MICROBIOLOGY AND IMMUNOLOGY

Peculiarities of Primary Humoral Immune Response in Mice with Cytostatic Disease

N. V. Masnaya and G. M. Ratner

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 129, No. 4, pp. 440-443, April, 2000
Original article submitted July 28, 1999

Single intraperitoneal injection of cyclophosphamide in a maximum permissible dose (250 mg/kg) followed by immunization with thymus-dependent antigen (sheep erythrocytes) markedly suppressed the formation of antibody-producing cells in the spleen, especially at early stages of cytostatic disease, and delayed the synthesis of specific antibodies in male CBA/CaLac mice. Platidiam (cisplatin) injected in comparable doses 4 days before immunization practically did not suppress the formation of antibody-producing cells and their functional activity, but being injected 30 days before immunization reduced the number of antibody-producing cells.

Key Words: *cyclophosphamide; Platidiam (cisplatin); antibody-producing cells; IgM and IgG antibodies*

Polychemotherapy is widely used in oncology [3]. Various antineoplastic drugs, including alkylating agents (cyclophosphamide, CP), are often applied in combination with platinum preparations (Platidiam). It is important to study the effects of these preparations not only on tumor cells, but also on normal and intensively proliferating cells (e.g. immune cells). Here we studied the formation of antibody-producing cells (APC) in the spleen during experimental cytostatic disease.

MATERIALS AND METHODS

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

Experimental mice were immunized with corpuscular thymus-dependent antigen (sheep erythrocytes, SE) by a single intraperitoneal injection of 15% erythrocyte suspension (0.2 ml) on days 4 (series I) or 30 (series II) after the 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 absolute number of APC in the spleen was counted as described elsewhere [14]. Serum antibody titers were determined by the reaction of hemagglutination [7]. To estimate the isotype of hemagglutinins, 50 μl 0.1 M 2-mercaptoethanol were added to 50 μl inactivated serum diluted 1:5 and incubated at room temperature for 1 h; the reaction of hemagglutination was then conducted [7].

The results were analyzed by standard methods of variational statistics.

Institute of Pharmacology, Tomsk Research Center, Siberian Division of the Russian Academy of Medical Sciences

0007-4888/00/0004-370$25.00

RESULTS

The dynamics of APC accumulation in the spleen was similar in all immunized mice (Fig. 1). The absolute number of APC was maximum on day 4 after administration of SE and returned to the control by the end of the 1st month. However, in immunized mice receiving CP this parameter was below the control over 1 month of observations, especially after immunization on day 4 after cytostatic treatment (Fig. 1, *a*).

In this case, the decreased number of APC was probably due to the toxic effects of CP on T and B-lymphocytes and macrophages involved in the formation of the primary humoral immune response. Therefore, the number of cells undergoing proliferation, differentiation, and blast-transformation sharply decreased. This assumption is confirmed by previous data that CP in MPD reduced the contents of various B cell subpopulations in the bone marrow and spleen [5,6,15] and T helper cells in the bone marrow [2] and suppressed functional activity of mononuclear phagocytes [9].

Reduced number of APC in the spleen of mice immunized on day 30 after injection of CP (Fig. 1, *b*) was probably related to impaired functional activity of cells involved in this process (macrophages and T1 helper cells) and the effects of suppressor cells, in particular T suppressors and erythroid cells, on antibody formation. Immunization on day 30 after injection of CP (series II) coincided with high content of erythroid cells in the spleen. It is known that erythropoiesis is stimulated by prostaglandin E_2. Prostaglandin E_2 suppressed production of interleukin-1 (IL-1) by macrophages [8] and T1 helper cells and reduced expression of IL-2 receptors. This prevents activation of T2 helper cells, which react with B lymphocytes and via cytokines IL-4 and IL-10 induce proliferative processes and synthesis of specific antibodies. Furthermore, stimulation of T suppressor cells with prostaglandin E_2 [11] can be the cause of weak immune response in mice. Erythroid cells can also produce suppressive effects: immature cells inhibit B lymphocyte proliferation, while mature cells affect APC formation [4,13].

