Early and Late Hemopoietic Precursor Cells in Patients with Chronic Myeloproliferative Diseases

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We evaluated the content of early and late cobblestone area-forming cells, immediate progeny of hemopoietic stem cells, and committed precursor cells in the bone marrow and peripheral blood of patients with chronic myeloproliferative diseases and healthy donors. In patients with essential thrombocythemia, the number of late cobblestone area-forming cells in the peripheral blood decreased, while other parameters did not differ from those in healthy donors. In patients with idiopathic myelofibrosis, we found a decreased number of late and early cobblestone area-forming cells in the bone marrow and late cobblestone area-forming cells in the peripheral blood, while the count of early cobblestone area-forming cells in the peripheral blood increased. In patients with chronic myeloid leukemia, the number of early cobblestone area-forming cells in the bone marrow decreased, but the count of late and early cobblestone area-forming cells in the peripheral blood increased. The number of endogenous committed precursor cells in the peripheral blood increased in all groups of patients with chronic myeloproliferative diseases and, particularly, in patients with idiopathic myelofibrosis and chronic myeloid leukemia. Functional characteristics of immediate descendants of hemopoietic stem cells probably reflect the level of damage and attest to the existence of various mechanisms underlying the defect of the hemopoietic stem cell during chronic myeloproliferative diseases.

Key Words: *hemopoietic precursor cells; hemopoietic stem cells; cobblestone area-forming cells; committed precursor cells; chronic myeloproliferative diseases*

Chronic myeloproliferative diseases represent a group of disorders associated with clonal disturbances in the hemopoietic stem cell [2]. These diseases are characterized by proliferation of one or several hemopoietic stems. It primarily concerns the granulocyte-erythroid stem. Chronic myeloproliferative diseases include essential thrombocythemia, erythremia, idiopathic myelofibrosis (IMF), and chronic myeloid leukemia. Functional activity of hemopoietic stem cells can be evaluated by the number of hemopoietic cobblestone area-forming cells (CAFC) in contact with stromal cells in *in vitro* system. In the hierarchy of human hemopoietic precursor cells, CAFC are closest to the hemopoietic stem cell [13]. Quantitative analysis of CAFC makes it possible to evaluate changes in hemopoietic precursor cells in patients with hemopoiesis abnormalities [8,11]. A relationship exists between the number of CAFC and repopulating stem cells in irradiated mice [5]. There are late and early CAFC. Late CAFC are more mature descendants of the hemopoietic stem cell; they appear in culture from the first to the fifth week. Early CAFC are present in the culture only

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after 5 weeks. They are primitive descendants of human hemopoietic stem cells [4].

Here we studied hemopoiesis (immediate progeny of the hemopoietic stem cell) in patients with chronic myeloproliferative diseases.

MATERIALS AND METHODS

Experiments were performed with cells of the bone marrow and peripheral blood from 24 patients with chronic myeloproliferative diseases (9 patients with essential thrombocythemia, 3 patients with IMF, and 12 patients with chronic myeloid leukemia) and 10 healthy donors. The study was conducted before the start of therapy. The cells were obtained from the sternal punctate of the bone marrow and peripheral blood. Mononuclear cells of the bone marrow and peripheral blood were isolated in a Ficoll density gradient (1.077 g/cm^2, Lymphoprep, Gibco/BRL).

Mononuclear cells of the bone marrow and peripheral blood from patients with chronic myeloproliferative diseases and healthy donors were cultured on the stromal sublayer in 96-well plates (Costar) using the method of limiting dilutions [3, 10]. MS-5 mouse cells maintaining the growth of human CAFC *in vitro* were used as the standard stromal sublayer [7]. Hemopoietic cells were explanted on MS-5 stromal cells in 0.2 ml nutrient medium. We used 4 consecutive dilutions of the bone marrow (25, 12.5, 6.0, and 3.0×10^3 cells per well) and blood (50, 25, 12.5, and 6.2×10^3 cells per well). Each concentration was used in 15 repetitions. The cells were cultured in α-MEM complete nutrient medium (ICN) containing 12.5% fetal bovine serum, 12.5% horse serum, 1% L-glutamine (200 mM), 1% 2-mercaptoethanol (10^{-4} mol/liter), 1% folic acid (Sigma), 1% inositol (Sigma), hydrocortisone succinate (10^{-6} mol/liter, Sigma), and antibiotics. The cells were cultured at 37°C, 5% CO_2, and 100% humidity for 5-9 weeks. The number of negative wells not containing cobblestone areas was evaluated weekly for 9 weeks. The ratio of precursors was estimated by the Poisson method for CAFC assay described by R. E. Ploemacher *et al*. [10]. The efficiency of CAFC cloning per 10^5 nucleated cells was determined using computer software created at the laboratory of O. A. Mamikonova. The focuses containing more than 5 cells were considered as CAFC. The medium was half-replaced weekly. The count of late CAFC was expressed as the mean number of CAFC from the 1st to the 5th week of culturing. The count of early CAFC was expressed as the average number of CAFC from the 6th to the 9th week of culturing.

