. Patients treated with the 1-mg myelin peptide skin patch showed an ARR significantly lower than in the placebo group ($P = .007$). The ARR in the 10-mg myelin peptide group was also significantly lower (ARR = 0.25) than in the placebo group. The ratio of relapse-free patients in the 1-mg myelin peptide skin patch group was 63% and was significantly higher compared with 10% in the placebo group ($P = .049$). In the 1-mg myelin peptide group, 19% of patients' EDSS scores worsened during the study vs 70% of patients in the placebo group ($P = .13$). In the 10-mg myelin peptide group, 1 patient of 4 had a worsened EDSS score during the study. Accordingly, during the year of the study, in the 1-mg myelin peptide group, 81% of patients remained disability progression free vs only 30% (3 of 10) in the placebo group ($P = .08$). The mean change in EDSS score between baseline and 12 months was lower in the 1-mg myelin peptide group than the placebo group (0.08 vs 0.75, respectively; $P = .09$).

Discussion

The results of this study using a skin patch of a mixture of 3 myelin peptides, MBP85-99, MOG 35-55, and PLP139-155, showed significant effect in reducing the MRI and clinical outcomes in patients with relapsing-remitting MS. The significant decrease of MRI activity in the 1-mg myelin peptide group during the study was shown for the number of Gd^+ lesions, changes in T2 lesion volume from baseline to study conclusion, and development of new T2 lesions. Thus, myelin peptide skin patch treatment showed an effect on blood-brain barrier opening and generation of new inflammatory lesions.[21] In addition, T1 lesion volume was also decreased in the 1-mg myelin peptide group, indicating that transdermal myelin peptides also showed an effect on the generation of established MRI lesions. The effect of myelin peptides was detected at as early as 3 months of treatment, as demonstrated by the reduction in the cumulative number of Gd^+ lesions vs the placebo group at this point of the study. The higher dose of myelin peptides (10 mg) was less efficacious than the lower dose. However, the low number of patients in the high-dose myelin peptide treatment group precluded statistical analysis of the data, and as indicated in the Methods section, this group was primarily used for safety determinations.

Patients treated with a myelin peptide skin patch had a significantly lower ARR compared with patients treated with placebo and the ratio of relapse-free patients during the study was higher in the myelin peptide group than the placebo group. We studied an MS cohort with active disease. Prior to study entry, the ARR in the 1-mg myelin peptide group was 1.1 and in the placebo group, 1.2. During the study, in the placebo group, the ARR remained at the same high level of 1.4, whereas in the 1-mg myelin peptide group, the ARR was reduced by 69.3% in comparison with the placebo group. In addition, in the 1-mg myelin peptide group, there was a significantly higher proportion of patients who remained disability progression free during the study. The low number of patients in the higher-dose myelin peptide group (10 mg) does not allow for direct comparison of clinical outcomes with the lower-dose myelin peptide group.

The clinical data for the 1-mg myelin peptide group were fully corroborated by the MRI findings, whereas in the 10-mg myelin peptide group, some MRI data did not differ from the placebo group. Two explanations might be proposed in this regard. The correlation between MS lesions observed on conventional MRI scans and the clinical manifestations of the disease remains weak,[22] and thus, the low number of patients in the high-dose myelin peptide group would not compensate for this discrepancy. In addition, it was shown in earlier studies on antigen-induced tolerance that lower antigen concentrations frequently were more efficacious than higher doses.[23] It was proposed that higher antigen concentrations could induce a number of specific T cells to differentiate into effector cells.

Both doses of myelin peptides showed an excellent safety profile. No serious adverse events were noted. Only a mild local skin reaction was observed in some patients receiving the myelin peptide patch. These events did not require any symptomatic treatment and were self-limited. Laboratory test results also did not show any abnormalities in blood cell counts,

liver enzyme levels, and kidney parameters. These results are in sharp contrast to the safety profile of many new drugs that have been developed recently for MS treatment. Most of these drugs inhibit a major immune function and often eliminate a wide array of immune cells. As a result, patients with MS treated with these powerful drugs are immune compromised and more vulnerable to attack by many pathogens that could lead to serious adverse events. Transdermal myelin peptide treatment offers a new approach that only regulates the pathological immune responses while leaving the rest of the immune system intact.