The effects of cisplatin on the content of APC in the spleen of immunized mice differed from those of CP (Fig. 1, *a*). The number of APC in these mice practically did not differ from the control probably due to lower sensitivity of resting cells to cisplatin and complete recovery of lymphoid elements in the spleen by the time of immunization (day 4 after cytostatic treatment). However, the content of APC in mice immunized 30 days after injection of cisplatin remained below the control over 3 months of observations. There were no statistically significant differences between animals of these groups by the 6th month of observations (Fig. 1, *b*). In this case, the low number of APC

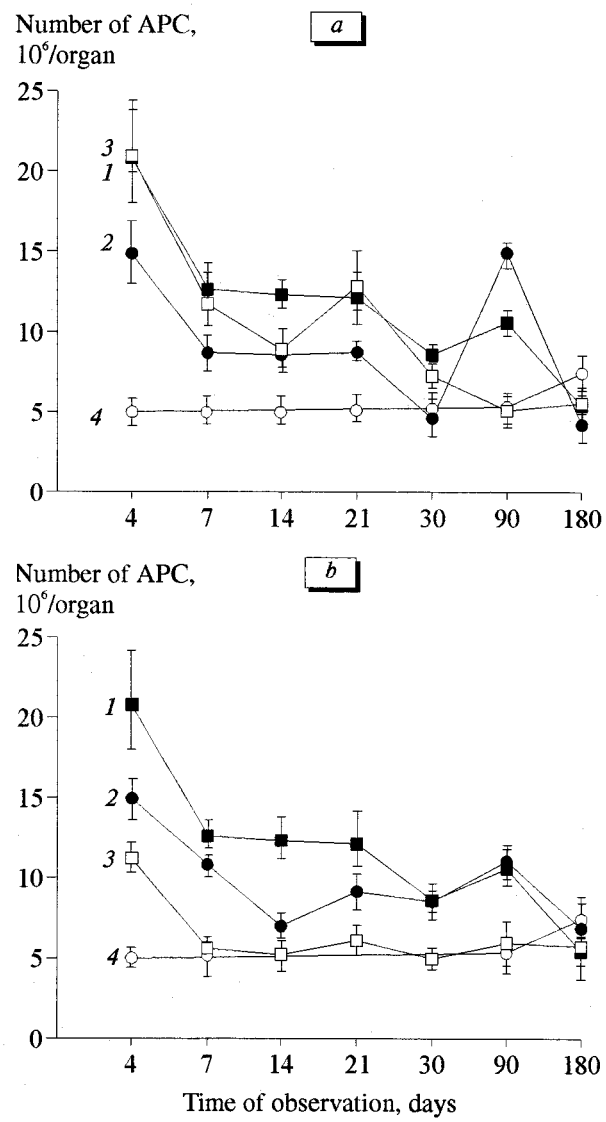

Fig. 1. Absolute number of antibody-producing cells (APC) in the spleen of CBA/CaLac mice immunized on day 4 (*a*) or 30 (*b*) after administration of cyclophosphamide or cisplatin in maximum permissible dose. Here and in Fig. 2: immunization (*1*), cyclophosphamide+immunization (*2*), cisplatin+immunization (*3*), intact animals (*4*).

can be attributed to suppression of proliferation of SE-stimulated target cells by intensively proliferating cells [10], because immunization coincided with considerable accumulation of lymphoid cells at various stages of maturation.

The level of IgM hemagglutinins in the serum of immunized mice peaked on day 4 after immunization. The only exception were the animals receiving SE at the early period after injection of CP; in these mice, serum content of IgM hemagglutinins peaked on day 4 after immunization (Fig. 2, *a*, *c*).

The content of IgG antibodies in control mice and in animals immunized after injection of cisplatin and CP was maximum on days 14, 7, and 21, respectively (Fig. 2, *b*, *d*).

