STUDY OF PERIPHERAL BLOOD T-LYMPHOCYTES BY THE METHOD OF SPONTANEOUS AND ACTIVE ROSETTES WITH LEVAMISOLE

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KEY WORDS: T-lymphocytes; rosette-forming cells; levamisole.

Fluctuation of the numbers of T- and B-cells in the blood of healthy persons of the same age and sex has been established by rosette-formation tests, the blast-transformation test (BTT), and detection of globulin receptors by a luminescence-serologic method [3, 5]. Such fluctuations have been shown to be due largely to hormonal disturbances in the body, connected with stress or the menstrual cycle [10]. It has been suggested that such fluctuations in indices of reactions for T- and B-cells may be due to a change both in the total number of T- and B-cells and to changes in the activity of their recirculation in the body [4].

The rosette-formation test, BTT, and determination of globulin receptors for B-lymphocytes by the luminescence method have been shown not to reveal all T- and B-cells. Some of these cells are not revealed, and the proportion of them differs, as regards both the age norm and in different pathological states. These cells constitute the so-called 0-subpopulation [6].

A method of stimulating lymphocytes with levamisole has recently been developed - under the influence of this drug cells of the 0-subpopulation begin to be detectable by the rosette-formation test and BTT, depending on whether they belong to T- and B-cells respectively [12]. This makes it possible to analyze the probable connection between variability of the number of immunocompetent cells in the peripheral blood by means of the rosette-formation test, BTT, and the other test, with a change in the 0-subpopulation of T- and B-cells, i.e., with a change in physiological activity of the immunocompetent cell population.

An attempt was made in the investigation described below to test this possibility by the use of a model of spontaneous and active rosette-forming cells (RFC) in healthy persons.

EXPERIMENTAL METHOD

Tests were carried out on 43 healthy subjects aged from 18 to 53 years, including 12 men and 21 women. In 12 women, mainly young, the tests were carried out repeatedly with allowance for the character of menstrual function, in the course of two menstrual cycles at intervals of 1 week. In all the women tested a two-phase menstrual cycle was verified by functional diagnostic tests.

The following methods were used to characterize the T-lymphocytes: The number of T-lymphocytes forming spontaneous "rosettes" (E-RFC) was determined by the method of Jondal et al. [9]; the number of T-lymphocytes forming active rosettes (A-RFC) was determined by the method suggested by Wybran et al. [13]; the effect of levamisole on the rosette-forming properties of T-lymphocytes was investigated in the test of spontaneous and active rosette-formation by the method described by Wybran et al [14], in the writers' modification [2].

To determine changes in the degree of activation of T-lymphocytes under the influence of levamisole, the index of stimulation (IS) of cellular activity was used; this index is the ratio of the difference in the number (in %) of RFC in the test with levamisole and in the test without levamisole to the relative percentage of RFC in the test without levamisole,

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multiplied by 100% for the index of stimulation of spontaneous T-erythrocytes:

$$IS_E = \frac{\text{E-RFC with levamisole - E-RFC without levamisole}}{\text{E-RFC without levamisole}} \times 100\%$$

For the index of stimulation of active T-lymphocytes:

$$IS_A = \frac{\text{A-RFC with levamisole - A-RFC without levamisole}}{\text{A-RFC without levamisole}} \times 100\%.$$

EXPERIMENTAL RESULTS

The results showed that the mean number of E-RFC was 60 ± 7.9%, with variation from 48 to 74%, and the mean number of A-RFC was 30 ± 4.2%, with variation from 24 to 38%, close to results obtained by other workers [1, 7, 9, 14].

The values of IS_E and IS_A were directly proportional to the degree of the stimulating effect of levamisole and varied between −21.2 and 46.2% and between 1 and +54.7% respectively. The negative value of IS indicates inhibition of the rosette-forming activity of the T-cells by levamisole.

Despite the fact that the fluctuations of the results varied significantly in different donors for both spontaneous and active RFC, analysis of the same donor showed that the variations were greater for spontaneous RFC: The maximum of fluctuations was 48% of the mean value (Fig. 1).

