tions — from 0.86 to 12 nM. The results are shown graphically in Fig. 2 in modified Lineweaver–Burk plots. Binding of ^{3}H-HC by thymocytes in these plots was described, both in the absence and in the presence of competitors, by two straight lines: One straight line (Fig. 2a) characterizes the unsaturable component, nonspecific binding; whereas the other straight line (Fig. 2b) reflects binding of ^{3}H-HC with the saturable component of the cells, i.e., specific binding. It will be clear from Fig. 2 that the extrapolated regions of the straight lines, reflecting binding of ^{3}H-HC by the saturable system without competitors and in the presence of 100 nM HC or PVP-HC, intersect on the ordinate at the same point, in agreement with the case of competitive inhibition.

Hence it follows that specific binding sites for HC on rat thymocytes are accessible for PVP-HC which does not penetrate into the cell, which means that the binding sites for the glucocorticoid are locatd in the plasma membrane.

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MECHANISM OF STIMULATION OF ANTIBODY-FORMING ABILITY OF BONE MARROW CELLS OF MICE IMMUNIZED WITH STAPHYLOCOCCI

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The writers showed previously that splenocytes and bone marrow cells (BMC) of mice immunized with staphylococci are able to enhance the immunoreactivity of intact syngeneic recipients of these cells to homologous antigen [3, 4]. It has also been shown that the stimulating effect of immune splenocytes is due to immunologic memory cells present in the transplant [2]. There is information in the literature on the ability of memory B cells to migrate from peripheral lymphoid organs into bone marrow tissue [10, 13]. Meanwhile the regulatory role of bone marrow in immunogenesis is well known [5, 8].

The aim of this investigation was to study the mechanism of formation of the ability of BMC of mice immunized with staphylococci. The research tasks included a study of the antibody-forming ability of bone narrow of primed animals and the effect of irradiation of BMC *in vitro* on their stimulating activity, and a study of the role of the thymus and spleen in the formation of this activity.

EXPERIMENTAL METHOD

Experiments were carried out on CBA and BALB/c mice and also on mice with congenital absence of the thymus (nude). Staphylococcal corpuscular antigen (SCA) was obtained as described previously [4]. The donors of BMC were immunized intravenously with SCA in a dose of $5 \cdot 10^9$ bacterial cells. After 5 days the animals were killed by cervical dislocation, bone marrow was taken from their femora, and a cell suspension with a density of $4 \cdot 10^7$ cells/ml was prepared by phosphate-salt buffer (pH 7.2). Mice of the experimental group were given an intra-

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TABLE 1. Effect of Gamma-Irradiation (20 Gy) *in Vitro* on Immunostimulating Activity of BMC of Mice Immunized with Staphylococci (M ± m)

Version of experiment	BMC	Number of recipients	Number of AFC in recipient's spleen	P_1	P_2
1	—	6	80 067±7 965	—	—
2	Not irradiated	6	258 259±4 880	<0,001	—
3	Irradiated	6	86 697±15 959	>0,05	<0,001

Legend. P_1) Significance of differences from version 1; P_2) significance of differences from version 2.

venous injection of 0.5 ml of the cell suspension ($2 \cdot 10^7$ BMC), whereas control animals received 0.5 ml of buffer. The animals were then immunized intravenously with SCA in a dose of $5 \cdot 10^9$ bacterial cells.

CMA mice were irradiated on the ÉKU-50 (^{60}Co) apparatus in a dose of 8 Gy, with a dose rate of 0.31 Gy/min. The irradiated animals were injected with $3 \cdot 10^7$ BMC obtained from intact or immunized donors, and were immunized with SCA.

The number of AFC specific for SCA in the spleens of the mice was determined 5 days after immunization by the immunofluorescence method on stained films [1]. Serum antibody titers were estimated by the agglutination test using a Takachi microtitrator.

The splenocytes were labeled with ^{51}Cr *in vitro* [15] and injected intravenously into intact syngeneic recipients together with SCA ($5 \cdot 10^9$ bacterial cells) or without the antigen. Three days later the level of radioactivity was determined in the spleen and fermora of the animals on a scintillation gamma-counter (Nuclear Chicago, USA). The fraction of cells sedimenting in the spleen or bone marrow (f) was calculated by the formula:

$$f = \frac{A}{B} \cdot 100\%,$$

where A is the level of the label in the test organ and B the level of label injected into the animals. Total radioactivity of the bone marrow was calculated by the method described previously [9].

The numerical results were subjected to statistical analysis by Student's t test.

EXPERIMENTAL RESULTS

Irradiation of BMC of immunized animals *in vitro* in a dose of 20 Gy was shown to abolish their stimulating effect on the humoral immune response of intact syngeneic recipients to SCA (Table 1). Consequently, the immunostimulating effect of BMC of SCA-primed animals, described by the writers previously [3], is realized through radiosensitive proliferating lymphoid cells of the transplant, and not by macrophages.

Dependence of the process of formation on the immunostimulating activity of BMC from immunized animals on the T system of immunity was studied in experiments in which congenitally athymic mice were used as cell donors. Injection of BMC obtained from nude mice primed with staphylococci was shown to induce a fivefold increase in the number of AFC specific for SCA in the spleen of congeneic recipients (Fig. 1). Meanwhile transplantation of BMC of intact nude mice did not affect the level of the immune response of the recipients of these cells to SCA. Thus the stimulating activityof BMC of animals immunized with staphylococci is not due to T lymphocytes, and the process of its formation is independent of the presence of a thymus in the BMC donors.

