SPLENIC CFU-S COLONY FORMATION BY LONG-TERM BONE MARROW AND EMBRYONIC LIVER CULTURE IN THE PRESENCE OF THYMOCYTES

O. V. Semina, T. N. Semenets, and A. M. Poverennyi

UDC 616.419-092.4-078.73

KEY WORDS: mouse thymocytes, bone marrow, embryonic liver, hematopoietic cells

Data on the effect of humeral and cellular preparations of the thymus on the hematopoietic stem cell (CFU-S) population are abundant but not always identical in their interpretation [3, 9]. Previously the writers described the possibility of restoring splenic colony formation, when inhibited by treatment of the bone marrow with mouse antibrain serum (MABS), by the additional injection of thymus cells [4]. However, these experiments, while demonstrating the role of thymocytes in splenic colony formation, have a certain disadvantage: the bone marrow is subjected to the action of a damaging agent (MABS), the polyclonal nature and toxicity of action of which prevent unambiguous interpretation of the results. We therefore decided to look for a system in which absence of thymocytes would not be associated with exposure to damaging agents. This stipulation was met by using cells of a long-term bone marrow culture, and also the liver of 13-16-day embryos [1, 7]. These also were chosen as the source of CFU-S in the present experiments. This paper is devoted to a study of the effect of thymus cells on the colony-forming ability of CFU-S of a long-term culture of bone marrow and embryonic liver.

EXPERIMENTAL METHOD

Female (CBA × C57BL)F_1, (C57BL × DBA)F_1, and CBA mice and male C57BL mice were used. The recipient mice were irradiated on the Luch-1 radiotherapy apparatus with ^{60}Co γ rays 18-24 h before transplantation of the cell suspensions. Colony-forming activity was determined by the splenic colonies method [10]. Settling factor was assessed by the method in [8] 24 h after the first transfer. Thymocytes ($2.0 \cdot 10^7$ cells/mouse, 0.5 ml) were injected intravenously 30 min before injection of the cell suspensions. To determine the kinetics of repopulation of CFU-S the method described in [11] was used. For this purpose, embryonic liver cells or bone marrow culture cells were transplanted with or without the addition of thymocytes into each group of primary recipients. A mixture of bone marrow from 5-15 animals was obtained after 4, 7, 11, and 14 days and injected into secondary recipients, in which the number of splenic macrocolonies was counted 10 days later. To prepare a suspension of embryonic liver cells, embryos (CBA × C57BL) aged 13-16 days were used. The day of appearance of a vaginal plug was taken as day 0. Cells of a long-term bone marrow culture were obtained 2-3 weeks after explanation of bone marrow from (C57BL × DBA)F_1 mice in a Dexter system by the method in [2]. In this case (C57BL × DBA)F_1 mice were used as recipients.

EXPERIMENTAL RESULTS

Table 1 gives data showing the stimulating action of thymocytes on colony formation when hematopoietic suspensions not containing T cells were injected (embryonic liver, long-term bone marrow culture). This effect was not connected with a change in the number of CFU-S injected and settling in the spleen, which could be judged by the absence of any difference in values of the settling factors for CFU-S from the two sources, in the presence and absence of thymus cells (Table 2). The stimulating action of thymocytes may perhaps be connected with acceleration of CFU-S proliferation. This hypothesis was based

Research Institute of Medical Radiology, Academy of Medical Sciences of the USSR, Obninsk. (Presented by Academician of the Academy of Medical Sciences of the USSR R. V. Petrov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 110, No. 8, pp. 176-178, August, 1990. Original article submitted April 10, 1989.

 0007-4888/90/0008-1080$12.50

TABLE 1. Effect of Thymocytes on Splenic Colony Formation by Cells of Long-Term Bone Marrow Culture and Embryonic Liver Cells ($M \pm m$)

Source of CFU-S	Thymocytes 2×10^7/mouse	Number of mice	Mean number of colonies per 3×10^5 injected cells	Stimulation factor	p
Bone marrow culture	—	52	8,15±0,7	—	
The same	+	57	12,5±1,03	1,5	<0,001
Embryonic liver	—	22	5,0±0,6	—	
The same	+	20	9,1±0,57	1,8	<0,001
Endogenous CFU-S	+	20	0,5±0,36	—	

TABLE 2. Values of Settling Factor for Cells of Long-Term Bone Marrow Culture and Embryonic Liver Cells in the Presence or Absence of Thymocytes (24 h after first transfer)

Source of CFU-S	Thymocytes	Value of settling factor, per cent
Bone marrow culture	—	8,5
The same	+	5,9
Embryonic liver	—	7,7
The same	+	5,6

TABLE 3. Values of CFU-S Doubling Time for Long-Term Bone Marrow Culture and Embryonic Liver in Femur (results obtained by determining kinetics of repopulation of bone marrow within the time interval 4-14 days)

Source of CFU-S	Doubling time, h	
	I	II
Bone marrow culture	42,5	—
The same	27,8	—
Embryonic liver	65,7	118,0
The same	48,0	41,3

Legend. I and II) Variants of experiment.

on a report that CFU-S regeneration from bone marrow enriched with T-cells can be intensified [11], and also by the results of experiments which demonstrated slowing of proliferation after exposure of the bone marrow cells to MABS, which could be returned to normal by the addition of thymocytes [5]. The kinetics of CFU-S regeneration revealed a reduction of the doubling time for CFU-S from both sources under the influence of thymus cells (Table 3). They may perhaps cause acceleration of proliferation of younger, polypotent CFU-S, for an increase in the number of colonies formed by embryonic liver CFU-S in the presence of thymus cells was observed both on the 7th and on the 12th day of growth (data not given).

