Changes in Erythropoiesis in Normal Mice Induced by Transplantation of Peritoneal Cells from Syngeneic Donors with Long-Term Sodium Arsenite Intoxication

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Chronic intoxication with sodium arsenite in a total dose of 320 mg/kg produced significant compensatory reparative changes in the erythropoiesis of inbred CBA mice. The effect on erythropoiesis of adoptive transfer of peritoneal cells from intoxicated donors to normal syngeneic recipients was similar to the effect of sodium arsenite.

Key words: *stress-erythropoiesis, peritoneal cells; sodium arsenite*

Adoptive transfer of peritoneal cells nonstimulated with inflammatory agents from anemic mice induces reparative erythropoiesis in intact syngeneic recipients [1-3]. However, there is no evidence on whether peritoneal cells can induce compensatory changes in the erythropoiesis in response to other factors impairing erythropoiesis. For example, long term exposure to arsenic disturbs erythropoiesis due to inhibition of proliferation of early erythroid precursor, hemolysis, *etc.* [5-11] that can be partly compensated [4].

Our aims were to study morphological features of the erythropoiesis in mice after a long-term exposure to sodium arsenite ($NaAsO_2$) and compensatory reparative erythropoiesis in syngeneic normal recipients induced by adoptive transfer of peritoneal cells from intoxicated mice.

MATERIALS AND METHODS

Experiments were performed on 36 male CBA mice weighing 21-24 g. Daily dose of sodium arsenite (10 mg/kg; 0.2% water solution) was administered through a tube to 21 mice for 32 days. A total dose of 320 mg/kg produces marked changes in the hemopoiesis [4]. Resident peritoneal cells [1] were isolated from mice on day 33 of treatment, washed 3-fold by centrifugation, and injected intraperitoneally to 9 normal syngeneic recipients in a dose of 10^7 viable cells. Suspension of transplanted cells contained only 2% dead cells suggesting high resistance of peritoneal cells to long-term arsenic intoxication. Control animals (n=6) received intraperitoneal injection of culture medium 199 (0.4 ml). Both the recipients and controls were killed 4 days after transplantation [1]. Bone marrow preparations and blood samples were obtained from these animals and 12 donor mice treated with $NaAsO_2$. Mitotic indices for basophilic (BE), polychromatophilic (PE), and oxyphilic erythroblasts (OE) per 1000 cells of each population, and erythrocyte/leukocyte ratio were determined using Giemza-stained bone marrow preparations. The number of reticulocytes per 1000 erythrocytes was counted in blood smears stained with brilliant cresyl blue. Erythrocytes containing fetal hemoglobin were identified in blood smears stained with standard reagents (Sigma). Blood smears from newborn mice were used as the positive control.

The experimental data were statistically analyzed by Student's t test.

RESULTS

Long-term exposure to $NaAsO_2$ induced compensatory and reparative processes in the bone marrow eryth-

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roid stem (Figs. 1 and 2). Similarly to extensive hemorrhage [1], chronic arsenic poisoning initiated proliferation of OE which is normally insignificant (Fig. 2, *c*). Mitotic index of OE increased to 48.3±3.3% *vs.* 7.7± 1.8% in the control ($p<0.001$). However, OE count in the bone marrow of arsenic-treated mice did not increase (Fig. 2, *c*). It can be hypothesized that after mitosis these cells undergo rapid terminal differentiation and enrich erythrocyte population. By contrast, mitotic index in BE population decreased significantly ($p<0.001$) compared with the control (Fig. 2, *a*), while the percentage of BE in the bone marrow did not differ from the control (Fig 2, *a*). The total number and mitotic index for PE population as well as erythrocyte/leukocyte ratio were also near the control. Our findings are consistent with the data obtained on pregnant mice treated with arsenic in the same doses and according to the same scheme, which is considered as a manifestation of compensatory erythropoietic response to long-term arsenic intoxication [4].

Other sign of reparative erythropoiesis process in the bone marrow of mice after long-term arsenic intoxication was a 2-fold increase in reticulocyte count

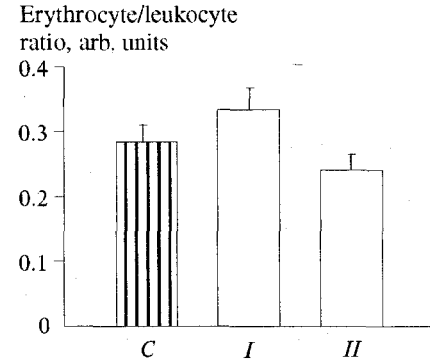

Fig. 1. Erythrocyte/eukocyte ratio in the bone marrow of donor mice after long-term arsenic intoxication (*I*), and recipients (*II*) of peritoneal cells compared with the control (*C*).

in the peripheral the blood (26.18±0.85% compared with 12.9±2.43% in the control). Moreover, a large number of non-reticulocytic macrocytes in peripheral blood of these mice were observed.

