Polish Journal of Pharmacology
Pol. J. Pharmacol., 2003, 55, 877–880
ISSN 1230-6002

EFFECT OF IMMUNOMODULATORY TREATMENT OF MULTIPLE SCLEROSIS ON LYMPHOCYTE SURFACE IMMUNOMARKERS

Grażyna Michałowska-Wender[1, #], Jacek Losy[1], Mieczysław Wender[1], Danuta Januszkiewicz-Lewandowska[2], Jerzy Nowak[2]

[1]Neuroimmunological Unit, Medical Research Center, Polish Academy of Sciences, Przybyszewskiego 49, PL 60-355 Poznań, Poland; [2]Institute of Human Genetics, Polish Academy of Sciences, Strzeszyńska 32, PL 60-479 Poznań, Poland

Effect of immunomodulatory treatment of multiple sclerosis on lymphocyte surface immunomarkers. G. MICHAŁOWSKA-WENDER, J. LOSY, M. WENDER, D. JANUSZKIEWICZ-LEWANDOWSKA, J. NOWAK. Pol. J. Pharmacol., 2003, 55, 877–880.

The aim of this study was to analyze the effect of immunomodulatory treatment of multiple sclerosis (MS) on lymphocyte surface immunomarkers. The special attention was given to TCR α/β, γ/δ and α/β HLA-DR markers. Peripheral blood was obtained from 39 patients with clinically definite R-R MS, fulfilling the criteria of McDonald et al.[5]. The group of 15 patients was treated with interferon β-1a (Avonex) intramuscularly once a week. The blood was obtained before and after two years of treatment. The other group of 10 patients was treated every day with 20 mg of glatiramer acetate (Copaxone) intracutaneously. Subsets of lymphocytes were analyzed by the method of flow cytometry, using monoclonal antibodies produced by Ortho Diagnostic System. The relative results were evaluated using Immuno Count II program. The frequency of the studied subsets in MS was markedly different from that in healthy persons. The higher number of CD4, TCR α/β positive cells and higher CD4/CD8 ratio was observed. In comparison to healthy individuals, in MS patients a decreased number of TCR γ/δ, and α/β HLA-DR was found. After therapy with glatiramer acetate, CD3 and CD8 positive lymphocytes were more frequently observed than before the drug administration. The CD4/CD8 ratio was markedly decreased. The effect of interferon β-1a treatment was similar as in the previous group, i.e. a slight increase in CD3 and CD8 was noticed after therapy. Despite the differences in action of both immunomodulatory drugs, which was established in several studies, we like to stress some similarity in their effect on CD3, CD8, α/β HLA-DR and γ/δ HLA-DR immunomarkers frequency in lymphocyte, and on the CD4/CD8 ratio. This may mean that there are some common immunological steps of special importance for the clinical effect in MS.

Key words: *lymphocyte surface immunomarkers, multiple sclerosis, interferon β, glatiramer acetate*

[#] *correspondence*; e-mail: mwender@amp.edu.pl

INTRODUCTION

Multiple sclerosis (MS) is an inflammatory disease of the central nervous system, where autoimmune processes play the central role. T lymphocytes of both subtypes Th1 and Th2 are markedly involved in this process [2]. The genes most likely involved in susceptibility to MS involve human leukocyte antigen (HLA) and T-cell receptor (TCR) gene. In our previous studies, we have established that V γ II- C γ TCR γ chain rearrangement is predominantly expressed on gamma/delta (γ/δ) lymphocytes of MS patients [9]. Taking into account the results indicating an increase in a number of activated γ/δ T cells, i.e. those expressing HLA-DR and CD25, it can be assumed that γ/δ lymphocytes may play a certain role in pathogenesis of MS [3].

Immunomodulatory agents: GA and interferon β play the main role in effective treatment of MS influencing positively natural course of the disease [1, 7, 10]. Therefore, the aim of this study was to analyze the effect of the immunomodulatory treatment of MS on lymphocyte surface immunomarkers. The special attention was given to TCR α/β, γ/δ and αβ/HLA-DR and γ/δ HLA-DR markers.

MATERIALS and METHODS

Peripheral blood was obtained from 39 patients (36 females and 3 males) with clinically definite MS, fulfiling the criteria of McDonald et al. [5] and from 20 control persons. The MS patients were in the relapsing-remitting phase of the disease, aged 20–43 years (mean 31 years). The mean duration of the disease was 48 months (ranging from 12 to 74 month). The control group comprised 20 healthy adults, aged 21–28 years.

The group of 15 patients was treated with interferon β-1a (Avonex) intromuscularly once a week. The blood was obtained before and after two years of treatment. The other group of 10 patients was treated every day with 20 mg of GA (Copaxone) intracutaneously. The blood for studies was taken before and after two years of treatment. The studies were approved by the Ethics Committee of the University of Medical Sciences in Poznań.

