EXPERIMENTAL BIOLOGY

Various Aspects of the Involvement of Peritoneal Exudate Cells in the Regulation of Apoptosis

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Peritoneal exudate cells are involved in the regulation of erythroid cell proliferation and hemoglobin synthesis. However, activation of these processes occurs independently of each other and is regulated by various mechanisms. Hemoglobin synthesis is initiated after changes in pH and/or water-electrolyte balance in the abdominal cavity. Peritoneal exudate cells gaining specific activity under conditions of hemorrhage play a role in stimulation of erythroblast proliferation.

Key Words: *peritoneal exudate cells; erythropoiesis; hemoglobin*

The regulation of erythropoiesis is associated with activation of erythropoietin production in the kidneys under conditions of massive hemorrhage. An alternative regulatory system including resident lymphoid cells and macrophages of the peritoneal exudate plays a role in the regulation of erythropoiesis in mice [2]. Over the first hours after external hemorrhage, peritoneal cells (PC) *in vivo* gain the ability to induce intensive proliferation of bone marrow erythroid cells and modify cell differentiation. PC are characterized by extreme potency in stimulating erythropoiesis under conditions of massive hemorrhage; adoptive transfer of PC into the abdominal cavity of intact syngeneic recipients causes an imbalance of erythropoiesis and the appearance of signs of "emergency" erythropoiesis typical of mice with hemorrhage [1]. Apart from the proliferative response of bone marrow erythroid precursors, activation of hemoglobin (Hb) synthesis is an important compensatory mechanism during hemorrhage.

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Here we studied whether PC are involved in modulation of *in vivo* Hb synthesis under conditions of specific (massive external hemorrhage) and nonspecific stimulation (change in pH and water-electrolyte balance). The relationship was evaluated between activity of PC and their ability to modulate proliferation of erythroid precursor cells.

MATERIALS AND METHODS

Experiments were performed on male inbred CBA mice weighing 23-27 g. Experimental conditions were selected taking into account published data [1]. The ability of PC to stimulate bone marrow erythropoiesis was maximum 2-18 h after blood withdrawal (2.5% body weight). The dose of PC was optimal for activation of erythropoiesis in healthy recipients (10^7 cells per mouse). Stimulation of bone marrow erythropoiesis and increase in the number of blood reticulocytes were observed starting from the 4th day after adoptive transfer of PC. The isolation of PC (5 h after hemorrhage) and intraperitoneal transplantation of cells to syngeneic recipients were performed using culture medium 199 on glutamine-free Hanks solution (pH 7.2; M. P. Chumakov Institute of Poliomyelitis and

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Viral Diseases, Russian Academy of Medical Sciences) [1].

In series I, the recipients received intraperitoneal injection of 0.5 ml suspension of PC or medium 199. Peripheral blood test was performed on day 4. In series II, the recipients received intraperitoneal injection of 0.2 ml suspension of PC or culture medium. The blood was sampled 3 and 6 days after administration of PC or culture medium. Blood samples from intact mice served as the control.

The blood was taken from the retroorbital sinus using a Pasteur pipette treated with EDTA. Blood samples were put in Microvette 200 EDTA K tubes (Sarstedt). Red blood cells were examined on a CELL-DYN 3500 analyzer (Abbot Lab.). We estimated Hb content, mean amount of Hb per erythrocyte (MCH), mean concentration of Hb per erythrocyte (MCHC), erythrocyte number, hematocrit (Ht), mean volume of erythrocytes, and erythrocyte heterogeneity by size.

The results were analyzed by Student's *t* test.

