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SUBPOPULATIONS OF T LYMPHOCYTES IN SUBACUTE SCLEROSING PANENCEPHALITIS (SSPE)*

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Subacute sclerosing panencephalitis (SSPE), a progressive encephalitis occurring in children and young adults, is regarded as a model of human slow virus infection associated with the measles virus[1]. It has been assumed that the host factors are involved in the pathogenesis of SSPE. The presence and multiplication of the measles virus despite a high titer of measles antibodies suggests that there may be an underlying cellular immune defect. The immune status of patients with SSPE has been studied and the results have been found to be variable. Some patients have demonstrated depression of cellular immune functions, whereas others showed normal cellular immunity[2-8].

Various immune responses are under the control of regulatory T cells. The balance between helper and suppressor T cells plays a significant role in the regulation of immune responses[9]. Abnormalities found in some immune functions in SSPE patients may be the result of an imbalance between the immunoregulatory T cells, and this imbalance may also play a part in the high measles antibody titers, besides the continuous antigenic stimulus[10].

Recently it has become possible to identify T lymphocyte subsets by using monoclonal antibodies. It has also been shown that the suppressor cells can be evaluated by theophylline modulation of E-rosette formation[11]. Pharmacologic agents that increase intracellular levels of cAMP inhibit the formation of E rosettes in peripheral blood lymphocytes (PBL), and a correlation has been found between

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the suppressor cells and the T-sensitive (Tsen) cells which lose their E-rosette forming capacity after theophylline incubation. Lymphocytes which still form E rosettes after theophylline incubation, that is T-resistant (Tres) cells, contain primarily helper T cells.

Although T and B lymphocyte counts have been determined in SSPE, to our knowledge there is no report in the literature in which the T lymphocyte subpopulations were investigated.

In this study we determined the helper and suppressor T cell subsets in patients with SSPE by theophylline modulation of E-rosette formation.

Material and Methods

T lymphocyte subsets were studied by theophylline modulation of the E rosettes in 12 patients with SSPE and 13 healthy age-matched controls. The diagnosis of SSPE was made on the basis of a typical clinical picture, characteristic electroencephalogram and was confirmed in five cases with elevated titers of measles antibody in the serum and cerebrospinal fluid*. Five patients were in the first stage, 6 in the second and one in the third according to Freeman's staging system[12].

The modulation of E-rosette formations was evaluated, with slight modifications, according to the method described by Shore et al[11]. Briefly, Ficoll Hypaque separated mononuclear cells[13] were washed three times with PBS (phosphate buffered saline) and the cell number was adjusted to 8×10^6/ml. Next, 0.125 ml of the cell suspension was incubated with 0.125 ml of 4×10^{-2} M theophylline or PBS at 37 °C for 2 hours. (This was done in duplicate.)

After the incubation period, 0.25 ml of 1% sheep red blood cell suspension, which had been prepared in a PBS solution containing 4% PBS, was added to the mononuclear cell suspensions and centrifuged at 800 rpm for 5 minutes. After an overnight incubation at 4 °C, at least 200 cells were counted for each sample and the percentage of rosette forming cells (RFC) was calculated.

Theophylline inhibition (TI) was calculated by means of the following formula :

$$\text{Theophylline inhibition (\%)} = \frac{(E - RFC) - Tres}{E - RFC}$$

E-RFC: the percentage of E-rosette forming lymphocytes incubated with PSB. Tres: the percentage of E-rosette forming lymphocytes after incubation with theophylline.

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* Measles antibody titers in CSF and serum were kindly determined by P. Lachmann, Laboratory of Molecular Biology, The Medical School, Hills Road, Cambridge, England.

Tsen cells were calculated as the difference between E-RFC and Tres cells. Since Tres cells (lymphocytes which are still forming E rosettes after theophylline incubation) will contain primarily helper T cells and Tsen cells represent suppressor T cells, the Tres/Tsen ratio was taken as the helper/suppressor T lymphocyte ratio. Student's *t* test and the Mann Whitney *U* test were used for statistical analysis.

Results

The mean percentage of E-RFC was significantly lower in SSPE patients than in controls (Tables I, II). There were some alterations in T cell subsets in some patients. Although no constant abnormality was observed, the Tres/Tsen ratio tended to be higher in patients than controls. The differences between patients and controls were not statistically significant in Tres and Tsen cells, theophylline inhibition or Tres/Tsen ratio (Tables I, II).

