Functional Activity of Hemopoietic and Stromal Cells in Various Types of Myelodysplastic Syndrome

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Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 127, No. 1, pp. 14-18, January, 1999
Original article submitted December 3, 1997

Using clonal methods for assessment of hemopoietic and stromal cells and long-term bone marrow cell cultures, we have demonstrated heterogeneity of myelodysplastic syndrome. Low content of stromal precursor cells in native bone marrow, peculiarities in the formation of the stromal layer and its hemopoiesis-stimulating capacity in long-term cultures, and altered properties of stromal precursor cells in long-term cultures indicate defect in the stromal microenvironment in myelodysplastic syndrome.

Key Words: *myelodysplastic syndrome; hemopoietic and stromal precursor cells; stromal microenvironment*

Myelodysplastic syndrome is a group of hemopoietic disturbances associated with abnormalities of polypotent hemopoietic stem cell [3]. Proliferation and differentiation of this defective stem cell suppress hemopoiesis and sometimes result in replacement of the bone marrow with a malignant cell clone. This clonal evolution is accompanied by accumulation of chromosome aberrations and results in acute leukemia [7].

Hemopoiesis requires close contact with stromal microenvironment which produces cytokines regulating proliferation and differentiation of the hemopoietic stem cell. Impaired interaction between hemopoietic and stromal cells causes severe hemopoietic disturbances. There are contradictory data on the disturbances in stromal microenvironment in patients with myelodysplastic syndrome: some authors reported changes in the stroma [4,14], while others observed no abnormalities [9].

Using long-term bone marrow cell cultures, we studied functional activity of hemopoietic and stromal cells in patients with myelodysplastic syndrome.

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MATERIALS AND METHODS

Bone marrow cells from 28 patients with different forms of myelodysplastic syndrome were used: refractory anemia (n=8), sideroblast anemia (n=3), refractory anemia with excess of blasts (n=4) and that in transformation (n=5), and chronic myeloleukemia (n=8). Bone marrow from 32 healthy donors obtained during sternal puncture or costectomy served as the control.

Sternal puncture samples (1-2 ml) were placed in a flask containing 50 U/ml heparin, puffy coat after erythrocyte sedimentation was gathered, washed with phosphate buffered saline, and nucleated cells were counted and cultured in an *in vitro* system.

Colony-forming unit granulocyte-macrophage (CFU-GM) and more mature cluster-forming cells (CFC) were studied in a bilayer agar system [11].

Bone marrow cells (10^5) were cultured in 1 ml 0.3% McCoy's 5A medium containing 1% sodium bicarbonate (7.5%), 1% sodium pyruvate (100 mM), 1% vitamins (100x), MEM amino acids: 1% essential (50x), 0.5% nonessential amino acids (100x) and 1% L-glutamine (200 mM), 15% fetal calf serum, 3% antibiotics (all reagents from ICN), and 10% bactoagar (Difco), on a feeder prepared from donor peripheral

0007-4888/99/0001-0010 $22.00

blood leukocytes (10^6 cell/ml) in 0.6% complete agar medium. Each sample was seeded to three Petri dishes.

Erythrocyte burst-forming units (BFU-E) were evaluated in methyl cellulose cultures of mononuclear cells isolated from the bone marrow in a Ficoll density gradient [7]. Bone marrow mononuclears (2×10^5/ml) were resuspended in α-MEM medium (ICN) containing 30% fetal bovine serum, 2% L-glutamine (200 mM), 10^{-4} M 2-mercaptoethanol (Serva), 1% bovine serum albumin (fraction V, Gibco), 2% HEPES, antibiotics, 10% U 5637 cell-conditioned medium, 2 U/ml erythropoietin (Boehringer Mannheim) and 1% methyl cellulose (Sigma) in α-MEM medium. The cultures were incubated in 24-well plates (0.5 ml/well, 4 wells for each sample).

