Malaysian J Pathol 1997; 19(2): 121 – 125

Lymphocyte subsets in systemic lupus erythematosus

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Abstract

Systemic lupus erythematosus (SLE) is an autoimmune disease characterised by increased B cell activity and depressed T cell function. However, the contribution of the immunoregulatory system to its pathogenesis is still unclear. The recent development in the production of monoclonal antibodies and the availablility of bench-top flow cytometers have allowed rapid quantitation of peripheral blood lymphocyte subsets. We analysed the distribution of the lymphocyte subsets in 24 patients with active SLE and 18 with inactive SLE. The distribution of immunoregulatory cells in 72 normal volunteers was used as control. Statistical analysis showed that there were significant differences between both the SLE groups and the normal controls, for total lymphocytes, T cells, B cells, T helper cells, T suppressor cells, T helper/suppressor ratio and natural killer cells. There was a significant difference for T helper cells between active and inactive SLE. T helper cells levels were found to be low in inactive SLE and lower in active SLE. It appears that treatment-induced remissions did not restore the levels of immunoregulatory cells to normal. Thus, T helper cell levels reflect disease activity and longitudinal assays of T helper cells may serve as an indicator of disease reactivation.

Key words: Immunoregulatory cells, autoimmune disease, flow cytometry, FACScan, SLE.

INTRODUCTION

Systemic lupus erythematosus (SLE) is an autoimmune disease characterised by increased B cell activity and depressed T cell function. However, the contribution of the immunoregulatory system to its pathogenesis is still unclear. Some workers found that T helper cells were decreased in active SLE, while others have found that both T helper cells and T suppressor cells were decreased.[1] The conflicting results could be due to the difference in techniques of quantitation of these cells in the peripheral blood. The recent development in the production of monoclonal antibodies and the availablility of bench-top flow cytometers have allowed rapid quantitation of peripheral blood lymphocyte subsets in a routine setting.

In this study, we have undertaken to determine the distribution of the lymphocyte subsets in patients with active SLE and inactive SLE. The distribution of immunoregulatory cells in normal volunteers was used as control.

MATERIALS AND METHODS

Duration. This study was carried out from July 1992 to June 1993.

Subjects. This study included 42 patients with SLE according to the 1982 American Rheumatic Association criteria of classification of SLE[2], who were under the care of one of the authors (KNCT). These patients were divided into two groups based on disease activity, according to the Physicians Global Assessment (PGA). Those who were asymptomatic were classified as inactive, whilst those who showed manifestations of the disease were classified as active. All patients were receiving immunosuppressive therapy. The control group comprised 72 normal adult volunteers who participated in establishing reference ranges of lymphocyte subsets in peripheral blood as reported elsewhere.[3]

Flow cytometry. Peripheral blood from patients and control subjects were obtained by venupuncture and collected in EDTA containers. Flow cytometric analysis of the blood samples was carried out using lysed the whole blood technique reported previously.[3] All samples were analysed within 24 hours of collection. Monoclonal antibodies used to identify the lymphocyte subsets were all supplied by Becton-Dickinson, USA. The lymphocyte subsets determined included T cell (CD3), B cell (CD19),

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T helper cell (CD4), T suppressor (CD8), and Natural Killer cell (CD16+56).

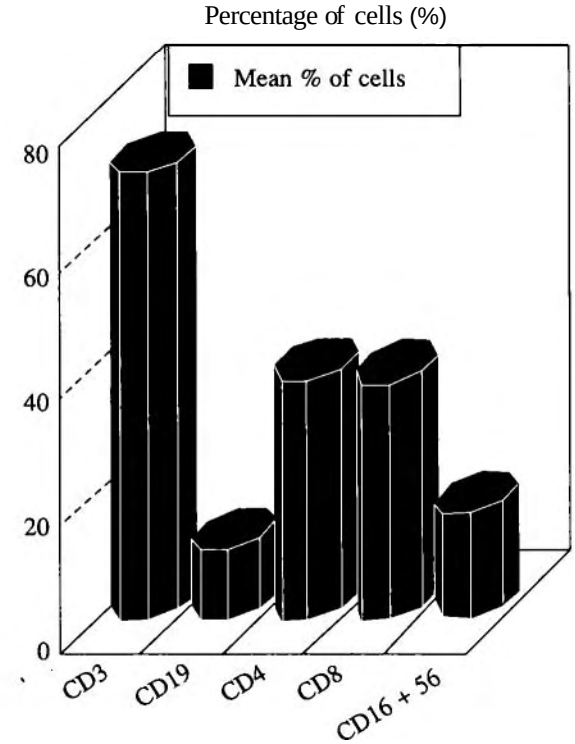
Statistical analysis. The results obtained were expressed as means ± 1 standard deviation. Analysis of Variance and Multiple Duncan Range Test were carried out on the data collected for the control and the SLE groups to see the effect of SLE on the levels of the lymphocyte subsets. Statistical analyses were performed using STATGRAPHICS program (Statistical Graphics Corporation, USA).

