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ERYTHROCYTE-DAMAGING AND IMMUNOMODULATING ACTION OF RETINOIDS

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UDC 615.356:577.161.11].065:616.151.1+
612.017.1.014.46:615.356:577.161.11

KEY WORDS: erythrocytes; retinoids; immunity.

The ability of vitamin A and structurally related compounds (retinoids) to stimulate immunity has been known for a relatively long time [5]. According to some reports, under the influence of compounds of this group both humoral and cellular immunity may be modified [6, 8, 9]. Attempts have been made to use vitamin A for immunostimulation in man [7]. However, the pathways of adjuvant action of vitamin A and retinoids have not yet been explained. The writers reported previously [1] that under the influence of vitamin A and retinoids lymphocyte formation is increased in the red bone marrow, the number of large and medium-sized lymphocytes in the blood and the number of small lymphoid follicles in the spleen are increased, and functional activity of the spleen cells is enhanced. Another investigation [4] showed that excessive doses of retinoids damage erythrocytes by reducing their osmotic resistance and stimulating erythropoiesis in the red bone marrow. These results served as the basis for the hypothesis that the adjuvant action of vitamin A and retinoids is mediated through damaged erythrocytes, which are expelled from the blood stream by an immunocellular mechanism [3].

The aim of this investigation was to study correlation between changes in the blood, splenic pulp, and functional activity of the liver macrophages of mice under the influence of retinoids.

EXPERIMENTAL METHOD

Experiments were carried out on adult mature mice of both sexes, either noninbred or pure-line CBA and (CBA × C57BL/6)F_1 (henceforward abbreviated to F_1) hybrid mice, weighing 16-20 g. The number of animals in each group, the doses and methods of administration of retinoic acid and its esterified cis- and trans-isomers, retinyl acetate (RA), and retinoids C_{15} and C_{20}, are given in Tables 1 and 2. The substances were obtained from the Laboratory of Chemistry of Polyenic Compounds (Head, Professor G. I. Samokhvalov), of the "Vitaminy" Research-Production Combine (USSR). The structures and trivial names of the compounds were given in [8]. The state of the erythrocytes was judged by the hemoglobin concentration in the blood, the number of erythrocytes in 1 mm^3 of blood, and the osmotic resistance of the erythrocytes. These parameters were determined as described previously [2]. The number of reticulocytes (in promille) was determined in blood films stained supravitally with bright cresyl blue.

To determine the delayed-type hypersensitivity reaction (DTHR) to purified tuberculin the animals were immunized by a single intraperitoneal injection of BCG vaccine in a dose of 0.5 ml of a 0.2% suspension in physiological saline. RA was injected intraperitoneally twice a week in a dose of 0.5 ml of a 0.2% oily solution. On the 21st day of the experiment (the optimal time for manifestation of the immunomodulating action of BCG vaccine and of vitamin A) 0.01 unit of purified tuberculin in 0.1 ml physiological saline was injected

Department of Histology, Cytology, and Embryology, I. M. Sechenov First Moscow Medical Institute. (Presented by Academician of the Academy of Medical Sciences of the USSR V. V. Kovanov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 100, No. 10, pp. 463-466, October, 1985. Original article submitted October 8, 1984.

 0007-4888/85/0010-1398$09.50

TABLE 1. Changes in Hemoglobin Concentration and Number of Erythrocytes and Reticulocytes in Peripheral Blood of Mice under the Influence of Retinoic Acid (M ± m)

Day of observation	Hemoglobin, g %	Erythrocytes, millions/mm³	Reticulocytes, %
Control (intact animals)	17,5±0,2	10,1±0,3	34,9±1,0
3-rd	17,9±0,2	11,2±0,3*	24,2±1,1**
7-th	16,7±0,2	10,0±0,2	35,7±1,7**
11-th	17,8±0,1	10,9±0,5	74,1±1,3

Legend. Male F_1 mice (n = 8) received a single intraperitoneal injection of 0.2 ml of 1% retinoic acid. *P < 0.05, **P < 0.001 compared with control.