The results thus demonstrate that thymocytes increase the efficiency of cloning in the spleen and shorten the proliferation time of CFU-S from embryonic liver and long-term bone marrow culture. Their action is evidently directed toward polypotent CFU-S. It is particularly important to note that this effect is observed in vivo without the additional action of damaging factors, possible evidence of its physiological character, that T cells are in fact involved in the regulation of hematopoiesis. In the irradiated animal, this action may be linked with lymphokine production by T cells, evidence of which is given by the increased production of interleukin-2 by lymphocytes after irradiation [6]. It will therefore be interesting to study the effect of interleukin-2 on CFU-S in the system used. Preliminary data already support the view that T-cell growth factor plays a role in the regulation of hematopoiesis. The facts described in this paper can evidently serve to confirm the existence of both these mechanisms of regulation of immunopoiesis and hematopoiesis.

The authors are grateful to Professor I. L. Chertkov and his colleagues for practical help and for discussion of the results.

LITERATURE CITED

1. U. A. Arkhipov, R. N. Khaitov, and V. G. Galaktionov, Outlines of Modern Immunology [in Russian], Tashkent (1981), pp. 195-212.
2. N. I. Drize and I. L. Chertkov, Probl. Gematol., No. 4, 19 (1984).
3. R. V. Petrov, V. M. Man'ko, R. M. Khaitov, et al., Tsitologiya, No. 2, 365 (1979).
4. A. M. Poverennyi, O. V. Semina, T. N. Semenets, et al., Byull. Éksp. Biol. Med., No. 6, 708 (1978).
5. O. V. Semina, T. N. Semenets, E. S. Kurilets, et al., Byull. Éksp. Biol. Med., No. 4, 444 (1987).
6. V. P. Shichkin and E. A. Protsak, Radiobiology of Stem and Clonogenic Cells [in Russian], Obninsk (1986), pp. 44-48.
7. T. M. Dexter, T. D. Allan, L. G. Lajtha, et al., Differentiation of Normal and Neoplastic Hematopoietic Cells, B. Clarkson et al. (eds.), New York (1978), pp. 63-80.
8. L. Siminovitch, E. A. McCulloch, and J. E. Till, J. Cell. Physiol., **62**, 327 (1963).
9. "T-cells as regulators of haematopoiesis. 1-er Forum d'Immunologie," Ann. Immunol., **135C**, 261 (1984).
10. J. Till and E. McCulloch, Radiat. Res., **14**, 213 (1961).
11. J. W. Visser and J. F. Elisson, Cell Tissue Kinet., **16**, 385 (1983).

SEROTONIN AND 5-HYDROXYINDOLEACETIC ACID LEVELS IN THE BRAIN AND IMMUNOCOMPETENT ORGANS AFTER IMMUNIZATION

O. V. Galkina, E. L. Al'perina, O. V. Suslyakova, and L. V. Devoino

UDC 612.822.015:
577.175.823].014.46:615.37].084

KEY WORDS: serotonin; 5-hydroxyindoleacetic acid; serotoninergic system of the brain; immunocompetent organs; adrenals

One aspect of the study of mechanisms of neuroimmunomodulation is the detection of neurochemical changes in specialized brain structures and changes in concentrations of those monoamines which perform the function of neurotransmitters in the brain in immunocompetent organs during formation of the immune response.

The serotoninergic system of the brain is known to be responsible for the inhibitory mechanism of immunomodulation [3, 5, 8]. Sporadic studies have demonstrated a fall in the serotonin concentration 20 min after immunization in the ventromedial part of the anterior hypothalamus [6] and an increase in the serotonin concentration in the hypothalamus of rats immunized with sheep's red blood cells (SRBC) on the 2nd and 4th days of the immune response, followed by a fall of the serotonin level on the 11th and 20th days [1]. So far as the immunocompetent organs are concerned, a fall of the serotonin concentration has been found in the thymus after immunization [2, 6, 7], and a very small increase in the serotonin concentration in the spleen on the 3rd day of the immune response [7].

The aim of this investigation was to analyze changes in the concentrations of serotonin (5-HT) and its principal metabolite, 5-hydroxyindoleacetic acid (5-HIAA) in brain structures related to the serotoninergic system of the mesencephalic nuclei raphe, the immunocompetent organs, and adrenals, which constitute the peripheral component in regulation of the immunosuppressive action of the serotoninergic system [5] in the early period after injection of the antigen.

Laboratory of Mechanisms of Memory Regulation and Laboratory of Mechanisms of Neurochemical Modulation, Institute of Physiology, Siberian Branch, Academy of Medical Sciences of the USSR, Novosibirsk. (Presented by Academician of the Academy of Medical Sciences of the USSR Yu. P. Nikitin.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 110, No. 8, pp. 178-180, August, 1990. Original article submitted August 30, 1989.

 0007-4888/90/0008-1082$12.50