Sensitivity of Immunocompetent Cells of DBA/2 and C57Bl/6 Mice to Cyclophosphamide

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Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 135, No. 4, pp. 427-431, April, 2003
Original article submitted October 22, 2002

T cells were most sensitive to cyclophosphamide in DBA/2 mice, while in C57Bl/6 mice both T and B cells were sensitive. The formation of antibody-producing cells and the production of specific antibodies were delayed in DBA/2 mice immunized after pretreatment with antitumor drug. Accumulation of antibody-producing cells in the spleen was more active in immunized C57Bl/6 mice treated with cyclophosphamide compared to animals not treated with cyclophosphamide.

Key Words: *DBA/2 and C57Bl/6 mice; cyclophosphamide; thymus-dependent antigen; immunocompetent cells*

The reactions of the hemopoietic and immune systems, the indicators of homeostasis in animals with different genotypes, are an interesting problem. Differences in immune reactions to the same antigen in mice of different strains [5] suggest quantitative and functional differences in the baseline pool of immunocompetent cells. It can be hypothesized that exposures to various factors (chemical, physical, *etc.*) can modulate the qualitative and quantitative composition of immune cells and the reactivity of the immune system characteristic of the strain.

Here we studied the reactions of immunocompetent cells of DBA/2 and C57Bl/6 mice to injection of antitumor drug cyclophosphamide.

MATERIALS AND METHODS

Experiments were carried out on 3-month-old DBA/2 (n=70) and C57Bl/6 (n=70) mice weighing 18-20 g (Laboratory of Biomedical Modeling, Institute of Pharmacology).

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Twenty animals of each strain were immunized with thymus-dependent antigen (TDA; single intraperitoneal injection of 0.2 ml 15% suspension of sheep erythrocytes, SE), 25 mice were injected with cyclophosphamide (Biokhimik Plant, Saransk) in a single dose of 250 mg/kg intraperitoneally, and 20 mice of each strain received TDA on day 4 after injection of 250 mg/kg cyclophosphamide. Five intact animals of each strain served as controls (baseline values).

The dose of cyclophosphamide was chosen on the basis of experimental data: this dose is the maximum tolerable dose (MTD) for CBA/Ca Lac mice (common biological model) determined by probit analysis. Single injection of cyclophosphamide in this dose to CBA/Ca Lac mice leads to suppression of the hemopoietic and immune systems, which can be characterized as immunodeficiency [4,5].

Material for investigation was collected on days 4, 7, 14, 21 after immunization and on days 4, 8, 11, 18, 25 after cyclophosphamide administration (corresponding to days 0, 4, 7, 14, and 21 after immunization). The mice were sacrificed by decapitation. Phagocytic activity of peritoneal macrophages (PAPM), number of antibody-producing cells (APC) in the spleen, specific IgM and IgG hemagglutinins [3], total counts of T and B cells [2] in the bone marrow and

0007-4888/03/1354-0365$25.00

spleen were determined. The data were compared with the baseline values in intact controls. The results were processed using Statistica software.

RESULTS

Cyclophosphamide injection to DBA/2 mice led to activation of PAPM at the initial stages of the experiment followed by its gradual reduction to the baseline level (Fig. 1, *a*). Analysis of the dynamics of quantitative composition of lymphocyte subpopulations in hemopoietic and lymphoid organs showed that the content of common T lymphocytes in the bone marrow (Fig. 1, *b*) and spleen (Fig. 2, *a*) in mice treated with cyclophosphamide was low throughout the observation period. The count of B cells in the bone marrow of these animals decreased on day 1 of the experiment and then virtually did not differ from the initial level. In the spleen the count of B cells transiently decreased in comparison with the initial level at the start and end of the experiment (Fig. 2, *b*). These data attest to high sensitivity of T cells and lower sensitivity of B cells of DBA/2 mice to cyclophosphamide.

For evaluation of the effect of cyclophosphamide on reactivity of immune cells, DBA/2 mice were immunized with TDA on day 4 after injection of the cytostatic. Cyclophosphamide produces a potent lymphocytotoxic effect, and therefore by the moment of antigen injection (point 0; Fig. 1, *b*, *c*; Fig. 2, *a*, *b*) the content of T and B cells in the bone marrow and spleen of mice treated with the antitumor drug alone considerably decreased. As a result, the content of APC in the spleen of cyclophosphamide-treated mice did not increase by day 4 after immunization in contrast to mice immunized without cyclophosphamide pretreatment (Fig. 3, *a*). However, injection of the antigen on day 4 after cytostatic treatment stimulated proliferation and differentiation of remaining lymphoid cells. The compensatory migration of lymphoid cells from the bone marrow into the spleen seemed to be also activated. As a result, on day 4 of the experiment the count of T and B cells in the spleens of animals immunized on day 4 after cyclophosphamide injection were higher than in mice treated with cyclophosphamide alone (Fig. 2, *a*, *b*), while the count of these cells in the bone marrow did not differ from the