TABLE 2. Effect of All-trans-Methylretinoate (MR), 13-cis-Methylretinoate (13-cMR), and Retinoid C_{15} (RC_{15}) on Hemoglobin Concentration and Number and Osmotic Resistance of Erythrocytes (M ± m)

Test conditions	Hemoglobin, g %	Erythrocytes, millions/mm³	Osmotic resistance of erythrocytes, % NaCl	
			Minimal	Maximal
Control (intact animals)	14,3±0,3	6,5±0,1	0,61±0,01	0,39±0,01
MR	11,0±0,2	4,3±0,7	0,65±0,02	0,42±0,02
13-cMR	13,6±0,1	5,8±0,2	0,63±0,01	0,41±0,01
RC_{15}	12,3±0,1	5,3±0,3	0,63±0,01	0,41±0,01

Legend. Results of experiments on male CBA mice receiving 0.5% oily solutions in a dose of 0.1 ml once a week, perorally, for 37 weeks, on 48th week of experiment are given (n = 18-20).

into the footpad of the left hind limb of the mice, and 0.1 ml of the solvent was injected into the right limb. The results were read after 24 h in both feet. For this purpose paraffin impressions were obtained of the foot, filled with water, and the volume of water was measured by means of a gas-liquid syringe.

To analyze the state of macrophage function, liver cells were chosen because the macrophages in this organ are distributed in the field of vision of the light microscope relatively uniformly, which facilitates counting. We reported previously [1, 2] that under the influence of retinoids changes in functional activity of macrophages in the liver, spleen, lymph nodes, lung, and skin similar. To estimate the number and phagocytic activity of liver macrophages labeled with colloidal carbon, the ratios between the areas of red and white pulp of the spleen of follicles, and the area of each lymphoid follicle, histological sections were prepared and morphometric analysis carried out by the method described previously [2]. The results were subjected to statistical analysis.

EXPERIMENTAL RESULTS

A single injection of retinoic acid led after 3 days to an increase in the number of erythrocytes and a decrease in the number of reticulocytes in the blood. By the 7th day these parameters had returned to their original level, but the number of reticulocytes subsequently continued to rise (Table 1). Prolonged administration of esterified isomers of of cis- and trans-retinoic acids and of retinoid C_{15} to the animals was accompanied by a

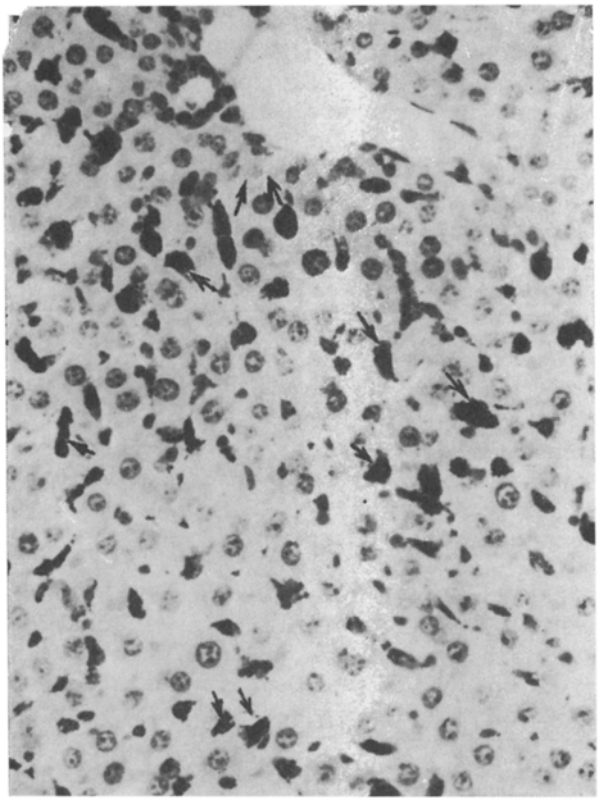

Fig. 1. Fragment of liver of mouse receiving retinoid C_{20}: macrophages, labeled with colloidal carbon, and with high intensity of phagocytosis (arrows). Hematoxylin and eosin. 400 ×.

