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PERIPHERAL BLOOD LYMPHOCYTE SUBPOPULATIONS IN CHILDREN WITH JUVENILE CHRONIC ARTHRITIS

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Peripheral blood lymphocyte subset levels were analyzed in 28 patients with active, polyarticular, juvenile chronic arthritis and in 12 healthy control children. The patients with a similar treatment were divided into two groups, at the beginning of disease and after five years' course. Ten children with juvenile chronic arthritis were treated by glucocorticosteroid.
After five years the percentage of active T cells, T suppressor cells and active / total T cells ratio were significantly elevated and there was a decrease in the T_4/T_8 ratio.
Glucocorticosteroids increased the proportion of active T cells but did not change the percentage of other lymphocyte subsets.
It is concluded that during the course of disease a drug-induced or spontaneous process appears which corrects the initial immunological disturbance.

INTRODUCTION

There have been conflicting reports on the alteration of lymphocyte subpopulations in rheumatoid arthritis /1, 2, 6, 8, 12/. In childhood the evaluation of the subset profile is not easy, as for the ratio of the subpopulations of lymphocytes is changing with age. At ages younger than 6-8 yr there is a dominance of helper T cells (T_4), which increases the ratio of T_4/T_8 /5/. Above six years of age, the values are similar to those for adults, so we can compare the different age groups.

In 1985 Oen et al /10/ proved that in the three subgroups of juvenile chronic arthritis (JCA), the percentage of suppressor

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T cells (T_8) decreases causing an increase of ratio of T_4/T_8. Other authors failed to show this change /3, 4, 9/. The present investigation was designed to study the numbers and percentages of lymphocyte subpopulations in patients with active JCA during the course of disease. The effect of long-term glucocorticoid therapy on lymphocyte subsets was also evaluated.

MATERIAL AND METHODS

The patient population consisted of 28 children (17 girls, 11 boys) with active JCA. Controls were 12 healthy children (8 girls, 4 boys) aged 6 to 19 years (mean $\pm$ SD = 13.2 $\pm$ 4.1 years). We divided the patients into two groups. In the first group there were fourteen patients aged 7-15 years (mean $\pm$ SD = 12.6 $\pm$ 3.3) whose disease has been started during the last six months. In the second group there were fourteen patients aged 6-18 years (mean $\pm$ SD = 13.4 $\pm$ 3.5), with a disease duration between five and seven years. JCA was considered to be active if 3 of following criteria were fulfilled: /1/ ESR $>$ 30 mm/h; /2/ serum haptoglobin level $>$ 2.0 g/l; /3/ the number of swollen and tender joints $>$ 5. All 28 patients belonged to the progressive polyarticular subgroup of JCA.

3 children in the first group, and 2 patients in the second group were seropositive based upon Rose-Waaler technique. The therapy was similar in both groups. In the first group 1 patient was given an immunosuppressive treatment (azathioprine), 5 patients were given steroid, 2-2 patients were given chloroquine and gold therapy and all patients were treated with nonsteroidal antiinflammatory drugs (NSAID). In the second group 2 children received an immunosuppressive treatment (azathioprine, cyclophosphamid), 5 patients were given steroid and 3-3 received chloroquine and gold therapy, all patients were given NSAID. The results of the patients who received corticosteroid were also evaluated separately. The patients received Prednisolon 7.5 - 10 mg/day, at least for three months before the study. Peripheral mononuclear cells were isolated from heparinized blood by means of Ficoll-Hypaque gradient centrifugation. The monocytes were removed by carbonyl iron treatment. The total count of T lymphocytes (T_T) was determined with E-rosette technique. The active E rosette (T_A) was applied by the method of Yu /15/. Surface immunglobulin positiv cells were identified by direct immunfluorescence applying fluorescein-isothiocyanate conjugated anti-human IgG, A, M (Hyland).

The helper and suppressor T cell subsets were determined by OKT_4 and OKT_8 monoclonal antibodies (Ortho Pharmaceutical Inc., Raritan, NJ).

Student's *t*-test was used for statistical analysis.

RESULTS

The various peripheral blood lymphocyte subpopulations of patients with JCA are shown in Tables I and II. In the second group there was a significant elevation as compared to the first one in the percentage of T_A ($p < 0.05$) and in the ratio T_A/T_T ($p < 0.01$). In the first group there was a marked increase in the percentage of surface immunoglobulin positive (SIg +) cells as compared to the second group and to control group, but the difference was not significant (Table I).

There was an increase in the percentage of T_8 cells in the second group of JCA as compared to the first group ($p < 0.01$) and the T_4/T_8 ratio decreased significantly ($p < 0.01$) in the second group (Table II). Table III and IV contain the laboratory parameters of patients who received glucocorticosteroid treatment. In the steroid treated group the absolute lymphocyte count, the percentage of T_A cells and the T_A/T_T ratio were significantly elevated. There was no significant change in other blood lymphocyte subpopulations.

