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SUBPOPULATIONS OF T LYMPHOCYTES IN PRIMARY IMMUNODEFICIENCY DISEASES*

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Various immune responses are under the control of regulatory T cells. Both positive and negative regulatory influences are mediated by distinct T cell subsets, namely by suppressor and helper T cells. Recently it has become possible to identify these subsets by means of surface marker reactivity with heteroantisera and by monoclonal antibodies[1-3]. It has also been shown that the suppressor cells can be evaluated by theophylline modulation of E-rosette formation[4,5]. 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 the suppressor cells and the T sensitive or T_{sen} cells which lose their E-rosette forming capacity after theophylline incubation. Lymphocytes which still form E rosettes after theophylline incubation, that is T resistant or T_{res} cells, contain primarily helper T cells[5].

Regulatory T cells have been studied in the context of various primary immunodeficiency diseases and it has been suggested that imbalances of T cell subpopulations may play an important role in the pathogenesis of certain immunodeficiency diseases as in common variable immunodeficiency (CVI) and selective IgA deficiency[6-14].

So far, it seems patients with ataxia-telangiectasia (AT) have rarely been included in studies in which suppressor and helper T cells have been evaluated through theophylline modulation of E rosette formation[15-17]. In this study of primary immune deficiency cases AT patients are in the majority.

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Material and Methods

Twelve patients with AT, three with chronic mucocutaneous candidiasis (CMCC), one with partial DiGeorge's syndrome, one with X-linked hypogammaglobulinemia and one with hyper-IgM syndrome, were studied along with 25 healthy, age-matched controls.

The modulation of E rosette formation was evaluated, with slight modifications, according to the method described by Shore et al[5]. Briefly, Ficoll-Hypaque separated mononuclear cells were washed 3 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% FCS, 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 percentages of rosette-forming cells (RFC) were calculated.

Theophylline inhibition was calculated by means of the following formula:

$$\text{Theophylline inhibition (\%)} = \frac{\text{(E-RFC)-}T_{res}}{\text{(E-RFC)}} \times 100$$

E-RFC = the percentage of E-rosette-forming lymphocytes incubated with PBS. T_{res} = the percentage of E-rosette-forming lymphocytes after incubation with theophylline.

T_{sen} cells were calculated as the difference between E-RFC and T_{res} cells. Since T_{res} cells (lymphocytes which are still forming E-rosettes after theophylline incubation) will contain primarily helper T cells and T_{sen} cells represent suppressor T cells, the T_{res}/T_{sen} ratio was taken to be the same as the suppressor/helper T lymphocyte ratio.

Student's t test and the Mann Whitney U test were used for statistical analysis.

Results

The percentages of E-RFC, theophylline inhibition, T_{res} and T_{sen} cells and the T_{res}/T_{sen} ratio values for both patient and control groups are given in Tables I-IV.

In patients with AT, the mean percentages of E-RFC and T_{res} cells were significantly low in comparison with the controls but there was no significant difference between the two groups regarding theophylline inhibition, T_{sen} cells and T_{res}/T_{sen} values (Tables I-II). Patients were divided into 2 groups according to their serum IgA levels. Five of the 12 patients were IgA deficient. There was no significant difference in any

TABLE I

T Lymphocyte Subpopulations in Patients with Ataxia-Telangiectasia

Patient	Sex	Age (yrs)	E (%)	T_{res} (%)	T_{sen} (%)	Theophylline inhibition (%)	T_{res}/T_{sen}
A.O.	M	13	65	12.0[b]	53[a]	81.5[a]	0.23[b]
S.A.	M	15	38[b]	21.5[b]	16.5[b]	43.4	1.30
M.B.	M	16	53[b]	23.0	30	56.6	0.76
İ.B.	F	10	65	21.5[b]	43.5	66.9	0.49
S.Ş.	F	9	65	28.5	36.5	56.2	0.78
E.Ç.	F	9	50[b]	13.5[b]	36.5	73.0[a]	0.37
S.AL.*	F	9	56[b]	18.0[b]	38	67.9	0.47
K.S.*	F	9	68	48.0[a]	20[b]	29.4[b]	2.40[a]
A.K.*	M	11	62	33.0	29	46.8	1.13
A.Ö.	M	9	70	29.0	41	58.6	0.70
M.Y.*	F	7	48[b]	16.0[b]	32	66.7	0.50
Y.T.*	M	11	60[b]	28.0	32	53.3	0.87
	Mean		58.3	24.3	34.0	58.4	0.83
	± SD		9.5	9.9	9.9	14.2	0.58
Control	Mean		71.1	34.2	36.8	51.7	0.98
	± SD		4.7	5.8	7.0	8.4	0.31

