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Peripheral blood cell immunomarkers in the course of methylprednisolone treatment of multiple sclerosis relapses

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Abstract

Intravenous methylprednisolone (MP) is the standard method in the treatment of acute relapses in multiple sclerosis (MS) and is believed to affect various immunological processes involved in the pathology of MS, including apoptosis and phagocytosis. Peripheral blood was obtained from 50 patients, with clinically definite MS, fulfilling the revised criteria of McDonald, a day before, after 5 days of MP treatment, and two weeks after conclusion of the treatment. Intravenous administration of 1.0 g daily of MP was used to treat the new relapses of the disease. The control group comprised 20 healthy blood donors. The subset of lymphocytes CD_3, CD_4, CD_8, CD_{16}, CD_{19}, CD95 /CD3 and CD95/ CD19 was studied using monoclonal antibodies by flow cytometry. Using the flow cytometric test the phagocytosis of granulocytes and caspase activity of granulocytes and lymphocytes were studied. In MS and in the course of MP treatment, both the absolute and relative count of lymphocytes were decreased, as well as the percentage of lymphocytes with CD_3, CD_4 and CD_8 antigen. The relative amount of lymphocytes CD_{16} and CD_{19} was increased. The lymphocytes CD95/CD3 and CD95/CD_{19}, representing markers of apoptotic activity, were not significantly changed. The phagocytosis of peripheral granulocytes before and after intravenous MP was increased.

The quantitative shift in the lymphocyte immunomarkers have an impact on the effect of methylprednisolone in the course of MS relapses. The increase in phagocytic activity in MS is a generalized one and is reflected in the peripheral blood granulocytes, but without marked changes after MP therapy.

Key words: *immunomarkers, methylprednisolone treatment, multiple sclerosis.*

Introduction

Multiple sclerosis (MS) is a subacute disease of the young population, characterized by inflammatory and demyelinating changes in the central nervous system, presumably of T-cell mediated autoimmune aetiology.

Methylprednisolone (MP) is used as a standard drug in the treatment of acute relapses in MS, and is believed to affect various immunological processes involved in the pathology of MS due to its significant anti-inflammatory and immunosuppressive action. However, the specific target involved in the course of MP therapy remains obscure.

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Suppression of immunological system genes by MP in exacerbations of MS was suggested by Airla et al. [1]. An interesting observation made by Mirowska et al. [8] revealed overexpression of matrix metalloproteinase-9 in peripheral blood of MS victims treated with IVMP. In our previous studies we have established that intravenous infusion of MP (IVMP) significantly diminishes the elevated expression of interferon γ inducible protein, one of the important chemoattractants for activated T cells, an effect which seems to be involved in the mechanism of the drug's action in MS relapses [7].

In order to elucidate the pathomechanism of MP therapy of relapses in MS patients, we have undertaken studies on lymphocyte subsets of crucial significance in immunopathology of MS relapses, before and after treatment. As more data become evident about the mechanism of MP action, it may be possible to find a more specific method of treatment for individual patients.

A failure of programmed cell death to eliminate potentially pathogenic autoreactive T-lymphocytes seems to be involved in the pathogenesis of MS. According to Sharief et al. [14], this failure may be provoked by many abnormalities in the cell death machinery. One important factor in this respect could involve overexpression of inhibition of apoptotic proteins occurring in stimulated T-lymphocytes. An increased susceptibility to apoptosis of peripheral blood T lymphocytes was postulated by Prieto et al. [13].

Therefore, the problem is whether or not in MS T-cell apoptosis can be induced by IVMP, an effect which has already been established in an animal model of experimental autoimmune encephalomyelitis. This particular question has been dealt only by Leussink et al. [4], who studied the immediate effect of MP on MS patients. The results led to the suggestion that corticosteroid therapy is a strong inducer of leukocyte apoptosis, and may contribute to the down-regulation of T-cell activity and thus influence the inflammation in the central nervous system. However, the problem seems to be still open and requires confirmation. An additional, still unresolved problem concerns the effect of IVMP on phagocytic activity of granulocytes in peripheral blood, functioning as a significant immunological link of MS reactions in the central nervous system.

Material and Methods

The study material comprised 50 patients (35 females and 15 males) with clinically definite MS, fulfilling the revised criteria of McDonald [12], and 20 control persons. The MS patients were in the relapsing-remitting phase of the disease. Their age ranged from 22 to 50 years (mean: 32.0 years). The mean duration of MS was 6.8 years (ranging from 2 to 18 years). Mean EDSS at the time before treatment was 3.0 (1.0 to 4.5). The control group comprised healthy adult blood donors (14 females and 6 males) aged 25 to 46 years (mean: 36.4 years).