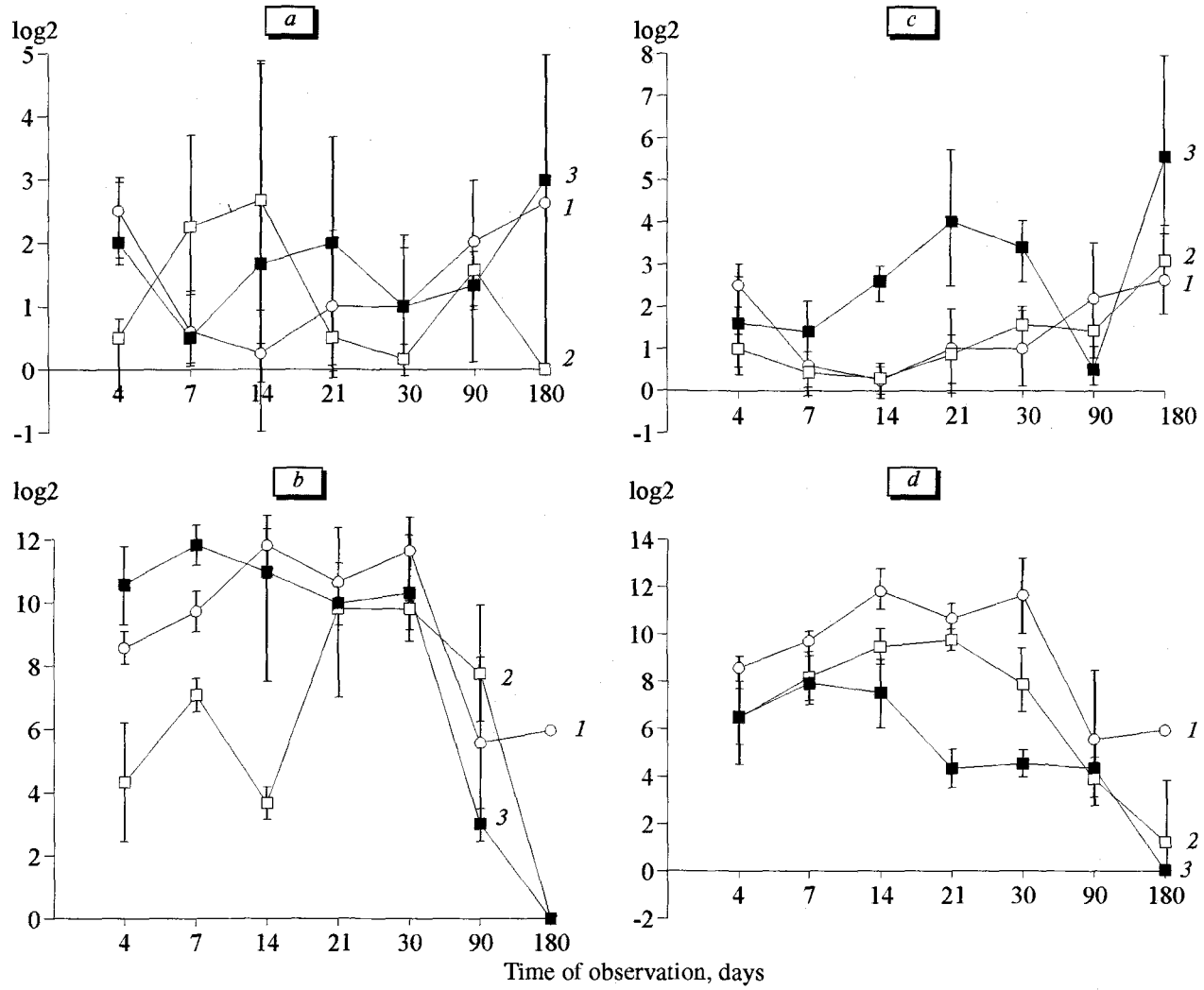

Fig. 2. Levels of specific IgM (*a*, *c*) and IgG antibodies (*b*, *d*) in the serum of mice immunized on day 4 (*a*, *b*) or 30 (*c*, *d*) after single injection of cyclophosphamide or cisplatin.