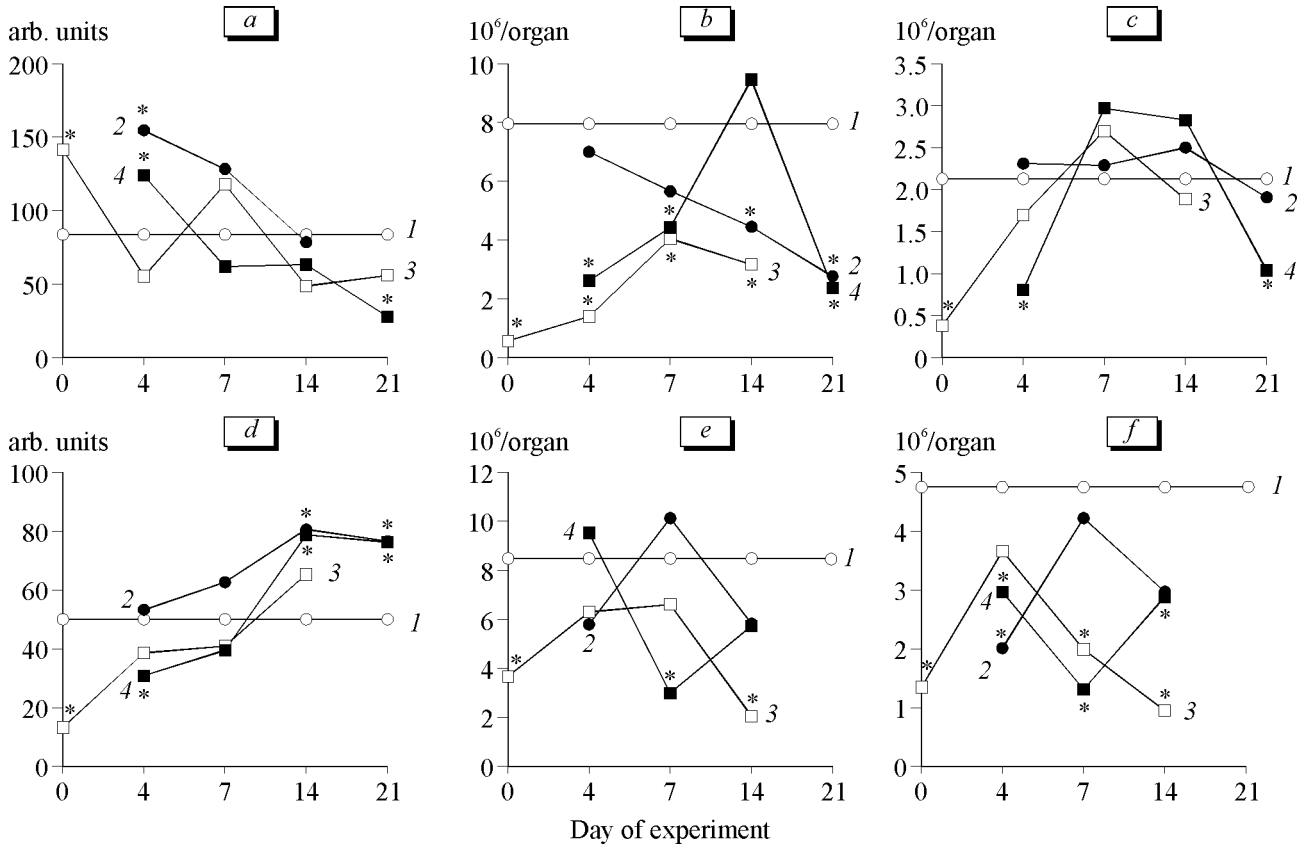

Fig. 1. Phagocytic activity (arb. units) of peritoneal macrophages (*a*, *d*), dynamics of absolute (10^6/organ) counts of total T (*b*, *e*) and B cells (*c*, *f*) in the bone marrow of DBA/2 (*a*, *b*, *c*) and C57Bl/6 mice (*d*, *e*, *f*): controls (*1*), immunized (*2*), after a single injection of cyclophosphamide in a dose of 250 mg/kg (*3*), immunized on day 4 after single injection of cyclophosphamide (*4*). Here and in Figs. 2-3: $*p_i<0.05$ compared to the control.

