Role of Leukocytes in Increased Vascular Permeability in Inflammation

N. A. Klimenko and E. A. Pavlova

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Carrageenan-induced acute aseptic peritonitis was used as a model of inflammation. In rats with irradiation-induced leukopenia, the immediate and delayed phases of increased vascular permeability in the inflammatory focus were mediated by leukocytes. In the immediate phase, lysosomal enzymes, reactive oxygen species, and cyclooxygenase and lipoxygenase metabolites of arachidonic acid are involved in the regulation of vascular permeability, while in the delayed phase, lysosomal enzymes and lipoxygenase derivatives play the major role. Dexamethasone sharply inhibits vascular permeability in both phases.

Key Words: *inflammation; vascular permeability; leukocytes; dexamethasone*

Our previous experiments showed that irradiation- or vinblastine-induced leukopenia modulates vascular permeability (VP) in the inflammatory focus during the exudative phase by reducing the immediate phase (IP) and delayed phase (DP) of its increase. However, in the late exudative phase, VP was higher than during natural inflammation. These changes in VP correlated with leukocyte count in the inflammatory focus and peripheral blood [5,8].

Here we studied the role of leukocytes in the regulation of VP during inflammation. VP in the inflammatory focus was determined in IP and DP of its increase during the recruitment of blood leukocytes in leukopenic animals and the use of lysosomal proteinase inhibitors, reactive oxygen species, cyclooxygenase, and lipoxygenase. The effects of dexamethasone on VP in the inflammatory focus were evaluated.

MATERIALS AND METHODS

Experiments were performed on 72 male Wistar rats weighing 180-200 g. Acute aseptic peritonitis induced by intraperitoneal injection of 5 mg λ-carrageenan (Sigma) in 1 ml isotonic NaCl was used as a model of inflammation [6]. Four days before peritonitis, leukopenia was induced by 5.5 Gy irradiation (0.442 Gy/min) on a RUM-17 device [8]. A leukocyte suspension (1 ml, 3×10^8cell/ml) was administered intravenously 2 h before modeling of acute peritonitis to replenish blood leukocytes in irradiated rats [13]. Leukocytes were isolated from the blood of intact rats using a Ficoll-Urotrast density gradient (1.073-1.079 g/ml).

Contrical, a lysosomal proteinase inhibitor, was injected intraperitoneally in doses of 10^4 U/kg and 6×10^4 U/kg 12 h and 30 min before modeling of acute peritonitis, respectively [1]. α-Tocopherol acetate (50 mg/kg) was injected intramuscularly 4 days before the experiment and 30 min before carrageenan injection to scavenge reactive oxygen species ($O_2^{\bullet}$ and $OH^{\bullet}$) and lipid peroxides [3]. The cyclooxygenase inhibitor indomethacin (5 mg/kg) was administered perorally 1 h before the experiment. Tween 80 served as the solvent [1]. The lipoxygenase inhibitor quercetin (100 mg/kg) was administered perorally 1 h before the experiment [10].

Dexamethasone (500 mg/kg) was injected intramuscularly 2.5 h before simulation of peritonitis [2].

Trypan blue (1%) was administered intravenously in a dose of 5 ml/kg 5 min before decapitation, and VP was determined by colorimetry of peritoneal lavage [5]. Peak VP was studied during IP and DP of its increase (15 min and 5 h after injection of carrageenan,

Department of Pathophysiology, Khar'kov State Medical University

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respectively) [5,8]. Peritoneal lavage was carried out with 5 ml isotonic NaCl containing 5 U/ml heparin.

RESULTS

The replenishment of blood leukocytes in leukopenic rats modulated both phases of VP increase in the inflammatory focus: during DP, it practically did not differ from that observed in natural inflammation, and 1.3-fold surpassed VP in irradiated rats during inflammation. IP was restored to a lesser extent, being 1.8-fold below this parameter in natural inflammation, and tended to exceed VP in irradiated rats 1.45-fold (Fig. 1).

Contrical inhibited the increase in VP during IP and DP by 1.3 and 1.4 times, respectively. Tocopherol and indomethacin 1.2-fold inhibited IP in comparison with natural inflammation. Quercetin reduced IP and DP by 1.6 and 1.5 times, respectively (Fig. 1).

Our findings confirm the major role of leukocyte-derived factors in DP of the VP increase. However, leukocytes are also involved in IP of this process.