This delay of peak IgM and IgG titers in mice treated with CP is probably related to suppression of mitotic activity and inhibition of DNA synthesis in cells by this cytostatic, which leads to lengthening of S and G2 phases of the cell cycle and, therefore, delayed production of IgM and IgG antibodies [1]. Furthermore, delayed transition to the synthesis of IgG antibodies can be associated with suppressed functional activity of T lymphocytes producing factors (BCDFμ and BCDFg) promoting this process [12].

Repeated increase in the content of APC in the spleen and IgM antibodies in the serum of mice (including control animals) at the late stage of observations is probably associated with impaired resistance of animals during long-lasting experiments and activation of opportunistic microorganisms. Some components of these microorganisms probably have antigens cross-reacting with SE and yielding false positive results in the analysis (local hemolysis) of APC in the spleen. In addition, bacterial polysaccharides induce T cell-independent polyclonal activation of B lymphocytes and, similarly to other thymus-independent antigens, initiate production of cells synthesizing IgM, but not IgG antibodies, which are detected in the reaction of hemagglutination with SE due to the presence of cross-reacting antigens.

Hence, CP suppresses the formation of APC in the spleen at the early and late periods after its stimulation with SE and delays the synthesis of specific IgM and IgG antibodies. However, suppressive effects of cisplatin on the humoral immune response are manifested in decreased number of APC in the spleen only at late terms after treatment.

REFERENCES

1. Z. P. Bulkina, *Antineoplastic Drugs* [in Russian], Kiev (1991), pp. 263-271.

2. E. D. Gol'dberg, A. M. Dygai, and V. V. Zhdanov, *Role of Hemopoietic Microenvironment in Regulation of Hemopoiesis during Cytostatic Myelosuppression* [in Russian], Tomsk (1999).
3. V. A. Gorbunova, N. I. Perevodchikova, A. M. Garin, *et al.*, *Drug Therapy of Malignant Tumors and Leukosis* [in Russian], Moscow (1991), pp. 94-98.
4. V. A. Kozlov, I. N. Zhuravkin, and I. G. Tsyrlova, *Stem Hemopoietic Cell and Immune Response* [in Russian], Novosibirsk (1982).
5. I. V. Kudaeva, *Morphological and Functional State of Plasma Cells during Cytostatic-Induced Blood Suppression and under Conditions of Antigenic Stimulation*, Abstract of Cand. Med. Sci. Dissertation, Tomsk (1997).
6. T. I. Leont'eva, N. E. Gladkova, and B. S. Uteshev, *Farmakol. Toksikol.*, **51**, No. 6, 60-65 (1988).
7. N. R. Ling and D. Ketti, *Hemagglutination and Reactions of Antibody-Dependent Hemolysis* [in Russian], Ed. D. Ketti, Moscow (1991), Vol. 1, pp. 238-243.
8. E. V. Markova, *Role of Prostaglandin E_2 in Regulation of IL-1 Production by Macrophages under Effects of Various Factors and during the Humoral Immune Response*, Abstract of Cand. Med. Sci. Dissertation, Novosibirsk (1991).
9. L. S. Mushtovatova, I. B. Tikhonova, Yu. V. Fedorov, and V. V. Novitskii, *Pathogenesis of Pathological Processes* [in Russian], Kemerovo (1994), pp. 76-78.
10. R. V. Petrov, R. M. Khaitov, R. I. Ataullakhanov, and I. G. Sidorovich, *Zh. Obshch. Biol.*, **39**, No. 4, 572-581 (1978).
11. A. F. Poveshchenko, *Mechanisms of the Involvement of Prostaglandin E_2 in the Humoral Immune Response*, Abstract of Cand. Med. Sci. Dissertation, Novosibirsk (1987).
12. A. Roit, *Principles of Immunology* [in Russian], Moscow (1991).
13. D. M. Samarin, G. V. Seledtsova, V. I. Seledtsov, *et al.*, *Byull. Eksp. Biol. Med.*, **123**, No. 1, 66-70 (1997).
14. A. J. Cunningham, *Nature*, **207**, 1106 (1965).
15. A. W. Thompson, C. A. McPhee, and H. F. Sewell, *Immunology*, **63**, No. 3, 477-482 (1988).