corresponding parameter in cyclophosphamide-treated nonimmunized mice (Fig. 1, *b*, *c*). Thus, lymphoid cells migrated into the spleen were involved in humoral immune response, which activated the process of APC formation: the maximum accumulation of APC in the spleens of mice immunized on day 4 after cyclophosphamide injection was observed on day 7 of the experiment (Fig. 3, *a*). Correspondingly, the peak of specific IgM antibodies was recorded on day 14 and of IgG antibodies on day 21 of the experiment (Fig. 3, *b*, *c*). In animals receiving only the antigen the peak of IgM antibodies was observed on day 4 and of IgG antibodies on day 14 of the experiment (Fig. 3, *b*, *c*). Delayed production of IgM and IgG antibodies can result from cyclophosphamide-induced inhibition of mitotic activity and inhibition of DNA synthesis in lymphoid cells [1]. Analysis of the dynamics of T and B cell counts in the studied organs of mice immunized on day 4 after cyclophosphamide injection showed accumulation of T and B cells in the bone marrow by days 7 and 14 of the experiment (Fig. 1, *b*, *c*) probably due to antigen-stimulated cell proliferation. Later on, the second wave of lymphoid cell migration to the spleen occurred: the number of T and B cells decreased in the bone marrow (Fig. 1, *b*, *c*) and increased in the spleen (Fig. 2, *a*, *b*).

Hence, injection of 250 mg/kg cyclophosphamide to DBA/2 mice decreased the counts of T and B cells in the bone marrow and spleen, decelerated accumulation of APC in the spleen, and delayed the production of specific antibodies by APC in comparison with animals immunized without cyclophosphamide pretreatment.

The study of the reaction of immunocompetent cells in C57Bl/6 mice to a single cyclophosphamide