According to data in the literature, the bone marrow of immunized animals may contain memory B cells [10]. To detect memory cells specific for SCA in bone marrow tissue a comparative study was made of the antibody-forming ability of BCM of immunized and intact animals in an adoptive transfer system. BMC of immunized mice were found to restore the ability of lethally irradiated syngeneic recipients to generate an immune response to SCA much more effectively than BMC of intact animals (Table 2). Similar results also have been obtained by other investigators studying other antigens [14]. Further evidence of the presence of memory cells in bone marrow tissue is given by data showing that sheep's red blood cells of primed mice preserve their immunologic memory after whole-body irradiation with shielding of the limb [6].

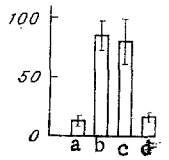

Fig. 1

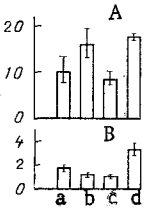

Fig. 2

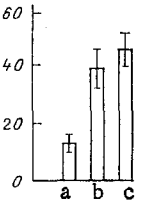

Fig. 3

Fig. 1. Effect of transplantation of splenocytes and BMC of immunized nude mice on number of AFC (M ± m) specific for SCA in recepients' spleen. Ordinate, number of AFC ($\times 10^3$): a) SCA (control); b) SCA + splenocytes of immunized mice; c) SCA + BMC of immunized mice; d) SCA + BMC of intact mice.

Fig. 2. Effect of SCA and previous immunization of cell donors on accumulation of intravenously injected ^{51}Cr-splenocytes in spleen (A) and bone marrow (B) of intact syngeneic recipients. Ordinate, number of ^{51}Cr-splenocytes (in %): a) intact splenocytes; b) intact splenocytes + SCA; c) splenocytes of immunized mice; d) splenocytes of immunized mice + SCA.

Fig. 3. Effect of splenectomy on formation of immunistimulating activity of BMC of mice primed with staphylococci. Ordinate, number of AFC ($\times 10^3$) specific for SCA in recipients' spleen (M ± m): a) SCA (control; b) SCA + BMC of splenectomized donors; c) SCA + BMC of donors undergoing mock operation.

The results, together with the independence of formation of the stimulating activity of BMC of immunized mice of the T system, which was found, are in agreement with data in the literature indicating that immunologic memory can be formed in the absence of T cells [12]. Thus bone marrow of mice immunized with SCA evidently contains memory cells capable of realizing their functions on repeated antigenic stimulation under adoptive transfer conditions.

According to some authorities, the ability of bone marrow of immunized animals to give an anamnestic response is associated with its colonization by memory cells which have migrated from peripheral lymphoid organs [13]. It must be pointed out, however, that the possibility of priming of B cells with antigen actually within bone-marrow tissue is still a matter for debate. We know, for example, that when animals are immunized intravenously virtually no antigen material reaches the bone marrow [8]. Our investigations of the pathways of migration of ^{51}Cr-labeled splenocytes obtained from intact mice and mice immunized with staphylococci showed that the number of cells entering the spleen of intact syngeneic recipients increases if splenocytes are injected together with homologous antigen (Fig. 2). These results are in agreement with data in the literature indicating that memory cells migrate mainly to sites where the antigen is located [7, 11]. We also found that splenocytes of immunized animals, by contrast with unimmunized splenocytes, if injected together with SCA into intact syngeneic recipients, accumulate in the bone narrow of the latter (Fig. 2), which suggests that the spleen may participate in repopulation of bone-marrow tissue by immunologic memory cells in the intact organism.

The role of the spleen in the formation of the stimulating activity of BMC from mice immunized with SCA was investigated in experiments in which the donors were splenectomized before immunization. It was shown that BMC of immunized splenectomized mice enhanced the immune response of the recipients to SCA to the same degree as BMC from immunized donors undergoing mock splenectomy (Fig. 3). Consequently, migration of primed B lymphocytes from the spleen is not essential for the bone marrow of immunized mice to acquire stimulating activity.

It can thus be concluded from these results that the mechanism of realization of the ability of BMC from donors immunized with SCA to enhance antibody formation is evidently analogous to the mechanism of potentiation of the immune response by transplantation of splenocytes of immunized mice into intact animals, namely by the development of an anamnestic response to staphylococci by transplanted memory B cells, specifically stimulated *in vivo* in the recipients.

TABLE 2. Immune Response of Irradiated Recipients, Restored with BMC of Intact and Immunized Donors, to Staphylococci

Donors of BMC	Parameters of immune response		
	number of AFC in spleen	number of AFC per 10^6 splenocytes	$-\log_2$ of antibody titer
Intact	$\frac{186}{\times}$ 1,32 (7)	$\frac{2,0}{\times}$ 1,35	Not found
Immunized	$\frac{4519}{\times}$ 1,29 (8)	$\frac{49,5}{\times}$ 1,29	4,75±0,32
P	<0,001	<0,001	—

Legend. Results given as geometric means. Number of mice shown in parentheses.

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