

EFFECT OF RETINOIC ACID ON ERYTHROCYTE AND LEUKOCYTE AGGREGATION IN THE BLOOD

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Vitamin A and retinoids are known to have the properties of adjuvants [1, 5, 7]. This effect is accompanied by moderate anemia [3]. It has been found [6] that rejection of damaged erythrocytes from the blood stream is effected by immunocompetent cells. These findings have served as the basis of a hypothesis on the mechanism of the immunostimulating action of vitamin A and retinoids, according to which the immune system is stimulated by autoerythrocytic antigens [2]. Immunocompetent cells have been shown to form rosettes *in vitro* with xenogeneic and syngeneic erythrocytes [4, 8-10].

The aim of this investigation was to study the effect of retinoic acid (RA) on aggregation of erythrocytes and leukocytes in the blood.

EXPERIMENTAL METHOD

Experiments were carried out on (CBA × C57B1/6)_{F1} mice of both sexes weighing 18 ± 1 g. RA was injected intraperitoneally in the form of its water-soluble product and of all-trans-methylretinoate (ATM). These substances were obtained from the Laboratory of Chemistry of Polyenic Compounds (Head, Professor G. I. Samokhvalov), "Vitaminy" Research-Production Combine (USSR). Blood for investigation of erythrocytes and leukocytes was taken from the caudal veins. Erythrocytes and leukocytes in 1 mm^3 of blood were counted and the ESR measured by the usual methods. The prothrombin time was determined after mixing 0.2 ml of citrated blood with 0.1 ml of a 10% solution of thromboplastin in the presence of calcium ions. Blood films were stained by Romanovsky's method and with paraldehyde-fuchsine (PAF), and the erythrocytes were counted by the method described previously [3]. To determine correlation between osmotic resistance and the staining properties of the erythrocytes, blood samples were taken from mice (10 mice in a group) on the 4th day after a single intraperitoneal injection of 0.3 ml of 0.75% ATM or solvent. The blood was incubated for 45 min at 25°C in 0.85, 0.58, and 0.50% NaCl solutions, centrifuged (1500 rpm, 10 min), and films of the residue were stained with PAF. In the blood films stained with PAF, a rosette with a large, round leukocyte, intensely stained with PAF, and surrounded by no fewer than six erythrocytes, was taken to be a leukocyte-erythrocyte aggregate (LEA). To determine the type of rosette-forming leukocytes, wet blood films were fixed as for staining with PAF, stained with azure II and eosin, and mounted in balsam. LEA was investigated in man during outpatient examinations of workers at a pharmaceutical factory, employed in the manufacture of RA. The results were subjected to statistical analysis by Student's *t* test.

EXPERIMENTAL RESULTS

Examination of peripheral blood films obtained from mice of the control group and stained with PAF showed that for every 1000 free erythrocytes there was about 0.15 LEA (0.15%). A single intraperitoneal injection of 0.2 ml of 0.85% NaCl into the animals did not change this proportion. The same injection of the oil which acted as the solvent for ATM increased the number of these aggregates to $0.23 \pm 0.09\%$. A single intraperitoneal injection of 0.3 ml of 1% ATM was accompanied by an increase in the number of LEA. On the 3rd day, for instance, it was $0.46 \pm 0.17\%$ and on the 5th day $1.48 \pm 0.37\%$ ($P < 0.01$). Subsequently the number of LEA fell to $0.77 \pm 0.09\%$ on the 6th day and to $0.72 \pm 0.09\%$ on the 7th day ($P < 0.001$). By

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TABLE 1. Number of LEA (rosettes), Leukocytes, Lymphocytes, and Monocytes, Number of Erythrocytes in an Aggregate, and Coefficient (K) of Aggregation in Aggregates of PAF-Negative Erythrocytes, and Prothrombin Time in Aggregates of PAF-Negative Erythrocytes, and Prothrombin Time in Peripheral Blood of Male (CBA × C57B1/6)F₁ Mice on 1st-7th Days after a Single Intraperitoneal Injection of 0.2 ml of 1% Water-Soluble Form of RA (M ± m, n = 8)

Treat- ment	Time of observa- tion, days	Number (per 1000 erythrocytes)				Number of erythrocytes per aggre- gate	K	Prothrombin time, sec	Control (solvent, 0.2 ml)
		of aggregates	of leukocytes	of lympho- cytes	of mono- cytes				
RA	1	0,12±0,01	1,09±0,02**	0,91±0,06	0,05±0,01	9,4±0,6	1,00	15±0,2	1—7
	2	0,36±0,16	1,09±0,02**	0,91±0,06	0,05±0,01	17,2±0,8*	1,07	21±1,8**	0,15±0,02
	4	0,43±0,08*	1,85±0,02**	1,45±0,09**	0,11±0,04	14,8±1,0	1,09	23±1,2**	0,95±0,01
	5	0,20±0,02	1,49±0,03**	1,23±0,04**	0,05±0,02	13,2±1,0	1,49	15±0,4	0,79±0,03
	7	0,21±0,02	1,49±0,01**	1,38±0,03**	—	12,8±0,5	1,22	15±0,4	0,03±0,02
									13,8±0,6
									0,81
									15±0,4