Lymphocyte subsets were analyzed by flow cytometry, using specific monoclonal antibodies produced by Ortho Diagnostic System. The evaluation was performed in Cytoron Absolute (Ortho Diagnostic System) at 488 nm wavelength. The relative results were evaluated using Immuno Count II program.

RESULTS

The frequency of the lymphocyte subsets in MS was markedly different as compared to healthy persons. The higher number of CD4, TCR α/β positive cell and higher CD4/CD8 ratio was observed. In comparison to healthy individuals, in MS patients a decreased number of TCR γ/δ, and α/β HLA-DR was found (Tab. 1).

Two year therapy of MS with GA leads only to minor changes in surface immunomarkers on peripheral lymphocytes. After therapy CD3 and CD8 positive lymphocytes were more frequently observed than before the drug administration (Tab. 2). The CD4/CD8 ratio was markedly decreased. The effect of interferon β1a treatment was similar as in the previous group, i.e. a slight increase in CD3 and CD8 frequency was noticed after the therapy (Tab. 3). It should be added that the observed differences in the frequency of TCR γ/β, TCR γ/δ, α/β HLA-DR and γ/δ HLA-DR positive lymphocytes were not statistically significant.

Table 1. Surface immunomarkers of peripheral blood lymphocytes in patients with multiple sclerosis (MS)

	Number of patients	% of lymphocytes in WBC count	CD3	CD4	CD8	CD4/CD8	TCR α/β	TCR γ/δ	α/β HLA DR	γ/δ HLA DR
MS	39	23.90 ± 6.29	72.30 ± 7.44	44.90 ± 9.79	26.40 ± 6.42	1.70 ± 0.61	67.90 ± 7.74	3.70 ± 1.78	7.80 ± 3.91	12.60 ± 10.91
Control group	20	30.70 ± 10.91	70.90 ± 5.88	37.70 ± 8.81	28.80 ± 6.05	1.31 ± 0.42	62.40 ± 10.00	7.90 ± 2.83	12.90 ± 7.68	14.90 ± 7.77
Statistical significance		$p < 0.05$	n.s.	$p < 0.01$	n.s.	$p < 0.05$	$p < 0.05$	$p < 0.05$	$p < 0.01$	n.s.

Table 2. The effect of treatment with glatiramer acetate on surface immunomarkers of peripheral lymphocytes in MS patients (n = 10)

	Before treatment	After 2 years of treatment	Statistical significance
% of lymphocytes in WBC count	33.02 ± 17.45	22.88 ± 6.25	n.s.
CD3	67.5 ± 6.56	72.3 ± 9.55	$p < 0.05$
CD4	42.6 ± 5.41	42.5 ± 8.29	n.s.
CD8	23.0 ± 5.92	27.7 ± 5.85	$p < 0.01$
CD4/CD8	1.85 ± 0.92	1.53 ± 0.81	$p < 0.05$
TCR α/β	62.6 ± 8.41	67.6 ± 9.12	n.s.
TCR γ/δ	4.2 ± 2.08	4.7 ± 1.79	n.s.
α/β HLA DR	14.2 ± 13.77	10.3 ± 6.45	n.s.
γ/δ HLA DR	20.0 ± 14.94	13.4 ± 4.42	n.s.

Table 3. The effect of treatment with interferon β1a (Avonex) on surface immunomarkers of peripheral lymphocytes in MS patients (n = 15)

	Before treatment	After 2 years of treatment	Statistical significance
% of lymphocytes in WBC count	33.01 ± 12.26	24.0 ± 6.69	$p < 0.05$
CD3	65.1 ± 11.22	71.7 ± 7.9	$p < 0.05$
CD4	37.7 ± 11.94	45.9 ± 10.47	n.s.
CD8	22.0 ± 6.85	26.1 ± 6.11	$p < 0.01$
CD4/CD8	1.71 ± 1.04	1.76 ± 0.66	n.s.
TCR α/β	58.1 ± 12.37	67.3 ± 7.50	n.s.
TCR γ/δ	3.9 ± 2.33	3.4 ± 1.56	n.s.
α/β HLA DR	8.1 ± 7.74	6.9 ± 2.41	n.s.
γ/δ HLA DR	11.7 ± 11.16	10.0 ± 8.12	n.s.

DISCUSSION

MS seems to be associated with an inbalance in the subsets of T cells, connected with the synthesis of pro- and antiinflammatory cytokines [4, 6]. Autoreactive T-cells are involved in the recruitment of peripheral macrophages and nonspecific activated T-cells responsible for the process of demyelination and neurodegeneration. The limited pattern of Vδ1, Vδ2 and Vδ3 to Cδ1 rearrangement on the cDNA level observed in the majority of MS patients suggests a significant role of γ/δ T cells with these rearrangement in MS [3, 9]. However, the results of studies of lymphocyte surface immunomarkers are inconclusive.

