Dynamics of Destructive and Repair Processes in the Immune System Organs during Acute Experimental Peritonitis under Different Conditions

V. Ya. Glumov, N. A. Kir'yanov, E. L. Bazhenov, and G. S. Ivanova

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The immune system (IS) is known to be on the whole an open, steady-state system. Its self-maintenance is realized by continuous processes of dying, proliferation, and migration of immunocompetent cells. The unequal contribution of these processes in response to various stimuli (antigens, hormones, toxins, etc.) may lead either to an enhancement of the immune response or to its inhibition and the development of immunosuppression [10]. However, the quantitative aspect of the processes of immunocompetent cell destruction and proliferation in the IS organs in different pathological situations has been insufficiently discussed in the literature.

This report presents data concerning the destruction and repair processes in the peripheral organs of the IS in acute experimental peritonitis (AEP) during its normal course, against the background of the action of immunomodulators (azathioprine, levamisole), and under conditions of hypertoxicosis.

MATERIALS AND METHODS

The experiments were carried out on 220 white rats, in which AEP was induced according to the method elaborated by S. S. Remennik and modified by us [5]. The animals were divided into 5 groups: 1st group (120 rats) with the usual course of AEP; 2nd group (35 rats) with AEP against the background of immunosuppressive treatment with azathioprine (a single dose of 4 mg/kg per os every day for 2 weeks); 3rd group (35 rats) with AEP proceeding under conditions of immunostimulatory treatment with levamisole (2-2.5 mg/kg every day in three-day courses, 3 courses at 6-day intervals); 4th group with AEP against the background of hypertoxicosis (intraperitoneal injection of 3% fecal mass in a volume of 1.5 ml repeated 2-3 times); and 5th group (10 rats, control) receiving intraperitoneally an equal volume of saline. The animals were decapitated under ether 12 to 24 hours and 2, 4, 6, 8, 10, ans 13 days following the start of the experiment. Twelve animals of the 1st group died on the first day, 10 rats of the 2nd group died within the first 3 days, no mortality was registered in the 3rd group, and 5 animals of the 4th group died at various times during the experiment. Specimens of peritoneum, lymph nodes (LN) of various localization, and spleen were embedded in paraffin and a histological examination was performed. The organ sections were stained using conventional histological methods. The histochemical determination of total nucleoproteins (after Einarson) and of DNA (after Feulgen) were performed. The preparations for transmission and scanning electron microscopy were processed routinely [3, 9]. In the

Department of Pathological Anatomy, Izhevsk Medical Institute. (Presented by D. S. Sarkisov, Member of the Russian Academy of Medical Sciences)

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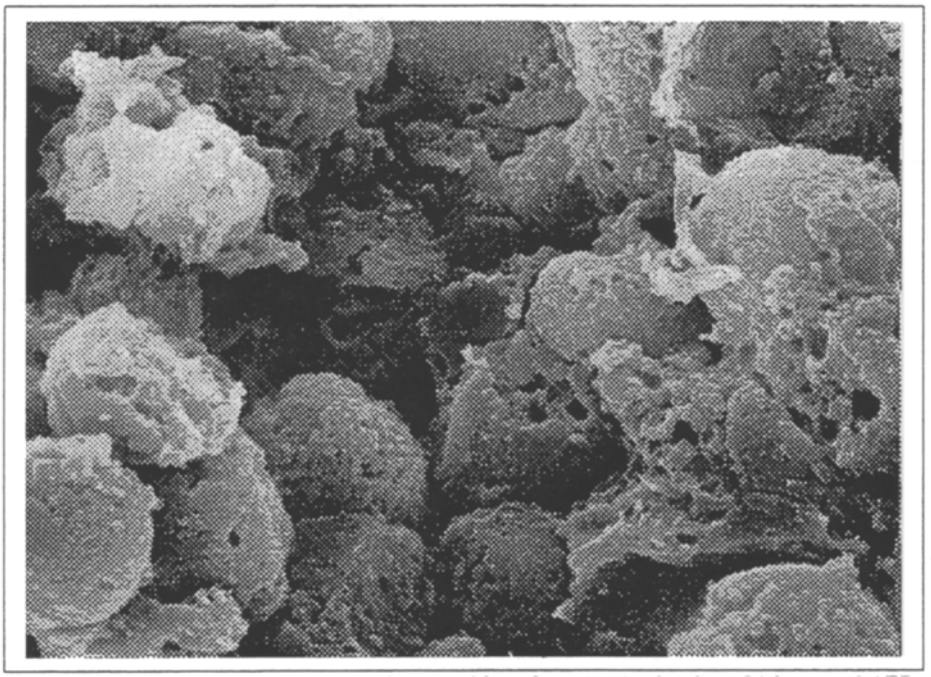

