Effect of α-Fetoprotein on the Count of Bone Marrow and Splenic Stromal Precursor Cells and Proliferation of Their Cultural Descendants

Yu. F. Gorskaya, O. V. Lebedinskaya, V. G. Nesterenko, R. Kh. Chailakhyan*, Yu. V. Gerasimov*, N. V. Latsinik*, A. I. Kuralesova*, and E. N. Genkina***

Translated from *Kletochnye Tehnologii v Biologii i Medicine*, No. 1, pp. 30-33, January, 2007
Original article submitted November 14, 2006

The efficiency of cloning of stromal precursor cell increased more than 2-fold in splenic cultures and more than 3-fold in bone marrow cultures 24 h after injection of Profetal preparation to mice *in vivo*. The number of nucleated cells did not change in the bone marrow and slightly increased in the spleen. Addition of Profetal *in vitro* 2-fold decreased the efficiency of stromal precursor cells colony formation in mouse splenic cultures and dose-dependently decreased this process in bone marrow cultures derived from these animals, the maximum (5-fold) inhibitory effect was observed in a dose of 50 μg/ml. Addition of Profetal to cultured human bone marrow fibroblasts did not change the content of stromal fibroblasts in cultures. These data indicate the possibility of indirect effect of α-fetoprotein on the number of stromal precursor cell in hemopoietic and lymphoid organs.

Key Words: *bone marrow and splenic stromal precursor cells; bone marrow fibroblasts; α-fetoprotein; Profetal*

The search for bioactive substances stimulating and inhibiting the immune reactions is now in progress. Serum α-fetoprotein (α-FP) capable of specific immunoregulation is one of natural substances of this class [1]. Previous studies demonstrated its high regulatory potential and wide spectrum of biological activities. α-FP participates in the complex regulation of cell proliferation processes, triggering of apoptosis mechanisms, induction of the regulatory signals through stimulation of receptor expression, provision of prostaglandin, thromboxane, and leukotriene synthesis, in interactions with the extracellular matrix structures, and in the realization of immunomodulating effects [14]. The effect of this protein on specific immunity was experimentally proven. The results of studies indicate that it possesses immunosuppressive and immunostimulating activities [17]. α-FP suppresses production of antibodies and maturation of cytotoxic T-lymphocytes, reduces the proliferative response of lymphocytes to mitogens, increases activity of specific T-suppressors, reduces phagocytic activity of macrophages, inhibits production of TNF-α and IL-1 by activated macrophages and activity of natural killers, and regulates synthesis of prostaglandins 2 [14,15, 17]. α-FP blocks the development of autoimmune processes, exhibits radioprotective effects, suppresses proliferative activity of tumor cells via inhibition of RNA, DNA, and protein synthesis and at the expense of activation of the cellular component of the immune system antitumor defense

Laboratory of Cellular Immunity, *Laboratory of Stromal Regulation of Immunity, N. F. Gamaleya Institute of Epidemiology and Microbiology, Russian Academy of Medical Sciences, Moscow; **Department of Histology, Perm State Medical Academy, Perm

0007-4888/07/1431-0140

[8,14,16]. α-FP directly modulates activity of immunocompetent cells, its effect being directed to proliferating cells, but not their mature forms [14].

We previously showed that human α-FP, as a component of Profetal preparation, in some doses stimulated cytotoxic activity of human peripheral blood mononuclear leukocytes (ML) towards K562 tumor cells and served as a mitogenic factor for donor blood ML, inducing blast transformation reaction in cultures comparable to that induced by IL-2 and phytohemagglutinin [7,12,13]. The stimulatory effect of α-FP on the growth, differentiation, and proliferation of fetal fibroblasts was demonstrated not once [14,16]. Treatment with this protein was associated with signs of stimulation of functional activity of immunocompetent organs (thymus, lymph nodes, macrophage system of the liver) [8,14].

High functional activity of α-FP *in vivo* prompted us to investigate possible effects of this protein on the colony-forming stromal precursor cells (CFC-F) of hemopoietic and lymphoid organs providing specific microenvironment for immunocompetent and hemopoietic cells [9,10]. The data on the immunomodulatory effects of α-FP seemed to be the most interesting, because lymphocyte activation by antigen injection significantly modified the count of CFC-F in the spleen and bone marrow [2-6].

We studied the effect of α-FP on the counts of mouse bone marrow and splenic of CFC-F and on the proliferation of cultured human bone marrow fibroblasts.

MATERIALS AND METHODS

Experiments were carried out on 2-3-month-old male CBA mice and 4-5-month-old male guinea pigs from Kryukovo Breeding Center. Some mice were intraperitoneally injected with Profetal (25 μg/animal in 0.5 ml saline) before the experiment. The main component of this drug is lyophilized and dextrane-stabilized human α-FP. Cell suspensions of the spleen and bone marrow (from the femur) were prepared as described previously [10] and explanted into 25-cm^2 flasks in 5 ml αMEM (Sigma) containing 15% FCS (Paneco) and antibiotics (100 μg/ml penicillin and 100 μg/ml streptomycin). A total of 1-3×10^6 bone marrow cells or 1-2×10^7 splenic cells were inoculated into each flask. Bone marrow cells (10^7) of guinea pigs irradiated in a dose of 60 Gy (^{60}Co, 10 Gy/min) served as the feeder for these cultures. Profetal in doses of 2, 10, or 50 μg/ml medium was added to some cultures after 1 h. After 9-11 days the cultures were fixed in ethanol, stained in Azur-eosin, and colonies containing ≥50 fibroblasts were counted. The efficiency of cloning (CFE-F; number of colonies formed per 10^6 explanted cells) was determined by the number of colonies. In order to obtain diploid strains of human bone marrow fibroblasts, 5×10^5 human bone marrow cells were explanted into 80-cm^2 flasks, after 10-12 days the cells were treated with 0.25% trypsin, washed, and inoculated into new larger flasks. At passage 5, the cells (10^4) were transferred into 25-cm^2 flasks. After 1 h Profetal (2, 10, or 50 μg ml) was added into some cultures. The cultures were fixed after 5 days, fibroblasts were counted in 15 visual fields (0.38 cm^2 area) situated diagonally, and the resultant cell number was converted to cell count per flask. All cultures were incubated at 5% CO_2.

