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EFFECT OF APPENDECTOMY ON THE ANTITUMOR EFFECT OF ALL-TRANS-METHYLRETINOATE

V. I. Nozdrin, M. Z. Bakhshinyan, and S. M. Subbotin

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Data have now been obtained on the role of the lymphoid tissue of the appendix in immune defense [4, 11, 13]. There is limited information on the results of statistical estimations of the degree of correlation between a previous appendectomy and the risk of malignant disease in man [5, 8, 9]. According to McVay [10], appendectomy was present in the history of 18.3% of persons dying from carcinoma of the large intestine and 12.6% dying from carcinoma elsewhere.

Vitamin A and its synthetic analogs, the retinoids, have the property of inhibiting growth of tumors mainly of epithelial nature and they have a protective action on epithelium against carcinogens. These compounds also have the property of inducing nonspecific stimulation of the immunocompetent system [3].

The aim of the present investigation was to study changes in the antitumor properties of the retinoid all-trans-methylretinoate (MR) after removal of the appendix, as an organ belonging to the immunocompetent system.

EXPERIMENTAL METHOD

Experiments were carried out on 70 adult mature Wistar rats whose appendix was removed under ether anesthesia. For this purpose an incision was made in the skin and abdominal wall measuring 2-2.5 cm in length on the left side of the midline. The appendix was removed and the stump ligated. The wound was closed in layers. Control animals underwent a laparotomy at the identical spot, followed by suture of the abdominal wall and skin. A carcinoma RS-1 was inoculated subcutaneously into the animals 15-18 days after the operation. The tumor was injected as a cell suspension in medium No. 199 in the ratio of 1:3 and in a dose of 0.5 ml. The day of inoculation of the tumor was counted as the beginning of the experiment. Changes in the dynamics of tumor growth were judged by reference to the carcinosomatic index (CSI), determined as the ratio (in %) between the weight of the tumor and the animal's body weight together with the tumor on the day of sacrifice.

A 1% oily solution of MR was used as the retinoid (the preparation was obtained from the Laboratory of Chemistry of Polyene Compounds, "Vitaminy" Scientific Production Combine, Ministry of the Oil Industry of the USSR), which was injected intraperitoneally in a dose of 0.5 ml once a week for 1 month. The effect of appendectomy on the CSI of carcinoma RS-1 was studied in a control experiment lasting 23 days. The animals were given an intraperitoneal injection of 2 ml of 50% colloidal carbon 2 h before sacrifice. Immediately before sacrifice

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TABLE 1. Effect of Appendectomy on Ratio between Areas of Cortex and Medulla of Thymus following Injection of MR into Animals

Experimental conditions	Area of lobule, conventional units	Ratio of area of cortex to total area of lobule, %
Carcinoma RS-1 + appendectomy	156±3,5 ($P<0,05$)	64,6
Carcinoma RS-1 + laparotomy	108±8,6	65,7
Carcinoma RS-1	124±12,6	—

TABLE 2. Effect of Appendectomy on Number of Follicles, Their Area, and Number of Plasma Cells in Red Pulp of Spleen following Injection of MR into Animals

Experimental conditions	Number of follicles	Area of follicles, conv. units	Number of plasma cells
Carcinoma RS-1 + appendectomy	2,1±0,3 $P<0,01$	10,3±0,4 $P<0,001$	19,1±0,3
Carcinoma RS-1 + laparotomy	1,2±0,15	14,6±0,5	20,1±0,1
Carcinoma RS-1	1,6±0,1	11,6±0,4	19,0±0,2

TABLE 3. Effect of Appendectomy on Relative Area of Paracortical and Follicular Zones and on Absolute Area of Follicles of Mesenteric Lymph Node following Injection of MR into Animals

Experimental conditions	Area of paracortical zone, %	Area of follicular zone, %	Area of follicles, conventional units
Carcinoma RS-1 + appendectomy	43,7 $P>0,05$	14,7 $P>0,05$	39,1±1,3 $P>0,05$
Carcinoma RS-1 + laparotomy	45,5	18,1	41,6±6
Carcinoma RS-1	38,8	23,6	47,9±1,6

the animals were weighed, and the tumor was weighed before fixation. The animals were killed with chloroform on the 30th day of the experiment. The thymus, spleen, mesenteric lymph node, liver, and lung were fixed in a mixture of 40% neutral formalin, 96° ethanol, and glacial acetic acid in the ratio of 9:3:1. Paraffin sections were stained with the routine dyes.

