REGULATION OF FUNCTION OF STEM CELL PRECURSORS OF GRANULO- AND MONOCYTOPOIESIS BY THYMIC AND BONE MARROW POLYPEPTIDE FACTORS

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UDC 612.419:612.112.91]-06:612.438

KEY WORDS: regulation of hematopoiesis; granulocytopoiesis; monocytopoiesis; stem cell; thymic factor; bone marrow factor.

Regulation of differentiation and function of the stem cells is an urgent aspect of some important practical problems awaiting solution at the present time in the field of immunology and hematology. In the modern view on the mechanism of histogenesis of bone marrow cells an important role in the regulation of stem cells is played by the microenvironment and, in particular, by stromal elements [8]. It has also been shown that processes of differentiation of stem cells are under lymphocytic and thymic control [2, 5, 6]. Cells of the thymus and bone marrow are known to produce humoral factors which affect differentiation of precursors of immunocompetent cells [9, 10, 12]. Processes of differentiation of stem cells into T lymphocytes under the influence of thymic factor and processes of differentiation of antibody-forming cells under the influence of bone marrow factor have been studied the most [7, 12]. Meanwhile the action of thymic and bone marrow factors on stem cells that are precursors for granulo- and monocytopoiesis has virtually not been studied.

The aim of this investigation was to study the effect of polypeptide factors of the thymus and bone marrow on function of stem cells which are precursors of granulocytopoiesis and monocytopoiesis.

EXPERIMENTAL METHOD

A double-layer agar system of Pike and Robinson [11] with the following modifications was used. A suspension of bone marrow cells was enriched with the stem cell fraction by separation of the cells in a Ficoll-Verografin gradient (specific gravity 1.076). Mononuclear cells isolated from freshly heparinized blood from group AB (IV) donors by centrifugation in a Ficoll-Verografin gradient were used as the feeder. A modified McCoy's 5A medium was prepared [1]. The bottom layer (feeder) of agar contained $4 \cdot 10^5$-$5 \cdot 10^5$ monocytes/ml; hematopoietic bone marrow cells were added to the top agar layer in a concentration of $1 \cdot 10^5$ cells/ml. Hematopoietic bone marrow cells were cultured in a double-layer agar system in three different versions: 1) control, 2) with the addition of thymic polypeptide factor [3] to the top agar layer (1 μg to 10^5 explanted cells), and 3) with the addition of bone marrow polypeptide factor [4] to the top agar layer (1 μg to 10^5 cells). The investigation was carried out on hematopoietic bone marrow cells from 15 healthy subjects. Agar cultures were prepared and cultivation carried out by Afanas'ev's method [1]. Hematopoietic bone marrow cells also were cultured in every case ($1 \cdot 10^5$ cells) in a single-layer agar system (without feeder) to test for any possible spontaneous colony formation. The results were assessed on the 8th day of culture under a microscope (magnification 40×). The colony-forming ability (CFA) of the bone marrow cells was determined by counting colonies, and the cluster-forming ability (ClFA) by counting the number of clusters corresponding to $1 \cdot 10^5$ explanted cells. Aggregates containing more than 20 cells were regarded as colonies and containing from 3 to 20 cells as clusters. The colonies were subdivided into small (20-40 cells), medium (40-100 cells), and large (over 100 cells).

S. M. Kirov Military Medical Academy, Leningrad. (Presented by Academician of the Academy of Medical Sciences of the USSR R. V. Petrov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 93, No. 4, pp. 94-95, April, 1982. Original article submitted September 4, 1981.

 0007-4888/82/9304-0502$07.50

TABLE 1. Effect of Thymic and Bone Marrow Factors on ClFA and CFA of Human Bone Marrow Precursor Cells of Granulo- and Monocytopoiesis (M ± m)

Experimental conditions	n	ClFA	CFA	Number of colonies of different sizes		
		per 1 × 10^5 myelokaryocytes		20-40 cells	40-100 cells	over 100 cells
Control	15	120,2±17,8	44,2±4,5	29,0±3,2	13,1±1,4	2,2±0,5
Addition of thymic factor	13	238,3±38,8†	83,1±8,5†	45,8±4,9*	27,5±2,9†	9,5±2,4†
Addition of bone marrow factor	10	130,8±9,9	49,5±7,0	29,4±3,9	14,8±2,5	5,3±1,6*

Legend. Differences from control statistically significant at *) $P < 0.01$ or †) $P < 0.05$ level.

EXPERIMENTAL RESULTS

Unlike double-layer agar systems, growth of colonies was not found in any single-layer agar system. Consequently, colony formation in the double-layer agar systems was due to the presence of a colony-stimulating factor in the feeder layer. Under the influence of thymic factor both the ClFA and CFA of precursor cells of granulo- and monocytopoiesis was found to be increased; an increase was observed, moreover, in the numbers of all types of colonies — small, medium, and large (Table 1).

Meanwhile the effect of bone marrow factor on the explanted hematopoietic bone marrow cells was much weaker. No significant change in ClFA or CFA was found under the influence of bone marrow factor.

Thymic factor was thus shown to have a stimulating action on cluster-forming and colony-forming cells.

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