Effect of Thymus-Dependent Antigen on Recovery of Hemo- and Lymphopoiesis Impaired by Antineoplastic Drugs

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Single injection of antineoplastic drugs cyclophosphamide and cisplatin (Platidiam) in maximum permissible doses to CBA/CaLac mice impaired morphological composition of hemopoietic and lymphoid organs (thymus and spleen). Drug-induced changes persisted for 6 months. Cyclophosphamide caused more severe disturbances in the lymphoid stem in the bone marrow, thymus, and spleen and more strongly stimulated erythropoiesis in the spleen compared to cisplatin. Immunization of mice with thymus-dependent antigen (sheep erythrocytes) after injection of cytostatics accelerated regenerative processes in hemopoietic and lymphoid organs. However, cell disorganization 3 and 6 months after immunization did not differ from that caused by antineoplastic drugs and was even more pronounced in the thymus by the 6th month of observations.

Key Words: *cyclophosphamide; Platidiam (cisplatin); bone marrow; peripheral blood; thymus; spleen*

There is a great body of data on the effects of antineoplastic drugs on lymphoid and hemopoietic tissues [2,5]. Suppression of practically all hemopoietic stems observed at the early terms after treatment (several hours or days) is followed by normalization of their functional state to the 30th day [2,3,11,14]. However, recovery of quantitative parameters of hemo- and lymphopoiesis does not necessarily indicate their normal functional activity. This can be verified by administration of thymus-dependent antigen to animals treated with cytostatics. These experimental conditions simulate the state of cancer patients receiving cytostatic therapy, which is often complicated by infectious processes due to low resistance of the body.

Here we studied the effects of thymus-dependent antigen injected to CBA/CaLac mice receiving cyclophosphamide (CP) or cisplatin (Platidiam) on the hemopoietic system and lymphoid organs.

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MATERIALS AND METHODS

Experiments were performed on male CBA/CaLac mice weighing 16-18 g and obtained from Rassvet nursery (Tomsk). To simulate cytostatic disease, the animals received single intraperitoneal injection of CP (Biokhimik, Saransk) or cisplatin (Platidiam, Lachema) in maximum permissible doses (MPD, 250 and 9 mg/kg, respectively) determined by a graphic probit analysis.

Experimental mice were intraperitoneally immunized with corpuscular thymus-dependent antigen (suspension of 15% sheep erythrocytes, 0.2 ml) on days 4 (series I) or 30 (series II) after treatment with antineoplastic drugs. The animals were decapitated on days 4, 7, 14, 21, and 30 after immunization, or 3 and 6 months after the administration of cytostatic drugs. Parameters of the immune system in treated mice were compared with those in intact animals and in mice receiving only the antigen (control). The total number of leukocytes and myelokaryocytes, the ratio between

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morphologically different cells in the blood, bone marrow, thymus, and spleen, and the weight and cellularity of lymphoid organs (spleen and thymus) were estimated by routine hematological methods [14].

The results were analyzed by method of variational statistics.

RESULTS

Previous studies revealed marked hyperplasia of hemopoietic tissues 1-4 days after single administration of CP in MPD [2,7,12]. In our experiments, we analyzed the reaction of hemopoietic and lymphoid cells on day 4 after immunization with thymus-dependent antigen. Since CP was used for as a reference drug, the mice receiving cisplatin in MPD were also injected with the antigen on day 4 of observations.

It was shown that immunization of animals activates monocytes and macrophages producing interleukin-1, which promotes entrance of hemopoietic multipotent stem cells and immature hemopoietic precursors (colony-forming units, CFUs-5, 8, and 11) into S phase of the cell cycle and their migration from the bone marrow into the spleen [1,8]. Quantitative parameters of the bone marrow, thymus, and spleen in mice immunized after administration of cytostatics returned to normal or increased 2-3 weeks earlier than in animals treated with CP or cisplatin. This was mainly due to intense regeneration of the lymphoid hemopoietic stem suppressed by CP (Figs. 1-3, *a*, *b*). At the same time, over the first 2-3 weeks the total cellularity of organs and the content of lymphoid elements in mice treated with cytostatics (in particular, with CP) were lower than in immunized animals (Figs. 1-3)

