Functions of Bone Marrow Hemopoiesis-Inducing Microenvironment during CCl_4-Induced Liver Cirrhosis

A. A. Zubakhin and S. N. Kutina

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 128, No. 9, pp. 283-285, September, 1999
Original article submitted August 17, 1998

During late stages of CCl_4-induced liver cirrhosis, production of factors stimulating the growth of erythroid and granulomonocytopoietic precursors by bone marrow adherent and nonadherent fractions of BALB/c mice decreased. Stimulation with the yeast polysaccharide zymosan decreased production of these activities by various bone marrow cells, especially by the adherent fraction.

Key Words: *liver; cirrhosis; bone marrow; hemopoiesis; macrophages*

Organotypic macrophages of various origins are involved in the regulation of hemopoiesis due to their ability to produce hemopoiesis-regulating factors [1-3]. Pathogenesis of irreversible CCl_4-induced liver fibrosis is accompanied by suppression of liver macrophages *in situ* [3,4] and a sharp decrease in functional activity of other resident macrophages and their precursors (blood monocytes) [6]. Liver fibrosis is also accompanied by functional insufficiency of bone marrow hemopoiesis (inadequate reactions of the blood system to stimulatory factors) [6]. At the same time, bone marrow hemopoiesis depends on the state of the hemopoiesis-inducing microenvironment formed by bone marrow macrophages [7,11].

Here we studied the production of factors stimulating the growth of myeloid precursors by bone marrow hemopoiesis-inducing microenvironment in cirrhotic animals.

MATERIALS AND METHODS

Experiments were performed on 75 male and female BALB/c mice weighing 20-25 g. In series I, the animals were divided into 3 groups. Group 1 mice served as the control (intact animals). Group 2 mice received intraperitoneal injections of vegetable oil in a dose of 0.15 ml 2 times a week for 16 weeks. In group 3 mice, liver cirrhosis was induced with CCl_4 (20% oil solution) according to the same scheme.

In series II, all mice were intravenously injected with a zymosan suspension in a dose of 100 mg/kg body weight (2 mg/mice in 0.25 ml 0.85% NaCl) 3 days after the last injection of CCl_4. Zymosan contains glucans and, therefore, activates macrophages and stimulates hemopoiesis [13]. The mice were killed 24 h after the injection of zymosan. The total, adherent (macrophage-containing), and nonadherent fractions of bone marrow cells were isolated [15]. Erythropoietic (EPA) and colony-stimulating (CSA) activities of isolated fractions and blood serum were determined by adding one-day-old supernatants (0.1 ml) to a culture of normal syngeneic bone marrow cells. Erythroid (CFU-E) and granulomonocytic (CFU-GM) precursors were counted on days 3 and 7 of culturing, respectively. The blood was taken from the retroorbital sinus.

In mice receiving CCl_4 for 2, 8, and 16 weeks, serum CSA was evaluated before and 24 h after zymosan injection. All experiments were performed in three (or two) replicates.

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

RESULTS

In the control group, all cell fractions produced EPA, the maximum EPA being observed in the total fraction (Table 1). EPA of the adherent fraction was lower

Laboratory of Pathophysiology, Institute of Human General Pathology and Ecology, Siberian Division of the Russian Academy of Medical Sciences, Novosibirsk

0007-4888/99/0009-0903$22.00

than that of the total faction and insignificantly surpassed that in the nonadherent fraction. Cell fractions from group 2 mice displayed similar activities. In group 1 mice, stimulation with zymosan increased EPA of all cell fractions, especially of the adherent fraction. Similar effects were observed in group 2 mice. After administration of zymosan, EPA of the total, adherent, and nonadherent fractions surpassed these parameters in unstimulated mice 2.4-, 2-, and 1.5-fold, respectively.

