ANTIBODY-FORMING CAPACITY OF MOUSE SPLEEN CELLS AFTER HYPOXIC HYPOXIA AND INJECTION OF ERYTHROPOIETIN

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The absolute and relative (per 10^6 spleen cells) number of antibody-forming cells (AFC) in the spleen of CBA mice was found to be reduced by half of the 1st, 4th, and 7th days after acute hypoxia (12 h, 6700 m) and on the 1st and 4th days after the end of exposure to chronic hypoxia (16 h daily for 16 days, 6700 m). By the 7th day after the end of exposure to chronic hypoxia the number of AFC in the spleen of the mice was back to normal. One of two injections of erythropoietin reduced the absolute and relative number of AFC in the spleen of the posthypoxic mice by 33-50% compared with control animals.

KEY WORDS: *antibody-forming cells; hypoxic hypoxia; erythropoietin.*

The regulatory role of erythropoietin in proliferation and differentiation of cells of the erythroid series has now been studied in detail [4] but the question of the direct influence of erythropoietin on the polypotent hematopoietic stem cell still remains open to debate [4]. This cell is known to be the precursor for mature cells of the erythroid, myeloid, megakaryocytic, and lymphoid series [6]. Different agents, inducing proliferation and differentiation of the hematopoietic stem cell, thus will evidently be reflected in the descendants of that cell, including immunocompetent cells.

In this investigation the effect of acute and chronic hypoxia and also of injection of erythropoietin into posthypoxic mice on the number of antibody-forming cells (AFC) was studied in the spleen of CBA mice immunized with sheep's red blood cells (SRBC).

EXPERIMENTAL METHODS

CBA mice obtained from the Stolbovaya Nursery of Laboratory Animals, Academy of Medical Sciences of the USSR, were used. Acute hypoxia was produced by exposing the mice in a pressure chamber to a pressure of 0.42 atm (6700 m) for 12 h; chronic hypoxia was produced by similar exposure to the same pressure for 16 h daily for 16 days. Erythropoietin (isolated from human urine), obtained from Dr. Peter P. Dukes, Hematology Research Laboratories, Children's Hospital of Los Angeles, USA, was injected intraperitoneally into posthypoxic mice on the 3rd day after the end of exposure to chronic hypoxia in a single dose of 6 units 24 h before immunization, or as two doses, each of 6 units, 24 and 8 h before immunization. On the 1st, 4th, and 7th days after the end of acute or chronic hypoxia the mice were immunized by intravenous injection of $2 \cdot 10^8$ SRBC. The number of AFC in the spleen was counted on the 4th day after immunization, using a modified Cunningham's method [3]. To assess the degree of polycythemia and the erythropoietic activity of the mice, the hematocrit index was determined and the number of reticulocytes in the blood counted by the usual methods. The results were subjected to statistical analysis by Student's t test.

EXPERIMENTAL RESULTS

After the end of exposure to chronic hypoxia the hematocrit index of the mice exceeded 70% and the reticulocyte count in the blood fell to 2-10‰ on the 4th-5th day, evidence of a high degree of inhibition of erythropoiesis. On the 5th day after one or two injections of erythropoietin the reticulocyte count in the blood rose to 30-40‰.

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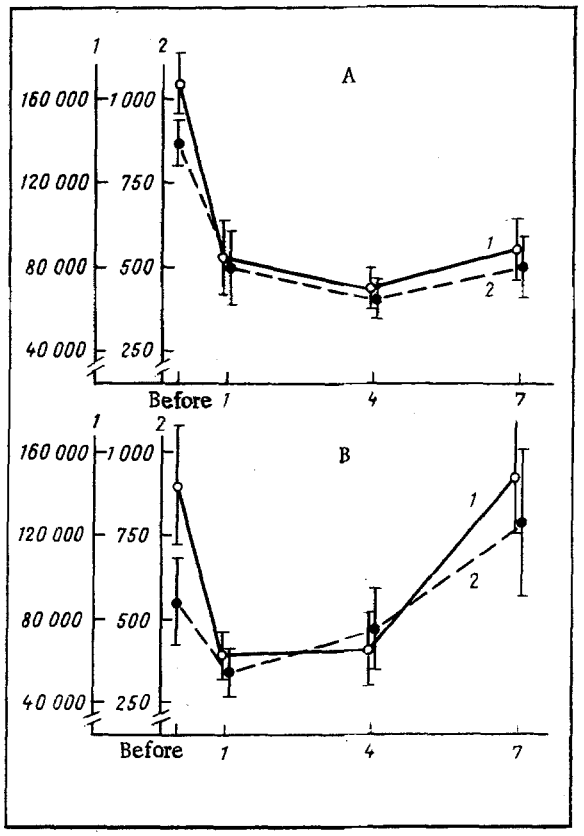

Fig. 1. Number of AFC in spleen of CBA mice at different times after acute (A) and chronic (B) hypoxia. 1) Absolute number of AFC; 2) relative number of AFC (per 10^6 spleen cells). Abscissa) time after end of hypoxia (in days); ordinate) here and in Fig. 2, number of AFC in spleen.

