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Formation of Immunodeficiency in Newborn Mice Exposed to Nicotine during Intrauterine Development

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Exposure to nicotine during intrauterine development leads to immunodeficiency manifested in inhibition of delayed-type hypersensitivity reaction and reduced number of antibody-producing cells forming in response to sheep erythrocytes in newborn mice. The number of splenic CFU in the bone marrow of newborn mice exposed to nicotine *in utero* is decreased compared to the control. By contrast, nicotine induced an increase in splenic CFU count in fetal liver. We concluded that nicotine modifying the hemopoietic microenvironment delayed the release of primitive precursors from fetal liver, which impaired colonization of fetal bone marrow and led to imbalance in the production of mature blood cell, including immune system cells.

Key Words: *immunodeficiency; nicotine; hemopoietic precursors; fetal bone marrow; pregnancy*

Cigarette smoke products easily penetrate through the placenta and are accumulated in fetal tissues: nicotine concentration in placental tissue, amniotic fluid, and umbilical blood is equivalent to or even surpasses its concentration in maternal plasma. The risk of spontaneous abortions, neonatal mortality, and subsequent psychomotor retardation in newborns is essentially higher for tobacco smoking mothers [8]. Epidemiological studies showed that intrauterine exposure to nicotine impairs migration, proliferation, and differentiation of embryonic cells, which increases the risk of coronary heart disease, hypertension, diabetes, and immunodeficiency [1-3]. Tobacco smoking modifies some parameters of immune reaction, including the proliferation of lymphocytes and neutrophils and functional activity of macrophages.

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The formation of committed precursors in the bone marrow and their subsequent differentiation into mature cells, including immune cells, depend on the number of primitive hemopoietic stem cells (HSC) in the bone marrow. In adult animals proliferation, differentiation, and maturation of HSC occur in the bone marrow; during fetal development the first primitive hemopoietic precursors are generated in the yolk sac and then migrate into fetal liver. The final and critical stage in the development of hemopoietic system is colonization of fetal bone marrow by stem cells; it occurs at the late stage of intrauterine development. Many pathophysiological factors, including cigarette smoke and its products, can disturb the process of hemopoietic system development during the ontogeny. We previously showed that nicotine can modify the function of hemopoietic microenvironment: reduced expression of CD44 adhesion molecule by stromal cells disordered their interaction with HSC and impaired bone marrow colonization by stem cells in lethally irradiated recipients [7].

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We studied the effect of nicotine on colonization of fetal bone marrow during intrauterine development in order to evaluate the mechanisms of the formation of immunodeficiency in newborns.

MATERIALS AND METHODS

The study was carried out on (CBA×C57Bl/6)F_1 mice from the Breeding Center of Siberian Branch of Russian Academy of Sciences, Tomsk. The mice were daily injected with nicotine (Sigma), nicotine metabolite cotinine, or normal saline during the entire course of pregnancy. Nicotine concentration in the blood of pregnant mice corresponded to its concentration in tobacco smokers (10^{-7}-10^{-8} M).

Delayed-type hypersensitivity (DTH) reaction was induced as follows: mice were intraperitoneally immunized with 0.5% sheep erythrocyte suspension (0.5 ml). The challenge dose of the antigen (0.05 ml 50% solution) was injected under the hind paw aponeurosis after 96 h. The formation of DTH reaction was evaluated 24 h after challenge injection by the degree of paw swelling (increase in paw thickness compared to contralateral paw of the same animal injected with RPMI-1640, positive control). The reaction index (RI, %) was evaluated for each mouse by the formula: $RI=(R_e-R_c)/R_c$, where R_e is the reaction in the experiment and R_c in the control.

The number of antibody-producing cells (APC) in the spleens was evaluated on day 4 after intraperitoneal immunization with 0.25 ml 10% sheep erythrocyte suspension by the number of local hemolysis zones in semisolid medium by a modified method [4].

The count of early hemopoietic precursors in the bone marrow and liver was evaluated by counting splenic CFU [9]. On day 8 after transplantation of bone marrow cells (10^5 cells/mouse) and liver (2×10^6 cells/mouse) to lethally irradiated syngeneic recipients, splenic CFU (CFUs-8) were counted in removed spleens. In order to evaluate the count of committed precursors in the liver, the cells in a concentration of 2×10^4/well were incubated in M-3434 methylcellulose medium (Stem Cell Technology) containing stem cell factor, erythropoietin, IL-3, and IL-6. Granulocytic macrophage CFU and burst-forming erythroid colonies (BFU-E) were counted under a microscope after 14-day incubation at 37°C in a humid atmosphere with 5% CO_2.

