

Natural killer cells in systemic lupus erythematosus (SLE)

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Natural Killer (NK) cells play an important role in nonspecific immune response. Blood samples were taken from 51 SLE patients' ages 15-56 (2 male, 49 female). Quantitative analyses of NK cells were evaluated with monoclonal antibodies and flow cytometry. Patients were grouped according to the clinic and laboratory data's and treatment (corticosteroid=CS and immunosuppressive=IS). Results were compared with control group, ages' 17-55 (mean age: 31 ±20). In active group the most common symptom was arthralgia (%100) and the least common was serozite (%13). In inactive group the commonest clinic manifestetion was renal involvement. In active patients NK cells were significantly decreased compared to the inactive patients and control group (P<0.001). There was no significant difference between inactive patients and control group. Natural killer cells in active and inactive patients with central nervous system (CNS) involvement were significantly decreased compared to patients without CNS involvement and control group (P<0.001). Natural killer cells in untreated patients were significantly decreased compared to patients taken CS or IS, and control group (P<0.001). Conclusion: In SLE, NK cells decrease in active period. [Turk J Med Res 1995 13 (5): 191-194]

Key words: SLE, NK cells, Immunosuppressive therapy

Cytotoxicity mediated by natural killer (NK) cell appears to be important in the elimination of virus-infected cells (1) and in resistance to tumors (2). There is a considerable amount of evidence indicating NK cell control of immune function. Indeed, NK cell abnormalities have described in a number of rheumatic disease, such as rheumatoid arthritis (3), Sjogren's syndrome (4), systemic sclerosis (5), mixed connective tissue disease (6), polymyositis (7) and systemic lupus erythematosus (SLE) (8-10).

Systemic lupus erythematosus has been associated with numerous immunologic aberrations, ranging from abnormalities of antibody formation to defects in cellular immunity. Many Drugs that are used in treatment in SLE have an effect on immune system.

In this study, we investigated the effects of disease activity on the number of NK cells.

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MATERIALS AND METHODS

All patients were followed at Immunology Department of Ankara University, İbn-i Sina Hospital. Fifty-one SLE patients (49 females, 2 males) were studied. Each patient satisfied the American Rheumatism Association (ARA) clinical criteria for the diagnosis of systemic lupus erythematosus (14) and had strongly positive diagnostic serologies. Patients were initially classified as having active and inactive disease. Inactive patients were evaluated according to Murray B. Urowitz et all's criterias (15). On the day of blood sampling, each patient's history was recorded and a physical examination was performed. Twenty-three patients, ages 15-56 (mean age: 28±10) were active and 28 patients, ages (mean age: 30±10) were inactive. Forty-three volunteers, ages 17-55 (40 females, 3 males, mean ages: 31 ±20) served as controls. Thirteen of active and 11 of inactive patients had renal involvement, 9 of active and 4 of inactive patients had central nervous system involvement (CNS). Patients were grouped according to the treatment. Fifteen patients were treated with CS, mean duration of treatment was 7 months, patients were taken 1mg/kg/day CS in the first three weeks, later dosage was gradually decreased and continued with 10-15 mg/day CS in inactive period. Thirteen patients were taken

Table 1. Symptoms in active patients

Symptoms	Patients (n=23)	%
Arthralgia	23	100
Oral ulcer	20	87
Skin lesion	17	73.9
Lymphadenopathy	14	60
Renal involvement	13	56.5
Hematological involvement	12	52.2
CNS involvement	9	39.1
Arthritis	9	39.1
Photosensitivity	9	39.1
Serozite	3	13

Table 2. Symptoms in inactive patients

Symptoms	Patients (n=28)	%
Renal involvement	11	39.8
Skin lesion	10	35.7
Arthralgia	7	25
CNS involvement	4	14.3
Photosensitivity	3	10.7
Oral ulcer	1	3.6

cyclophosphamide (100mg/day) and CS (10 mg/day), mean duration of treatment was 9 months.

Quantitative value of NK cells was studied with Facscan Model Consort 32 Flow Cytometry according to the method of Landay et al(16). Monoclonal antibodies; Smultest leucogate CD 45, FITC/CD 14 PE, smultest control IgG1, FITC+CD 56 PE (NK) (Becton-Dickinson products) was marked with Fluorescein-isothiocyanate (FITC) and Phycoerthrin (PE). One hundred pi blood samples were taken from all patient and control group, than mixed with 20ul monoclonal antibodies and incubated at room temperature for 15 minutes. Later, 2 pi monoclonal of FACS lysing solution was added to all tubes and incubated for 10 minutes. After centrifugation (300xg, 5 minutes) supernatant layer was excluded, and washed with PBS. Later 0.5 ml Facsflow was used to make a suspension with the cells left behind. Quantitative values of NK cells were evaluated with facscanning and results were taken with percentage value.

