Stimulating Effects of a Polypeptide Agent (Kuban Stimulant) on Hemopoiesis and the Formation of Humoral Immunity to Thymus-Dependent Murine Antigen

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The polypeptide agent Kuban stimulant has a dose-dependent stimulating effect on hemopoiesis regeneration in sublethally irradiated mice and on the proliferation of hemopoietic stem cells in the bone marrow of mice; it also stimulates T cells, improving the colony-forming function of colony-forming units in the spleen. Kuban stimulant exhibits a pronounced dose-dependent adjuvant property which manifests itself in the stimulation of the generation of antibody-producing cells in the spleen of mice in response to thymus-dependent antigen: sheep red cells.

Key Words: *colony-forming units of the spleen; antibody-forming cells; Kuban stimulant*

Kuban stimulant (KS) is a bioactive agent derived from koumiss. It represents a mixture of polypeptides with molecular weights of 500,000 to 4,000,000 D and is characterized by pronounced immunostimulating activity [1,2].

This study examines the effects of KS on the content of colony-forming units in the spleen (CFUs), blood, and hemopoietic organs of mice, on the proliferative function of CFUs, the functional activity of thymocytes, and the formation of humoral immunity to thymus-dependent antigen.

MATERIALS AND METHODS

Male CBA mice and (CBA×C57Bl/6) F_1 hybrids weighing 18 to 20 g from the *Stolbovaya* Breeding Center, Russian Academy of Medical Sciences, were used.

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KS prepared as described previously [2] was dissolved in normal saline and injected to mice intraperitoneally in doses of 0.2, 1, and 1.5 μg per mouse. The content of KS was measured routinely by endo- and exocolonization of the spleen [4]. Mice were sacrificed by decervication 9 days after exposure and transplantation of hemopoietic cells, the spleens were fixed in Bouin's solution, and macroscopically visible colonies were counted. In the control without transplantation there were 0 to 0.5 colonies per spleen. The content of CFUs in the synthetic phase (S-phase) of the cell cycle was determined by incubating murine bone marrow cells in RPMI-1640 medium with 1% HEPES, and 2% fetal calf serum with (or without) hydroxyurea (Serva) in the final concentration of 1 mg/ml (1 h at 37°C), after which the CFUs content in the suspension was assessed in the exotest [4]. The magnitude of cellular thymidine-dependent suicide, reflecting the number of CFUs in the S-phase of the cell cycle (A), was calculated according to the formula: $A=(a-b/a)\times100\%$, where a is the number of

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TABLE 1. Effect of KS on Endogenous Colony Formation in the Spleen of Mice Injected One Day before Irradiation in a Dose of 6.0 Gy

Group	Day			
	5		9	
	colonies per spleen	number of mice	colonies per spleen	number of mice
Control	4.34±1.07	29	3.23±0.86	17
0.2 μg	13.04±1.99*	26	9.47±1.64*	17
1 μg	4.1±1.25	10	5.0±0.87	17
5 μg	-	-	4.7±1.3	6

Note. *$p<0.01$ in comparison with the control.

colonies formed by the cells incubated without hydroxyurea and *b* of those incubated with hydroxyurea.

To assess the effect of KS on the functional activity of thymocytes vis-a-vis hemopoietic cells, syngeneic bone marrow cells together with thymocytes of syngeneic donors preinjected (1, 3, and 7 days before irradiation of recipients) intraperitoneally with KS in a dose of 0.2 μg per animal were injected to recipient mice irradiated with 9.2 Gy.

The effect of KS on the generation of antibody-producing cells (APC) in mouse spleen was assessed as follows: KS was injected in doses of 0.2, 5, and 25 μg/mouse in 0.2 ml normal saline intraperitoneally one day before, simultaneously with, and one day after immunization. The animals were immunized intraperitoneally with a 5% suspension of sheep red cells in 0.5 ml normal saline. The immune response was assessed on day 5 after immunization by estimating the number of direct (IgM) APC in the spleen as described elsewhere [3].

