

STUDIES ON T AND B LYMPHOCYTES IN THE PERIPHERAL BLOOD OF DISCOID LUPUS ERYTHEMATOSUS PATIENTS WITH AND WITHOUT CHLOROQUINE TREATMENT

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Abstract. The proportions of T-B cells of peripheral lymphocytes in DLE patients as well as the effect of chloroquine treatment on T cell count were studied. In DLE, both the active and the total rosette-forming cell counts decreased, whereas the B cell count did not differ from normal values. Long-term chloroquine administration causes a further reduction in T cell count.

Key words: T and B lymphocytes; Peripheral blood; Lupus erythematosus, discoid; Chloroquine

Discoid lupus erythematosus (DLE) belongs to the group of autoimmune diseases. Its pathomechanism is not completely elucidated in spite of widespread investigations concerning autoimmune phenomena. It is known that DLE might be transformed into systemic lupus erythematosus (SLE) in a certain proportion of cases.

The T (thymus-derived) and B (bursa-dependent) marker characteristics of the peripheral lymphocytes of SLE patients have been studied by several authors (3, 8, 11) but nothing is known about the T-B distribution of the peripheral lymphocytes in DLE patients.

This report contains our observations on the proportions of T-B lymphocytes derived from the peripheral blood of DLE patients as well as the effect of chloroquine treatment on the T cells.

MATERIAL

1. The proportions of active (16) and of total rosette-forming cells (total-E.RFC) were determined as well as those of cells carrying surface immunoglobulins in the peripheral blood of 15 patients with florid DLE. Before and at the time of the investigation the patients did not

receive any treatment. The absolute number of lymphocytes was also counted.

2. In the blood of 12 DLE patients to whom chloroquine was administered in daily doses of 0.25-0.5 g for one month or more, the number of T cells were determined. At the same time the proportions of active rosette-forming cells and the cell counts of total rosette-forming cells were also investigated, as well as absolute lymphocyte counts.

The results obtained were compared with the data of 16 healthy control persons.

METHODS

Determination of T cells

For determination of the proportion of T cells in the peripheral blood the rosette formation method was used. The total number of rosette-forming cells (total-E.RFC) was determined as well as the number of the active rosette-forming cells.

The lymphocytes were separated from heparinized blood by means of Ficoll-Uromiro, and washed three times in PBS. Then the cells were incubated with inactivated bovine serum for 1 hour (cell count 10^6 lymphocytes/ml). After incubation, 0.1 ml sheep red blood cells was added to 0.1 ml lymphocyte suspension (1:20, erythrocyte/lymphocyte). Following centrifugation (200 g) for 5 min, determination of the active rosette-forming cells was carried out immediately (16), while the determination of the total number of rosette-forming cells followed at least 1 hour after incubation at 4°C (5).

Determination of B cells

This procedure was carried out by demonstration of immunoglobulin on the surface of the lymphocytes. For determination of the surface immunoglobulins, fluorescein bound anti-human-immunoglobulin was used. 0.05 ml of the lymphocyte suspension in PBS (cell count: 10^7 lymphocytes per ml) was incubated with goat anti-human IgG-IgA-IgM serum bound with fluorescein-isothiocyanate (Hyland product) for 60 min at 4°C. After incubation the cells were centrifuged (200 g), then washed twice

Table I. Proportions of active and total rosette-forming cells and cells carrying surface immunoglobulins as well as absolute counts of lymphocytes, T and B cell in controls and DLE patients (mean $\pm$ S.E.)

	Active rosette (%)	Total rosette (%)	Cells with surface		Immuno-globulin IgM	Lymphocyte (mm ³)	T cell (mm ³)	B cell (mm ³)
			IgG	IgA (%)				
Control	39.9 $\pm$ 2.8 ^a	70.3 $\pm$ 1.2 ^a	15.4 $\pm$ 2.0	5.9 $\pm$ 1.1	5.5 $\pm$ 1.2	2 100 $\pm$ 110 ^a	1 470 $\pm$ 55 ^a	540 $\pm$ 30
DLE patients	20.8 $\pm$ 3.1 ^a	53 $\pm$ 5 ^a	15.4 $\pm$ 1.4	5.7 $\pm$ 0.9	6.8 $\pm$ 1.1	1 710 $\pm$ 154 ^a	846 $\pm$ 83 ^a	480 $\pm$ 28

^a The difference is significant (with the Student's *t*-test).

in TC-199, and resuspended in 0.1 ml glycerol buffer. Determination of the cells in suspension was carried out by means of Zeiss (Jena) microscope (light-source HBO 200, filter BG 12, barrier filter OG 1). For evaluation, at least 200 cells were counted on each occasion.