Statistical analysis of results was performed using Duncan's test and one-way analysis of variance.

RESULTS

Evaluation of symptoms according to the activation of disease, in active patients are shown in table 1. In active group the most common symptom was arthralgia (%100) and the least common was serozite (%13). In inactive patients, renal involvement%39.3 (11/28), skin lesions %35.7 (10/28), arthralgia %25 (7/28), CNS in-

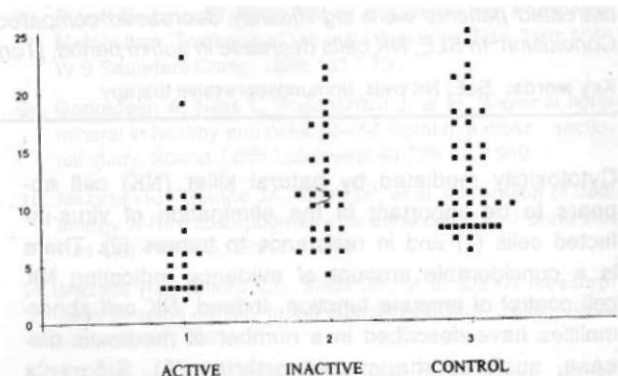
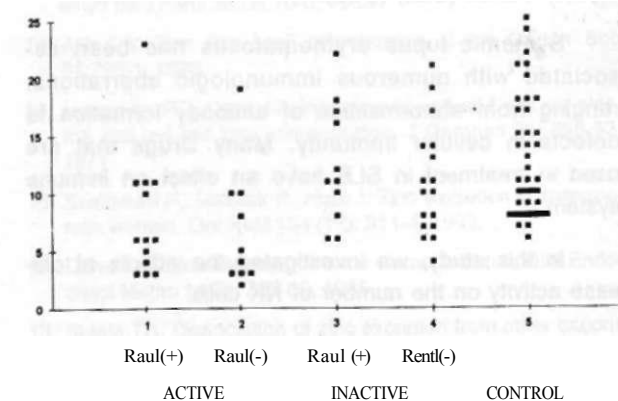
volvement %14.3 (4/28), photosensitivity %10.7 (3/28), oral ulcer %3.6 (1/28) were seen (Table 2).

Natural killer cells in active patients were significantly decreased compared to inactive patients and control group ($P<0.001$). There was no systatistical difference between inactive patients and control group ($P>0.05$).

In active group; NK cells in active patients both with renal involvement and without renal involvement were significantly decreased compared to control group ($P<0.001$). There was no difference between active patients with renal involvement and without renal involvement ($P>0.05$). Natural killer cells patients with CNS involvement were significantly decreased compared to patients without CNS involvement and control group ($P< 0.001$).

Inactive group; there was no difference in NK cells between patients with or without renal involvement compared to control group ($P>0.05$). Natural killer cells in patients with CNS involvement were significantly decreased compared to patients without CNS involvement and control group ($P<0.001$).

Natural killer cells in untreated active patients were significantly decreased compared to patients taken CS and IS ($P<0.001$). Natural killer cells were lower in patients taking CS than patients taking IS, but

**Figure 1.** Comparison of control group with active and inactive patients**Figure 2.** Comparison of control group with the patients according to the renal involvement

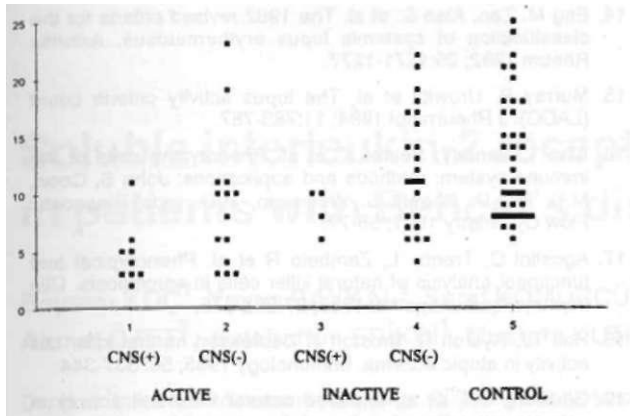


Figure 3. Comparison of control group with the patients according to the central nervous system involvement

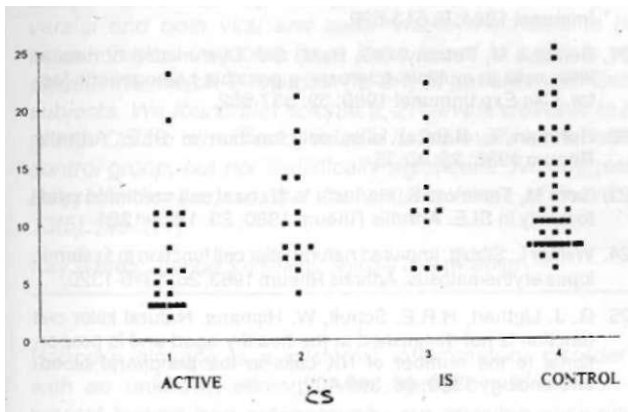


Figure 4. Comparison of the groups according to the treatment

it has no systatistical importance. Results are shown in graphics 1, 2, 3, 4.

