of the results of the present investigation. Most likely the greater part of the antigen-dependent NIG is produced by certain other cells. The fact that the effect of MAAS on antigen-dependent NIG synthesis was less marked than its effect on accumulation of RFC in immunized animals suggests that some of the B lymphocytes, which carry aggregated immunoglobulins on their surface, can be activated by these aggregates and can participate in the synthesis of nonspecific immunoglobulins.

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VALUE OF THE SPONTANEOUS ROSETTE FORMATION TEST WITH MOUSE ERYTHROCYTES IN MULTIPLE ASSAY OF HUMAN B LYMPHOCYTES

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KEY WORDS: lymphocytes; T and B systems of immunity; receptors; immunologic insufficiency; leukemia.

Identification of lymphocyte subpopulations is one of the foremost problems in human immunology. To detect T lymphocytes spontaneous rosette formation with sheep's erythrocytes (SE-RFC) is used. To detect B lymphocytes several variants of rosette formation are used, based on detection of receptors for the C'_3 component of complement (EAC-RFC) or of receptors for the Fc fragment of IgG (EA-RFC).

The phemenon of spontaneous rosette formation of human lymphocytes with mouse erythrocytes (ME-RFC) was described in 1974 [10]. It has been shown that B lymphocytes have the property of binding mouse erythrocytes. Some particular features of the phenomenon have been studied and the ME-RFC level has been determined in certain pathological processes [6-8].

In the present investigation ME-RFC were investigated along with other tests for detecting human T and B lymphocytes. The role of these markers in the reaction of rosette formation between B lymphocytes and mouse erythrocytes was studied by the method of inhibition of surface receptor structures.

EXPERIMENTAL METHOD

Lymphocytes were isolated from the blood of patients and healthy blood donors by centrifugation in a Ficoll-Hypaque density gradient by the method of Böyum [5]. The pure suspension of lymphocytes was diluted with Hanks' solution to a concentration of 2×10^6 cells/ml.

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TABLE 1. Multiple Assay of Peripheral Blood Lymphocyte Populations in Healthy Donors (n = 23)

Index	Relative percentages of RFC							
	ME-RFC	EAC-RFC	EA-RFC (n=8)	Ig-carrying lympho-cytes	E-RFC	Lymphocytes not carrying markers of B or T lymphocytes (null cells)		
						100 - (ME-RFC + E-RFC)	100 - (EAC-RFC + E-RFC)	100 - (Ig-carrying cells + E-RFC)
M	7,7	16,7	19,4	17,9	65,2	27,1	18,1	16,9
$\pm m$	0,48	0,85	2,35	0,65	2,6	2,44	2,91	2,7
Maximal	13,5	27,0	28,0	23,0	80,0	50,0	45,0	45,5
Minimal	4,5	10,0	9,0	12,0	42,5	10,0	1,0	1,0

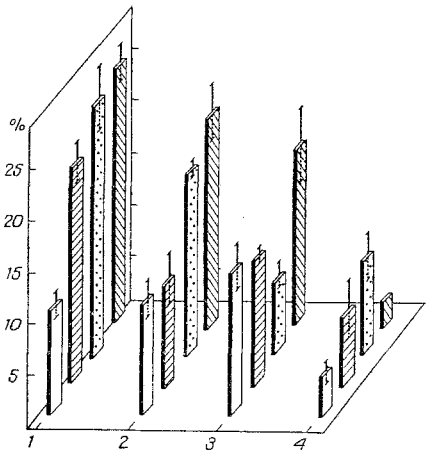

Fig. 1. Effect of treatment of lymphocytes with various factors on rosette formation. Unshaded columns – ME-RFC; horizontally shaded columns – EA-RFC; dotted columns – EAC-RFC; obliquely shaded columns – SmIg. 1) Control, 2) aggregated gamma-globulin, 3) complement, 4) anti-immunoglobulin serum.

T-lymphocytes were identified in the E rosette-formation test by the method of Mendes [9], modified by Stenina [3]. Lymphocytes binding mouse erythrocytes spontaneously (ME-RFC) were detected by a test similar to E rosette formation. EA-RFC and EAC-RFC were detected by Mendes' method [9]. B lymphocytes carrying surface immunoglobulins were detected by the method described by Andreeva [1].

