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CORRELATION BETWEEN MAST-CELL AND LEUKOCYTIC ACTIVITY AND PERMEABILITY OF MESENTERIC VENULES IN RATS WITH EXPERIMENTAL PERITONITIS

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Disturbance of permeability of the walls of microvessels, manifested as increased outflow of protein into the tissues through the walls of venules, is one of the most obvious signs of inflammation in the early stages of its development [2]. An important role in the increase of permeability is played by mast cells and also, perhaps, by leukocytes, a change in the functional state of which in the early phase of inflammation is a well known phenomenon [13]. These cell groups produce factors which are interpreted as the most active "permeability mediators." They include histamine and active forms of oxygen [12, 14].

The aim of this investigation was to assess dependence of increased protein transport from blood into tissue in the region of inflammation on the level of functional activity of mesenteric mast cells and on adhesion of leukocytes circulating in the mesentery.

EXPERIMENTAL METHOD

Experiments were carried out on albino rats weighing about 300 g. Experimental peritonitis was induced in the animals under ether anesthesia, by irrigating the surface of the mesentery with a filtrate of contents of the large intestine [3]. The peritoneal cavity was closed without drainage. Observations began 1, 2, 4, and 8 h after induction of peritonitis. The state of the mast cells and leukocytes was analyzed by intravital microscopy: under pentobarbital (50 mg/kg) anesthesia laparotomy was performed, and a loop of small intestine with segments of the mesentery were laid on a special stage, irrigated continuously with warm (37°C) Hanks' solution (pH 7.4), and examined in a "Leitz" intravital microscope under ordinary and luminescent illumination. Adhesion of leukocytes to the luminal surface of the venular endothelium was evaluated by counting the number of cells in a 100-μ length of a vessel about 50 μ in diameter [4]. Next, the mast cells

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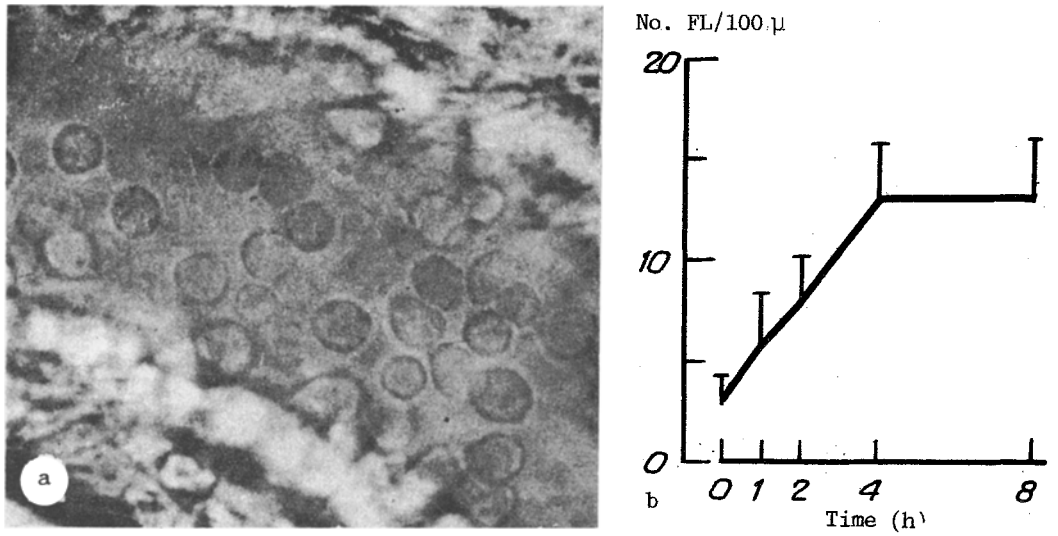

Fig. 1. Adhesion of leukocytes to walls of mesenteric venules during inflammation. a) Group of cells fixed to luminal surface of vessel; b) change in number of fixed cells after induction of peritonitis. Ordinate, number of fixed leukocytes per 100 μ length of vessel; abscissa, here and in Figs. 2 and 3, time (in h).

were stained by the method in [15], modified for intravital microscopy. The fluorescent dye berberine sulfate in a concentration of 0.02% (pH 4.0), was applied to the surface of the mesentery for 1 min, after which the object was photographed on RF-3 film. To study permeability of the venules FITC-albumin was injected into the animals' blood stream in a dose of 25 mg/100 g body weight, and 20 min after the beginning of the injection the mesentery in situ was fixed with liquid nitrogen; freeze-dried total preparations of the mesentery were then made [1].

EXPERIMENTAL RESULTS

With high enough resolution (objective 55×) leukocytes were clearly visible in the luman of the venules (Fig. 1a). They rolled slowly along the inner surface of the vessel in the direction of the blood flow, and some of the cells were fixed quite strongly to the endothelium. In the control the average number of fixed cells was 2.9 ± 0.8/100 μ. Since the number of these fixed cells may increase spontaneously during prolonged exposure of the mesentery (over 20-30 min), the period of observation in these experiments did not exceed 10-20 min.

