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Lymphocyte Subset T4/T8 Ratio in Systemic Lupus Erythematosus: Correlation with Disease Activity, Laboratory Abnormalities and Treatment.

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SUMMARY **Lymphocyte subsets were studied in forty-nine patients with SLE using monoclonal antibodies and flow cytometry. A decrease in T4+ reactive cells (helper/inducer) was the most frequent observation. Decreased T4/T8 ratios were seen in patients with increasing clinically active disease, patients with positive anti-DS-DNA, positive anti-RNP antibodies and patients with low CH50 activity. However, low T4/T8 ratios were seen in patients with negative anti-Sm. Low T4/T8 ratios were also observed in patients taking prednisone at more than 10 mg/day and in patients treated with immunosuppressive drugs.**

Systemic lupus erythematosus (SLE) is a multisystem disease characterized by the presence of multiple autoantibodies and depressed suppressor cell activity.[1-4] Although significant progress has been made regarding the pathogenesis of SLE over the past decade, the etiology of the disease is still not known. Existing evidence suggests that SLE may not be a single entity, but a constellation of signs and symptoms produced by a variety of etiologic agents.[5]

Recent developments in flow cytometry[6] and hybridoma methodology for the production of monoclonal antibodies[7] have led to the analysis of human lymphocyte subpopulations. A few studies on T-cell subpopulations in SLE patients have been reported with conflicting results.[8,12] Morimoto and co-worker[8] found percentages of OKT3$^+$ cells (total T-cells) and OKT8$^+$ cells (T-suppressor/cytotoxic cells) to be decreased in SLE patients with active disease. These findings were not confirmed by Smolen *et al.*[9,12] who found that a decrease in OKT4$^+$ cells (T-helper/inducer cells) was the most frequent observation in active SLE. Smolen *et al.*[9] found that SLE patients could be separated into 3 clinically distinguishable groups based on the T4/T8 ratios. Group 1 patients had severe renal disease, thrombocytopenia, and early stage of onset and low T4/T8 ratios. Group 2 patients were characterized by lymphadenopathy, myopathy, sicca syndrome, and central nervous system (CNS) involvement but rarely renal disease and high T4/T8 ratios. Group 3 patients had widespread multisystem disease including renal and CNS manifestations and normal T4/T8 ratios. No correlation of T4/T8 ratio with disease activity, duration of illness, or corticosteroid therapy was seen in these patients. Bakke *et al.*[10] also found the most frequent observation to be a decrease of OKT4$^+$ cells. However, the association was seen in active SLE patients on no medication as well as in patients with inactive disease receiving greater than 10 mg of prednisone per day. Melendro *et al.*[11] found OKT3$^+$, OKT4$^+$, and OKT8$^+$ cells to be decreased in active SLE patients.

In this study, T-lymphocyte subset ratios were correlated with clinical disease activity, serological abnormalities, and treatment.

SUBJECTS AND METHODS

Patients

All patients selected for this study fulfilled at least 4 out of 11

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criteria for the diagnosis of SLE according to the American Rheumatism Association.[13] They were patients followed in the lupus clinic at the University of Mississippi Medical Center (UMC) from February 1983 to February 1986. Forty-nine patients agreed to participate in this study (46 females, 3 males). Mean age and standard deviation (SD) at the time of study was 33.9 ± 11.0 years (range 16 to 69); mean age of onset was 28.5 ± 9.5 years (range 15 to 52); mean duration of disease was 78.1 ± 85.9 months (range 1 to 389); and mean duration of follow-up was 43.8 ± 46.7 months (range 1 to 182). There were five patients treated with immunosuppressive drugs; four patients were taking 100 mg of azathioprine; one patient was taking 2 mg of chlorambucil.

Patients were divided into groups according to clinical disease activity, serologic abnormalities, and treatment at the time lymphocyte subpopulations were studied. Scoring of clinical disease activity was done using the arbitrary scoring on a 0 to 3 scale described previously.[14] Briefly, activity 0 = inactive; activity 1 = arthralgia, mild rashes; activity 2 = polyarthritis, serositis; activity 3 = multisystem disease with renal and/or CNS involvement.

Controls

Because T cell subpopulations may vary with age,[15] forty-two normal individuals matched for age with SLE patients were used as controls with a mean age of 36.7 ± 13.5 years (range 19 to 65).

