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EFFECT OF ANTIGENIC STIMULATION ON PROLIFERATIVE ACTIVITY OF HEMATOPOIETIC STEM CELLS IN SPLENECTOMIZED MICE

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The solution to the problem of the mechanisms of regulation of proliferation and differentiation of the bone marrow hematopoietic stem cell (BMC) during antigenic stimulation depends on the elucidation of the role of the peripheral lymphoid organs and their cells in these processes. We know that the spleen plays an important role in the development of the immune response to thymus-dependent (SRBC — sheep's red blood cells) and thymus-independent (PPS — pneumococcal polysaccharide SS 111) antigens [4, 9]. Meanwhile it has been shown that after injection of another thymus-independent antigen — *E. coli* lipopolysaccharide (LPS) into mice, a marked immune response to this antigen is formed even in the absence of the spleen [11]. Immunization of mice with SRBC, PPS, and LPS also caused stimulation of proliferation of hematopoietic stem BMC [3, 6].

Since the role of the hematopoietic stem cell (CFUs — colony-forming unit of the spleen) in immunopoiesis is no longer disputed [3], it was considered important to determine the proliferative activity of CFUs in splenectomized mice in response to injection of various antigens.

EXPERIMENTAL METHOD

Experiments were carried out on male (CBA × C57BL/6)F_1 mice weighing 18-20 g, obtained from the Svetlye Gory nursery, Academy of Medical Sciences of the USSR. Antigenic stimulation consisted of intraperitoneal injection of SRBC in a dose of $2 \cdot 10^8$, PPS, generously presented by P. J. Baker (USA), in a dose of 100 μg, and *E. coli* LPS (from Difco, USA) in a dose of 100 μg. Immunization was carried out 72 h before determination of proliferative activity of CFUs in the bone marrow of splenectomized mice and mice undergoing a mock operation, by the method in [5]. The recipients (20-25 mice) of each group were given an intravenous injection of BMC from three to five donors. The results of two or three experiments are pooled in Tables 1 and 2. Splenectomy was performed under ether anesthesia 45 days before the experiment. Immune spleen cells (ISC) were obtained 48 h after intravenous immunization of normal animals with SRBC in a dose of $2 \cdot 10^8$. Cell populations of ISC, enriched with macrophages and lymphocytes, were obtained by the method in [10].

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TABLE 1. Proliferative Activity of Bone Marrow CFUs in Splenectomized (CBA × C57BL/6)F_1 Mice during Antigenic Stimulation

Experimental conditions (treatment of bone marrow donors)	No. of CFUs per 10^5 BMC (M ± m)		Percentage of CFUs in S phase
	without ^{3}H-thymidine	^{3}H-thymidine	
Splenectomy			
Physiological saline	21±0,9	18,7±0,8	10,9
SRBC	22,2±1,6	19,7±1,3	11,2
PPS	22±1,9	19,2±1,8	12,7
LPS	22,5±1,0	16,4±1,4	27,1
Mock splenectomy			
Physiological saline	18,8±1,1	16,8±1,1	10,6
SRBC	20,4±1,2	14,4±1,2	29,4
PPS	20,8±1,1	15,4±1,0	27,8
LPS	13,4±1,4	8,6±1,2	35,8

TABLE 2. Proliferative Activity of Bone Marrow CFUs in Splenectomized (CBA × C57BL/6)F_1 Mice after Transfer of Spleen Cells from Syngeneic Animals, Intact and Immune to SRBC

Treatment of bone marrow donors	No. of CFUs per 10^5 BMC (M ± m)		Percentage of CFUs in S phase
	without ^{3}H-thymidine	^{3}H-thymidine	
Physiological saline	18,1±1,8	15,8±1,5	12,2
SC	15,8±1,4	14,1±1,3	10,7
ISC	18,5±1,3	13,1±0,8	29,1
Physiological saline	15±0,9	13,6±1,7	9,3
Nonadherent			
SC	19,8±1,0	17,7±0,9	10,6
ISC	23,8±0,7	17,2±0,9	27,7
Adherent:			
SC	21,4±0,8	19,2±0,8	10,2
ISC	19,8±0,5	18±1,1	9

EXPEIMENTAL RESULTS

After intraperitoneal injection of SRBC and PPS into the splenectomized mice no increase was observed in the level of CFUs proliferation, determined on the 4th day after immunization, whereas injection of LPS caused stimulation of proliferation of CFUs by 2.5 times (Table 1). In mice undergoing the mock operation, injection of all three antigens was accompanied by an increase in proliferative activity of CFUs in the bone marrow. Splenectomy itself did not affect proliferation of CFUs (Table 1). In addition, 45 days after splenectomy, erythropoiesis and granulopoiesis were restored in the mice [7, 8], and no disturbances were observed of differentiation and colony formation by CFUs, determined among the BMC [1].

The results indicate that the spleen plays an important role in the regulation of proliferative activity of the hematopoietic stem BMC in response to injection of SRBC and PPS, i.e., of antigens to which the development of the primary immune response in mice is mainly undertaken by the spleen [4, 9]. After injection of LPS, the immune response to which also is formed in the absence of the spleen [11], stimulation of proliferation of CFUs was observed, confirming that regulation of proliferation of the hematopoietic stem BMC in response to injection of LPS is not dependent on the presence of the spleen.

Since regulation of the proliferative response of the bone marrow CFUs during antigenic stimulation is effected with the participation of T and B lymphocytes [2, 3], and since the spleen plays an important role in this process, it can be tentatively suggested that transfer of splenic immune cells into splenectomized animals would be accompanied by stimulation of CFUs proliferation in the bone marrow of these mice. In our investigations the number of CFUs in the S phase of the cell cycle was determined in splenectomized mice 72 h after intravenous injection of ISC into them in a dose of $80 \cdot 10^6$.

These results are evidence that the transfer of syngeneic ISC into splenectomized animals induces stimulation of proliferative activity of bone marrow CFUs after 72 h by 2.7 times compared with the control (intact spleen cells — SC — were injected; see Table 2).

To discover which spleen cells transfer the observed stimulating effect, ISC were separated into a cell fraction adherent to the plastic (enriched with macrophages) and a nonadherent fraction (containing mainly lymphocytes) [10]. The nonadherent ($60 \cdot 10^6$) and adherent ($10 \cdot 10^6$) ISC were injected intravenously into splenectomized mice. On the 4th day the proliferative activity of the CFUs of these animals was determined in the bone marrow.

The results show that the nonadherent fraction of ISC has a stimulating (by 2.6 times) effect on the process of proliferation of CFUs, whereas the adherent ISC had no such effect (Table 2).

It can be concluded from these data that spleen cells immune to SRBC have a stimulating effect on proliferation of bone marrow CFUs 72 h after transfer into splenectomized animals.

Thus the spleen and its lymphoid cells play an essential role in the realization of the stimulating effect of antigenic stimulation on proliferative activity of the hematopoietic stem BMC. This conclusion shed light on the hithero unknown phenomenon of participation of spleen cells in the process of regulation of proliferation of bone marrow CFUs during antigenic stimulation, and it provides a new approach to the problem of control of the initial stages of reactions of immunogenesis and hematopoiesis.

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