into the spleen and an increase in the number of helper cells in it, an increase in the number of helper cells in the bone marrow, activation of amplifiers [1], and increased activity of T cells and macrophages [5]. Histamine, on the other hand, has a suppressive action on many immunologic phenomena, including inhibition of lymphocyte proliferation in response to stimulation by an antigen or mitogen, antibody formation, and lymphocytotoxicity, and it depresses cutaneous delayed-type hypersensitivity, release of lymphokines, T-helper cell generation, and effector functions, followed by a decrease in polyclonal activation of B cells [5, 7]. It has been suggested [8] that the suppressor action of histamine is due to stimulation of H_2 receptors on the surface of the lymphocytes and it is mediated by a raised intracellular cAMP level. The possibility that manifestation of the opposite effects of histamine may depend on its concentrations in vitro has been described [8] in the lymphocyte blast transformation reaction.

The results confirm data in the literature on the possible effect of the biogenic amines system on immunity and they indicate that the monoaminergic system contains components capable of exerting opposite effects (both stimulating and inhibitory) on lymphocyte function.

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CHANGES IN FUNCTION AND KINETICS OF MACROPHAGES AND LYMPHOCYTES CAUSED BY RETINOIC ACID

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Along with retinol esters, when it enters the human or animal body in high doses, retinoic acid (RA) has a positive immunomodulating action [4, 6, 7]. It has been suggested that, when present in the blood unbound with transport protein, RA causes damage to the blood cells and, in particular, erythrocytes. Subsequent stimulation of the immune response immediately after such an event develops like the response to antigenic conversion of erythrocyte membrane proteins [2]. On experimental testing of this hypothesis several features of erythrocyte damage, increased phagocytosis of altered erythrocytes by macrophages, and activation of interaction between macrophages and lymphocytes were found [1, 3]. However, observations in vivo give only indirect evidence of the mechanisms of the immunomodulating action of RA. Experiments in vitro in this respect have several advantages, relating primarily to the possibility of studying the effect of RA on the composition and functions of individual populations and types of immunocompetent cells.

The aim of this investigation was to study the action of RA in vitro on quantitative parameters and function of human macrophages and T and B lymphocytes.

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EXPERIMENTAL METHOD

To study the transformation activity of macrophagal precursor cells and the state of their function, heparin (250 U/ml) was added to freshly obtained blood from 10 clinically healthy men aged 20-27 years, and the blood was allowed to stand for 1 h at 4°C to enable the cells to be sedimented. A primary culture of white blood cells was prepared from the buffy coat in medium 199 with the addition of 10% embryonic calf serum and antibiotics. RA was added in the water-soluble form in the following amounts of a 10^{-6} M solution to 100 ml of medium: 1st dose 0.05 ml, 2nd dose 0.25 ml, 3rd dose 1 ml. The substance was obtained from the Laboratory of Chemistry of Polyene Compounds (Head, Professor G. I. Samokhvalov), Vitaminy Research and Production Combine, Ministry of the Medical Industry of the USSR. The cells were incubated at 37°C for 24 h on coverslips. Functional activity of the macrophages was evaluated by the bacterial phagocytosis test, by loading macrophages in culture with inactivated staphylococci for 45 min at 37°C and counting the mean number of staphylococci phagocytosed by one cell. Transformation activity of macrophagal precursors was analyzed by counting 200 cells in preparations with adherent white blood cells, fixed and stained with Romanovsky's stain, and estimating the relative number of untransformed cells and of macrophages with the typical cytological structure [5]. At the same time, the state of the erythrocytes and the character of intercellular interactions were assessed in the preparations.

The size of the populations and functional activity of the lymphocytes were judged from the presence of marker receptors on the membrane and their proliferative activity. Medium 199 (in the rosette formation test) and medium 199 made up with Hanks' solution with the addition of 20% bovine serum and antibiotics (for the blast transformation reaction) were used for the lymphocyte cultures. Before carrying out the test the cells were incubated for 24 h with a water soluble form of RA, used in the same concentrations as to study the properties of macrophages. The receptor-membrane properties of T and B lymphocytes were characterized by spontaneous and complementary rosette formation tests (E-RFC and EAC-RFC, respectively) tests. Lymphocytes were isolated from heparinized blood by centrifugation in a Ficoll—Verografin density gradient. Rosettes with sheep's erythrocytes were fixed with glutaraldehyde and films of the supernatant were dried and fixed with methanol. The proliferative activity of the lymphocytes was estimated by determining their ability to undergo blast transformation under the influence of phytohemagglutinin (PHA) and tuberculin PPD. Removal of factors blocking transformation of precursor cells into macrophages was not carried out. After incubation for 72 h with mitogens the erythrocytes were hemolyzed with 10% acetic acid. The supernatants were fixed with ethanol and films prepared, dried, and stained with Romanovsky's stain. The number of rosette-forming and blast forms of cells was determined in per cent after counting 200 to 300 lymphocytes.

The effect of RA on macrophages and lymphocytes was assessed by comparing the results with data obtained in cell cultures whose medium did not contain RA.

EXPERIMENTAL RESULTS

When cell suspensions enriched with macrophages and lymphocytes were cultured, addition of RA to the culture medium was accompanied by changes in the erythrocytes: they were enlarged, became uneven and developed multiple processes, and they aggregated together and formed complexes measuring 200-300 μ. Similar changes in erythrocytes, accompanying the action of active forms of vitamin A, have been described in experiments in vivo and in vitro [2, 3, 7], and they are evidently due to the surface-active properties of the vitamin A molecules. Addition of RA to the culture medium was followed by only a tendency toward increased ability of the precursor cells to be transformed into macrophages. The phagocytic activity of the macrophages was not significantly changed by the action of RA. Addition of RA to the culture fluid was accompanied by increased ability of the macrophages to interact with granulocytes and lymphocytes with the formation of compact clusters of five to 12 cells. In a high dose RA often caused damage to the macrophages. The nuclei of these cells were flattened, the cytoplasm vacuolated and fragmented, and the surface contours became indented.

RA inhibited the blast transformation reaction of the lymphocytes. The ability of the cells to undergo spontaneous and complementary rosette formation also was reduced (Table 1). However, in the experiments with macrophages in which RA was used in the same doses, the lymphocytes preserved their normal functions and took part in interaction with macrophages.

Comparison of the experimental results shows that RA damages erythrocytes and potentiates interaction of macrophages with leukocytes and, more especially, with lymphocytes. If the

TABLE 1. Effect of RA on Blast Transformation Reaction of Lymphocytes (BTRL) and Number of E-RFC and EAC-RFC in Man (in %, M ± m)

Parameter	Untreated (control)	Doses of RA per 100 ml medium: 0.05 ml of 10^{-6} M solution	0.25 ml of 10^{-6} M solution	1 ml of 10^{-6} M solution
BTRL	56±18	42±1*	—	42±3,6
E-RFC	44±1,3	32±2,6**	27±1,1*	—
EAC-RFC	33±1	24±0,8*	21±1,2*	21±2,7**

Legend. *p < 0.001, **p < 0.02 compared with control.

precursors of the blood cells were not washed before culture to remove serum inhibitors and, for that reason, could not be transformed into macrophages, despite the presence of damaged erythrocytes, the functional activity of the lymphocytes was inhibited, and no aggregates of lymphocytes and macrophages were formed. These data are evidence in support of a role for macrophages in the realization of the immunomodulating action of RA. It was shown previously [1] that damage to erythrocyte membranes by RA is necessary for activity of the immunocompetent cells to be potentiated. The results of the present experiments do not contradict the view that RA may have a positive immunomodulating action through modification of erythrocytic antigens.

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