ROLE OF T LYMPHOCYTES IN MECHANISMS OF ACTIVATION OF THE HEMATOPOIESIS-INDUCING MICROENVIRONMENT IN INFLAMMATION

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A decisive role in the regulation of hematopoiesis under normal and pathological conditions is played by the hematopoiesis-inducing microenvironment (HIM). Theoretically, all elements in the parenchyma of the hematopoietic organs could participate in the formation of HIM. An important role of T lymphocytes, macrophages, stromal mechanocytes, etc. in the regulation of processes of proliferation and differentiation of myeloid precursors has been demonstrated [2-4]. During exposure to extremal factors migration of T lymphocytes into the bone marrow, activating the function of the cellular elements of HIM, is observed [4]. The role of T lymphocytes in the regulation of myelopoiesis during inflammation still remains virtually unstudied [6].

The aim of this investigation was to study the role of T lymphocytes in the mechanisms of activation of HIM in mice with acute infectious peritonitis.

EXPERIMENTAL METHOD

Experiments were carried out in the fall and winter on 172 CBA mice (from the "Rassvet" Nursery, Tomsk) weighing 18-20 g. Peritonitis was produced by intraperitoneal injection of 0.5 LD_{50} of *E. coli* strain ATCC 25922 in 0.3 ml isotonic sodium chloride solution. The animals were killed by decapitation at various times during the experiment. The bone marrow was removed and divided into two parts, one of which was treated with antibodies to Thy 1,2 antigen [5]. The total number of myelokaryocytes per femur (TNM) and the myelogram were counted, and the number of granulocyticmacrophagal (GM-CFU) [10] and of erythroid (E-CFU) [8, 9] precursors in the bone marrow was determined by in vitro cloning in methylcellulose. The colony-stimulating (CSA) and erythropoietic (EPA) activity of the bone marrow supernatants obtained from lipopolysaccharide (LPS)- and ConA-stimulated myelokaryocytes was tested as in [10]. Adherent and nonadherent cells were incubated for 24 h in medium RPMI 1640, containing 10% fetal calf serum, in the presence of LPS of *E. coli* (10 μg/ml) and ConA (5 μg/ml) respectively.

EXPERIMENTAL RESULTS

The development of inflammation was accompanied by marked activation of medullary hematopoiesis. The total number of myelokaryocytes rose regularly to reach peak values of up to 180% of the initial level on the 6th and 7th days of the experiment. Analysis of the myelograms showed that the increase in TNM was due to stimulation, not only of granulocytopoiesis, but also of erythropoiesis. The number of cells of these branches in the bone marrow increased to 200% (6 days) and 180% (5 days) respectively. As the experiments showed, the changes discovered in the

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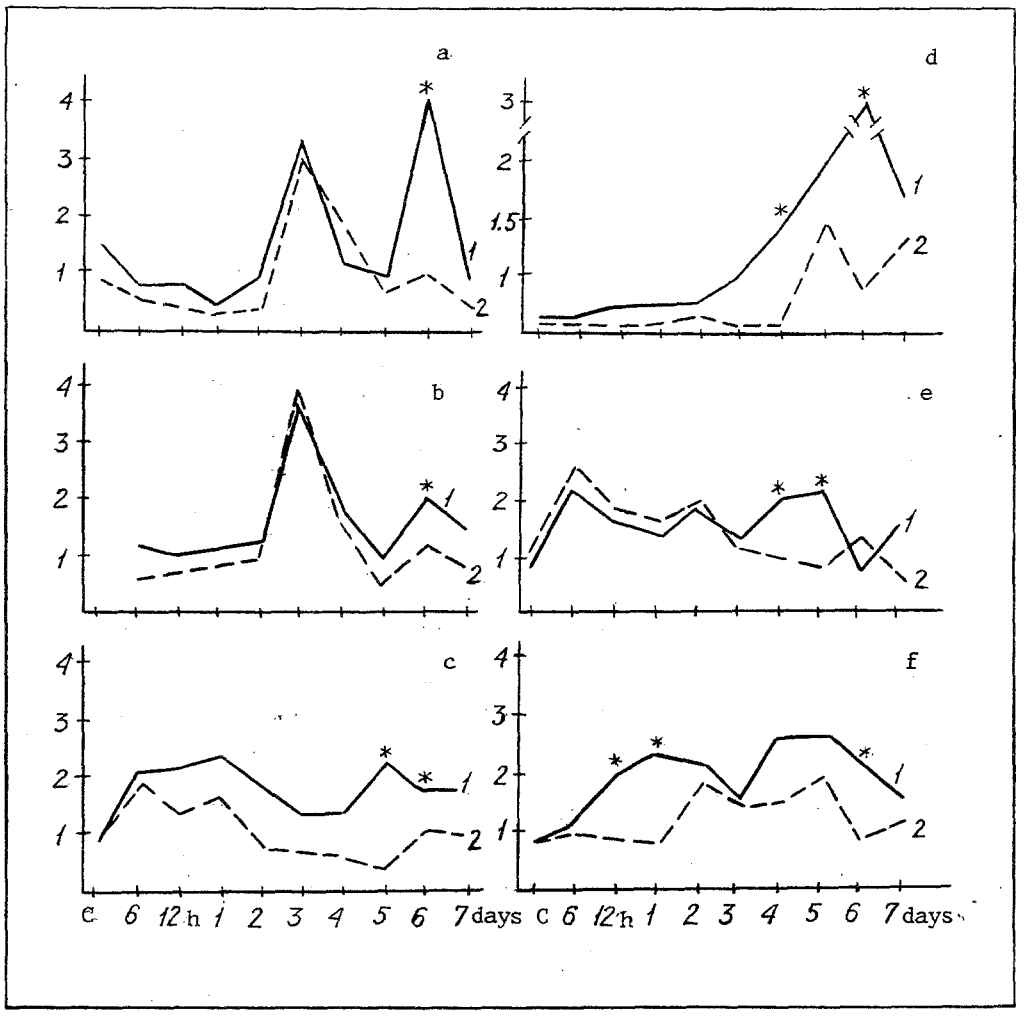