Committed granulocyte-macrophage precursor cells (CFU-GM) were studied in semisolid agar medium by the standard method [1]. Hemopoietic cells were cultured in agar in the presence and absence of colony-stimulating factor (CSF). Medium conditioned by U5637 cells served as CSF [14].

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

RESULTS

CAFC were found in the bone marrow and peripheral blood after 9-week culturing of hemopoietic cells from patients with chronic myeloproliferative diseases and healthy donors (Fig. 1). CAFC num-

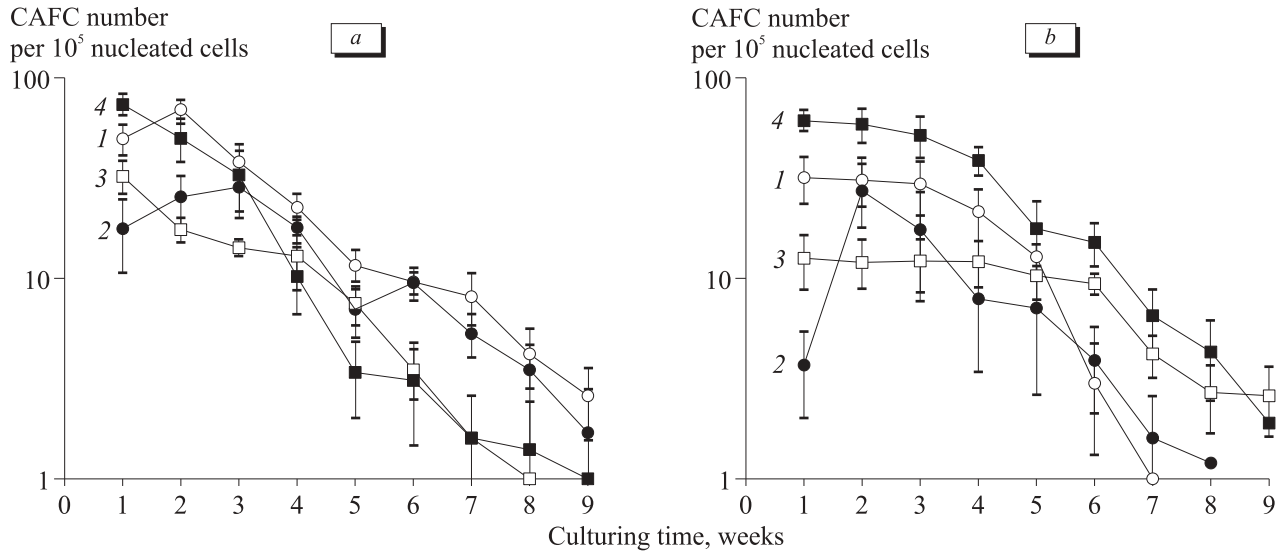

Fig. 1. Number of CAFC in the bone marrow (*a*) and peripheral blood (*b*) of patients with chronic myeloproliferative diseases and healthy donors. Here and in Fig. 2: healthy donors (*1*), essential thrombocythemia (*2*), IMF (*3*), and chronic myeloid leukemia (*4*).

ber in the bone marrow and peripheral blood from healthy donors did not differ over the first 5 weeks of culturing. In the follow-up period, the number of CAFC in the peripheral blood was lower than in the bone narrow ($p<0.001$). CAFC number in the bone marrow and peripheral blood from patients with essential thrombocythemia decreased over the first weeks of incubation. In the follow-up period, the number of CAFC in the bone marrow and peripheral blood from patients with essential thrombocythemia did not differ from that in healthy donors. Over the 1st week of culturing, the number of CAFC in the bone marrow from patients with IMF and chronic myeloid leukemia did not differ from that in healthy donors. As differentiated from healthy donors, the number of CAFC in the bone marrow of these patients sharply decreased after the 4th week of culturing. Over the first weeks of culturing, CAFC number in the peripheral blood from IMF patients was lower than in healthy donors ($p<0.05$). After the 6th week, this parameter in patients was higher than in healthy donors. From the 1st to the 9th week of culturing, CAFC number in the peripheral blood from patients with chronic myeloid leukemia was higher than in healthy donors. CAFC number in the peripheral blood was higher than in the bone marrow of these patients.