* : Patients with decreased IgA levels

a : above 2 SD of normal mean values

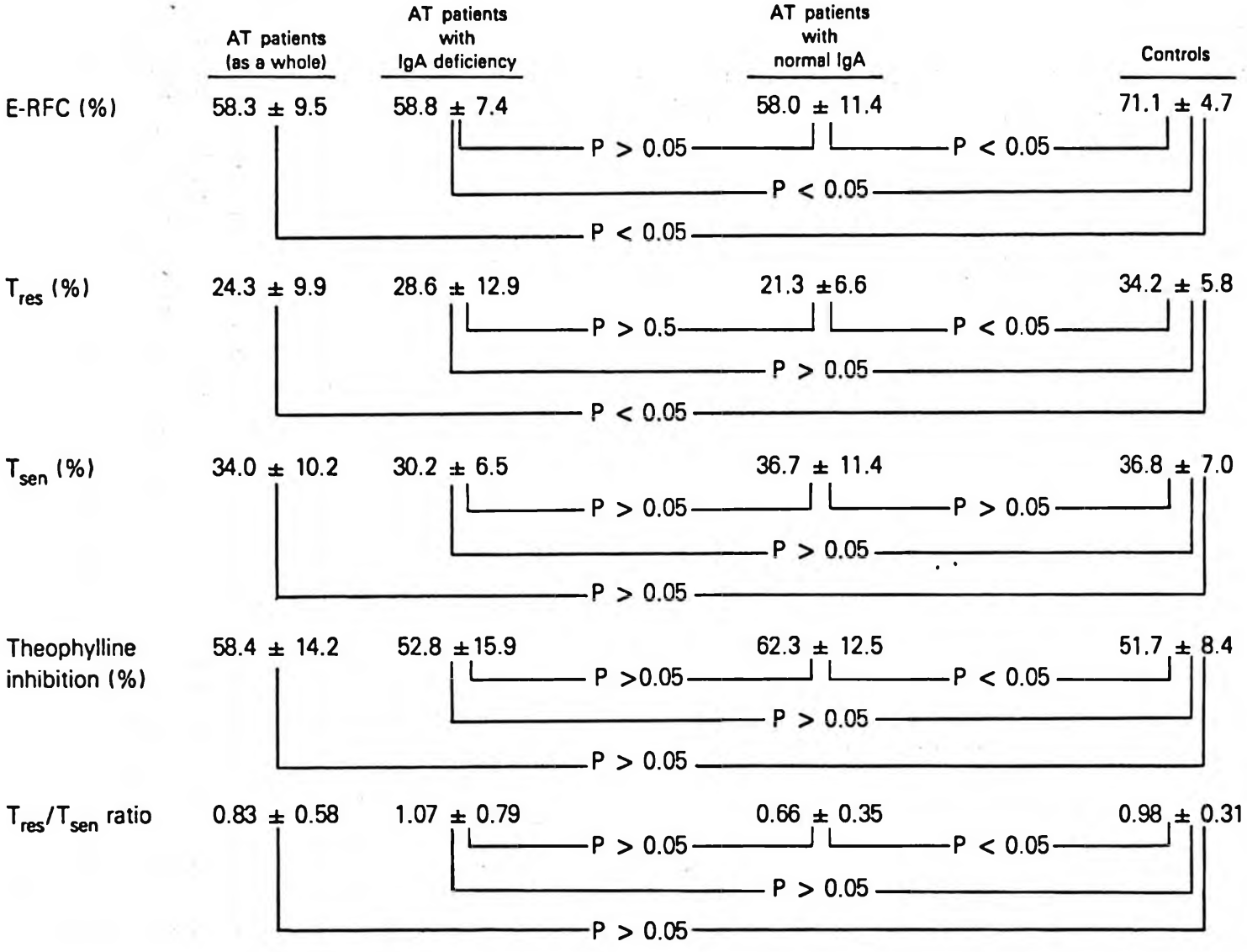
b : below 2 SD of normal mean values

of the parameters between the IgA deficient group and the IgA normal group. In the IgA deficient group, only the E-RFC was lower than in the controls, while in patients with normal serum IgA levels the mean percentages of E-RFC, T_{res} cells and T_{res}/T_{res} ratios were lower, and the theophylline inhibition was higher than in the controls (Table II). However, these deviations were not constant in every patient. Individual evaluation of patients with a normal IgA level showed that three out of 7 had T_{res} cells below and one had T_{sen} cells above the 2 SD of the control mean values.

The 2 patients with CMCC (Y.O. and O.O.) were siblings and both had endocrinopathy (hypoparathyroidism and adrenal insufficiency). All parameters were within

TABLE II

Statistical Analysis of T Lymphocyte Subpopulations in Patients with Ataxia-Telangiectasia



normal limits in two of these patients (M.K. and Y.O.) but the T_{res} cells and the T_{res}/T_{sen} ratio was high in one (O.O.) (Table III).

TABLE III

T Lymphocyte Subpopulations in Patients with Chronic Mucocutaneous Candidiasis

Patient	Sex	Age (yrs)	E (%)	T_{res} (%)	T_{sen} (%)	Theophylline inhibition (%)	T_{res}/T_{sen}
M.K.	M	2.5	62.0	26.0	36	57.3	0.72
Y.O.	M	16	66.0	36.0	30	45.5	1.20
O.O.	M	13	74.0	46.0[a]	28	37.8	1.64[a]

a : above 2 SD of normal mean values.

