

Alterations in Immunoregulatory T Cell Subsets in Active Systemic Lupus Erythematosus

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ABSTRACT To determine whether imbalance among subsets of human T cells exists in patients with systemic lupus erythematosus (SLE), we analyzed peripheral blood lymphocytes in SLE patients during active and inactive stages of disease. For this analysis, we used monoclonal antibodies to the surface antigens of inducer (T4) and suppressor (T5/T8) T cell subsets, as well as a common T cell antigen (T3). In contrast to normal and inactive SLE patients, the percentage of T3⁺ cells was reduced in all active SLE patients. More importantly, there was a selective decrease in T5⁺/T8⁺ suppressor T cells in 12 of 14 active patients, including 1 of 2 patients with drug-induced SLE. Serial analysis of three SLE patients showed a significant correlation between the presence of T5⁺/T8⁺ subset and clinical disease activity in all patients. We conclude that aberrations in suppressor T cell subsets are an important correlate of disease in patients with SLE.

INTRODUCTION

Systemic lupus erythematosus (SLE)¹ has long been considered a prototypic autoimmune disease because of its association with B cell hyperactivity (1, 2), resulting in production of multiple autoantibodies, and with disorders of cell-mediated immunity (3, 4). Previous studies have suggested that many of these abnormalities

occur as a consequence of defects in suppressor T cell function (5–9). To determine whether imbalance among subsets of human T cells exists in patients with SLE, we analyzed peripheral blood lymphocytes in SLE patients during active and inactive stages of disease. For this analysis, we used monoclonal antibodies to cell surface antigens restricted to inducer (T4) and suppressor (T5/T8) T cell subsets, as well as to a common T cell antigen (T3) defining all peripheral T cells (10–14). The results show that the T5/T8 suppressor subset is selectively decreased during active disease and is reconstituted after the cessation of disease activity.

METHODS

Isolation of lymphocytes. Human peripheral blood mononuclear cells were isolated from heparinized venous blood by Ficoll-Hypaque (Pharmacia Fine Chemicals, Inc., Piscataway, N. J.) density centrifugation.

Production of monoclonal antibodies and analysis of lymphocyte populations with a fluorescence-activated cell sorter. Four monoclonal antibodies, termed anti-T3, anti-T4, anti-T5, and anti-T8, were used in the present study. The production and characterization have been described elsewhere (10–14). In brief, anti-T4 was shown to react with 55–60% of peripheral T cells, representing the human inducer population (11, 12), whereas anti-T5 and anti-T8 defined ~20–30% of T cells with cytotoxic/suppressor functions (13, 14). A monoclonal antibody, termed anti-T3 and reactive with 100% of peripheral T cells, was used to enumerate T cells (10). It should be noted that all four monoclonal antibodies were restricted in their cellular expression to normal lymphocytes of T lineage. All monoclonal antibodies were reactive by indirect immunofluorescence and/or complement-mediated lympholysis to a titer of from 1:150,000 to 1:1,000,000. Cytofluorographic analysis of cell populations was performed by indirect immunofluorescence with fluorescein-conjugated goat anti-mouse immunoglobulin (IgG (G/M FITC; Meloy Laboratories, Inc., Springfield, Va.) on a fluores-

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¹Abbreviation used in this paper: SLE, systemic lupus erythematosus.

cence-activated cell sorter (B-D FACS Systems, Mountain View, Calif.) as previously described (10). Background fluorescence reactivity was determined with a control ascites obtained from mice immunized with nonsecreting hybridoma clones.

Patients. The sample population consisted of 29 patients with SLE satisfying the diagnostic criteria of the American Rheumatism Association.

All patients were monitored at the Arthritis Branch of the National Institute of Arthritis, Metabolism, and Digestive Disease, Massachusetts General Hospital, and the Robert B. Brigham Hospital; and clinical activity was assessed by two physicians at the times of blood drawing on the basis of the following signs and symptoms: active rash, serositis, arthritis, active central nervous system disease, and active renal disease. The active patients in this study had at least three of the above criteria of activity. In addition, they all had high titers of antibodies to native DNA. Patients lacking these symptoms or detectable signs of activity were categorized as inactive. At the times of blood drawing, patients with active disease had not yet received any treatment. Patients with inactive SLE had received moderate to low doses of corticosteroids.

RESULTS

Table I summarizes the surface characteristics of lymphocyte subpopulations from active and inactive SLE patients and compares them with those from healthy control individuals. The normal lymphocyte population is composed of $67 \pm 3\%$ T cells, as determined by reactivity with anti-T3. Moreover, some lymphocytes are reactive with anti-T4 ($41 \pm 2\%$), which defines inducer T cells, while smaller percentages are reactive with anti-T5 ($20 \pm 1\%$) and anti-T8 ($22 \pm 1\%$), which define suppressor T cells. The lymphocyte population was abnormal from 14 patients with active SLE (Tables I and II). First, there was an absolute diminution in the total T cell population. Second, the most striking abnormality was the decreased number of the T5⁺/T8⁺ T cell subset. Interestingly, a patient with hydralazine-induced active SLE (case 2) also showed a markedly decreased number of the T5⁺/T8⁺ subsets (Table II). A second patient with procaineamide-induced SLE (case 1) had only a slight reduction in the suppressor subset.