RESULTS

The CFE-F in mouse bone marrow and splenic cultures increased 24 h after *in vivo* injection of Profetal (Table 1). The number of nucleated cells did not change in the bone marrow and somewhat increased in the spleen. Hence, the count of CFC-F increased 3-fold in the spleen and bone marrow of mice 24 h after *in vivo* injection of Profetal. *In vitro* treatment with Profetal 2-fold reduced cloning efficiency in mouse splenic cultures and dose-dependently decreased this parameter in bone marrow cultures, the maximum inhibitory effect (more than 5-fold drop of CFE-F) was observed after the dose of 50 μg/ml. We previously showed that dextrane, a component of Profetal, had no effect of this kind on stromal tissue, hence, the effect of Profetal was presumably due to α-FP. Addition of Profetal into cultures of human bone marrow fibroblast after several passages did not lead to changes in the stromal fibroblast count in the cultures. The number of fibroblasts in the culture was 6.5±0.1×10^4 after addition of 2 μg/ml Profetal, 6.7±0.3×10^4 after 10 μg/ml, and 6.2±0.2×10^4 after addition of 50 μg/ml Profetal. Before addition of Profetal, fibroblast count was 7.4±0.4×10^4.

According to some data, the effect of human α-FP is not species-specific. However, we can hypothesize that the increase in CFC-F count in mouse spleen and bone marrow was due to reaction to human α-FP as a foreign protein. It is known that *in vivo* injection of antigens causes a drastic elevation of CFC-F content in hemopoietic and lymphoid organs. For example, injection of *S. typhimurium* to mice *in vivo* causes a 10-fold increase in the content of CFC-F in the spleen as early as after 24 h; their content in the lymph nodes of guinea pigs increases more than 30-fold 24 h after injection of diphtheria toxoid, and immunization of mice with

TABLE 1. CFE-E in Mouse Splenic and Bone Marrow Cultures 24 h after Injection of α-FP (Profetal)

Organ		Number of nucleated cells/organ, $\times 10^7$	CFE-F per 10^6 explanted cells	Number of CFC-F/organ
Spleen	without α-FP	11.6±0.4	0.3±0.1	37±10
	after α-FP injection	14.4±0.6	0.7±0.2	101±24
Bone marrow	without α-FP	1.4±0.2	10.0±0.1	135±15
	after α-FP injection	1.3±0.2	35.0±4.1	438±53

TABLE 2. CFE-F in Mouse Splenic and Bone Marrow Cultures after *In Vitro* Treatment with α-FP as a Component of Profetal

Organ	α-FP dose, μg/ml	CFE-F/10^6 explanted cells
Spleen	—	0.4±0.1
	2	0.3±0.1
	10	0.2±0.0
	50	0.2±0.0
Bone marrow	—	5.7±1.7
	2	4.0±1.0
	10	1.5±0.3
	50	1.0±0.2

group A (type 5) streptococcus vaccine significantly increased (by 3.5 times) the content of CFC-F in the bone marrow [2-6]. However, this reaction of CFC-F pool to injection of α-FP can be due to the immunostimulating effect of this protein on the lymphoid tissue [7,12-14]. The decrease in mouse splenic and bone marrow CFE-F after addition of α-FP directly into culture medium supports this hypothesis (Table 2). At least two phenomena of stromal cell inhibition in cultures are described by the present time: one of them is observed after addition of mitogens or antibodies *in vitro* into splenic cell cultures or after addition of the corresponding conditioned media into splenic and bone marrow cultures (presumably, due to the effect of activated lymphocytes) [2,5,6]. These data are in line with the results obtained in studies of Profetal effect on cultured human peripheral blood ML. Profetal in concentrations of 0.01-10.00 μg/ml activated ML by inducing lymphoid cell differentiation into cytotoxic lymphocytes and natural killer cells, increasing their activity and proliferative potential [7,12, 13]. The other phenomenon of stromal cell inhibition *in vitro* is observed in rat and rabbit bone marrow and splenic cell cultures and seems to be due to macrophage activation in the presence of FCS [5,6]. Presumably, reduction of CFE-F in bone marrow and splenic cultures after addition of α-FP *in vitro* is due to activation of a certain category of non-stromal cells (lymphocytes or macrophages), present in the cultures by this fetal serum protein. It was previously shown that the number of cells expressing CD14 macrophagic markers in donor peripheral blood ML cultures incubated with Profetal reached 45% [13].

Hence, the decrease in CFE-F in bone marrow and splenic cultures after addition of α-FP *in vitro* seems to be due to activation of lymphocytes and/or macrophages by this protein. The fact that addition of α-FP into cultured human bone marrow fibroblasts after several passages did not change the content of stromal fibroblasts in cultures also confirmed this hypothesis.

On the whole, our data attest to the possibility of indirect effect of α-FP on the stromal cell population of hemopoietic and lymphoid organs.

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