INTERACTION BETWEEN HUMAN PERIPHERAL BLOOD LYMPHOCYTES AND AUTOLOGOUS ERYTHROCYTES

Academician V. P. Kaznacheev* and
L. A. Trunova

UDC 612.112.94-06:612.111

Interaction between lymphocytes and autologous erythrocytes was discovered in patients with autoimmune diseases. The interaction was manifested macroscopically as hemagglutination and microscopically as rosette formation. Interaction between lymphocytes and autologous erythrocytes was not found in a group of healthy subjects or in patients free from autoimmune disturbances.

KEY WORDS: autologous lymphocytes and erythrocytes; autoimmune diseases; rosette formation.

In 1970 the writers [1] suggested a test for the detection of hypersensitivity of delayed type in vitro for use in rheumatic fever: The test is based on adsorption of autologous erythrocytes loaded with connective-tissue antigen by peripheral blood lymphocytes and is conventionally abbreviated to AELT. Macroscopically the test was manifested as hemagglutination of varied intensity, and microscopically as rosette formation, due to the attachment of autologous erythrocytes, loaded with antigen, to lymphocytes.

One of the controls to the AELT was interaction between autologous lymphocytes and erythrocytes only; in a certain proportion of cases this also was manifested as hemagglutination and the phenomenon of rosette formation.

The object of this investigation was to study interaction between lymphocytes and autologous erythrocytes without antigen in certain diseases.

EXPERIMENTAL METHOD

Blood was taken from the cubital vein of patients and healthy subjects in a volume of 4-5 ml. The method used to separate the white blood cells, lymphocytes, and erythrocytes was described previously [2].

Interaction between lymphocytes and autologous erythrocytes took place either when white blood cells were used or with the lymphocytes isolated from them. In variant 1, 0.3 ml physiological saline contained from 10^6 to $1.5 \cdot 10^6$ white blood cells, whereas in variant 2 the same volume contained from 400,000 to 600,000 lymphocytes. An equal volume of a 1% suspension of autologous erythrocytes in physiological saline was added to the white blood cells or lymphocytes. The total volume of the mixture was made up to 1 ml with physiological saline. The test was carried out in wells on agglutination tiles, incubated for 30 min, and then kept for 18-20 h in a refrigerator at 4°C.

The test was evaluated by a 4-point system such as is used for assessing the hemagglutination test. A result estimated at ++ was regarded as positive. Rosettes formed by lymphocytes with three or more autologous erythrocytes adhering to them were identified in films stained by the Romanovsky–Giemsa method (Fig. 1).

* Academy of Medical Sciences of the USSR.

Laboratory of Immunology, Institute of Clinical and Experimental Medicine, Siberian Division, Academy of Medical Sciences of the USSR, Novosibirsk. Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 82, No. 12, pp. 1469-1471, December, 1976. Original article submitted May 10, 1976.

TABLE 1. Results of Interaction of Lymphocytes with Autologous Erythrocytes Test on Healthy and Sick Subjects

Group of subjects	Total number of subjects tested	Interaction between lymphocytes and autologous erythrocytes	
		number of positive results	% of positive results (M ± m) and confidence interval (for P ≤ 0.05)
Donors	100	0	0±1 (0—4)
Healthy men aged 20-22 years	26	0	0±2 (0—8)
Patients with myocardial infarction	42	0	0±2 (0—8)
Children with:			
pneumonia	65	1	2±2 (0—8)
chronic tonsillitis	45	2	4±3 (0—14)
chronic tonsillitis and suspected rheumatic fever	30	9	30±9 (15—49)
rheumatic fever:			
active phase	35	24	69±8 (51—83)
inactive phase	10	1	10±1 (0—44)
Adults with:			
rheumatic fever:			
active phase	54	12	22±6 (12—36)
inactive phase	23	0	0±4 (0—15)
rheumatoid arthritis, active phase	95	27	28±5 (20—39)

EXPERIMENTAL RESULTS

The results of the tests are summarized in Table 1. The phenomenon of interaction between lymphocytes and autologous erythrocytes could not be detected in groups of healthy subjects or patients with myocardial infarction and pneumonia. The phenomenon was discovered in 4 ± 3% of cases in a group of children with chronic tonsillitis, and in as many as 30 ± 9% of children with chronic tonsillitis suspected of having rheumatic fever.