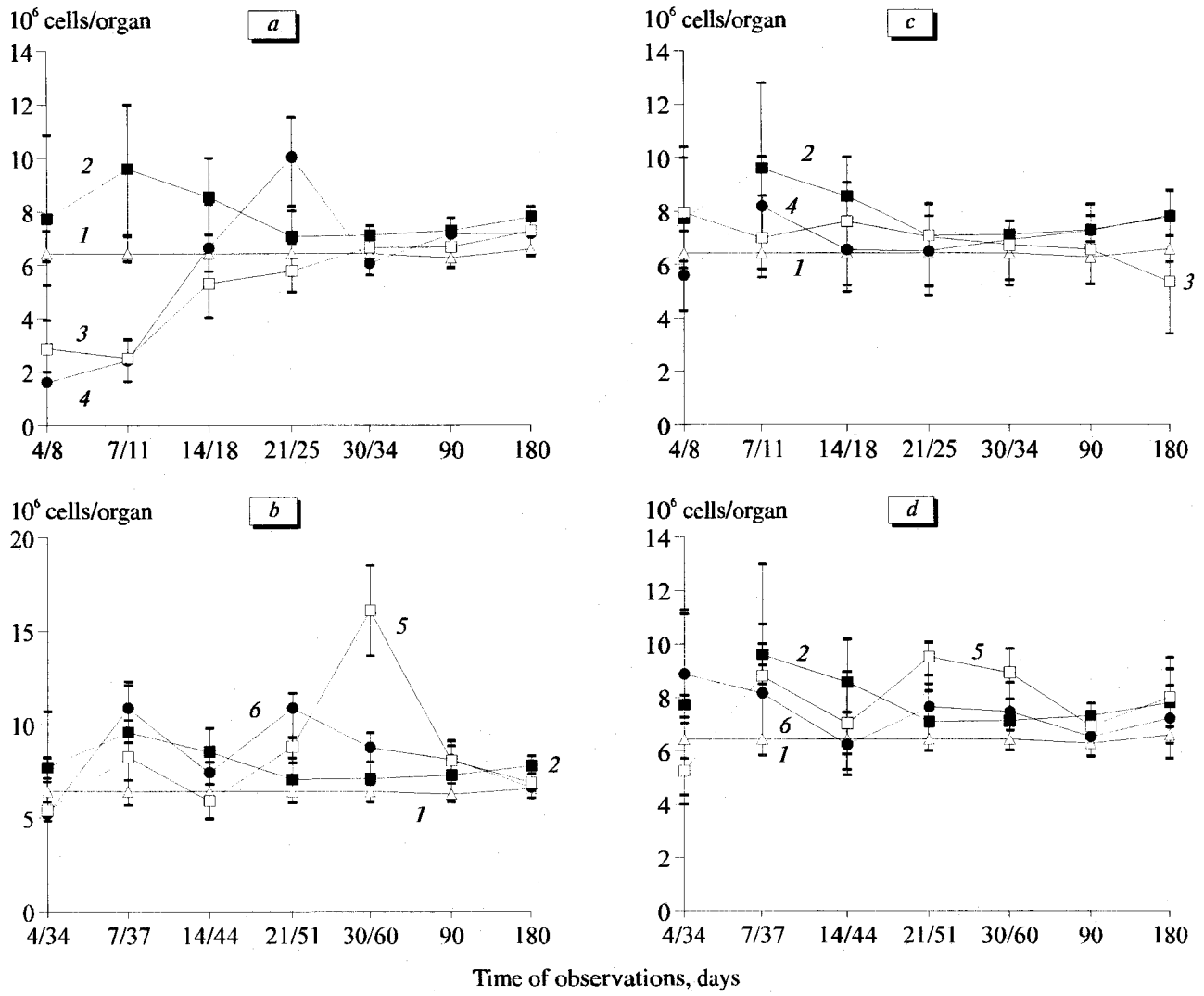

Fig. 1. Absolute content of lymphoid cells in mouse bone marrow. Here and in Figs. 2 and 3: intact animals (*1*), immunization (*2*), cyclophosphamide (*3*), immunization+cyclophosphamide (*4*), cisplatin (*5*), immunization+cisplatin (*6*). Immunization on day 4 (*a*, *c*) or 30 (*b*, *d*) after cytostatic treatment.

probably due to depletion of these cell population by antineoplastic drugs.

