ESTIMATION OF THE NUMBER OF T LYMPHOCYTES AND OTHER ROSETTE-FORMING CELLS IN HUMAN BLOOD IN HEALTH AND DISEASE

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When the spontaneous rosette-formation test is used to assess the T system of immunity it is not sufficient simply to determine the relative percentage of rosette-forming cells. To obtain a true picture of the immune status the number of immunocompetent cells in 1 mm^3 of the particular donor's blood must be determined. If the test is carried out with a leukocyte suspension, not only lymphocytes but also other cells with the property of binding sheep's red cells can also be taken into account.

KEY WORDS: rosette-forming cells; T lymphocytes.

The spontaneous rosette-formation test has been used in recent immunologic investigations to evaluate the T system of immunity [2]. In this test the number of lymphocytes able to bind sheep's red cells on their surface, and thus to form spontaneous rosettes, is determined. Usually the rosette-formation test is performed with a pure suspension of lymphocytes isolated from the blood and the relative number (in per cent) of rosette-forming lymphocytes is determined without staining the cells. In this case the absolute number of rosette-forming cells in the blood is not actually determined and the possible participation of cells other than lymphocytes in rosette formation is not allowed for.

The object of this investigation was to determine the conditions for carrying out and analyzing the results obtained by the rosette-formation test to enable it to provide the fullest quantitative evaluation of the T system of immunity of the sick person.

EXPERIMENTAL METHOD

The rosette-formation test was carried out by a modified method of Jondal [2] with a suspension of peripheral blood leukocytes. The number of leukocytes was determined in heparinized blood (25 units heparin to 1 ml blood) and films were made for the differential blood count. Red cells were sedimented with 3% gelatin solution in medium No. 199. The supernatant containing leukocytes was drawn up into silicone-treated tubes and the cells were sedimented by centrifugation at 750 rpm for 10 min. Red cells present as an impurity were removed by brief osmotic shock by the addition of 0.1 ml distilled water to the residue and pipeting for 15 sec. An excess of medium No. 199 was then quickly added. The cells were washed twice by centrifugation at 750 rpm for 10 min. The cell residue obtained after a second centrifugation was diluted with medium No. 199 and the leukocyte concentration was measured in a Goryaev counting chamber. Next, 0.1 ml of the suspension containing $0.2 \cdot 10^6$ leukocytes was incubated with 0.1 ml of 0.4% washed sheep's red cells at 37°C for 5 min. The cells were then sedimented for 5 min at 200 g and allowed to stand for 60 min at 12°C. To obtain stained films of the rosettes, the cells were fixed with glutaraldehyde (in a final concentration of 0.6%) for 20 min at room temperature after the end of incubation. Distilled water was then

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TABLE 1. Relative and Absolute Numbers of RFC in Human Peripheral Blood in Health and Disease

Donor	State	RFC (in %)	Number of leukocytes in 1 mm³ blood	Lymphocyte in blood formula (in %)	Absolute number of RFC in 1 mm³ blood
S.	Healthy	69	4 700	23	745
M.	»	39	4 700	45	825
K.	»	56	4 900	41	1120
R.	»	73	6 200	33	1500
K*	»	20	16 000	56	1800
O.	Chronic lymphatic leukemia	7	30 000	70	1460
Kr.	The same	9	3 200	84	2400
R.	Partial red-cell aplasia	13	2 300	7	21
Sh.	Congenital immunodeficiency	57	8 000	8	370