GA (Copaxone) is a synthetic copolymer, composed of L-alanine, L-lysine, L-glutamic acid and L-tyrosine, acting as immunomodulatory agent in MS, with known effect: reduction of exacerbation rate in patient with relapsing-remitting form of the disease. Interferon β is another important immunomodulatory agent used in the therapy of MS. The mechanisms of action of GA as well as interferon β in MS are not yet fully explained. Mix et al. [8] emphasized that interferon β suppresses the T cell immunity and stimulates the foetal type lymphocytes. The clinical effect may be atributed to action on particular T-cell subset, and subsequently the influence on cytokine production [7] as well as specific and time-dependent changes in multiple mRNAs in lymphocytes [11]. Despite some differences in action of both immunomodulatory drugs, which was established in several studies, we would like to stress that Copaxone, similarly to interferon β, may have some effect on frequency of CD3, CD8 and CD4/CD8 ratio. The effect on α/β HLA-DR and γ/δ HLA-DR was not proved with certainly. This may mean that these two drugs affect some common immunological pathways of special importance for the clinical effect in MS.

However, it is difficult to hypotethise whether the effect of Copaxone on lymphocyte subsets has some clinical relevance. The observed differences in lymhocyte subsets in patients treated with Copaxone may constitute just side effect of the drug which has nothing to do with the immunological defence against some MS-related etiological agent. In our opinion, the obtained results indicate mostly that some immunological disturbances observed in MS may by modulated by the Copaxone treatment.

REFERENCES

1. Coyle P., Hartung M.: Use of interferon beta in multiple sclerosis: rationale for early treatment and evidence for dose and frequency-dependent effect on clinical response. Mult. Scler., 2002, 8, 2–6.
2. Giovanoni G., Hartung P.: The immunopathogenesis of multiple sclerosis and Guillain-Barre syndrome. Curr. Opin. Neurol., 1996, 9, 165–171.
3. Januszkiewicz D., Pernak H., Rembowska J., Nowicka K., Lewandowski K., Hertmanowska H., Wender M., Nowak J.: Non functional TCR gamma gene rearrage-

ment in multiple sclerosis. Folia Neuropathol., 2001, 39, Suppl. A, 17–21.
4. Losy J., Michałowska-Wender G., Wender M.: Interleukin 12 and interleukin 10 are affected differentially by treatment of multiple sclerosis with glatiramer acetate (Copaxone). Folia Neuropathol., 2002, 40, 173–175.
5. McDonald W., Compston A., Edan G.: Recommended diagnostic criteria for multiple sclerosis; guidelines from the international panel on the diagnosis of multiple sclerosis. Ann. Neurol., 2001, 50, 121–127.
6. Michałowska-Wender G., Losy J., Wender M.: Biological markers to confirm diagnosis and monitor the therapy in multiple sclerosis patients. Folia Neuropathol., 2001, 39, 1–5.
7. Miller A., Shapiro S., Gershtein R., Kinarty A., Rawashdek M., Honigman S., Lahat T.: Treatment of multiple sclerosis with copolymer 1 (Copaxone): implicating mechanisms of Th1 to Th2/Th3 immunedeviation. J. Neuroimmunol., 1998, 92, 113–121.
8. Mix E., Stefan K., Hoppner J., Klauer T., Zettl U.K., Strauss U., Meyer-Rienecker H.J., Rolfs A.: Lymphocyte subpopulations, oxidative burst and apoptosis in peripheral blood cells of patients with multiple sclerosis – effect of interferon-β. Autoimmunity, 2003, 36, 291–305.
9. Nowak J., Januszkiewicz D., Pernak H., Hertmanowska H., Nowicka-Kujawiak K., Rembowska J., Lewandowski K., Nowak T., Wender M.: Limited pattern of TCR delta gene rearragement on the RNA level in multiple sclerosis. J. Appl. Genet., 2001, 42, 531–540.
10. Rudick R.: Disease-modifying drugs in relapsing-remmiting multiple sclerosis and future directions for multiple sclerosis therapeutics. Arch. Neurol., 1999, 56, 1076–1084.
11. Weinstock-Guttman B., Badgett D., Patrick K., Hartrich L., Santos R., Hall D., Baier M., Feichter J., Ramanathan M.: Genomic effects of INF-β in multiple sclerosis patients. J. Immunol., 2003, 171, 2694–2702.

Received: September 29, 2003; in revised form: November 3, 2003.