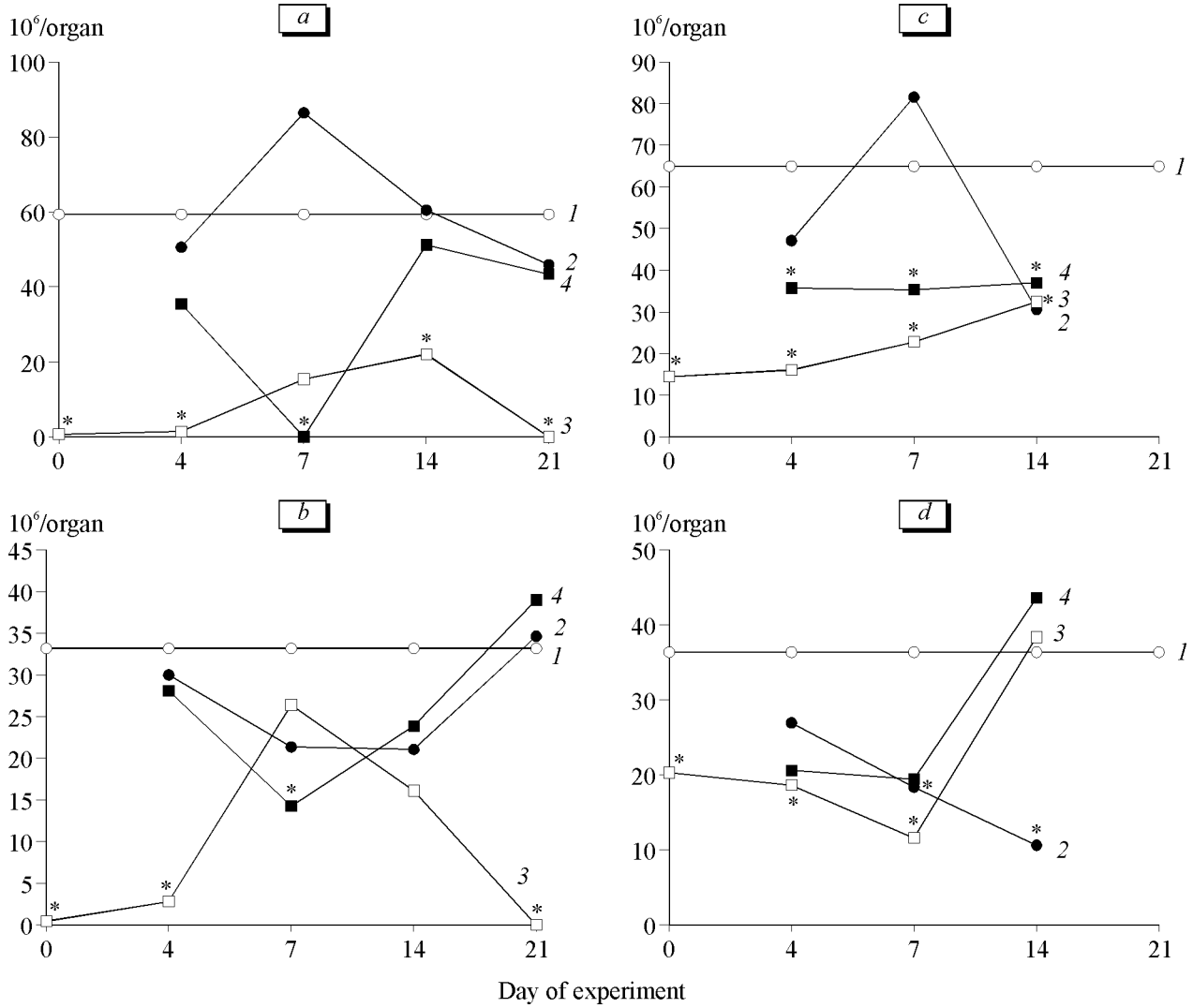

Fig. 2. Dynamics of absolute (10^6/organ) counts of total T (*a*, *c*) and B cells (*b*, *d*) in the spleens of DBA/2 (*a*, *b*) and C57Bl/6 mice (*c*, *d*): control (initial level, *1*), immunized (*2*), treated with 250 mg/kg cyclophosphamide (*3*), and immunized on day 4 after a single injection of cyclophosphamide (*4*).