The absence of any significant parallel between the number of E-RFC and of their A-RFC subpopulation will be noted. For instance, in donors with a high value of E-RFC in their blood (67.3 ± 2.4%) the number of A-RFC was 28.0 ± 0.2% whereas in donors with a low value of E-RFC (50.9 ± 3.1%) the number of A-RFC was 29.0 ± 0.3%. This last fact indicates that A-RFC is not a stable fraction of all the T-cells detectable as E-RFC.

Treatment of the lymphocytes with levamisole in the overwhelming majority of cases gave an increase in the number of detected rosettes on average by 18.5 ± 2.7%. A large increase in their number was observed in association with a lower normal value of RFC, which leads to stabilization of indices of both E-RFC and A-RFC. This indicates that variability of the values of E-RFC depends substantially not on a change in the number of T-lymphocytes circulating in the blood, but on a change in the proportion of T-cells among them that cannot form rosettes during tests under the conditions used, i.e., on a change in the activity of the receptors on their surface membranes.

Levamisole treatment of lymphocytes from donors with high indices of the rosette-formation test often gave a reduction and not an increase in the number of RFC, on average by 13.6 ± 5.8%. Considering data showing that the effect of levamisole on the body differs depending on its degree of activity [11], it can be tentatively suggested that levamisole in equal doses may suppress maximally activated T-lymphocytes, by contrast with stimulation of lymphocytes if the body is in a less active state. Observations of this kind provide a basis for the hope that levamisole can be used *in vitro* in rosette-formation tests for predicting the efficacy of treatment of patients with this drug.

In the group of women investigated the dependence of the number of rosette-forming T-lymphocytes on phases of the menstrual cycle was studied. The lowest percentages of numbers of E-RFC and A-RFC were found in the middle of the menstrual cycle, between the 12th and 16th days, and in the lutein phase of the cycle, between the 21st and 24th days. The highest numbers were found at the beginning of the follicular phase, before the 3rd-4th day, and at the end of the lutein phase, on the 27th-28th days of the cycle.

The index of stimulation of cellular activity by levamisole was inversely proportional to the number of circulating T-lymphocytes (Table 1).

Since equalization of the indices of E-RFC and A-RFC between the different phases of the menstrual cycle was observed after the inclusion of levamisole in the rosette-formation test, this suggests that fluctuations in the number of RFC in the peripheral blood of healthy women are largely connected with changes in the functional properties of T-lymphocytes, expressed as a change in the size of the O-subpopulation of these cells.

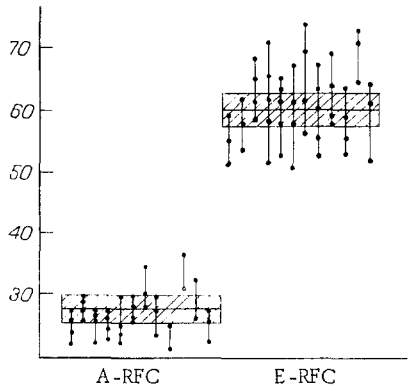

Fig. 1. Changes in number of T-lymphocytes in peripheral blood. Points on vertical lines represent results of repeated tests on the same donor at intervals of 2 weeks; horizontal lines represent mean values of A-RFC and E-RFC. Ordinate, number of RFC (in %).

TABLE 1. Percentages of E-RFC and A-RFC on Days of Menstrual Cycle

Days of cycle	No. of observations	No. of E-RFC		IS_{E}, %	No. of A-RFC		IS_{A}, %
		<28%	>28%		<55%	>55%	
26—6-th	12	1	12	15,1	4	7	11,3
12—16-th	10	8	2	46,2	7	3	26,4
20—23-rd	8	6	2	29,1	4	4	21,2

Since the decrease in the number of circulating T-lymphocytes coincided with physiological raising of the estrogen level, and an increase in the number of T-cells coincided with the period of least production of sex hormones, the pattern thus discovered can evidently be explained by the influence of sex hormones which, according to the data of Hellig and Gerneke [8], inhibit the function of lymphoid tissue.

As Table 1 shows, dependence of the number of RFC on phases of the menstrual cycle was detected most clearly by counting lymphocytes forming active rosettes. The A-RFC subpopulation is evidently more sensitive than other populations of T-cells to the action of hormones.

