MORPHOLOGICAL CHARACTERISTICS OF MILK SPOTS OF THE RAT OMENTUM DURING INFLAMMATION

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The greater omentum in man and animals can actively produce antibodies in response to intraperitoneal injection of an antigen. This fact correlates with the presence in the omentum of milk spots (MS), whose cells produce antibodies, as has been shown by various methods [1, 4]. The principal cells of milk spots are macrophages and lymphocytes. Nowadays MS are regarded by some investigators as an anatomically and functionally organized system, which they call the lymphoid organ of the omentum [3]. Since the structure and function of MS of the omentum has not been adequately examined in the literature, and since there is virtually no information about cellular proliferation in the organ, the aim of this investigation was a morphological and functional study of MS of the omentum in response to their stimulation by an inert material (carbon) and by a biologically active polysaccharide lamellae (zymosan). Carbon and zymosan, injected into the peritoneal cavity, induce aseptic inflammation, and zymosan also, being a biologically active polysaccharide, stimulates macrophages.

EXPERIMENTAL METHOD

Experiments were carried out on 60 male Wistar rats with an average weight of 120-140 g. The rats were divided into four groups: 1) control group, receiving 1 ml of physiological saline; 2) animals receiving an intraperitoneal injection of finely dispersed coal particles measuring less than 5 μm in a dose of 50 mg/ml, suspended in 1 ml of physiological saline. These animals were killed 2, 7, 16, and 30 days later. Rats of group 3 were given an intraperitoneal injection of 2 ml of 0.1% zymosan and were sacrificed 2 and 7 days later. Rats of group 4 received coal dust by intraperitoneal injection in a dose of 50 mg/ml and these animals were killed after 7, 16, and 30 days. They were given an injection of 2 ml of 0.1% zymosan 7 days before sacrifice. To determine their mitotic activity, 3 h before sacrifice the animals were given an intraperitoneal injection of a solution of colchamine in a dose of 5 mg/kg. To evaluate the fraction of DNA-synthesizing cells, 1 h before sacrifice the animals were given an injection of ^{3}H-thymidine in a dose of 40 MBq/g body weight. The greater omentum of the rats was removed and fixed in Carnoy's fluid. The length and width of MS were calculated by means of an ocular micrometer, but the area only of circular MS was determined. Histological sections and films of peritoneal exudate were stained with hematoxylin and eosin and with methyl green and pyronine by Brachet's method, while semithin sections were stained with toluidine blue and examined under the light microscope. A "Hitachi-12A" instrument was used for the electron-microscopic study of ultrathin sections. To determine the index of C-mitoses (MI_{colch}) and the index of labeled nuclei (RI), all MS were examined in serial sections. Values of MI_{colch} and RI were expressed in promille. The numerical results were subjected to statistical analysis by the Fisher–Student method.

EXPERIMENTAL RESULTS

The histological study showed that besides macrophages and lymphocytes, and mast cells. In the control the area of the circular MS varied from 228 μm^2 to 0.4 mm^2, the mean length of the elongated MS was 0.424 ± 0.050 mm, and their width 0.288 ± 0.053 mm. The results of the study of MI_{colch} and RI in the control and experimental animals are given in Table 1. Mitoses were found extremely rarely in films of peritoneal exudate, and for that reason only labeled nuclei were counted (Table 1). Injection of coal particles induced an inflammatory reaction in the peritoneal cavity with an increase in the area of MS 2 days

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TABLE 1. MI_{colch} and RI in MS of Rat Omentum and RI in Peritoneal Exudate in Different Experimental Groups

Group of rats	MI_{colch} in MS, %	RI in MS, %	RI in peritoneal exudate, %
1-	5,9±0,8	36,7±8,4	15,8±3,3
2-	1,0±0,2**	21,4±4,7	22,1±10,0
3-	12,4±3,5**	21,2±3,7	46,0±10,3*
4-	3,7±0,5	33,9±8,7	42,6±9,6*

Legend. *$p < 0.05$ Compared with control, **$p < 0.05$ compared with other experimental groups.