The results of the study of the effect of acute and chronic hypoxia on the number of AFC in the spleen of the mice are given in Fig. 1. Clearly on the 1st to the 7th days after acute hypoxia the absolute and relative (per 10^6 spleen cells) number of AFC in the spleen of the mice was reduced by 44-50% compared with the control animals. On the last and 4th days after the end of exposure to chronic hypoxia the relative number of AFC in the spleen was reduced by 38 and 23%, respectively, and the absolute number was reduced by 55%. However, unlike acute hypoxia, on the 7th day after the end of chronic hypoxia the absolute number of AFC in the spleen of the mice was back to normal and the relative number of AFC was actually a little higher than in the control animals (by 40%).

The results of experiments to study the effect of exogenous erythropoietin on the antibody-forming capacity of the spleen cells are given in Fig. 2. After one and two injections of erythropoietin the absolute number of AFC in the spleen was found to be 47,314±6557 and 37,086±4450, respectively (the number of AFC in the control posthypoxic mice was 63,435± 11,600) and the relative number of AFC was 243±37 and 222±34, respectively (431±68 in the control).

The results thus indicate that stimulation of erythropoiesis by means of hypoxia and injection of exogenous erythropoietin leads to a decrease in the number of AFC in the spleen of the mice.

One of the mechanisms of the decrease in the number of AFC in the spleen of the mice after exposure to factors stimulating erythropoiesis is evidently a decrease in the production of cells belonging to other series of hematopoiesis, including immunocompetent cells. The results obtained by other workers, to show that injection of erythropoietin into mice reduces the ability of their spleen cells to induce the graft versus host reaction, are good confirmation of this hypothesis [5]. However, the possibility cannot be ruled out that hypoxia may have a direct toxic action on the immunocompetent cells. The decrease in erythropoietic activity after chronic hypoxia, and the consequent liberation of additional powers of differentiation of the polypotent hematopoietic stem cell were probably the reason why on the 7th day after the end of exposure to chronic hypoxia the number of AFC in the spleen of the polycythemic mice was restored to the normal level, whereas at the same times after acute hypoxia their number was considerably smaller than the number of AFC in the control animals (Fig. 1). This hypothesis is confirmed by the writers' earlier observations, indicating that posttrans-

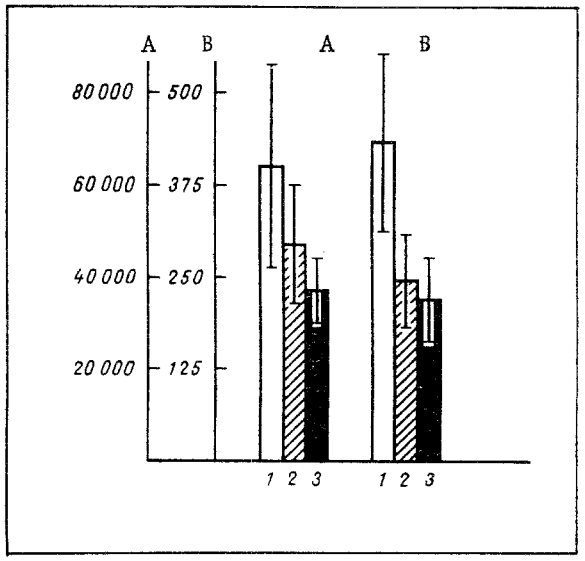

Fig. 2. Effect of erythropoietin on absolute (A) and relative (B) number of AFC in spleen of posthypoxic CBA mice. 1) Control; 2) one injection of 6 units erythropoietin 24 h before immunization; 3) two injections, each of 6 units, of erythropoietin 24 and 8 h before immunization.

fusion polycythemia leads to an increase in the number of AFC in the mouse spleen [1], and by the results of other workers, according to whom transfusion of syngeneic erythrocytes increases the antibody titer whereas injection of erythropoietin abolishes this effect [2]. These data and the results of the present investigation are evidence that differentiation of the polypotent hematopoietic stem cell can be regulated in a certain direction.

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