Stromal precursor cells, fibroblast colony-forming units (CFU-F) were analyzed in monolayer cultures as described elsewhere [9] with our modifications [1]. Bone marrow cells (10^5) were transferred to a 35-mm Petri dish in 2 ml α-MEM medium containing 2% L-glutamine (200 mM), 5% fetal calf serum, 5% heat-inactivated human serum, and antibiotics (3 dishes for each sample).

All cultures were incubated at 37°C, 100% humidity, and 5% CO_2 for either 10 (CFU-GM, CFC, CFU-F) or 14 days (BFU-E).

Aggregates containing 50 or more cells were considered as colonies, while those containing less than 50 cells were counted as clusters. Aggregates containing 500 or more cells or 3 or more colonies were considered as bursts. The efficiency of cloning of hemopoietic and stromal precursor cells per 10^5 nucleated cells was calculated.

Long-term bone marrow cell cultures were maintained in 25-cm^2 flasks as described previously [5]. Myelokaryocytes ($1.5\text{-}2\times10^7$ cells) were placed in 8 ml complete McCoy's 5A medium containing 1% sodium bicarbonate (7.5%), 1% sodium pyruvate (100 mM), 1% MEM vitamins (100x), MEM amino acids: 0.8% essential (50x), 0.4% nonessential amino acids (100x), 12.5% fetal calf serum, 12.5% horse serum (ICN), 1% L-glutamine (200 mM), 10^{-6} M hydrocortisone hemisuccinate (Sigma), and antibiotics. The cells were cultured at 33°C for 9 weeks. Half of the culture medium was weekly replaced with fresh portion, the number of viable cells, CFU-GM and CFU-F in the supernatant was determined. CFU-GM were assayed in 24-well plates using U 5637 cell-conditioned medium as a colony-stimulating factor.

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

RESULTS

Bone marrow cultures from 28 patients with myelodysplastic syndrome and 32 donors contained the same number of CFU-GM (Table 1), while the number of CFC was higher in patients.

The content of BFU-E and CFU-F in the bone marrow from patients was considerably below the normal, which agrees with previous data on a defect in the population of committed cells in patients with myelodysplastic syndrome.

Different types of myelodysplasia considerably varied in the content of bone marrow stromal and hemopoietic precursor cells (Fig. 1). Low content of CFU-GM was typical of patients with refractory anemia. Patients with sideroblast anemia had low or normal content of CFU-GM and CFC. In patients with refractory anemia with excess of blasts and that in transformation both increased and decreased contents of CFU-GM were found. This anemia was often accompanied by increased number of CFC, which usually was associated with aggravation of the disease. Sometimes the content of CFC in the bone marrow 30-40 times surpassed the number of colonies. In patients with chronic myelomonocytic leukemia the bone marrow content of CFU-GM was considerably increased ($p<0.001$). The number of BFU-E in the bone marrow was below normal in all types of myelodysplastic syndrome ($p<0.001$). The content of stromal precursor cells determined by the number of CFU-F did not differ from normal in refractory anemia and refractory anemia with excess of blasts, but was decreased in sideroblast anemia, refractory anemia with excess of blasts in transformation, and in chronic myelomonocytic leukemia ($p<0.001$).

TABLE 1. Content of Hemopoietic and Stromal Precursor Cells in the Bone Marrow of Donors and Patients with Myelodysplastic Syndrome ($M\pm m$)

Group	CFU-GM	CFC	BFU-E	CFU-F
Donors (n=32)	43.0±3.0 (25.0-84.0)	51.0±5.0 (21.0-114.0)	18.8±1.6 (12.0-28.8)	13.0±2.0 (6.0-26.0)
Myelodysplastic syndrome (n=28)	38.4±10.6 (0-170.6)	202.9±88.0 (1.4-1405.0)*	2.8±0.9 (0-12.0)*	6.5±1.8 (0-27.2)*

Note. *$p<0.01$ compared with donors.