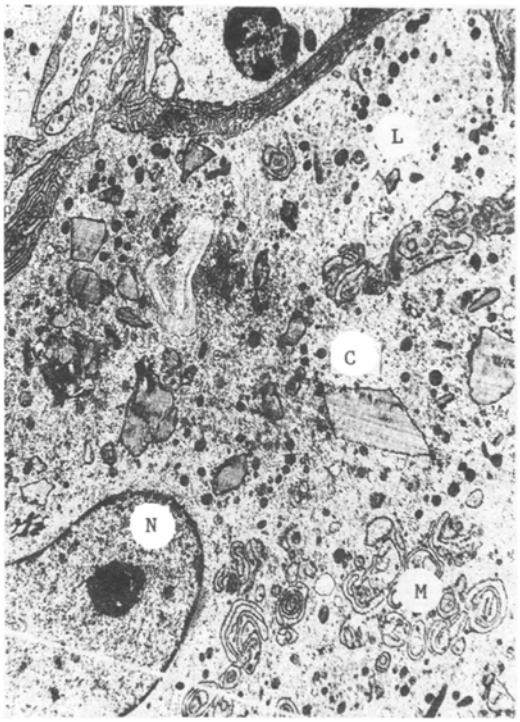

Fig. 1. Cytoplasm of giant cell with ingested coal particles. N) Nucleus, L) lysosomes, C) coal particles, M) membranes. 5000×.

after the beginning of the experiment. After 14 days the area of their circular forms attained a size of between 0.2 and 0.8 mm, and the length and width of the elongated MS were increased (0.702 ± 0.08 mm and 0.511 ± 0.04 mm, $p < 0.01$). The increase in the size of MS was accompanied by an increase in the number of macrophages and, to a lesser degree, of lymphocytes and polymorphonuclear leukocytes. Macrophages with ingested coal particles could be seen in the peritoneal cavity and also in MS. On electron-microscopic investigation coal particles, of varied geometric shapes, were found lying freely in the cytoplasm of the macrophages (Fig. 1). After 30 days nodules consisting of macrophages appeared in MS. These were mature phagocytic cells, occasionally containing the label, and preserved particles of ingested material could be seen in their cytoplasm. Giant macrophages with numerous small lysosomes appeared around the unphagocytosed particles. These cells cooperated to form a multinuclear cell, containing numerous interdigitating membranes. As the coal particles accumulated they were encapsulated within MS, which underwent fibrosis and involution. It will be noted that MI_{colch} fell 2 days after injection of the coal particles and remained unchanged thereafter for more than 7 and 14 days, but the decrease in RI was not significant.

In animals killed 2 days after injection of zymosan alone, the area of the circular MS also was increased compared with the control from 0.3 to 1.02 mm^2; the mean length and width of the elongated MS wa 0.627 ± 0.06 and 0.463 ± 0.05 mm respectively, $p < 0.05$. The area of MS after 7 days was unchanged, but their number was increased. The number of nodules

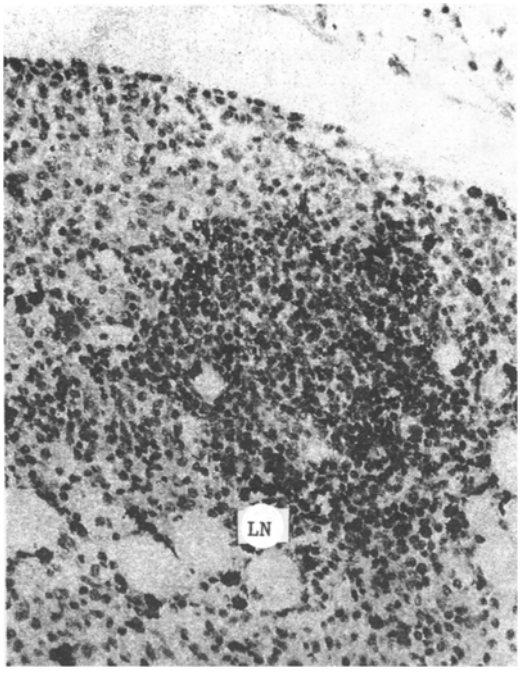

Fig. 2. Lymphoid follicle-like structure of MS. LN) Nuclei labeled with ^{3}H-thymidine. 100×. Stained with hematoxylin and eosin.

formed by macrophages with different degrees of maturity was increased. Lymphocytes were concentrated around them, mixed with many lymphoblasts and plasma cells. Sometimes the concentrations of lymphocytes resembled primary lymphoid follicles, but no germinative centers could be found in them (Fig. 2). The parameters of proliferation in MS did not differ significantly from the control. RI was increased only in peritoneal exudate cells.

