RELATIONS BETWEEN THE HEMATOPOIETIC MICROENVIRONMENT, OSTEOGENESIS, AND HEMATOPOIESIS UNDER THE INFLUENCE OF ESTRONE

V. A. Almazov, I. I. Ivasenko, and A. Yu. Zaritskii

UDC 612.751.1:612.65]+ 612.119].014.46:[615.357:577.175.644

KEY WORDS: estrone; hematopoiesis; experimental myelofibrosis

The use of estrone in order to produce myelofibrosis in experimental animals has provided a model of the basic phenomena of human myelofibrosis, namely fibrosis of the bone marrow, extramedullary myelopoiesis, an increase in the number of hematopoietic cells (precursors of blood cells), and the potential reversibility of the process [1, 10]. It can accordingly be concluded that the model is adequate for the experimental study of myelofibrosis associated with diseases of the blood system in man.

It was shown previously, during the use of heterotopic bone marrow transplantation, that reduction of hematopoiesis developing parallel with the increase in volume of the bone tissue is unconnected with mechanical displacement of hematopoietic cells [1]. The aim of this investigation was to continue the analysis of the possible mechanisms of the disturbance of hematopoiesis during the development of experimental myelofibrosis.

EXPERIMENTAL METHOD

The investigation was conducted on (C57BL × CBA)F_1 mice. Altogether 140 mice were used in three series of experiments. Heterotopic bone marrow transplantation was carried out by the method developed in the laboratories of Professor I. L. Chertkov [4] and A. Ya. Fridenshtein [3]. Estrone was dissolved in olive oil and injected into the experimental animals in a dose of 0.5 mg/kg once a week for 4-6 weeks. Control animals received injections of olive oil alone. The cell content and mass of the femoral bone tissue and of the heterotopic focus were estimated 4-6 weeks after the beginning of the estrone injections and also 60 days after the last injection. In a separate series of experiments a single injection of estrone was given into the donors of the bone marrow, after which their femoral marrow was transplanted. Transplantation of bone marrow was impossible after a large number of injections, due to the development of fibrosis of the bone marrow.

The results were subjected to statistical analysis by the Wilcoxon—Mann—Whitney nonparametric test.

EXPERIMENTAL RESULTS

Just as in the original work, when the method of estrogenic myelofibrosis was suggested [10], clear dependence of the reduction of the cell content of the bone marrow on the number of estrone injections (4-6) was revealed (Fig. 1). This indicates that the model of myelofibrosis thus produced is adequate. The same rule as a whole also was observed in a heterotopic focus of hematopoiesis (Fig. 2). Compared with the femoral bone marrow, its cell content after five or six injections of estrone did not differ significantly, probably due to the extremely low values of the cell content of the bone marrow of the heterotopic focus after five injections of estrone. Incidentally, in the control animals, with an increase in the duration of the experiment from 4 to 6 weeks, the cell content of the heterotopic focus increased regularly. Parallel with the reduction of the cell content of the femoral marrow and of the heterotopic focus of hematopoiesis, an increase in the mass of the femur and of the bony capsule of the heterotopic focus was observed in the experimental animals. In the heterotopic focus, unlike in the femur, significant differences in the mass

I. P. Pavlov First Leningrad Medical Institute. Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 109, No. 2, pp. 140-142, February, 1990. Original article submitted August 29, 1989.

0007-4888/90/0002-0160$12.50

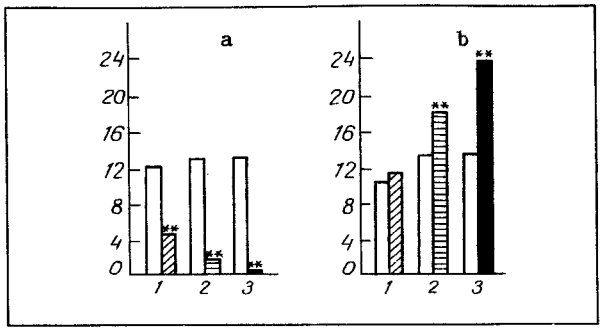

Fig. 1. Dynamics of cell content (a) and mass (b) of femur after injection of estrone for 4 (1), 5 (2), and 6 (3) weeks. Here and in Fig. 2, ordinate: a) cell content 10^6, b) mass, mg; asterisks indicate significance of differences between parameter studied and that estimated immediately after 4-6 estrone injections ($p < 0.01$). Here and in Figs. 2-4: unshaded columns— control group, obliquely shaded columns — four injections, horizontally shaded columns — five injections; black columns — six injections of estrone.