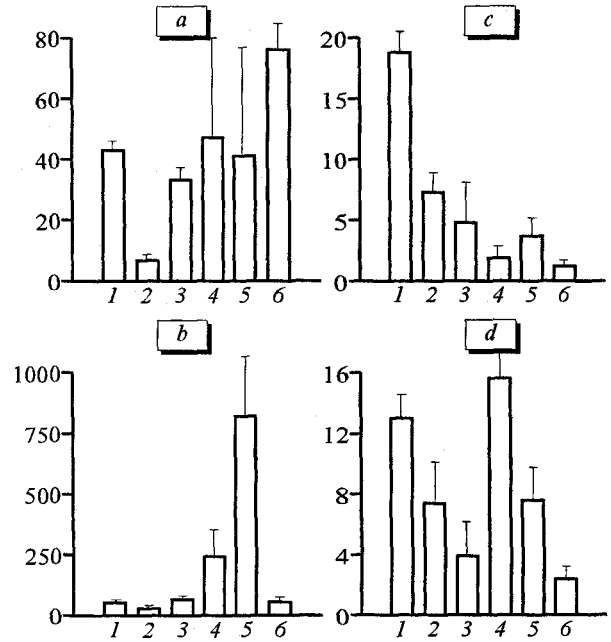

Fig. 1. Content of hemopoietic and stromal precursor cells in the bone marrow of donors and patients with myelodysplastic syndrome. Ordinate: number of hemopoietic and stromal precursor cells per 10^5 nucleated cells. *a*) granulocyte-macrophage colony-forming units; *b*) cluster-forming cells; *c*) erythrocyte burst-forming units; *d*) fibroblast colony-forming units. Here and on Fig. 2: *1*) donors; *2*) refractory anemia; *3*) sideroblast anemia; *4*) refractory anemia with excess of blasts; *5*) refractory anemia with excess of blasts in transformation; 6) chronic myelomonocytic leukemia.

Thus, these data prove heterogeneity of myelodysplastic diseases and quantitative abnormalities in the bone marrow committed hemopoietic and stromal precursor cells in patients with these diseases.

Further experiments were carried out on long-term cultures (*in vitro* modeled hemopoiesis) maximally approximating *in vivo* conditions. Formation of stromal sublayer and proliferation of hemopoietic cells were observed in all long-term cultures. However, there were some peculiarities of the stroma and hemopoiesis in different types of myelodysplastic syndrome. In donors and patients with refractory anemia, stromal sublayer attained confluence on weeks 3-4 in culture, while in chronic myelomonocytic leukemia this process took about 2-3 weeks and the stromal sublayer contained no adipocytes. In 3 out of 4 patients with refractory anemia the stromal sublayer attained no confluence and consisted of solitary fibroblast colonies. Stromal sublayer in refractory anemia and chronic myelomonocytic leukemia as well as in donors maintained proliferation and differentiation of hemopoietic cells for a long time, whereas in refractory anemia with excess of blasts hemopoiesis decayed at the 5th-6th week in culture. During the first 5 weeks in culture the number myelokaryocytes among nonadherent cells in donors and patients with chronic myelomonocytic leukemia, refractory and sideroblast anemia was the same, while later high content of hemopoietic cells was noted only in cultures from donors and patients with chronic myelomonocytic leukemia and refractory anemia (Fig. 2, *a*).

In all cultures, suspension of nonadherent cells contained CFU-GM (Fig. 2, *b*). Cultures from patients with refractory and sideroblast anemia and refractory anemia with excess of blasts contained few CFU-GM, while in patients with chronic myelomonocytic leukemia the content of CFU-GM was high throughout the 9-week culturing.