The phenomenon was constantly found when a marked autoimmune process was present, in the active phase of the disease. It was present in a particularly high percentage of children with rheumatic fever in the active phase (up to 69 ± 8%).

Initially to explain the mechanism of interaction between lymphocytes and autologous erythrocytes two working hypotheses were put forward. 1) Considering the high adsorptive power of the erythrocyte membrane, the hypothetical autoantigen could have been adsorbed on to the surface of the erythrocytes where it could react with sensitized lymphocytes. 2) Cross-reacting antigens on the membrane of autologous erythrocytes and autoantigens of the affected organs and tissues could have been present.

With these hypotheses in mind, it might be supposed that the phenomenon would be manifested in the presence of a well-defined autoimmune process. This was confirmed by comparing the presence of the phenomenon with the clinical course of the illness: In 27 patients with rheumatoid arthritis and in whom the phenomenon was present, the activity of the process was assessed as second and third degree. In children with the active phase of rheumatic fever a positive result was detected only if the activity of the process was of the second or third degree. To confirm the connection between the phenomenon and the presence of a true autoimmune disease, a further and closer study of the problem is necessary.

From an analysis of the data published in recent years a further hypothesis could be put forward to explain the mechanism of this phenomenon. It has been shown that 80-90% of lymphocytes of the healthy human thymus can form rosettes with autologous erythrocytes. Peripheral lymphocytes have lost this ability completely [4]. However, other workers have found that under particularly cold conditions 5-10% of the subpopulation of peripheral blood T lymphocytes of healthy subjects can form rosettes with autologous erythrocytes [6].

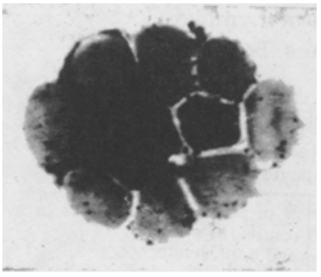

Fig. 1. Rosette formation by autologous erythrocytes around a lymphocyte. Magnification: ocular 7, objective 90. Romanovsky–Giemsa.

Since healthy human peripheral blood contains 5-10% of so-called null cells, their role in the phenomenon of interaction with autologous erythrocytes seems acceptable. An increase in the percentage of "null" cells in autoimmune processes [5] should be accompanied in that case by the more intensive manifestation of the phenomenon of interaction between lymphocytes and autologous erythrocytes.

There is also information in the literature that the thymus factor in vitro can induce the transformation of immature precursors (according to the authors cited, "null" cells) into functionally active T cells [3]. It is also known that autoimmune diseases are accompanied by considerable changes in the thymus.

Comparison of these facts suggests that during the development of an autoimmune disease, accompanied by disturbance of the regulatory ability of the thymus, there is an increase in the number of undifferentiated T lymphocytes (possibly "null" cells), capable of interacting in vitro with autologous erythrocytes, into the peripheral blood.

This type of interaction can be prevented in vivo by many factors and, in particular, by serum blocking factors and by T suppressors.

LITERATURE CITED

1. V. P. Kaznacheev, L. A. Trunova, V. D. Peterson, et al., Vopr. Revm., No. 3, 3 (1970).
2. L. A. Trunova, "The study of the cellular reactions of immunity in man in health and disease," Doctoral Dissertation, Novosibirsk (1970).
3. F. Aiuti, V. Schirrmacher, P. Ammirati, et al., Clin. Exp. Immunol., 20, 499 (1975).
4. G. Baxley, C. B. Bishop, A. G. Cooper, et al., Clin. Exp. Immunol., 15, 385 (1973).
5. A. Brenner, M. Scheinberg, and E. Cathcart, Arthritis Rheum., 18, 297 (1975).
6. I. Kaplan, Clin. Immunol., 3, 471 (1975).