INHIBITION OF DNA SYNTHESIS IN NORMAL AND LEUKEMIC SPLEEN CELLS IN VITRO BY LYOPHILIZED AQUEOUS EXTRACT FROM NORMAL SPLEEN

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Loss of their tumor properties by leukemic cells in culture is the basis for the study of the effect of factors concerned in the regulation of hematopoiesis on leukemic cells. Body tissues are the natural source for obtaining substances with a growth-regulating action. In particular, tissue-specific (but not species-specific) substances which have been isolated from tissues, inhibit proliferative processes in the original tissues [2, 6]. Whether or not these substances can be used to influence tumor growth is determined by many factors, among which one of the most important is the degree of sensitivity of tumor and normal cells to the action of agents isolated from normal tissues.

The object of this investigation was to study the effect of a lyophilized aqueous extract from normal spleen on DNA synthesis in leukemic and normal spleen cells.

EXPERIMENTAL METHOD

Extracts were obtained from bovine spleen tissue [4, 5] after mincing of the organ and dehydration and defatting of the tissue with acetone, by mixing for 24 h with 15 volumes of water. All operations were conducted in a cold chamber at 4°C. The extracts were dialyzed against large volumes of water for 24 h in the cold. Before lyophilization of the preparation it was passed through membrane filters. The preparations were added to cultures in concentrations of 50, 100, 500, and 1000 μg/ml.

Normal cells were isolated from the spleen of AKR and C57BL mice and leukemic cells from the spleen of AKR mice with spontaneous leukemia and from the spleen of C57BL mice with transplanted La leukemia. More than 50% of cells from the spleen of AKR mice with spontaneous leukemia and under 3% of cells from the spleen of C57BL mice with La leukemia reacted with anti-θ-serum. The cells were cultured in nutrient medium 199 with the addition of 10% human blood group AB (IV) serum, 1% L-glutamine, 100 i.u./ml penicillin, and 100 μg/ml streptomycin. The total volume of the samples was 1 ml. The cell concentration was 5×10^6 cells/ml. Leukemic and normal cells were cultured in the presence of the preparation as a rule for 4 h. Under these circumstances the preparation was added to the culture of leukemic cells immediately after setting up the samples, for the leukemic cells had high spontaneous DNA-synthesizing ability. Normal cells were usually cultured beforehand for 68 h with PHA-M (from CBI or Gibco, USA). ^{3}H-thymidine was added to all samples simultaneously with the preparation in a dose of 2 μCi. The total number of experiments was 54. Each test was conducted on five parallel samples. The viability of the cells was determined by their resistance to staining with trypan blue. After the end of culture the cells were cooled and sedimented by centrifugation, washed twice with cold physiological saline, and treated with a 5% solution of TCA. The cell precipitates were transferred to "Synpor" filters, rinsed with TCA and ethanol, dried in air, and transferred to small flasks for determination of radioactivity on liquid scintillation counters. The intensity of incorporation of ^{3}H-thymidine into the experimental samples was calculated as a percentage of incorporation into samples not containing the preparation. Changes in DNA synthesis were calculated by the equation

$$\% \text{ of inhibition of synthesis} = 100 - \frac{\text{cpm in experiment}}{\text{cpm in control}}.$$

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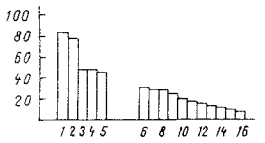

Fig. 1

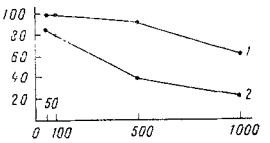

Fig. 2

Fig. 1. Effect of addition of preparation in dose of 1000 μg/ml on incorporation of ^{3}H-thymidine into PHA-stimulated normal and leukemic mouse spleen cells. Abscissa: 1-5) cultures of normal PHA-stimulated cells, 6-16) cultures of leukemic cells; ordinate, incorporation of isotope in experimental samples relative to control not containing preparation (in %).

Fig. 2. Effect of dose of preparation on incorporation of ^{3}H-thymidine into PHA-stimulated normal and leukemic spleen cells. Abscissa, concentration of preparation (in μg/ml); ordinate, incorporation of isotope into experimental samples relative to samples not containing preparation (in %). 1) PHA-stimulated normal cells, 2) leukemic cells.

