The Role of Leukocytes in the Reaction of Mast Cells from an Inflammatory Focus

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Biologically active products of different leukocytes (lysosomal enzymes, nonenzymatic cationic proteins, cytokines, active oxygen metabolites, lymphokines) administered from the outside or studied *in vitro* are able to induce degranulation of mast cells (MC) and in this manner to release potential inflammatory inductors contained or synthesized de novo in the MC [9,16-19]. However, the actual role of leukocytes in the reaction of MC in inflammation is not clear.

The aim of the present study was to reveal the morphofunctional state of the peritoneal MC in the dynamics of acute peritonitis in rats for the natural development of an inflammation and for inflammation accompanied by leukopenia.

MATERIALS AND METHODS

Experiments were carried out on 311 male Wistar rats weighing 180-200 g. To induce peritonitis 2 billion (1/2 LD_{50}) microbic bodies of a 24-h culture of E.coli isolated from a patient with peritonitis or 5 mg carrageenin in 1 ml saline were administered i.p. [1,4-6]. The animals were decapitated at different time periods after an inflammation had been induced. Counts and morphological examination of the peritoneal MC were performed in a counting chamber with neutral red staining [3]; the mesenteric MC were counted on pellicular preparations stained with toluidine blue [14]. A peritoneal flush was obtained by irrigating the peritoneal cavity with 5 ml cold Tirode solution containing 5 U/ml heparin. Histamine as a biochemical marker of MC functional state was measured by a modified fluorometric technique [2] in the extracellular fraction of the peritoneal flush and mesentery [7] and also in the blood. The total number of leukocytes and the composition of their populations were calculated in the peritoneal cavity and in the blood. Leukopenia was induced by a single i.v. injection of 0.75 mg/kg sulfate vinblastine (Richter, Hungary) 4 days before simulation of peritonitis [13].

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RESULTS

Leukopenia itself (4 days after vinblastine administration) resulted in a decrease of the count of MC in rat peritoneal fluid and mesentery and in an increase of their degranulation (Figs. 1, 2). Infectious peritonitis induced against a background of leukopenia was accompanied by a phase reaction of the MC just as with its natural development [6]. The first phase, noted for half an hour after the action of the infectious agent, was characterized by a more intensive MC degranulation and by a fall of the MC count in the peritoneal fluid. For example, a significant decrease of the MC count was already noted toward the 15th min and by the 30th min this index differed nearly 4-fold from the original one, whereas a mere 1.5-fold decline of the MC number was revealed in the natural course of inflammation and only in

