The Role of Peritoneal T Lymphocytes and Macrophages in the Initiation of Stress Erythropoiesis *In Vivo* Following a Single Massive Blood Loss in Mice

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Peritoneal cells of anemic donors enriched with macrophages at the expense of T-cell lysis using anti-Thy-1.2 monoclonal antibodies are capable of triggering various routes of terminal erythroid differentiation characteristic of stress erythropoiesis during their adoptive transfer to normal syngeneic recipients. Stimulation of the proliferation of polychromatophilic erythroblasts and initiation of mitoses in oxyphilic erythroblasts are regulated by interacting T cells and macrophages, and the "reserve erythropoiesis" mainly by macrophages.

Key Words: *stress erythropoiesis; peritoneal cells; reserve erythropoiesis; blood loss*

In mammals with severe anemias the erythroid precursors, on reaching the terminal stages, form red cells as a result of terminal differentiation by various routes. One of these routes, "reserve erythropoiesis," is an emergency route of accelerated differentiation of cells providing for the survival of the organism during the early periods after massive blood loss. It consists in the omission of several terminal stages and the rapid release of morphologically immature cells into the blood [2,3,6-8]. A transitory increase in the content of basophilic erythroblasts (BE) in the bone marrow paralleled by a decrease of their mitotic activity may be considered as a bone marrow marker of "reserve erythropoiesis" [3,7]. Moreover, blood loss enhances polychromatophilic erythroblasts (PE) amplification and initiates mitoses in oxyphilic erythroblasts (OE), which normally almost never divide [1,3].

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Under conditions of massive blood loss resident peritoneal cells (PC) acquire the ability to trigger stress erythropoiesis in normal syngeneic recipients during the adoptive transfer of these cells [1,4]. Their effect on recipient erythropoiesis manifests itself by activation of various routes of terminal erythroid differentiation characteristic of anemic animals. The PC population has been demonstrated to be an important regulatory system which reacts swiftly to massive blood loss [4].

Our aim in this research was to estimate the contribution of peritoneal T lymphocytes and macrophages to the triggering of various routes of stress erythropoiesis and to elucidate whether they interact during this process.

MATERIALS AND METHODS

Experiments were carried out with male CBA mice weighing 17 to 20 g. Anemia was induced by bleeding from the retroorbital sinus under Nembutal narcosis, the volume of blood loss being 2.5% body weight individually for each mouse. PC not stimulated with an inflammatory agent

