Effects of Prenatal Hypoxia on the Formation of Immune Deficiency in Newborn Mice

V. Yu. Matrosova, I. A. Orlovskaya, D. V. Kozlova, and V. A. Kozlov

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 129, No. 6, pp. 664-666, June, 2000
Original article submitted May 15, 2000

Fetal hypoxia in the II trimester of pregnancy caused immunodeficiency in newborn mice: inhibition of antibody production to sheep erythrocytes and disturbances in migration of early hemopoietic precursors from the bone marrow to the spleen.

Key words: *chronic hypoxia; spleen colony-forming unit; antibody-producing cell*

Implantation of the embryo and its intrauterine development, as well as the postnatal period are characterized by active immune processes. Identification of factors regulating the intrauterine development will help to improve diagnosis, prognosis, and treatment of pathological states accompanied by immune compromise in the early infancy. Prenatal hypoxia is one of these factors. Published data on the effects of hypoxia on the hemopoietic and immune systems [2-4,8] stimulate further studies of the mechanisms responsible for immune deficiency in newborn animals exposed to intrauterine hypoxia. These studies will open prospects for correction of this disorder.

MATERIALS AND METHODS

Experiments were performed on (CBA×C57Bl/6)F1 mice aged 3-6 months.

Pregnant females in the second trimester of gestation were placed in a pressure chamber and "elevated" to an altitude of 6700 m above sea level for 14-16 h. The procedure was repeated daily for 7 days.

The number of antibody-forming cells (AFC) in the spleen was determined on day 4 after intraperitoneal immunization with 10% sheep erythrocyte suspension (0.25 ml) by counting local hemolysis zones in a semiliquid medium as described elsewhere [6] with some modifications.

Institute of Clinical Immunology, Siberian Division, Russian Academy of Medical Sciences, Novosibirsk

The number or early hemopoietic precursors in the bone marrow, spleen, and liver was determined by the number of spleenic colony-forming units (CFUs) [9]. CFUs were counted on day 8 after transplantation of bone marrow cells (10^5 cells per mouse), spleen cells (2×10^6 cells per mouse), or liver cells (2×10^6 cells per mouse) to lethally irradiated syngenic recipients (CFUs-8).

The offspring was examined at the age of 2, 4, and 8 weeks.

Significance of differences was evaluated by Student's *t* test.

RESULTS

We found a considerable decrease in the relative and absolute AFC counts in mice exposed to chronic hypoxia (test groups) compared to control mice in all age groups (Fig. 1, *a*).

Hematocrit in 2-and 4-week-old test mice was considerably higher than in age-matched controls: 39.8±1.4 and 46.5±1.11 *vs.* 36.4±1.8 and 43.9±1.0, respectively. There were no significant differences between 8-week-old test and control mice (44.9±1.0 and 45.6±1.1, respectively).

The test mice were characterized by higher weights of the thymus, spleen, and liver on week 8 (Table 1), and reduced number of nucleated cells in the thymus on weeks 2 and 4 and in the liver on weeks 2, 4, and 8 (changes on weeks 2 and 8 were significant) (Table 1).

In all age groups, the number of CFUs in the bone marrow of the test mice were considerably higher than

0007-4888/00/0006-564$25.00

in age-matched controls (Fig. 1, *b, c*). The number of nucleated cells in the bone marrow of test mice also surpassed that in controls, and this difference was significant on week 8 (Table 1). The numbers of CFUs in the spleen of 4- and 8-week-old test mice were lower than in age-matched controls (Fig. 1, *c*).

Studies of the colony-forming activity of liver cells found an insignificant decrease in the number of liver CFUs in test groups in comparison with the control (Fig. 1, *d*).

The decrease in the number of AFC formed in the spleen in response to immunization with sheep erythrocytes can be explained by immune deficiency in the offspring of mice that experienced chronic hypoxia in the second trimester of gestation.

Enhanced colony-forming activity of bone marrow cells and reduced content of CFUs in the spleen accompanying the formation of immune deficiency in newborn mice probably reflect disturbed migration of hemopoietic precursors from the bone marrow to the spleen. The involvement of hemopoietic precursors in the formation of immunodeficiency in newborn mice is confirmed by accumulation of nucleated cells in the bone marrow and their reduced content in peripheral immune organs.

Previous studies showed that factors stimulating proliferation of hemopoietic stem cells, in particular hypoxia, can cause immunosuppression. A possible mechanism of hypoxia-induced immunosuppression is predominant erythroid differentiation of hemopoietic stem cells at the expense of other hemopoietic lineages, in particular, lymphoid elements [1]. Erythroid suppressor cells inhibiting B cells precursors were identified [7]. There is evidence that erythroblasts obtained from embryonic liver produce some inhibitors of lymphocyte functional activity [5].

