Regulatory Activity of Kupffer Cells in Acute Blood Loss

A. A. Zubakhin, S. N. Kutina, D. D. Tsyrendorzhiev, and D. N. Mayanskii

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 119, № 3, pp. 258-261, March, 1995
Original article submitted March 23, 1994

The scavenger function of Kupffer cells and the erythropoietinlike, granulocyte-macrophage colony-stimulating, and leukocyte- and lymphocyte-stimulating activities of extracts of Kupffer cells obtained before and at different times after acute massive blood loss were experimentally evaluated on mice. Extracts of Kupffer cells from normal mice are shown to exhibit all types of the studied activities. Acute blood loss reduces the scavenger function of Kupffer cells during the first few hours, especially erythropoietinlike activity. The activities return to normal levels 5 days after blood loss and, after a relatively stable period, they rise again to the end of the recovery period. This is accompanied by an increase in the number of phagocytizing cells and is evidently related to the renewal of their population.

Key Words: *blood loss; hemopoiesis; Kupffer cell*

Outcomes of acute blood loss largely depend on the system of liver nonparenchymal cells, primarily Kupffer cells (KC), which are a component of the mononuclear phagocyte system responsible for the removal of old and partially destroyed erythrocytes [10]. It has been shown in our previous studies that prestimulation of the mononuclear phagocyte system with bacterial or yeast polysaccharides, prodigiosane or zymosan, 1-2 days prior to acute blood loss primarily hastened the recovery of erythron [2], while the corresponding potentiation of the granulocyte-macrophagal component of the bone marrow occurred later.

Apart from their excellent capacity to scavenge erythrocytes, KC produce and secrete erythropoietin and other cytokines regulating hemopoiesis (interleukin-1, tumor necrosis factor-α - TNF-α, granulocyte-macrophagal colony-stimulating factor, etc.) [8,12]. This function, normally weak, becomes markedly enhanced after partial hepatectomy or liver damage with CCl_4 [9,14,17]. Moreover, it is considerably more pronounced in the prenatal and early postnatal periods [15].

This suggests that KC also play a key role in the recovery of hemopoiesis after blood loss. However, their regenerative activity in this situation is poorly understood. In the present investigation we studied hemopoiesis-, leukocyte-, and lymphocyte-stimulating activities of KC in different phases of the posthemorrhagic period.

MATERIALS AND METHODS

The experiments were carried out on 45 CBA mice of both sexes weighing 20-25 g. Blood (2.5% body weight) was drawn from the retroorbital sinus.

Blood clearance from a colloid was determined as described earlier [6]. Colloid carbon (0.2 mg/g body weight, Gunter Wagner) was injected intravenously. The half-life and the rate constant of blood clearance from the colloid (C index, min^{-1}) were calculated from the curves. The mean number of KC with phagocytized carbon particles was determined on liver sections stained with hematoxylin and eosin at ×1000 in 10 visual fields.

Laboratory of Pathological Physiology, Institute of Human General Pathology and Ecology; Siberian Division of the Russian Academy of Medical Sciences, Novosibirsk. (Presented by V. P. Kaznacheev, Member of the Russian Academy of Medical Sciences)

0007-4888/95/0003-0248$12.50

KC were isolated from the liver of intact mice and 6 hours and 2, 5, and 9 days after blood loss as described elsewhere [13]. Two hours before isolation of the cells the animals were injected intravenously with carbonyl iron R100F (1.0-1.5 mg/g body weight). The liver was perfused with cooled 0.85% NaCl and homogenized, and then iron-loaded KC were pelleted using a magnet. The obtained cells were destroyed in a hypotonic medium at 3000 rpm during 2 min and then centrifuged for 20 min at 12,000 rpm and 4°C. The protein concentration in the supernatants was determined after Lowry. In each experiment liver tissue from 5-6 mice was pooled.

Granulocyte-macrophagal colony-stimulating (GM-CSA) and erythropoietinlike (ELA) activities of KC extracts were assessed by their ability to stimulate the growth of granulocyte-macrophagal (colony-forming units - CFU-GM) and erythrocyte (CFU-E) precursors in cultured bone marrow cells. To this end the extracts of KC (100 μg protein/ml) were added to 10^5 syngeneic bone marrow cells cultured in RPMI-1640 medium supplemented with 20% fetal calf serum, 2×10^{-2} M 2-mercaptoethanol (for CFU-GM), L-glutamine (200 mg/liter), gentamicin (80 mg/liter), and 0.8% methylcellulose. Serum from anematized or zymosan-stimulated animals (for CFU-E and CFU-GM, respectively) was used as positive controls [3]. CFU-E consisting of no less than 50 cells were counted after 3 and CFU-GM after 7 days in culture at 37°C, 5% CO_2, and a humidified atmosphere in a CO_2-incubator (GPI-01, Leningrad).