EXPERIMENTAL RESULTS

The results of multiple assay of healthy human peripheral blood lymphocyte subpopulations are given in Table 1. Under the conditions provided for the ME rosette-formation test, the number of ME-RFC in healthy subjects was 7.7 ± 0.48%. These values agree with those obtained by most other workers [4, 6, 8].

There is evidence that B lymphocytes carrying surface immunoglobulins of the M class participate in ME-rosette formation [6, 8]. By the use of an approach of successive selective inhibition of Fc- and C'_3 receptors and of surface immunoglobulins, data on the degree of participation of these structures in the phenomenon of ME-rosette formation was obtained (Fig. 1). After treatment of the lymphocytes with aggregated rabbit gamma-globulin the EA-RFC level was sharply reduced (from 17.4 ± 2.1 to 7.8 ± 1.4%). On the addition of complement to lymphocytes with blocked Fc-receptors, drastic inhibition of EAC-RFC was observed (from 19.0 ± 1.7 to 5.6 ± 0.9%). The number of ME-RFC was unchanged in both cases. Subsequent treatment of the lymphocytes with polyvalent anti-immunoglobulin serum had a marked action on the level of Ig-carrying cells (which was reduced from 19.0 ± 1.7 to 2%). Under the same conditions ME-rosette formation was inhibited (reduced from 8 ± 0.8 to 3.5 ± 0.6%). Treatment of the lymphocytes with monospecific anti-IgM- or anti-IgG-sera led to a decrease in the number of ME-RFC only in experiments in which anti-IgM-serum was used. The E-RFC level was unchanged during subsequent treatment of the lymphocytes.

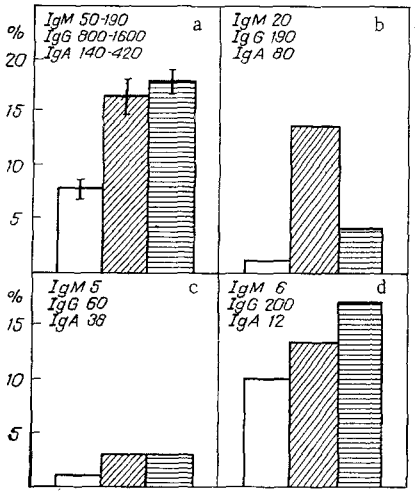

Fig. 2

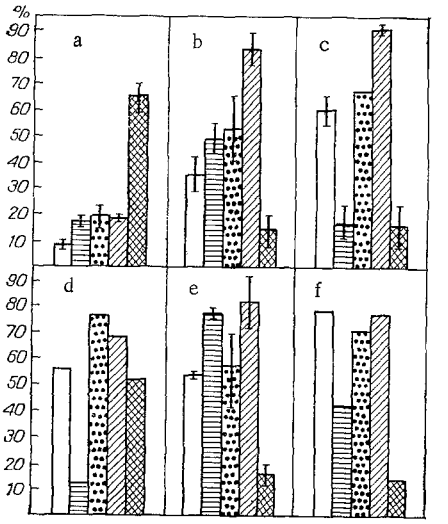

Fig. 3

Fig. 2. Investigation of B lymphocytes in patients with dys- and agammaglobulinemia. Unshaded column – ME-RFC, obliquely shaded column – EAC-RFC, horizontally shaded column – SmIg. a) Normal, b) patients with dysagammaglobulinemia, c and d) patients with agammaglobulinemia.

Fig. 3. Distribution of lymphocytes in chronic lymphatic leukemia. Unshaded column – ME-RFC, horizontally shaded columns – EAC-RFC, dotted columns – EA-RFC, obliquely shaded columns – SmIg, cross-hatched columns – E-RFC. a) normal, b-f) variants 1-5 respectively.