RESULTS

Table I shows the proportions of active and total-E.RFC and those of B cells as well as the absolute lymphocyte counts and the absolute T and B cell counts of control persons and DLE patients. Among the peripheral lymphocytes of the DLE patients, both the active and the total-E.RFC occurred in a significantly lower percentage than in the healthy persons. The proportion of cells carrying surface immunoglobulins is approximately the same in controls as in DLE patients.

The numbers of cells carrying surface immunoglobulins (B cell) do not differ significantly in the peripheral blood of controls and of DLE patients. The total T cell count is significantly lower in the peripheral blood of the DLE patients than in that of the controls.

Table II shows the proportions of active and total-E.RFC in the DLE patients with and without chloroquine treatment, and the corresponding change in the absolute T cell count. As regards the percentage proportions, there is no significant difference between active and total rosette-forming cells in the treated and in the non-treated patients, whereas the absolute T cell count is significantly lower in treated than in non-treated patients.

DISCUSSION

On the basis of the above results it can be established that the proportion of cells responsible for both the active and the total rosette formation in the blood of DLE patients decreases. The T cell

count (the count of the rosette-forming cells) calculated by means of the absolute lymphocyte count is significantly lower than in the controls. The number of B cells carrying surface immunoglobulins in DLE patients does not differ significantly from that of the controls, either in amount or according to type of immunoglobulin (IgG, IgA, IgM).

It is also apparent from our data that the type (marker) of a rather large percentage of the lymphocytes of the DLE patients cannot be determined. The proportion of these so-called "null" cells is about 19% in the DLE patients, as against only 3-4% in the controls. The "null" cells were found in similar, sometimes even greater proportions in our SLE patients (14). By means of these investigations it cannot be established whether these cells are actually either T or B cells. To decide this, more lymphocyte-marker characteristics would need to be investigated.

It is of interest that in agreement with other authors, the investigations on the proportions of T-B cells in SLE patients (3, 8, 11) revealed that the T lymphocyte count is low, as in patients with DLE. A relative increase in B cells could be demonstrated

Table II. Proportions of active and total rosette-forming cells with corresponding changes in absolute T cell count in DLE patients with and without chloroquine treatment (mean $\pm$ S.E.)

	Active rosette (%)	Total-rosette (%)	Lymphocyte (mm ³)	T cell (mm ³)
Untreated DLE patients	20.8 $\pm$ 3.1	53 $\pm$ 5	1 710 $\pm$ 154	846 $\pm$ 83 ^a
DLE patients treated with Chloroquine	19 $\pm$ 3.8	43 $\pm$ 5.2	1 510 $\pm$ 147	642 $\pm$ 102 ^a

^a The difference is significant.

among the peripheral lymphocytes of the SLE patients, which was not observed in DLE. The T cell depletion in SLE may be the result of several causes: lymphocytotoxic antibodies occur in a rather high percentage in the sera of the SLE patients (6, 12, 13, 15). Concerning these antibodies, a T cell specificity could be demonstrated by Lies (7) as well as in our investigations (2). For this reason they can be held responsible for T cell depletion. On the other hand, the possible accumulation of T lymphocytes in the inflammatory target organ may also contribute to the decrease of the T cell count in SLE patients.

In our previous investigations we have demonstrated lymphocytotoxic antibodies in DLE (9). These antibodies may be implicated in T cell depletion in DLE as well.

To elucidate the nature of the so-called "null" cells observed in rather large numbers in the peripheral blood of the DLE patients, further studies are needed. Maybe these cells, or at least part of them, are really neither T nor B in character but belong partly or entirely to the group of so-called K cells.