Fig. 1. Alterations in the outer membrane of lymphocytes in the first 24 hours of AEP. Scanning electron microscopy, ×3500.

histoautoradiographic study, 1.5 hours preceding sacrifice animals received subcutaneously in the thigh ^{3}H-thymidine in a dose of 0.5 μCi per gram body weight. The deparaffinized sections were covered with an M type photoemulsion and exposed at 4°C for 3-4 weeks, after which they were developed and stained with hematoxylin and eosin or with gallocyanin. The morphometric study, carried out according to our modification, included the calculation of: a) the index of lymphocyte destruction (ratio of apoptotic to living cells, expressed in percent); b) the labeling index (percent of ^{3}H-thymidine-labeled cells); c) the proliferation index (ratio of mitotic pictures to apoptotic cells) [7], and d) the mitotic index (%).

RESULTS

In its various courses AEP is attended by the development of pronounced vascular alterations in the IS organs, destruction and repair of immunocompetent cells, and immunological restructuring. Expressed in varying degrees, these alterations make up a polymorphic picture.

For instance, swelling of the vascular wall occurs, together with a marked rise of permeability. Hemostasis and microcoagulation events develop, especially in the microcirculatory bed. Under conditions of hypertoxicosis the alterations of the vascular wall attain a considerable level, often culminate in fibrinoid necrosis, and are accompanied by microhemorrhage and subsequent hemosiderosis of the surrounding tissue. In the case of azathioprine-induced immunosuppression the blood and lymphatic vessels exhibit, as a rule, a picture of suppurative-destructive vasculitis with massive miltiplication of microorganisms.

The initial stages of lymphocyte destruction are easily distinguished under the scanning electron microscope due to the occurrence of oval holes in the outer membrane (0.4-1.1 μ) (Fig. 1), followed by cell corrugation.

Under the transmission microscope dying cells can be recognized by the marked induration of the nucleus. At the level of light microscopy dying cells are distinguished as apoptotic bodies. Cell disintegration can be observed in all the functional areas of the IS and results in a marked loss of lymphocytes in the latter.

When AEP proceeds in the usual way, the destructive processes rapidly escalate in the first 12-24 hours; they are particularly pronounced in the B-dependent areas of the peripheral organs of the IS (Fig. 2). At this time a serous-fibrinous inflammation is registered in the peritoneum. Subsequently, the destructive processes subside and reach the initial level by the end of the experiment. An encapsulation of peritoneal exudate is observed at this time. A spe-

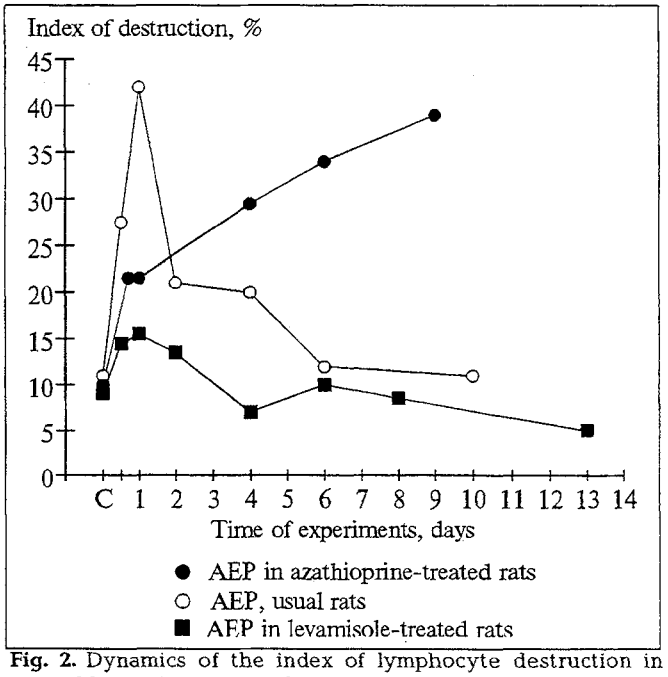

Fig. 2. Dynamics of the index of lymphocyte destruction in vertical layer of mesenteric lymph node in AEP proceeding under different conditions.