Laboratory studies

Anti-DS-DNA antibodies were performed by the modified Millipore filter technique.[16] Antigen ^{14}C-labelled DNA was purchased from Amersham Searle. Normal levels are 0 to 1.4 μg/DNA bound per milliliter of serum. Complement levels were measured as CH50 according to Kent and Fife.[17] Normal values are 150 to 250 units. Anti-Sm and anti-RNP antibodies were done by counter-current immunoelectrophoresis.[18] Antigens were extracted from rabbit thymus.

T cell and T cell subset values were quantitated using the monoclonal antibodies from Ortho Diagnostics, Raritan, NJ. T3 and T11 monoclonal antibodies react with 95% of normal human peripheral T-cells. T4 monoclonal antibodies react with the helper/inducer subset which represents approximately 65% of peripheral T-cells in normal subjects. T8 monoclonal antibodies react with approximately 35% of peripheral T cells and represent the suppressor/cytotoxic cells.[19-21]

Purified mononuclear cell populations were prepared from 20 ml samples of whole fresh heparinized venous blood using the standard Ficoll-Hypaque technique.[22,23] The purified lymphocytes were resuspended in 0.15 M phosphate-buffered saline (PBS) pH 7.2 and adjusted to 1×10^6 cells/ml. One milliliter aliquots of the cells were suspended in PBS and placed on ice. Resuspended cells were incubated with 100 μl of a 1:50 dilution of monoclonal antibody with specificity for each cell type. Control cells were incubated with 100 μl of PBS. Incubation was carried out in an ice bath for 20 to 30 min. The cells were washed twice with 2 ml of PBS. The resuspended pellet was incubated again for 20 to 30 min at 4° C with 100 μl of a 1:20 dilution of fluorescein-conjugated $F(ab')_2$ goat anti-mouse IgG (Tago, Inc., Burlingame, CA). The cells were washed again three times with 2 ml of PBS and resuspended in 1 ml of PBS containing 5% FCS and 0.1% sodium azide for flow cytometric analysis.

Flow cytometry was performed using a cell sorter equipped with an argon-ion laser which operated at a 488-nm wave-length (Ortho Cytofluorograf System, HH50 with a 2150 computer). The stained cells were analysed for 3 parameters: the narrow forward angle light scatter which measures cell size; the 90° angle light scatter which discriminates the internal structure of cells; and the fluorescence emissions.[23] Results were expressed as a percentage of total T cells($T3^+$,$T11^+$),T helper/inducer cells ($T4^+$), T suppressor/cytotoxic cells ($T8^+$), and as the ratio of T-helper/inducer to T suppressor/cytotoxic cells (T4/T8 ratios). To avoid potential inaccuracies in calculation of the T4/T8 ratios, assays in which the percentage of $T3^+$ cells were equal to or less than 10% were excluded from analysis.[24]

Statistical analysis

All mean values are given with standard deviations. Student's *t*-test was used to compare the difference between mean values for various groups of SLE patients and controls.

RESULTS

The mean percentages of peripheral blood T lymphocytes reactive with T11, and T4 antibodies in patients with SLE were decreased when compared to the control population (Table 1). Conversely, the mean percentage of T8 antibody reactive cells was increased when compared to controls. Thirteen patients (27%) had T4/T8 ratios below 95% confidence limits of the normal controls (mean ± 2SD); 33 patients (67%) had T4/T8 ratios within the normal range; and 3 patients (6%) had T4/T8 ratios above these limits.

There were five patients treated with immunosuppressive drugs: three patients were disease activity 3; two patients were disease activity 1. The mean percentages of T lymphocyte subsets in patients treated with or without immunosuppressive drugs are shown in Table 2. There was a statistically significant decrease in the mean percentage of $T4^+$ cells in patients treated with or without immunosuppressive drugs when

Table 1. T–cell subsets in patients with SLE and control groups.

	% of reactive cells		
T–cell subsets	SLE (49)	Control (42)	p value
T_3	62.4 ± 15.6	65.7 ± 12.3	0.28
T_{11}	67.3 ± 16.5	74.6 ± 12.5	0.02
T_4	36.7 ± 11.3	43.6 ± 9.5	0.002
T_8	28.9 ± 11.4	24.5 ± 6.6	0.03
T_4/T_8	1.50 ± 0.83	1.85 ± 0.46	0.02

All values are means ± SD

compared to controls. However, only in patients treated with immunosuppressive drugs was the T4/T8 ratio statistically significantly lower when compared to controls. Because of this significance, patients treated with immunosuppressive drugs were excluded from all other groups for statistical analyses.

