In Vivo Production of Cytokines by Bone Marrow Stromal Cells and Macrophages from Patients with Myelodysplastic Syndrome

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We studied functional disturbances in hemopoietic microenvironment and cytokine production by stromal sublayer in long-term bone marrow cultures and peripheral blood macrophages from patients with various forms of myelodysplastic syndrome. Production of factors stimulating the growth of normal erythroid and granulocytic precursors by cells of the stromal sublayer from patients with refractory sideroblast anemia and refractory anemia with excess blasts is impaired compared to cells from healthy donors. The medium conditioned by macrophages from patients with chronic myelomonocytic leukemia displayed a higher ability to stimulate the growth of granulocytes and macrophages compared to media conditioned by cells from donors and patients with refractory sideroblast anemia and refractory anemia with excess blasts. Cultured stromal cells and macrophages produced tumor necrosis factor-α and interleukin-6. Their content in media conditioned by cells from patients with myelodysplastic syndrome surpassed that in healthy donors. Our results suggest that production of cytokines by stromal microenvironmental cells is impaired in patients with various forms of myelodysplastic syndrome.

Key Words: *hemopoietic microenvironment; stromal cells; macrophages; cytokines; myelodysplastic syndrome; erythroid burst-forming precursors; granulocyte-macrophage precursors; tumor necrosis factor-α; interleukin-6*

Hemopoietic disturbances in patients with myeloproliferative disorders result from either abnormalities in hemopoietic stem cells or functional disturbances in stromal microenvironment. Myelodysplastic syndrome (MDS) is a heterogeneous group of diseases with clonal defects of hemopoiesis characterized by inefficient hemopoiesis and refractory cytopenia [9]. These diseases are characterized by bone marrow hypercellularity and peripheral blood cytopenia due to apoptosis of hemopoietic cells, which can be induced by hemopoietic and stromal cells [13]. Little is known about dysfunction of the hemopoietic microenvironment in patients with MDS. Recent studies revealed functional abnormalities of stromal cells in patients with MDS. These abnormalities manifested in impaired formation of stromal sublayers in long-term bone marrow cultures, decreased ability of these sublayers to maintain *in vitro* proliferation and differentiation of hemopoietic cells [1,2,16], and defects in mechanisms regulating cytokine production [5,8,17]. Macrophages, the major producer of cytokines in the body, are an important component of hemopoietic microenvironment [3]. Here we studied the ability of hemopoietic cells from patients with MDS to produce cytokines involved in the regulation of hemopoiesis. To this end, the effects

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of cytokines produced by stromal cells and macrophages on proliferation and differentiation of normal hemopoietic precursors was evaluated. We also evaluated production of tumor necrosis factor-α (TNF-α) and interleukin-6 (IL-6) involved in apoptosis and regulation of immature hemopoietic precursors in the medium conditioned by stromal cells from long-term bone marrow cultures and cultured macrophage from patients with various forms of MDS.

MATERIALS AND METHODS

Bone marrow and peripheral blood were obtained from 10 healthy donors and 32 patients with various forms of MDS (FAB classification): refractory anemia (RA), refractory sideroblast anemia (RSA), RA with excess blasts (RAEB), RA with excess blasts in transformation (RAEBT), and chronic myelomonocytic leukemia (CMML). Mononuclear cells were isolated from the bone marrow aspirate and peripheral blood by centrifugation in a Ficoll density gradient (1.077 g/cm^3). Bone marrow cells were maintained in long-term cultures for 3-5 weeks [6]. Cells of the stromal sublayer were treated with trypsin and cultured in a complete nutrient medium in 24-well plates (10^5 cells/well) for 24 h. For isolation of macrophages, peripheral blood mononuclear cells were cultured in 24-well plates for 24 h (10^6 cells/well) and then the adherent cells were cultured for 3 days. Macrophages constitute 97.0± 1.2% adherent mononuclears, which was confirmed by positive reaction for nonspecific α-naphthyl acetate esterase with NaF inhibition. Conditioned medium was collected after culturing of stromal cells or macrophages in a serum-free medium for 1 day. Cytokine production was evaluated by the effects of this medium on the growth of normal bone marrow hemopoietic precursors, erythroid burst-forming units (BFU-E) and granulocyte-macrophage colony-forming units (CFU-GM), in semisolid media [1]. Bone marrow cells from healthy donors stimulated with supernatant of U5637 cells (standard growth factor) served as the control. The contents of TNF-α and IL-6 were estimated by enzyme-linked immunosorbent assay and calculated per 10^5 macrophages taking into account the percent of adherent peripheral blood mononuclear cells. The results were analyzed by Student's *t* test.

