Changes in Erythropoiesis during Liver Regeneration after Partial Hepatectomy

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Changes in erythropoiesis during liver regeneration after partial hepatectomy depended on functional activity of phagocytosing mononuclear cells.

Key Words: *regeneration; macrophages; erythroblastic islets; erythropoiesis*

Postresection regeneration of the liver serves as a convenient model for studies of the mechanisms of repair in damaged tissues [9,10]. It proceeds in two stages: destructive-reactive and proliferative. Each phase is characterized by specific proliferative and metabolic processes [6]. The system of phagocytosing mononuclear cells in the liver presented by Kupffer cells plays a role in the regulation of regeneration in both phases. Our previous studies showed that pharmacological stimulation of macrophages before surgery increases the weight of the regenerating liver part during destructive-reactive phase and intensifies cellular and intracellular regeneration during the proliferative phase [2,7].

The system of mononuclear phagocytes is present in various organs, including hemopoietic organs. For example, macrophage is a central cell in the erythroblastic islet. Macrophages in this structure have high affinity for erythroid cells, play a role in ropheocytosis, produce regulators of erythropoiesis, and form a specific hemopoietic microenvironment for erythropoiesis [5,8]. Changes in the system of mononuclear phagocytes during liver regeneration can affect the intensity of erythropoiesis. Here we studied changes in erythropoiesis in the bone marrow under conditions of activation of liver regeneration and modulation of macrophage function with Tamerit and carrageenan.

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MATERIALS AND METHODS

Experiments were performed on male outbred rats weighing 180-200 g. The animals were kept in a vivarium under standard conditions. Two-thirds partial hepatectomy was performed as described elsewhere [11]. We used Tamerit (2 mg/kg, Russian-made macrophage modulator) [1] and carrageenan (10 mg/kg, inhibitor of macrophage phagocytic activity, synthetic polygalactose derivative). The test drugs were administered 1 h before surgery. Control animals received single injection of physiological saline in an equivalent volume during the same period.

Activity of hemopoiesis was studied in the destructive and proliferative phase of liver regeneration (4 and 17 h after surgery, respectively). The animals were killed by ether overdosage during these periods. Peripheral blood indexes were studied on a Micros-60 blood analyzer (ABX-diagnostic). Erythroblastic islets (EI) in the bone marrow were counted and typed by the method developed at the laboratory of Yu. M. Zakharov [3]. EI of maturity classes I, II, and III, involuting EI, and reconstructing EI were distinguished. The intensity of erythropoiesis in bone marrow islets was determined by the total number of differentiating erythroid colony-forming units (CFUe), index for EI maturity, and coefficient for macrophage reinvolvement into EI. The results were analyzed by parametric statistical methods. Samples were selected using Student's *t* test.

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RESULTS

Hematocrit, hemoglobin concentration, and erythrocyte count in rats receiving physiological saline in the destructive-reactive phase of liver regeneration did not differ from the corresponding parameters in intact animals (Table 1).

The total number of EI in the femoral bone marrow remained unchanged (Table 2). The absolute and relative number of maturity class I, II, and III islets increased (Table 2), while the count of involuting islets decreased. The observed changes were probably associated with *de novo* formation and rapid maturation of islets. The index of CFUe involvement into differentiation and coefficient of macrophage reinvolvement into erythropoiesis increased (Table 3), while the degree of EI maturity decreased.

Functional changes in EI were accompanied by an increase in blood reticulocyte count (Table 1). It was probably related to acceleration of EI maturation and release of reticulocytes into the blood.

Peripheral blood indexes underwent more significant variations under conditions of liver resection and treatment of macrophages with Tamerit. Qualitative changes in erythrocytes included the increase in the mean cell volume and the width of erythrocyte volume distribution. The mean concentration of hemoglobin in erythrocytes decreased. The absolute number of bone marrow EI significantly decreased compared to intact and untreated animals (control). It was associated with changes in the number of maturity class I and III islets. Maturity class III islets underwent transformation to the pool of involuting islets. The index of islet maturity increased less significantly in these animals. The absolute number of reconstructing islets increased, while the index of macrophage reinvolvement did not differ from that in intact rats. No significant changes were revealed in the number of reticulocytes. Our results indicate that stimulation of macrophages in the reactive-destructive phase of regeneration (4 h postoperation) contributes to the involvement of CFUe and macrophages into erythropoiesis, but decelerates maturation of EI and reinvolvement of macrophages into the formation of islets.