Table 3 shows results when SLE patients were divided into groups with various disease activities and serologic abnormalities. Only patients

Table 2. The mean percentages of T–cell subsets in patients treated with or without immunosuppressive drugs.

	No.	% of reactive cells				T_4/T_8	p values§
		T_3	T_{11}	T_4	T_8		
Immunosuppressive drugs	5	49.9 ± 18.0	61.6 ± 29.9	30.9 ± 14.4 #	31.5 ± 11.7	0.95 ± 0.14	< 0.001
No immunosuppressive drugs	44	63.9 ± 14.7	67.8 ± 15.2	37.3 ± 10.9 #	28.6 ± 11.5	1.56 ± 0.85	0.05

All values are means ± SD.
p value < 0.01 compared with control group.
§ p value of T_4/T_8 ratio compared with control group.

Table 3. The mean percentages of T–cell subsets as classified by clinical disease activity and serologic abnormalities.

Groups	No.	% of reactive cells			T_4/T_8	p values§
		T_{11}	T_4	T_8		
Activity 0	16	64.7 ± 14.3¶	37.2 ± 9.5	26.8 ± 11.3	1.7 ± 1.0	0.43
Activity 1	2	71.4 ± 13.6	50.8 ± 13.2	23.5 ± 1.6	2.1 ± 0.4	0.40
Activity 2	4	67.4 ± 11.2	34.4 ± 11.5	28.8 ± 12.8	1.5 ± 1.0	0.15
Activity 3	22	69.8 ± 17.0	36.8 ± 11.6¶	30.3 ± 12.1¶	1.4 ± 0.7	0.006
+ Anti–DNA	21	71.3 ± 15.9	36.8 ± 11.9¶	31.9 ± 11.5#	1.4 ± 0.8	0.003
– Anti–DNA	23	64.5 ± 14.2#	37.8 ± 10.3	25.6 ± 10.9	1.7 ± 0.9	0.49
+ CH_{50}	24	70.0 ± 17.1	35.9 ± 12.3#	30.9 ± 12.8#	1.4 ± 0.8	0.008
– CH_{50}	20	65.1 ± 12.6#	39.1 ± 9.1	25.8 ± 9.2	1.7 ± 0.9	0.47
+ Anti–Sm	10	71.4 ± 11.7	46.3 ± 10.2	24.3 ± 7.0	2.1 ± 1.1	0.19
+ Anti–Sm	32	67.2 ± 15.6	34.9 ± 10.0#	29.5 ± 12.5	1.4 ± 0.7	0.002
+ Anti–RNP	20	70.1 ± 15.8	39.5 ± 11.3	28.6 ± 11.1	1.6 ± 0.7	0.04
– Anti–RNP	22	66.4 ± 13.8	35.8 ± 10.8#	27.9 ± 12.3	1.6 ± 1.0	0.24

All values are means ± SD.
¶ p value < 0.02 compared with control group.
# p value < 0.01 compared with control group.
§ p value of T_4/T_8 ratio compared with control group.

Table 4. The mean percentages of T–cell subsets according to dosages of prednisone per day.

	No.	% of reactive cells				T_4/T_8	p values§
		T_3	T_{11}	T_4	T_8		
Pred. < 10 mg/dl	21	64.4 ± 15.7	65.7 ± 16.6	37.0 ± 9.2¶	27.0 ± 11.7	1.64 ± 0.93	0.24
Pred. 11–40 mg/d.	14	67.7 ± 12.3	74.1 ± 10.8	38.1 ± 11.8¶	31.2 ± 11.8¶	1.49 ± 0.89	0.05
Pred. > 40 mg/d.	9	57.4 ± 15.5	62.8 ± 16.2¶	36.9 ± 9.0	28.3 ± 10.9	1.48 ± 0.65	0.05

All values are means ± SD
¶ $p < 0.02$ compared with control group.
§ p value of T_4/T_8 ratio compared with control group.

with disease activity score of 3 had statistically significant decrease of $T4^+$ cells and increase of $T8^+$ cells ($p < 0.02$) resulting in a low T4/T8 ratio ($p = 0.006$) when compared to controls.

The mean daily doses of prednisone in patients with various activity scores are: activity 0 = 7.6 ± 9.8 mg; activity 1 = 15.6 ± 4.3; activity 2 = 29.4 ± 22.0; activity 3 = 33.3 ± 24.1. The daily doses of prednisone increased with increasing disease activity. The difference of doses taken by patients with activity 0 and activity 3 was significant at $p < 0.001$.