It can thus be concluded from the results of this investigation that fluctuations in the number of spontaneous and active RFC take place largely on account of depression or restoration of the physiological properties of these cells, dependent on the functional state of the body, rather than on account of a decrease or increase in the size of the populations of T-cells in the peripheral blood.

Inclusion of levamisole in the rosette-formation test can effectively be used to determine the number of T-zero cells which are a physiologically inactive subpopulation of circulating immunocompetent cells, and it may perhaps also be used to assess the action of levamisole on the lymphoid tissue of a given patient or experimental animal.

LITERATURE CITED

1. K. A. Lebedev, R. V. Petrov, Yu. M. Lopukhin, et al., Vest. Akad. Med. Nauk SSSR, No. 10, 130 (1976).
2. A. O. Lyapon, Lab. Delo, No. 1, 12 (1979).
3. R. V. Petrov and K. A. Lebedev, in: Proceedings of an All-Union Conference on General and Applied Immunology [in Russian], Part 2, Moscow (1974), pp. 18-20.

4. R. V. Petrov, M. A. Stenina, and K. A. Lebedev, Byull. Eksp. Biol. Med., No. 12, 8 (1975).
5. G. Asma, R. Henrica, and H. Schmit, Clin. Exp. Immunol., 20, 286 (1977).
6. J. M. Dwyer, Prog. Allergy, 21, 178 (1976).
7. H. R. Helling and W. H. Gerneke, Onderstepoort J. Vet. Res., 42, 53 (1975).
8. M. Jondal, G. Holm, and H. Wigzell, J. Exp. Med. 136, 207 (1972).
9. M. Raptopoulou and G. Goulis, Clin. Exp. Immunol., 28, 458 (1977).
10. H. Verhaegen, J. De Cree, W. De Cock, et al., Clin. Exp. Immunol., 27, 313 (1977).
11. J. Wybran and H. H. Fudenberg, J. Clin. Invest., 52, 1026 (1973).
12. J. Wybran and A. Govaerts, Clin. Exp. Immunol., 27, 319 (1977).

BLOODLESS ESTIMATION OF BIOMECHANICAL PROPERTIES OF HUMAN CAPACITIVE VESSELS

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Determination of the state of the veins has not yet found its true place in functional investigations of the human cardiovascular system. Information on the elasticity of capacitive vessels is particularly deficient. The writer has perfected an apparatus and method for the bloodless determination of the elasticity of the limb veins [1, 2], and the results obtained are described below.

EXPERIMENTAL METHOD

The subject was placed on a table which could be tilted into the orthostatic (vertical) position. Rubber ring electrodes, connected to a two-channel rheoplethysmograph, were secured to the upper and lower thirds of the legs of the subject in the horizontal position. After measurement of the original values of interelectrode resistance, the two legs, which were at the foot end of the table, were simultaneously lowered; under these circumstances the lower limbs were flexed at the knee practically to a right angle (test 1). Changes taking place under these conditions in the interelectrode resistance were recorded for 2 min, after which the limbs were returned to their initial positions. Similar measurements were carried out during lowering of the limbs alternately (test 2). The investigation was completed with the orthostatic test (test 3). During this procedure the subject, together with the table, was tilted passively into the near-vertical position (an angle of 75° to the horizontal). Typical tracings of rheoplethysmograms are illustrated in Fig. 1.

Elasticity was determined immediately after the subject or his limbs had been tilted into the vertical position, and 30, 60, and 120 sec later. The calculation was done by the following equation:

$$B = \frac{\Delta R}{R \Delta P},$$

where B is elasticity; R and ΔR interelectrode resistance and its change; and ωP is the change in hydrostatic pressure caused by the change in position of the limb in the verticle direction from the level of the heart.

This method was used to investigate 45 clinically healthy persons and 35 patients with a clinical diagnosis of varicose veins. The healthy subjects included 25 men aged 15 to 43 years and 20 women aged from 15 to 42 years; the patients included 15 men aged from 18 to 49 years and 20 women aged from 15 to 64 years.

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