Tests for fetal hemoglobin in erythrocytes of arsenic-treated mice, recipients, and control animals were negative. At the same time, fetal hemoglobin was observed in some erythrocytes from newborns.

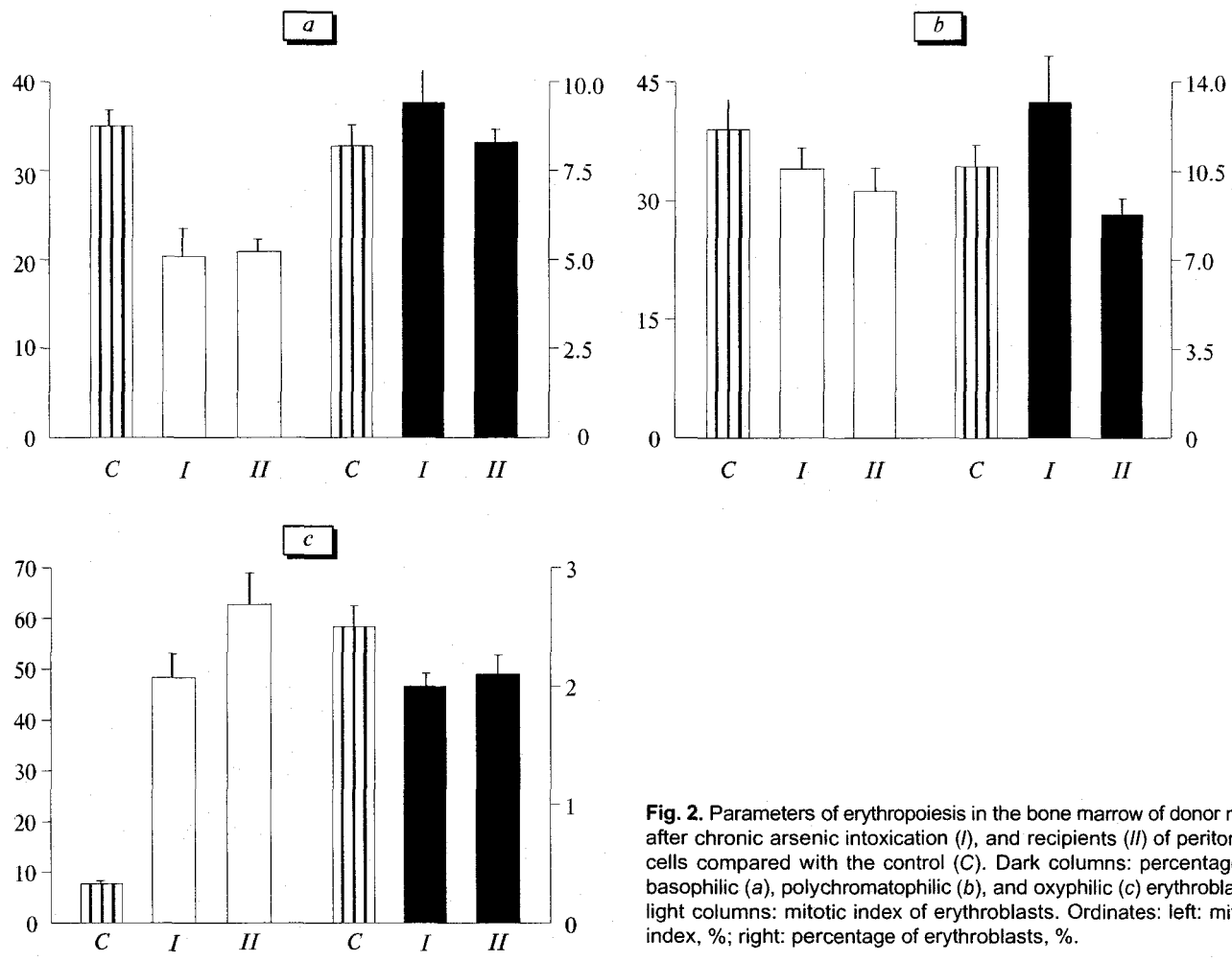

Fig. 2. Parameters of erythropoiesis in the bone marrow of donor mice after chronic arsenic intoxication (*I*), and recipients (*II*) of peritoneal cells compared with the control (*C*). Dark columns: percentage of basophilic (*a*), polychromatophilic (*b*), and oxyphilic (*c*) erythroblasts; light columns: mitotic index of erythroblasts. Ordinates: left: mitotic index, %; right: percentage of erythroblasts, %.