Intravenous administration of MP (Solu-Medrol) at a dose of 1.0 g over a period of 5 days was used to treat the new relapse of the disease 2 to 7 days after the first signs and symptoms of the relapse. The pyramidal and cerebellar systems were usually affected. The definition of relapse included appearance of a new neurological abnormality or worsening of previously stable or improving pre-existing neurological abnormality, separated by at least 30 days from the onset of a preceding clinical demyelinating event. The abnormality had to be present for at least 24 hours and to occur in the absence of fever (<37.5°C) or known infection. None of the patients was in the primary or secondary progressive course of the disease. The peripheral blood samples were taken one day before the onset of treatment (50 patients), after 5 days of IVMP treatment (49 patients) and two weeks after conclusion of the treatment (43 patients). The lower number of studied individuals in the group two weeks after conclusion of the treatment was due to refusal of some patients to undergo repeated blood collection. Lymphocyte subsets (CD_3, CD_4, CD_8, CD_{16}, CD_{18}, $CD95/CD_3$, $CD95/CD_{19}$) were studied by flow cytometry using Facscan produced by Becton Dickinson Company, with the use of specific monoclonal antibodies produced by Ortho Diagnostic System. The phagocytosis of granulocytes and caspase activity of lymphocytes and granulocytes were also tested by flow cytometry using FAM Caspase Activity Kit produced by Imgenex. The phagocytosis of granulocytes was studied using Phagotest produced by Becton Dickinson Company. The study protocol was approved by the Ethics Committee of the Medical University in Poznań.

The analysis of variances (ANOVA) and, subsequently, the significance of differences were tested by means of post hoc Dunnett's test in the group of results with a normal distribution. The post hoc Dunn's test was used for determination of significance of differences in the group of non-parametric distribution.

Results

In multiple sclerosis patients and in the course of MP treatment the absolute and relative count of

lymphocytes as well as the percentage of lymphocytes with CD_3, CD_4 and CD_8 antigen was decreased when compared with control values, while the relative amount of CD_{16} and CD_{19} lymphocytes was increased. The $CD95/CD_3$ and $CD95/CD_{19}$ lymphocytes, representing markers of apoptotic activity, were not significantly changed, demonstrating only an insignificant increase of $CD95/CD_3$ and a decrease of $CD95/CD_{19}$. Also the differences in activity of caspases in lymphocyte cells and granulocytes were insignificant. In MS patients before and after IVMP treatment the phagocytosis of peripheral granulocytes was significantly increased, in comparison with control material. Detailed results are presented in Tables I and II.

Discussion

T lymphocytes differ in their antigen recognition, have various functions and provoke different immunological reactions. Upon antigenic stimulation they express various signalling factors and initiate different immunological responses. T cells can also be cytotoxic and exert a mediating effect on macrophage activation. When compared with healthy persons, in MS there are some lines of evidence for significant differences in the pattern of mononuclear subsets in the peripheral blood. CD4+ T cells are the decisive factors in the immunopathology of MS. Clinical improvement of MS relapses was observed in parallel with a decrease in CD3 and CD19 helper inducer T cells in CSF [15]. The tendency (though not reaching statistical significance) of the expression of chemokine receptor 5 (CCR5) on CD4 and CD8 lymphocytes led to the hypothesis expressed by Elevaora et al. [2] suggesting an inhibited potential of the cells to be transported into the central nervous system. When the mononuclear subsets were compared to results of brain gadolinium contrasted imaging, indicating ongoing activity (gadolinium positive) or stabilization (gadolinium negative images), significant

Table I. The effect of treatment with Solu-Medrol on lymphocyte surface immunomarkers in MS patients in relative (%) and in absolute values (the number of cells in x10E9/L)

	Controls n=30	MS before treatment n=50	After 5 days of IVMP treatment n=49	2 weeks after the IVMP relapse treatment n=43
	x±SD	x±SD	x±SD	x±SD
lymphocytes	2122±489	1315±525*	1205±510*	1255±512*
lymphocytes %	25.0±5.0	25.8±7.9	17.4±9.7*	26.1±8.6
CD3	1643±665	979±439*	822±391*	882±377*
CD3%	77.5±6.0	74.4±8.3	67.3±9.5*	70.3±7.6*
CD4	977±448	623±280*	516±259*	563±259*
CD4%	46.3±7.8	48.0±7.7	42.0±8.4	44.7±7.2
CD8	572±304	335±172*	282±157*	295±143
CD8%	26.5±8.2	25.0±8.1	23.0±8.2*	23.5±7.4*
CD16	248±154	167±99	151±90*	192±109
CD16%	11.6±6.7	13.2±7.1	13.9±8.3*	16.3±8.5*
CD19	238±152	172±108	231±147	178±128*
CD19%	10.8±3.2	13.1±6.3	18.8±7.8*	13.2±5.6
CD95/CD3	1.98±1.29	3.07±2.37	3.20±2.44	2.96±2.34
CD95/CD19	3.62±1.70	2.94±2.73	2.84±3.47	2.53±2.61