We examined 12-day embryos (fetal liver), 19-day embryos, newborn mice, and mice aged 1.0-1.5 months. The significance of differences was evaluated by Student's *t* test.

RESULTS

A significant reduction (by 2.3 times) in the number of APC forming in response to sheep erythrocytes (normalized to splenocyte count) was observed in 1.0-1.5-month-old mice exposed to nicotine *in utero*; cotinine only slightly reduced this parameter (Fig. 1, *a*). Injection of nicotine and cotinine to pregnant mice caused a negligible reduction in DTH reaction in mice aged 1.0-1.5 months in comparison with the control (Fig. 1, *b*). Hence, parameters of the immune response decreased in animals exposed to nicotine *in utero*.

Simce HSC are common precursors for mature immune cells, we studied the effect of intrauterine exposure to nicotine on the number of CFUs and committed precursors (CFUc) in the liver of 12-day embryos. A significant increase in the number of CFUs in the liver of embryos exposed to nicotine was detected (Fig. 2, *a*); the numbers of granulocyte macrophage and erythroid precursors also surpassed the control level, the number of BFU-E increased significantly (Fig. 2, *b*). The content of CFUs in the liver of

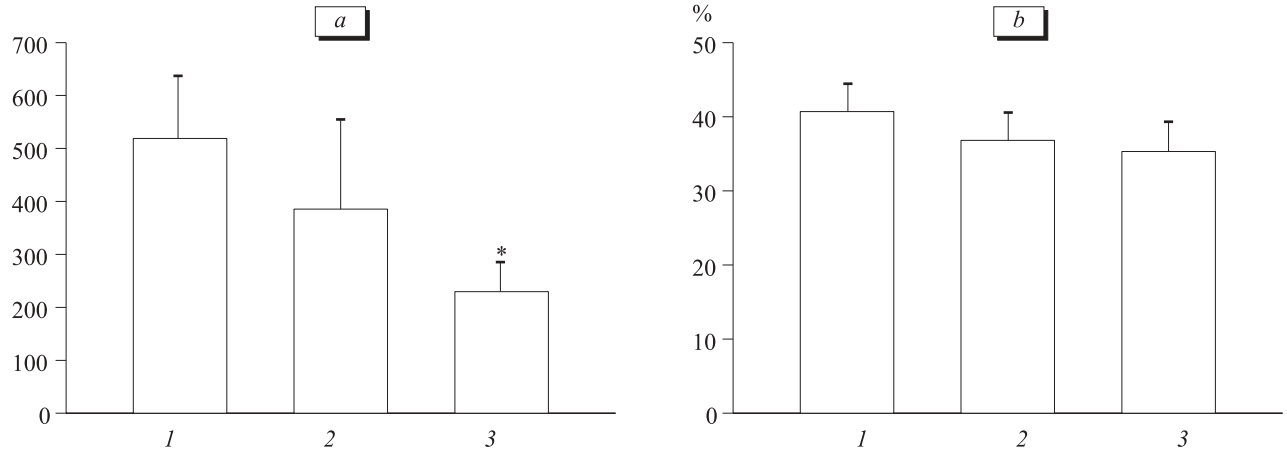

Fig. 1. Number of antibody-producing cells forming in response to sheep erythrocytes (*a*) and index of delayed-type hypersensitivity reaction (*b*) in mice aged 1.0-1.5 months exposed to nicotine *in utero*. *1*) control; *2*) cotinine; *3*) nicotine. Here and in Figs. 2, 3: *$p<0.05$ compared to the control.