During the development of inflammation the juxtamural pool of strongly adherent leukocytes increased gradually. A tendency to increase appeared as early as the end of the first hour after induction of peritonitis (Fig. 1b). After 4 h the number of "adherent" leukocytes reached its peak (13.3 ± 2.5/100 μ), and later it remained virtually unchanged. By this time a state of some kind of equilibrium has evidently become established between the number of cells fixed to the endothelial surface and the number of them which have migrated into the perivascular tissue. As inflammation of the peritoneum develops, ever-increasing leukocytic infiltration of the mesenteric tissue can be observed.

Mast cells were quite numerous in the mesentery of the rat's small intestine. They were distributed more or less uniformly in the tissue, but in a few cases a characteristic concentration of cells could be seen near vessel walls of venular type (Fig. 2a). Under high power degranulation of the cells could be reliably identified. In the "resting" state the fluorescent cells had quite clear outlines and were oval in shape (Fig. 2b). During degranulation a marked change took place in the shape of the cells (Fig. 2c). Complete degranulation led to the formation of a fluorescent spot, indistinctly outlined and consisting of the granules of a mast cell. In the control the total number of mast cells in an area of 1 mm was 187 ± 43. The fraction of cells with clear signs of degranulation did not exceed 9-10% (Fig. 2d). The number of such cells 1 h after the beginning of experimental peritonitis was a little greater than in the control (16.9 ± 4.9 and 9.2 ± 3.5% respectively); later (after 8 h) the total number of these cells in the mesentery fell, whereas the fraction of degranulated forms remained comparatively high, but constant.

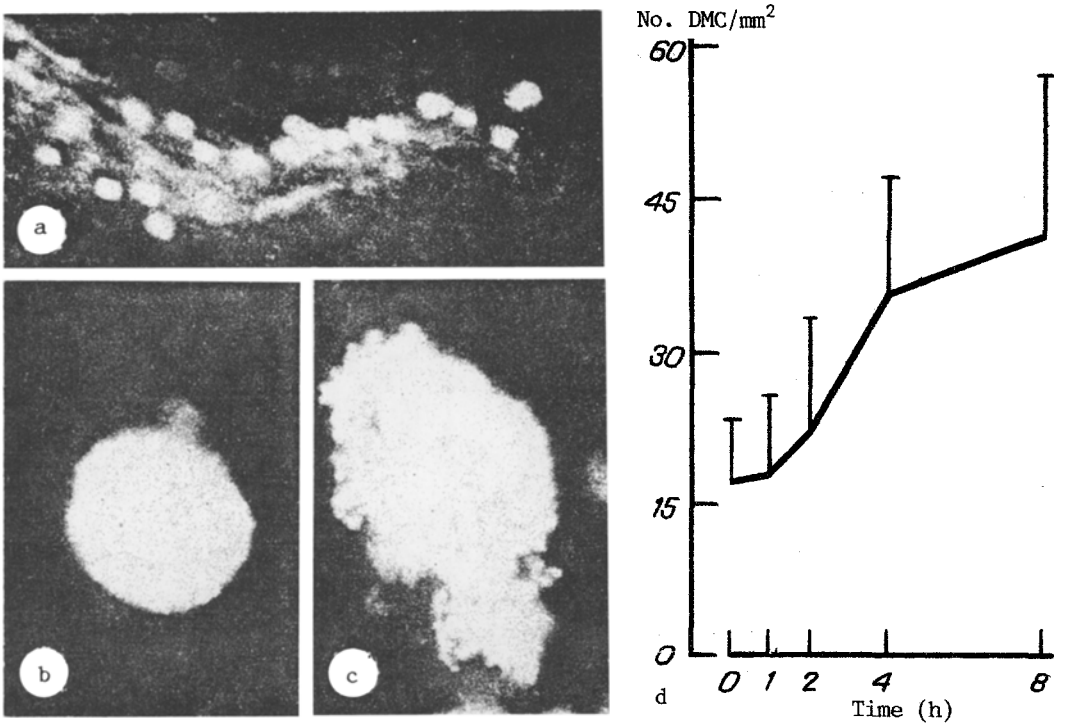

Fig. 2. State of mesenteric mast cells during inflammation. a) Characteristic localization of cells near wall of venule; b) mast cell with no signs of degranulation; c) release of granules into surrounding tissue; d) increase in number of degranulating cells during development of inflammation. Ordinate, number of degranulating mast cells (DMC) per 1 mm area.

The study of freeze-dried material confirmed a previously ascertained fact: the outflow of protein from blood into tissue takes place mainly through the walls of vessels of venular type.