The selective decrease in T5⁺/T8⁺ cells in most SLE patients was more apparent from analysis of ratios of

T4⁺ cells to T5⁺/T8⁺ cells in any given individual with active SLE. In normal individuals, the ratio of T4⁺:T5⁺ and T4:T8⁺ is $\sim 2:1$ and $1.9:1$, respectively. In contrast, as shown in Table II, 12 of 14 patients with active SLE had a ratio of $>3:1$. All 15 patients with inactive SLE had no significant alterations in T3⁺ and T4⁺ subsets, but unexpectedly, the T5⁺/T8⁺ suppressor population was increased when compared with healthy controls.

To determine whether there were variations in T cell subsets of a given individual during periods of differing disease activity, three patients were sampled on two or more occasions. Fig. 1A shows a fluorescence-activated cell sorter analysis of lymphocytes from case 7 at the onset of active symptoms. As shown in Fig. 1A, there was a decreased number of T8⁺ suppressor cells. After 1 wk, with further clinical progression, there was an absence of detectable T8⁺ suppressor cells (Fig. 1B). In contrast, 4 wk later, with marked reduction in disease activity, there was a return of the T8⁺ subset (Fig. 1C). In this case, the patient did not receive corticosteroid or immunosuppressive agents. Moreover, identical patterns were seen in two other individuals (cases 8 and 11) during active and inactive stages of disease, although in these two cases, both patients received corticosteroids after their initial cell characterization.

DISCUSSION

In the present study, we have characterized T lymphocytes from SLE patients during clinically active and inactive stages of disease by means of a series of monoclonal antibodies. Patients with active SLE, including two drug-induced SLE cases, had a decreased number of T cells (T3⁺) when compared with healthy individuals. More importantly, there appeared to be a selective decrease in the T5⁺/T8⁺ suppressor cell subset in 12 of 14 active patients, but 0 of 15 inactive patients. In contrast, patients with inactive SLE had a normal number of T cells (T3⁺) and an increase in the T5⁺/T8⁺ suppressor cell subset. Serial analysis of individual SLE patients showed that the decrease in number of suppressor cells correlated with disease activity. It

TABLE I
Cell Surface Characteristics of T Cells in SLE Patients and Normal Controls

| Condition | Number | Mean age | Sex ratio | Reactivity with monoclonal antibodies | | | | Ratio | |
|--------------|--------|------------|-----------|---------------------------------------|---------|---------|---------|----------------------------------|----------------------------------|
| | | | | Anti-T3 | Anti-T4 | Anti-T5 | Anti-T8 | T4 ⁺ :T5 ⁺ | T4 ⁺ :T8 ⁺ |
| | | yr | M/F | % | | | | | |
| Active SLE | 14 | 32 (21-57) | 2:12 | 51±3* | 40±3 | 9±1* | 11±1* | 4.4±0.6* | 3.6±0.3* |
| Inactive SLE | 15 | 28 (19-51) | 1:14 | 65±4 | 36±2 | 27±2* | 28±2* | 1.3±0.1* | 1.3±0.1* |
| Normal | 30 | 29 (18-65) | 15:15 | 67±3 | 41±2 | 20±1 | 22±1 | 2.0±0.2 | 1.9±0.2 |

Results are mean±SEM.

* Significant difference ($P < 0.05$) on the basis of 95% of confidence limits between patients and normal control groups.

TABLE II
Analysis of Lymphocyte Populations in Patients with Active SLE

| Disease state | Age | Sex | Absolute T cell number | Reactivity with monoclonal antibodies | | | | Inducer/suppressor* |
|-----------------------|-----|-----|------------------------|---------------------------------------|---------|---------|---------|---------------------|
| | | | | Anti-T3 | Anti-T4 | Anti-T5 | Anti-T8 | |
| | yr | | mm ³ | % | | | | |
| Active (drug-induced) | | | | | | | | |
| Case 1† | 57 | M | 494 | 56.3 | 38.0 | 13.0 | 14.7 | 2.6 |
| 2‡ | 51 | M | 354 | 59.9 | 50.8 | 7.6 | 11.4 | 4.5 |
| Active (spontaneous) | | | | | | | | |
| Case 3 | 34 | F | 144 | 36.2 | 31.3 | 5.4 | 9.1 | 3.4 |
| 4 | 28 | F | 360 | 45.3 | 32.9 | ND | 7.8 | 4.2 |
| 5 | 31 | F | 224 | 55.9 | 33.5 | 9.6 | ND | 3.5 |
| 6 | 32 | F | 438 | 52.8 | 40.0 | 11.0 | 13.0 | 3.1 |
| 7 | 26 | F | 384 | 57.0 | 49.0 | 5.9 | 8.0 | 6.1 |
| 8 | 24 | F | 411 | 53.5 | 42.0 | 10.6 | 11.5 | 3.7 |
| 9 | 27 | F | 184 | 41.2 | 34.0 | ND | 9.2 | 3.7 |
| 10 | 32 | F | 394 | 57.7 | 49.5 | ND | 11.0 | 4.5 |
| 11 | 27 | F | 284 | 57.9 | 46.3 | ND | 10.9 | 4.2 |
| 12 | 21 | F | 120 | 27.6 | 18.0 | 9.0 | ND | 2.0 |
| 13 | 37 | F | ND | 59.0 | 48.9 | 9.7 | 11.0 | 4.4 |
| 14 | 27 | F | 424 | 57.5 | 51.0 | ND | 11.8 | 4.3 |