* The material for investigation was cored blood

TABLE 2. Morphological Composition of RFC

Donor	RFC (in %)				
	lymphocytic	neutrophilic	eosinophilic	monocytic	other
Sh.	98	2	—	—	—
S.	91	8,4	—	0,3	Plasma cells 0,1
Sl.	73	26	1	—	—
V.	95	2	3	—	—
N.	91	4,5	—	—	Plasma cells 4,5

added to the tube and the cells were sedimented by centrifugation for 3 min at 200 g. Most of the supernatant was removed and the remaining suspension was carefully pipeted and a drop of it was applied to a slide. The specimen was dried, postfixed with methanol, and stained with Methyl Green and pyronine. The reaction was read in a light microscope. A cell binding at least three red cells was taken to be a rosette. In the stained film, the number of rosette-forming cells (RFC) was calculated as a percentage of all types of leukocytes. Knowing the absolute number of leukocytes in 1 mm³ blood from a given donor (counted in a Goryaev's chamber) and the relative proportions of lymphocytes, neutrophils, monocytes, and other cells (examination of blood films stained by the Romanovsky–Giemsa method), the absolute number of the corresponding white blood cells in 1 mm³ could be determined. By multiplying this value by the relative proportion of RFC of the particular type, as counted in films stained with Methyl Green and pyronine, the absolute number of the corresponding RFC in 1 mm³ was determined.

EXPERIMENTAL RESULTS

On the average 53 ± 6% of peripheral blood lymphocytes of healthy donors (34 persons) formed rosettes. The relative number of RFC varied in individual donors from 40 to 73% (Table 1). The absolute number of RFC in 1 mm³ blood also varied within wide limits among normal donors and changed sharply in some normal or pathological states accompanied by changes in the total leukocyte count or a shift in the blood formula.

It can be concluded from analysis of the results in Table 1 that during evaluation of the immune status it is not sufficient simply to determine the relative percentage of RFC. This relative percentage defines only the fraction of T cells among the other lymphocytes. Their absolute number in 1 mm³ blood, however, reflects the size of the population of immunocompetent cells in circulation in a given individual. A characteristic example was the case with chronic lymphatic leukemia (B leukemia). The fraction of RFC in the peripheral blood of such patients is sharply reduced (7-9%). However, when converted to absolute numbers the results indicated a normal or even increased population of circulating T lymphocytes. Another example confirming the inadequacy of determining only the relative percentage of RFC is given in Table 1: The relative number of RFC in patient Sh. was normal, but because of the severe lymphocytopenia the absolute number of T cells in 1 mm³ blood was lowered.

When the test was carried out by the method adopted, counting the number of rosettes in the stained films showed that not only lymphocytes but also other types of leukocytes give rosettes with sheep's red cells. The overwhelming majority of RFC were in fact lymphocytes (Table 2). However, the percentage of neutrophilic rosettes fluctuated within wide limits (from 2 to 8%, 26% of all RFC). When expressed as absolute numbers, the neutrophilic rosettes averaged one third of all rosette-forming lymphocytes circulating in the blood. Besides neutrophils and lymphocytes, rosettes also were found by eosinophils, monocytes, and plasma cells. The significance of this phenomenon is unknown.

Morphological heterogeneity of RFC in lymphoid organs of animals has been described by Pavlovsky et al. [5]. Cells of human embryonic liver and spleen and also reticulum cells and macrophages [7] can bind sheep's red cells. Red cells sensitized with γG-globulin can give rosettes with human monocytes [3] and neutrophils [4]. In the present experiments practically all types of white blood cells in the stained films had unsensitized sheep's red cells bound to a varied degree to their surface. It is impossible at present to state definitely whether the mechanism of adhesion of erythrocytes with T cells (lymphocytes) and non-immunocompetent cells (neutrophils, eosinophils, monocytes, etc.) differs. It is likewise unknown whether

this is nonspecific adhesion or the result of the transfer of receptors (IgT) from T lymphocytes to other cells. However, there is no dispute about the need to take all cell forms giving rosettes into account. Adhesibility, determined by rosette formation, may reflect the functional state of the cell (lymphocyte, neutrophil, etc.). When they bind certain particles, antibodies, and complement on their surface, neutrophils can secrete the enzymes which they contain and which can cause direct injury to the tissue [1]. The degree of adhesion of erythrocytes to a neutrophil may reflect the phagocytic activity and the functional readiness of the cell to discharge its enzymes. Changes in the adhesibility of neutrophils have been described by Prehal [6]. In the case of Behcet's syndrome at the height of the attack neutrophils acquired what for them is the unusal property of binding platelets to form rosettes.

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