CP produced a more prolonged suppression of the erythroid hemopoietic stem in the bone marrow of mice than cisplatin. Immunization of CP-treated animals aggravated this state probably due to the activation of hemopoietic cells, *e.g.*, due to enhanced production of interleukin-1 suppressing late stages of erythropoiesis, decreasing the content of erythroid CFU in the bone marrow, and promoting recovery of granulocyte-macrophage CFU and mature granulocytes in the bone marrow [1,8]. In addition, it was shown that antigen-stimulated T lymphocytes produce colony-stimulating factor and interleukin-3 promoting differentiation of CFU towards granulocytopoiesis and inhibiting the formation of erythroid colonies [6].

Immunization of cisplatin-treated mice sharply decreased the content of erythroid cells in the bone marrow and promoted normalization of this parameter compared to mice receiving CP (days 7 and 21, respectively). Stimulation of erythropoiesis following its suppression decreased the content of bone marrow granulocytes probably due to competition between erythroid and granulocyte hemopoietic stems at the level of multipotent stem cells [10,13].

Immunization on day 30 after administration of CP or cisplatin did not stimulate cell proliferation and differentiation in the bone marrow (Fig. 1, *c*, *d*) and spleen (Fig. 3, *c*, *d*): the cellularity and content of lymphoid cells did not differ from those in cytostatic-treated animals. However, changes in the thymus were even more pronounced: the content of small lymphocytes in immunized mice was lower than in animals treated with CP and cisplatin over 1 month and 2 weeks of observations, respectively (Fig. 2, *c*, *d*).

Probably, immunization against the background of regenerative processes in hemo- and lymphopoiesis induced by antineoplastic drugs leads to their inhibi-