In patients with rheumatoid arthritis (7 patients) a significant decrease was found in the ME-RFC level to 2.8 ± 0.4%. Different values for the lymphocyte subpopulations were found in one patient with dysimmunoglobulinemia and in 20 patients with hypoimmunoglobulinemia (Fig. 2). A sharp fall in the ME-RFC level (to 1%) and in the number of lymphocytes carrying surface immunoglobulins (to 4%) was found in the patient with dysimmunoglobulinemia, whereas the number of EAC-RFC was indistinguishable from normal (13.5%). A sharp reduction in the number of B lymphocytes was observed with respect to all indices for one patient with hypogammaglobulinemia, evidence of a block to the differentiation of B lymphocytes in this form of primary immunodeficiency. In another patient with hypoimmunoglobulinemia the B-lymphocyte level was within normal limits, although the immunoglobulin concentration in the peripheral blood was sharply reduced, possible evidence of a disturbance of immunoglobulin production by B lymphocytes and their progenies. In all three patients the number of cells carrying IgG and the number of ME-RFC showed positive correlation with each other.

Among the nine patients with chronic lymphatic leukemia at least five variants of distribution of lymphocyte subpopulations were distinguished (Fig. 3). It must be emphasized that during investigation of patients with chronic lymphatic leukemia, the most constant and demonstrative results were given by the ME-rosette formation test. Detection of lymphocytes with ME-receptors in lymphatic leukemias has advantages over the immunofluorescence method because first, it is much simpler to perform and more acceptable, and second, the concentration of surface immunoglobulins on the cell is sharply reduced in lymphatic leukemias, so that the fluorescence of these cells is much weaker during immunofluorescence microscopy [2].

Determination of ME-RFC is thus a simple and convenient method for identifying subpopulations of human B lymphocytes. The ME-RFC level fluctuates considerably in different human diseases with disturbance of immunologic mechanisms, and in certain diseases, such as chronic lymphatic leukemia, this test may have advantages over all other methods for the identification and counting of B lymphocytes in man.

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RELATIONSHIP BETWEEN SEVERITY OF TETANUS INTOXICATION AND THE LEVEL OF HOST RESISTANCE

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Tetanus still remains high on the list of infections with high mortality. Successful management of the paroxysmal syndrome, which dominates the pathogenesis of the disease [3], has given urgency to the study of secondary infectious complications. Accordingly the need has arisen for a method of determining the level of the natural protective forces of the host, its general resistance, and the state of immunity and of the nervous system in tetanus intoxication.

Nonspecific factors of defense and the specific response were studied in the present investigation at different times after injection of tetanus toxin (TT) in doses causing tetanus intoxication with a slow and nonfatal course (1/3 or 2/3 MLD) or with a more severe course leading rapidly to death (1 MLD).

EXPERIMENTAL METHOD

Experiments were carried out on 110 rats weighing 200-240 g and on 21 rabbits weighing 2.5-3 kg. Experimental tetanus was produced by administration of TT, batch 21 "Leningrad," in doses of 1, 1/3 and 2/3 MLD. The TT was injected intramuscularly into the left leg in a volume of 0.2 ml physiological saline into rats and in a volume of 2.5 ml into rabbits. TT inactivated by heating to 56°C for 2 h was injected into the control animals.

Nonspecific factors of defense were assessed on the basis of a number of indices: phagocytic activity of the leukocytes, plasma-cell response of the lymph nodes and spleen, serum concentrations of complement [1], lysozyme [2], and properdin [5], and the anticomplementarity and cytotoxicity of the serum [4]. The last was judged from the percentage of adrenalectomized mice which died. The specific response was assessed by the passive hemagglutination test with an erythrocytic diagnostic serum for tetanus. The numerical results were subjected to statistical analysis and the significance of differences was estimated by Student's t-test [7].

EXPERIMENTAL RESULTS

Statistically significant activation of the cellular factors – an increase in the phagocytic index, an active plasma-cell response, and general morphological changes in the regional and contralateral lymph nodes and spleen (Fig. 1, 1), and also of humoral factors – complement, lysozymes, and properdin, were clearly observed 24 h after injection of 1 MLD TT (Fig. 1).

Representation of the plasma-cell response in graphic form emphasized the rapid changes in response to injection of the toxin affecting all cell forms tested from this point of view. It will be clear from Fig. 1 that cells of the plasma-cell series were found infrequently in the lymph nodes and spleen. However, one day after injection of TT a sharp increase in their number was noted in the regional lymph node: twofold in the case of

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