Administration of zymosan to animals receiving intraperitoneal injections of coal particles led to the greatest increase in size of MS. After 14 days the area of the circular MS varied from 0.2 to 5 mm^2. Compared with injection of coal particles alone, in this case there was a noticeable inflow of large numbers of monocytes and lymphocytes. Hypertrophy of the organelles and the appearance of numerous phagolysosomes and secondary lysosomes were observed in the large, branching macrophages. The number of macrophagal nodules in this case was greater than after injection of coal particles alone. Coal particles lying freely in the form of geometric figures could be seen in the cytoplasm of the macrophages. After 30 days giant macrophages and multinuclear cells, whose nuclei were arranged in the form of a crown around the periphery of the cytoplasm (Fig. 3), appeared in MS. In their structure they resembled giant multinuclear cells of the tuberculous granuloma. Investigation of the proliferative activity showed that MI_{colch} was lower after 14 days than after administration of zymosan, but higher than after injection of coal particles, whereas RI did not differ from its value in the other groups.

This investigation thus did not reveal positive correlation between the level of cell proliferation in MS and variations in their size. The considerable increase in size and number of MS was not accompanied by increased proliferative activity in any series of the investigation, but in the group receiving an injection of coal particles, on the contrary, proliferation was reduced, due to an increase in the functional load on the phagocytic cells. These data are in agreement with conclusions with the effect that the cellular composition of MS varies depending on the state of the peritoneal cavity, and the size and number of MS are increased on account of an inflow of cells from the peritoneal cavity and bloodstream [1]. Under conditions of inflammation, an active inflow of monocytes and lymphocytes was observed and was particularly marked in the 3rd and 4th series of experiments. Zymosan is known to accelerate renewal of the macrophagal population on account of an inflow of monocyte-like cells from the bone marrow [2]. The increase in the number of immature phagocytes and also of lymphocytes in the present experiments also evidently took place on account of an inflow of these cells from the blood and bone marrow. Administration of zymosan also had a stimulating effect on phagocytic activity of the macrophages, as shown by hypertrophy of their intracellular organelles and the appearance of secondary lysosomes and autophagosomes. Zymosan-induced inflammation leads to the formation of macrophagal granulomas in the liver, lungs, and spleen [2]. The appearance of numerous macrophagal nodules resembling granulomas, and also of giant multinuclear cells, in MS can be regarded as a response of the macrophages to inflammation. According to ob-

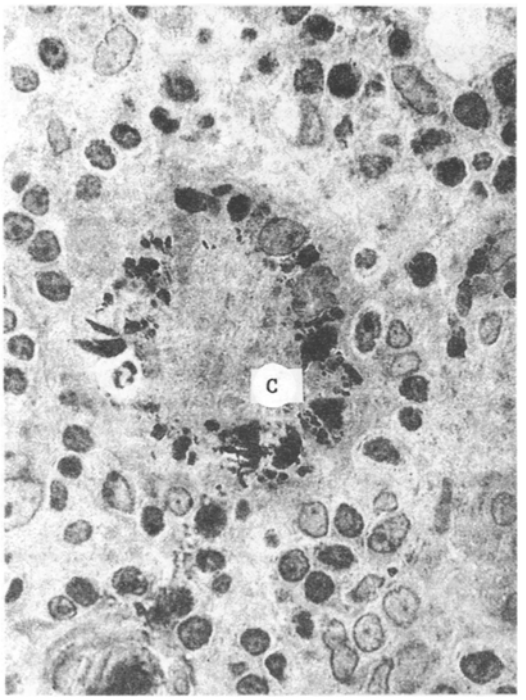

Fig. 3. Giant multinuclear cell with ingested coal particles. C) Coal particles. Semithin section. 625×. Stained with toluidine blue.

servations made by several investigators, the giant multinuclear cell is the final stage of differentiation of the monocyte [5]. MS of the omentum must be regarded not simply as depots for migrating mononuclear phagocytes, but also as the site of their maturation. Lymphoid structures found after injection of zymosan resemble the primary lymphoid follicles observed in lymph nodes after antigenic stimulation. Despite the absence of germinative centers, the appearance of a large number of lymphoblasts and plasma cells is evidence of active antibody formation, aimed at protecting the peritoneum. Thus the investigation demonstrates the important role of MS of the omentum in the immunologic protection of the peritoneal cavity.

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