

Lymphocyte subsets in patients with systemic lupus erythematosus

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Systemic lupus erythematosus (SLE) is a chronic inflammatory disease with unknown etiology. Some investigators have described quantitative and qualitative abnormalities in lymphocyte subsets in patients with active SLE. In this trial, we studied the percentage of peripheral blood lymphocyte subsets (CD4, CD8, CD3 and CD 19) with flow cytometry in blood samples taken from 51 SLE patients (23 active, 28 inactive) and 51 healthy controls. SLE patients were divided into 3 groups; a) untreated patients with active SLE, b) the patients with inactive SLE who have been treated with prednisone (P) and c) the patients with inactive SLE who have been treated with cyclophosphamide (CY). In the patients with active disease, the peripheral blood total CD3+, CD4+ lymphocytes and CD4/CD8 ratio were decreased and the number of CD8+ and CD19+ lymphocytes were increased compared to control group. In the patients with inactive disease treated with P and CY the number of total CD3+ cells were increased and CD 19+ cells were decreased compared to the untreated patients with active SLE. CD 19+ cells were significantly low in the patients taking CY than the patients taking P. As a result, we found that the number of CD3+ cells and CD4/CD8 ratio were decreased, CD 19+ cells increased in the patients with active SLE compared to controls. There was no difference in CD4/CD8 ratio between active and inactive patients. [Turk J Med Res 1996; 14(3): 92-96]

Keywords: Systemic lupus erythematosus, Lymphocyte subsets, Cyclophosphamide, Prednisone

Systemic lupus erythematosus (SLE) is a chronic inflammatory disease with unknown etiology, and the presence of autoantibodies may affect multiple tissue and organs. The clinical course of SLE is characterized by periods of remission and acute relapses (1-6).

Although the cause of the disease is unknown, several investigations have revealed immunoregulatory abnormalities that may underlie the autoimmune reactivity. Multiple defects of the functions of T and B cells functions are found in patients with SLE. The numbers of T lymphocytes are decreased and T cell functions are impaired. T cell responses to mitogens and lymphokines are abnormal. The most commonly

observed abnormality is a deficiency of suppressor cell activity (7-9). Some investigators have described increased percentage of CD 4+ T cells in patients with active SLE that may correlate with the disease activity (6).

B-cell hyperactivity and the production of multiple autoantibodies are the key immune function abnormalities in SLE, independent of whether this is related to an intrinsic B cell defect or not (10-12).

We analyzed peripheral blood lymphocytes of patients with SLE during their active and inactive stages .

MATERIALS AND METHODS

All patients were followed at Medical School of Ankara University, Department of Immunology. Fiftyone SLE patients (49 female, 2 male) were studied. Each patient satisfied the American Rheumatism Association (ARA) clinical criteria for the diagnosis of SLE (13). Patients

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were initially classified as having active and inactive disease. Twenty-three patients, ages 15-56 (mean age: 28 ± 10) were active and 28 patients, ages 18-55 (mean age: 30 ± 10) were inactive. The lupus activity was evaluated according to Murray B. Urowitz et al's criteria (14). Fiftyone volunteers, ages 17-55 (49 female, 2 male, mean ages: 31 ± 20) served as controls. All active patients were diagnosed at the time of the study and were not on therapy. Inactive patients were divided into two subgroups according to the mode of drug treatment. Among the 28 inactive SLE patients, 15 had received P, and 13 had received CY. Mean duration of treatment for P was 7 months, those patients were given 1 mg/kg/day P in the first three weeks, later dosage was gradually decreased and continued with 10-15 mg/day P in inactive period. Thirteen patients were given cyclophosphamide (100 mg/day), in the first three weeks of treatment, CY was combined with 1 mg/kg/day P, later P dosage was gradually decreased and ceased, mean duration of treatment for CY was 9 months. Among the 13 treated with CY patients, 11 of them had renal involvement, 2 had both central nervous system (CNS) involvement and severe skin lesions. Among the 23 active patients, 13 had renal involvement and 9 had CNS involvement.

We assessed renal involvement with renal biopsy and urine analyses. Patients with lupus nephritis were considered to have active nephritis by the presence of abnormal urinary sediment, including hematuria and/or cellular casts, with or without abnormal proteinuria and deteriorating renal function. All the active patients with renal involvement had hypocomplementemia and high titers of antibody to native DNA. Central nervous system involvement was assessed with psychiatric evaluation, neurological examination, EEG, computed tomography and magnetic resonance.