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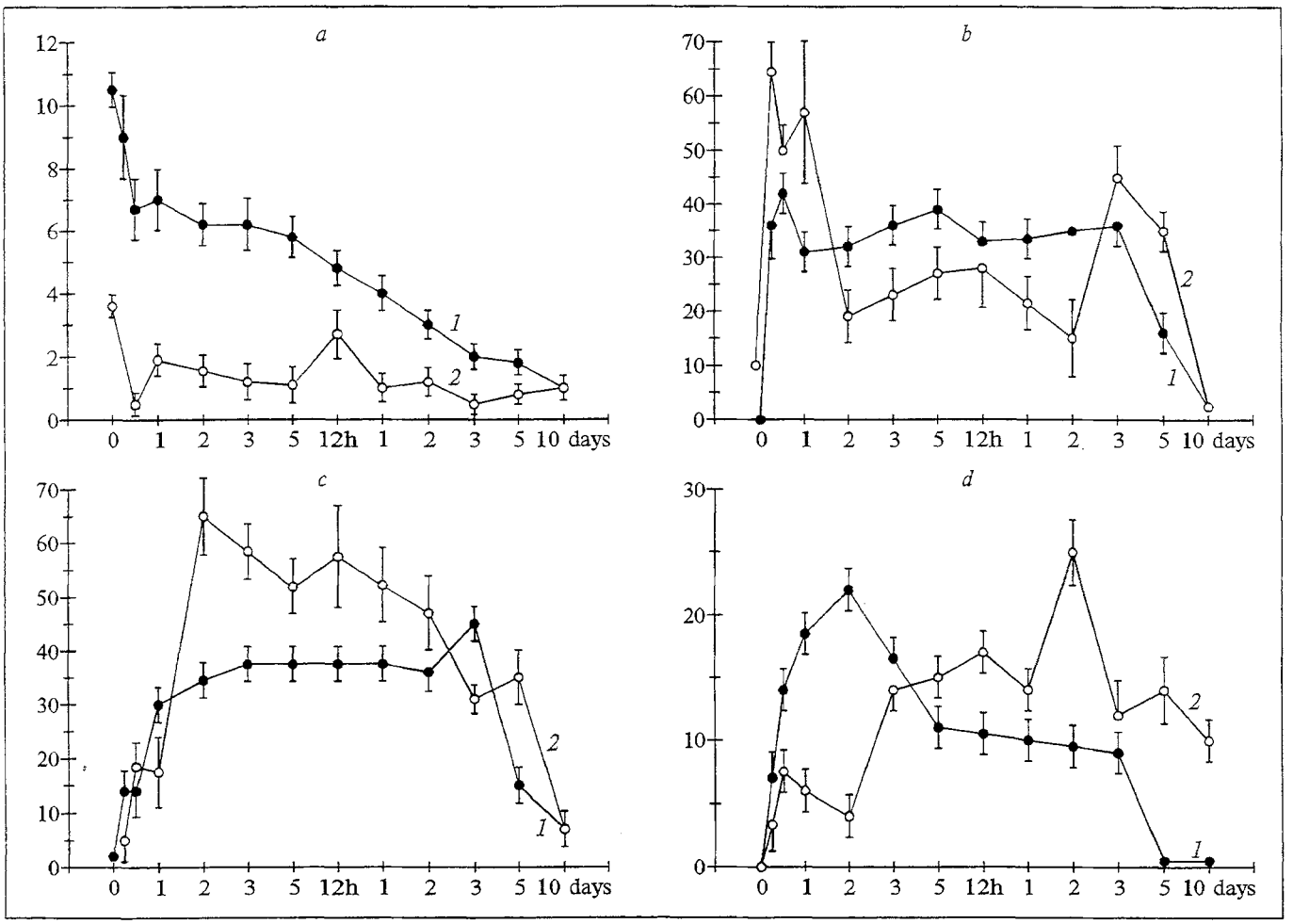

Fig. 1. Number of MC ($\times 10^5$) in the peritoneal flush (*a*) and number of degranulated cells of the 1st (*b*), 2nd (*c*), and 3rd (*d*) degree of degranulation (%) in the course of acute infectious peritonitis (days) in rats under natural conditions (*1*) and against the background of leukopenia (*2*).

the 30th min (Fig. 1). During the first 5 h the content of degranulated MC was not essentially different in natural or leukopenia-accompained inflammation, but the intensity of degranulation in the latter case was higher and a prevalence of MC with second-degree degranulation was noted. The leukopenic animals also demonstrated a predominance of the total percent content of degranulated cells in the period from 12 h to 5 days. Degranulation was more pronounced in the mesentery of the rats with leukopenia (compared to the natural development of peritonitis) throughout the experiment (10 days), as was manifested in the predominance of third-degree MC degranulation and resulted in a more marked decrease of the MC count, i.e., a significant slope as early as after 30 min versus 2 h in the ordinary course of peritonitis (Fig. 2). The content of free histamine in the peritoneal flush and mesentery of leukopenic rats achieved its first peak only 5 min after flogogen administration, versus 30 min and 1 h, respectively, with the natural development of inflammation (Fig. 3). The concentration of free histamine in the leukopenic mesentery exceeded that in the natural inflammation both after 5 min and after 2-5 h, in spite of a lower initial amine level. The average values of histamine in the blood after 15 min, 3-5 h, and 12 h were also above the levels obtained in the natural course of inflammation. These findings attested to an advanced release of histamine in peritonitis induced against the background of leukopenia. An intensified depletion of the reduced histamine reserve resulted in its lower level in exudate, mesentery and blood of the leukopenic rats 1-5 days after the simulation of inflammation. It should be noted that an elevated histamine release on the 10th day coincided with a higher degree of MC degranulation, particularly in the mesentery, probably due to a prolongation of the inflammatory reaction up to the point where the MC population under the original leukopenia conditions began to be restored.