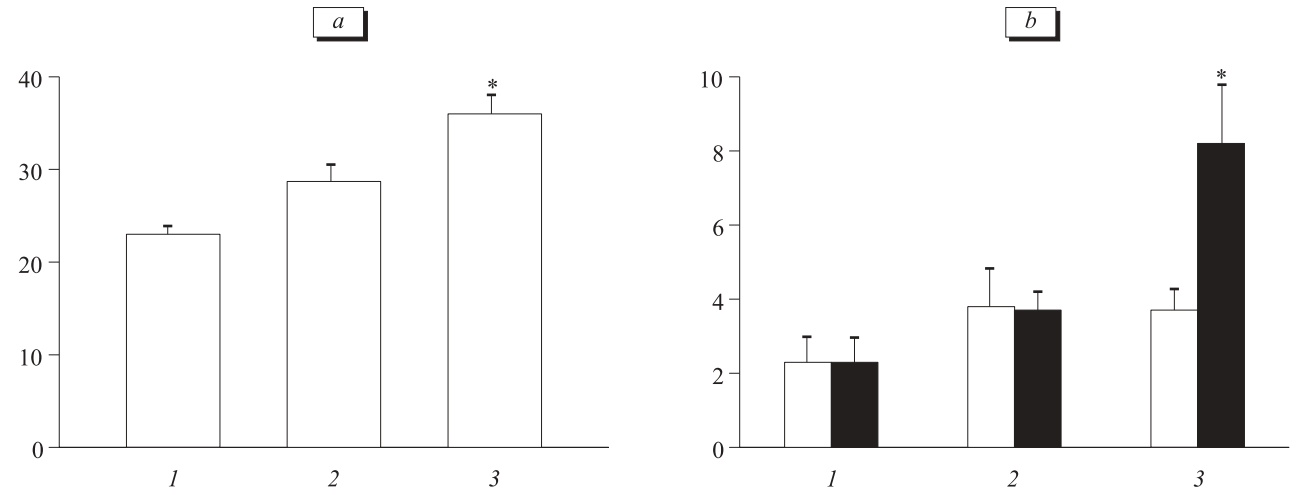

Fig. 2. Number of CFUs-8 (*a*) and committed precursors (*b*) in the liver of 12-day embryos exposed to nicotine during intrauterine development. *1*) control; *2*) cotinine; *3*) nicotine. Dark bars: granulocyte-macrophage CFU; light bars: burst-forming erythroid units.

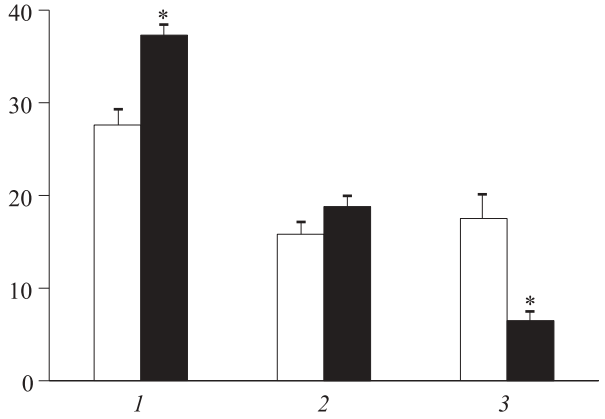

Fig. 3. Number of CFUs-8 in the liver of 19-day embryos (*1*), in the liver (*2*) and bone marrow (*3*) of newborn mice exposed to nicotine *in utero*. Dark bars: control; light bars: nicotine.

19-day embryos treated *in utero* with nicotine remained significantly increased (Fig. 3).

We hypothesized that intrauterine exposure to nicotine can inhibit the release of primitive precursors from the liver. The number of CFUs-8 in the bone marrow of newborn (1 week) mice exposed to nicotine *in utero* increased (Fig. 3).

Presumably, nicotine modifying the hemopoietic environment initiates a cascade of reactions inhibiting the release of primitive HSC from embryonic liver, which leads to decreased colonization of embryonic bone marrow and subsequent imbalance in the production of mature blood cells, including immune cells. Nicotine stimulates the production of IFN-γ and suppresses production of IL-10 [5]. The increase in the production of proinflammatory cytokines (IL-1 and TNF-α) under the effect of nicotine was previously demonstrated [6]. Expression of SDF-1 (chemokine stimulating migration activity of HSC, including embryonic) markedly decreased under the effect of proinflammatory cytokines, including IL-1 and TNF-α [5]. Hence, nicotine-induced increase in the production of proinflammatory cytokine can decrease the level of SDF-1 and subsequent delay of fetal bone marrow colonization.

The detected mechanism of the formation of immunodeficiency in newborns resulting from nicotine-induced delay of fetal bone marrow colonization can open new approaches to prevention and correction of diseases of infancy.

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