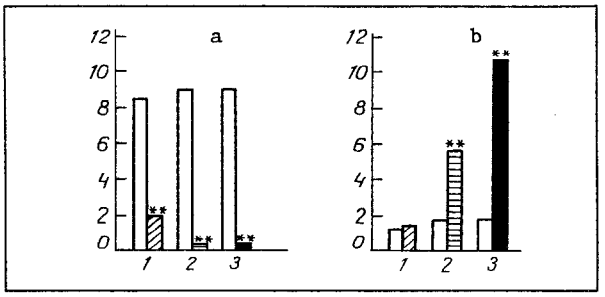

Fig. 2. Dynamics of cell content (a) and mass (b) of heterotopic focus following injections of estrone for 4 (1), 5 (2), and 6 (3) weeks.

of bone tissue were discovered only after five injections of estrone. These results raise the question of the causes of suppression of hematopoiesis in the medullary organs and the increase in mass of the bone tissue. Since estrone has no inhibitory action on hematopoietic stem cells [10], the results can be explained either by stimulation of development of osteosclerosis and fibrosis of the bone marrow and the reciprocal relations of these processes and hematopoiesis, or by a disturbance of the stromal microenvironment.

Analysis of the development of bone marrow fibrosis suggests that reciprocal relations exist between the bone tissue and hematopoiesis. However, this was not fully confirmed by the next series of experiments. The mice were not killed immediately after four or six injections of estrone, but not until 60 days had elapsed. The mass of the femur 60 days after the fourth injection of estrone did not differ from that in mice killed immediately after four injections of estrone, and mice of the corresponding control group (Fig. 3). Conversely, the mass of the bony capsule of the heterotopic focus in this situation continued to rise and it differed significantly from that in mice killed immediately after four injections of estrone. It must be recalled that there was also a parallel increase in the mass of the bony capsule in animals of the control group. No significant differences could be found between them (Fig. 4). A definite trend of the cell content of the femoral marrow and heterotopic focus could be distinguished. The number of nucleated cells in the femur increased, but did not reach the level in the control group (Fig. 3). A similar situation also was observed in the heterotopic focus (Fig. 4).

Changes in the cell content of the bone marrow and the weight of the bone when investigated 60 days after six injections of estrone were rather different. For instance, the mass of the femur became slightly less although, however, significantly greater than in animals of the control group (Fig. 3). The mass of the bony capsule of the heterotopic focus declined significantly and did not differ from that in mice of the control group. The character of the changes in the cell content of the medullary organs also

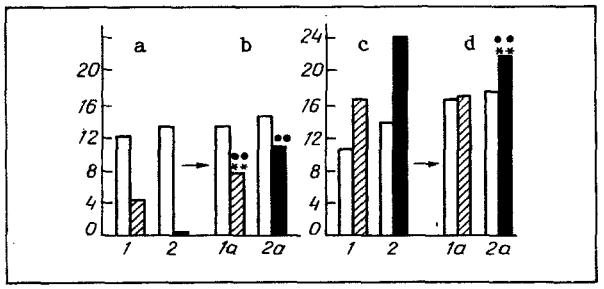

Fig. 3. Delayed effect of estrone injections 8 weeks after last injection of estrone on cell content (a — before, b — after injection of estrone) and on mass (c, d respectively) of femur. 1) Four injections, 2) six injections of estrone. Ordinate: a, b) cell content, $\times 10^6$; c, d) mass, in mg. Asterisks indicate significance of difference between parameter studied and corresponding value in control group ($p < 0.01$). Dots indicate significance of trend of parameters immediately after injections of estrone and 8 weeks later ($p < 0.001$).

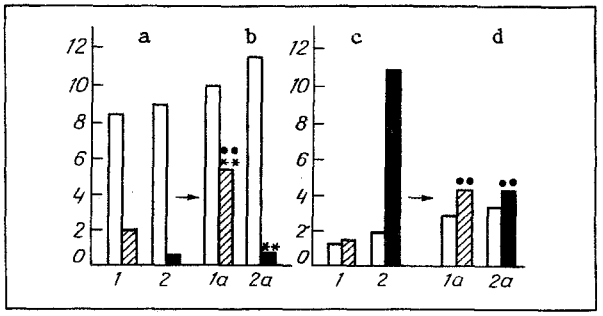

Fig. 4. Delayed effect of estrone injections after 8 weeks on cell content and mass of heterotopic focus.

differed. A statistically significant increase in the cell content of the bone marrow took place in the femur, but it was comparable with the corresponding parameter in the control group. The cell content of the heterotopic focus showed no significant change and remained below the control level.