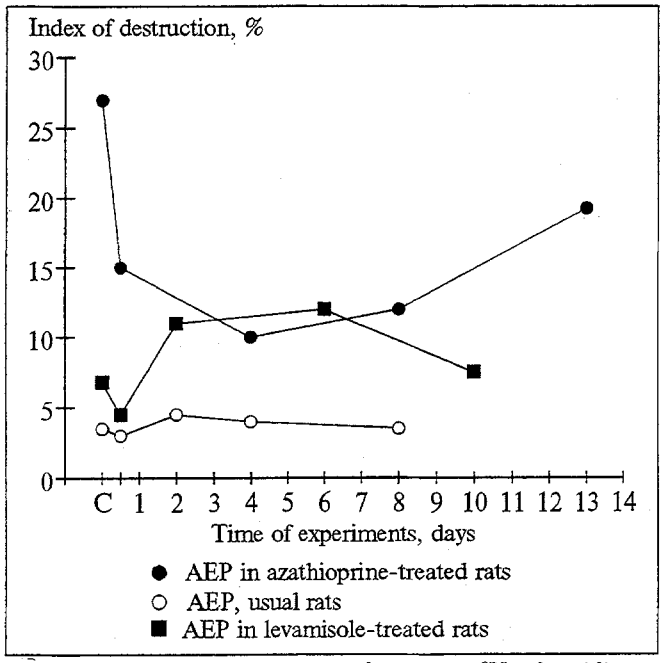

Fig. 3. Dynamics of quantitative changes in ^3H-thymidine-labeled lymphocytes in cortical layer of mesenteric lymph node in AEP proceeding under different conditions.

cific feature of the immunosuppressive drug-aggravated destructive processes in AEP is their increase during the whole course of the experiment. This, as a rule, causes almost total depletion of the lymphoid organs and a rapid purulent development of the intraperitoneal inflammation.

Quite another picture is seen in the animals treated with levamisole. During the whole course of the experiment the specialized IS cells do not exhibit as pronounced signs of destructive processes as in the usual course of AEP and for the combined action of AEP and azathioprine treatment.

Hypertoxicosis is accompanied by a dramatic increase in the index of lymphocyte destruction 12-24 hours after the repeated intraperitoneal injection of fecal suspension. This leads to a very rapid lymphoid depletion of the IS organs.

The repair processes also show quantitative differences in different forms of AEP. For instance, in the usual course of AEP the synthetic processes decline during the first 24 hours (Fig. 3). However, by the end of the second day these processes undergo a two- to threefold rise, particularly in the B-dependent areas of the peripheral organs of the IS. This value remains on the same level for 3-4 days and then gradually decreases. A similar dynamics of the synthetic processes is seen in AEP aggravated by azathioprine treatment and hypertoxicosis; however, in the latter cases the synthetic processes do not reach the level observed in the conventional course of AEP. The indexes of proliferation and mitotic activity are also low in the case of aggravated AEP.

In the levamisole-treated rats with AEP a striking fall of the labeling index is revealed in the first 24 hours. However, this parameter still remains at a much higher level than in the azathioprine-treated animals with AEP. Another peculiarity of the levamisole-treated group is a delayed (by 4-5 days) rise of the synthetic processes in comparison with the other groups; however, subsequently the labeling index increases steadily at all time points of the experiment (Fig. 4). The values of the indexes of proliferation and mitotic activity change in parallel with the labeling index.

The described specific balance between the destruction and repair processes in the IS organs in AEP animals treated with levamisole precludes a considerable depletion of the IS organs. Moreover, an increase occurs in the number of activated lymphocytes, immunoblasts, plasmablasts, and plasmacytes with a high secretory activity.