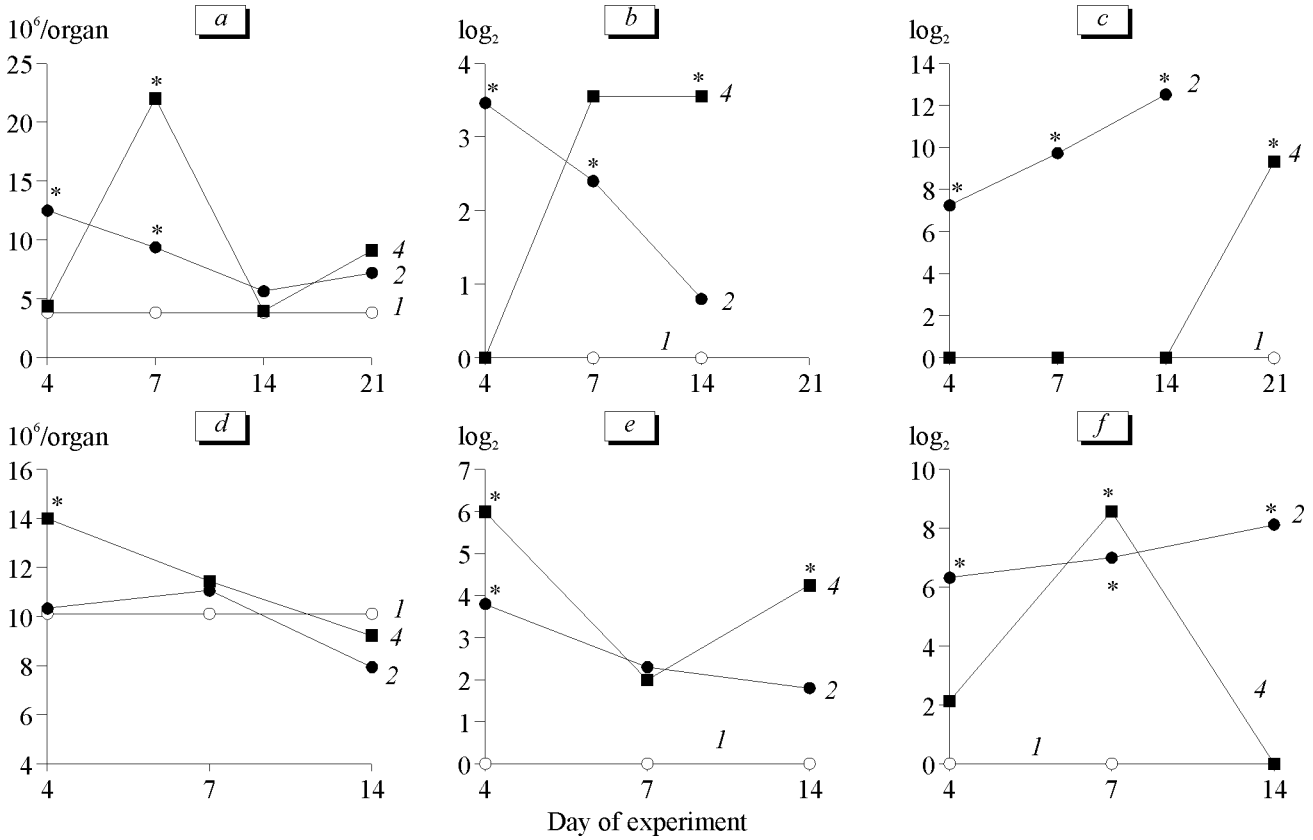

Fig. 3. Dynamics of absolute (10^6/organ) counts of APC (*a*, *d*) and hemagglutinating IgM (*b*, *e*) and IgG antibodies (*c*, *f*) in the serum of DBA/2 (*a*, *b*, *c*) and C57Bl/6 mice (*d*, *e*, *f*): control (initial level, *1*), immunized (*2*), treated with 250 mg/kg cyclophosphamide (*3*), and immunized on day 4 after single injection of cyclophosphamide (*4*).

injection showed a decrease in PAPM at the initial stages of the experiment and its subsequent normalization (Fig. 1, *d*). Cyclophosphamide also decreased the total count of T cells in the bone marrow and, to a greater extent, in the spleen (Fig. 1, *e*; Fig. 2, *c*). The content of B cells in the bone marrow and spleen of cyclophosphamide-treated mice (Fig. 1, *f*, Fig. 2, *d*) decreased throughout the experiment except day 14, when the count of B cells in the peripheral lymphoid organ was virtually normal. These findings attest to high sensitivity of both subpopulations of lymphoid cells in C57Bl/6 mice to the alkylating compound.

Injection of the antigen to C57Bl/6 mice on day 4 after cyclophosphamide treatment seemed to activate proliferation and differentiation of immune cells, which manifested in increased T cell counts in the bone marrow and spleen by day 4 of the experiment in comparison with animals treated with the cytostatic alone (Fig. 1, *e*; Fig. 2, *c*). By day 7 of the experiment the counts of T and B cells in the bone marrow decreased (Fig. 1, *e*, *f*). This can be due to migration of the studied cells into peripheral lymphoid organs, while repeated increase of their count (by day 14, Fig. 1, *e*, *f*) was associated with compensatory activation of proliferation of lymphoid cells in the bone marrow and their subsequent migration into the spleen (Fig. 2, *d*: increased count of B cells by day 14).

Taking into account decreased counts of immunocompetent cells (T and B cells, Fig. 1, *e*, *f*; Fig. 2, *c*, *d*) and low functional activity of macrophages (Fig. 1, *d*) in C57Bl mice at the initial terms after cyclophosphamide treatment, we expected that TDA-stimulated primary humoral response would be markedly reduced. Moreover, the count of APC in the spleens of animals treated with the antigen alone was close to baseline (Fig. 3, *d*). However, we found that immunization on day 4 after cyclophosphamide treatment promoted accumulation of APC in the spleen by day 4 of observation (Fig. 3, *d*) and stimulated active production of specific antibodies. The peak of IgM antibodies was observed on day 4 and of IgG hemagglutinins on day 7 (Fig. 3, *e*, *f*). In immunized mice not treated with the cytostatic the peak of IgM antibodies in the serum was also recorded on day 4 (Fig. 3, *e*) and of IgG hemagglutinins on day 14 (Fig. 3, *f*). It can be hypothesized that cyclophosphamide known for its lymphocytotoxic action and injected to C57Bl/6 mice in a dose of 250 mg/kg eliminated T and B suppressor lymphocyte subpopulations responsible for

suppression of the immune response. This hypothesis is based on the data obtained on CBA/Ca Lac mice. Cyclophosphamide in a dose of 250 mg/kg (MTD for these mice) produced a potent lymphocytotoxic effect on all lymphocyte subpopulations [1,5], while in lower doses it selectively affected (eliminated) suppressor lymphocytes and exhibited an immunomodulating effect [1]. In our study the dose of 250 mg/kg for C57Bl/6 mice is lower than MTD (323 mg/kg).

Hence, injection of cyclophosphamide in a dose of 250 mg/kg to C57Bl/6 mice reduced the counts of T and B cell in the bone marrow and spleen. The drug stimulated primary humoral immune response, presumably via elimination of T and B suppressor cells. Hence, T cells proved to be the most sensitive to cyclophosphamide in DBA/2 mice, while in C57Bl/6 mice both T and B cells were sensitive to the cytostatic. Moreover, the formation of antibody producing cells and production of specific antibodies were delayed in DBA/2 mice immunized after pretreatment with the alkylating agent. Activation of APC accumulation in the spleen after cyclophosphamide injection was observed in C57Bl/6 mice.

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