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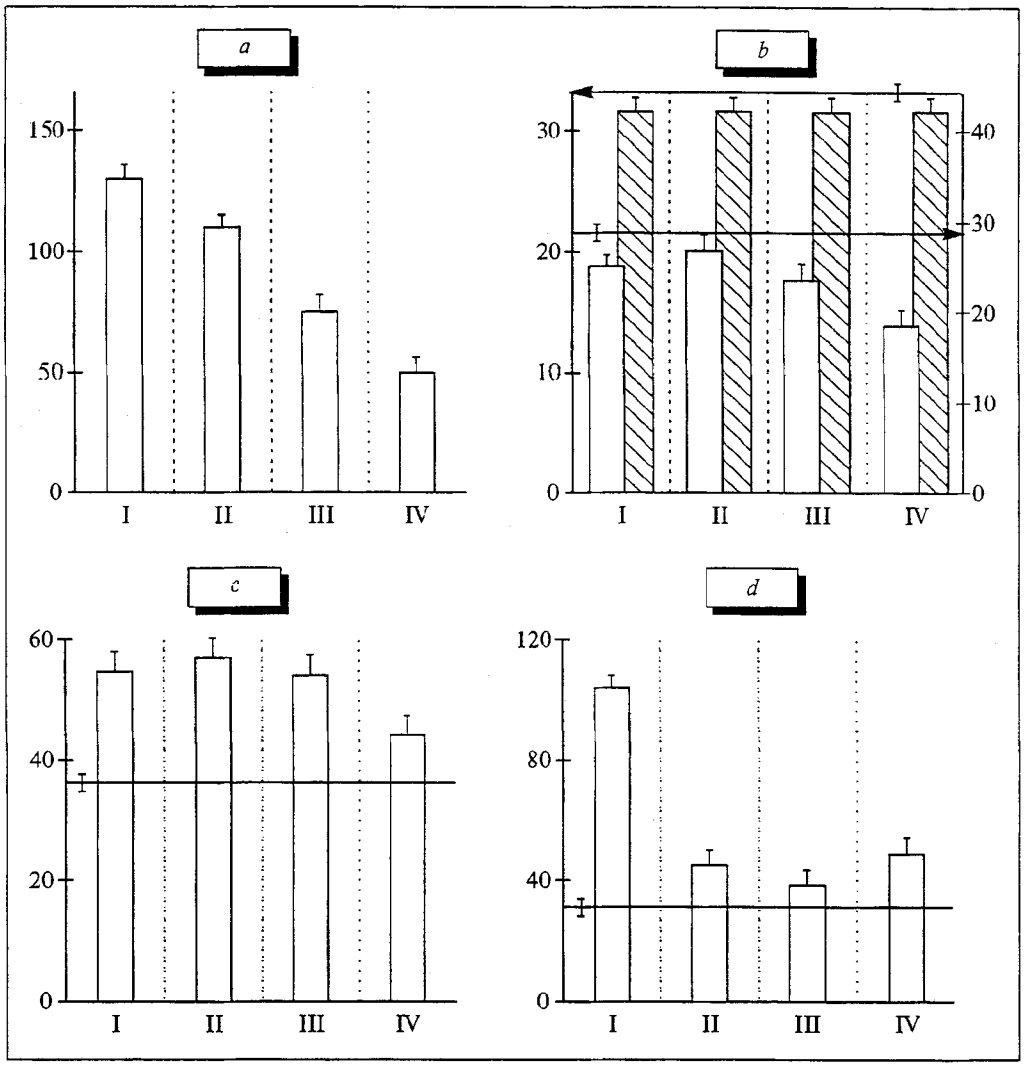

Fig. 1. Changes in erythropoiesis of normal recipients under the influence of transplantation of PC from anemic donors enriched with macrophages at the expense of T-cell lysis ($M \pm m$). *I*) anemic mice; *II*) recipients of intact PC; *III*) recipients of complement-treated PC; *IV*) recipients of PC treated with anti-Thy-1.2 monoclonal antibodies and complement; horizontal lines: normal control (injection of medium 199). *a*) MI(‰) of bone marrow OE; *b*) mitotic activity of bone marrow BE (white bars) and BE count in the bone marrow *vs.* total count of erythroblasts (hatched bars). Left ordinate: MI (‰) of BE; right ordinate: BE count (%); *c*) MI (‰) of bone marrow PE; *d*) reticulocyte content (‰) in peripheral blood.

were isolated 4 h after bleeding as described previously [1] and enriched with macrophages by complement-dependent lysis of T cells using murine antimurine anti-Thy-1.2 monoclonal antibodies (Cedarlane, Canada) (1:100) and rabbit serum as complement (1:10). The indicated dilutions of the serum and monoclonal antibodies were optimal and caused the death of 89.3% thymocytes. PC enriched with macrophages at the expense of T-cell lysis were injected to recipients intraperitoneally in a dose of 10^7 live cells. To control animals complement-treated or intact PC were transplanted, or 0.4 ml of culture medium 199 was injected. Recipients were sacrificed 4 days after cell transplantation. The mitotic indexes (MI) in populations of BE, PE, and OE per 1000 cells of each population and the percent share of BE in the bone marrow were assessed in bone marrow impressions stained after Giemsa, and the counts of reticulocytes were measured in blood smears.

The results were statistically processed using the Student t test.