TABLE I

T Lymphocyte Subpopulations in SSPE Patients

E–RFC	Tres	Tsen	TI	Tres / Tsen
56.5**	9.5[b]	47	83.1[a]	0.20[b]
59.0*	21.0	38	64.7	0.55
59.0	11.0[b]	48	81.3[a]	0.22
65.5*	39.5	26	39.6	1.51[a]
63.0**	24.0	39	61.9	0.61
47.0**	8.0[b]	39	82.9[a]	0.20[b]
62.0**	42.5	20	31 4[b]	2.12[a]
61.0*	49.0[a]	12[b]	19.6[b]	4.08[a]
49.0**	31.0	18[b]	36.0	1.72[a]
51.0*	17.0[b]	34	66.0	0.50
75.0*	32.0	43	57.3	0.74
64.0**	38.0	26	41.0	1.46[a]
Mean±SD 59.2±7.7	26.8±13.8	32.5±11.8	55.4±21.5	1.1±1.1
Controls 70.1±6.0	30.7±6.8	39.3±10.2	55.2±11.8	0.8±0.3

* SSPE patients in stage I
** SSPE patients in stage II
a More than 2 SD above normal mean values
b More than 2 SD below normal mean values

TABLE II

Statistical Analysis of T Lymphocyte Subsets in SSPE Patients and Controls

	Patients		Controls
E–RFC	59.2±7.7	$P < 0.05$	70.1±6.0
Tres	26.7±13.8	$P > 0.05$	30.7±6.8
Tsen	32.5±11.8	$P > 0.05$	39.3±10.2
TI	55.4±21.5	$P > 0.05$	55.2±11.8
Tres / Tsen	1.1±1.1	$P > 0.05$	0.8±0.3

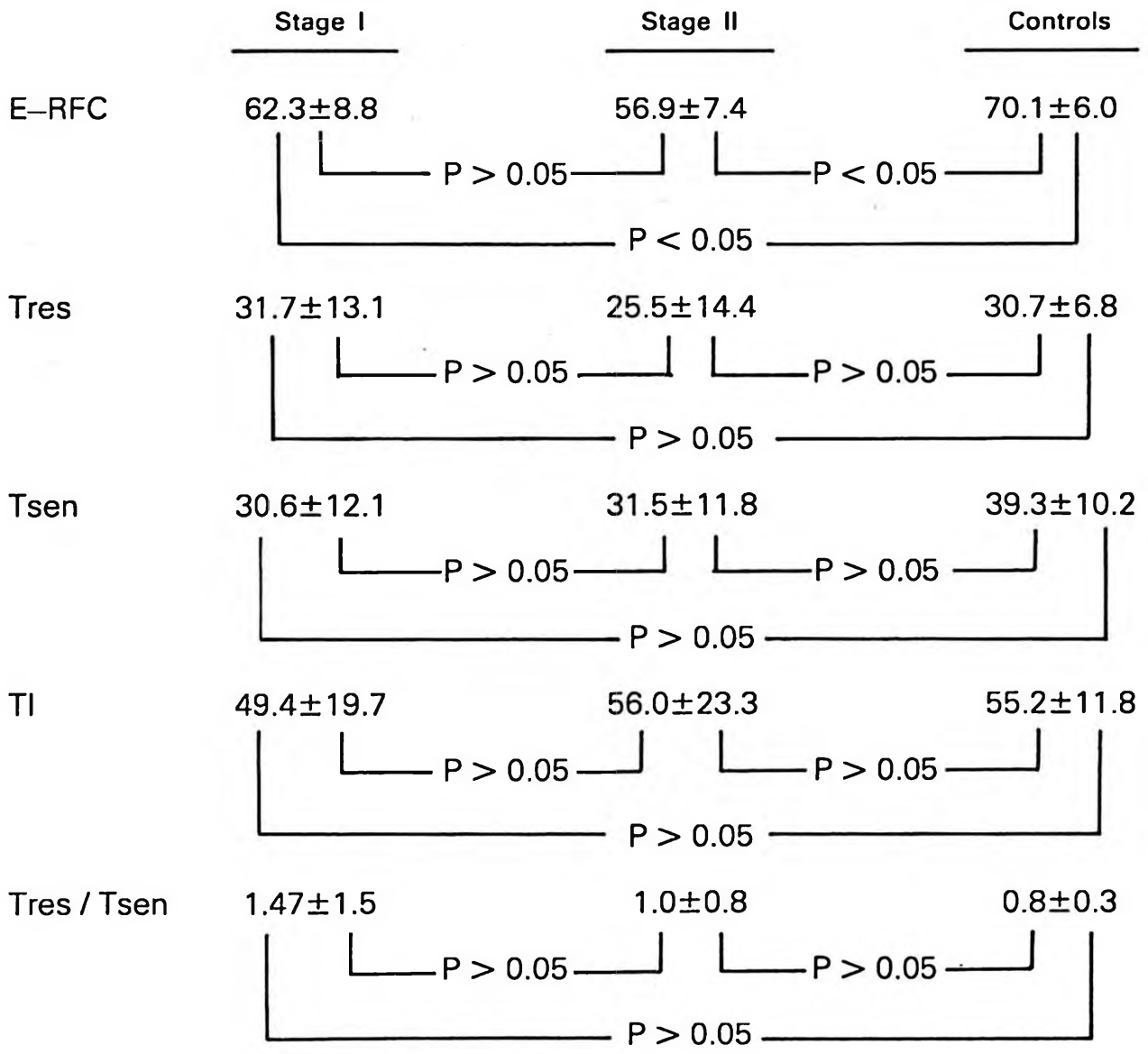
The E-RFC values were significantly lower in both stage I and II patients than in controls but only one out of five patients in stage I, and three out of six in stage II had E-RFC values 2 SD below normal values (Tables II, III). None of the other parameters showed any difference between patients in stages I or II and controls (Tables II, III).

