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EFFECT OF B LYMPHOCYTES FROM DIFFERENT ORGANS ON HEMATOPOIETIC COLONY FORMATION IN THE SPLEEN BY BONE MARROW CELLS

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T lymphocytes, activated by isoantigens, influence the process of hematopoietic colony formation in the spleen of irradiated recipients and alter the ratio between colonies of different types through their action on the control of differentiation of syngeneic hematopoietic cells [2]. B lymphocytes are involved in the realization of this effect, and serve as helper Tα-cells of the T lymphocytes realizing a differential effect [1]. Other forms of action of lymphoid cells and their products on colony formation also are known. It has been shown, for instance, that hematopoietic bone marrow precursor cells cannot form splenic colonies in the absence of precursors of T lymphocytes (PTL) or their products [4-6, 9]. It is interesting to study the possibility of a similar helper action of B lymphocytes in a syngeneic system, and also their possible participation in the mechanism of the PTL effect. The investigation described below was devoted to the study of this problem, and involved the study of the effect of B lymphocytes arising from different organs on the formation of hematopoietic colonies by stem cells, freed from PTL, mature lymphocytes, and cells adherent to plastic, as well as on interaction of stem cells and PTL during colony formation.

EXPERIMENTAL METHOD

The donors and recipients of the cells and their fractions were male (CBA × C57BL/6)F_1 mice weighing 18-20 g, obtained from the Stolbovaya nursery (Academy of Medical Sciences of the USSR) and the Central nursery (Academy of Sciences of the USSR). The bone marrow cells were flushed out of the femora of the mice with medium 199 and fractionated, by methods based on the panning method [7] and mass cytolysis [3]. The fractionation scheme shown in Fig. 1 was used for the bone marrow cells. During fractionation of spleen and lymph node cells (pooled inguinal, popliteal, and axillary lymph nodes) only cells carrying membrane immunoglobulins (Ig^+-cells) (fraction 1) were isolated.

Expression of surface markers was assessed by immunofluorescence (Thy-1,2, Ig) and cytotoxic (SC-1, MBLA) tests. Monoclonal antibodies to Thy-1 2 antigen (G4), immune sera to PTL SC-1 antigen (brain antiserum exhausted with liver and thymocytes), and immune rabbit serum to mouse MBLA antigen (provided by Professor N. A. Kraskina) were used. Affinity-purified IgG-antibodies to immunoglobulins of classes M and G (specificity verified by Ouchterlony's method), isolated from immune rabbit serum, also were used.

Colony formation in the spleen was assessed by the exotest in [8]. For this purpose recipient mice were irradiated in a dose of 8.5 Gy on a "Stebel'" ^{137}Cs γ-ray source The doses of cells injected corresponded to their yield during fraction-

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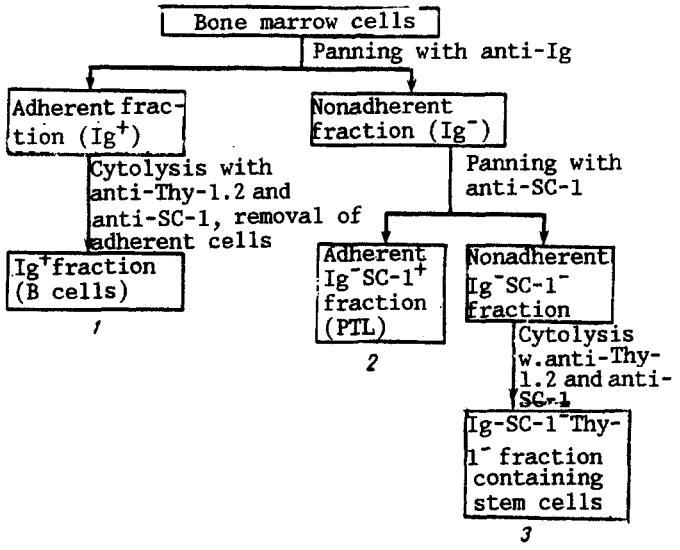

Fig. 1. Scheme showing fractionation of bone marrow cells.

ation of 10^5 bone marrow cells, The experimental results were subjected to statistical analysis with calculation of the arithmetic mean, the error of the mean, and Student's "t."