The involvement of leukocytes in DP (correlating with their accumulation in the inflammatory focus [5,8]) and IP of the increase in VP is clear. Activation of leukocytes begins in the blood flow and results from their chemotactic stimulation. Margination of leukocytes develops after their contact with the inflammatory agent. Migrating leukocyte passes through the endothelium over 2-12 min. The number of leukocytes in the inflammatory focus considerably increases as soon as on the 10th min of inflammation [12,15]. It can be assumed that VP in inflammation is regulated by migrating, rather than migrated leukocytes, since the inflammatory focus contains primarily depleted cells, which undergo further degranulation and elimination.

These data indicate that lysosomal enzymes, reactive oxygen species, and cyclooxygenase and lipoxygenase metabolites of arachidonic acid are involved in the leukocyte-mediated regulation of VP during IP of its increase. In DP, lysosomal enzymes and lipoxygenase derivatives play the major role in this process.

The fact that tocopherol modulates only IP of a VP increase are probably due to short life-span of reactive oxygen species. Considering significant individual variations of this parameter, effects of reactive oxygen species on VP are most pronounced when the rate of leukocyte accumulation in the inflammatory focus is sufficiently high (the first 2 h of inflammation). Cyclooxygenase products induce arterial hyperemia and play an important role in IP. Lipoxygenase products (leukotrienes C_4, D_4, and E_4) elevate VP due to contraction of the endothelium and are probably involved in IP, whereas leukotriene B_4, a neutrophil-

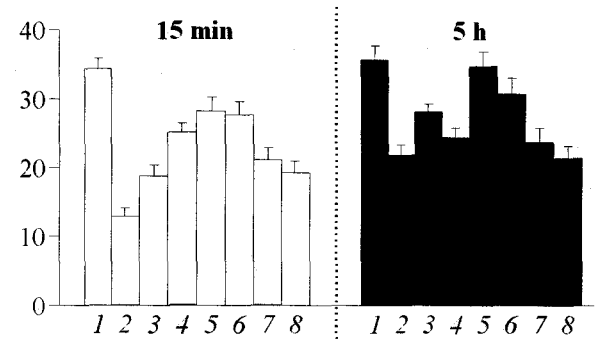

Fig. 1. Vascular permeability in the inflammatory focus during natural inflammation (*1*), inflammation in irradiated rats (*2*), replenishment of blood leukocytes in irradiated rats (*3*), and effects of contrical (*4*), tocopherol (*5*), indomethacin (*6*), quercetin (*7*), and dexamethasone (*8*). Ordinate: concentration of trypan blue ($*10^{-6}$ g/ml) in peritoneal lavage.

depended inductor of the VP increase, acts primarily during DP [4,16].

Neutrophil-derived cationic proteins that increase VP by contracting the endothelium are probably involved in IP of VP increase. Cationic proteins, lysosomal enzymes, and reactive oxygen species cause degranulation of mast cells [4]. Leukocytes were shown to control the intensity of mast cell degranulation and the release of mediators and modulators. Thus, leukocytes perform a combined function: protection from lytic effects of pathogenic microorganisms and activation [6]. Leukocyte-derived products interact with other vasoactive systems involved in IP of VP increase, such as the complement and kallikrein-kinin systems [4,15]. Neuropeptides, enzymes, reactive oxygen species, nitric oxide, and eicosanoids of other origins (resident cells) probably play role in this phase.

On the other hand, nonleukocytic mechanisms regulate VP during the exudative phase and mediate or modulate leukocytic functions (C5a des Arg, vasoactive amines, acetylcholine, etc.) [7,9,16]. Various leukocyte-derived products (lysosomal enzymes, reactive oxygen species, and cyclooxygenase and lipoxygenase derivatives) modulate the leukocytic response [7].

Dexamethasone inhibited both IP and DP of VP increase by 1.79 and 1.67 times, respectively. VP did no differ from that observed during inflammation in irradiated rats (Fig. 1).

Such a strong action of dexamethasone in both phases is obviously due to diverse antiinflammatory effects of glucocorticoids, which inhibit the release of transmitters from various cells, in particular leukocytes, mast cells, platelets, endothelial cells, and resident macrophages. Dexamethasone and other glucocorticoids are potent inhibitors of inducible (macrophagic) NO synthetase, and their considerable antiinflammatory effects are related to the role of nitric oxide as the inflammatory transmitter [14].

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