RESULTS

The data indicate that injection of KS in doses of 0.2, 1, and 5 μg/mouse one day before exposure to

TABLE 2. Time Course of Proliferation of Mouse Bone Marrow CFUs after Injection of KS in Doses of 0.2, 1, and 5 μg

Experimental conditions	Number of mice	Number of colonies	Content of CFUs in S-phase, %
One day postirradiation			
Control	8	8.0±1.41	
Control+HU	10	8.3±1.3	0
KS, 0.2 μg	9	10.8±2.1**	33.8
KS, 0.2 μg+HU	10	7.2±1.3	
KS, 1 μg	4	25.75±1.8*,**	51.07
KS, 1 μg+HU	5	12.6±3.38	
KS, 5 μg	5	25.2±2.6*,**	45.2
KS, 5 μg+HU	5	13.8±4.08	
Three days postirradiation			
Control	10	5.1±1.4	-
Control+HU	10	4.7±1.16	7.8
KS, 0.2 μg	10	8.8±1.15*,**	47
KS, 0.2 μg+HU	9	4.7±1.06	
KS, 1 μg	4	17.75±2.59*	8.47
KS, 1 μg+HU	11	16.36±1.84	
KS, 5 μg	12	13.45±2.15*	0
KS, 5 μg+HU	15	14.26±1.02	
Seven days postirradiation			
Control	7	6.57±0.97	5.6
Control+HU	6	6.2±0.95	
KS, 0.2 μg	6	6.5±0.72	0
KS, 0.2 μg+HU	9	7.8±1.42	
KS, 1 μg	5	25.6±2.16	4.69
KS, 1 μg+HU	10	24.4±1.91	
KS, 5 μg	9	19.44±2.68*	5.14
KS, 5 μg+HU	9	18.44±2.62	

Note. HU: hydroxyurea. $p<0.05$: *in comparison with the control, **in comparison with HU groups.

TABLE 3. Effect of KS on the Number of CFUs in the Peripheral Blood

Experimental conditions	Day					
	1		3		7	
	CFUs per ml blood	number of mice	CFUs per ml blood	number of mice	CFUs per ml blood	number of mice
Control: normal saline	42.8±9.4	7	35.7±18.0	7	80.0±14.1	9
KS, μg:						
0.2	60.0±17.8	4	162.7±26.0*	11	264.0±6.0*	5
1.0	15.0±2.2*	6	121.2±23.6*	8	88.5±7.7	7
5.0	76.2±12.7	8	102.2±15.0*	9	118.0±25.0	5

Note. Exposure control <0.5 CFUs/ml blood; *$p \leq 0.02$ in comparison with the control.

a sublethal dose of radiation has a dose-dependent stimulating effect on the formation of endogenous hemopoietic foci (Table 1) forming 5 days (transitory colonies) and 9 days after exposure. Injection of KS in a dose of 0.2 μg resulted in a threefold increase in the number of colonies forming on day 5 postirradiation. The dose of 1 μg did not influence this parameter.

On day 9 the maximal number of endogenous colonies was observed after injection of KS in the same dose, 0.2 μg (threefold increase of the baseline level). KS doses of 1 and 5 μg did not appreciably increase the number of endogenous colonies.

In order to elucidate the effect of KS on the level of CFUs proliferation in the bone marrow, KS was injected in doses of 0.2, 1, and 5 μg per animal 1, 3, and 7 days before irradiation (8.3 Gy).

Analysis of the results (Table 2) shows that injection of KS in the above doses one day before exposure results in an increase of CFUs content in the bone marrow. A reliable increase of this parameter is observed after injection of 1 and 5 μg of KS. The maximal number of CFUs in the DNA synthesis phase is observed after injection of KS in a dose of 1 μg.

Three days after injection of KS all three doses of the agent (0.2, 1, and 5 μg) promoted a reliable increase of the content of CFUs in the bone marrow. The level of CFUs in the S-phase appreciably increased after injection of 0.2 μg of KS (47% inhibition). The doses of 1 and 5 μg did not influence this parameter.