Thus, AEP, causing extensive peritoneal involvement, massive release of toxins in the circulation, and

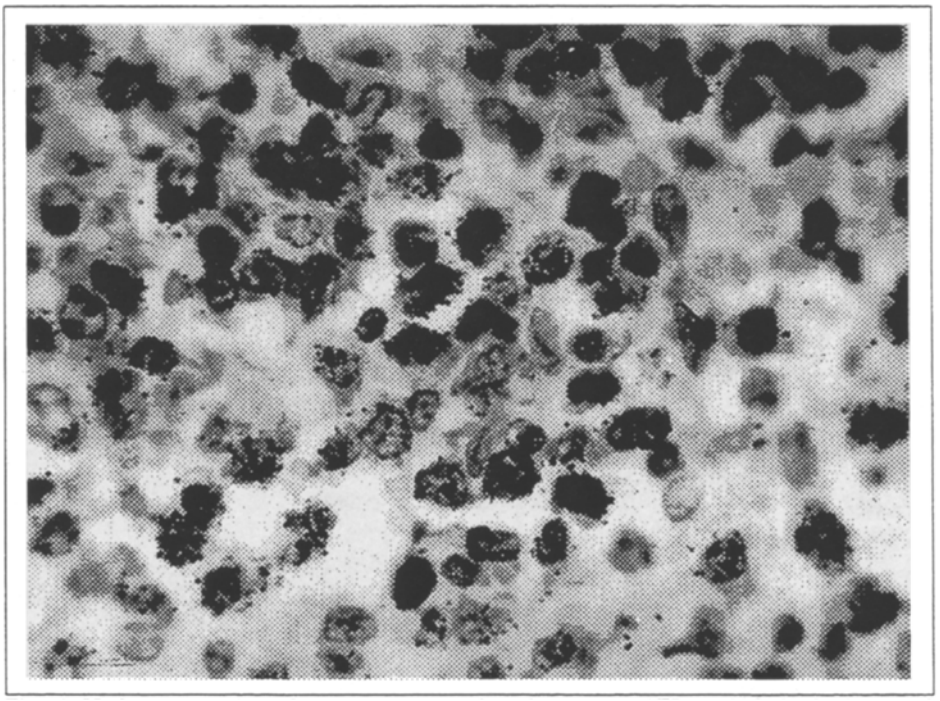

Fig. 4. High activity of synthetic processes in lymphocytes in AEP against the background of levamisole treatment. Histoautoradiograph, ×400.

a stress reaction of the organs of immunogenesis, is accompanied by vascular, destructive, reparative, and immunological processes. As the peritoneal inflammation becomes extracerbated, the vascular and destructive alterations also intensify. However, as the inflammation in the peritoneal cavity subsides, these gradually wane and give way to enhanced processes of repair, with the result that a significant lymphoid depletion of the IS organs does not take place.

Among the possible causes of cell death the lymphoid organs, one should point to the following factors: endogenous toxicosis inherent in acute peritoneal inflammation; marked vascular disorders [6, 8]; high blood concentration of glucocorticoids caused by the stress and toxicosis [1, 2]. The drop in the number of lymphocytes in the IS organs is also related to increased lymphocyte migration and redistribution in the organism [4]. Perhaps T-helpers are especially prone to destruction, as their quantity dramatically falls in the peripheral IS organs in the toxic phase of peritonitis [11].

The peritoneal inflammation developing against the background of immunosuppression is associated with a marked rise of the destructive processes in the specialized cells of the IS, together with a drop of the indexes of their proliferation. Such a situation leads to a depletion of the IS organs, which may be considered to be the morphological equivalent of a secondary immunodeficiency. The inflammation in the peritoneal cavity has an unfavorable course under these conditions, resulting in a high mortality of the animals. On the other hand, treatment with levamisole slows down the destructive processes in the IS organs and at the same time markedly boosts the processes of synthesis and repair. Such a dynamic equilibrium of destructive and reparative processes prevents the depletion of the IS organs and promotes the stimulation of immune reactions, thereby providing a basis for an accelerated arrest of the inflammation in the peritoneal cavity.

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