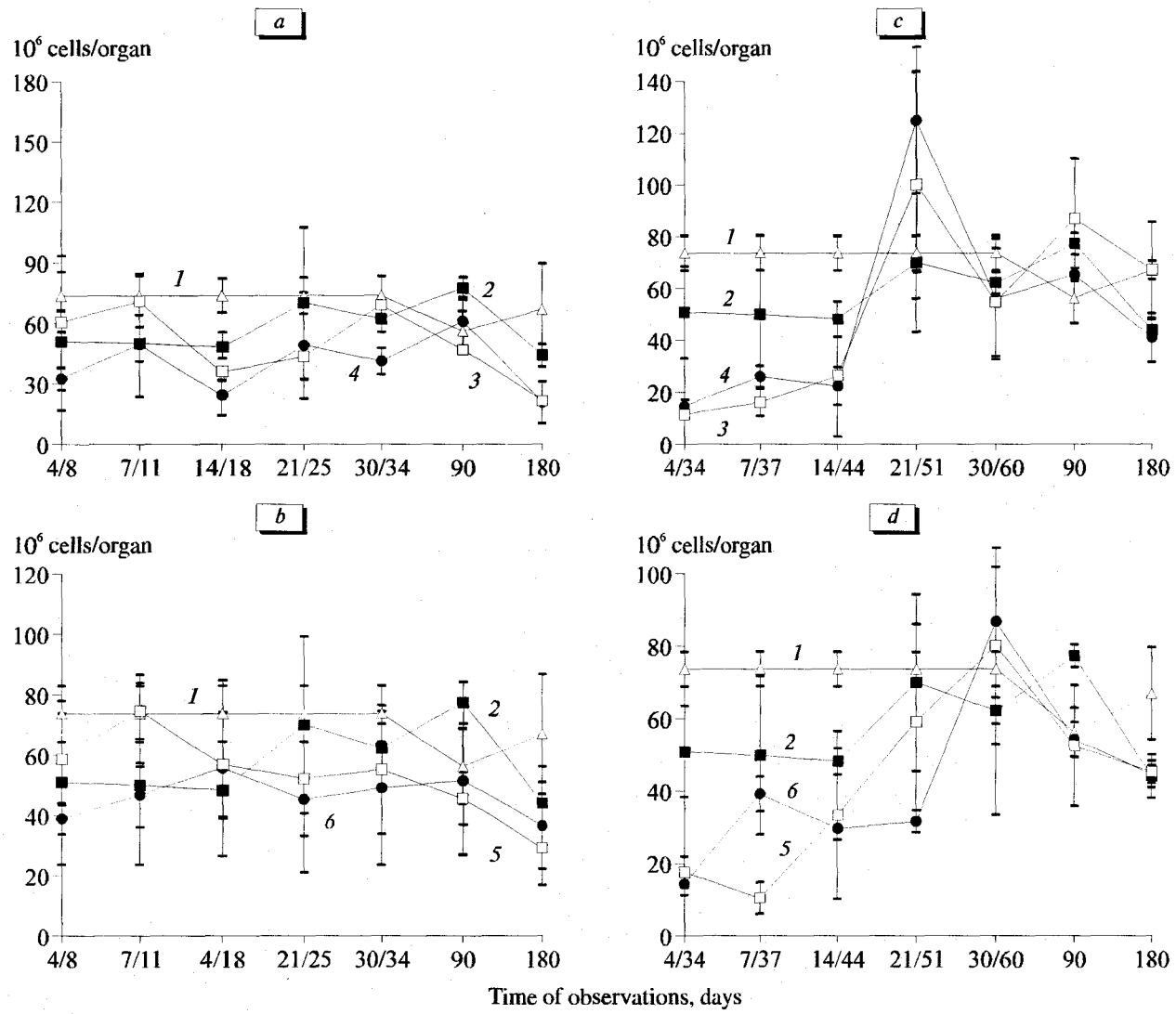

Fig. 2. Absolute content of small lymphocytes in the thymus of mice.