TABLE 1. DNA Synthesis in Leukemic Cells in Presence of Preparation

Leukemic cells	Dose of prep. μg/ml	No. of living cells, %	Incorporation of ^{3}H-thymidine, ppm	P
Spontaneous leukemia	0	10	58 550±12 130	
	50	8	52 630±5 230	>0,05
	100	15	39 650±2 580	>0,05
	500	19	11 730±1 060	<0,05
	1000	17	6 689±890	<0,05
Transplanted leukemia	0	7	206 920±8 170	
	50	8	156 120±17 870	>0,05
	100	10	163 160±9 820	>0,05
	500	10	54 120±1 980	<0,05
	1000	12	33 260±2 710	<0,05

EXPERIMENTAL RESULTS

It will be clear from Fig. 1 that the preparation thus obtained, in a concentration of 1000 μg/ml, inhibited DNA synthesis in leukemic cells by 69-92% (in spontaneous leukemia cells by 79%, in transplanted leukemia cells by 83%). DNA synthesis in normal lymphocytes stimulated with PHA was reduced under these conditions by only 16-52%. A decrease in the dose of the preparation to 500 μg/ml caused a reduction but not disappearance of the inhibitory effect on DNA synthesis in the leukemic cells. Incorporation of ^{3}H-thymidine in this case was reduced by 40-80% (mean 62-65%). Smaller doses of lyophilized extract from normal spleen as a rule caused less constant inhibition of DNA synthesis in leukemic cells (Table 1). Estimation of DNA synthesis in PHA-stimulated cells treated with the preparation in doses of 500 μg/ml or less revealed no appreciable effect on the intensity of incorporation of the labeled DNA precursor into the cells.

Significant inhibition of DNA synthesis in leukemic cells (by 72%) was found after incubation of the cells with the preparation in a dose of 1000 μg/ml for 1 h. If the normal cells were treated with the preparation for 1 h, rinsed to remove it, treated with PHA, and then cultured for 3 days, stimulation of DNA synthesis in these cells in fact corresponded to stimulation of DNA synthesis in untreated spleen cells (92% of the control). Small doses of the preparation, even if incubated for 72 h, did not prevent activation of DNA synthesis in normal cells under the influence of PHA; larger doses inhibited it by half (Table 2).

Aqueous extract from bovine spleen thus contains substances capable of inhibiting DNA synthesis in normal and leukemic mouse spleen cells. Because of this property, these substances can be compared with lymphocytic chalone [3]. However, leukemic cells were found to be more sensitive to the action of the inhibitor of DNA synthesis isolated from spleen than were normal cells (Fig. 2).

TABLE 2. Transformation of Normal Mouse Spleen Cells under the Influence of PHA in the Presence of Different Doses of Preparation (PHA and preparation were added at the same time)

PHA	Dose of prep., μg/ml	No. of viable cells, %	Incorporation of ^{3}H-thymidine, cpm	P
—	0	40	360±40	
+	0	42	7144±500	
—	50	41	345±45	
+	50	47	6600±470	>0,05
—	0	54	300±20	
+	0	54	6050±960	
—	1000	62	220±30	
+	1000	55	2900±140	<0,05

The preparation isolated from spleen also resembles chalones in its action on cell viability. For instance, if the preparation was present in the medium, in whatever concentration, it did not reduce the number of viable normal (Table 2) or leukemia (Table 1) cells. Consequently, the stronger inhibiting activity of the preparation on leukemic cells was not connected with any cytocidal action on them. The results of these investigations do not contradict previous data [1] showing that the normal spleen of animals may be the source of substances with cytotoxic activity on leukemic cells, for this effect was found during culture of leukemic cells under special conditions. There is no doubt that fractionation of the substances isolated, a change in the methods of extraction, or a change in the morphofunctional state of the organ at the time of extraction would enable substances with a different biological action to be obtained from the spleen. A particularly promising line of investigation in this direction is the search for methods of obtaining substances from the tissue of an organ with a more marked differential action on normal and leukemic cells.

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