DISCUSSION

The aim of our study was twofold; first to provide data on lymphocyte subpopulation distribution in blood of patients with JCA at the beginning of disease and after five years progression, and second, to study the effect of long-term corticosteroid treatment on subpopulations. Our data have demonstrated parameters of a so called "immunological destabilisation" (at the beginning of disease). They were as follows: a./ the percentage of active T cells decreased b./ T_A/T_T was low c./ there was an elevation in proportion of SIg + cells d./ the percentage of T suppressor cells decreased and the T_4/T_8 ratio increased.

These alterations suggest an inadequate regulation of the

TABLE I

T and B lymphocytes in peripheral blood of patients with juvenile chronic arthritis (mean ± SD)

Diagnosis	Age, years	Absolute lymphocyte count/l	T_A percent	T_T percent	T_A/T_T	SIg + cell percent
JCA group I n = 14	12.6±3.3	2.972×10^9 ±0.714	25.0±3.90	56.0±4.80	0.44±0.08	17.1±5.32
JCA group II n = 14	13.4±3.5	2.510×10^9 ±0.991	31.6±8.84	55.7±7.20	0.57±0.13	13.8±2.00
healthy controls n = 12	13.2±4.1	2.315×10^9 ±0.918	29.8±7.00	61.6±7.30	0.50±0.10	14.3±3.38
Significance						
JCA I - II		NS	$p < 0.05$	NS	$p < 0.01$	NS
JCA I - control		NS	NS	NS	NS	NS
JCA II - control		NS	NS	NS	NS	NS

NS = non-significant

TABLE II

T lymphocyte subsets in peripheral blood of patients with juvenile chronic arthritis (mean ± SD)

Diagnosis	Age, years	Absolute lymphocyte count/l	OKT_4 percent	OKT_8 percent	T_4/T_8
JCA group I n = 14	12.6±3.3	2.972×10^9 ±0.714	54 10±6.80	19.00±2.60	2.96±0.57
JCA group II n = 14	13.4±3.5	2.510×10^9 ±0.991	45.10±13.60	27.70±7.10	1.83±0.79
healthy controls n = 12	13.2±4.1	2.315×10^9 ±0.918	52.30±8.30	23.10±3.70	2.30±0.60
Significance					
JCA I - II		NS	NS	$p < 0.01$	$p < 0.01$
JCA I - control		NS	NS	$p < 0.05$	$p < 0.05$
JCA II - control		NS	NS	NS	NS

NS = non-significant

TABLE III

T and B lymphocytes in peripheral blood of patients with steroid treated juvenile chronic arthritis (mean ± SD)

Diagnosis	Age, years	Absolute lymphocyte count/l	T_A percent	T_T percent	T_A/T_T	SIg + Cell percent
1. patients with steroid treatment n = 10	12.4±4.1	3.628×10^9 ±0.706	32.70±6.90	57.60±6.80	0.58±0.12	14.70±4.40
2. patients without steroid treatment n = 18	13.8±3.1	2.248×10^9 ±0.689	25.80±6.26	55.20±5.01	0.46±0.10	15.80±2.98
healthy controls n = 12	13.2±4.1	2.315×10^9 ±0.918	29.80±7.00	61.60±7.30	0.50±0.10	14.30±3.38
Significance						
1. - 2.		$p < 0.001$	$p < 0.05$	NS	$p < 0.05$	NS
1. - control		$p < 0.02$	NS	NS	NS	NS
2. - control		NS	NS	NS	NS	NS

NS = non-significant

TABLE IV

T lymphocyte subsets in peripheral blood of patients with steroid treated juvenile chronic arthritis (mean ± SD)

Diagnosis	Age, years	Absolute lymphocyte count/l	OKT_4 percent	OKT_8 percent	T_4/T_8
1. patients with steroid treatment n = 10	12.4±4.1	3.628×10^9 ±0.706	46.70±13.90	23.90±9.47	2.21±0.78
2. patients without steroid treatment n = 18	13.8±3.1	2.248×10^9 ±1.689	51.20±10.20	23.10±6.48	2.33±0.88
healthy controls n = 12	13.2±4.1	2.315×10^9 ±0.918	52.30±8.30	23.10±3.70	2.32±0.60
Significance					
1. - 2.		$p < 0.001$			
1. - control		$p < 0.02$	The differences are not significant		
2. - control					

immunological system even if the results comparing with the control group are not always significant. The results in the second group are at variance with previous results. The number of T_A cells increased, the proportion of suppressor cells was elevated and the T_4/T_8 ratio was decreased. (Fig. 1, 2)