The absolute number of T lymphocytes from active SLE patients ($0.12\text{--}0.5 \times 10^6$ cells/cm³) was less than that from inactive SLE patients ($0.6\text{--}1.8 \times 10^6$ cells/cm³) and that from normal controls ($0.7\text{--}2.1 \times 10^6$ cells/cm³). ND, not done.

* T4⁺/T8⁺ inducer/suppressor ratio given in all cases except 5 and 12, in which T4⁺/T5⁺ was used.

† Procaineamide-induced SLE.

‡ Hydralazine-induced SLE.

was interesting to note that patients with inactive SLE appeared to have some compensatory increase in suppressor cells when compared with normals. Whether this increase is a result of therapy or a physiological response to the disease process will have to be determined in future studies.

Earlier studies demonstrated that patients with SLE had a diminished concanavalin A-induced suppressor T cell function during active stages (6–9). Inasmuch as

recent studies showed that only the T5⁺ subset of T cells was inducible by concanavalin A to suppress (13), the present results were not unexpected. Furthermore, the *in vitro* findings that polyclonal immunoglobulin production by B cells is markedly enhanced when T5⁺/T8⁺ T cells are removed from the unfractionated T cell preparation (15), correlate closely with the *in vivo* loss of T5⁺/T8⁺ T cells subset in patients with active SLE, and the associated B cell hyperactivity (1, 2). In contrast to the above findings, it should be noted that some investigators have been unable to observe a defect in suppressor cell function (16, 17). The reason for these discrepancies is unclear, but may be due to differences in patient selection or the *in vitro* techniques used. Several studies have emphasized the importance of anti-T cell antibodies in altering T cell number and function in patients with active SLE (18–23). The mechanism for selective loss of T5⁺/T8⁺ is not entirely clear. In this regard, recent studies demonstrated that anti-T cell antibodies found in the sera of active SLE patients were selectively reactive with the T5⁺/T8⁺ suppressor T cell populations (24). Sera from patients studied in this paper also contained autoantibodies to T5⁺/T8⁺ T cells during active stages (data not shown). Thus, the anti-T5⁺/T8⁺ autoantibody in active SLE may play an important physiological role in the reduction of these cells. Given the fact that we can account for the decrease in T5⁺/T8⁺ T cells by a relative increase in T4⁺ T cells,

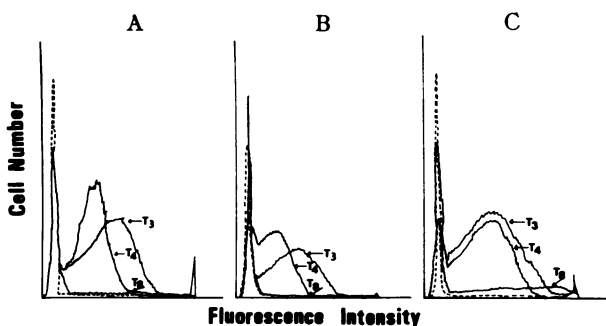


FIGURE 1 Serial analysis of lymphocytes during the clinical course of SLE. Fluorescence-activated cell sorter profile of lymphocytes from case 7 at onset (panel A), at 1 wk (panel B), and at 4 wk (Panel C) with disease. Analyses were performed using monoclonal antibodies anti-T3, anti-T4, and anti-T8, and indirect immunofluorescence. We determined background fluorescence by using an ascites from a nonproducing hybridoma clone.

it seems unlikely that the lower number of T5⁺/T8⁺ T cells observed in active SLE is due to blocked determinants by naturally occurring antissuppressor antibodies. In this regard, the sum of T4⁺ and T5⁺/T8⁺ T cells accounts for the total T cell population as defined by anti-T3 in normal individuals and patients with active or inactive SLE.

It still remains to be determined whether the loss of this major immunoregulatory T cell subset is primary or secondary to the pathophysiologic process in SLE; however, the observation that patients with drug-induced SLE can demonstrate the same cellular defect as naturally occurring SLE, namely loss of T5⁺/T8⁺ T cells, suggests that aberrations in these subsets may be critical for pathogenesis. In addition, it implies that drug-induced, as well as naturally occurring immunopathologic states may initiate immunoregulatory aberrations that could result in similar clinical disease.

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