Comparative analysis of late and early CAFC in patients with chronic myeloproliferative diseases and healthy donors (Fig. 2) showed that the number of late CAFC in the bone marrow and peripheral blood from healthy donors was higher than the count of early CAFC (by 6 and 23 times, respectively). The number of late CAFC in the bone marrow from patients with essential thrombocythemia was lower than in healthy donors ($p<0.001$). However, the number of late CAFC in patients with chronic myeloid leukemia did not differ from normal. The number of early CAFC in the bone marrow from patients with essential thrombocythemia did not differ from than in healthy donors. The number of early CAFC in patients with IMF and chronic myeloid leukemia was lower than in healthy donors ($p<0.01$). We revealed decreased number of early CAFC and low or normal content of late CAFC in the bone marrow of patients with chronic myeloid leukemia. Our results are consistent with published data that malignant hemopoietic stem cells exhibit low self-maintenance ability and high differentiation activity [9]. The number of late CAFC in the peripheral blood of patients with essential thrombocythemia and IMF was lower than in patients with chronic myeloid leukemia. The number of late CAFC in patients with chronic myeloid leukemia 6-fold surpassed the normal ($p<0.001$). The number of early CAFC in the peripheral blood increased in patients with IMF and chronic myeloid leukemia. The number of early CAFC in patients with essential thrombocythemia did not differ from normal.

The decreased number of late CAFC and normal content of early CAFC in the bone marrow and peripheral blood of patients with essential thrombocythemia indicate that disturbances in hemopoietic stem cells mainly concern late CAFC.

No correlation was found between the numbers of CFU-GM and CAFC in patients with chronic myeloproliferative diseases. Endogenous CFU-GM in the bone marrow and peripheral blood were identified in some individuals in each group of patients with chronic myeloproliferative diseases. The number of CFU-GM in these patients was much higher than in healthy donors. High content of spontane-

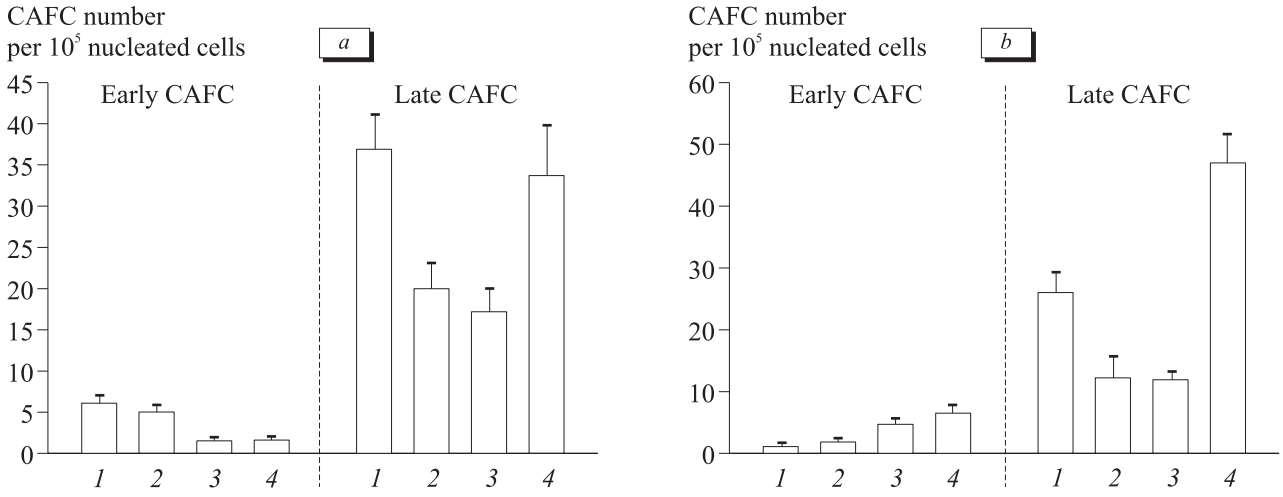

Fig. 2. Number of early and late CAFC in the bone marrow (*a*) and peripheral blood (*b*) from patients with chronic myeloproliferative diseases and healthy donors.