By means of an ocular micrometer the area of the cortex and medulla of the thymus were measured in sections. In sections of the spleen the mean area of the lymphoid follicles and their number per field of vision of the light microscope (objective 90, ocular 10) was counted. In sections of the lymph node the mean area of the lymphoid follicles and the ratio between the follicular and paracortical zones and the medulla were determined. In sections of the liver, spleen, lung, and mesenteric lymph nodes the mean number of macrophages labeled with carbon was counted. In addition, phagocytic activity of the macrophages was determined in sections of the spleen by counting the mean number of carbon granules in one cell and also the number of cells with exceptionally intensive phagocytosis, in which the number of granules could not be counted because of their high concentration. The mean number of plasma cells also was counted in the same preparations stained with methyl green and pyronine. The morphometric analysis was carried out under standard magnification of the microscope. The results were subjected to statistical analysis and were expressed in conventional units.

TABLE 4. Effect of Appendectomy on Number of Macrophages in Lung, Liver, Mesenteric Lymph Node, and Spleen and also on Phagocytic Intensity (PI) and Number of Cells with Exceptionally Intensive Phagocytosis (EIP) in Spleen following Injection of MR into Animals

Experimental conditions	Lung	Liver	Mesenteric lymph node	Spleen	PI	EIP, %
Carcinoma RS-1 + appendectomy	1,1±0,02	4,9±0,2	0,7±0,04	1,8±0,03	8,2±0,3	6,5
Carcinoma RS-1 + laparotomy	1,3±0,02	6,5±0,1	1,3±0,04	2,4±0,05	15,2±0,2	21,6
Carcinoma RS-1	1,2±0,02	7,2±0,1	1,5±0,06	2,8±0,08	16,0±0,5	18,2

EXPERIMENTAL RESULTS

Removal of the appendix reduced the survival period of the animals by 15% and, compared with laparotomy, increased the rate of growth of the tumor. For instance, on the 23rd day of the experiment CSI for intact animals inoculated with carcinoma RS-1 was 3.9 ± 0.4%, in the laparotomized animals it was 5.1 ± 0.4%, and in the appendectomized animals 8.4 ± 0.8% ($P < 0.01$). Administration of MR was followed by inhibition of tumor growth but did not change the tendency. For instance, in intact animals with carcinoma RS-1, CSI on the 30th day was 1.7 ± 0.2%, in the laparotomized rats it was 2.0 ± 0.2%, in the appendectomized rats 3.1 ± 0.4% ($P < 0.05$); the survival rate of the animals in this case fell to 80%.

The results thus indicate that the presence of an appendix, together with the other lymphoid organs, is probably an essential condition for inhibition of tumor growth by the immune system. Incidentally laparotomy accelerated tumor growth, although to a less marked degree. The functional strain on the immune system due to the necessity to regulate regeneration evidently deflects part of the effector components of the immune defense system from their participation in the restraining of tumor growth. A similar effect of laparotomy on tumor growth was observed by other workers [14].

Appendectomy led to a significant increase in the size of lobules of the thymus. The ratio of cortex to medulla of the lobule was unchanged (Table 1), evidence of a disturbance of the recirculation of lymphocytes with delay in their departure from the organ. Appendectomy led to an increase in the number of lymphoid follicles in the spleen and a small decrease in their area. The number of plasma cells in the red pulp was unchanged under these circumstances (Table 2). A decrease in the absolute and relative areas of the lymphoid follicles was observed in the mesenteric lymph node after removal of the appendix (Table 3). This correlates with the notion of the appendix as the site of formation of precursors of plasma cells producing IgM [7].

There is evidence in the literature that the appendix is connected with the repopulation of the periarteriolar zones in the spleen in thymectomized mice [12]. At the same time, we know that retinoic acid and its analogs stimulate proliferative and functional activity of T lymphocytes [6]. It is therefore possible that appendectomy led to a decrease in the number of T lymphocytes as the point of application of the adjuvant action of the retinoid, and that this at least partly determined the reduction in the antitumor effect. This explanation is supported also by the fact that the paracortical zone of the mesenteric lymph node, containing a large concentration of T lymphocytes, did not respond to the action of retinoids in appendectomized animals, although in intact animals an increase in the accumulation of these cells was observed in response to injection of the retinoid [3].

Removal of the appendix was accompanied by a decrease in the number of macrophages which had ingested particles of ink in the lungs, liver, spleen, and mesenteric lymph node (Table 4). In the spleen, moreover, a decrease was observed in the phagocytic intensity of the macrophages.

The results thus showed that removal of the appendix is accompanied by acceleration of growth of carcinoma RS-1 and a decrease in the ability of MR to restrain tumor growth. Under these conditions administration of the retinoid led to functional changes in the lymphocytes and macrophages and was accompanied by quantitative changes in these cells in the thymus, spleen, mesenteric lymph nodes, liver, and lungs.

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