IMMUNOLOGY AND MICROBIOLOGY

Effect of Serum from Mice Immunized with Group A Streptococcus Antigens on the Efficiency of *In Vitro* Cloning of Stromal Precursor Cells

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Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 146, No. 12, pp. 663-666, December, 2008
Original article submitted April 9, 2008

The efficiency of cloning of stromal precursor cell in mouse bone marrow culture increases significantly (2-3-fold) in the presence of serum from mice immunized with type 5 group A streptococcus antigens (5-20 μl serum/ml culture medium) in comparison with intact animal serum. The levels of TNF-α and IFN-γ are significantly reduced (2.7 times and more than 6-fold, respectively) in the sera of immunized mice in comparison with normal serum. Serum levels of IL-2, -4, -5, -10, and -12 were about the same in both groups; no granulocyte-macrophage CSF was detected. These data attest to appreciable effect of immunization with streptococcal antigens on the bone marrow stromal tissue; this effect is presumably mediated through serum cytokines.

Key Words: *bone marrow stromal cells; immune response; streptococcus antigens; cytokines*

Immunization of animals with antigens leads to a drastic increase in the counts of stromal precursor cells (CFU-F) in the spleen and lymph nodes [2]. We previously showed that the efficiency of cloning (CFE-F) and content of CFU-F in the bone marrow of mice immunized with killed type 5 group A Streptococcus vaccine also increased 2-4-fold in comparison with normal (intact) mouse bone marrow [3]. It is also known that the immune process is associated with changes in the blood cytokine spectrum and content. According to published data, some of these cytokines can appreciably modify the growth and proliferation of bone marrow stromal precursor cells and their cultured descendants (fibroblasts from strains after several passages in culture) *in vitro*. It was shown, for example, that IL-1 suppressed and TNF-α had a biphasic effect on *in vitro* growth of stromal cells. Low TNF-α doses slightly (50%) stimulated the increase in the content of CFU-F and their cultured descendants, while higher doses of IL-3 and TNF-α completely suppressed the growth of stromal cells in cultures [1].

We studied possible effect of serum from mice immunized with streptococcal antigen on the growth of bone marrow CFU-F in cultures; evaluated the levels of cytokines TNF-α, IFN-γ, IL-2, -4, -5, -10, -12, and granulocyte-macrophage CSF (GM-CSF) in the sera of mice immunized with streptococcal antigens.

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MATERIALS AND METHODS

Experiments were carried out on 2-3-month-old CBA mice and 4-5-month-old guinea pigs from Kryukovo Breeding Center. The mice were immunized with killed type 5 group A Streptococcus vaccine [3] over 3 weeks with ascending doses of the vaccine. The vaccine was injected during 3 weeks intraperitoneally: 1 billion bacterial cells per injection during week 1, 2 billion cells during week 2, and 3 billion cells during week 3. The presence of antibodies to streptococcal antigens in the sera of immune animals was confirmed by the gel precipitation test with hydrochloric acid extract of group A Streptococcus.

The blood was collected on day 8 after the end of immunization. The sera from normal (NS) and immune (IS) mice were collected by the standard method, divided into aliquots, and frozen at -20°C. Directly before use, the serum was defrosted and filtered through HA millipore filter. Filtered NS and IS were added into some intact bone marrow cultures 10 min after cell explantation (5-20 μl/ml culture medium). Mouse and guinea pig bone marrow suspensions were prepared with a syringe as described previously [5]. Mouse bone marrow cells ($1\text{-}7.5\times10^6$) were explanted into 25-cm^2 flasks in 5 ml α-MEM with 20% FCS (Paneco) and antibiotics (penicillin and streptomycin, 100 μg/ml each). Guinea pig bone marrow cells (10^7) irradiated in a dose of 60 Gy (^{60}Co, 10 Gy/min) were added some cultures as a feeder. The cultures were incubated for 10-12 days in a CO_2 incubator at 37°C, fixed with ethanol, stained with azur and eosin, and the colonies containing at least 50 fibroblasts were counted. CFE-F (number of colonies formed by 10^5 explanted cells) was evaluated.

Cytokines (TNF-α, IFN-γ, IL-2, -4, -5, -10, -12, and GM-CSF) were measured using a Bio Plex device with a set of appropriate reagents for mice (Bio Rad). The results were processed using Bio Plex Manager Software.

