MORPHOLOGICAL AND FUNCTIONAL CHANGES ARISING IN THE MOUSE SPLEEN BY THE ACTION OF CYCLOPHOSPHAMIDE

M. A. Yumasheva, L. N. Fontalin,
and A. M. Poverennyi

UDC 615.277.3.015:612.411

For the first 3 or 4 days after injection of cyclophosphamide considerable inhibition of all hematopoietic cells with an immunological function in the spleen was observed. By the end of this period the population of hematopoietic cells was completely, and immunological competence partly, restored. Later observations showed hyperregeneration of the myeloid and erythroid cells and also of the stem cells, accompanied by continued depression of the lymphoid and plasma-cell series. The colony-forming activity was restored much faster than after γ-ray irradiation. The results are interpreted in the light of the hypothesis of potential reparable lesions of the cell induced by cyclophosphamide.

It is now known that the action of cyclophosphamide (CP) on the immunological functions of lymphoid tissue differs in many respects from the effects of other alkylating agents or of irradiation [5, 6, 8-10, 17]. Differences have also been found when hematopoiesis has been tested in animals exposed to the action of CP [11, 15, 18]. However, the results obtained by the authors cited above are difficult to compare because of the dissimilarity between the experimental models. The objects of this investigation were: a) to investigate morphological changes and the immunological potential of the mouse spleen in parallel tests after administration of CP, and b) to compare the restoration of the hematopoietic function of the spleen after CP administration and γ-ray irradiation.

EXPERIMENTAL METHOD

(CBA × C57B1/6)F_1 or noninbred albino male mice weighing 18-25 g were used. CP was injected intraperitoneally in a single dose of 200 mg/kg. The animals were killed at various times after the injection and impressions made of sections through the spleen, fixed with methanol, and stained by the May – Gruenwald method and counter-stained with azure–eosin. The differential cell count of the spleen was determined by identification of 3000-5000 cells. Absolute numbers of cells of each line for the whole spleen were determined. The proportions of living and dead cells were obtained by staining with water-soluble 0.1% eosin. At each time of the investigation 15 to 20 mice were examined. Some animals were immunized at appropriate periods after injection of CP by intravenous injection of 5×10^8 sheep's red cells. The number of antibody-forming cells in the spleen was determined 4 days later by the method of Jerne and Nordin [16].

The number of hematopoietic stem cells in the spleen was studied by the method of Till and McCulloch [19]. Donor (CBA × C57B1/6)F_1 mice were injected with CP or irradiated in a dose of 500 R from a cobalt source at the rate of 157 R/min. After various intervals of time the mice sacrificed and their spleen cells

Laboratory of Immunological Tolerance, N. F. Gamaleya Institute of Epidemiology and Microbiology, Academy of Medical Sciences of the USSR, Moscow. Laboratory of Radiation Biochemistry, Institute of Medical Radiology, Academy of Medical Sciences of the USSR, Obninsk. (Presented by Academician of the Academy of Medical Sciences of the USSR V. G. Vygodchikov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 75, No. 5, pp. 64-67, May, 1973. Original article submitted June 27, 1972.

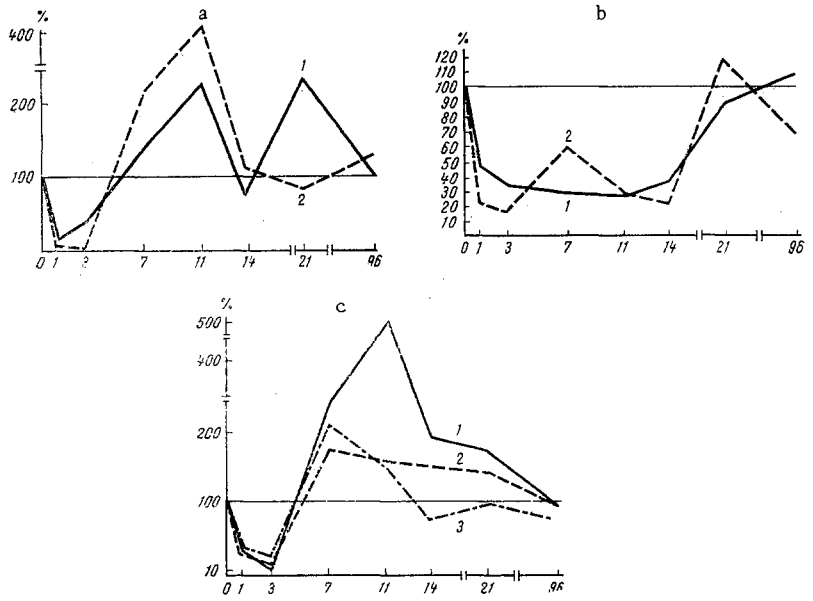

Fig. 1. Number of nucleated cells in spleen of mice at various times after injection of CP: a) blast cells (1) and dividing cells (2); b) lymphocytes (1) and plasma cells (2); c) myeloid (1), erythroid (2), and reticulum (3) cells. Abscissa, days after injection of CP; ordinate, number of cells (percentage of control).