RESULTS

The distribution of the lymphocyte subsets in normal subjects is shown in Figure 1 and in SLE groups, in Figure 2. Results of Analysis of Variance and Multiple Duncan Range Test on the difference between means of the control and the SLE groups are shown in Table 1. Only T helper cell levels showed statistically significant difference between control and SLE groups. The distribution of T helper cells in normal and SLE groups is illustrated in Figure *3*.

DISCUSSION

Before the advent of flow cytometry, lymphocyte subsets were studied using manual immunofluorescence techniques. The methods used were laborious and the number of patients studied was usually small. It is therefore understandable that the results were quite variable. In the early eighties, lymphocyte subsets were determined with early models of flow cytometers which allowed larger numbers of patients to be studied and has improved the precision of analysis. However, the results were still conflicting.[1,4-7] Some of the results produced by flow cytometry are summarised in Table 2. Morimoto ***et al*** reported that the alteration of lymphocyte subsets was in T suppressor cells. Others reported otherwise. They reported that T helper cells were decreased in active SLE with or without treatment. We undertook this project



Mean % of cells	71	11.4	38.3	37.7	17.2
SD	8	4.1	8.1	8.4	7.2

Lymphocyte subsets

FIG. 1: The distribution of lymphocyte subsets in normal subjects showing mean and one standard deviation (SD) of cells in %.

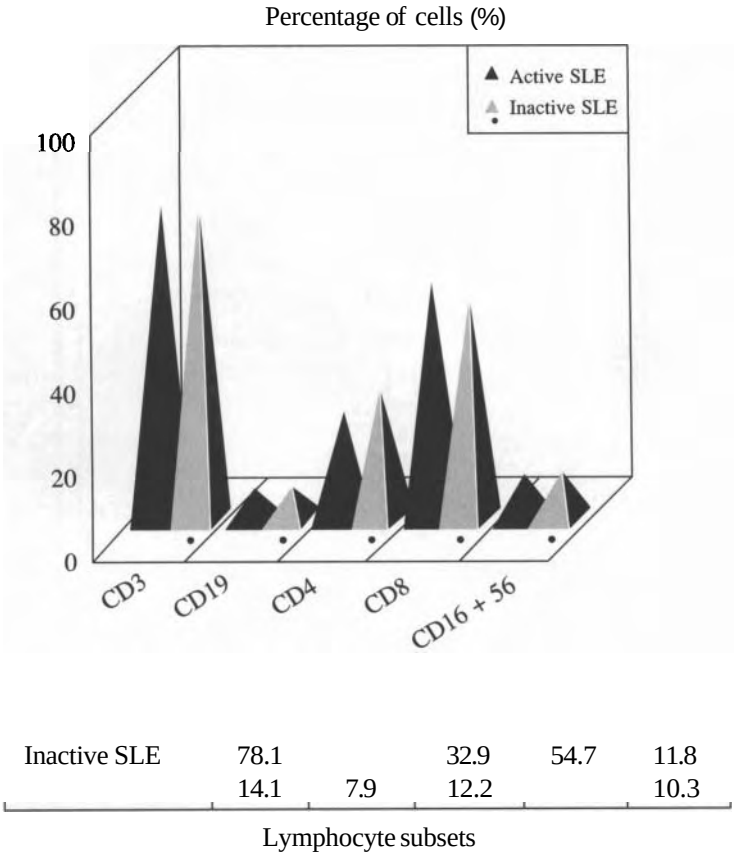


FIG. 2: The distribution of lymphocyte subsets in active and inactive SLE groups showing mean and one standard deviation (SD) of cells in %.

to examine the alteration of lymphocyte subsets in **SLE** with a newer generation of bench-top flow cytometers and commercially available monoclonal antibodies.

Morimoto *et al* reported that untreated active **SLE** patients had low T suppressor cells and normal **T** helper cells. Melendro *et al* reported otherwise. They found that both T helper and T suppressor cell levels were low. We are unable to clarify this in our sudy as the majority of cases referred to the lupus clinic were already commenced on steroid therapy.

In the treated active **SLE** group, Nilganuwonge *et al* reported that the levels of T

TABLE 1: Difference between means of normal controls, active SLE and inactive SLE.

Parameters	Difference between means (%)		
	Normal vs Active	Normal vs Inactive	Active vs Inactive
T cells	6.29 *	7.05 *	0.76
B cells	3.90 *	3.33 *	0.57
CD4 cells	11.81 *	5.32 *	6.49 *
CD8 cells	20.13 *	17.00 *	3.13
CD4/CD8 ratio	0.57 *	0.40 *	0.17
Natural Killer cells	5.99 *	5.42 *	0.57

* Denotes a statistically significance difference where the significance level is set at $p<0.05$.

