INCREASE IN THE BLOOD LEVEL OF α-FETOPROTEIN IN MICE AFTER A SINGLE DOSE OF CYCLOPHOSPHAMIDE

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UDC 615.277.3.015.45:612.124:162.64

KEY WORDS: α-fetoprotein; cyclophosphamide; hematopoiesis in the liver.

The work of Barkhotkina et al. [1] showed that in response to injection of cyclophosphamide into adult mice the number of hematopoietic stem cells in their liver increases and foci of hematopoiesis appear. These workers, however, did not record an increase in the α-fetoprotein (α-FP) level in the blood of the experimental animals. Since in all cases of hematopoiesis in mouse liver known to us there is an accompanying increase in the blood α-FP concentration [3], it appeared that this model was exceptional in this respect. However, the method used by Barkhotkina et al. to determine α-FP, namely double immunodiffusion in gel, was unable to detect less than a five-tenfold increase in its concentration.

Accordingly, in the investigation described below, the α-FP level was assessed in the blood serum of mice receiving cyclophosphamide by a highly sensitive radioimmunologic method.

EXPERIMENTAL METHOD

Experiments were carried out on male CBA - C3H/He, DD, C57BL/6, and CC57BR/Mv mice, aged 3 months, reared at the Institute of Cytology and Genetics, Siberian Branch, Academy of Sciences of the USSR. Cyclophosphamide (from the Saransk Medical Preparations Factory) was dissolved in distilled water in a concentration of 20 mg/ml and injected intraperitoneally in a dose of 200 mg/kg body weight. The CBA and C3H/He mice were killed 2, 4, 6, and 9 days, and the remaining mice 2 and 4 days after injection of the drug. Blood for obtaining serum and determing α-FP and the liver for obtaining squash preparations were taken from all the animals. The air-dried squash preparations of the liver were stained by Pappenheim's method and examined under the microscope, no fewer than 500 cells being identified in each preparation. Hematopoietic cells were classified according to the nomenclature of Chertkov and Vorob'ev [4]. α-FP was determined by the radioimmunologic method described by Blank-Liss et al. [5]. A highly purified preparation of α-FP was obtained from mouse embryonic serum by preparative electrophoresis in polyacrylamide gel, as described previously [2], with subsequent concentration of the isolated protein and re-electrophoresis under the same conditions. The protein was iodinated by the chloramine method, using ^{125}I from the Radiochemical Centre, Amersham (England) and chloramine T from Merck (West Germany). The specific radioactivity of the ^{125}I-α-FP thus obtained was 1.4 μCi/μg protein. The preparation of labeled protein was diluted to the working dilution (approximately 300,000 cpm/ml) with a 0.5% solution of bovine serum albumin in 0.05 M phosphate buffer, pH 7.6, and stabilized with 0.01% ammonia solution. The source of antibodies against α-FP was a rabbit immune serum obtained previously [2] and diluted 10,000 times.

Preliminary experiments showed that such a solution contains the minimal quantity of antibodies which completely binds labeled α-FP in an equal volume of working solution of the antigen. To set up the reaction, to 0.1 ml of 0.05 M phosphate buffer (pH 7.6) were added 0.1 ml of the test serum (diluted beforehand if necessary with this same buffer), 0.1 ml of the working solution of labeled α-FP, and 0.1 ml of a solution of the antibodies. After incubation at room temperature for 20 h, 0.1 ml of a 1% solution of bovine serum albumin and 0.5 ml of a 16% solution of polyethylene glycol (mol. wt. 6000-7500, from Serva, West Germany) were added to each tube. The mixture was thoroughly mixed, kept for 30 min, and centrifuged for 1 h at 2500g. The supernatant was removed and the residue containing the precipitated immune complexes was dissolved in 0.5 ml of a 1% solution of sodium dodecylsulfate, alkalified with NaOH to pH 9.8, and transferred to scintillation

Laboratory of Genetics of Cancer, Institute of Cytology and Genetics, Siberian Branch, Academy of Sciences of the USSR, Novosibirsk. (Presented by Academician of the Academy of Medical Sciences of the USSR N. V. Vasil'ev.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 91, No. 5, pp. 562-564, May, 1981. Original article submitted October 21, 1980.