The changes obtained in the MC reaction in the leukopenic animals were to a high degree con-

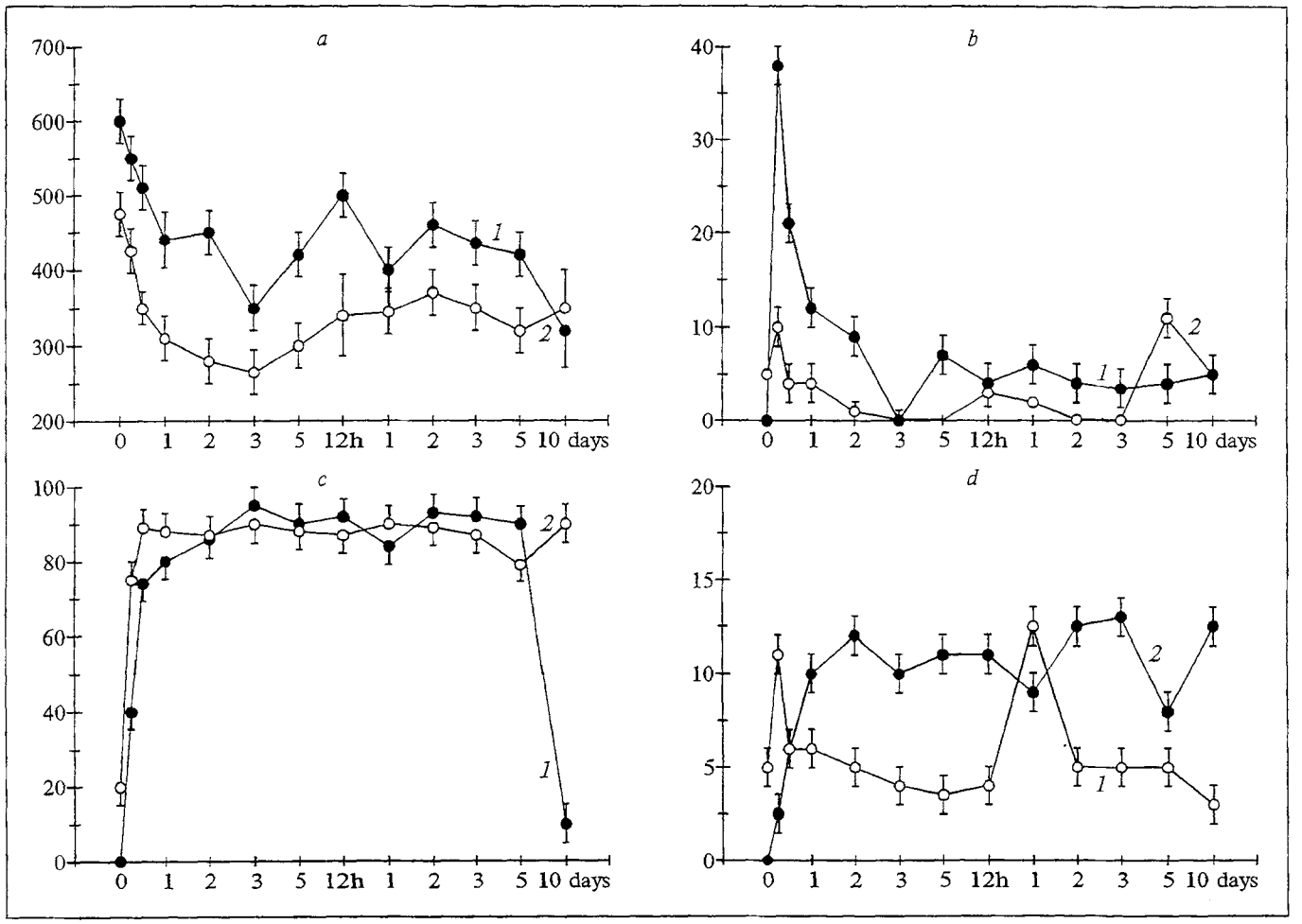

Fig. 2. MC count (in 100 visual fields, ×400) in mesentery (*a*) and number of degranulated cells of the 1st (*b*), 2nd (*c*), and 3rd (*d*) degree of degranulation (%) in the course of acute infectious peritonitis (days) in rats under natural conditions (*1*) and against the background of leukopenia (*2*).