The patient with partial DiGeorge's syndrome who had been treated with thymic humoral factor one year previously had a low T_{sen} cell count. The X-linket hypogammaglobulinemic patient had a high T_{res} cell count, a high T_{res}/T_{sen} ratio and low theophylline inhibition and the patient with hyper-IgM had a low T_{res} cell count and T_{res}/T_{sen} ratio, high theophylline inhibition and a high T_{sen} cell count (Table IV).

TABLE IV

T Lymphocyte Subpopulations in Various Immunodeficient Patients

Type of Immunodeficiency	Patient's Age (yrs)	E (%)	T_{res} (%)	T_{sen} (%)	Theophylline inhibition (%)	T_{res}/T_{sen}
DiGeorge syndrome	2	56.0[b]	35	21[b]	37.5	1.47
X-linked hypogammaglobulinemia	12	80.5	57.5[a]	23	28.8[b]	2.50[a]
Hyper IgM	7	71.5	16.5[b]	55[a]	76.9[a]	0.30[b]

a : above 2 SD of normal mean values

b : below 2 SD of normal mean values

Discussion

In recent years studies have been carried out to determine the T cell subpopulations in patients with primary immunodeficiency diseases[4,11-17]. However, these studies have included few patients with AT. In this study T lymphocyte subpopulations were studied in 12 patients with AT by theophylline modulation of E rosette formation. The mean percentages of E-RFC and the T_{res} cells were found to be low in AT patients while other parameters did not show any significant difference when compared with the controls. Evaluation of AT patients according to serum IgA levels disclosed higher theophylline inhibition, low T_{res} cells and a low T_{res}/T_{sen} ratio in the group with normal IgA while these parameters were within normal limits in the group with decreased IgA.

Although hyperactivity of suppressor T cells has been postulated in the pathogenesis of decreased levels of immunoglobulins[6,7-10], in this study we did not find any significant imbalance of T subpopulations in AT patients with decreased IgA levels. Earlier studies on T cell subpopulations which have made use of theophylline modulation of E-rosette formation did not include patients with AT. However, $T\gamma$ and $T\mu$ cells were checked in three patients with AT by Gupta and Good[12]. They found low $T\mu$ in two of the three and high $T\gamma$ in all. Fiorilli et al[18] also evaluated T cell subsets in five patients with AT with monoclonal antibodies. All patients had a significantly reduced proportion of helper and two had an increased proportion of suppressor T cells. In our study 5 of the 12 patients had T_{res} cell counts below and only one had T_{sen} cells above 2 SD of the control mean values. This discordance may be due to various factors, as for instance the different methods used in evaluating the lymphocyte subpopulations, the inclusion of relatively few AT patients in the study and a possible heterogeneity in lymphocyte subpopulations in these patients. Further, in previous studies, the serum IgA levels of the patients were not referred to[12,18]. In this study, it was noted that patients with normal IgA levels had a higher theophylline inhibition, low T_{res} cells and a low T_{res}/T_{sen} ratio.

We also observed alterations of regulatory T cell subsets in some patients with CMCC, the DiGeorge's syndrome and the hyper-IgM syndrome but, as was also the case in previous reports, there was no constant pattern[11-13, 16]. In contrast to earlier studies which revealed a high proportion of suppressor cells in some patients with X-linked hypogammaglobulinemia[11-13], our patients had a low proportion of T_{sen} cells and theophylline inhibition and a high proportion of T_{res} cells and a high T_{res}/T_{sen} ratio. Sirianni et al[17] also, through theophylline modulation of E-rosette formation, found a low proportion of T_{sen} cells in one out of 3 patients with X-linked hypogammaglobulinemia.

In conclusion, very much in accordance with previous studies, we found that patients with various immunodeficiency diseases showed a deviation from the normal proportion of T cell subsets, but without any constant pattern. Although all

these deviations may not be of any pathogenic importance as such, they could be of importance for the patients themselves in the selection and outcome of certain forms of immunotherapy.

Summary

Suppressor and helper cell populations were studied in patients with various immunodeficiency diseases (most of them were AT patients) by using theophylline modulation of E-rosette formation in order to detect regulatory T cell abnormalities.

The mean E-RFC and T_{res} cell counts were found to be low in patients with AT while other parameters did not show any significant differences when compared with the controls. However, some patients also showed deviations from the normal range in other parameters. Evaluation of AT patients according to serum IgA levels disclosed higher theophylline inhibition, lower T_{res} cells and a low T_{res}/T_{sen} ratio in the group with normal IgA levels while these parameters were within normal limits in the group with decreased IgA. In line with previous studies, we found no consistency in the abnormalities of subpopulation distribution in any of the other forms of immunodeficiency.

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