In all cultures from donors and patients with myelodysplastic syndrome, the nonadherent cell suspension contained some CFU-F, which persisted up to the 3rd-

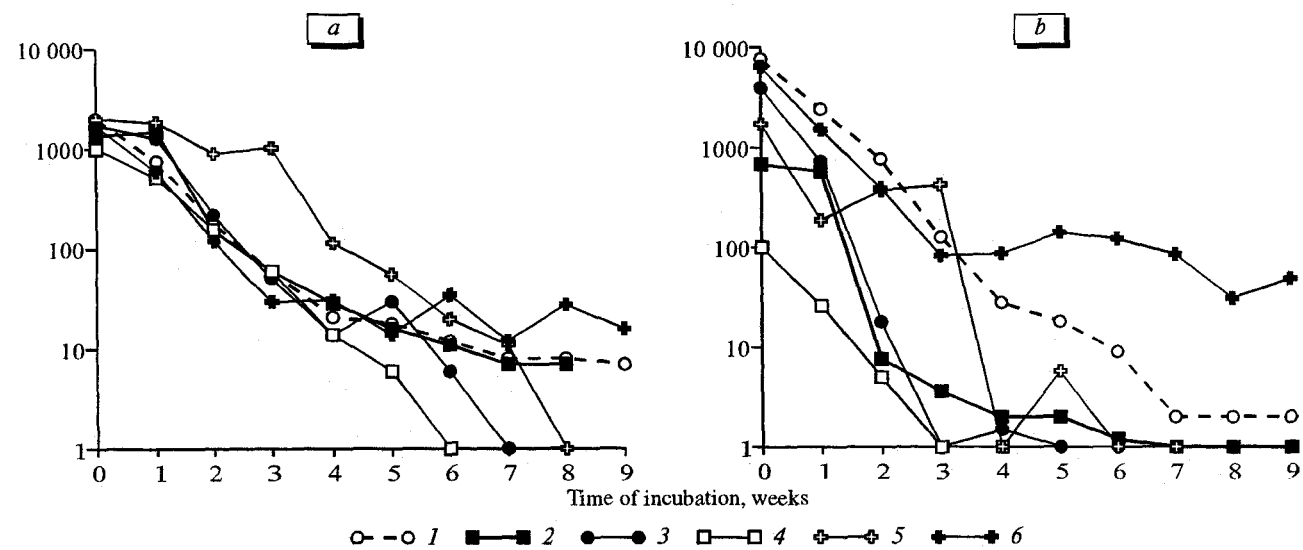

Fig. 2. Number (per flask) of hemopoietic cells (*a*) and granulocyte-macrophage precursor cells (*b*) in long-term bone marrow cell cultures from donors and patients with myelodysplastic syndrome.

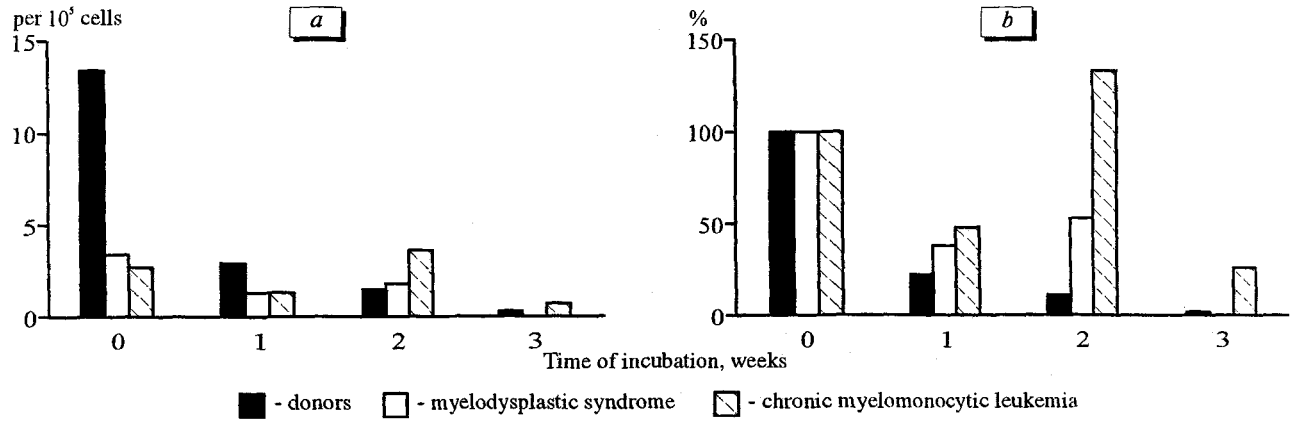

Fig. 3. Content of fibroblast colony-forming units (CFU-F) in long-term bone marrow cell cultures from donors and patients with myelodysplastic syndrome. *a*) number of CFU-F per 10^5 nonadherent cells (median); *b*) nonadherent CFU-F, %.