Discussion

The T lymphocyte counts and cell mediated immune competence of patients with SSPE have been determined previously by various investigators and the results have been variable[14-18]. In some patients no impairment of cell mediated immunity was observed while in others deficient cellular immunity was demonstrated. In the present study, when the patient group was evaluated as a whole, T cell numbers were found to be low in SSPE patients. The patients in both stage I and stage II had significantly lower numbers of T cells than controls. However, one out of five in stage I and three out of six in stage II had E-rosette values 2 SD below the mean value for controls.

TABLE III

Statistical Analysis of T Lymphocyte Subsets in SSPE Patients with Stage I and II SSPE



The thymus is essential for the differentiation of T cells and their helper, suppressor and cytotoxic subsets by its microenvironment and thymic hormones[19]. Varsano et al[15] proposed that impairment of the cell mediated immune competence observed in some SSPE patients could be due to immaturity of a part of the T cell system and treated two SSPE patients with thymic humoral factor (THF). The patients showed reconstitution of T cell function. The authors explained the improvement of the cell mediated immune capacity after THF administration by the assumption that these two patients lacked mature T cells

and that the THF had converted immature T lymphocytes into mature T lymphocytes. They also proposed that THF may be effective by modulating the activity of suppressor T cells. Wijermans et al[17] measured the activity of thymus dependent serum factor in 11 patients with SSPE and found significantly decreased activity in six. Agnarsdottir and Valdimarsson[10] suggested that suppressor T lymphocytes may be infected by measles virus, resulting in increased measles antibody response. All these data may suggest the presence of an imbalance between the immunoregulatory cells that regulate cellular and humoral immune functions in these patients. We could not find any study in the literature about T lymphocyte subpopulations in SSPE patients. In the present study, although the Tsen/Tres ratio tended to be higher in SSPE patients, there was no constant pattern and no statistically significant difference could be found between patients and controls. However, our results do not rule out functional T cell defect. Besides, SSPE patients may have impaired measles specific suppressor cell functions. Further studies are needed to clarify this subject.

Summary

Suppressor and helper T cell subpopulations in 12 patients with SSPE (5 patients in stage I, 6 in stage II and 1 in stage III) were studied by using theophylline modulation of E rosette formation in order to detect regulatory T cell abnormalities. The percentages of Tres (helper), Tsen (suppressor) cells and the Tres/Tsen ratio were not significantly different in patients and controls. When patients were evaluated according to their clinical stages, again no significant difference was found. However, variations from the normal range were observed in some patients and the Tres/Tsen ratio tended to be higher in SSPE patients than in controls. Only the mean percentage of E rosettes was significantly lower in patients in both stage I and stage II than in controls.

These results suggest that SSPE patients do not have any consistent abnormality in regulatory T cells measured by theophylline modulation of E rosette formation.

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