Adoptive transfer of peritoneal cells from arsenic-treated mice to intact syngeneic recipients caused dramatic changes in terminal stages of the erythropoiesis typical of chronic arsenic intoxication (Figs. 1 and 2). Four days after transplantation of peritoneal cells, mitotic index of OE increased to 62.7±4.9%, while mitotic activity of BE significantly decreased ($p<0.001$). Similar to intoxicated mice, in recipients the percentage of each type of erythroblasts and erythrocyte/leukocyte ratio did not differ the control.

In recipients, reticulocyte count increased 1.6-fold (21.14±2.27%, $p<0.03$) compared with the control, and non-reticulocytic macrocytes appeared in the peripheral blood. These phenomena can not be explained only by changes in some kinetic parameters in the erythroid compartment. Peritoneal cells taken from animals, in which erythropoiesis was reparative with signs of compensation can induce similar reparative and compensatory erythropoietic changes in recipients. These effects of adoptive transfer can not be caused by arsenic remaining in the peritoneal exudate after 3 cycles of washing, because the effect of arsenic was not observed below a cumulative dose of 150 mg/kg [4].

The peculiarities of erythropoiesis typical of arsenic intoxication result not only from disturbances in early erythroid precursors and inhibitory effect of arsenic on their proliferation [8], changes in terminal stages of erythropoiesis can be induced by transplantation of peritoneal cells from donors exposed to arsenic intoxication. So-called ineffective erythropoiesis characterized by production of macrocytes is an essential reparative mechanism triggered by regulatory peritoneal cells.

Changes in erythropoiesis induced by arsenic intoxication and adoptive transfer of peritoneal cells differ from those induced by excessive hemorrhage and by adoptive transfer of peritoneal cells from anemic animals [1,2]. Reserve erythropoiesis induced by massive blood loss and adoptive transfer of peritoneal cells is characterized by accumulation of BE in the bone marrow with simultaneous inhibition of their mitotic activity, increased PE and OE counts and corresponding mitotic indices, and a higher erythrocyte/eukocyte ratio [1-3]. Thus, peritoneal cells play an important role in the regulation of immediate response of erythropoiesis to various stress factors. Regulation of erythroid differentiation and proliferation by peritoneal cells is very specific and depends on the nature of the stress factor.

REFERENCES

1. A. G. Babaeva and E. I. Belan, *Ontogenez*, **19**, No. 2, 125-131 (1988).
2. E. I. Belan, *Role of Cell-Cell Interactions in Transduction of Regenerative Information under Conditions of Reparative Erythropoiesis in the Bone Marrow after Hemorrhage*, Abstract of Cand. Med. Sci. Dissertation, Moscow (1992).
3. E. I. Belan and L. L. Golovkina, *Byull. Eksp. Biol. Med.*, **118**, No. 9, 287-290 (1994).
4. M. G. Skal'naya, *Morphological and Functional Characteristics of the Thymus and Hemopoietic Organs in Pregnant and Newborn Mice Exposed to Sodium Arsenic*, Abstract of Cand. Med. Sci. Dissertation, Moscow (1994).
5. P. C. Blair, M. B. Thompson, M. Bechtold, *et al.*, *Toxicology*, **63**, No. 1, 25-34 (1990).
6. E. A. Conner, H. Yamauchi, and B. A. Fowler, *Chem. Biol. Interact.*, **96**, No. 3, 273-285 (1995).
7. H. L. Hong, B. A. Fowler, and G. A. Boorman, *Toxicol. Appl. Pharmacol.*, **97**, No.1, 173-182 (1989).
8. B. S. Morse, M. Conlan, D. G. Giuliani, M. Nussbaum, *Am. J. Hematol.*, **8**, No. 3, 273-280 (1980).
9. H. Pralle, F. Manz, *Blut*, **50**, No. 1, 51-54, (1985).
10. D. D. Westhoff, R. J. Samaha, A. Jr. Barnes, *Blood*, **45**, No. 2, 241-246 (1975).
11. S. L. Winski, D. S. Barber, L. T. Rael, D. E. Carter, *Fundam. Appl. Toxicol.*, **38**, No. 2, 123-128 (1997).