Concerning the therapeutic effect of chloroquine, numerous experiments have been carried out, according to which chloroquine has an enzyme inhibiting, melanine binding, DNS linking capacity, as well as lysosome stabilizing, anti-inflammatory and sun-screening effect (10). Hurwitz & Hirschhorn (4) found that chloroquine inhibits blast transformation of the lymphocytes in vitro. Forbes & Smith (1) noted that chloroquine, similarly to immunosuppressive agents, inhibits the protein synthesis of lymphocytes.

Our present study established that chloroquine administered in 0.25–0.5 g daily doses for a month or more, reduces the number of the total rosette-forming cells as well as the absolute number of T cells.

It seems possible that in addition to its other properties, this effect of chloroquine also contributes to the therapeutic results obtained in DLE patients.

REFERENCES

1. Forbes, I. J. & Smith, J. L.: Effect of antiinflammatory drugs on lymphocytes. *Lancet* 2: 334, 1967.
2. Gergely, P., Szegedi, Gy., Fekete, B., Stenszky, V. &

Petrányi, Gy.: Studies concerning lymphocyte cytotoxins (Hung.). *Acta Med Hung Acad Sci*, Unpubl. data.

3. Gergely, P., Szegedi, Gy., Fekete, B., Szabó, G. & Petrányi, Gy.: Lymphocyte surface immunoglobulin in autoimmune diseases. *Lancet* 1: 482, 1973.
4. Hurwitz, D. & Hirschhorn, K.: Suppression of in vitro lymphocyte response by chloroquine. *N Engl J Med* 273: 23, 1965.
5. Jondal, M., Holm, G. & Wigzell, H.: Surface markers on human T and B lymphocytes. I. A large population of lymphocytes forming non-immune rosettes with sheep red blood cells. *J Exp Med* 136: 207, 1972.
6. Kreisler, M., Naito, S. & Terasaki, P. I.: Cytotoxins in disease. Various diseases. *Transplant Proc* 3: 1301, 1971.
7. Lies, R. B., Messner, R. P. & Williams, R. C., Jr: Relative T-cells specificity of lymphocytotoxins from patients with systemic lupus erythematosus. *Arthritis Rheum* 16: 365, 1973.
8. Messner, R. P., Lindström, F. D. & Williams, R. C., Jr: Peripheral blood lymphocyte cell surface markers during the course of systemic lupus erythematosus. *J Clin Invest* 52: 3046, 1973.
9. Nagy, E., Stenszky, V. & Szerze, P.: Das Verhalten der lymphocytotoxischen Antikörper während Chloroquine-Behandlung. *Derm Monsch*, unpubl. data.
10. Sams, W. M.: Chloroquine mechanism of action. *Mayo Clin Proc* 42: 300, 1967.
11. Scheinberg, M. A. & Cathcart, E. S.: B- and T-cell lymphopenia in SLE. *Arthritis Rheum* 16: 566, 1973.
12. Stastny, P. & Ziff, M.: Antibodies against cell membrane constituents in systemic lupus erythematosus and related disease. *Clin Exp Immunol* 8: 543, 1971.
13. Stenszky, V., Szegedi, Gy., Aszódi, L. & Petrányi, Gy.: HL-A and systemic lupus erythematosus. *Haematologia* 7: 211, 1973.
14. Szegedi, Gy., Gergely, P., Fekete, B., Szabolcsi, M. & Pálóczi, K.: Investigations on T-B lymphocytes in peripheral blood of SLE patients (Hung.). *Acta Med Hung Acad Sci*, unpubl. data.
15. Terasaki, P. I., Mottironi, V. D. & Barnett, E. V.: Cytotoxins in disease. Autocytotoxins in lupus erythematosus. *N Engl J Med* 283: 724, 1970.
16. Wybran, J., Levin, A. S., Spitler, L. E. & Fudenberg, H. H.: Rosette-forming cells, immunologic deficiency diseases and transfer factor. *N Engl J Med* 288: 710, 1973.

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