ONCOLOGY

Reactions of the Immune and Hemopoietic Systems to Antigenic Stimulation in the Cytostatic Disease

N. V. Masnaya and G. M. Ratner

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny,* Vol. 126, No. 11, pp. 576-578, November, 1998
Original article submitted December 2, 1997

A single intraperitoneal injection of cyclophosphamide in the maximum tolerated dose to male CBA mice followed by immunization with thymus-dependent antigen suppressed the humoral immunity reactions (the number of antibody-producing splenocytes and their functional activity) and hematological parameters at the early stages of the cytostatic disease, this suppression persisting at the late stages of the disease.

Key Words: *cyclophosphamide; antibody-producing cells; IgM, IgG antibodies; peritoneal macrophages; lymphoid organs; peripheral blood*

Cytostatic drugs are toxic for tumor cells and for normal cells capable of active proliferation and differentiation or blastogenesis [2,3,6,7]. Disorders in animal and human organs and systems occur both at the early and remote periods after antitumor drug injection [5,12]. We compared the reactions of the immune and hemopoietic systems of mice after injection of the antigen at the early stages of the cytostatic disease (CD) caused by a single dose of the alkylating compound cyclophosphamide (CP).

MATERIALS AND METHODS

Experiments were carried out on 230 male CBA mice weighing 16-33 g (Rassvet Breeding Center). The cytostatic disease was induced by CP (Biokhimik, Saransk)/ The drug was injected intraperitoneally in a single maximum tolerated dose (250 mg/kg) which was determined by graphical probit analysis [11].

Experimental mice were immunized by corpuscular thymus-dependent antigen in a single dose of 0.2 ml 15% sheep erythrocyte suspension intraperitoneally on days 4 (group 1) or 30 (group 2) after CP. The animals were decapitated on days 4, 7, 14, 21, and 30 after immunization and 3 and 6 months after CP injection. The parameters of the immune and hemopoietic systems of experimental animals were compared with those of intact mice (intact control) and of those injected with the antigen alone (control). The total leukocyte count, ratio of individual morphological forms in peripheral blood, bone marrow cell count, and weight and cell count of lymphoid organs (spleen and thymus) were studied by common hematological methods [4]. The absolute (10^6 cells/organ) count of antibody-producing cells (APC) in the spleen was estimated by the local hemolysis method [10] and serum antibodies were tittered by the hemagglutination test [8]. For identifying the hemagglutinin isotype the test was carried out after incubation of 50 μl inactivated serum diluted 5-fold with 50 μl 0.1 M 2-mercaptoethanol at 18-20° for 1 h.

The phagocytic activity of peritoneal macrophages was studied by measuring the light absorbence of the lyzing solution after destruction of phagocytes which digested neutral red corpuscles [13].

The data were processed by the variational statistics method.

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

0007-4888/98/0011-1152$20.00

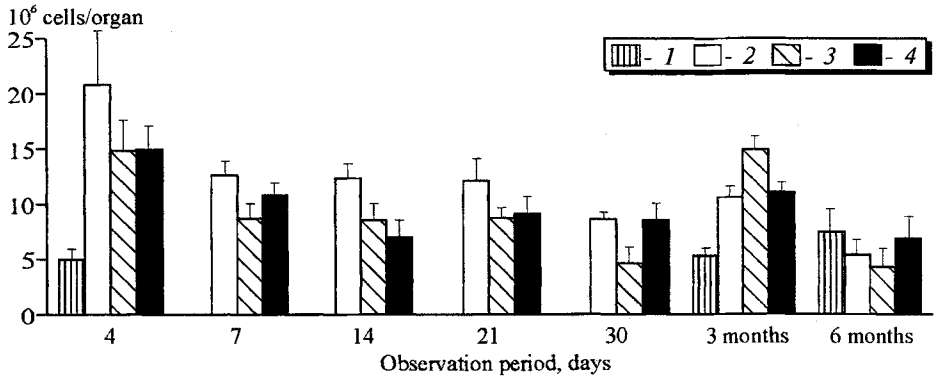

Fig. 1. Time course of absolute (10^6 cells/organ) count of antibody-producing cell in the spleen of intact control (*1*), immunized control mice (2), and mice immunized on days 4 (*3*) and 30 (*4*) after a single injection of cyclophosphamide. Here and in Fig. 2: confidence interval at p=0.05.