Lymphocyte subpopulations (CD4, CD8, CD19 and CD3) were studied with Flow Cytometry (FacsCan Model Consort 32) according to the method of Landay et al (15). Monoclonal antibodies were purchased from Becton Dickinson. Quantitative values of lymphocyte subsets were evaluated with facscaning and results were taken with percentage value. Statistical analysis of the results was performed using Student's t test, one-way ANOVA and Duncan's multiple range test.

RESULTS

The symptoms of patients with active disease are shown in Table I. In active group the most common

Table I. Symptoms in active patients.

Symptoms	Patients (n=23)	%
Arthralgia	23	100
Oral ulcers	20	87
Skin lesions	17	74
Lymphadenopathy	14	60
Renal involvement	13	57
Hematological involvement	12	52
CNS involvement	9	39
Arthritis	9	39
Photosensitivity	9	39
Serositis	3	13

symptom was arthralgia (100 %) and the least common was serositis (13 %). In patients with active disease, the percentage of CD3+ lymphocytes was significantly low ($p < 0.05$) and CD19+ lymphocytes was significantly high ($p < 0.001$) compared to the inactive patients and control group. The percentage of CD8+ lymphocytes was significantly high both in active and inactive patients ($p < 0.05$, $p < 0.05$). Both active and inactive patients, the percentage of CD4+ cells was significantly low and CD4/CD8 ratio was decreased compared to control group ($p < 0.001$, $p < 0.001$, $p < 0.001$). In the inactive patients, there were no difference between the percentages of CD3+ and CD19+ lymphocytes compared to control group ($p > 0.05$, Table 2).

In patients treated with P and CY, CD3+ cells were significantly high ($p < 0.05$), and CD 19+ lymphocytes were low ($p < 0.001$, $p < 0.001$ respectively) compared to the active patients. CD 19+ lymphocytes were higher in patients treated with P compared to the patients treated with CY ($p < 0.001$). There was no difference either in the number of CD3+, CD4+, CD8+ cells and CD4/CD8 ratios between patients treated with P and CY, or between the patients with or without renal involvement ($p > 0.05$).

DISCUSSION

SLE is thought by many investigators to result from the interaction of an environmental agent, perhaps a virus, with a genetically and hormonally susceptible host (5). Patients with SLE exhibit multiple manifestations of au-

Table 2. Comparison of parameters in patients with active or inactive disease and controls.

Subjects	n	CD3 (%)	CD 19 (%)	CD4 (%)	CD8 (%)	CD4/CD8 ratio
Control	50	75.0±5.7*	13.7±4.1**	47.2±5.5**	32.8±6.5*	1.51±0.56**
Active	23	69.8±12.1*	22.1±12.5**	35.8±8.6**	39.8±13.1*	1.07±0.60**
Inactive	28	76.3±9.2*	13.8±7.6	36.2±9.8**	43.3±11.0*	0.90±0.36**
P(prednisone)	15	74.9±9.3	16.9±8.2	36.4±9.2	40.9±9.6	0.96±0.38
CY (Cyclophosphamide)	13	78.0±9.1	10.3±5.0	35.5±10.7	46.2±12.1	0.83±0.34

:P<0.001

toimmunity, some of which may be related to abnormal T lymphocyte function and number (17).

There are many different results about T cell subsets. Some researchers explained that T cell auto-antibodies could be reason for this situation (13, 16-18). In patients with active SLE, CD4+ cell counts have been found to be increased, normal or decreased (2,4,6,8,19,20). The most consistent finding in SLE is impairment of CD8+ cell functions and a decrease in the number of CD8+ cells (4, 8,19, 21).

Raziuddin et al reported that the percentage of CD4+ cells was high and CD8+ T cells was low before treatment, but with control of the disease, CD4+ T cells were low and CD8+ T cells were high (2). Jang-Jia-Lin et al reported that the CD4/CD8 ratio in 73% of 26 SLE patients was abnormal, with either CD4+ cell or CD8+ cell deficiency (20). Besides, all these cases had clinical symptoms. SLE patients with a normal CD4/CD8 ratio were in moderately active or inactive stages of the disease. McInerney et al found that all SLE patients had normal percentages of CD8+ cells, and 45% of the SLE patients had significantly low CD4+ cell numbers (18). Bakke et al have reported decreased CD4+ cells and CD4/CD8 ratio, and relatively increased CD8+ cells (11). The patients who had decreased CD4/CD8 ratio, had arthritis, rash and increased serological activation. In our study, CD4+ cell subset was decreased both in active and inactive patients and CD8+ cell subset was increased in SLE patients compared to the control group. Nine of the active patients had CNS involvement. Six of them were under 21 years. They had both multisystem involvement and increased serological activation. Four of them had very high levels of

CD8+ cells.

