ROLE OF BONE MARROW GLYCOSAMINOGLYCANS IN RESPONSE OF HEMATOPOIETIC TISSUE TO EXTREMAL INFLUENCES

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In recent years an important role in the regulation of hematopoiesis has been ascribed to the hemopoiesis-inducing microenvironment (HIM). However, its study is hindered by the lack of any methods capable of changing its qualitative composition in the required direction and of reliable criteria for its assessment when exposed to different factors. The biochemical components of HIM remain virtually unstudied.

This paper describes an attempt to act upon HIM by various extremal influences (hyoxia, hyperbaric oxygenation, irradiation, inflammation, cooling, blood loss). As marker of qualitative changes in the composition of HIM we used determination of glycosaminoglycans (GAG). These compounds, as components of HIM, occupy a special place in the regulation of hematopoiesis, for they are components of the glycocalyx, which controls many processes of cellular activity, including proliferative activity. Their content in hematopoietic tissue varies considerably under the influence of hematopoiesis-disturbing influences. GAG affect mitotic activity of early erythroid and granulocytic precursors [3, 5].

EXPERIMENTAL METHOD

Experiments were carried out on male CBA mice weighing 20-22 g and male Wistar rats weighing 140-170 g. Hypoxic conditions were created in a pressure chamber by reducing the air pressure to 40.98 kPa in the course of 6 h; hyperbaric oxygenation was carried out in a BKI-192 pressure chamber by supplying oxygen under a pressure of 303.9 kPa (3 atm) for 1 h. Inflammation was produced in the mice by a single subcutaneous injection of turpentine in the dorsal region [2]. Mice were irradiated on the IGUR-1 apparatus with cesium-137 gamma-ray source, with dose rate of 0.516 A/kg. To study the action of cold the rats were kept in a VT-1000 temperature chamber (East Germany) at 0°C for 23 h daily, with an interval of 1 h for care and feeding. Acute blood loss in rats was produced by bleeding from the jugular vein at the rate of 2% of body weight. The GAG concentration in the bone marrow was determined at intervals after the end of exposure to the extremal factor: acid (uronic acids) by the method in [4], neutral as in [6]. The state of hematopoiesis was assessed by the reticulocyte level in the peripheral blood and the absolute number of erythroid cells in the bone marrow. DNA synthesis in the cells of individual branches of hematopoiesis was assessed by determining incorporation of ^{3}H-thymidine by in vivo autoradiography [1].

EXPERIMENTAL RESULTS

During exposure of the mice to extremal factors, four types of response of HIM could be distinguished with respect to the GAG content in the bone marrow (Table 1). Type I was formed under the influence of hypoxia, an erythropoiesis-inducing factor, and was characterized by an increase in the content of acid and neutral GAG. In type II, observed as a result of expsoure to hyperbaric oxygenation (HBO), which inhibits erythropoiesis, the concentration of neutral GAG was reduced while that of acid GAG showed no significant change. Type III was observed during inflammation, which activates granulocytopoiesis, and was characterized by a sharp increase in the content of acid and a decrease in the content of neutral GAG in the period of maximal

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TABLE 1. GAG Content (in mg/g dry weight) in Bone Marrow of CBA Mice Exposed to Extremal Conditions

Procedure	GAG			Acid/neutral ratio
	acid	neutral	total	
Intac (30)	4,9±0,5	11,3±1,0	16,16	0,44
Acute hypoxia (20)	10,7±1,1	32,4±2,6	43,10	0,33
HBO (10)	7,0±1,8	7,3±1,0	14,27	0,95
Inflammation (10)	16,4±3,2	6,9±0,8	23,30	2,38
Irradiation (10)	3,9±1,4	20,2±1,5	24,09	0,19

TABLE 2. Effect of Blood Loss on GAG Concentration (in $\mu g/10^6$ cells) in Bone Marrow of Rats

Group of rats	GAG	
	acid	neutral
Intact	136,2±4,3	24,0±1,8
After blood loss without previous cooling		
2 h	153,5±3,9*	17,6±1,0*
1 day	19,2±5,1*	38,5±1,7*
3 days	216,5±6,4*	34,6±1,8*
After blood loss preceded by cooling for 14 days	58,6±3,9	51,2±2,7
2 h	110,5±6,1*	18,9±0,8*
1 day	242,6±11,0*	23,6±0,8*
3 days	210,3±13,2*	22,0±0,6*
6 days	221,6±9,9*	26,9±0,4*