RESULTS

In mice immunized on the 4th day after CP injection the count of peripheral blood leukocytes decreased on days 7 and 14 and of bone marrow karyocytes on days 4-14 in comparison with intact and immunized animals. In group 2 these parameters were virtually the same as in both control groups. Analysis of hemograms showed that leukopenia was caused by a significant decrease in the absolute count of lymphocytes and at early stages of CD was associated with an increase in the absolute count of segmented neutrophils, while at late stages it was paralleled by a decrease in the count of eosinophils and an increase in the counts of monocytes and segmented neutrophils in peripheral blood.

In experimental animals cell count and the spleen weight increased significantly. On days 4-30 of experiment, the spleen weight in both groups was higher than in intact animals, but in group 2 this parameter was significantly lower than in group 1 on days 7-21. Cell count was higher than in intact controls on days 14 and 21 in group 1 and on days 7-21 after immunization in group 2. In both experimental groups spleen weight and cell count were virtually the same as in immunized controls.

In group 1, the thymus weight and cell count were lower than in intact and immunized controls for two weeks; by the third month of experiment these parameters gradually increased and normalized. In group 2, they were lower in comparison with both control groups during the first month of experiment. A more pronounced involution of the thymus was observed in mice at the early stage of CD.

The absolute count of APC in the spleen was significantly lower in groups 1 and 2 than in immunized controls for 30 days. The greatest depression of this parameter was observed in mice immunized with sheep erythrocytes on day 4 after CP injection (Fig. 1).

In immunized controls and group 2, the measurements of serum specific hemagglutinins revealed

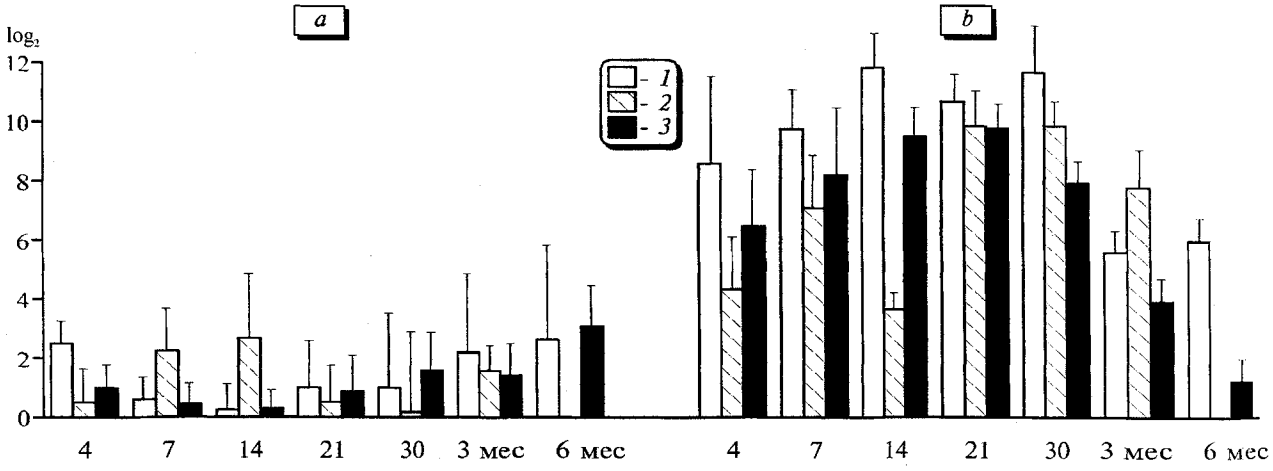

Fig. 2. Level of serum IgM (*a*) and IgG (*b*) antibodies in immunized control mice (*1*) and mice treated with an antigen on day 4 (2) or 30 (3) after cyclophosphamide injection.

the peak of IgM antibodies on day 4 after immunization, while in mice injected with the antigen at the early stages of CD it was recorded only on day 14 after immunization. The titer of these antibodies in both experimental groups was lower than in the control (Fig. 2, *a*). Transfer to the production of IgG antibodies in experimental groups lagged behind the controls for one week (Fig. 2, *b*).