Inhibition of macrophages with carrageenan decreased the absolute number of bone marrow EI compared to that in operated rats. It was associated with changes in the count of maturity class II and III islets, involuting islets, and reconstructing islets. These changes were accompanied by a decrease in the number of CFUe and differentiating islets. The rate of their maturation increased 2-fold. Blood reticulocyte count decreased. These data indicate that inhibition of macro-

TABLE 1. Red Blood Parameters in Rats after Liver Resection (*M*±*m*)

Parameter	Intact	Resection, 4 h			Resection, 17 h		
		without preparation	Tamerit	carrageenan	without preparation	Tamerit	carrageenan
Hematocrit, %	36.78±0.50	35.38±0.96	25.10±2.22*	34.08±1.60	45.50±2.75+	41.58±2.26	33.08±2.51*
Hemoglobin, g/liter	129.7±3.3	130.80±3.41	95.50±4.71*	124.00±4.41	144.00±7.06+	131.000±0.705	108.47±9.69*
Erythrocytes, g/liter	6.85±0.16	7.60±0.21	5.165±0.280*	7.25±3.23	8.60±0.82	8.30±0.38	7.39±0.96
Mean FL volume	53.68±0.60	46.58±0.40+	51.73±0.71*	47.00±0.58+	56.67±0.84+	52.00±0.82*	48.98±0.44*
Erythrocyte hemoglobin concentration, pg	18.96±0.40	17.26±0.21	18.41±0.17	17.18±0.56	17.95±0.37	16.47±0.37	17.38±0.46
Mean hemoglobin concentration in erythrocytes, g/liter	35.32±0.90	37.06±0.23	33.90±1.48*	36.74±0.50	31.68±2.77	31.56±0.24	35.53±0.93
Erythrocyte volume distribution, %	12.88±0.10	13.22±0.19	15.07±0.75*	12.28±0.30	15.370±0.217+	15.55±0.92	13.72±0.55*
Reticulocytes, g/liter	102.001±2.000	207.76±13.30+	170.76±36.00	152.70±20.53	101.00±40.00	150.00±60.00	206.90±37.77
Reticulocytes, %	1.730±0.270	2.95±0.23	3.24±0.60	1.475±0.380*	1.38±0.62	2.02±0.90	2.575±0.230*

Note. Here and in Tables 2 and 3: $p<0.05$: *compared to control animals; +compared to intact animals.

TABLE 2. Absolute Number of EI in the Femoral Bone Marrow of Rats after Liver Resection

Parameter	Intact	Resection, 4 h			Resection, 17 h		
		without preparation	Tamerit	carrageenan	without preparation	Tamerit	carrageenan
EI, $\times 10^3$ per femur	90.00±2.35	133.33±6.08*	97.80±2.16	79.34±5.51*	61.66±6.73	47.80±2.20$^+$	58.33+8.37
EI-I, $\times 10^3$ per femur	3.87±0.41	27.99±1.39*	22.78±1.14$^+$	25.83±1.80	25.90±1.55*	19.31±0.96$^+$	6.73±0.47*
%	4.3±0.6	21.0±1.5	23.3±0.9$^+$	37.7±1.9*	42.2±2.1	40.40±2.02	11.8±0.7*
EI-II, $\times 10^3$ per femur	12.78±1.54	22.21±1.33*	28.42±1.42$^+$	21.33±1.49*	12.63±0.76*	10.08±0.50	13.39±1.07
%	14.2±0.9	16.7±1.0*	29.3±1.7$^+$	27.0±1.6	20.7±1.0	21.1±1.0	23.5±1.4
EI-III, $\times 10^3$ per thigh	10.44±0.83	36.04±2.16*	20.95±1.26$^+$	15.70±1.25*	6.83±0.40	7.60±0.48$^+$	16.76±1.17*
%	11.6±1.8	27.1±0.4	21.6±1.9$^+$	19.9±1.1	11.2±0.5	15.10±0.75	29.4±1.7*
EI_{inv}, $\times 10^3$ per femur	47.61±2.25	35.11±1.75*	15.13±1.05$^+$	9.64±0.67*	11.41±0.79*	6.31±0.28$^+$	13.39±0.97*
%	52.8±3.7	26.4±1.3*	15.6±0.9$^+$	12.20±0.73*	18.7±0.9*	13.2±0.7$^+$	23.5±1.6*
EI_{rec}, $\times 10^3$ per femur	15.39±1.37	11.70±0.58	9.89±0.51$^+$	6.08±0.43*	4.39±0.31	4.87±0.32$^+$	6.73±0.54*
%	17.1±2.1	8.8±0.5	10.2±0.6$^+$	7.7±0.4*	7.2±0.3	10.2±0.8$^+$	11.8±0.5