Although the most common immunoregulatory abnormality in SLE is a deficiency in suppressor cell activity, hyperactive B cells, defective helper T cells, CD4+ cells deficient in IL-2 production, impaired T-cell activation and responsiveness to antigens have also been reported (1,7,18,22). Such defects do not necessarily correlate with the activity and severity of the disease; several cellular immune dysfunctions persist during inactive disease.

Corticosteroids have marked effects on the number and relative ratios of circulating lymphocytes (6,7,18,23). Using Fc receptors for IgM and IgG to measure helper T cells and suppressor T cells respectively. Haynes and Fauci reported that corticosteroids markedly decreased helper T cell counts but had only a modest effect on suppressor T cell counts(22). Likewise, Bakke et al reported that in some patients with SLE, prednisone therapy reduced the number of total CD4+ helper T cells in the circulation, which correlated with disease activity (11). Smolen et al found no difference in lymphocyte subsets between patients treated with low dose of corticosteroid and untreated inactive patients (23). Cyclophosphamide has different effects on immune system in patients with SLE. In animal models, different data are taken according to dose and duration of treatment (22). Its effect on T and B lymphocytes is progressive lymphopenia. With low dose of CY, the number of CD4+ and B lymphocytes decrease. It has the same effect on CD4+ and CD8+ lymphocytes with high dose. In our study, the patient group who was treated with P and CY, T cells were higher compared to the untreated ones

($p < 0.05$). CD4+ cell counts were similar between patients who were treated with P and CY. There were no difference in CD8+ cell numbers between untreated active and inactive patients who were treated with P. CD8+ cells were increased in patient taking CY compared to patients taking P and untreated active patients had the lowest. Patients treated with CY.

In summary, patients with active SLE had lymphopenia and with treatment lymphocyte counts reached to normal values. This result may be related to decrease of the antilymphocytes antibodies. In active patients CD4+ cells were low, CD8+ cells were high. Prednisone and CY had no significant effect on CD4+ and CD8+ cells and CD4/CD8 ratio.

Sistemik lupus eritematozuslu hastalarda lenfosit alt grupları

Sistemik lupus eritematozus (SLE) etyolojisi bilinmeyen kronik inflamatuvar bir hastalıktır. Bazı araştırmacılar aktif SLE'li hastalarda lenfosit alt gruplarında kalitatif ve kantitatif anormallikler tarif etmişlerdir. Biz bu çalışmada, 51 SLE'li (23 aktif, 38 inaktif) ve 51 sağlıklı kontrolden alınan kan örneklerinden flow sitometri ile periferik kan lenfosit alt gruplarının (CD4, CD8, CD3 ve CD19) yüzdesini çalıştık. SLE'li hastalar 3 gruba ayrıldı; a) aktif SLE'li tedavisiz hastalar, b) daha önceden prednisone (P) ile tedavi edilmiş inaktif SLE'li hastalar ve c) daha önceden siklofosamid (CY) ile tedavi olmuş inaktif SLE'li hastalar. Aktif hastalığı olan grupta, kontrollerle karşılaştırıldığında periferik kanda total CD3+, CD4+ lenfosit ve CD4/CD8 oranında azalma ve CD8+, CD19+ lenfositlerde artma tespit edilmiştir. P ve CY ile tedavi görmüş inaktif SLE'li hastalar, tedavi almamış aktif SLE'li hastalarla karşılaştırıldığında, CD3+ hücreler artmış ve CD19+ hücreler aza mıştır. CD19+ hücrelerin seviyesi CY alan hastalarda P alanlara göre anlamlı derecede düşüktü. Sonuç olarak aktif SLE'li hastalarda kontrol grubu karşılaştırıldığında CD3+ ve CD4/CD8 oranı düşmüş, CD19+ artmış olduğu bulundu. Aktif ve inaktif hasta gruptan ara-sında CD4/CD8 oranı arasında anlamlı fark bulunmadı. [Turk J Med Res 1996, 14(3): 92-96]

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