sistent with the reported variations of leukocyte kinetics in the peritoneal cavity and blood [4,5]. There was an initial drop of the leukocyte count in the peritoneal cavity, with a minimal level after 5 h, probably owing to the loss of resident cells without equivalent compensation from the blood leukocytes. Later there were two influxes of leukocytes to the peritoneal cavity: after 12 h and 2 days. The first leukocyte influx was virtually composed of monocytes, while the second one consisted in part of neutrophils. A leukocyte influx in the blood was also noted only after 12 h and derived from an increase of the number of monocytes and lymphocytes. A noticeable elevation of neutrophils was observed 1-2 days after inflammation was induced, but it was insignificant in comparison with that in the natural course of inflammation.

The experimental results showed that leukopenia has a marked influence on the morphofunctional state of the MC in an inflammatory focus. It leads to intensified MC degranulation both before a substantial number of leukocytes enter the focus (during the first 5 h after induction of inflammation) and during their massive influx (between the 5th and the 12th h, and the 1st and the 2nd day). The increased degranulation of MC in the first period may be due to excessive action of bacterial factors in connection with the abolition of the microbicidal properties of the leukocytes, while in the second period there was an influence of the surplus of leukocyte products due to the simultaneous development of local leukocytosis.

The results obtained allow us to assume that the MC reaction is delayed in the leukopenic animals before the appearance of an appreciable number of leukocytes in comparison with the natural development of inflammation. For this reason, the rat MC reaction was studied under carrageenin peritonitis progressing naturally and against a leukopenia background. The initial MC reaction (to the 30th min) was found to be similar in leukopenic animals both in aseptic and in septic in-

