action also will differ, and in particular, it will be much smaller for SIII than for PVP_{350} and Vi-antigen.

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EFFECT OF ANTIGEN ON COLONY-FORMING ACTIVITY OF HEMATOPOIETIC STEM CELLS IN TOLERANT MICE

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KEY WORDS: hematopoietic stem cell; immunologic tolerance; regulation.

The polypotent hematopoietic stem cell (PHSC) is known to participate in formation of the immune response [1]. Injection of the most widely differing antigens causes an increase in the number of colony-forming cells and in their proliferative activity [1, 7]. Meanwhile, problems connected with the concrete mechanisms of participation of PHSC in immunogenesis remain largely unsolved.

To determine the character of the cellular mechanism of involvement of PHSC in the immune response, in the investigation described below the effect of an antigen (sheep's red blood cells — SRBC) on the colony-forming activity of PHSC was studied in mice tolerant to that antigen.

EXPERIMENTAL METHOD

Experiments were carried out on male (CBA × C57BL/6)F_1 mice weighing 18-20 g. To induce a state of tolerance SRBC were injected intraperitoneally in a dose of $5 \cdot 10^9$ per mouse 48 h before intraperitoneal test injection of SRBC in a dose of $2 \cdot 10^8$, and the number of antibody-forming cells (AFC) against SRBC was determined in the spleen [5]. The colony-forming activity of PHSC was determined by the method described previously [8], by testing the number of splenic colony-forming units (CFUs) on the 8th day after injection of donors' spleen cells (SC) into the recipients in a dose of $5 \cdot 10^5$ 24 h after, and injection of bone marrow cells (BMC) in a dose of $5 \cdot 10^4$ 72 h after antigenic priming. SC and BMC of intact animals served as the antigenic priming control.

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TABLE 1. Change in Number of Exogenous CFUs in Spleen and Bone Marrow of Mice Tolerant to SRBC in Response to Antigenic Priming

Preliminary treatment of donors	Test injection	Number of recipients	Number of CFUs per 5×10^4 BMC
—	—	27	10,9±0,6
—	SRBC	26	16,2±0,5
SRBC + CP	—	31	11,6±0,5
SRBC + CP	SRBC	29	10,6±0,5
—	HRBC	27	16,9±0,6
SRBC + CP	HRBC	29	16,9±0,6

TABLE 2. Change in Number of Exogenous CFUs in Spleen of Mice Tolerant to SRBC in Response to Antigenic priming

Preliminary treatment of donors	Test injection	Number of recipients	Number of CFUs per 5×10^5 BMC
—	—	23	10±0,5
—	SRBC	27	14,7±0,6
SRBC + CP	—	26	9,7±0,6
SRBC + CP	SRBC	29	12,9±0,7
—	HRBC	16	16,1±0,7
SRBC + CP	HRBC	17	16,1±0,9

The number of endogenous colonies in the spleen of tolerant mice was determined by the method in [3]. Mice receiving a test injection of SRBC 24 h before the experiment were used.

EXPERIMENTAL RESULTS

The colony-forming activity of BMC and SC was determined in mice after injection of SRBC into animals tolerant toward this antigen. The development of a state of tolerance was tested 12-14 days after injection of cyclophosphamide (CP). In the control animals after the test injection of SRBC there were 88 AFC, compared with 4 AFC in the tolerant mice per 10^6 splenocytes. The development of a state of tolerance to SRBC was therefore observed.

As Table 1 shows, no increase in the column-forming activity of PHSC was observed in the bone marrow of the tolerant mice, based on the number of exogenous splenic colonies in response to the test injection of SRBC, whereas injection of another antigen, namely human red blood cells (HRBC), caused an increase in the number of CFUs in donors tolerant toward SRBC.

By contrast with those of bone marrow, splenic PHSC responded to injection of SRBC into the tolerant mice in the same way as to injection of HRBC, by an increase in colony formation (Table 2). The same picture also was observed in the system for endogenous column formation, when injection of SRBC into tolerant animals caused an increase in the number of endogenous splenic colonies to twice the control level (Table 3).

Injection of SRBC into animals tolerant toward this antigen thus caused an increase in colony-forming activity of PHSC of bone marrow, whereas the same mice responded to injection of HRBC by an increase in the number of PHSC in the bone marrow. The colony-forming activity of splenic PHSC in tolerant mice was increased by injection of both SRBC and HRBC. Injection of SRBC and HRBC into intact donors caused an increase in the number of CFUs in both bone marrow and spleen.

It was shown previously that after antigenic priming, migration of T cells into the bone marrow is observed [4]. We also know that the increase in proliferative activity of bone marrow PHSC in response to injection of thymus-dependent antigens is attributable to T lymphocytes [7]. At the same time it has been shown that tolerance induced by CP is due to a deficiency of antigen-reactive T lymphocytes [2]. Since no increase in the colony-forming activity of CFUs of bone marrow was observed in the present experiments in mice tolerant to SRBC in response to the test injection of SRBC, it can be postulated that T lymphocytes participate in the stimulation of colony-forming activity of bone marrow PHSC in response to antigenic priming. This participation is evidently antigen-specific in character, for after injection of HRBC into mice tolerant to SRBC, their bone marrow PHSC responds by an increase in colony formation.

In all probability the increase in colony formation by CFUs in tolerant mice, discovered in the present experiments by methods of exogenous and endogenous cloning, in response to test injection of SRBC, indicates the nonspecific character of the mechanism of activation of splenic PHSC during this antigenic priming. The possibility cannot be ruled out that this effect is produced by macrophages, for we know that splenic macrophages take an active part in enhancement of the colony-forming activity of splenic PHSC in response to injection of SRBC [1]. Again, when the same model of tolerance was used, a persistent decrease in the number of T lymphocytes [2], but not of macrophages [6], was observed in the spleen.

TABLE 3. Change in Number of Endogenous CFUs in Mice Tolerant to SRBC in Response to Antigenic Prining

Preliminary treatment of animals	Number of animals	Test injection	Number of CFUs per spleen
—	14	—	3±0,3
—	15	SRBC	6,7±0,7
SRBC + CP	11	—	2,7±0,4
SRBC + CP	22	SRBC	5,6±0,6

The mechanism of activation of PHSC in response to antigenic priming thus differs for the bone marrow and spleen. In bone marrow it evidently takes place with the participation of T lymphocytes, but in the spleen with the participation of macrophages.

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STIMULATION OF PHAGOCYTIC FUNCTION BY LEUKOCYTE AND MACROPHAGE MIGRATION INHIBITION FACTORS IN MAN

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An important trend in immunocorrection is the search for preparations with selective action on different cell populations. Recent years have seen a beginning of the use of biological factors secreted by lymphocytes, namely lymphokines, for the treatment of certain immunodeficiency states. These substances include a group of factors selectively influencing macrophages and polymorphonuclear leukocytes, such as macrophage migration inhibition factors (MMIF), leukocyte migration inhibition factor (LMIF), and macrophage activating factor. The effective use of these factors in the treatment of small-cell forms of cancer spontaneous metastases of melamonas, and so on, under experimental conditions, has been reported [4, 5]. With the opening up of these prospects for the widespread clinical use of these mediators, the task of their isolation and purification had the more detailed study of the mechanism of their action on target cells are of great urgency.

This paper describes the simultaneous isolation of MMIF and LMIF from supernatants of lymphocyte cultures stimulated by phytohemagglutinin (PHA), and the effect of the isolated factors on migration and phagocytic activity of target cells was studied.

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