ACTION OF RETINOIDS ON THE RAT SPLEEN

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The discovery of adjuvant properties in vitamin A and its synthetic analogs (retinoids) has aroused the interest of research workers in compounds of this group as possible stimulators of immunity. The spleen occupies a leading place among organs of the blood system concerned with the formation of immunity. However, its role in the mechanism of the immunostimulating action of vitamin A and retinoids has received little attention in the literature. Isolated communications have shown that, under the influence of retinoids, the lymphatic follicles of the spleen become smaller, and immunocompetent cells become functionally more active [6]. Prolonged percutaneous application of methyl retinoate to mice was accompanied by an increase in the number and phagocytic intensity of macrophages in the spleen [3, 4].

This paper gives the results of microscopic, light-optical, and ultramicroscopic study of changes in the white and red pulp of the spleen in rats receiving retinoids.

EXPERIMENTAL METHOD

Experiments were carried out on 72 male Wistar rats weighing 110 ± 10 g, receiving a single intraperitoneal injection of 1% oily solution (peach oil was the solvent) of 2 and 4 ml of all-trans-methylretinoate (MR) or methyl-7,8-dihydroretinoate (MDHR). The substances were obtained from the Laboratory of Chemistry of Polyene Compounds (Head, Professor G. I. Samokhvalov), "Vitaminy" Research-Production Combine, Ministry of the Medical Industry of the USSR. To label macrophages, on the 10th day of the experiment, 1 h before the animals were killed with ethyl ether, they were given an intramuscular injection of 2 ml of 50% colloidal carbon. The spleen was weighed and fixed for light microscopy in a mixture of a 40% solution of formalin, 96° ethanol, and glacial acetic acid (9:3:1) for 2 h. Paraffin sections were stained with ruthenium red. The area of the lymphatic follicles was determined

TABLE 1. Effect of Single Intraperitoneal Injection of 1% Oily Solution of MR and MDHR on Weight of Spleen, Number and Area of Lymphatic Follicles, Mitotic Activity of Lymphocytes, Phagocytic Intensity of Macrophages, and Number of Macrophages with Superintensive Phagocytosis in Male Wistar Rats on 10th Day of Experiment (M ± m)

Group of animals	Experimental conditions	Weight of spleen, g	Number of follicles in field of vision of light microscope	Area of follicle (M ± m), conventional units	Phagocytic intensity of macrophages	Number of macrophages with superintensive phagocytosis	Mitotic activity of lymphocytes promille
1	Intact animals	1,4±0,4	1,3±0,3 (—1,0)	3,0±0,4 (—1,0)	9,7±0,2	2,3±0,9 (—2,3)	1,1
2	Solvent (oil)	1,6±0,3	2,0±0,4 (—1,5)	3,3±0,5 (—1,1)	11,2±0,03	3,0±1,2 (—2,9)	1,1
	MR:						
3	2 ml	5,0±0,9*	2,6±0,7 (—2,0)	2,5±0,6 (—0,8)	7,7±0,3	3,0±0,6 (—2,9)	2,1
4	4 ml	4,7±0,8*	3,1±0,6 (—2,4)	2,6±0,7 (—0,9)	14,3±0,1*	5,3±0,9 (—5,2)	4,9
	MDHR:						
5	2 ml	4,0±0,3*	2,8±0,8 (—2,2)	2,4±0,6 (—0,8)	21,1±0,8*	17,0±2,0 (—14,5)	2,0
6	4 ml	3,7±0,7**	3,0±0,7 (—2,3)	2,9±0,4 (—1,0)	10,7±0,1	10,3±1,7 (—9,3)	2,2

Legend. *P < 0.001, **P < 0.01 compared with control (5th group of animals). Comparison factor shown in parentheses.