An increase in the phagocytic activity of peritoneal macrophages in mice immunized with sheep erythrocytes on days 4 and 30 after CP (in comparison with intact controls) during the first month of experiment is in line with the published reports [9].

From our results it can be concluded that the reactions of the immune and hemopoietic systems are suppressed to a greater extent at the early stages after CP injection, but the toxic effect of this antitumor drug is observed in later periods; this effect is due to suppression actively proliferating and quiescent cells [1].

The relative and absolute counts of B lymphocytes in the spleen of CBA mice decrease after a single injection of CP in the maximum tolerable dose [7], thus inhibiting the generation of APC in splenocytes of animals immunized after the CP injection, in comparison with mice injected only with an antigen.

Suppression of the functional activity of splenic APC in CP-treated animals and immunized with sheep erythrocytes may be due to the ability of CP to decrease the mitotic activity and inhibit DNA synthesis, which prolongs the S and G2 phases [1] and, as a result, delays the production of IgM and IgG antibodies.

Impairments of hemopoiesis, lympho- and immunopoiesis in remote periods after CP treatment may be associated with persistent instability of the genetic system of hemopoietic bone marrow cells [5].

REFERENCES

1. Z. P. Bulkina, in: *Antitumor Drugs* [in Russian], Kiev (1991), pp. 263-271.
2. V. E. Gol'dberg, A. M. Dygai, and V. V. Novitskii, *Lung Cancer and Blood System* [in Russian], Tomsk (1992).
3. D. I. Gol'dberg and L. S. Dedova, *Vopr. Onkol.*, **15**, No. 4, 82-85 (1969).
4. E. D. Gol'dberg, A. M. Dygai, and V. P. Shakhov, *Cell Culture Methods in Hematology* [in Russian], Tomsk (1992).
5. E. D. Gol'dberg, V. V. Novitskii, O. B. Zapuskalova, and M. Yu. Klimova, *Vestn. Akad. Med. Nauk SSSR*, No. 9, 52-57 (1990).
6. G. V. Karpova, V. E. Gol'dberg, T. I. Fomina, and T. V. Vetoshkina, *Byull. Tomsk. Nauch. Tsentra Akad. Med. Nauk SSSR*, No. 2, 90-101 (1990).
7. I. V. Kudaeva, *Morphological and Functional Status of Plasma Cell Pool in Cytostatic Hemodepression and under Conditions of Antigenic Stimulation*, Author's Synopsis of Cand. Med. Sci. Thesis, Tomsk (1997).
8. N. R. Ling and D. Catty, in: *Hemagglutination and Antibody-Dependent Hemolysis Tests. Antibodies. Methods* [in Russian], D. Catty, ed., Moscow (1991), Book 1, pp. 238-243.
9. S. L. Potapov and M. M. Vyadro, *Byull. Eksp. Biol. Med.*, **108**, No. 9, 330-332 (1989).
10. A. J. Cunningham, *Nature*, **201**, 1106-1111 (1965).
11. J. Litchfield and F. J. Wilcoxon, *J. Pharmacol. Exp. Ther.*, **96**, 99-113 (1949).
12. J. E. Talmadge, J. D. Jeckson, C. D. Borgeson, and G. A. Perry, *Cancer Immunol. Immunother.*, **39**, No. 1, 59-67 (1994).
13. B. Vrav, J. Hoebeke, M. Saint-Guillain, *et al.*, *Scand. J. Immunol.*, **11**, 147-153 (1980).