Bone marrow cell fractions obtained from group 3 mice with CCl_4-induced liver cirrhosis displayed considerably lower EPA than the corresponding fractions obtained from groups 1 and 2 animals. This was especially typical of the total and adherent cell fractions obtained from cirrhotic mice: stimulatory effects of supernatants of these fractions on the growth of erythroid colonies were 2.3- and 2.2-fold lower, respectively, in comparison with those of fractions obtained from control animals. EPA of nonadherent cells from cirrhotic mice was lower than in control animals. The effects of zymosan on EPA of various cell fractions were different. Slight stimulatory effect was observed in the total fraction, and more pronounced in nonadherent cells, whereas EPA of the adherent fraction did not increase.

Supernatants of the total fraction obtained from intact mice induced growth of 95±9.58 CFU-GM per 10^5 bone marrow cells. CSA of supernatants of adherent and nonadherent cells was 1.6- and 1.9-fold lower than that of the total fraction (Table 1). Supernatants of bone marrow cells obtained from group 2 mice displayed insignificantly lower activity than those obtained from control animals. In group 1 mice, there was an increase in CSA of various bone marrow cell fractions 24 h after the administration of zymosan. The maximum CSA (2-fold surpassing the initial level) was noted in the adherent cell fraction. CSA of the total and nonadherent fractions increased 1.7- and 1.5-fold, respectively, compared with the initial levels. Similar changes were observed in group 2.

However, supernatants of the total and adherent fractions obtained from cirrhotic mice displayed considerably lower stimulatory effects on the growth of CFU-GM. CSA of the total, adherent, and nonadherent fractions from these mice were 1.9-, 1.7-, and 1.3-fold lower, respectively, than in group 1 animals. Zymosan injected to cirrhotic mice (group 3) decreased CSA of the total, adherent, and nonadherent fractions by 4.3, 2.5, and 3.2 times, respectively, compared with the initial levels.

In special experimental, we determined serum CSA in control animals and mice with different stages of CCl_4-induced hepatitis before and 24 h after stimulation with zymosan. CSA was not detected in the sera of groups 1 and 2 mice, while zymosan sharply increased CSA in these animals 24 h postinjection (Table 2). By contrast, this activity was present in the serum of unstimulated mice with CCl_4-induced hepatitis and decreased at the late stages of the disease. In mice injected with CCl_4 for 2, 8, and 16 weeks, zymosan enhanced this activity 7-, 5.3-, and 2.3-fold, respectively, 24 h postinjection.

Thus, at the late stages of CCl_4-induced hepatitis (liver cirrhosis), production of factors stimulating erythropoiesis and granulomonocytopoiesis by microenvironmental elements sharply decreases. These changes were most pronounced in the fraction of adherent cells containing mainly bone marrow resident macrophages [7,11] (stimulation with zymosan did not enhance by even reduced these activities) and less pronounced in the nonadherent fraction. Bone marrow macrophages and other mononuclear phagocytes are involved in the regulation of proliferation and maturation of hemopoietic precursors [3,11,12]. At the same time, functional activity of tissue macrophages in the

TABLE 1. EPA and CSA of Supernatants of Bone Marrow Cell Fractions in Mice Injected with Zymosan (ZS, *M±m*)

Cell fractions		EPA, number of CFU-E per 10^5 bone marrow cells			CSA, number of CFU-GM per 10^5 bone marrow cells		
		groups					
		1 (*n*=7)	2 (*n*=8)	3 (*n*=6)	1 (*n*=7)	2 (*n*=8)	3 (*n*=6)
Total	without ZS	68.4±5.33	61.3±5.02	26.2±5.43**	95.0±9.58	78.6±5.13	51.0±2.90**
	with ZS	161.1±7.09	146.0±5.69	34.5±3.11	162.0±9.07	131.5±16.43	11.7±0.96
Adherent	without ZS	49.6±4.13	46.5±4.65	19.7±2.70**	58.9±3.50	49.6±2.50	29.0±1.16**
	with ZS	121.3±7.20	94.0±9.87	15.0±2.90	117.1±5.43	87.2±5.43	11.5±0.96
Nonadherent	without ZS	36.0±3.33	37.9±6.01	23.0±2.32*	51.3±3.12	45.0±3.48	39.4±2.12*
	with ZS	79.0±6.12	56.1±2.12	38.0±3.48	77.0±4.27	60.9±4.27	12.2±0.38

Note. *p<0.05 and **p<0.01 compared with group 1 mice. The number of zymosan-stimulated and unstimulated mice is shown in parentheses.