(from 5×10^5 to 1×10^7) were injected intravenously into recipient mice irradiated in a dose of 850 R. On the 8th day the recipients were killed, the spleens were fixed in a mixture of alcohol and glacial acetic acid (1 : 3), and the number of colony-forming units (CFU) was counted 30 min later. The number of CFU was counted per million spleen cells and also per whole Donor's spleen.

The content of three polydeoxynucleotides (PDN) was determined by Dische's method [2] in the spleens of mice irradiated in a dose of 500 R or receiving CP, by fractionation as described by Cole and Ellis [12] and extraction with hot 5% TCA (90°C, 10 min). The fraction of the total DNA in the acid-insoluble fraction accounted for by PDN was estimated at various times.

EXPERIMENTAL RESULTS

The cell composition of the spleen of the intact mice (control) in these experiments was as follows (mean data, millions): lymphocytes 144.3, myeloid cells 19.8, erythroid cells 59.2, reticulum cells about 17, plasma cells 1.8, blast cells 1.1, dividing cells 2.9.

The results given in Fig. 1 show that during the first days (1-4) after injection of CP there was a sharp decrease in the total number of all types of cells. The maximal depopulation, accompanied by a sharp decrease in weight of the spleen (from 130 mg in the control to 49 mg) was observed on the 4th day. The greatest decrease occurred in the dividing and blast cells.

From the 7th to the 11th days hyperplasia of all types of cells except lymphocytes and plasma cells was observed. The greatest rise in mitotic activity was observed on the 11th day, and it was followed by a gradual decrease to normal. During this period the weight of the spleen increased considerably (177-280 mg).

By the 14th day a secondary, comparatively smaller, decrease in the number of blast cells and cells of the myeloid series took place. By the 21st day a fresh wave of hyperplasia of the blast cells was observed. The lymphocyte count was gradually restored. The number of myeloid cells fell. By the 3rd month the cell composition of the spleen was on the whole back to normal, and only the numbers of reticulum cells and plasma cells were a little below the control level.

Investigation of death of the cells and accumulation of PDN in the spleen at different times after exposure to CP or ionizing radiation gave the following results. Whereas during the 5 h after irradiation the number of dying cells rose to 54% (10% in the control), after administration of CP during this period and

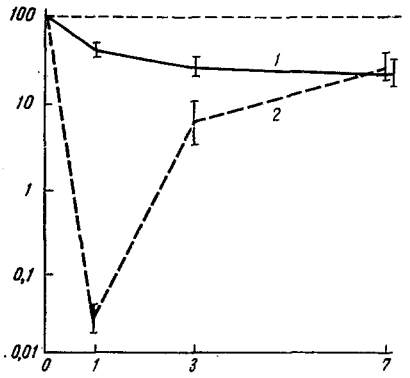

Fig. 2

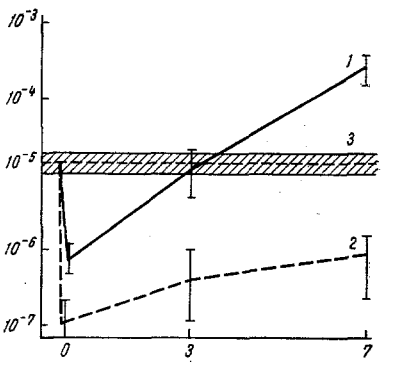

Fig. 3

Fig. 2. Morphological and functional changes in splenic lymphoid tissue induced by CP; 1) lymphocytes; 2) antibody-forming cells 4 days after immunization with 5×10^8 sheep's red cells. Abscissa, day of immunization and of determination of number of lymphocytes after injection of CP; ordinate, number of cells (in % of control).

Fig. 3. Concentration of CFU after injection of CP (1) or irradiation (2) and in intact mice (3). Abscissa, time of investigation (in days); ordinate, CFU concentration.

later (until 24 h) the number of dying cells showed no significant change (not more than 20%) and no PDN could be detected.

It can be concluded from these results that CP does not cause interphase death of the cells such as is observed in lymphoid organs after exposure to ionizing radiation.

This conclusion is in agreement with the dynamics of the cytological changes after injection of CP.

It will be clear from Fig. 2, in which the total number of lymphocytes in the spleen and changes in immunological reactivity are compared at different times after injection of CP, that the compound causes depression of the lymphoid tissue with respect to both parameters, but that there is considerable disproportion between them, especially in the early period after injection of CP. Later (after 3-7 days) this disproportion disappears because of the rapid restoration of immunoreactivity.

In the last series of experiments the number of hematopoietic stem cells was investigated in the spleen of mice receiving CP or irradiated in a dose of 500 R. The results are given in Fig. 3. A sharp decrease in the number of CFU was observed 3 h after injection of CP, followed by a return to normal on the 3rd day. On the 7th day the number of CFU per million cells increased (18 times above normal, or 34 times if counted for the whole donor's spleen). After irradiation in a dose of 500R, a marked decrease in the number of CFU took place in the course of 7 days.