4th week in culture presumably until the formation of the stromal sublayer was completed (Fig. 3, *a*). However, the ratio of CFU-F in the suspension of CFU-F initially seeded (in %) was higher in patients (Fig. 3, *b*) particularly in the group with chronic myelomonocytic leukemia (48-133% on weeks 2-3 in culture). These findings attest to detachment of CFU-F from the stromal sublayer to the suspension. High content of CFU-F in cultures from patients with chronic myelomonocytic leukemia probably reflects changes in stromal microenvironment, in particular in CFU-F adhesiveness.

Thus, our experiments revealed abnormalities in the closest offspring of the hemopoietic stem cell in patients with myelodysplastic syndrome. The low content of CFU-F in native bone marrow, some peculiarities of the formation of the stromal sublayer and its hemopoiesis-stimulating capacity as well as altered properties of CFU-F in long-term bone marrow cultures indicate disturbances in stromal microenvironment in myelodysplastic syndrome. Other investigators also described defects of stromal microenvironment, in specifically impaired cytokine secretion [10] and inability to maintain normal hemopoiesis in long-term bone marrow cultures [2,6,13].

The observed functional changes in hemopoietic and stromal cells in different types of myelodysplastic syndrome are most pronounced in patients with chronic myelomonocytic leukemia: intense proliferation of CFU-GM in original bone marrow and long-term cultures together with low content of CFU-F in the original bone marrow and high functional activity of the stromal sublayer in long-term cultures. Our findings prove heterogeneity of myelodysplastic diseases artificially combined in a common group, improve our understanding of pathogenesis of these diseases, and can be useful in the development of new approaches to the treatment of preleukemia.

The study was supported by the Russian Foundation for Basic Research (grant No. 96-04-49063).

REFERENCES

1. L. P. Gerasimova, N. S. Kislyak, E. A. Mamedova, and N. A. Finogenova, *Gematol. Transfusiol.*, No. 3, 16-18 (1991).
2. L. J. Bendall, K. Kortlepel, and D. J. Gottlieb, *Exp. Hematol.*, **25**, 132-139 (1997).
3. J. M. Bennett, D. Catovsky, M. T. Daniel, and G. Flandrin, *Br. J. Haematol.*, **33**, 189-195 (1982).
4. L. H. Countinho, C. G. Geary, J. Chang, *et al.*, *Ibid.*, **75**, 1-10 (1990).
5. S. Gartner and H. S. Kaplan, *Proc. Natl. Acad. Sci. USA*, **77**, 4756-4759 (1980).
6. L. Holmberg, K. Seidel, W. Leisenring, and B. Torok-Storb, *Exp. Hematol.*, **22**, 741 (1990).
7. N. V. Iscove and F. Sieber, *Ibid.*, 3, 32-43 (1975).
8. A. Jacobs, *Eur. J. Cancer.*, **1142**, 1113-1115 (1990).
9. S. Kaneko, S. Motomura, H. Ibayashi, *et al.*, *Br. J. Haematol.*, **51**, 217-225 (1982).
10. M. Mencoboni, G. Castello, R. Lerza, *et al.*, *Eur. J. Haematol.*, **56**, 148-152 (1996).
11. P. L. Pike and W. A. Robinson, J. *Cell. Physiol.*, **76**, 77-84 (1970).
12. T. Ruutu, S. Partanen, R. Lintula, *et al.*, *Scand. J. Haematol.*, **32**, 395-402 (1984).
13. G. B. Tennat, L. N. Truran, and A. K. Burnet, *Br. J. Haematol.*, **93**, 66-67 (1996).
14. D. Zipori, N. Reichman, L. Arcavi, *et al.*, *Exp. Hematol.*, **13**, 603-609 (1985).