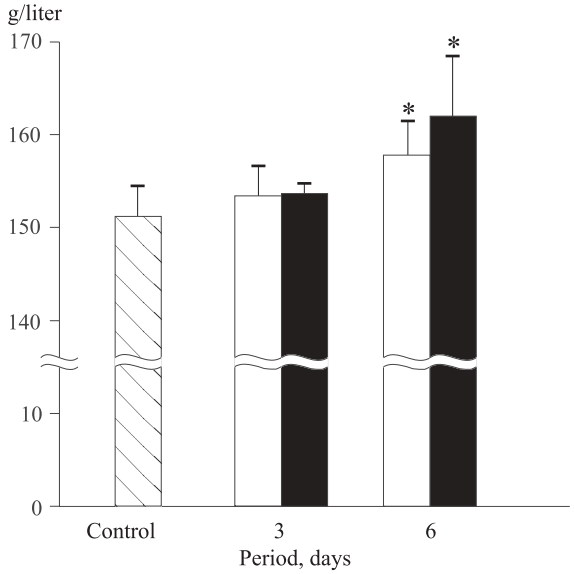

Fig. 1. Hemoglobin content in the peripheral blood of mice after intraperitoneal injection of culture medium (light bars) or PC suspension (dark bars). *p<0.02 and **p<0.007 compared to the control.

RESULTS

Series I was performed by a scheme resulting in maximum stimulation of bone marrow erythropoiesis after transplantation of PC from anemic donors [1]. Transplantation of 0.5 ml suspension of PC was followed by a significant increase in MCHC and MCH compared to the intact control (Table 1). However, stimulation of Hb synthesis in recipients was not related to activity of PC under conditions of hemorrhage. A similar increase in the contents of Hb, MCH, and MCHC in the blood of animals was observed on day 4 after administration of the culture medium (Table 1). Other parameters of red blood cells did not differ in intact and treated mice under both conditions.

Previous experiments with adoptive transfer of PC from animals with massive hemorrhage to healthy syngeneic recipients showed that changes in the mitotic index of erythroblasts and percentage of cells in the bone marrow is related to activity of PC in response to hemorrhage [1], but not to administration of the culture medium.

In series II, the volume of PC suspension or pure culture medium was decreased to 0.2 ml to exclude possible role of hemodilution in stimulation of erythropoiesis. Characteristics of Hb increased similarly on day 6 after transplantation of PC suspension or culture medium (Figs. 1-3), but did not differ from those in intact controls 3 days after treatment. Other parameters of red blood cells remained unchanged.

TABLE 1. Parameters of Red Blood Cells on Day 4 after Intraperitoneal Treatment with 0.5 ml Culture Medium or PC Suspension ($M \pm m$)

Parameter	Control	Day 4 after administration of medium 199	Day 4 after administration of PC suspension
Hb, g/liter	151.2±3.4	160.3±2.0**	153.0±3.7
MCH, pg	14.9±0.2	15.6±0.2***	15.3±0.1*
MCHC, g/liter	184.8±2.6	195.5±1.5***	191.8±1.7*
Erythrocyte number, million/μl	10.2±0.2	10.3±0.2	10.0±0.2
NBT	81.8±1.7	82.0±1.5	79.7±2.4
Mean volume of erythrocytes, fl	80.6±0.6	79.9±0.8	79.5±0.5
Erythrocyte heterogeneity by size, %	17.2±1.1	17.4±0.8	16.6±0.7

Note. *p<0.01, **p<0.001, and ***p<0.0001 compared to the control.

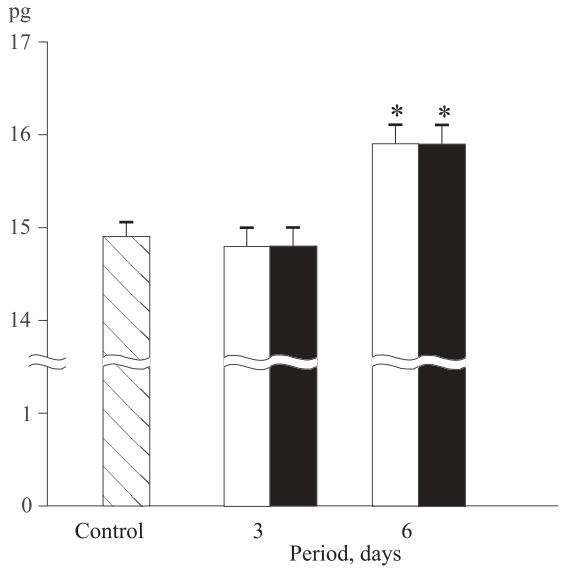

Fig. 2. MCH in the peripheral blood of mice after intraperitoneal injection of culture medium (light bars) or PC suspension (dark bars). Here and in Fig. 3: *$p<0.0001$ compared to the control.