RESULTS

Adoptive transfer of 10^7 PC obtained from donors 4 h after bleeding (that is, when their morphogenetic activity was maximally expressed) to intact syngeneic recipients caused changes in their bone marrow which were characteristic of anemic animals 4 days after bleeding (Figs. 1 and 2). Intensively dividing OE were found in recipient bone marrow. After transfer of PC from anemic donors the MI of polychromatophilic erythroblasts was reliably higher ($p<0.05$) than in the bone marrow of intact animals (injected culture medium), and the value of this index was the same as in anemia. PC transfer from anemic donors initiated changes characteristic of "reserve erythropoiesis" in the bone marrow of recipients. This was manifested as a reliable ($p<0.05$) drop of MI in the BE population

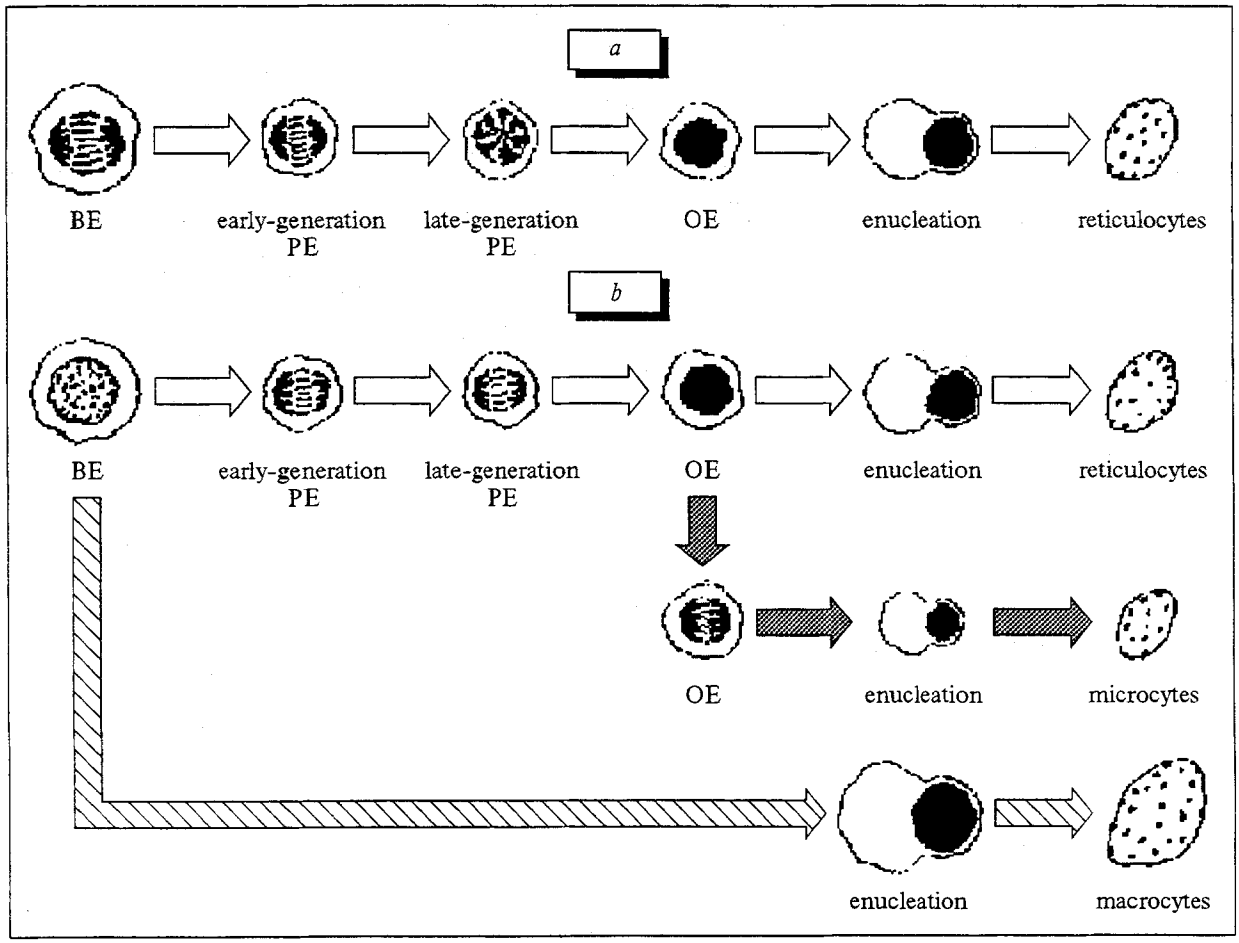

Fig. 2. Routes of terminal differentiation of erythroid cells during normal and repair regeneration of the blood. *a*) stable erythropoiesis; *b*) repair erythropoiesis. Actively proliferating populations with cells in mitosis are shown. Obliquely hatched arrows show "reserve erythropoiesis". Cross-hatched arrows show red cell replenishment due to initiated proliferation of OE.

in parallel with a reliable increase of the percent share of these cells in recipient bone marrow.