On the basis of these results a fundamental conclusion can be drawn: there is neither positive nor negative correlation between the change in mass of the bone tissue of the hematopoietic organ and activation or inhibition of hematopoiesis in it. Consequently, the decrease in the cell content of the medullary organs in estrogenic myelofibrosis cannot be explained by the presence of reciprocal relations between hematopoiesis and osteogenesis. The reason for excessive production of connective tissue may be the stimulating influence of estrogens on osteoblast proliferation [7]. To elucidate the causes of depression of hematopoiesis, an additional experiment was carried out. Heterotopic transplantation of bone marrow was undertaken 1 week after injection of estrone. The results showed that the focus thus formed has a much smaller cell content than the control, whereas its mass does not differ from that in the control (Fig. 5). This contradicts the idea of the primary stimulating effect of estrone on proliferation of osteogenic cells as the cause of depression of hematopoiesis. Since retransplantation of bone marrow from animals receiving an injection of estrone did not lead to the construction of a more massive heterotopic focus, it must be concluded that the increase in mass of the bone tissue under the influence of estrone took place on account of nontransplantable (committed) osteogenic precursor cells. These data are in agreement with results obtained by other workers [8], who showed that in myelofibrosis in man no increase in the number of fibroblast precursor cells takes place in the bone marrow.

Considering that estrone has no direct inhibitory action on hematopoiesis the result of this experiment can be explained only by the direct influence of estrone on interaction between the bone marrow stroma and hematopoietic cells. One possibility is weakening of the homing effect of hematopoietic precursor cells for stromal cells [5]. In fact, after injection of estrone there is a rapid increase in the number of hematopoietic precursor cells in the blood [10]. Another possibility may be disturbance of

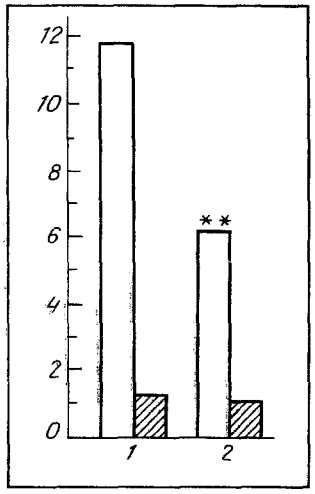

Fig. 5. Effect of one injection of estrone on results of subsequent heterotopic bone marrow transplantation (1 — control animals, 2 — animals receiving one injection of estrone before heterotopic transplantation). Unshaded columns — cell content, $\times 10^6$; shaded columns — mass, mg. $^*p < 0.01$.

the ability of the bone marrow stromal cells to maintain hematopoiesis. Both these mechanisms lead to reduction in the cell content of the bone marrow. Depression of myelopoiesis has been shown to lead to the development of fibrosis [9]. The direct mechanism leading to the development of fibrosis of bone marrow may be a decrease in its content of cells of the monocytic-macrophagal series, capable of exerting an inhibitory action on proliferation of connective-tissue cells [2]. The possibility cannot be ruled out that estrogens in experimental myelofibrosis, and the growth factor secreted by platelets in human chronic myelofibrosis may lead to a disturbance of production of the extracellular matrix, which is fundamental for interaction between hematopoietic cells and growth factors [6].

The general conclusion can be drawn that the pathogenesis of myelofibrosis is associated, not so much with excessive proliferation of connective-tissue cells as with a disturbance of the hematopoietic microenvironment.

LITERATURE CITED

1. V. A. Almazov, N. B. Koz'min-Sokolov, M. A. Kulik, et al., Byull. Éksp. Biol. Med., No. 9, 347 (1984).
2. V. A. Almazov, G. E. Arkad'eva, A. Yu. Zaritskii, and I. N. Ivasenko, Byull. Éksp. Biol. Med., No. 6, 753 (1986).
3. A. Ya. Fridenshtein and E. A. Luriya, Cellular Bases of the Hematopoietic Microenvironment [in Russian], Moscow (1980).
4. I. L. Chertkov and O. A. Gurevich, The Hematopoietic Stem Cell and Its Microenvironment [in Russian], Moscow (1984).
5. S. Aisawa and M. Tavassoli, Exp. Hematol., **14**, 492 (1987).
6. M. J. Gordon, G. P. Riley, and M. F. Greaves, Nature, **326**, 403 (1984).
7. T. K. Gray, S. Mohan, T. A. Linkhardt, and D. J. Baykink, Biochim. Biophys. Res. Commun., **158**, 407 (1989).
8. T. Hotta, M. Utsumi, T. Kato, et al., Scand. J. Haematol., **34**, 251 (1985).
9. K. Kagawa, K. Hayashi, and M. Awai, Acta Path. Jpn., **37**, 999 (1986).
10. B. S. Morse, D. Guilliani, and M. Soremekun, Cell. Tissue Kinet., **7**, 113 (1974).