*Mean ±SD; * Significant differences between particular MS groups versus controls.*

Table II. The effect of treatment with Solu-Medrol on phagocytosis and markers of apoptosis in MS patients in relative values (%)

	Controls n=30	MS before treatment n=50	After 5 days of IVMP relapse treatment n=49	2 weeks after IVMP relapse treatment n=43
	med. (min-max)	med. (min-max)	med. (min-max)	med. (min-max)
Phagotest mean (+)	88.2 (56.0-11.8)	116.1 (21.8-177.5)*	124.4 (34.2-184.1)*	131.7 (15.7-185.4)*
Phagotest R%	62.4 (41.9-80.8)	72.3 (13.6-98.1)	78.6 (15.6-97.3)*	81.5 (3.3-99.0)*
Living lymphocytes	99.61 (97.57-99.96)	99.37 (93.25-99.94)	99.27 (94.36-99.91)	99.51 (87.28-99.96)
Caspase+ lymphocytes	0.21 (0.04-2.20)	0.42 (0.06-3.07)	0.28 (0.00-2.20)	0.23 (0.00-2.29)
Caspase++ lymphocytes	0.08 (0.00-0.40)	0.15 (0.00-5.98)	0.21 (0.00-5.34)	0.16 (0.00-12.57)
Necrotic lymphocytes	0.00 (0.00-0.05)	0.00 (0.00-0.67)	0.00 (0.00-1.26)	0.00 (0.00-0.52)
Living granulocytes	96.45 (89.55-98.53)	95.04 (72.98-98.55)	95.91 (80.36-99.53)	94.80 (60.72-99.22)
Caspase+ granulocytes	1.84 (0.78-9.68)	2.18 (0.43-11.50)	1.98 (0.36-12.44)	2.85 (0.58-17.07)
Caspase++ granulocytes	0.95 (0.09-4.74)	1.06 (0.23-25.84)	1.15 (0.00-17.05)	0.99 (0.00-20.68)
Necrotic granulocytes	0.19 (0.00-0.85)	0.28 (0.00-2.14)	0.19 (0.00-41.00)	0.31 (0.00-3.43)

*Median (minimum – maximum); * significant differences between particular MS groups versus control; phagotest R+ percent of cells participating in phagocytosis.*

differences between the two stages of MS were found. The relative percentages of white blood cell count as well as the absolute number of CD3 lymphocytes were significantly lower in the group of gadolinium negative MS cases. The CD4+ relative content was higher in the total group of MS patients. The absolute number of CD4 lymphocytes was the lowest in gadolinium negative MS patients [6]. We have established that the treatment of MS relapses with IVMP exerts an effect on mononuclears in the peripheral blood, provoking a decrease in absolute and relative counts of lymphocytes and a relative decrease in CD3, CD4 and CD8 lymphocyte subsets. The relative amount of CD16 and CD14 lymphocytes increases as a result of MP treatment of MS relapses. It seems therefore justified to assume that the quantitative shift in the lymphocyte immunomarker subpopulations has an impact on the effect of methylprednisolone in the course of treatment of MS relapses.

The effect of corticoid therapy on the apoptotic process in the course of MS relapses is more complex, and partly also controversial. Zipp et al. [16] have observed an undesirable effect of glucocorticoid treatment of MS on apoptosis, i.e. a down-regulation of BCL-2 inhibition of CD95-mediated T-cell apoptosis. Petelin et al. [10] have observed that pulse therapy influences the CD95/Fas expression of CD_8 and CD_4 T-lymphocytes in MS patients. It should be added that also in MS patients treated with other immunomodulatory drugs, such as interferon β-1α, an evident effect was noted on apoptosis in peripheral blood cells [9].

In our material, we have been unable to find any significant differences either in CD95/CD_3, pertaining to T lymphocytes, or in CD95/CD_{19} ratio, pertaining to B cells, or in the activity of caspases in peripheral lymphocytes. The controversial results found in various studies imply that changes in the process of apoptosis in peripheral lymphocytes are very small only, if discernible at all, in the course of corticoid therapy of MS relapses.

In some studies of phagocytic reactivity in granulocytes circulating in peripheral blood of patients with MS, enhanced activity was noted [3], whereas in other ones a depressed function of polynuclears was observed [11]. In our studies, an increase in phagocytic activity in MS before and after IVMP has also been observed but without marked changes after the IVMP therapy.

In appears from our results that the differences in the process of phagocytosis, even when representing a significant factor in pathological events occurring in the acute phase of MS in the central nervous system [5],

in which they are connected with microglial activity, are generalized ones, and correspond to changes in activity of the peripheral blood granulocytes.

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