Figure 1 shows that treatment of PC with the complement alone leads to just a partial reduction of their transfer capacity, that is, the capacity to trigger stress erythropoiesis in recipients. The capacity to trigger OE proliferation was 1.5-fold reduced in comparison with that after treatment with intact PC. Lysis of T lymphocytes in the PC suspension led to a marked reduction ($p<0.02$) of the transfer ability of PC as regards triggering the mitosis of OE and stimulating of mitosis in PE in comparison with the transfer capacity of complement-treated PC (Fig. 1). The adoptive transfer of PC enriched with macrophages by T-cell lysis did not change the MI or the percent content of BE *vs.* transfer of intact or complement-treated PC. This fact may be interpreted as follows: removal of T lymphocytes from the PC suspension does not alter the ability of PC from anemic donors to transfer signs of "reserve erythropoiesis" to recipients; in other words, the triggering of "reserve erythropoiesis" seems to be regulated mainly by peritoneal macrophages.

As is seen in Fig. 1, treatment of PC from anemic donors with rabbit serum alone reduced their transfer capacity in respect of OE and PE, but not BE. It is possible that some components of rabbit serum interacted with surface receptors of PC and this might have reduced their transfer capacity vis-a-vis OE and PE, but did not affect the transfer capacity of PC to trigger "reserve erythropoiesis." Hence, these data may serve as additional proof that during the triggering of the reserve route, on the one hand, and stimulation of OE and PE proliferation, on the other, various mechanisms of regulation in the "macrophage-lymphocyte-erythroblast" system may be involved in stress erythropoiesis.

Hence, during adoptive transfer, the macrophage-rich PC population is capable of regulating the development of erythroid cells by different routes at the terminal stages which are characteristic of repair erythropoiesis. However, with regard to the stimulation of PE proliferation and triggering of

mitosis in OE, the maximal effect was observed during interaction of peritoneal macrophages and T lymphocytes activated by anemia factors in the early periods after hemorrhage. Our data indicate the presence of T-cell-macrophage interaction but not their sum effect, because the deficit of eliminated T cells was compensated for during transfer of up to 10^7 cells by macrophages.

The data indicate that the PC population is an extremely important component of the regulatory system, because the transfer of these cells to intact recipients with a normal level of erythropoiesis is capable of upsetting stable erythropoiesis and triggering repair erythropoiesis. This makes it easier to understand why the development of peritonitis in rats after blood loss selectively blocked the macrocytic regeneration [5] despite a high level of erythropoietin production. The usefulness of a regulatory system of rapid response to blood loss in the organism becomes clear if we bear in mind that peritoneal macrophages control the "reserve erythropoiesis" which ensures survival at the early period of blood recovery.

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The Phenomenon of Mouse Death after Parenteral Administration of Mink Blood Serum

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Parenterally administered mink blood serum is found to kill mice. The lethal factor of the serum is shown to be thermolabile, not dialyzed, stable during prolonged storage at -20°C and repeated freezing-thawing, destroyed by trypsin, and detected in globulin fractions after fractionation of the serum with ammonium sulfate.

Key Words: *mink blood serum; mice; protein*

The object of this research was to study the rapid death of mice after parenteral administration of low amounts of mink blood serum (MBS).

MATERIALS AND METHODS

Hybrid mice (CBA×C57B1/6)F_1 and outbred male mice weighing 16-18 g were used in the study. Blood was collected from minks of both sexes weighing 1-1.5 kg. Blood was taken from the heart after the animals had been sacrificed under ether anesthesia. MBS was injected to mice intraperitoneally or under ether into the retroorbital venous plexus. The volume of material injected was brought to 0.5 ml with PBS. MBS was treated with trypsin in 0.05 M phosphate buffer, pH 8.0, for 2 h at 37°C, with 5 mg trypsin (Serva) added per 2 ml MBS. After incubation, 4 mg of soybean trypsin inhibitor (Spofa) as a 1% solution in the aforesaid buffer

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