Leukocyte-stimulating activity of KC was assessed by the ability of their extracts to induce a chemiluminescent response of syngeneic leukocytes. To this end 0.1 ml whole blood was mixed with 0.8 ml Hanks solution and 0.1 ml 10^{-4} M luminol and, after measurement of the background values, 0.1 ml extract of KC containing 100 μg protein or zymosan granules in a dose of 0.5 mg/ml blood were added as the positive control. The results were assessed by the peak amplitude of the chemiluminescent response and expressed in 10^{-1} cpm/granulocyte.

The lymphocyte-activating ability of the KC extracts was evaluated by proliferation of concanavalin A-treated thymocytes. To this end 0.05 ml thymocyte suspension from CBA mice (5×10^5 cells) was added to 0.05 ml 0.001% concanavalin A solution and cultured for 72 hours in the humidified atmosphere at 37°C and 5% CO_2, after which 0.05 ml KC extract (100 μg) and 0.5 μCi ^{3}H-thymidine were added 24 hours before the end of incubation. The result was assessed by incorporation of the label into proliferating thymocytes and expressed in stimulation indexes as the ratio:

$$\frac{\text{(thymocytes + concanavalin A) + KC extracts}}{\text{thymocytes+concanavalin A}}$$

The numerical data were processed statistically using the Student t test.

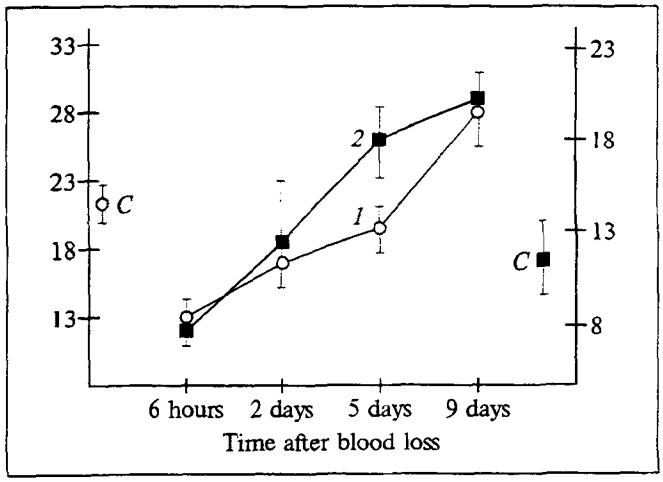

Fig. 1. Rate of blood clearance from colloid (*1*) and scavenging by KC (*2*) at different times after blood loss. Ordinates: left — rate constant of blood clearance, min^{-1}; right — number of KC scavenging carbon particles. Here and on Figs. 2 and 3: *C* = control.

RESULTS

The maximal (1.7-fold) decrease of the rate of blood clearance from the colloid in comparison with the control was observed 6 hours after blood loss ($p<0.001$, Fig. 1). The rate increased slightly on the 2nd day, though still remaining 1.3-fold lower than the control value, and reached the level

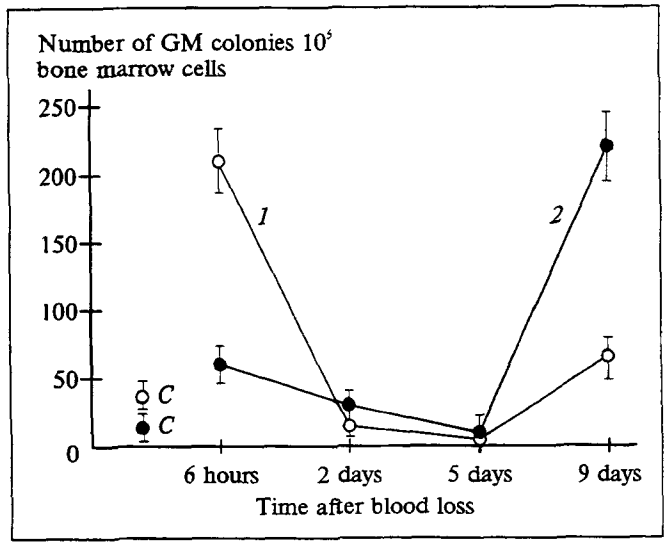

Fig. 2. ELA (*1*) and CSA (*2*) of KC extracts obtained at different times of the posthemorrhagic period.