EXPERIMENTAL RESULTS

It will be clear from Table 1 and Figs. 1 and 2 that when bone marrow cells of fractions 1, 2, and 3 were injected into irradiated mice separately (Fig. 1) colony formation was virtually absent. In full agreement with previous findings [6], ability to form colonies was largely restored when fraction 3, containing stem cells, was mixed with fraction 2, containing PTL serving as helpers in colony formation [4, 5]. However, as will be clear from Fig. 2, colony formation was not restored to the level of unfractionated bone marrow. This may be connected with losses of cells arising when the separate fractions were obtained.

A similar but weaker helper effect was found with bone marrow Ig^+-cells when cells of fractions 3 and 1 were mixed. Since only 20% of the cells in fraction 1 are Ig^+ B lymphocytes, additional proof of a connection of the helper effect with B lymphocytes was necessary. This proof was obtained in experiments in which the helper effect was abolished by cytolysis of cells of fraction 1 by antibodies to the B-cell marker MBLA.

An effect in the same direction, but weaker, was obtained for the Ig^+-fraction of lymph node cells, which also was abolished by treatment with anti-MBLA-serum. Conversely, the Ig^+-fraction of spleen cells had no stimulating action on colony formation by bone marrow SC-1 cells (fraction 3). However, treatment of splenic Ig^+-cells with anti-MBLA before their addition to fraction 3 of the bone marrow cells revealed a helper effect. Treatment of the Ig^+-cells (including splenic) with anti-MBLA did not induce colony-forming ability when injected alone.

Thus when interaction of the Ig^+-fractions of bone marrow and lymph node cells was studied, helper activity of Ig^+-cells specific for MBLA-serum in relation to the colony-forming ability of bone marrow SC-1 cells was demonstrated. The Ig-fraction of the spleen also possessed this activity, but it was exhibited only after treatment with antiserum to MBLA antigen. In other words, the B lymphocytes (Ig^+) of this fraction were characterized by suppressor activity, and helper activity was exhibited by cells that differed from B lymphocytes, i.e., they did not carry the MBLA antigen on their membrane.

Phenomenologically the effect of $SC\text{-}1^-Ig^+MBLA^+$ bone marrow and lymph node cells (Fig. 2, fractions 3 + 1a and 3 + 1b) was analogous to the action of $SC\text{-}1^+$-cells (PTL) in this system (Fig. 2, fractions 3 + 2), although it was weaker. In this connection the action of the Ig^+-fractions of the organs studied on colony formation was tested also during interaction of the $SC\text{-}1^-$ and $SC\text{-}1^+$ fractions of bone marrow. When Ig^+-cells from all three sources were used, inhibition of the colony-forming activity of a mixture of fractions 3 and 2 was observed. The inhibitory effect was manifested weakest of all when bone marrow Ig^+-cells (sometimes bone marrow was absent) fractions of Ig^+-cells from the three sources

TABLE 1. Action of Ig^+-Lymphocytes of Different Origin on Colony-Forming Activity of Bone Marrow Cell Fractions

Origin of Ig^+-cells	Number of colonies after addition of isolated fractions (M ± m)				Number of colonies after addition of mixture of $SC\text{-}1^-$ and Ig^+-cells				Number of colonies after addition of mixture of $SC\text{-}1^-$ and $Sc\text{-}1^+$ cells	Number of colonies after addition of mixture of $SC\text{-}1^-$, $SC\text{-}1^+$, and Ig^+-cells			
	Ig^-	Ig^+ MBLA	SC-1	CS-1+	calculated	actual	II (inhibitory interaction)	after treatment with Ig^+-anti-MBLA		calculated	actual	II (inhibitory interaction)	after treatment with Ig^+ anti-MBLA
Bone marrow	0,3±0,2	0,3±0,2	0,4±0,2	0	0,7	4,0±0,3	5,7	0,4±0,3	6,8±0,6	7,1	3,4±0,3	0,48	0,6±0,3
Lymph nodes	0,2±0,2	0	0	0	0,2	1,9±0,3	9,5	0	4,7±0,5	4,9	1,2±0,3	0,25	0
Spleen	0,3±0,2	0	0,3±0,2	0,2± 0,2	0,6	0,3±0,2	0,5	3,0±0,3	4,0±0,5	4,3	0,1±0,3	0,02	4,0±0,3

Legend. Ig^+) Ig^+-fraction (B lymphocytes); $SC\text{-}1^-$) fraction $Ig^+Thy\text{-}1^-SC\text{-}1^-$ (stem cells); $SC\text{-}1^+$) fraction of precursors of T lymphocytes (PTL).