In our previous publication,[24] we demonstrated that administration of a myelin peptide skin patch in patients with MS led to immunologic tolerance to myelin antigens. We found that myelin peptides applied transdermally in patients with MS activated dendritic Langerhans cells in the skin at the site of patch application and induced a unique population of granular dendritic cells in local lymph nodes. In the periphery, transdermal application of myelin peptides resulted in the generation of type 1, interleukin 10–producing regulatory T cells, suppression of specific autoreactive proliferative responses, and suppression of interferon-γ and transforming growth factor-β production. The current data demonstrate that induction of immune tolerance with transdermal application of myelin peptides translates into attenuation of disease activity as evidenced by MRI and clinical measures. This is of particular interest in light of the results with previous trials with antigen-specific therapy in MS, where despite induction of immune tolerance the clinical efficacy was limited.[25-27]

In summary, the efficacy and safety profiles that have emerged from this study make the transdermal application of a mixture of 3 myelin peptides, MBP 85-99, MOG35-55, and PLP 139-155, an attractive and promising therapeutic approach in patients with relapsing-remitting MS. In particular, this antigen-specific therapy using a myelin peptide skin patch offers selective immune intervention targeting MS-related antigens while sparing other mechanisms critical for immune protection.

ARTICLE INFORMATION

Accepted for Publication: March 28, 2013.

Published Online: July 1, 2013. doi:10.1001/jamaneurol.2013.3022.

Author Contributions: *Study concept and design:* Szczepanik and Selmaj.
Acquisition of data: Walczak, Siger, Ciach, and Selmaj.
Analysis and interpretation of data: Walczak, Siger, and Selmaj.
Drafting of the manuscript: Walczak, Siger, Ciach, and Selmaj.
Critical revision of the manuscript for important intellectual content: Szczepanik and Selmaj.
Statistical analysis: Walczak, Siger, and Selmaj.
Obtained funding: Szczepanik and Selmaj.
Administrative, technical, and material support: Walczak and Ciach.
Study supervision: Selmaj.

Conflict of Interest Disclosures: Dr Selmaj has served on scientific advisory boards for Novartis, Biogen Idec, ONO Pharma, Genzyme, Roche, Synthon, and Teva and has received payment for lectures for Novartis, Biogen Idec, ONO Pharma, and Merck Serono. Dr Walczak has received consulting fees from Novartis.

Additional Contributions: We thank Celia Brosnan, PhD, Albert Einstein College of Medicine, Bronx, New York, for helpful advice and editing the manuscript.

REFERENCES

1. McFarland HF, Martin R. Multiple sclerosis: a complicated picture of autoimmunity. *Nat Immunol*. 2007;8(9):913-919.

2. Bhat R, Steinman L. Innate and adaptive autoimmunity directed to the central nervous system. *Neuron*. 2009;64(1):123-132.

3. Codarri L, Fontana A, Becher B. Cytokine networks in multiple sclerosis: lost in translation. *Curr Opin Neurol*. 2010;23(3):205-211.

4. Franciotta D, Salvetti M, Lolli F, Serafini B, Aloisi F. B cells and multiple sclerosis. *Lancet Neurol*. 2008;7(9):852-858.

5. Hafler DA, Slavik JM, Anderson DE, O'Connor KC, De Jager P, Baecher-Allan C. Multiple sclerosis. *Immunol Rev*. 2005;204(1):208-231.

6. Brinkmann V, Billich A, Baumruker T, et al. Fingolimod (FTY720): discovery and development of an oral drug to treat multiple sclerosis. *Nat Rev Drug Discov*. 2010;9(11):883-897.

7. Giacomini PS, Darlington PJ, Bar-Or A. Emerging multiple sclerosis disease-modifying therapies. *Curr Opin Neurol*. 2009;22(3):226-232.

8. Coles AJ, Wing MG, Molyneux P, et al. Monoclonal antibody treatment exposes three mechanisms underlying the clinical course of multiple sclerosis. *Ann Neurol*. 1999;46(3):296-304.

9. Clifford DB, De Luca A, Simpson DM, Arendt G, Giovannoni G, Nath A. Natalizumab-associated progressive multifocal leukoencephalopathy in patients with multiple sclerosis: lessons from 28 cases [published correction appears in *Lancet Neurol*. 2010;9(5):463]. *Lancet Neurol*. 2010;9(4):438-446.

