Ability of Peritoneal Exudate Cells to Trigger Various Mechanisms of Erythroid Differentiation at Different Terms after Massive Blood Loss in Mice

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Thirty minutes after massive blood loss, peritoneal cells gained the ability to trigger reparative erythropoiesis in the bone marrow of normal syngeneic recipients. This was manifested in proliferation of oxyphilic erythroblasts and activation of the reserve pathway of erythroid differentiation 4 days after cell transplantation. The maximum transfer capacity of peritoneal cells was observed 4 h after blood loss, but 4 days later they only initiated mitoses in oxyphilic erythroblasts.

Key Words: *stress erythropoiesis; reserve erythropoiesis; erythroid differentiation; peritoneal cells*

After severe anemia, erythrocyte pool is restored by 3 various differentiation pathways [1-5,7]. At the terminal stage of anemia, the reserve (emergency) pathway of erythropoiesis is manifested in transitory accumulation of basophilic erythroblasts (BE) in the bone marrow accompanied by a decrease in their mitotic activity [5,7]. Some BE enter S phase of the cell cycle (duplication of DNA and cytoplasm), but do not enter mitosis and undergo rapid differentiation [7,8]. Hence, immature cells (primarily macrocytes in mammals) are recruited into the blood and maintain the function of various body systems until the formation of mature erythrocytes due to intense and accelerated erythroid differentiation. This is manifested in the elevated mitotic index of polychromatophilic erythroblasts (PE) and the increase in their bone marrow content. Furthermore, a great number of proliferating oxyphilic erythroblasts (OE), which normally do not enter mitosis, appears in the bone marrow [1,3,4]. Their proliferation can also be regulated by anemia factors [3]. All these changes in erythroblast differentiation are considered as reparative erythropoiesis.

Unstimulated with inflammatory antigen, peritoneal exudate cells (60-70% macrophages and 25% lymphocytes [6]) obtained from mice with massive blood loss gain the ability to trigger reparative erythropoiesis in the bone marrow of syngeneic recipients [1,2]. In this case, specific features typical of reserve erythropoiesis, initiation of OE proliferation, and enhancement of PE proliferation are observed.

Here the ability of peritoneal cells to trigger various reparative processes in the erythroid compartment was studied at different terms after blood loss using the model of adoptive cell transfer.

MATERIALS AND METHODS

Experiments were performed on 247 male CBA mice weighing 19-23 g. Peritoneal cells were taken 0.5, 1, 2, 4, 18, 48, and 96 h ("donor interval") after massive blood loss (2.5% body weight) by the method described elsewhere [1]. The time from blood loss to isolation of peritoneal cells was strictly controlled. Peritoneal cells from anemic donors were resuspended and injected intraperitoneally in a dose of 10^7 cells/0.4 ml

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medium 199 to normal syngeneic recipients. The recipients were euthanized by cervical dislocation 96 h after cell transfer *i.e.*, at the period corresponding to maximum intensity of reparative erythropoiesis in the bone marrow of anemic donors. Normal mice receiving intraperitoneal injection of 0.4 ml medium 199 and anemic animals examined 96 h after blood loss served as the control. Morphological examination of the bone marrow from recipients and control mice and statistical analysis of the results were performed as described previously [1].

RESULTS

Peritoneal cells obtained from anemic animals 0.5 h after massive blood loss possessed the ability to initiate proliferation of OE in the bone marrow of recipient mice (Table 1), while peritoneal cells from intact donors do not display such properties [1].

Furthermore, at this period peritoneal cells gained the ability to change mitotic activity of BE and their percentage in the bone marrow (Table 1). Our previous studies showed that peritoneal cells transfered from intact donors elevate the mitotic index in the population of BE and decrease the relative content of BE in the bone marrow of recipients [1], while peritoneal cells from anemic donors produced opposite effects. Mitotic activity of BE significantly decreased compared not only to that in recipients of normal peritoneal cells, but also to control mice injected with medium 199. At the same time, the count of BE considerably increased.

The transfer of peritoneal cell obtained from mice 30 min after blood loss produced changes in the PE population of recipients, which were similar (but less pronounced) to those observed in the bone marrow of anemic animals: the mitotic index of PE significantly increased compared to that in normal mice (Table 1), but the percentage of PE did not differ from the control. The erythrocyte-leukocyte index in treated mice (as well as that in anemic animals) was higher than in normal mice due to elevated BE content.

Hence, severe 30-min anemia markedly changes the properties of peritoneal cells and triggers reparative erythropoiesis (primarily, the reserve pathway and proliferation of OE). However, the morphogenetic activity of these cells 30 min after blood loss is still below maximum.

One hour after massive blood loss, the ability of peritoneal cells to trigger reparative erythropoiesis did not differ from that observed during the preceding period (Table 1).

Peritoneal cells obtained 2 h after blood loss displayed well-pronounced transfer properties (Table 1). The ability of peritoneal cells to trigger reparative erythropoiesis was most pronounced 4 h after blood loss (Table 1).