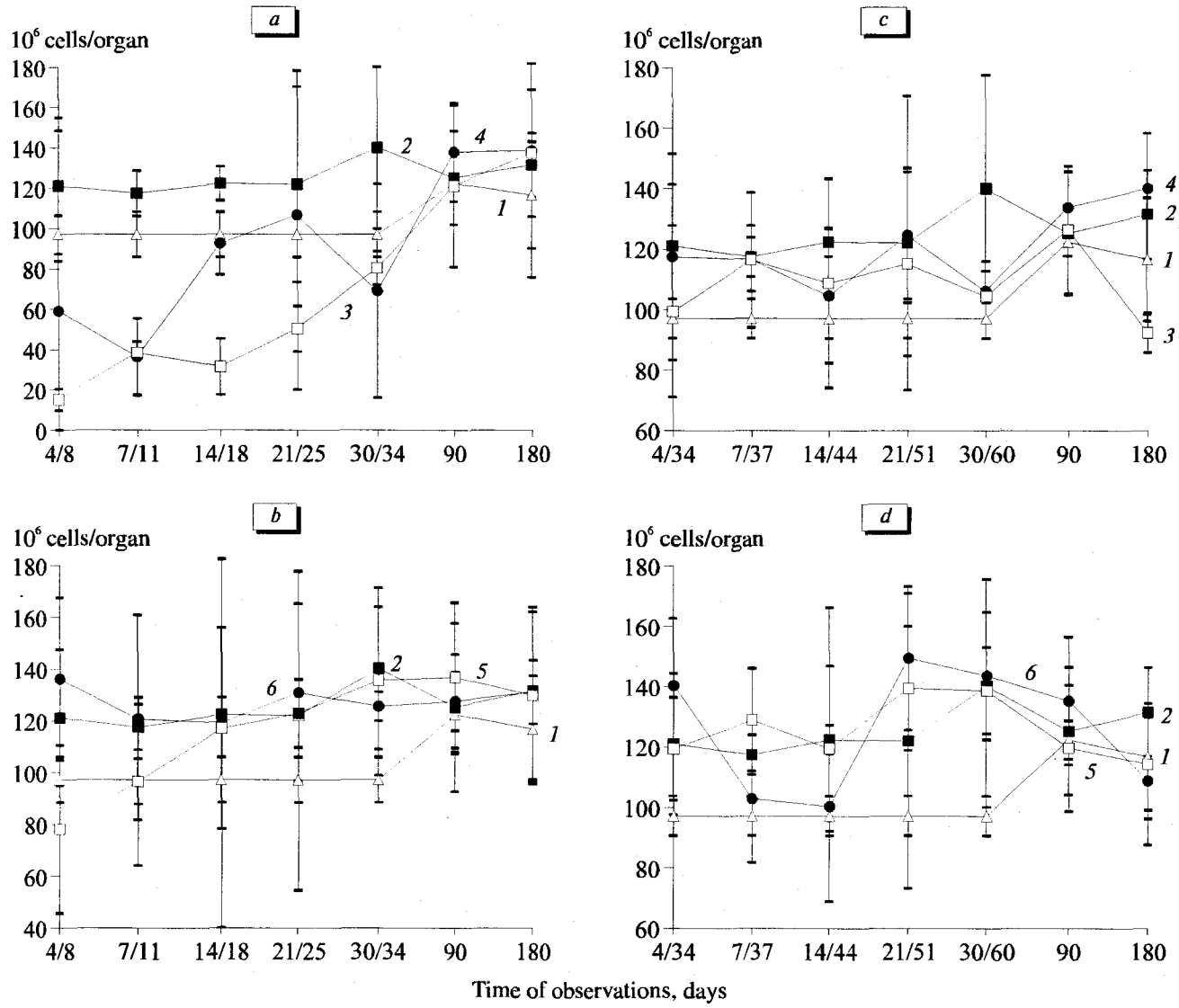

Fig. 3. The absolute content of small lymphocytes in the spleen of mice.

tion due to depletion of regulating cells, stimulates production of suppressor factors, or triggers a feedback mechanism. This effect can be also related to adaptive inhibition of hemopoietic cells by the antigen and cytostatic.

In the late stages of observations (months 3 and 6), cell rearrangement in the studied organs persisted in all mice probably due to toxic effects of preparations on the genome of bone marrow cells, including hemopoietic cells, and the presence of defective cells with impaired functional activity. It was shown that even 6 months after treatment with CP in MPD, adherent bone marrow cells were less potent in maintaining growth of normal bone marrow cells [9]. At the same time, hemopoietic cells play an important role in the regeneration of hemopoiesis under conditions of a pathological process.

Decreased thymic cellularity and the reduction of the absolute content of small lymphocytes to the 6th month of observations (Fig. 2, *a*, *b*), in particular in mice immunized 4 days after CP administration, were probably related to the effects of the antigen. Activation of proliferative and differentiation processes in the thymus induced by immunization was followed by exhaustion of reserve capacities of lymphoid cells and the decrease in their number. This probably impaired the thymic control over proliferation and differentiation of hemopoietic cells. Similar changes were revealed after immunization of mice 30 days after cytostatic treatment (Fig. 2, *c*, *d*).

At the same time, high content of lymphocytes in the spleen of mice immunized 4 days after injection of CP or cisplatin (Fig. 3, *a*, *b*) observed from the 3rd to 6th month was probably associated with regenera-

tive processes in the spleen and cell migration from the bone marrow to the spleen. These changes were also revealed in animals immunized 30 days after cytostatic treatment (Fig. 3, *c*, *d*).

Hence, single injection of CP and cisplatin in MPD caused a long-term impairment (for 6 months) of morphological composition of hemopoietic and lymphoid organs (thymus and spleen) in CBA/CaLac mice. CP produced marked and long-term suppression of lymphoid and erythroid hemopoietic stems in the bone marrow. In addition, this preparation (compared to cisplatin) caused more severe disturbances in the lymphoid stem of the thymus and spleen and markedly stimulated erythropoiesis in the spleen. Immunization with thymus-dependent antigen accelerated regenerative processes in hemopoietic and lymphoid organs. However, cell disorganization 3 and 6 months after immunization did not differ from that caused by antineoplastic drugs and was even more pronounced in the thymus by the 6th month of observations.

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