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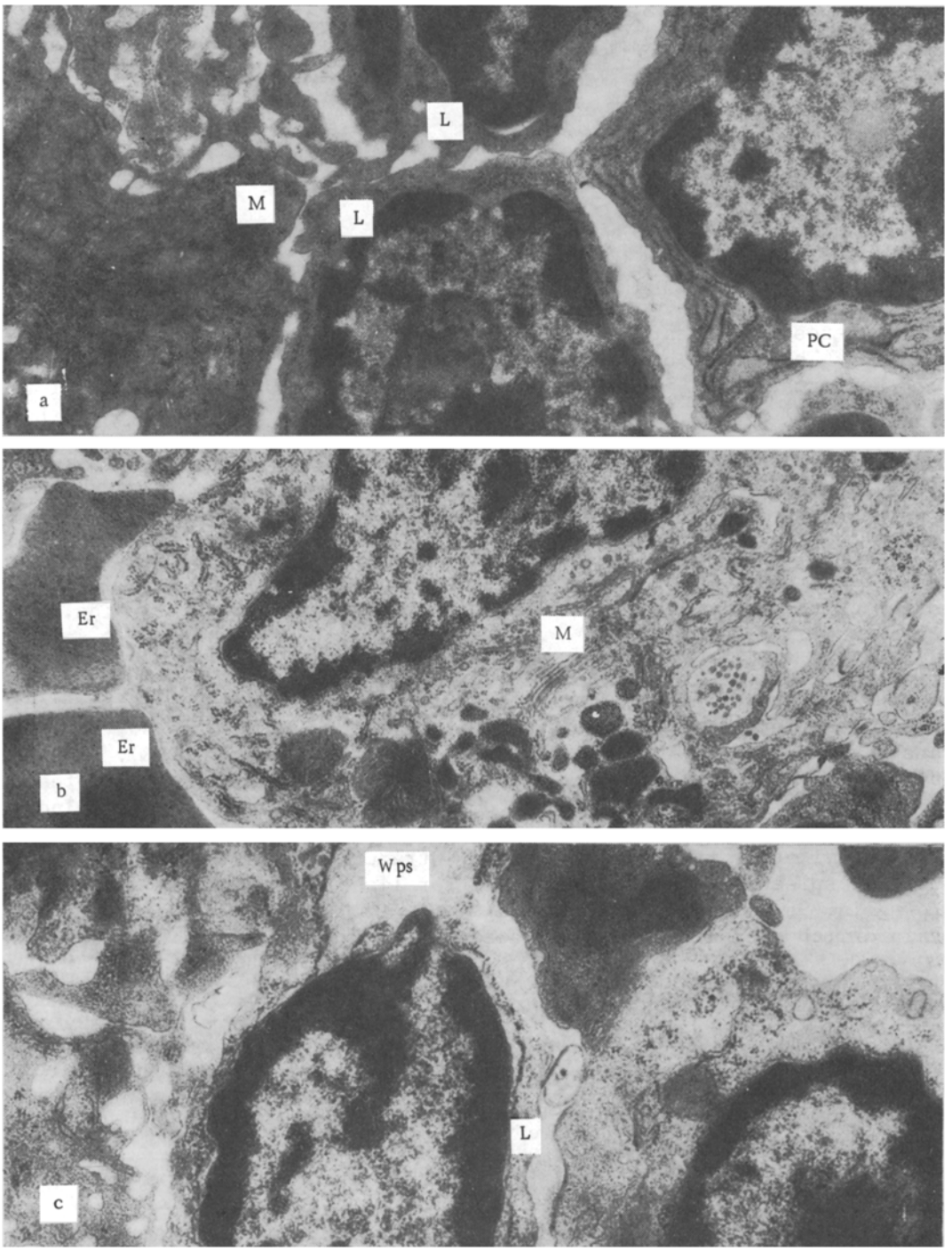

Fig. 1. Rat spleen: intercellular interaction of macrophage, lymphocytes, and plasma cells (a), contact of macrophage with deformed erythrocytes (b), lymphocyte with widened perinuclear space (c); 12 days after single intraperitoneal injection of 2 ml (a, b) and 4 ml (c) of MDHR. M) Macrophage; PC) plasma cell; L) lymphocyte; Er) erythrocyte; Wps) widened perinuclear space. Magnification: a, b) 21,000 ×; c) 30,000 ×.

in conventional units in spleen preparations by projection drawing on squared paper. Only those follicles in which the reactive center, central artery, and marginal and thymus-dependent zones appeared simultaneously in the section were chosen for measurement. The number of lymphatic follicles per unit area was determined by scanning the organ, cut along its long axis, under standard magnification of the microscope. Mitotic activity was counted in promille in preparations of the organ stained with iron-hematoxylin, taking account of cells in prophage, metaphase, and anaphase stages of mitosis. The results were subjected to