The T4/T8 ratio in patients with positive anti-DS-DNA, CH50 below 150 units (+CH50), negative anti-Sm and positive anti-RNP was statistically significantly lower than in normal controls. There was no statistical significant difference between the mean doses of prednisone taken by patients with a positive and negative serologic tests (positive anti-DNA vs negative anti-DNA, *etc.*).

Table 4 shows the mean percentages of T cell subsets according to dosages of prednisone per day. Patients taking more than 10 mg of prednisone per day had decreases of $T4^+$ cells and lower T4/T8 ratios which approached statistical significance at $P = 0.05$ when compared to controls.

DISCUSSION

The present study of T-cell subsets in SLE patients using monoclonal antibodies and flow cytometry revealed that a decrease of $T4^+$ cells (T-helper/inducer) was the most frequent abnormality. This observation was in agreement with the previous reports by Smolen *et al.*[9,12] and by Bakke *et al.*[10] Our study was also in agreement with Bakke *et al.* in finding decreased T4/T8 ratios in SLE patients with increasing disease activity and in patients taking more than 10 mg of prednisone daily.

In our study, we found that patients with lower disease activity scores were taking lower doses of prednisone per day. Therefore, it appeared as though low T4/T8 ratios might be due to prednisone treatment itself. However, there were 3 out of 6 patients with activity score of 3 who were taking less than 10 mg per day of prednisone with low T4/T8 ratio. Also, there were 4 out of 14 patients with activity score of 0 who were taking less than 10 mg per day of prednisone with low T4/T8 ratio. These findings indicated that the mechanism causing low T4/T8 ratio in SLE patients was not due to treatment alone. The mechanism by which SLE affects T cell subpopulations needs further studies.

A low T4/T8 ratio was found to be associated with positive anti-DS-DNA, low serum complement (CH50), and positive anti-RNP. The presence of anti-Sm was associated with normal T4/T8 ratios, with its absence being associated with low T4/T8 ratios. Data regarding anti-Sm as a prognostic indicator have been controversial. Winn *et al.*[25] reported the association of anti-Sm with a subset of SLE patients with milder CNS and renal disease as compared to patients with positive anti-DNA. Barada *et al.*,[26] on the other hand, found patients with anti-Sm to have a comparable incidence of severe renal and CNS disease when compared to patients without anti-Sm antibody. Our findings of association of low T4/T8 ratios in SLE patients without anti-Sm antibody seem to indicate a favorable effect of anti-Sm. However, this conclusion requires further study on this group of patients.

The association of low T4/T8 ratios with abnormal serologic tests appeared to be independent of doses of prednisone taken by patients at the time of study. The mechanism of low ratios in these patients was unclear but probably was associated with active disease.

The results of 5 patients on immunosuppressive drugs were striking, with reduction of $T3^+$, $T11^+$ and $T4^+$ cells and with a relative increase of the percentage of $T8^+$ cells. A selective reduction of $T4^+$ cells in peripheral blood had been shown in patients with renal allografts treated with immunosuppressive drugs.[27,28] The decrease

in $T4^+$ cells with a resulting low T4/T8 ratio might serve as an indicator of effective immunosuppression in patients treated with immunosuppressive drugs. The number of patients in our study was small, and therefore, the result should not be considered conclusive. Further studies will be carried out in the future.

Smolen *et al.* [9,12] observed the distribution of T4/T8 ratios into 3 peaks in SLE patients as compared to only one peak in controls. They hypothesized that this finding probably represented the heterogeneity of pathogenetic mechanisms of SLE. Our study revealed low T4/T8 ratio to be associated with multiple variables. It was seen in patients treated with higher doses of prednisone and immunosuppressive drugs. It was also seen in patients treated with low doses of prednisone with or without active disease. Based on our findings, the distribution of T4/T8 ratios into 3 peaks observed by Smolen *et al.* [9,12] may not be due to intrinsic immunologic abnormalities in SLE patients. Further study should be done on untreated patients with or without active disease.

Lastly, the finding of low T4/T8 ratios in SLE patients was important because this finding was frequently used to diagnose AIDS. [29] Recent data showed that patients with SLE were also found to have positive tests for HTLV-I and HTLV-III antibodies. [30,31] A patient presented with rashes, arthritis, low T4/T8 ratio and positive HTLV-III antibody test may have SLE but could be labelled as having AIDS. Further study needs to be done to distinguish these patients.

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