TABLE 1. Content of CFU-GM in Bone Marrow and Peripheral Blood of Patients with Chronic Myeloproliferative Diseases and Healthy Donors (per 10^5 Nucleated Cells)

Group		Bone marrow		Blood	
		-CSF	+CSF	-CSF	+CSF
Healthy donors (*n*=6)	median	0	108.0	3.3	5.6
	M±*m*	1.3±0.9	103.0±18.0	3.7±0.6	6.1.±3.4
	CI	0-6.7	27.2-172.0	0-7.7	0.2-9.3
Essential thrombocythemia (*n*=9)	median	0	40.7	2.6	2.6
	M±*m*	4.3±2.8	71.3±21.1	3.7±1.5	9.9±5.7
	CI	0-35.5	0-259.0	0-14.4	0-53.0
IMF (*n*=3)	median	0	139.0	23.4	30.3
	M±*m*	4.2±4.1	100.7±49.2	35.4±23.7	55.2±39.7
	CI	0-12.5	4.0-165.0	0-80.0	2.2-133.0
Chronic myeloid leukemia (*n*=9)	median	0	130.7	18.7	72.4
	M±*m*	30.5±22.2	188.3±52.6	36.0±11.4	77.4±13.9
	CI	0-200	14.7-455.0	0-70.6	23.7-135.0

Note. CI, confidence interval.

ous granulocytic colonies in patients with chronic myeloproliferative diseases reflects the presence of malignant hemopoietic cells, whose proliferation is impaired and does not depend on normal regulatory factors. The number of spontaneous and stimulated CFU-GM in the peripheral blood increased in most patients with IMF and chronic myeloid leukemia. This parameter also increased in the bone marrow of patients with chronic myeloid leukemia (Table 1), which is consistent with published data [6,12]. A sharp increase in the number of bone marrow CFU-GM is an unfavorable prognostic sign. Our results suggest that the peripheral blood of patients with chronic myeloid leukemia and IMF carries malignant hemopoietic stem cells produced in focuses of extramedullary hematopoiesis, but not in the bone marrow.

In patients with chronic myeloid leukemia, the number of CFU-GM and late CAFC in the bone marrow and peripheral blood increases, while the count of early CAFC in the bone marrow decreases. This attests to high level of damage to hemopoietic stem cell. All early hemopoietic precursor cells enter differentiation, which leads to the formation of numerous late CAFC, CFU-GM, and mature cells. The excess number of early CAFC and spontaneous CGU-GM in the peripheral blood from patients with chronic myeloid leukemia and IMF is probably a result of intensive proliferation and differentiation of malignant hemopoietic stem cells and/or changes in their interaction with stromal microenvironment leading to migration of early CAFC, primitive descendants of the defective hemopoietic stem cell, into the blood.

Functional characteristics of primitive descendants of hemopoietic stem cells probably reflect different levels of damage and different mechanisms underlying the defect in hemopoietic stem cells during chronic myeloproliferative diseases.

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REFERENCES

1. L. P. Gerasimova, T. E. Manakova, N. V. Tsvetaeva, *et al*., *Byull. Eksp. Biol. Med*., **127**, No. 1, 14-18 (1999).
2. B. Anger, J. W. Janssen, H. Schrezenmeier, *et al*., *Leukemia*, **4**, No. 4, 258-261 (1990).
3. L. Benboubker, C. Binet, G. Gartron, *et al*., *Exp. Hematol*., **30**, No. 1, 74-81 (2002).
4. D. A. Breems, E. A. Blokland, S. Neben, and R. E. Ploemacher, *Leukemia*, **8**, No. 7, 1095-1104 (1994).
5. R. H. Cho and C. E. Muller-Sieburg, *Exp. Hematol*., **28**, No. 9, 1080-1086 (2000).
6. M. D. Colovic, P. H. Wiernik, G. M. Jankovic, *et al*., *Eur. J. Haematol*., **62**, No. 3, 155-159 (1999).
7. L. Kobari, A. Dubart, F. Le Pesteur, *et al*., *J. Cell. Physiol*., **163**, No. 2, 295-304 (1995).
8. R. Pettengell, T. Luft, R. Henschler, *et al*., *Blood*, **84**, No. 11, 3653-3659 (1994).
9. A. L. Petzer, C. J. Eaves, M. J. Barnett, and A. C. Eaves, *Ibid*., **90**, No. 1, 64-69 (1997).
10. R. E. Ploemacher, J. P. van der Sluijs, J. S. Voerman, and N. H. Brons, *Ibid*., **74**, No. 8, 2755-2759 (1989).
11. L. Ponchio, E. Conneally, and C. Eaves, *Ibid*., **86**, No. 9, 3314-3321 (1995).
12. V. Sagaster, E. Jager, A. Weltermann, *et al*., *Haematologica*, **88**, No. 11, 1204-1212 (2003).
13. H. J. Sutherland, P. M. Lansdorp, D. H. Henkelman, *et al*., *Proc. Natl. Acad. Sci. USA*, **87**, No. 9, 3584-3588 (1990).
14. K. Welte, E. Platzer, L. Lu, *et al*., *Ibid*., **82**, No. 5, 1526-1530 (1985).