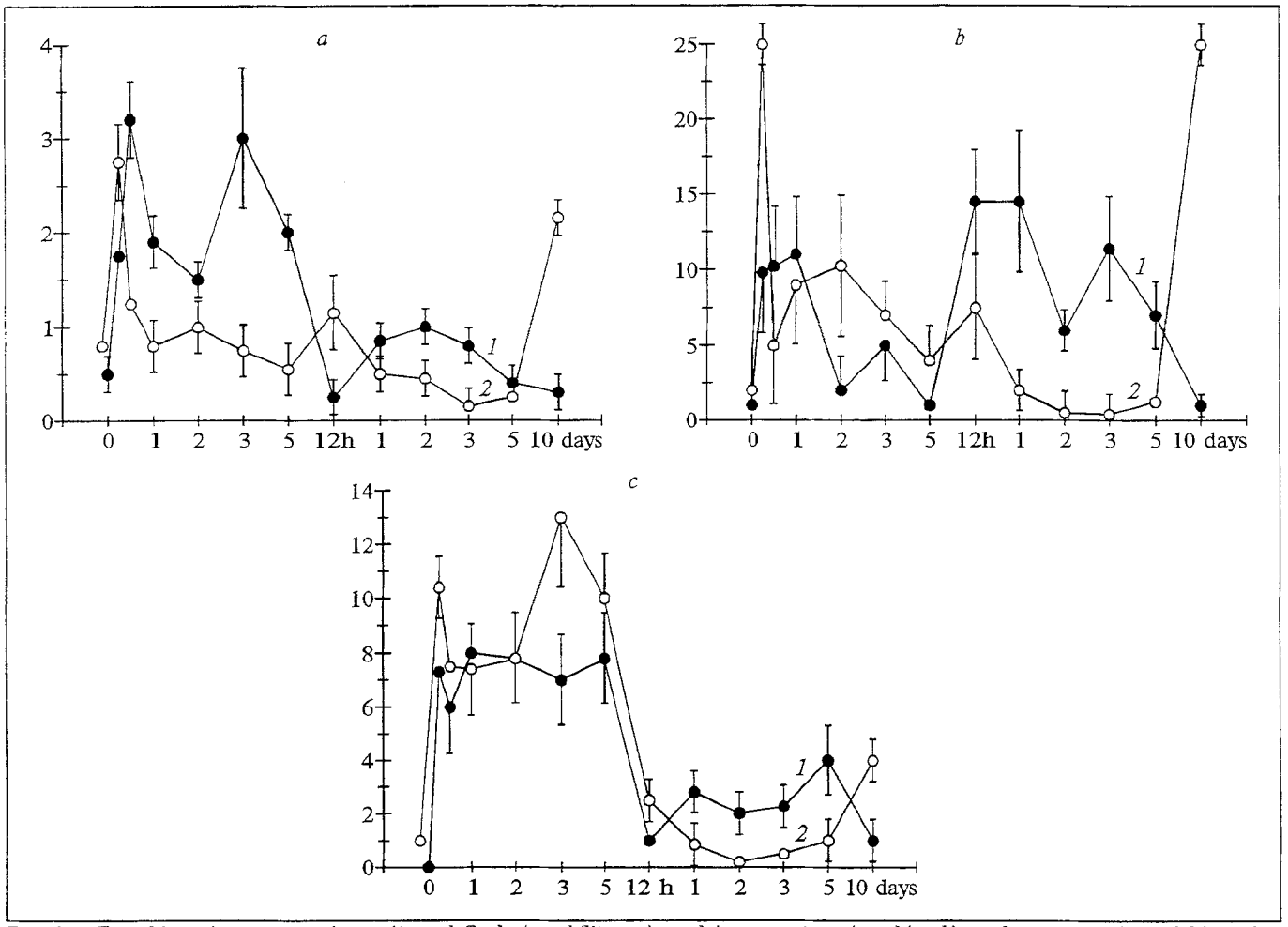

Fig. 3. Free histamine content in peritoneal flush (μmol/liter; a), and in mesentery (nmol/g; *b*), and concentration of histamine in blood (μmol/liter; *c*) in the course of acute infectious peritonitis (days) in rats under natural conditions (1) and against the background of leukopenia (2).

flammation and was more pronounced than in the native state (Fig. 4). However, the percentage of degranulated cells after 3 and 5 h was markedly decreased, but the absolute MC number was higher than in the preceding periods of inflammation, whereas in natural aseptic inflammation the degranulation was further stepped up and the MC count was diminished. The leukopenic animals exhibited a repeated elevation of the number of degranulated cells, which in turn resulted in a new decrease of the absolute number of MC on the 1st and 2nd days. One day later and then throughout the experiment the number of degranulated MC in the leukopenic animals was higher then that in the original inflammation. The changes in MC morphofunctional state were also parallel to the variations of the leukocyte count in the exudate and blood, which were governed by the same regularities as those under septic inflammation in animals with leukopenia.

Thus, while an infectious inflammation induced against a leukopenia background is characterized by intensified degranulation of MC independently of the leukocyte number in the focus and blood, an aseptic inflammation results in a delay of the MC reaction until a pronounced leukocyte influx and massive degranulation of the MC take place.

The shifts in the morphofunctional state of the MC in inflammation induced against the background of leukopenia testified that in natural inflammation a significant role in the MC reaction belongs to the leukocytes, namely neutrophils and monocytes. They seem to protect the MC (from the effects of the pathogenic microorganisms) and, at the same time, to activate them and thereby regulate the MC reaction. The degranulating effect of leukocytes on the MC is not exclusively lytic and is mainly related to a nontoxic type of degranulation arising from the nonspecific effect of cationic proteins on the MC and also from the possible modulatory effects of lysosomal enzymes, cytokines, and active metabolites of oxygen in inflammation [8,11,12,15].