Note. EI-I, EI-II, and EI-III: erythroblastic islets of classes I, II, and III, respectively; EI_{inv}, involuting islets; EI_{rec}, reconstructing islets.

TABLE 3. Estimated Functional Parameters of Erythropoiesis in Bone Marrow EI

Parameter	Intact	Resection, 4 h			Resection, 17 h		
		without preparation	Tamerit	carrageenan	without preparation	Tamerit	carrageenan
Total number of differentiating CFUe, $\times 10^3$ per femur	41.52±3.50	99.02±5.28	89.12±3.50$^+$	69.64±4.86*	49.99±5.43	46.90±3.66	47.9±06.7
Involvement of CFUe into differentiation, $\times 10^3$ per femur	19.32±1.31	39.41±1.39	39.15±3.40$^+$	32.32±4.13	30.97±4.65	25.00±1.34$^+$	14.78±2.06*
Period of EI maturation, rel. units	1.88±0.31	1.15±0.09*	0.58±0.01$^+$	0.350±0.043*	0.390±0.025	0.360±0.019$^+$	1.120±0.001*
Macrophage reinvolvement into erythropoiesis, rel. units	0.33±0.03	0.35±0.04	0.66±0.11$^+$	0.347±0.043	0.431±0.04*	1.013±0.050$^+$	0.502±0.001*

phages with carrageenan attenuates changes in the ratio of bone marrow EI compared to control animals.

Hematocrit, blood hemoglobin concentration, mean cell volume, and the width of erythrocyte volume distribution increased in animals receiving physiological saline 17 h after surgery.

The ratio of bone marrow EI changed significantly in this period. The number of class I islets remained high, while the absolute number of EI, count of mature islets, and period of maturation decreased. The number of reconstructing islets decreased by 2 and 3 times compared to the early stage of observations and intact rats, respectively. The index of macrophage reinvolvement into erythropoiesis increased. Blood reticulocyte count did not differ from that in intact rats. Probably, activation of proliferative processes in the liver is accompanied by a decrease in the number of bone marrow EI and acceleration of their maturation.

Peripheral red blood parameters in rats exposed to macrophage activation 17 h postoperation did not differ from those in control animals. The absolute number of bone marrow EI decreased less rapidly compared to untreated animals. The distribution of islets did not differ from that observed 4 h after resection.

Hepatectomy and inhibition of macrophages with carrageenan were followed by a sharp decrease in hematocrit and blood hemoglobin concentration (17 h postoperation). We revealed a decrease in the mean erythrocyte volume and the width of erythrocyte volume distribution. During this period the absolute number of EI did not differ from that in rats not receiving the test preparation, but was much lower than in intact animals. The number of peripheral blood reticulocytes increased. The structure of islets in these rats differed from that in untreated animals. The number of maturity class I islets decreased, while the count of involuting and reconstructing islets slowly increased. The index of CFUe involvement into differentiation decreased. Maturation of EI was decelerated. The index of macrophage reinvolvement into erythropoiesis did not differ from that observed in the early stage after treatment. However, this index was 2-fold lower compared to rats with unchanged activity of macrophages.

Our findings indicate that postresection regeneration of the liver in experimental animals significantly modulates erythropoiesis, which depends on the stage of reparative processes in the liver. The degree of changes depends on functional activity of macrophages. Activation of macrophages with Tamerit stimulates the formation of new EI in both phases of postresection liver regeneration. The rate of maturation decreases in the destructive phase. The number of newly formed islets is lower under conditions of blockade with carrageenan. The rate of islet maturation decreases in the proliferative phase. Both preparations prevent macrophage reinvolvement into erythropoiesis.

These data indicate that liver regeneration after damage is accompanied by changes in the system of erythropoiesis. This reaction depends on functional activity of mononuclear phagocytes.

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