Regions of intensive transport could be identified starting from postcapillaries about 10 μ in diameter and ending with venules of collecting type (50 μ or more) (Fig. 3a). The appearance of FITC-albumin was not observed in the walls of arterioles and arterial segments of capillaries in the control. The number of "leaking" vessels was virtually unchanged 1 h after the beginning of peritonitis (Fig. 3b). Later the number of such vessels rose appreciably. By 4 h after the beginning of inflammation the fraction of venules permeable for protein reached its peak of 12.0 ± 2.25%. After 8 h, however, the number of these vessels decreased, although it remained higher than in the control (9.0 ± 2.7 and 3.45 ± 1.5% respectively). By this time signs of disturbance of the peripheral blood flow appeared quite clearly: some parts of the mesenteric vascular bed were difficult to distinguish in the field of vision, because of the absence of tracer in the vessels. Incidentally, slowing and complete arrest of the blood flow were observed during this period on intravital microscopy also. Conglomerates consisting of aggregated platelets, leukocytes, and erythrocytes, which partly or completely concealed the blood flow, could be observed in the lumen of some venules.

The results of this investigation show that the dynamics of the functional state of the leukocytes during the development of inflammation has characteristic differences from changes in mast cell activity. An increase in the number of leukocytes fixed to the walls of venules was observed virtually immediately after the induction of peritonitis, whereas changes in the mast cell population were considerably delayed. During the first 4 h the number of active forms of leukocytes increased at a constant rate (the slope of the curve relative to the abscissa did not change). During this same period the rate of appearance of new mast cells with evidence of granulation rose gradually. The increase in the number of "leaking" vessels also followed a similar pattern. This suggests that during the first few hours of this model of inflammation the principal role in disturbance of the transport function of the vessel walls is played by mast-cell activation products. The free histamine level in the tissue is known to depend on the degree of degranulation of the mast cells [11], and the morphological basis for the disturbance of vascular permeability during its action is reversible reactive changes in the endothelium [10]. Later, when the number of leukocytes fixed to the walls of the venules reaches a certain peak value,

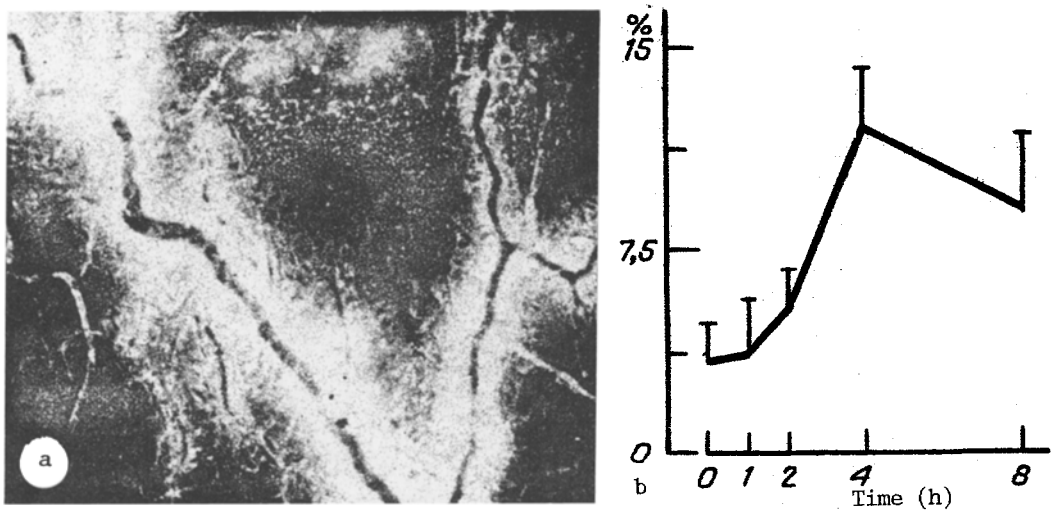

Fig. 3. Disturbance of mesenteric vascular permeability for protein in peritonitis. a) Appearance of FITC-albumen near venules 20 min after injection of tracer into blood stream; b) changes in number of "leaking" vessels in early stages of development of peritonitis. Ordinate, number of "leaking" vessels (in %).

factors of a different kind evidently begin to acquire supreme importance in the genesis of permeability disturbances. Metabolites of activated leukocytes (free oxygen radicals and hydroperoxides [7]) exert a marked cytotoxic action, including on endothelial cells [14]. Destructive changes caused by them in endotheliocytes and platelets, and also the appearance of lipid peroxidation products, are evidently the initial stages of thrombus formation [6]. At this stage of the development of inflammation we therefore observed a decrease in the number of "leaking" vessels due to the exclusion of part of the microvascular bed from the blood flow. Of course the endothelium of the venules is not the only, although for various reasons it is probably the main, target for the action of activation products of mast cells and leukocytes. These reasons include specific features of the organization of the venular endothelium, namely the comparatively large number of histamine receptors on its surface [8]. In addition, it is in the region of the venules that concentrate those activated leukocytes which, in the process of inflammation, become fixed to the walls of these vessels and migrate into the tissue. Under these circumstances not only the endothelium, but also the mast cells, which lie next to the vessels, must be exposed to the action of active forms of oxygen and release their granules into the surrounding tissue. Those components of the granules that can activate leukocytes may perhaps also pass into the surrounding medium [9]. Consequently, the functional state of the mast cells and leukocytes during inflammation largely depends on their interaction.

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