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T-Lymphocyte Subpopulations in Multiple Sclerosis – Do They Help to Judge Immunosuppressive Therapy?

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Summary. T-cell subpopulations were tested in multiple sclerosis (MS) patients before and after cyclophosphamide ($n = 38$) and corticotropin ($n = 37$) treatment and physiotherapy ($n = 30$). There were no specific changes of subset ratios immediately after immunosuppressive treatment. However, T-cell subpopulations showed great day-to-day variations in MS patients.

Key words: T-lymphocyte subpopulations – Multiple sclerosis – Immunosuppressive therapy

Introduction

It is accepted that multiple sclerosis (MS) is a disease of the immune system. Immunosuppressive therapy has been used in many cases. Cyclophosphamide (CY) has been shown to be beneficial [4, 6, 20] after careful studies in vitro [16, 19] and in the animal model of MS, experimental allergic encephalomyelitis (EAE) [13]. At least in mice, this effect may not be simply due to destruction of immunocompetent cells, but to induction of suppressor cells [11].

T-lymphocyte subpopulations in the peripheral blood are altered during the course of the disease. Especially the ratio of helper/suppressor cells [1, 15] and the numbers of activated T-cells [5] have been shown to be elevated in MS patients compared with controls. Recently the correlation of these parameters with the activity of the disease has again been discussed [2, 12].

In Ulm University Hospital CY has been given to MS patients in the last 18 months in an open trial. Although side-effects are dramatically diminished by individually adjusted dosage [8], we tried to establish a laboratory parameter for the activity of the disease [7].

Therefore we looked for any differences in the T-lymphocyte subpopulations of patients groups after CY and corticotropin (ACTH) treatment or physiotherapy. When we failed to find any difference, we examined the reliability of these values by testing the day-to-day variations of patients over 1–2 weeks.

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Methods

Patients treated by CY (Endoxan, Degussa, Bielefeld, FRG), received 8 mg/kg body weight i.v. Four days later total lymphocyte counts were determined, and the dose was repeated until the lymphocyte counts dropped to half the initial value (usually after 3–4 doses) as an indicator of effect.

Patients before and 3 days after CY ($n = 38$) and ACTH ($n = 37$) treatment or physiotherapy ($n = 32$) were tested. The groups were matched according to age and sex. Diagnosis was determined by using the McDonald criteria [10]. Each group contained about two-thirds relapsing/remitting cases and one-third chronic progressive cases.

Blood was drawn between 8 and 9 a.m. T-lymphocyte subpopulations were differentiated by monoclonal antibodies in an indirect immunofluorescence assay. Briefly, lymphocytes were separated by density centrifugation (Ficoll-Paque, Pharmacia, Stockholm). 1×10^6 cells were incubated for 30 min at 4°C in an appropriate dilution of monoclonal antibodies (T11 = pan-T, T8 = T-suppressor/cytotoxic cells, and Ta_1 = activated T-cells were generous gifts from Prof. B. Fleischer, Immunology, Ulm; Leu3a = T-helper cells were purchased from Becton and Dickinson, Rödermark, FRG). An incubation with the second antibody (FITC-conjugated goat anti-mouse IgG, Ortho Diagnostic Systems, Raritan, NJ, USA) followed (30 min at 4°C). Two hundred cells per sample were evaluated blind with the help of a fluorescence microsocpe.

To test the day-to-day variations we examined the lymphocyte subsets in 13 further unmedicated MS patients (McDonald criteria, relapsing/remitting course) daily for 1–2 weeks. Concomitantly 6 healthy donors were examined to test the reliability of the method.

Table 1. Mean values of T-cell subsets in patients before and after therapy (per cent of counted cells). Standard deviations in brackets. T11 = pan-T cells, Ta1 = activated T-cells, T4 = T-helper cells, T8 = T-suppressor cells; T4/T8 = helper/suppressor ratio

	Cyclophosphamide ($n = 38$)		Corticotropin ($n = 37$)		Physiotherapy ($n = 32$)		
	Before	After	Before	After	Before	After	
T11	43.2 (22.8)	44.6 (20.5)	41.8 (22.0)	44.1 (16.6)	34.8 (17.4)	38.6 (17.6)	T11
Ta1	22.0 (19.4)	22.0 (17.2)	20.8 (18.3)	19.7 (16.5)	16.3 (14.6)	16.8 (10.6)	Ta1
T4	36.8 (20.4)	37.0 (18.5)	32.1 (17.2)	32.1 (13.9)	28.3 (10.8)	32.3 (14.7)	T4
T8	20.4 (14.3)	21.9 (14.8)	19.8 (15.7)	20.9 (13.7)	15.9 (10.3)	17.9 (11.0)	T8
T4/T8	2.23 (1.13)	2.07 (0.94)	1.94 (0.85)	1.85 (1.06)	2.28 (1.07)	2.15 (0.97)	T4/T8