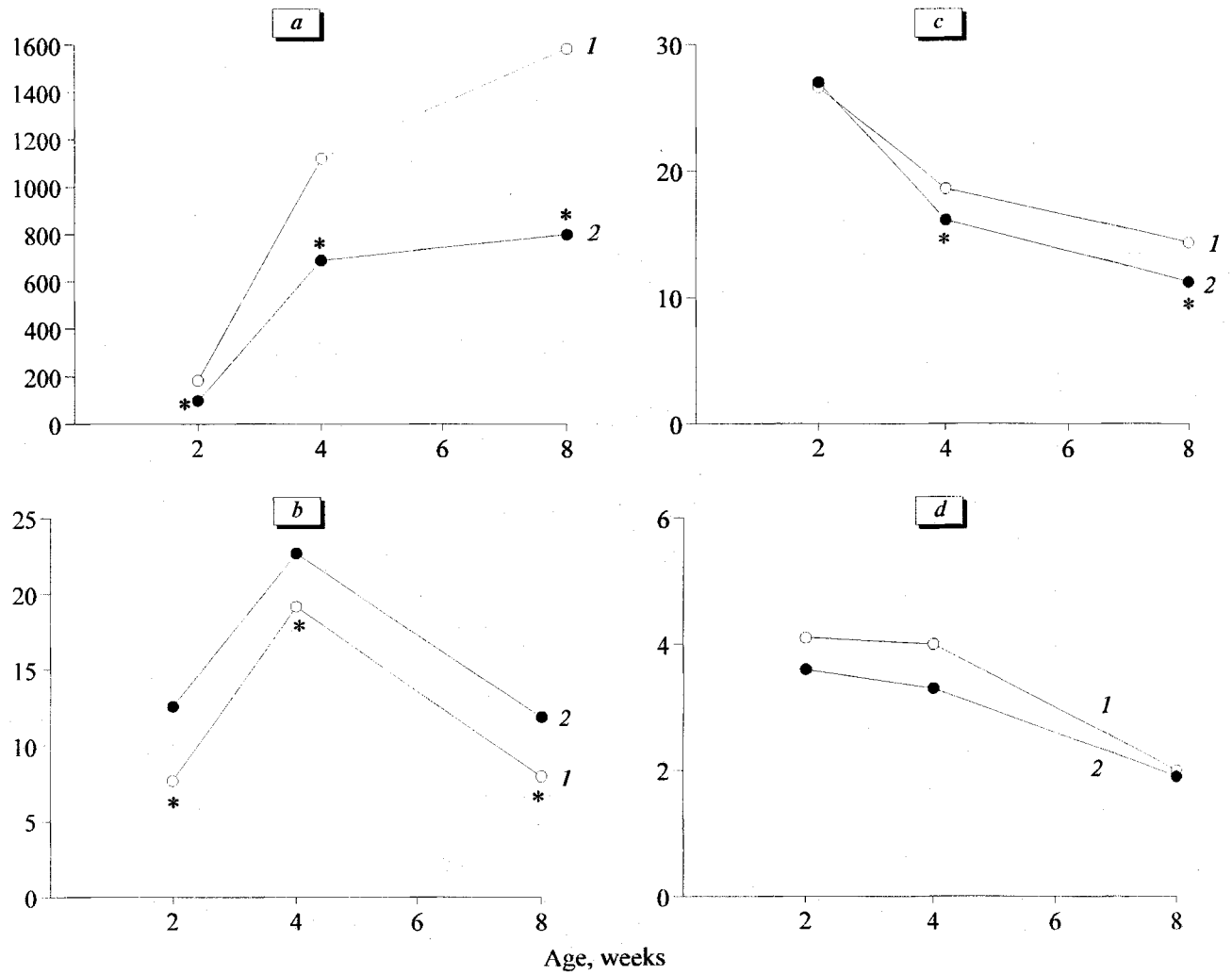

Fig. 1. Number of antibody-forming cells (*a*) after immunization with sheep erythrocytes, and number of CFUs in the bone marrow (*b*), spleen (*c*), and liver (*d*) in control newborn mice (*1*) and in offspring of mice exposed to chronic hypoxia (*2*). *Significant difference from the control.

TABLE 1. Effects of Chronic Hypoxia in Trimester II of Gestation on Weight of Immune Organs and the Number of Nucleated Cells (NC) in the Offspring (*M*±*m*)

Organ		Age, weeks					
		2		4		8	
		control	test	control	test	control	test
Thymus	weight, mg	27.1±1.0	29.1±1.5	50.0±3.4	49.1±3.4	68.1±4.4	76.3±5.3
	NC, 10^6	99.0±3.2	83.1±1.5	178.5±17.6	145.0±9.9	74.2±7.7	76.3±6.8
Spleen	weight, mg	12.0±0.6	21.2±2.8	41.3±5.6	42.9±1.6	40.0±1.9	46.0±2.8
	NC, 10^6	32.6±0.9	35.9±2.3	90.1±8.4	79.3±4.1	89.9±8.7	87.1±5.4
Liver	weight, mg	101.1±9.3	98.2±2.8	394.8±15.1	413.8±14.3	523.2±11.1	589.7±19.7
	NC, 10^6	74.3±1.4	55.1±0.9	106.5±23.7	97.4±11.2	185.3±13.3	123.2±9.8
Bone marrow	NC, 10^6	8.4±0.8	9.1±0.6	9.3±0.7	9.6±0.6	25.5±1.6	27.3±1.4

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