These data indicate that intraperitoneal administration of small amounts of the culture medium initiates Hb synthesis in erythroid precursor cells. "New" erythrocytes migrate to the blood starting from the 4th day. The formation of these erythrocytes was accompanied by increased synthesis of Hb. A similar number of circulating erythrocytes are eliminated from the circulation due to disintegration or accumulation in stores. These changes are followed by a significant increase in the total content of Hb in the blood. The elevation of MCH and MCHC was not accompanied by changes in the mean volume of erythrocytes or increase in heterogeneity of circulating erythrocytes. These data illustrate the increase in Hb synthesis. It should be emphasized that treatment with a small amount of the culture medium had no effect on mitotic activity of erythroblasts and relative number of these cells in the bone marrow.

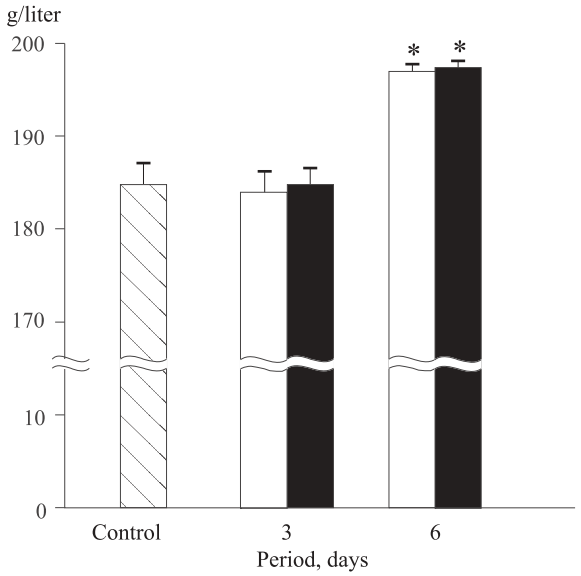

Fig. 3. MCHC in the peripheral blood of mice after intraperitoneal injection of culture medium (light bars) or PC suspension (dark bars).

The presence of PC in a small volume of the culture medium did not result in the increased stimulation or inhibition of Hb synthesis. However, these cells stimulate the terminal stage of erythroblast proliferation and modify cell differentiation [1]. Our findings indicate that PC are involved in the regulation of erythroid cell proliferation and Hb synthesis. However, activation of these processes occurs independently of each other and is regulated by different mechanisms. Severe anemia over several hours modifies the effect of lymphoid cells and macrophages from the peritoneal exudate on proliferative activity of bone marrow erythroid cells. PC exhibit a specific response to specific stimulation. As regards to stimulation of Hb synthesis, we observed a specific response to nonspecific stimulation (change in water-electrolyte balance and/or pH in the abdominal cavity).

pH of the peritoneal fluid in healthy and anemic mice is <7.2 (culture medium 199 containing phenol turned yellow during peritoneal lavage). PC gain the ability to stimulate Hb synthesis due to changes in water-electrolyte balance, acid-base balance, and the presence of components from the culture medium in the abdominal cavity. Activity of PC can be also associated with a combined effect of these factors. The mechanism mediating stimulation of Hb synthesis with PC remains unclear. However, this nonspecific mechanism can play a physiological role. It is unknown which cells of the abdominal cavity (lymphocytes and macrophages of the peritoneal exudate or peritoneal mesothelial cells) are involved in stimulation of Hb synthesis.

Our findings provide a new interpretation of clinical data that illustrate stimulation of erythropoiesis in anemic patients with severe renal failure on outpatient peritoneal dialysis. Many researches believe that this effect is associated with washout of erythropoiesis inhibitors [4]. It should be noted that stimulation of erythropoiesis during peritoneal dialysis can be related not only to removal of erythropoiesis inhibitors, but also to the increased synthesis of erythropoiesis stimulators. One of these factors is probably erythropoietin [3]. Further studies of the intraperitoneal mechanisms for stimulation of Hb synthesis are required to improve the quality of dialysis solutions.

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