Legend. $K = \frac{\text{Number (in \%) of PAF-negative erythrocytes inside aggregates}}{\text{Number (in \%) of PAF-negative erythrocytes outside aggregates}}.$

*P < 0.01, **P < 0.001 compared with control.

TABLE 2. Number of PAF-Negative Erythrocytes among Cells Resisting Osmotic Shock (M ± m, n = 5)

Experimental conditions	NaCl in in- cubation medium	Number of PAF-nega- tive erythro- cytes in film made from residue, %	Index of com- parison with 0,85% NaCl solution
Intact animals	0,85 %	15,1±1,8	1,0
	0,60 %	7,1±1,6	0,5
ATM	0,85 %	21,6±1,5	1,0
	0,60 %	9,6±1,9*	0,4

the 18th day it was $0.15 \pm 0.10\%$. These results show that the maximal increase in the number of LEA in the blood was observed during the first 5-7 days after injection of RA. A detailed analysis of these aggregates was therefore carried out during the first week after a single injection of the water-soluble form of RA. It will be clear from Table 1 that injection of the water-soluble form of RA led to an increase in the number of LEA, but the increase was not so great as after injection of ATM. The number of erythrocytes per aggregate also increased, to reach a maximum by the 2nd day. Mononuclear leukocytes developed in the blood, and was most marked on the 4th day, which evidently reflects the known ability of RA to stimulate immunity [5, 7]. Comparison of the numbers of leukocytes, lymphocytes and monocytes in the blood with the number of LEA showed that lymphocytes could occupy the center of the aggregates in 93-96% of cases, and monocytes in 4-7% of cases. It follows from Table 2 that on the 4th day after a single injection of ATM the number of osmotically unstable PAF-negative erythrocytes in the blood of the mice was increased. Analysis of the change in the coefficient of aggregation of PAF-negative erythrocytes showed that these cells accumulated in aggregates towards the 5th-7th days of the experiment. Injection of ATM was accompanied by a dose-dependent increase in ESR.

For instance, on the 4th day after a single intraperitoneal injection of the substance ESR for the 0.25% solution was 2.4 ± 0.2 mm/h, for the 0.5% solution 3.1 ± 0.2 mm/h, for the 0.75% solution 4.1 ± 0.3 mm/h, and for the 1% solution 4.8 ± 0.4 mm/h.

The increase in the number of LEA in the blood was evidently not connected with activation of the clotting system: The prothrombin time after a single injection of the water-soluble form of RA increased (Table 1). All this may mean that the increase in the number of PAF-negative erythrocytes in the blood, their concentration in LEA, and the increase in the size and number of these aggregates were the result of the damaging action of an excess of RA on the erythrocytes. It may reflect both elimination of the injured erythrocytes and their repair through the participation of blocking immunoglobulins [6].

LEA similar to those observed experimentally, and with a similar time course of their changes under the influence of RA, also were found in man. For instance, the number and size

TABLE 3. Number of LEA, Leukocytes, Lymphocytes, and Monocytes, Number of Erythrocytes per Aggregate, and Coefficient of Aggregation (K) of PAF-Negative Erythrocytes in Leukocytic-Erythrocytic Aggregates in Peripheral Blood of Women Working on RA Production

No. of case	Total duration of contact with RA, h	Number (per 1000 erythrocytes)				Number of erythrocytes per aggregate	K
		of aggregates	of leukocytes	of lymphocytes	of monocytes		
1	12	0,47	1,75	0,50	0,14	15,7	1,30
2	18	0,30	2,17	0,54	0,13	15,2	1,44
3	120	0,50	8,22	1,00	0,06	16,7	1,65

of LEA and the coefficient of aggregation of PAF-negative erythrocytes in them were increased in three women working in a pharmaceutical factory on RA production, proportionally to the duration of contact with the compound (Table 3). These patients developed lymphocytic leukocytosis, and up to 50% of lymphocytes aggregated PAF-negative erythrocytes around themselves.

Aggregates of erythrocytes and leukocytes (mainly lymphocytes) were thus discovered in blood films, fixed in chromate mixture, from experimental animals and human subjects. The action of RA led to an increase in the number of PAF-negative erythrocytes gathered in aggregates, and the number of the latter also increased.

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