DISCUSSION

Natural killer cell number function have been reported to be abnormal in a number of disease, such as sarcoidosis (17), atophical eczema (18), inflammatory bowel disease (19), amyloidosis (20), multiple sclerosis (21). In SLE, the number and activity of NK cells were reduced (5, 22, 23, 24, 25). The mechanism by which NK cell function is impaired in SLE is unknown .
 •Depressed NK cell activity in SLE appears to be related to decreased number of active NK cells (26,27), intrinsic defects or serum factors (28).

Immune complexes (29), antilymphocyte antibodies (4, 23, 25, 30) and a relative defect in IL-2 production (31) have been implicated in depressed NK function in some SLE patients. In some studies, IL-2 production was found to be decreased (32) or normal (33). Incubations of NK cells with IFN- gamma and IL-2 have been demonstrated different results. NK activation becomes normal with IL-2 and IFN-gamma (8), but in some studies it was reported that it becomes

partially normal (24). It has been reported that immature NK cells increase in SLE and multiple sclerosis (8).

In our study, in untreated active patients NK cells were significantly decreased compared to inactive patients and control group. In inactive patients, NK cells were decreased compared to control group, but it has no statistical importance. Our results were similar to literature (26,27).

Lipnick at al found that the numbers of NK cells were increased in active patients, but they could not explain its reason (30).

In SLE, important variations are seen in lymphocytes with different drugs, used in treatment (10, 12,34 35, 36). The analysis of NK cell activity in patients with autoimmune disease is difficult as these patients often receive medicaments, e.g. prednisone, azathioprine and cyclophosphamide, known to influence NK cell activity (25). Egan et al found no difference in the number and function of NK cells with duration or dosage of prednisone therapy (8).

Struyf et al found that IS and CS treatments have no effect on the number of NK cells and lymphocytes in SLE, but stimulated NK cell activation with IFN- gamma was found to be decreased (10).

In our study, NK cells, in patients who treated with CS and IS were decreased compared to control group, but it has no systatistical importance. In addition, there is no difference between patients who treated CS and IS. Active SLE patients, with treatment NK cells reached to normal values. This result may be related to decrease of the antilymphocytes' antibodies.

In summary, NK cells decrease in active SLE patients and come to normal levels with treatment. Corticosteroid and IS therapies probably reduce NK cell antibodies. There is no difference in the number of NK cells between patients with renal involvement and without renal involvement. Natural killer cells are very low in SLE patients with CNS involvement. The number of NK cells and their function are effected by many factors. It will be for out benefit to investigate these factors together.

Sistemik lupus eritematozusda "natural killer" (nk) hücreleri

"Natural Killer" hücresi, spesifik olmayan immün cevapta önemli rol oynamaktadır. Sistemik lupus eritematozuslu yaşları 15 ile 56 arasında değişen 2'si erkek, 49'u kadın 51 hastadan periferik kan örneği alındı. NK hücrelerinin sayısal değeri monoklonal antikorlarla flow sitometrik olarak çalışıldı. Hastalar klinik ve laboratuvar aktivitelere ve tedavi protokolüne (kortikosteroid ve immünsüpre-

sif) göre gruplandırıldı. Sonuçlar hasta grupları arasında ve yaşları 17 ile 55 arasında değişen 43 sağlıklı kişi ile karşılaştırıldı. Klinik olarak aktif grupta en sık semptom artralji (%100) idi ve çok az sayıda serözit (% 13) saptandı. İnaktif grupta en sık bulgu renal tutulum idi. Aktif hasta grubunda NK hücre sayısı, kontrol ve inaktif hasta grubuna göre anlamlı derecede düşük bulundu ($P<0.001$). İnaktif hasta grubu ile kontrol grubu arasında anlamlı farklılık bulunmadı. SSS tutulumu olan hem aktif hem de inaktif gruptaki hastalarda NK hücre sayıları, SSS tutulumu olmayan hastalara ve kontrol grubuna göre anlamlı derecede düşük bulundu ($P<0.001$). Tedavi almayan aktif grupta NK hücre sayıları, kortikosterooid ve immünsüpresif alan hastalar ile kontrol grubuna göre anlamlı derecede düşük bulundu ($P<0.001$). Sonuç olarak; SLE'li hastalarda NK hücre sayısı aktif hastalık sırasında azalır. [TurkJMedRes 1995 13(5): 191-194]

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