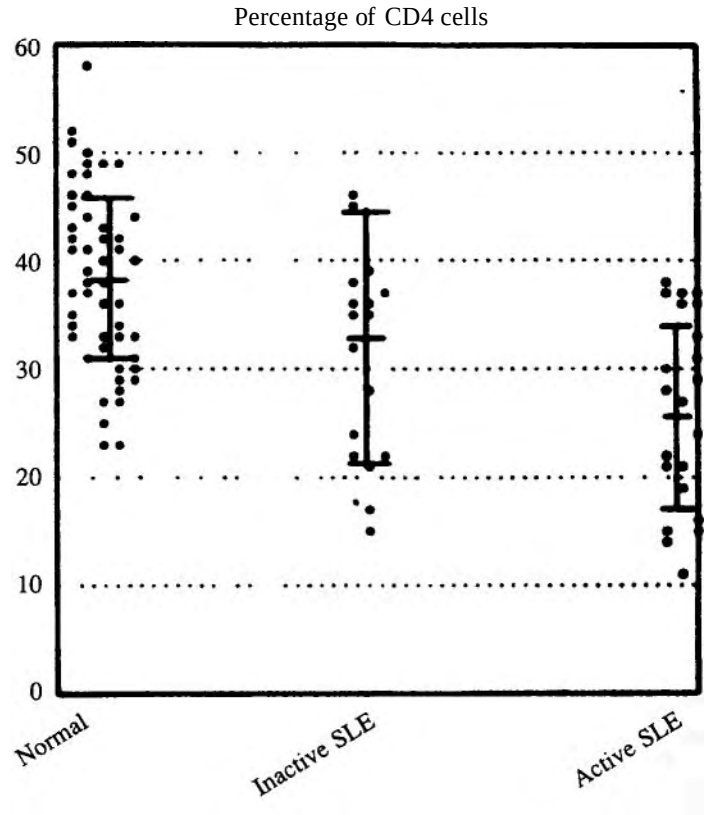


FIG. 3: Dot plot of CD4 cells in normal subjects, inactive and active SLE groups showing mean and one standard deviation of cells in %

TABLE 2: Lymphocyte subsets by flow cytometry – literature review

Authors / Disease status (No. of cases studied)	Total T Cells	CD4 Cells	CD8 Cells	CD4: CD8 Ratio
Nilganuwonge *et.al.*				
Active with treatment (22)	Normal	Decrease	Decrease	Decrease
Inactive with treatment (16)	Decrease	Normal	Normal	Normal
Bakke *et.al.*				
Active with/without treatment (12)	Decrease	Decrease	Decrease	Decrease
Inactive with/without treatment (16)	Decrease	Normal	Decrease	Decrease
Smollen *et.al.*				
Active with /without treatment (32)	Normal	Decrease	Normal	Decrease
Morimoto *et.al.*				
Active without treatment (14)	Decrease	Normal	Decrease	Increase
Inactive with treatment (15)	Normal	Normal	Increase	Decrease
Melendro *et.al.*				
Active without treatment (17)	Decrease	Decrease	Decrease	Normal
Inactive .without treatment (24)	Normal	Normal	Normal	Normal
Cheong *et.al.*				
Active with treatment (18)	Decrease	Decrease	Decrease	Decrease
Inactive with treatment (24)	Decrease	Decrease	Decrease	Decrease

helper cells were low and T suppressor cells were normal. In a mixed population of active SLE patients with or without treatment, Smolen *et al* found a similar change of low T helper cells but normal T suppressor cells. Our study supported Nilganuwonge's findings. However, Bakke *et al* found that T helper and T suppressor cells were both low in a mixed population of active SLE patients with or without treatment.

In the treated inactive SLE group, the reported results were mostly conflicting. Nilganuwonge *et al* reported that both T helper and T suppressor cells were normal. Morimoto *et al* showed that T helper cells were normal and T suppressor cells were increased. Our study completely differed from their findings as both T helper and T suppressor cells were low. On the other hand, Bakke *et al* showed that T helper cells were normal and T suppressor cells were low in a mixed population of inactive SLE with or without treatment. In fact, Melendro *et al* had shown that in the untreated inactive SLE patients, the T helper and T suppressor cells were both normal. Thus, it is likely that the observed differences were due to the dosing of immunosuppressives in these patients, as immunosuppressive therapy is a well-known cause of lymphopenia.

Our results and those reported by other workers appear to indicate that T lymphocyte subsets are affected by disease activity and also therapy of SLE. The changes could return to normal when the disease is under control and therapy is discontinued. However, under circumstances of treatment with prednisolone or other immunosuppressives, measurement of T helper cells appears to be a useful marker to predict activity of SLE. Improvement of T helper cell count seems to indicate control of disease, whilst decrease of T helper cell count, deterioration or relapse of SLE.

Non-flow cytometry studies have indicated that B cell levels are low in untreated SLE and normal in untreated inactive SLE.[8] Our study showed that levels of B cells were low in both active and inactive SLE. Similarly, we have also observed that NK cells were low. We are uncertain whether these observations were due to disease activity or therapy of SLE. A prospective study that includes new active untreated and inactive untreated SLE patients is indicated and may provide some necessary answer. This prospective study should also be performed with newer flow cytometry technique that allow absolute quantitation of lymphocyte subsets.

ACKNOWLEDGEMENT

This research project was partly supported by IRPA grant No: 3-07-03-059 from the Ministry of Science, Technology and Environment, Malaysia.

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