Table 2. CSA of Blood Serum from Mice of Various Groups ($M \pm m$, $n \geq 5$)

Group		CSA, number of CFU-GM per 10^5 bone marrow cells	
		before zymosan	24 h after injection of zymosan
Intact		0	108.0±5.09
2 weeks	oil	0	114.6±8.37
	CCl_4	17.6±1.25	125.0±5.40
8 weeks	oil	0	135.0±9.76
	CCl_4	11.0±0.37	59.0±2.99
16 weeks	oil	0	82.0±5.11
	CCl_4	8.3±0.53	22.0±3.20

liver, lungs, and peritoneum and clearing functions of the mononuclear phagocyte system were reported to decrease in animals with developed CCl_4-induced liver cirrhosis [5]. These data suggest that the reduced capacity of bone marrow macrophages to maintain proliferation and maturation of hemopoietic precursors reflects depression of the mononuclear phagocyte system. Production of CSA is typical of all components of the mononuclear phagocyte system [9,10]. Hence, the sharp decrease serum in CSA in zymosan-stimulated and unstimulated mice with CCl_4-induced liver cirrhosis can result from inhibition of the hemopoiesis-regulating activity of mononuclear phagocytes.

REFERENCES

1. E. D. Gol'dberg, A. M. Dygai, and V. P. Shakhov, *Methods of Tissue Culture in Hematology* [in Russian], Tomsk (1992).
2. A. A. Zubakhin, S. N. Kutina, and D. N. Mayanskii, *Byull. Eksp. Biol. Med.*, **113**, No. 5, 225-233 (1992).
3. S. A. Ketlinskii and N. M. Kalinina, *Immunologiya*, No. 3, 30-44 (1995).
4. S. N. Kutina and D. N. Mayanskii, *Byull. Eksp. Biol. Med.*, **92**, No. 9, 366-369 (1981).
5. D. N. Mayanskii, *Chronic Inflammation* [in Russian], Moscow (1991).
6. D. N. Mayanskii, Ya. Sh. Shvarts, D. D. Tsyrendorzhiev, and S. N. Kutina, *Byull. Eksp. Biol. Med.*, **105**, No. 2, 214-216 (1988).
7. H. Mayani, L. J. Guibert, and A. Janowska-Wieczorek, *Eur. J. Haematol.*, **49**, No. 5, 225-233 (1992).
8. D. N. Mayanski, Y. S. Schwartz, S. N. Kutina, *et al.*, *Int. J. Exp. Pathol.*, **74**, 229-236 (1993).
9. D. Metcalf, *C. R. Acad. Sci. [III]*, **316**, No. 9, 316-319 (1993).
10. D. Metcalf, *Leukemia*, **11**, No. 10, 1599-1604 (1997).
11. T. Nagao, *Tokai J. Exp. Clin. Med.*, **20**, No. 2, 121-130 (1995).
12. M. Ogawa, *J. Allergy Clin. Immunol.*, **94**, No. 3, 645-650 (1994).
13. M. L. Patchen and T. J. MacVittie, *Int. J. Immunopharmacol.*, **7**, No. 6, 921-932 (1985).
14. P. J. Quesenberry and P. A. Lowry, *Cancer*, **70**, No. 4, 343-347 (1992).
15. E. D Zanjani, P. B. McGlave, E. S. Davies, *et al.*, *Br. J. Haematol.*, **50**, No. 3, 479-490 (1982).