TABLE 3. Effect of Cooling on Hematologic Parameters of

Parameter	Outcome	Cooling, 0°C		
		3 days	7 days	14 days
Reticulocytes, %	1,5±0,3	2,8±0,4*	3,3±0,2*	1,9±0,3
Erythroid branch of bone marrow, $\times 10^6$/femur	31,0±2,1	43,2±4,6*	34,8±3,9	38,3±3,1
Erythroid cells capable of proliferation, $\times 10^6$/femur	8,3±0,8	16,7±1,7*	10,3±1,0	7,6±0,8
Thymidine labeling index for erythroid cells capable of dividing, %	42,4±1,9	47,9±5,9	57,6±6,1*	33,1±1,3*
Neutrophil series of bone marrow, $\times 10^6$/femur	47,9±3,1	34,0±3,0*	34,8±3,9*	34,2±2,3*
Thymidine labeling index in neutrophil series, %	4,0±0,6	2,0±0,5*	1,7±0,3*	1,4±0,1*

increase in the bone marrow cell population. Irradiated mice (4.5 Gy) are characterized by a type IV response: an increase in the content of neutral and a small decrease in the content of acid GAG at the time of maximal cell population in the bone marrow. These types of responses were confirmed by differences in the total and relative concentrations of acid and nebtral GAG. Since irradiation damages hematopoietic cells directly, an attempt was made to obtain a type IV response by the action of other factors on the animals, among which the one with the closest effect as regards the direction of changes in GAG was cooling. The content of acid GAG on the 7th day of cooling fell to 98.9 ± 3.1 $\mu g/10^6$ cells, and on the 14th day to 58.6 ± 3.9 $\mu g/10^6$ cells, compared with 136.2 ± 4.3 $\mu g/10^6$ cells in intact animals. The content of neutral GAG on the 7th day of cooling increased to 77.8 ± 2.1 $\mu g/10^6$ cells, and fell a little on the 14th day, while remaining significantly increased, to 51.2 ± 2.7 $\mu g/10^6$ cells compared with 24.0 ± 1.8 $\mu g/10^6$ cells in intact animals. These changes in HIM were accompanied by a characteristic reaction of the hematopoietic tissue, manifested as stimulation of erythropoiesis, accompanied by a decrease in the available area for granulocytopoiesis (Table 2). Correlation between the different types of responses was discovered during exposure to factors affecting several branches of hematopoiesis simultaneously. For instance, in acute blood loss there was a successive interchange of types of response of HIM: II (2 h after bleeding) to IV (1 day), and later to type I (3 days: Table 3). This phasic pattern also was reflected in changes in the parameters of hematopoiesis: on the first and third days after blood loss the reticulocyte count increased to 11.3 ± 2.3 and 14.4 ± 1.3% respectively compared with 1.5 ± 0.3% in the control, and the number of erythroid cells

in the bone marrow capable of proliferation rose to $12.6 \pm 1.7 \cdot 10^6$ per femur and $12.0 \pm 1.2 \cdot 10^6$ per femur compared with $8.3 \pm 0.8 \cdot 10^6$ per femur (all differences between experiment and control are statistically significant), whereas the number of cells of the neutrophil series in the femur fell to $37.4 \pm 3.9 \cdot 10^6$ ($p < 0.05$), but later, by the 3rd day, it rose to $44.7 \pm 4.0 \cdot 10^6$ compared with $47.9 \pm 3.1 \cdot 10^6$ in the control. Predominance of acid GAG over neutral in the first hours after blood loss and of neutral until the first day evidently has a definite significance, for in experiments. With administration of exogenous preparations of GAG it was shown that the response of the granulocytic series develops more slowly than that of the erythroid series and reaches its peak value one day later than the peak activity of erythropoiesis [3]. The hypothesis of a connection between phasic changes in GAG and the state of hematopoiesis has been expressed previously, but it was not confirmed experimentally. Meanwhile changes in the duration of any of the above phases were reflected in the state of hematopoiesis and the rate of recovery of the blood cells. For instance, after blood loss in animals exposed to cooling for the two weeks before the operation, the type III of HIM, characteristic of early responses of hematopoietic tissue, lasted until the 6th day. Considerable delay was observed in restoration of the erythroid series in this case, the absolute number of erythroid precursors capable of proliferation in the femur on the 1st, 3rd, and 5th days after the operation showed no significant change, and was $9.5 \pm 1.2 \cdot 10^6$, $8.7 \pm 1.1 \cdot 10^6$, and $10.7 \pm 1.3 \cdot 10^6$ respectively compared with $7.9 \pm 1.0 \cdot 10^6$ at the time of the operation. The release of neutrophils into the peripheral blood, characteristic of exposure to this factor, was not present on the 1st day: the absolute number of neutrophils in the femur was $35.1 \pm 1.9 \cdot 10^6$ compared with $34.2 \pm 2.3 \cdot 10^6$ at the time of operation, whereas after blood loss not preceded by cooling, there was a considerable decrease in the number of cells of the corresponding series in the bone marrow.

The results demonstrate the important role of GAG in the maintenance of adaptive reactions of hematopoietic tissue.

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