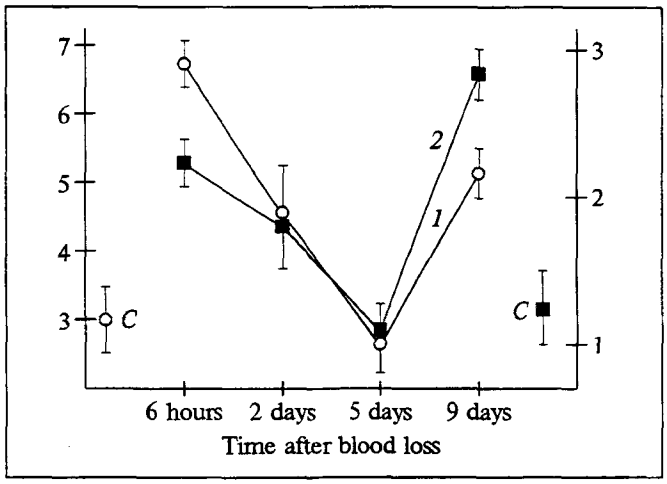

Fig. 3. Lymphocyte-activating (*1*) and leukocyte-stimulating (*2*) activities of KC extracts isolated at different times of posthemorrhagic recovery. Ordinates: left – index of stimulation of thymocyte proliferation; right – absolute values of peak of chemiluminescent response of leukocytes, $\times 10^{-1}$ cpm/granulocyte.

in control by the 5th day of recovery. After that, on day 9 the rate of blood clearance rose and surpassed the control value 1.3-fold ($p<0.01$).

The number of KC which absorbed the carbon particles was minimal 6 hours after blood loss and 1.6-fold lower than the control value ($p<0.05$, Fig. 1). By the 2nd day it reached the normal level and increased further. Five days after the blood loss the number of phagocytizing cells surpassed the control values 1.5-fold and after 9 days the increase was 1.8-fold ($p<0.01$).

Extracts of KC obtained from normal mice exhibited erythropoietinlike activity and induced the growth of 34.7±7.78 colonies per 10^5 bone marrow cells on average. Six hours after blood loss ELA of KC increased dramatically and exceeded

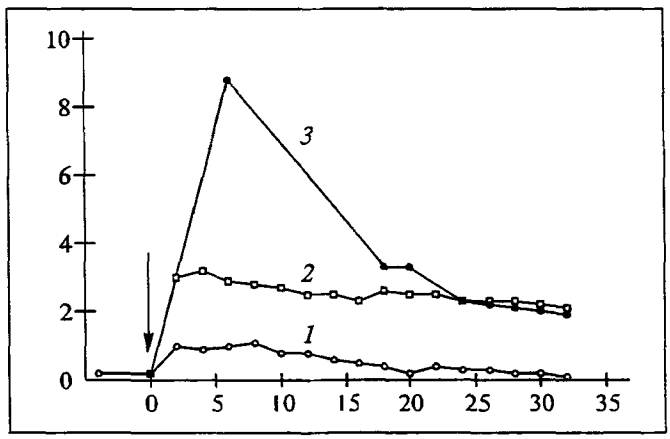

Fig. 4. Effect of KC extracts on luminol-dependent chemiluminescence of blood leukocytes. Ordinate: absolute value of leukocyte chemiluminescence, $\times 10^{-1}$ cpm/granulocyte; abscissa: time of chemiluminescent response, min. *1*) extracts of normal KC; *2*) KC extracts 9 days after blood loss; *3*) zymosan granules. Arrow indicates the time of injection of KC.

the normal level 6.3-fold ($p<0.001$). However, on the 2nd day this activity decreased, being twice as low as that in the control, and remained at this level on day 5 (Fig. 2). Toward the end of observation ELA of the extracts rose again.

Extracts of KC obtained from intact animals induced the growth of 14.3±3.99 GM colonies per 10^5 bone marrow cells (Fig. 2). Six hours after acute blood loss GM-CSA increased 4.2-fold in comparison with the norm but then this activity dropped and on day 5 did not differ from the norm. However, on day 9 of the recovery period CSA of KC extracts sharply rose again and exceeded the norm 17.1-fold.

Lymphocyte-activating ability of extracts of KC increased 2.1-fold 6 hours after blood loss. After 2 days it decreased and on day 5 did not differ from the normal value. Then on day 9 it rose again and was 1.6-fold higher than in the control (Fig. 3).

Leukocyte-stimulating activity of KC extracts obtained 6 hours after blood loss was 1.8-fold higher than that of extracts of normal KC (2.2±0.33 *vs.* 1.2±0.17×10^{-1} cpm/granulocyte, $p<0.01$). After 2 days it decreased and after 5 days practically did not differ from the normal level (Fig. 3). However, toward the end of observation it increased again and surpassed the norm 2.3-fold ($p<0.001$). In this case, the peak of the chemiluminescent response of leukocytes was 3-fold lower than that induced by zymosan, while the stimulating effect was more prolonged (Fig. 4).