10. Lindå H, von Heijne A, Major EO, et al. Progressive multifocal leukoencephalopathy after natalizumab monotherapy. *N Engl J Med*. 2009;361(11):1081-1087.

11. Muir VJ, Plosker GL. Cladribine tablets: in relapsing-remitting multiple sclerosis. *CNS Drugs*. 2011;25(3):239-249.

12. Lutterotti A, Sospedra M, Martin R. Antigen-specific therapies in MS: current concepts and novel approaches. *J Neurol Sci*. 2008;274(1-2):18-22.

13. Kaushansky N, Eisenstein M, Zilkha-Falb R, Ben-Nun A. The myelin-associated oligodendrocytic basic protein (MOBP) as a relevant primary target autoantigen in multiple sclerosis. *Autoimmun Rev*. 2010;9(4):233-236.

14. Fissolo N, Haag S, de Graaf KL, et al. Naturally presented peptides on major histocompatibility complex I and II molecules eluted from central nervous system of multiple sclerosis patients. *Mol Cell Proteomics*. 2009;8(9):2090-2101.

15. Greer JM, Pender MP. Myelin proteolipid protein: an effective autoantigen and target of autoimmunity in multiple sclerosis. *J Autoimmun*. 2008;31(3):281-287.

16. Honma K, Parker KC, Becker KG, McFarland HF, Coligan JE, Biddison WE. Identification of an epitope derived from human proteolipid protein that can induce autoreactive CD8+ cytotoxic T lymphocytes restricted by HLA-A3: evidence for cross-reactivity with an environmental microorganism. *J Neuroimmunol*. 1997;73(1-2):7-14.

17. Zhang H, Podojil JR, Chang J, Luo X, Miller SD. TGF-beta-induced myelin peptide-specific regulatory T cells mediate antigen-specific suppression of induction of experimental autoimmune encephalomyelitis. *J Immunol*. 2010;184(12):6629-6636.

18. Bynoe MS, Evans JT, Viret C, Janeway CA Jr. Epicutaneous immunization with autoantigenic peptides induces T suppressor cells that prevent experimental allergic encephalomyelitis. *Immunity*. 2003;19(3):317-328.

19. Tutaj M, Szczepanik M. Epicutaneous (EC) immunization with myelin basic protein (MBP) induces TCRalphabeta+ CD4+ CD8+ double positive suppressor cells that protect from experimental autoimmune encephalomyelitis (EAE). *J Autoimmun*. 2007;28(4):208-215.

20. Polman CH, Reingold SC, Edan G, et al. Diagnostic criteria for multiple sclerosis: 2005 revisions to the "McDonald Criteria". *Ann Neurol*. 2005;58(6):840-846.

21. Harris JO, Frank JA, Patronas N, McFarlin DE, McFarland HF. Serial gadolinium-enhanced magnetic resonance imaging scans in patients with early, relapsing-remitting multiple sclerosis: implications for clinical trials and natural history. *Ann Neurol*. 1991;29(5):548-555.

22. Filippi M, Agosta F. Imaging biomarkers in multiple sclerosis. *J Magn Reson Imaging*. 2010;31(4):770-788.

23. Bynoe MS, Bonorino P, Viret C. Control of experimental autoimmune encephalomyelitis by

CD4+ suppressor T cells: peripheral versus in situ immunoregulation. *J Neuroimmunol*. 2007;191(1-2):61-69.

24. Juryńczyk M, Walczak A, Jurewicz A, Jesionek-Kupnicka D, Szczepanik M, Selmaj K. Immune regulation of multiple sclerosis by transdermally applied myelin peptides. *Ann Neurol*. 2010;68(5):593-601.

25. Hafler DA, Kent SC, Pietrusewicz MJ, Khoury SJ, Weiner HL, Fukaura H. Oral administration of myelin induces antigen-specific TGF-beta 1 secreting T cells in patients with multiple sclerosis. *Ann N Y Acad Sci*. 1997;835:120-131.

26. Warren KG, Catz I, Wucherpfennig KW. Tolerance induction to myelin basic protein by intravenous synthetic peptides containing epitope P85 VVHFFKNIVTP96 in chronic progressive multiple sclerosis. *J Neurol Sci*. 1997;152(1):31-38.

27. Genain CP, Abel K, Belmar N, et al. Late complications of immune deviation therapy in a nonhuman primate. *Science*. 1996;274(5295):2054-2057.