RESULTS

Addition of normal and immune serum to bone marrow cell culture led to significant changes in CFE-F: CFE-F in cultures containing IS was 2-3-fold higher than in cultures containing NS irrespective of the presence of irradiated feeder in the cultures (Table 1). Addition of sera in different doses (5, 15, and 20 μl/ml culture medium) induced similar relative increase in CFE-F in cultures with IS and NS: IS CFE-F:NS CFE-F remained virtually constant (2.4, 1.9, and 3, respectively). Interestingly, that in cultures with the feeder, CFE-F sharply increased after addition of NS and IS in comparison with cultures containing only FCS, CFE-F in cultures with IS remaining 2-2.5 times higher than in cultures with NS (Table 1). CFE-F in control cultures (without IS or NS) was low [3]. These results are in line with our previous data indicating that addition of serum from adult animal to cultures containing FCS can increase CFE-F because the effects inhibiting the growth of CFU-F are blocked by nonstromal cells present in the culture [4]. It was previously shown that bone marrow cell populations contained cells stimulating and inhibiting CFU-F proliferation and CFU-F of different animals are characterized by different sensitivity to the growth-inhibitory factors [4]. Platelets and megakaryocytes are responsible for the colony-stimulating effect of the bone marrow feeder on CFU-F [5]. Cells inhibiting the growth of CFU-F in the presence of FCS carry macrophage marker F4-80; T cells seem to be not involved in this process [4]. The inhibitory effect is not abolished by irradiation of cells in a dose of 60 Gy and is largely reduced if the culture medium apart from FCS contains a serum autologous

TABLE 1. CFE-F in Mouse Bone Marrow Cultures in the Presence of Serum from Mice Immunized with Streptococcus Antigens and from Intact Mice

Serum	Volume of initial serum per culture, μl	Presence of irradiated feeder, 10^7 cells/culture	Number of explanted cells per culture, $\times10^6$	CFE-F, per 10^{-5} explanted cells
—	—	—	7.5	0.19±0.03
NS	100	—	7.5	0.07±0.02
IS	100	—	7.5	0.20±0.03
—	—	+	1	0.4±0.1
NS	25	+	1	1.0±0.2
IS	25	+	1	2.4±0.8
NS	75	+	1	3.6±1.4
IS	75	+	1	6.8±1.8

TABLE 2. Cytokine Levels in the Sera of Intact Mice and Mice Immunized with Killed Group A Streptococcus Vaccine (pg/ml; *M*±*m*)

Cytokine	Experiment 1		Experiment 2	
	NS	IS	NS	IS
IL-2	1.28±0.04	0.86±0.04	1.73±0.10	1.40±0.08
IL-4	0.28±0.01	0.27±0.01	0.43±0.06	0.46±0.03
IL-5	2.44±0.29	2.56±0.31	3.35±0.06	3.75±0.65
TNF-α	15.11±1.17	4.27±1.07	20.98±0.39	11.92±1.86
IL-12	7.46±1.25	4.76±0.69	16.45±2.12	28.02±9.97
GM-CSF	N.d.	N.d.	N.d.	N.d.
IL-10	2.38±0.17	1.97±0.27	5.05±0.26	3.42±0.95
IFN-γ	5.28±0.53	N.d.	8.18±0.19	1.32±0.58

Note. N.d.: not determined. Two independent experiments were made.

for the cultured cells or adult animal serum [4]. It seems that the presence of adult mouse serum (both NS and IS) is responsible for CFE-F increase in bone marrow cultures with the feeder. However, CFE-F was 2-2.5 times higher in cultures with IS than in those with NS (Table 1).

Since immunization was associated with changes in the cytokine spectrum and content in the blood, we compared the cytokine profiles of IS and NS. The results of measurements are presented in Table 2. The levels of TNF-α and IFN-γ were reduced significantly (2.7 times and more than 6-fold, respectively) in the sera of mice receiving repeated immunization with streptococcal antigens in comparison with intact mouse serum. Comparison of IL-2, -4, -5, -10, and -12 levels in the sera of the two groups showed about the same levels, while GM-CSF was not detected. Hence, the cause of the stimulatory effect of low concentrations of immune serum (from 0.5 to 2%) on CFU-F growth in cultures is not clear. It is known that the bone marrow CFU-F population contains osteogenic precursor cells. Retransplantation of bone marrow fragments and cultured bone marrow CFU-F descendants (stromal fibroblasts after passages in culture) into the body leads to the development of a bone marrow organ populated with hemopoietic cells [5]. It was reported that TNF-α and IL-1 not only absorbed available bone tissue, but inhibited the formation of new bone tissue [6]. Our previous data, according to which the increase of TNF-α and IL-1 concentrations in culture medium leads to inhibition of CFU-F growth and even completely suppresses it, suggests that reduced TNF-α concentration in immune serum leads to CFE-F increase due to blockade of the inhibitory effect of this cytokine on the stromal tissue. In this case, our results can be explained not by the presence of factors stimulating the growth of CFU-F in the immune serum, but reduced inhibition of the growth of these cells by cytokines. However, it is also possible that the immune serum contains some heretofore unknown factors stimulating the proliferation of CFU-F.

On the whole, our findings attest to significant effect of immunization with streptococcal antigens on the bone marrow stromal tissue, this effect being presumably mediated through serum cytokines.

The authors are grateful to N. A. Borodiyuk, Doct. Med. Sci. (N. F. Gamaleya Institute), for vaccine used in the study.

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