Thus, during the first few hours after acute blood loss the scavenging capacity of KC was inhibited. This is possibly due to enhanced phagocytosis of inherent destroyed erythrocytes and products of hemolysis [4]. It is known that phagocytosis of IgG-erythrocytes reduces the expression of FcR and CR3 by liver macrophages [11]. Absorption of erythrocyte products leads to complex functional rearrangements of KC, among them inhibition of the respiratory burst and of the attendant production of such key inductors of destruction as reactive oxygen metabolites. This is caused by inhibited release of arachidonic acid in the course of Fc-dependent phagocytosis of opsonized IgG-erythrocytes [16].

In addition, the inhibition of the scavenging activity of KC during the first few hours after blood loss is attended by a rise of their ELA. In turn, the increased ELA suggests the involvement of KC into emergency restoration of erythron, since KC are the main producers of extrarenal erythropoietin [15]. In our experiments this function of KC could be enhanced by the action of

both tissue hypoxia and products of the partial destruction of erythrocytes (erythrodieresis). After transient normalization, ELA increased again after 1.5 weeks, i.e., to the end of the recovery period. This is most likely due to renewal of the KC population. As for GM-CSA of KC, its kinetics was similar to that of ELA. Of particular interest is the sharp rise of GM-CSA toward the end of the recovery period.

Lymphocyte- and leukocyte-stimulating activity changed in a similar manner. During the first few hours after acute blood loss they were considerably elevated. This was probably due to enhanced adsorption of endotoxin from the intestine on account of its disturbed permeability [1]. The endotoxin stimulates production of interleukin-1, TNF-α, and other leukocyte stimulators [7]. Moreover, some evidence has appeared that immediately after blood loss KC actively produce interleukin-1, TNF-α, and other regulatory proteins (cytokines) [5]. It cannot be ruled out that an endotoxin-related mechanism plays an important role not only in the stimulation of the periphery but also in the regeneration of the bone marrow.

REFERENCES

1. A. M. Gorbashko, *Diagnosis and Treatment of Blood Loss* [in Russian], Leningrad (1982).
2. A. A. Zubakhin and D. N. Mayanskii, *Pat. Fiziol.*, № 5, 62-65 (1986).
3. A. A. Zubakhin, S. N. Kutina, and D. N. Mayanskii, *Byull. Eksp. Biol. Med.*, **114**, № 7, 24-26 (1992).
4. Ya. G. Uzhanskii, *Physiological Mechanisms of Blood Regeneration* [in Russian], Moscow (1968).
5. W. Ayala, M. Perrin, P. Wang, *et al., J. Immunol.*, **147**, № 12, 4147-4154 (1991).
6. G. Biozzi, B. Benacerraf, and B. N. Halpern, *Brit. J. Exp. Path.*, **34**, 441-457 (1953).
7. S. Chensue, P. Terebuh, D. Remick, *et al., Amer. J. Pathol.*, **138**, 395-402 (1991).
8. K. Decker, in: *Cells of the Hepatic Sinusoid,* Eds. E. Wisse et al., Vol. 2, Rijswijk (1989), pp. 171-175.
9. W. Freid, J. Barone, S. Schade, *et al., J. Lab. Clin. Med.*, **93**, № 5, 700-705 (1979).
10. H. Kondo, K. Saito, J. P. Grasso, *et al., Hepatology*, **8**, № 1, 32-38 (1988).
11. D. J. Loegering, L. M. Commins, F. L. Minnear, *et al., Infect. Immun.*, **55**, 2074-2079 (1987).
12. R. N. Moore, J. T. Hoffeld, J. Farrar, *et al.*, in: *Lymphokines,* Ed. E. Pick, Vol. 3, New York (1990), pp. 119-148.
13. D. J. Nabours, D. L. Berliner, and T. F. Dougherty, *J. Reticuloendothel. Soc.*, **4**, 237-253 (1967).
14. B. A. Naughton and A. S. Gordon,in: *Reticuloendothelial System, Comparative Treatment,* Vol. 7A, New York-London (1984), pp. 147-188.
15. I. N. Rich, in: *Molecular and Cellular Aspects of Erythropoietin and Erythropoiesis.* Ed. I. N. Rich, Berlin-Heidelberg (1987), pp. 291-310.
16. M. G. Schwacha, P. W. Gudewicz, J. A. Snyder, *et al., J. Immunol.*, **150**, № 1, 236-245 (1993).
17. G. B. Zuckerman, B. A. Naughton, A. Gaito, *et al., Proc. Soc. Exp. Biol. Med.*, **176**, 197-202 (1984).