Fig. 1. Dynamics of GM-CFU (a), CSA of adherent (b) and nonadherent (c) cells, E-CFU (d), EPA of adherent (e), and EPA of nonadherent (f) cells in bone marrow of mice with acute infectious peritonitis, after treatment of myelokaryocytes with Thy $1,2^+$ antibodies (2) or without them (1). Asterisk indicates points with significant differences between series of experiments. C) Control.

pattern of medullary hematopoiesis were based on the activating effect of elements of HIM on proliferation and differentiation of hematopoietic cells. For instance, a significant increase was observed in the number of myeloid precursors of the GM-CFU type in the bone marrow, which was phasic in character. The number of GM-CFU reached peak values on the 3rd and 6th days of the experiment (Fig. 1). On the whole, there was general agreement between the dynamics of the granulocytic-macrophagal precursors described above and levels of CSA production by adherent and nonadherent fractions of medullary nuclears. For instance, CSA of the adherent cells was significantly increased on the 3rd and 6th days, whereas that of the nonadherent cells was similarly increased after 12 h and on the 1st and 5th days of the experiment. Thus the processes of stimulation of medullary granulocytopoiesis during infectious inflammation are based on intensification of production of colony-stimulating activities by the cells composing HIM – groups of factors responsible for proliferation and differentiation of granulocytic elements [7].

Largely similar general principles were found in a study of the role of the cells of HIM and the cytokines produced by them in the regulation of erythropoiesis. For instance, the maximal increase in the number of erythroid precursors on the 6th day of the investigation was preceded by increased EPA production by adherent and nonadherent medullary nuclears on the 4th and 5th days of the experiment. The changes affecting medullary hematopoiesis during inflammation probably have largely nonspecific features characteristic of the stress reaction [1]. For instance, we previously demonstrated similar changes in the blood system under the influence of immobilization [4], also due to activation of the function of HIM. It was shown that under the conditions of immobilization stress, T

lymphocytes migrate into the bone marrow tissue and, in cooperation with resident bone marrow macrophages, stimulate proliferation and differentiation of committed precursors (GM-CFU and E-CFU).

The experiments showed that the development of inflammation is accompanied by marked accumulation of T lymphocytes (Thy $1,2^+$ cells) in the bone marrow. For instance, the number of cells with the above-mentioned phenotype on the 4th and 5th days of the experiment exceeded the control values by 150 and 200% respectively. It is logical to suggest that under the conditions of acute inflammation, T lymphocytes migrating into bone marrow tissue stimulate the processes of hematopoiesis.

Preliminary treatment of myelokaryocytes with antibodies led to disappearance of the second peak of colony formation (GM-CFU, preceded by migration of T lymphocytes into the bone marrow), disappearance of the phenomenon of intensification of CSA production by adherent cells on the 6th day, and to considerable inhibition of CSA production by nonadherent cells (Fig. 1). Largely similar data were obtained also in a study of the effect of Thy $1,2^+$ cells on medullary erythropoiesis. For instance, removal of the Thy $1,2^+$-positive cells from the bone marrow was accompanied by a significant decrease in the output of erythroid precursors (E-CFU), of EPA production by adherent cells on the 5th and 6th days of the experiment, and of the EPA level of the supernatants of the nonadherent fraction of bone marrow cells.

Thus the results are unambiguous evidence of the important role of T lymphocytes in regulation of medullary hematopoiesis in acute infectious inflammation. Thy $1,2^+$ cells migrating into the bone marrow under these circumstances stimulate processes of proliferation and differentiation of myeloid precursors directly (through the lymphokines produced by them) and in cooperation with other elements of HIM (in particular, with monocytes and macrophages).

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