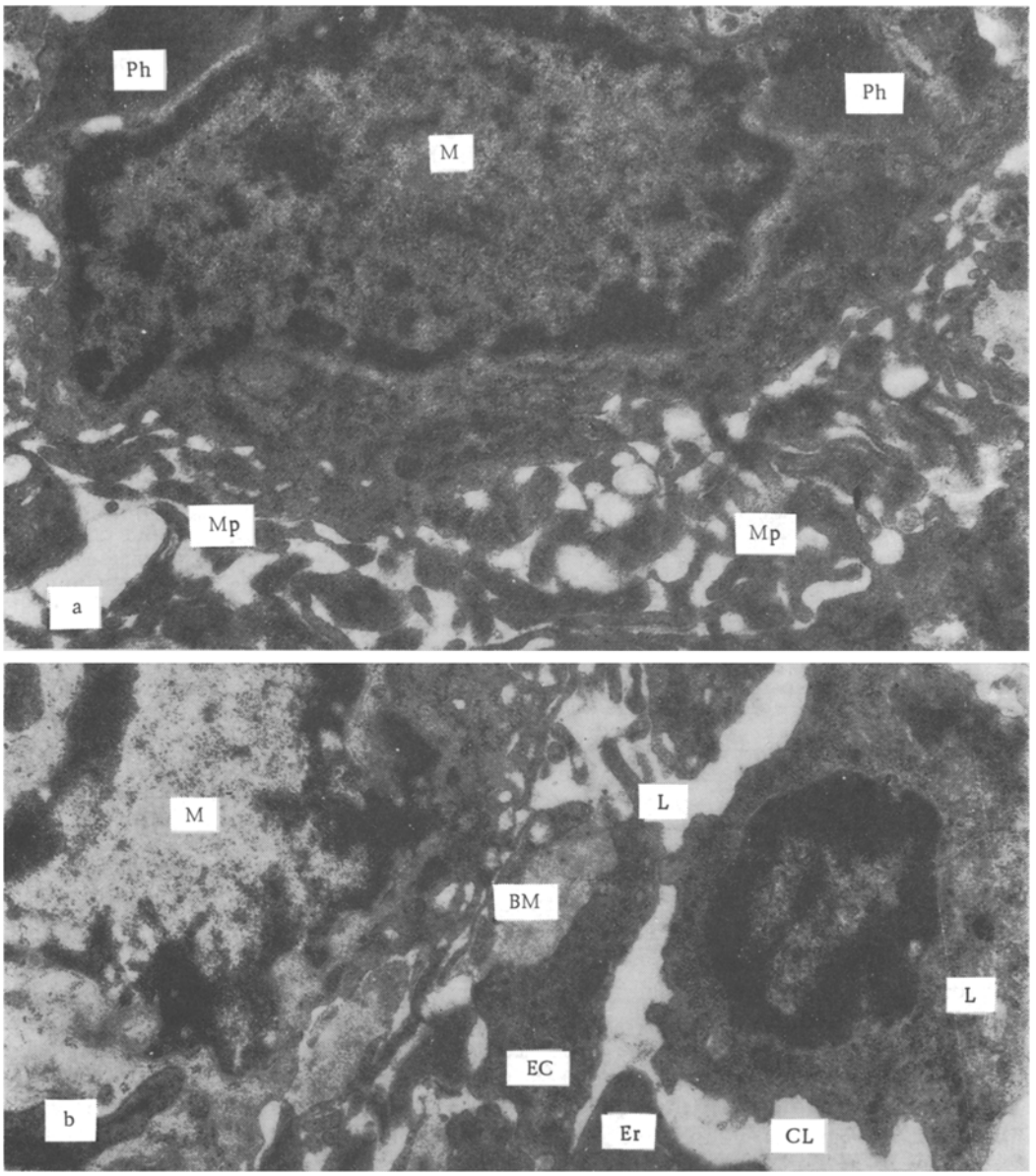

Fig. 2. Rat spleen: macrophage with intensively developed cell periphery (a), fragment of blood capillary (b), 10 days after single intraperitoneal injection of MR (a) and MDHR (b). CL) Capillary lumen; L) lymphocyte; Er) erythrocyte; EC) fragment of cytoplasm of endothelial cell; BM) fragment of basement membrane; P) pore in blood capillary wall; M) macrophage; Ph) phagosome; Mp) microprocesses. 21,000 ×.

statistical analysis. Phagocytic intensity of the macrophages was determined as described previously [3, 4]. Pieces of the organ for electron-microscopic investigation were fixed in glutaraldehyde, postfixed in osmium tetroxide, dehydrated, and embedded in polymerized resins; ultrathin sections were stained and examined in the IEM-100B electron microscope.