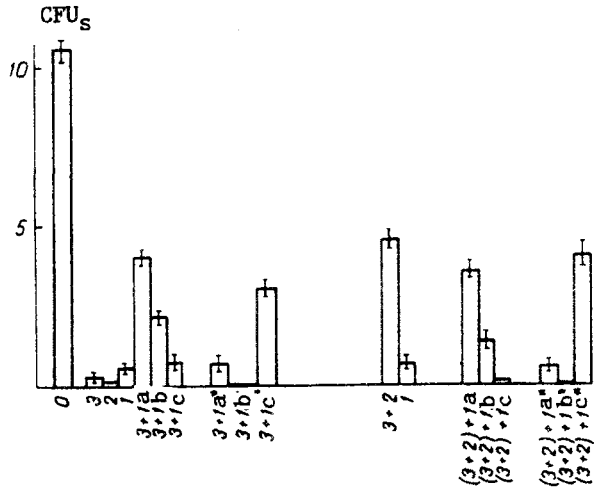

Fig. 2. Action of B lymphocytes from bone marrow, lymph nodes, and spleen on colony-forming activity of bone marrow stem cells and their cooperation with PTL. **Legend.** Types of cells: 0) unfractionated bone marrow cells; 3) $SC\text{-}1^-$ fraction; 2) $SC\text{-}1^+$ fraction; 1) Ig^+-fraction. Sources of Ig^+-cells: a) bone marrow, b) lymph nodes, c) spleen. *) Cells of Ig^+ fraction treated beforehand with antiserum to MBLA and complement. Ordinate, number of colonies in spleen of irradiated recipients after introduction of cells of the above-mentioned types.

studied contained cells inhibiting interaction of stem cells and PTL; in the spleen, moreover, the inhibitory effect was connected with $MBLA^+$-cells, whereas in the bone marrow and lymph nodes it was connected with certain other cells contained in the Ig^+-fraction in the form of contamination or with Ig^+ $MBLA^-$-cells. One hypothesis which can explain the effect recorded may be that in a population of Ig^+ lymphocytes from bone marrow and lymph nodes there are cells of the B series with countersuppressor activity against unidentified suppressor cells, contained in the Ig^+-cell population. Removal of the B countersuppressors with the aid of anti-MBLA-serum was accompanied by abolition of their action on the unidentified suppressors and, as a result of that, marked inhibition of the colony-forming function of the stem cells.

The results can be summed up as follows. MBLA$^+$-lymphocytes from the Ig$^+$fraction of bone marrow and lymph nodes have a helper action on bone marrow stem cells, expressed as ability of cells of the SC-1$^-$ fraction of bone marrow to induce colony formation in the spleen. Splenic Ig$^+$ MBLA$^+$-cells do not possess this action and suppress the helper effect of SC-1$^+$-PTL and of unknown cells contained in the Ig$^+$-fraction. The Ig$^+$-fraction of bone marrow and lymph nodes contains MBLA$^-$-cells, abolishing the helper action of SC-1$^+$. Consequently, in these experiments effects of Ig$^+$ MBLA$^+$-helpers of colony formation (in the bone marrow and lymph nodes), of suppressors (in the spleen), and also of helpers (in the spleen) and suppressors (in the bone marrow and lymph nodes) of unknown nature, containing cells of these organs in the Ig$^+$-fractions, were recorded. Their nature, their physiological role, the mechanisms of realization of these effects, and also the biological significance of the opposite effects of spleen cells await elucidation by further analysis of Ig$^+$ MBLA$^{+/-}$-cells.

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REGULATORY PROPERTIES OF RAT HEART ADENYLATE CYCLASE DURING THE COURSE OF TOXICOINFECTIOUS SHOCK INDUCED BY *Yersinia pestis* TOXIN

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Previously the writers found weakening of the contractile function of the heart and a decrease in the number of voltage-dependent Ca^{2+}-channels and beta-adrenergic receptors of the cardiomyocytes in the course of poisoning with murine plague toxin [3]. At the present time the effects of catecholamines responsible for activation of beta-receptors are linked with elevation of the intracellular cAMP level, which causes an increase in adenylate cyclase activity. Hormone-dependent adenylate cyclase (AC) is a key enzyme responsible for the regulatory effect of many hormones and biologically active substances, such as glucagon, histamine, prostaglandins of the E group, and so on, whose levels change sharply in toxicoinfectious shock due to plague, on the heart [3, 4, 7].

In the investigation described below the effect of murine plague toxin on AC activity of the rat heart and also on its regulation by isoproterenol and glucagon was studied.

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