Table 2. Day-to-day variations of helper/suppressor ratios in patients and healthy donors. $\bar{x}$ = mean value of tests performed in one patient on consecutive days; s = standard deviation; d = number of tests; $s/\bar{x}$ = parameter for the variance

Patient	$\bar{x}$	s	d	$s/\bar{x}$	Normals	$\bar{x}$	s	d	$s/\bar{x}$
1	3.13	1.06	10	0.34	5	1.50	0.28	9	0.19
2	1.87	0.39	9	0.21	6	1.32	0.12	9	0.09
3	1.92	0.42	10	0.22	12	1.28	0.20	6	0.16
4	1.64	0.38	8	0.23	17	1.20	0.22	9	0.18
7	1.68	0.37	9	0.22	18	1.06	0.15	9	0.14
8	2.92	1.12	9	0.38	19	0.93	0.18	8	0.19
9	1.12	0.20	9	0.18		$s/\bar{x} = 0.16$ ($s = 0.04$)			
10	1.21	0.37	9	0.30					
11	1.83	0.36	9	0.20					
13	1.38	0.45	9	0.33					
14	1.41	0.35	9	0.25					
15	1.80	0.76	9	0.42					
16	1.73	0.39	9	0.23					
$s/\bar{x} = 0.27$ ($s = 0.08$)									

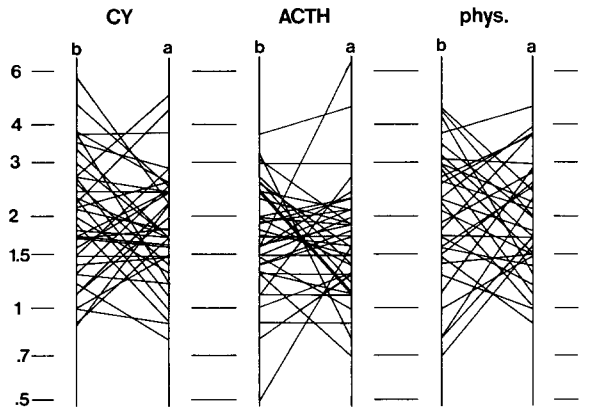

Fig. 1. Helper/suppressor ratios of multiple sclerosis patients before *(b)* and after *(a)* therapy. CY = cyclophosphamide; ACTH = corticotropin; phys. = physiotherapy. (In our laboratory ratios of healthy controls ranged between 0.8 and 2.2)

Results

When patients were tested before and after CY, ACTH or physiotherapy no differences could be found among the three groups (Table 1). In all of the groups the helper/suppressor ratios showed great variation with a slight tendency to fall after therapy without reaching significant differences. We could not, however, find any specific influence of CY treatment on the behaviour of the tested surface antigens. Courses of helper/suppressor ratios are shown in Fig. 1.

There were marked fluctuations in patients. Thus, we wondered whether T-cell surface antigens might show a great day-to-day variability in MS patients. Table 2 shows the mean values of helper/suppressor ratios drawn from consecutive days. It is evident that patients showed significantly greater variations ($P < 0.001$) of these values than healthy donors.

Discussion

When monoclonal antibodies for the subclassification of T-cells were developed, they seemed to give a measure of the activity of MS [1, 15]. Later this was doubted [14, 17, 18], but recently again two groups have reported a correlation between changes in T-cell subsets and the course of the disease [2, 12].

Therefore, we tried to use the determination of T-cell subpopulations for an early evaluation of immunosuppressive treatment. We did not find any correlation between T-cell subsets and treatment course.

Others have described changes of helper/suppressor ratios after a longer time interval between treatment and blood sampling [3]. However, we cannot exclude the possibility that the dose of CY we used might have been insufficient to provoke those changes. We adjusted the therapy to the total lymphocyte count, but we applied smaller doses than others [4, 6, 20], never producing hair loss. However, the clinical success (stable course in 80% of patients; Kornhuber, to be published) is equivalent to results of other studies (77%, Gonsette et al. [4]; 80%, Hauser et al. [6]).

Because of the great day-to-day variations in some of our patients we cannot accept a connection between clinical course and T-cell subpopulations as reported by Mickey et al. [12]. Day-to-day variations have already been mentioned by Hauser et al. [6], but they described variations also in 4 healthy controls. In contrast, our results in 6 healthy persons who served to confirm the reliability of our methods are in agreement with Kuś et al. [9], who did not find day-to-day variations in 13 healthy volunteers. Whether the observed difference between healthy persons and MS patients is due to immunological changes in MS remains to be elucidated.

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