Eighteen hours after blood loss, morphogenetic activity of peritoneal cells remained high, but they produced less distinct responses (Table 1). Forty-eight hours after blood loss, peritoneal cells still triggered proliferation of OE, increased the mitotic index of PE, and decreased the mitotic index of BE (compared to those in the intact bone marrow). At the same time,

TABLE 1. Induction of Reparative Erythropoiesis in Intact Recipients by Peritoneal Cells from Anemic Donors as a Function of Donor Interval ($M \pm m$)

Donor interval, h		PE		BE		OE		Erythrocyte-leukocyte index
		%+	Mitotic index, ‰	%+	Mitotic index, ‰	%+	Mitotic index, ‰	
0.5	control	6.8±0.4	31.7±2.8	9.7±0.5	28.1±1.2	2.4±0.2	1.4±0.3	0.234±0.014
	experiment	9.1±0.3*	49.3±2.7*	10.0±0.4	17.0±1.2*	3.6±0.5	62.3±5.8*	0.294±0.014*
1	control	5.2±0.4	34.5±2.5	9.5±0.1	31.8±0.9	3.3±0.6	1.8±0.5	0.219±0.005
	experiment	9.2±0.9*	52.5±2.8*	10.3±1.1	19.3±0.5*	4.3±0.6	60.0±6.5*	0.313±0.030*
2	control	2.3±0.3	41.6±2.0	9.9±0.5	32.0±1.7	1.2±0.2	1.5±0.4	0.156±0.009
	experiment	4.9±0.3*	61.2±5.0*	11.2±0.9	23.2±1.4*	1.4±0.2	82.0±7.5*	0.207±0.014*
4	control	2.3±0.3	41.6±20	9.9±0.5	32.0±1.7	1.2±0.2	1.5±0.4	0.156±0.009
	experiment	5.8±0.6*	64.4±2.0*	10.3±0.8	22.4±1.8*	1.7±0.2	90.0±2.7*	0.217±0.018*
18	control	2.3±0.3	41.6±2.0	9.9±0.5	32.0±1.7	1.2±0.2	1.5±0.4	0.156±0.009
	experiment	4.5±0.6*	51.8±3.1*	8.3±0.8	22.2±2.2*	1.8±0.7	78.3±13.3*	0.174±0.016
48	control	7.7±0.8	36.8±2.8	8.7±0.9	27.4±1.2	2.3±0.2	1.2±0.4	0.230±0.022
	experiment	8.1±0.9	49.0±2.4	8.8±0.5	20.8±1.8*	3.1±0.5	68.0±2.7*	0.251±0.020

Note. *$p<0.05$ compared to the corresponding control group; +from the total count of hemopoietic cells.

we observed no statistically significant differences (compared to the control) in the percentage of erythroblasts at various stages (Table 1). Therefore, peritoneal cells lost their ability to trigger reserve erythropoiesis and to stimulate PE proliferation.

It should be emphasized that the increase in the mitotic index of PE after the transfer of peritoneal cells from anemic donors obtained at various periods after blood loss was not accompanied by the rise of PE count in the bone marrow of recipients (Table 1). It can not be excluded that some PE underwent mitosis, lost their nuclei, and were rapidly washed out from the bone marrow.

Peritoneal cells obtained 96 h after blood loss (the peak of reparative processes at morphologically identified stages of erythropoiesis) triggered only proliferation of OE in the bone marrow of recipients (mitotic index 50%). This indicated that 96 h after blood loss, the ability of peritoneal cells to trigger reparative erythropoiesis was lower than that observed 48 h after blood loss.

Thus, the population of peritoneal cells is an important regulatory system, which rapidly react to massive blood loss. Peritoneal cells obtained from anemic donors even 30 min after blood loss stimulated OE proliferation and triggered reserve erythropoiesis in the bone marrow of intact recipients. Previous studies showed that plasma erythropoietin content increased by 10 times only 12 h after massive blood loss [9]. Probably, the role of this rapidly reacting regulatory system is that peritoneal cells control reserve erythropoiesis responsible for organism survival at the early terms after sublethal blood loss.

Intensively proliferating OE also contribute to the recovery of erythrocyte count in the early posthemorrhagic period. Proliferation of OE probably yields microcytes typical (similarly to macrocytes) of the recovery period in mammals.

Published data indicate that proliferation of OE is observed even in moderate anemia [3,4]. Our experiments suggest that the onset of OE proliferation is a rapid response of the erythroid system to the regulatory influences from the macrophage-lymphoid system. Peritoneal cells retain their ability to induce proliferation of OE in recipients even 96 h after blood loss, when blood volume is partially restored and anemia factors produce less pronounced effects. Compared to proliferation of PE, OE proliferation is induced by weaker regulatory influences. Our preliminary experiments showed that peritoneal cells from anemic donors in a low dose (10^6) initiate mitosis of bone marrow OE in recipients (32.2±4.9%), but did not stimulate mitosis of PE.

The ability of peritoneal cells from anemic donors to activate the mechanisms of cell differentiation in the erythroid compartment appears and then weakens at various periods. These data indicate that various pathways of terminal erythroid differentiation are independently triggered by regulatory factors.

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