A reliable increase of the number of CFUs in the bone marrow was observed 7 days after injection of 1 and 5 μg of KS.

An increase of the number of circulating CFUs in the peripheral blood (Table 3) in response to KS was observed after 3 and 7 days. The maximal increase in the count of circulating CFUs was caused by the agent in a dose of 0.2 μg, the peak (surpassing the control value 4.5 times) being observed after 3 days. KS in doses of 1 and 5 μg had a less pronounced stimulating effect during this period of the investigation, and after 7 days the stimulating effect of these doses was null. Moreover, KS did not stimulate the content of circulating CFUs in the peripheral blood 1 day postinjection.

These data permit us to conclude that KS has a marked dose-dependent effect on the functional activity of bone marrow hemopoietic stem cells, the dose of 0.2 μg being the most effective.

In order to assess the effect of KS on the functional activity of T cells capable of modifying hemopoiesis at various times (1, 3, and 7 days) after injection of KS, thymic lymphocytes of mice were isolated and their effects on the formation of exogenous colonies in the spleen of lethally (8.3 Gy) irradiated syngeneic recipients were studied (Table 4).

TABLE 4. Formation of Exogenous Colonies in the Spleens of Recipient Mice on Day 9 after Injection of Syngeneic Intact Bone Marrow Cells with Thymocytes of Donor Syngeneic Mice Injected KS

Experimental conditions	Day after KS injection to thymocyte donors					
	1		3		7	
	number of colonies	number of mice	number of colonies	number of mice	number of colonies	number of mice
Control	8.0±1.41	8	5.1±1.4	10	7.5±1.24	8
Injection of thymocytes (5×10^5):						
Control	9.81±0.96	11	5.9±1.1	11	5.12±1.16	8
KS	12.43±1.13*	7	9.47±0.88*,**	17	3.1±0.7*	7

Note. $p<0.05$: *in comparison with the control, **in comparison with control injection of thymocytes.

TABLE 5. Effect of KS on APC Generation in Spleens of (CBA×C57Bl/6) F_1 Hybrids

Group	KS injected					
	1 day before immunization	stimulation index	simultaneously with immunization	stimulation index	1 day after immunization	stimulation index
Control			148.4±15.03			
KS, μg:						
0.2	277.2±38.8*	1.87	128.7±24.2	0.87	129.6±26.2	0.87
5	223.3±22.2*	1.5	210.4±16.2	1.4	-	-
25	346.7±36.8*	2.3	178.8±30.28	1.2	250.4±39.8	1.68*

Note. *$p<0.05$ in comparison with the control.

The results indicate that injection of KS in a dose of 0.2 μg 1 and 3 days before irradiation statistically reliably stimulates the T cells increasing the colony-forming function of CFUs. When injected 7 days before exposure, KS did not influence this parameter.

For a study of the effect of KS on the generation of APC in the spleen of hybrid mice (CBA× ×C57Bl/6) F_1, KS in doses of 0.2, 5, and 25 μg was injected one day before, simultaneously with, and one day after immunization (Table 5).

KS in doses of 0.2, 5, and 25 μg reliably stimulated the formation of IgM antibodies when injected one day before immunization. The maximal count of APC was observed after injection of 25 μg per mouse, the stimulation index being 2.3. The number of APC was somewhat lower after injection of KS in a dose of 0.2 μg (stimulation index 1.87), and after 5 μg of KS the stimulation of antibody production was the lowest. Injection of KS in doses of 5 and 25 μg simultaneously with immunization gave rise to a tendency for the number of APC to increase, whereas the dose of 0.2 μg did not have any effect on the number of APC in mouse spleen. Injected in a dose of 25 μg one day after immunization, KS reliably stimulated the generation of APC. Lower doses of the agent did not influence this parameter.

Hence, KS exerts a dose-dependent stimulating effect on the regeneration of hemopoiesis in sublethally irradiated mice and on the proliferation of CFUs in their bone marrow, stimulates T-helper function towards the proliferation of syngeneic CFUs, and increases the generation of APC in mouse spleen in response to thymus-dependent antigen (sheep red cells).

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