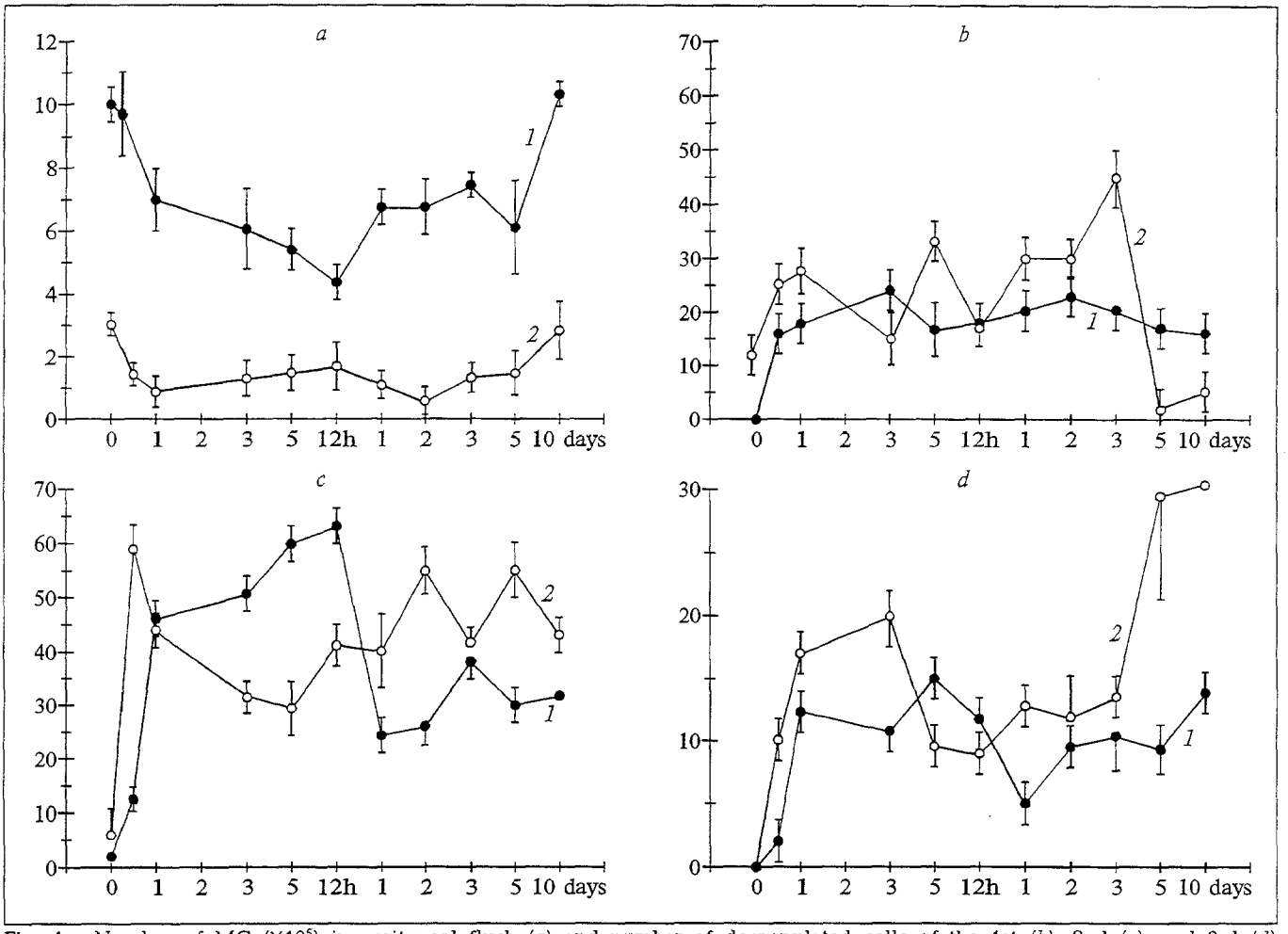

Fig. 4. Number of MC ($\times 10^5$) in peritoneal flush (*a*) and number of degranulated cells of the 1st (*b*), 2nd (*c*), and 3rd (*d*) degree of degranulation (%) in the course of acute aseptic peritonitis (days) in rats under natural conditions (*1*) and against the background of leukopenia (*2*).

It should be noted that the stepped-up MC degranulation following the action of the infectious agent in leukopenic rats may be due to a high initial MC degranulation in such animals. However, an increased degranulation and a diminished MC number in the initial state may be a compensatory reaction derived from the depletion of circulating leukocyte factors which may interact with MC products interfering with the mechanisms of homeostasis in health and pathology, for instance, in the regulation of the vascular permeability in inflammation [4,20]. A low original number of MC may be due to a decreased bone-marrow production of the MC precursor, which is reportedly the same as the granulocyte precursor [10].

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