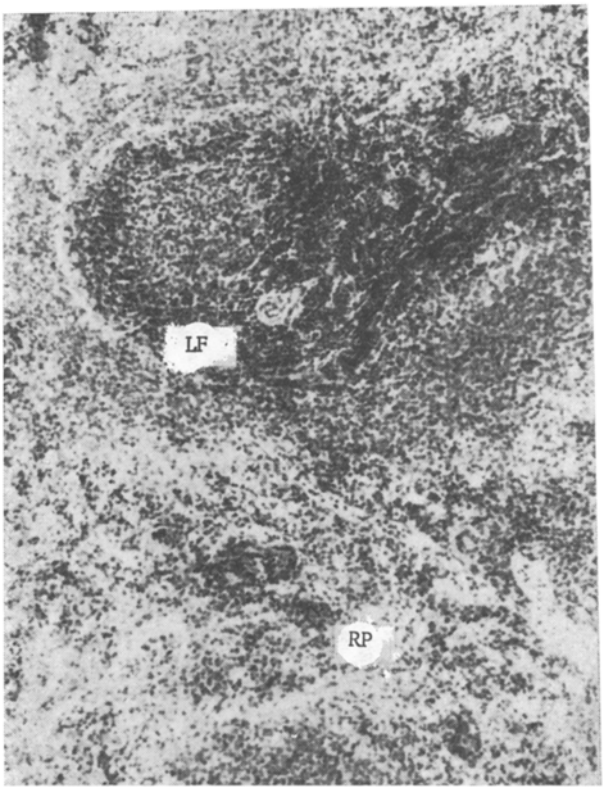

Fig. 2. Fragment of spleen of mouse receiving retinoid C_{20}: large lymphoid follicle (LF). RP) Red pulp. Hematoxylin-eosin. 75 ×.

tendency for the hemoglobin concentration and the number and osmotic resistance of the erythrocytes to fall (Table 2). Injection of excessive doses of retinoic acid evidently caused moderately severe anemia, followed by intensification of erythropoiesis. The small increase in the number of erythrocytes, accompanied by a decrease in the number of reticulocytes, evidently reflected compensatory release of erythrocytes into the circulation from the blood depots in response to the erythrocyte-damaging action of the substance. The increase in the number of reticulocytes until the 11th day of observation could be evidence in support of activation of erythropoiesis.

Investigation of the state of the immune system showed that in animals receiving excessive doses of the vitamin the intensity of DTHR was increased: in mice receiving RA the volume of the foot into the pad of which turberculin was injected was much greater (91.8 ± 0.8 in the control, 192.1 ± 3.7 in the experiment). Under the influence of retinoid C_{20} the number of macrophages in the liver and their phagocytic activity (by a lesser degree) increased (Fig. 1). For instance, after four intramuscular injections of 0.1 ml of 0.3% oil solution of retinoid C_{20} on alternate days, the number of macrophages per unit area on the 12th day of the experiment was 32 ± 0.4 (14.2 ± 1.3 in the control; $P < 0.001$), and the phagocytic intensity was 6.4 ± 0.6 (5 ± 0.7 in the control). In the same experiment, injection of retinoid C_{20} was followed by a tendency for the area of the white pulp of the spleen and the total number of lymphoid follicles and the area of each follicle to increase (Fig. 2). For instance, the area of the white pulp was 21.1 ± 2.4% (11.3 ± 1.8% in the control; $P < 0.001$), the area of one lymphatic follicle was 8.8 ± 1.4 conventional unit (6.3 ± 0.4 conventional unit in the control; $P < 0.05$), and the number of lymphatic follicles in conventional units of area was 3.07 (1.3 ± 0.4 in the control; $P < 0.001$).

In our view these changes reflect the adjuvant properties of the test substances and they develop in the course of expulsion of the damaged erythrocytes. Enhancement of functional activity of the liver macrophages may be linked with phagocytosis of the damaged erythrocytes. The increase in area of the white pulp of the spleen was probably due to the formation of new clones of lymphocytes in response to autoantigenic stimulation. Intensification of DTHR may reflect increasing immunity, based on the increase in number and functional activity of immunocompetent cells.

Injection of RA and retinoids thus causes damage to erythrocytes in mice, and increase in the number of macrophages in the liver, an increase in area of the white splenic pulp, and intensification of the local reaction to turberculin. Changes in the red blood produced by compounds of this group and their adjuvant properties may be interconnected.

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