EFFECT OF EXOGENOUS DNA ON ERYTHROPOIETIN-SENSITIVE CELLS

V. M. Luzanov

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Transfusion polycythemia was reproduced in Wistar rats. Preparations of homologous and heterologous DNA were injected intraperitoneally in a dose of 5 mg. On the 1st, 3rd, 5th, 10th, 15th, and 20th days after injection of DNA the erythrocytes production was determined from the Fe^{59} content in these cells in the peripheral blood. Despite the sharp inhibition of erythropoiesis in the polycythemic animals, injection of the preparations of exogenous DNA restored the formation of the red blood cells. The effect from injection of heterologous DNA was less than from homologous DNA. The restoration of erythropoiesis in polycythemic animals in response to injection of DNA preparations was due to the effect of the DNA on differentiation and proliferation of erythropoietin-sensitive cells.

KEY WORDS: exogenous DNA; erythropoietin-sensitive cells; transfusion polycythemia; erythropoiesis.

High-polymer homologous DNA, if injected into healthy animals, increases the total number of cells in the bone marrow and spleen and increases their mitotic activity; in the peripheral blood the number of reticulocytes, leukocytes, and platelets increases [2]. The prolonged changes in the hematopoietic tissues observed suggest that DNA acts on hematopoietic stem cells.

The object of this investigation was to study the effect of high-polymer homologous and heterologous DNA on erythropoietin-sensitive cells.

EXPERIMENTAL METHOD

An experimental model of transfusion polycythemia, as suggested by Gurney et al. [5] to study the kinetics of the stem cells, was used. Polycythemia was induced as described previously [4]. One series of experiments was carried out on rats in which the polycythemic state was maintained by additional injections of red cells every 4th day until the 30th day.

An intraperitoneal injection of 5 mg DNA was given to the experimental animals 3 days after the final transfusion of red cells. DNA preparations were obtained by a modified method of Kay et al. [6] from the spleens of young rats and from the calf thymus. The molecular weight of the DNA preparations was between $25 \cdot 10^6$ and $30 \cdot 10^6$ dalton, the RNA content was $2 \pm 0.7\%$, and the protein content $0.2 \pm 0.01\%$. The animals received standard salt and sodium citrate solution (SSC). An injection of a solution of Fe^{59} in a dose of 6.6 μCi/100 g body weight was given to the animals 1, 3, 5, 7, 10, 15, and 20 days after the injection of DNA or SSC. Three days later the quantity of Fe^{59} in 1 mm^3 washed and hemolyzed peripheral blood red cells was determined. The number of erythropoietin-sensitive (EPS) cells present on the day of injection of the isotope was estimated from the content of Fe^{59} in 1 mm^3 of red cells.

Erythropoietin-rich serum was obtained from anemic rats. Anemia was induced by injection of 2% phenylhydrazine solution.

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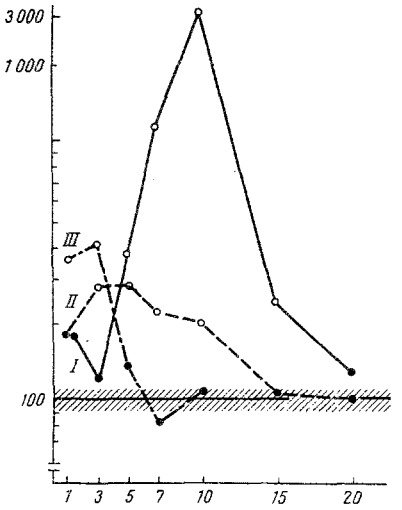

Fig. 1

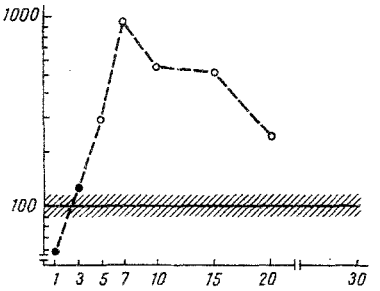

Fig. 2

Fig. 1. Number of Fe^{59}-labeled red cells in the peripheral blood of polycythemic animals receiving preparations of homologous (I) or heterologous DNA (II) and erythropoetin-rich serum (III) as percentages of number of labeled red cells of control polycythemic animals. Combined data of 3 repeated experiments are shown; each point in the control corresponds to 25 animals, in the experimental series to 15 animals. Empty circles indicate statistically significant differences ($P < 0.05$). Abscissa, days after injection of DNA or serum; ordinate, incorporation of Fe^{59} (in percent of control).

Fig. 2. Effect of homologous DNA on number of Fe^{59}-labeled red cells in peripheral blood of rats with prolonged polycythemia. Combined data of 3 repeated experiments given; each point in the control and experimental series corresponds to 15 animals. Empty circles indicate statistically significant differences ($P < 0.05$). Abscissa, days after injection of DNA; ordinate, incorporation of Fe^{59} (in percent of control).

EXPERIMENTAL RESULTS

In the polycythemic animals red cell produced by the hematopoietic tissues was significantly inhibited on account of the cessation of formation of endogenous erythropoietin [7]. A single injection of exogenous DNA into the polycythemic rats restored red cell production (Fig. 1), on account of the effect of the DNA preparations on differentiation of the EPS-cells. It must be noted that the response of the hematopoietic tissue to the injection of homologous DNA was more marked than to the injection of heterologous DNA. For example, after injection of homologous DNA the greatest effect was seen 10 days later, when the number of Fe^{59}-labeled red cells was more than 30 times higher than in the control. The maximal response to the injection of heterologous DNA occurred on the 3rd and 5th days, when the number of labeled red cells in the peripheral blood of the experimental animals was 3 times greater than in the control polycythemic animals.

The effect of exogenous DNA on the EPS-cells observed in these experiments may be due to the assimilation of the biopolymer by the hematopoietic cells. There is evidence that DNA is incorporated into the chromatin of bone marrow cells [1]. The smaller effect from the injection of heterologous DNA was evidently due to its less marked utilization by the recipient's cells than that of homologous preparations [3].

Comparison of the response of the polycythemic animals to a single injection of exogenous DNA and of erythropoietin-rich serum shows that the action of DNA on the EPS-cells was not confined to stimulating their differentiation. The observed differences in the character of the response to injection of the serum of anemic rats and of exogenous DNA (Fig. 1) suggests that this biopolymer also influences the proliferation of EPS-cells. This hypothesis was confirmed by the more prolonged erythropoietic response to DNA in the polycythemic rats, in which red cell production was inhibited until the 30th day (Fig. 2).

High-polymer DNA thus stimulates the differentiation and proliferation of EPS-cells and this is evidently one mechanism of the action of this biopolymer on hematopoiesis.

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