TABLE 1. Cell Composition of Squash Preparations of Liver from C3H/He Mice after a Single Injection of Cyclophosphamide (M ± m)

Time after injection of cyclophosphamide, days	Promyelocytes	Myelocytes	Metamyelocytes	Neutrophils		Eosinophils	Blast cells	Erythroid cells	Lymphocytes	Monocytes	Hepatocytes	Other cells
				stab cells	polymorphs							
2	0	0	0	3,1±0,5	16,9±3,7	1,5±0,2	0	0	18,5±2,6	6,6±1,5	449,6±18,6	3,8±0,5
3	0	0	0	2,7±0,9	19,4±2,5	1,1±0,3	0	0	22,4±1,8	5,7±2,8	442,4±16,3	6,3±1,1
4	1,7±0,4	3,5±1,6	12,3±2,7	12,6±1,9	24,4±3,1	2,3±0,5	0,8±0,2	7,6±1,5	32,8±3,4	7,4±2,4	389,5±11,9	5,1±1,0
6	3,9±1,2	9,8±2,2	22,1±3,8	30,4±2,9	52,8±5,8	2,1±0,5	4,6±2,1	51,5±10,1	28,8±3,9	6,1±3,0	280,1±14,8	7,8±2,3
9	1,1±0,6	5,3±1,7	11,5±3,1	14,4±1,8	58,1±4,7	4,1±0,8	2,1±0,9	38,4±6,9	34,9±4,1	13,4±5,5	318,5±20,5	8,1±3,9

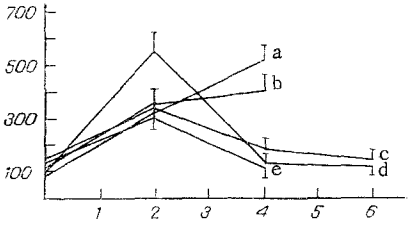

Fig. 1. Trend of changes in serum α-FP concentration of mice after a single injection of cyclophosphamide: a) C57BL/6, b) CC57BR, c) C3H/He, d) CBA, e) DD mice. Abscissa, time after injection of cyclophosphamide (in days); ordinate, α-FP concentration (in ng/ml serum).

cuvettes with Bray's scintillator. The radioactivity of the samples was determined on a Mark-3 counter (Nuclear Chicago, USA). The α-FP concentration in the samples were determined from a calibration curve plotted by means of a series of dilutions of a standard protein preparation kindly supplied by Dr. A. K. Yazova. The sensitivity of the method as we used it was between 10 and 300 ng α-FP/ml. The protein concentration was determined in each animal and, on the basis of the individual data, mean values and standard errors were calculated. The results given in Fig. 1 were averaged from data for 6-8 animals.

EXPERIMENTAL RESULTS

The results of analysis of the cell composition of squash preparations from the liver of C3H/He mice are given in Table 1. The first hematopoietic cells (of both myeloid and erythroid series) were found in 50% of animals on the 4th day after injection of cyclophosphamide. Later hematopoietic cells at different levels of differentiation were discovered in all the animals studied. Hematopoiesis in the liver reached its highest degree on the 6th day. By the 9th day it was still present, although a tendency was observed for the number of young cells to decrease. A similar trend in the development of hematopoiesis in the liver also was observed in the CBA mice. In the DD mice hematopoietic cells appeared in the squash preparations of the liver on the 4th day after injection of cyclophosphamide also. By contrast, in C57BL/6 and CC57BR mice, hematopoiesis had not yet begun in the liver at this time. Mice of the last two strains were not investigated later, but there is no doubt that hematopoiesis in the liver is activated in these animals also after injection of cyclophosphamide. This is confirmed, in particular, by the data described by Barkhotkina et al. [1].

Investigation of the serum α-FP concentration showed that it was increased in all mice in response to injection of cyclophosphamide (Fig. 1). In CBA, C3H/He, and DD mice it reached a maximum on the 2nd day, and thereafter began to fall, whereas in C57BL/6 and CC57BR mice it continued to rise until the 4th day after injection of cyclophosphamide. In mice of all strains the increase in the α-FP concentration was statistically significant ($P < 0.05$–0.001). However, in absolute terms the increase was small (not more than 560 ng/ml) and was outside the threshold of sensitivity of immunodiffusion methods.

A single injection of cyclophosphamide in these experiments thus caused foci of hematopoiesis to appear in the mouse liver and the α-FP concentration in the blood to rise. Under these circumstances, hematopoiesis was observed to begin earlier in mice with an early rise in the α-FP level (than in animals with a late α-FP peak (C57BL/6 and CC57BR). It is unlikely that the increase observed in the serum α-FP concentration could have been due to a decrease in the rate of its degradation. It can be tentatively suggested that the reason for this was intensification of α-FP synthesis by hepatocytes, as is observed in the case of the action of all hepatotoxins which have so far been studied. However, the question of what really is the mechanism of the elevation of the blood α-FP level of mice after injection of cyclophosphamide and how it is related to the hematopoiesis which develops under these circumstances in the liver is a matter which requires special investigation.

The authors are grateful to A. D. Amosov for iodinating the protein and to A. K. Yazova (Oncologic Scientific Center, Academy of Medical Sciences of the USSR) for providing the standard α-FP preparation.

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