EXPERIMENTAL RESULTS

Both retinoids increased the weight of the spleen by two to three times on account of enlargement of both the white and red pulp (Table 1). Injection of the retinoids was accompanied by new lymphoid follicle formation, reflected in a two- to fourfold increase in mitotic activity of the lymphocytes and the appearance of multiple small follicles. The density of macrophages in the visual field did not increase in either red or white pulp, but under the influence of MDHR their functional activity increased considerably, with a resulting increase in phagocytic intensity and in the number of cells with superintensive phagocytosis.

On the basis of the results of the electron-microscopic study, four types of cells were identified in the parenchyma of the spleen. The most numerous type consisted of lymphocytes located in the lumen of the sinusoidal capillaries and outside them in close contact with other cells. Lymphocytes of all types and plasma cells had their characteristic ultrastructure (Fig. 1). Next in size of cell population were the macrophages, with a well-developed branching periphery of the cell. The multiple microprocesses of these cells interwove with each other, interacted with processes of neighboring macrophages, and often surrounded erythrocytes and their fragments and attracted them into the interior of their cytoplasm. With the aid of such processes the macrophages made contact with lymphocytes and other types of cells. The macrophages had a relatively well-developed lysosomal apparatus. Often fragments of erythrocytes could be seen in secondary lysosomes (Figs. 1 and 2a). Endothelial cells (the third type of cells) were located on a thin basement membrane, they had their usual structure, they were loosely connected with each other, they passed blood cells outside the lumen of the vessel and back again, and made contact with macrophages and lymphocytes (Fig. 2b).

The fourth type of cells was erythrocytes. Erythrocytes were arranged singly or in groups, whole or fragmented, with uneven outlines in vacuoles of the macrophages. The types of cells identified above in the parenchyma of the spleen of rats receiving retinoids showed certain distinguishing structural features. The lymphocytes were loosely in contact with each other and neighboring cells. Their developed cell periphery formed microprocesses through which the lymphocytes made contact with each other and with macrophages and plasma cells. The lymphocytes had a whole cell and nucleus of irregular shape, and regions of their perinuclear space were widened, sometimes considerably so (Fig. 1a, b). Macrophages were distinguished by a well-developed cell periphery, with numerous long processes, by multiple contacts with neighboring cells, a well-developed lysosomal apparatus, and marked phagocytic activity (Figs. 1c and 2a). The erythrocytes were often fragmented, with an irregular outline, and with multiple evaginations, invaginations, and vacuoles in the cytoplasm (Fig. 1c). The changes described were similar in character when retinoids were given in a dose of 4 ml, but their intensity was greater.

Taking into account the results of the light-optical study, the data now obtained were interpreted as the ultrastructural reflection of utilization of erythrocytes, damaged by retinoids, by macrophages and activation of lymphocyte–macrophage interaction with subsequent proliferation of lymphocytes. These and other data published previously [1] suggest that when administered in excess, vitamin A and retinoids appear in the blood, organs, and tissues in the free state, and damage the cell membranes. In this situation erythrocytes are among the first and the most numerous cells to be damaged. Lymphocytes and macrophages are responsible for removing the injured cells from the bloodstream and destroying them [2, 5], and their number in the blood and lymphoid organs increases with an increase in the number of cells so utilized. This leads to the accumulation of effector immunocompetent cells.

A single intraperitoneal injection of MR and MDHR in high doses was thus followed by an increase in weight of the spleen, the appearance of erythrocytes of altered shape in it, enhanced phagocytic activity of the macrophages, activation of intercellular interaction, proliferation of lymphocytes, and the formation of new lymphoid follicles.

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