ROSETTE FORMATION BY VIRUS-TREATED HUMAN LEUKOCYTES (V ROSETTES)

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Human lymphocytes can react in vitro with heterologous cells, such as sheep's erythrocytes (E rosettes) or mouse erythrocytes (M rosettes), and also with heterologous or allogeneic cells loaded with antibodies (EA rosettes) or with antibodies and complement (EAC rosettes). On the basis of this property, T and B populations of lymphocytes can be differentiated and estimated quantitatively under normal and pathological conditions [2, 3].

The object of the present investigation was to study the ability of immunocompetent cells, treated with Sendai virus, to form rosettes with autologous or allogeneic erythrocytes (V rosettes).

EXPERIMENTAL METHOD

Lymphocytes were isolated from peripheral blood from healthy persons, stabilized with heparin (10 units/ml), by centrifugation in a Verografin–Ficoll gradient [4]. Monocytes were isolated from the resulting cell suspension containing 75% lymphocytes, 20% monocytes, and 5% granulocytes, by making use of their ability to adhere to the surface of the plastic [5]. The original cell suspension was enriched with B cells by removing cells forming rosettes with sheep's erythrocytes (T cells) [1].

The three cell suspensions thus obtained – a suspension of monocytes, a suspension enriched with B cells, and the original suspension – were incubated with Sendai virus in a subagglutinating dilution* for 30 min at 4°C. The cells were then washed 3 times with Hanks' solution and suspended in that solution in a concentration of 2×10^6 cells/ml. The cell suspension was transferred in volumes of 0.1 ml into conical centrifuge tubes, and to each tube was then added 0.1 ml of a 1% suspension of allogeneic or autologous erythrocytes. The mixture was centrifuged for 5 min at 200g, kept for 15 min at 4°C, and the residue was resuspended by gentle agitation of the tubes. A drop of the cell suspension was transferred to a Goryaev's counting chamber. The result was expressed as the number of rosette-forming cells as a percentage of the total number of leukocytes in the chamber.

The cells were subjected to parallel tests of rosette formation with sheep's erythrocytes, both intact and sensitized with antierythrocytic antibodies, and the number of cells carrying surface immunoglobulins also was determined.

EXPERIMENTAL RESULTS

The largest number of V rosettes (57%) was found when a suspension of monocytes was used (Table 1). Under the conditions of testing (short-term incubation of the cells with virus in the cold), when the first stage of interaction between virus and cell (adsorption) takes place, monocytes are evidently the most sensitive cell population binding the virus. Characteristically, not all monocytes form V rosettes. From 10 to 30% (on average about 15%) of monocytes did not take part in the reaction of virus rosette formation. This state of affairs can evidently be attributed to the existence of at least two qualitatively different populations of monocytes, one of which does not possess receptors for binding viruses.

*Subagglutinating development of the virus was determined by the agglutination test with human erythrocytes.

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TABLE 1. Rosette-Forming Properties of Human Blood Monocytes

Type of cells	Number of rosette-forming cells, %			Number of Ig^+-cells*, %
	E	*EA*	*V*	
Initial suspension	39.3 (28—48; *n*=7)	13.5 (9—17; *n*=5)	7.6 (5—15; *n*=6)	10.3 (3—19; *n*=5)
Suspension enriched with B cells	19.8 (11—28; *n*=4)	—	24.8 (15—33; *n*=6)	23.5 (11—29; *n*=6)
Monocytes	2.5 (0.5—5; *n*=8)	69.9 (57—81; *n*=7)	45.2 (28—57; *n*=6)	—

Legend. Limits of variations and number of blood samples tested shown in parentheses. *Determination carried out by the direct immunofluorescence test.

T cells did not participate in the reaction of V rosette formation. Their selective removal from the total fraction of monocytes not only did not cause any decrease in the number of V rosettes but, on the contrary, led to an increase in their number. Enrichment of the cell suspension with B cells was accompanied by an increase in the number of V rosettes from 7.6 to 24.8%, so that B cells can be included equally with monocytes in the cell population involved in interaction with the virus.

The reaction of virus rosette formation belongs by its nature to the cold category. With an increase in the temperature of incubation of the virus-treated cells with erythrocytes to 37°C the number of V rosettes fell virtually to zero. This is in agreement with data on the optimal temperature for the action of virus agglutinins (4°C) and it indicates that the form of reaction of monocytes studied is due to the presence of virus on the surface of these cells.

By the methods used it was thus possible to detect populations of monocytes binding Sendai virus. The data on participation of monocytes and B lymphocytes in this interaction can be explained not only by the choice of virus for these target cells. The phenomenon is probably due more particularly to the characteristics of the surface receptors, including immunoglobulin receptors, which determine the ability of the cells to adsorb virus.

Although the data on binding of Sendai virus by monocytes and B lymphocytes contradict previous findings indicating preferential interaction of T lymphocytes with paramyxoviruses [6], it must be remembered that, as was stated above, only the first phase of virus–cell interaction, namely adsorption, was studied, and this stage takes place in the cold, during only a short period of time, and it does not require active functioning of the cell apparatus. However, the possibility cannot be ruled out that during cooperative cellular interaction in vivo B lymphocytes bind the virus and then "hand it on" to T lymphocytes.

The choice of Sendai virus in the investigation described above was fortuitous. Evidently any virus possessing agglutinating properties with respect to human erythrocytes can be used in the V rosette formation test.

It is difficult to decide as yet what is the importance of this in vivo reaction. However, the ability of immunocompetent cells, treated with virus, to interact with autologous erythrocytes suggests that the immunologic phenomenon described in this paper may lie at the basis of the development of autoimmune hemolytic anemia and other autoimmune diseases.

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