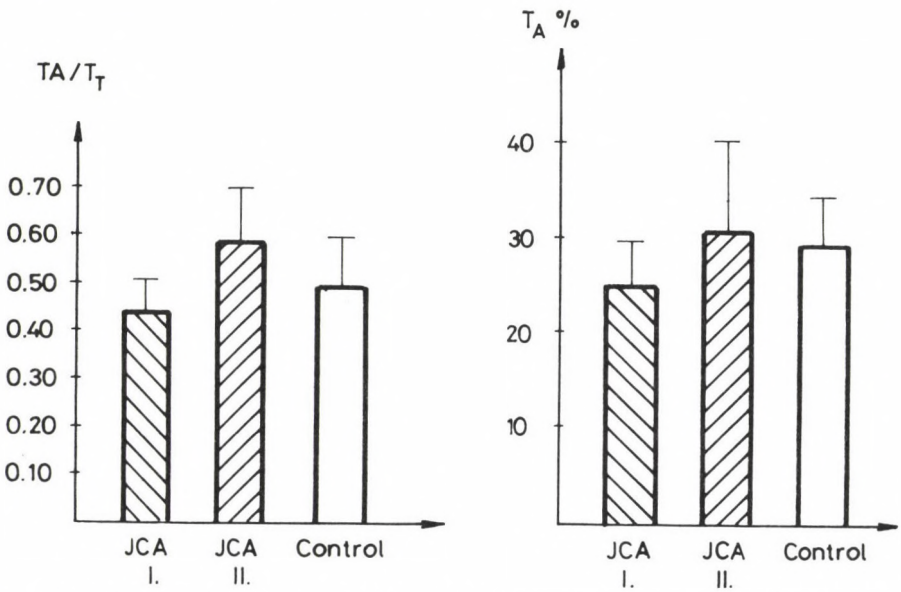

Fig. 1. The ratio of T_A/T_T and percentage of T_A at the beginning (JCA I.) and after five years progression (JCA II.) of juvenile chronic arthritis

The changes of the lymphocyte subpopulations in the second group could be regarded as compensatory. The changes may be in connection with the long-term treatment, since all patients have received drugs which slow the progression of JCA. The antirheumatic drugs influence immunological state in various ways. And after longterm treatment the combined effects of the drugs are seen.

Alternatively there are some previous studies, which suggest that the alteration of lymphocyte subsets may not correlate directly with the drug treatment of the disease /11, 13, 14/. So, the change in the distribution of T cell subpopulations is due to the changing immunological activity which appears after five years of the disease process in the individual patients.

The difference between the patients with and without steroid

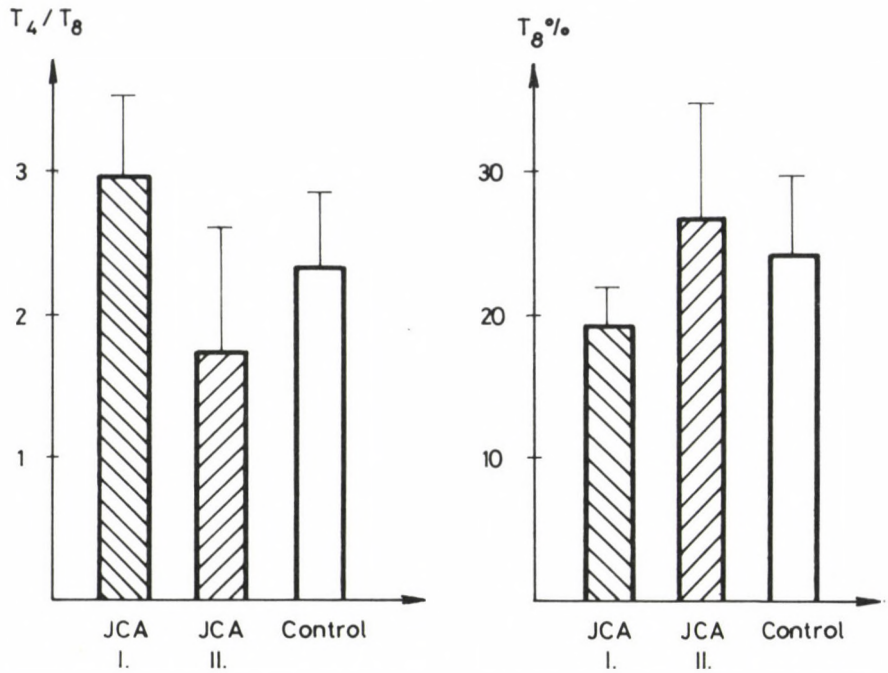

Fig. 2. The ratio of T_4/T_8 and percentage of T_8 at the beginning (JCA I.) and after five years progression (JCA II.) of juvenile chronic arthritis

treatment was less marked. The glucocorticosteroid therapy increased the number of active T cells and decreased the percentage of T helper lymphocytes, but it was unable to produce a strong immunological correction. However, the increase in the percentage of T_A cells indicated a tendency towards the correction of the immunological deficiency. Haynes, Katz and Fauci /7/ reported that T helper cells were preferentially depleted from peripheral blood after hydrocortison administration. We found a similar decrease in the percentage of the T_4 cells, but the change was not significant.

In summary our report suggests there is an advantageous modification in the proportion of lymphocyte subpopulations after many years of onset of disease, but further longitudinal studies are required to clarify the relationship between the effect of antirheumatic drugs, the alteration of lymphocyte subsets and the immunological activity of JCA.

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