Comparison of the histological data with the number of CFU after injection of CP shows that the decrease in the number of lymphocytes at the times of investigation did not correlate with the number of CFU, whereas restoration of the myeloid and erythroid series and of the blast cells correlated with the increase in the number of CFU in the spleen.

Hyperregeneration of CFU and activation of hematopoiesis in the mouse spleen 7-10 days after injection of CP were also observed by Fried et al. [13]. Hyperplasia of the myeloid series in the bone marrow of rats after injection of CP and slower restoration of the lymphoid cells have also been observed [1, 4, 14, 15]. Rapid repair of disorders of myelopoiesis and lymphopoiesis (compared with the changes induced by whole-body irradiation or nitrogen mustard) has also been reported [15, 18].

Bruce et al. [11] attribute the unique nature of the hematological changes induced by CP to the selective action of the compound on cells of the proliferative pool (and also to the weaker action on resting stem cells) compared with other alkylating agents or irradiation. Other workers [13, 20] suggest that CP induces reversible cell damage. Immunomorphological evidence can be adduced in support of both the first [5, 8-10] and the second [5] points of view. The results of the present investigation indicating the very rapid restoration of the number of hematopoietic stem cells (their number is doubled in 15-17 h) and of the immunologically competent cells (number doubled in 7.5 h) during the first 3 days after injection of CP seem

to favor the second point of view. On the other hand, the relative resistance of interphase lymphocytes to the action of CP noted above and the special sensitivity of the proliferating cells can be interpreted as evidence in support of the first point of view. If the proportion of reparable injuries to DNA induced by CP is significantly higher than is found after the action of other alkylating agents or irradiation on the cell [20], this may explain the special sensitivity of the proliferating cells to CP.*

The relative reversibility of the lesions induced by CP and the relative resistance of the resting cells (compared with the blast cells) may thus be interconnected. This hypothesis is supported by results obtained in the present experiments and illustrated in Fig. 2. Since an antigenic stimulus induces proliferation of immunocompetent cells, the latter will evidently die selectively under the influence of CP whereas other lymphocytes, not responding to the particular antigen, will repair the damage inflicted on them by CP. Death of the cell clone selectively involved in proliferation may lead to the formation of immunological tolerance [7, 8, 10]. It is interesting to note that other alkylating agents (sarcolysin, dipin) or whole-body irradiation are far less suitable for the induction of immunological tolerance than CP [8, 10].

The unique features of the effect of CP on lymphoid tissue are also connected, evidently, with differences in the sensitivity of lymphocytes of different origin to CP. This factor may explain the differences observed above between the dynamics of the lymphocyte population and the population of immunocompetent cells (Fig. 2). However, this problem requires special study.

LITERATURE CITED

1. V. A. Grishko, in: Cyclophosphamide [in Russian], Riga (1965), p. 71.
2. Z. Dische, in: Nucleic Acids, Academic Press (1968).
3. N. P. Dubinin and A. P. Akif'ev, Uspekhi Sovr. Biol., 69, No. 2, 272 (1970).
4. A. A. Zidermane et al., in: Cyclophosphamide [in Russian], Riga (1965), p. 51.
5. K. A. Kazaryan et al., Byull. Éksperim. Biol. i Med., No. 11, 58 (1971).
6. L. A. Pevnitskii et al., Byull. Éksperim. Biol. i Med., No. 10, 59 (1969).
7. L. A. Pevnitskii et al., Byull. Éksperim. Biol. i Med., No. 8, 71 (1971).
8. L. N. Fontalin et al., Vestn. Akad. Med. Nauk SSSR, No. 7, 75.
9. L. N. Fontalin et al., in: Proceedings of the 15th All-Union Congress of Epidemiologists, Microbiologists, and Infectious Diseases Specialists [in Russian], Part 2, Moscow (1970), p. 134.
10. A. C. Aisenberg and C. Murray, J. Exp. Med., 133, 877 (1971).
11. W. R. Bruce et al., J. Nat. Cancer Inst., 37, 233 (1966).
12. L. J. Cole et al., Radiat. Res., 7, 507 (1957).
13. W. Fried et al., Exp. Hematol., No. 20, 11 (1970).
14. K. Hambusch and W. Otto, Z. ges. inn. Med., 21, 243 (1966).
15. H. Host, Acta Radiol. Ther. (Stockholm), 4, 337 (1966).
16. N. K. Jerne and A. A. Nordin, Science, 140, 405 (1963).
17. T. W. Makinodan et al., Pharmacol. Rev., 22, 189 (1970).
18. D. E. Pegg, in: Cyclophosphamide, Baltimore (1965), p. 35.
19. J. E. Till and E. A. McCulloch, Radiat. Res., 14, 213 (1961).
20. W. D. De Wys and N. Kight, J. Nat. Cancer Inst., 42, 155 (1969).

* Potential injuries to DNA can be recorded in proliferating cells before repair at one phase of the cell cycle (see [3], etc.).