RESULTS

Media conditioned by stromal cell from healthy donors and patients with MDS were equally potent in stimulating the growth of normal CFU-GM (Table 1). Conditioned media of long-term bone marrow cultures from patients with RSA and RAEB+RAEBT added to cultured bone marrow cells from healthy donors significantly decreased the number of BFU-E ($p<0.05$).

The media conditioned by macrophages from patients with MDS and healthy donors stimulated proliferation of normal CFU-GM (Table 2). The media conditioned by macrophages from patients with RSA (2 of 7) and RAEB (4 of 6) were less potent than the medium conditioned by donor macrophages in stimulating the growth of granulocytic colonies. However, the media conditioned by macrophages from 3 of 7 patients with CMML produced a more potent stimulating effect on CFU-GM growth compared to media conditioned by donor macrophages. Generally, the media conditioned by macrophages from patients with CMML induced the growth of a higher number of granulocytic colonies compared to RSA ($p<0.02$) and RAEB ($p<0.05$) groups.

Thus, the media conditioned by stromal cells and macrophages stimulated the growth of normal hemopoietic precursors. Cells from patients with RSA and RAEB produced a less pronounced stimulatory effect compared to cells from healthy donors and patients with CMML. This is consistent with previous data obtained on combined cultures, when cells of the stromal sublayer and macrophages from patients with MDS served as the source of growth factors [2]. The peculiarities of stimulation of CFU-GM growth indicate the existence of various mechanisms underlying func-

TABLE 1. *In Vitro* Effects of Media Conditioned by Stromal Cells from Patients with MDS on the Growth of Normal Hemopoietic Precursors (% of Control)

Group	BFU-E		CFU-GM	
	Me (extremes)	*M*±*m*	Me (extremes)	*M*±*m*
Healthy donors (*n*=5)	44.0 (37.5-68.6)	49.8±6.1	48.0 (44.1-112.8)	61.5±13.1
RA (*n*=4)	40.0 (22.8-45.7)	37.4±5.6	52.5 (34.6-70.9)	52.6±8.2
RSA (*n*=4)	20.3 (4.3-48.0)	23.2±9.4	55.3 (43.0-82.0)	58.7±14.3
RAEB+RAEBT (*n*=5)	25.0 (6.3-46.0)	25.2±7.2	60.0 (29.6-76.0)	56.4±9.7

Note. Control: number of hemopoietic precursors per 10^5 normal bone marrow nuclear cells after stimulation with a standard factor (21.3±4.8 and 56.1±7.2 for BFU-E and CFU-GM, respectively).

Table 2. *In Vitro* Effects of Media Conditioned by Macrophages from Patients with MDS on the Growth of Normal Hemopoietic Precursors (% of Control)

Group	CFU-GM	
	Mean (extremes)	*M*±*m*
Healthy donors (*n*=5)	29.9 (16.3-64.5)	36.1±8.4
RA (*n*=4)	27.1 (6.0-59.4)	29.9±13.1
RSA (*n*=7)	18.8 (8.9-37.0)	22.8±4.6
RAEB+RAEBT (*n*=6)	8.6 (0-66.1)	19.9±10.4
CMML (*n*=7)	46.0 (24.0-116.5)	61.1±13.3

Note. Control: number of CFU-GM per 10^5 normal bone marrow nuclear cells after stimulation with a standard factor (97.6±23.0, range 21.0-267.3).

tional disturbances in the stromal microenvironment during various forms of MDS.

Secretion of growth factors by stromal cells from patients with MDS in conditioned media was evaluated by the content of TNF-α and IL-6 in conditioned media. TNF-α is involved in apoptosis. Previous studies showed increased content of TNF-α in the plasma and bioptates from the majority of patients with MDS [12,14,15]. The antiinflammatory cytokine IL-6 stimulates proliferation of multipotent hemopoietic precursors [7]. Stromal cells from patients with RA (2 of 6) and RAEB (1 of 4) more intensively produced TNF-α than cells from healthy donors (Fig. 1, *a*). Production of TNF-α by macrophages from patients with MDS did not differ from that in healthy donors (except 1 patient with RAEB and 2 patients with CMML, in whom cytokine content markedly surpassed the control, Fig. 1, *c*). Macrophages from healthy donors and patients with MDS less intensively secreted TNF-α compared to stromal cells. Only in some patients the concentrations of TNF-α in media conditioned by stromal cells and macrophages were similar. Little is known about TNF-α production by stromal cells from patients with MDS. L. Molnar *et al*. [11]

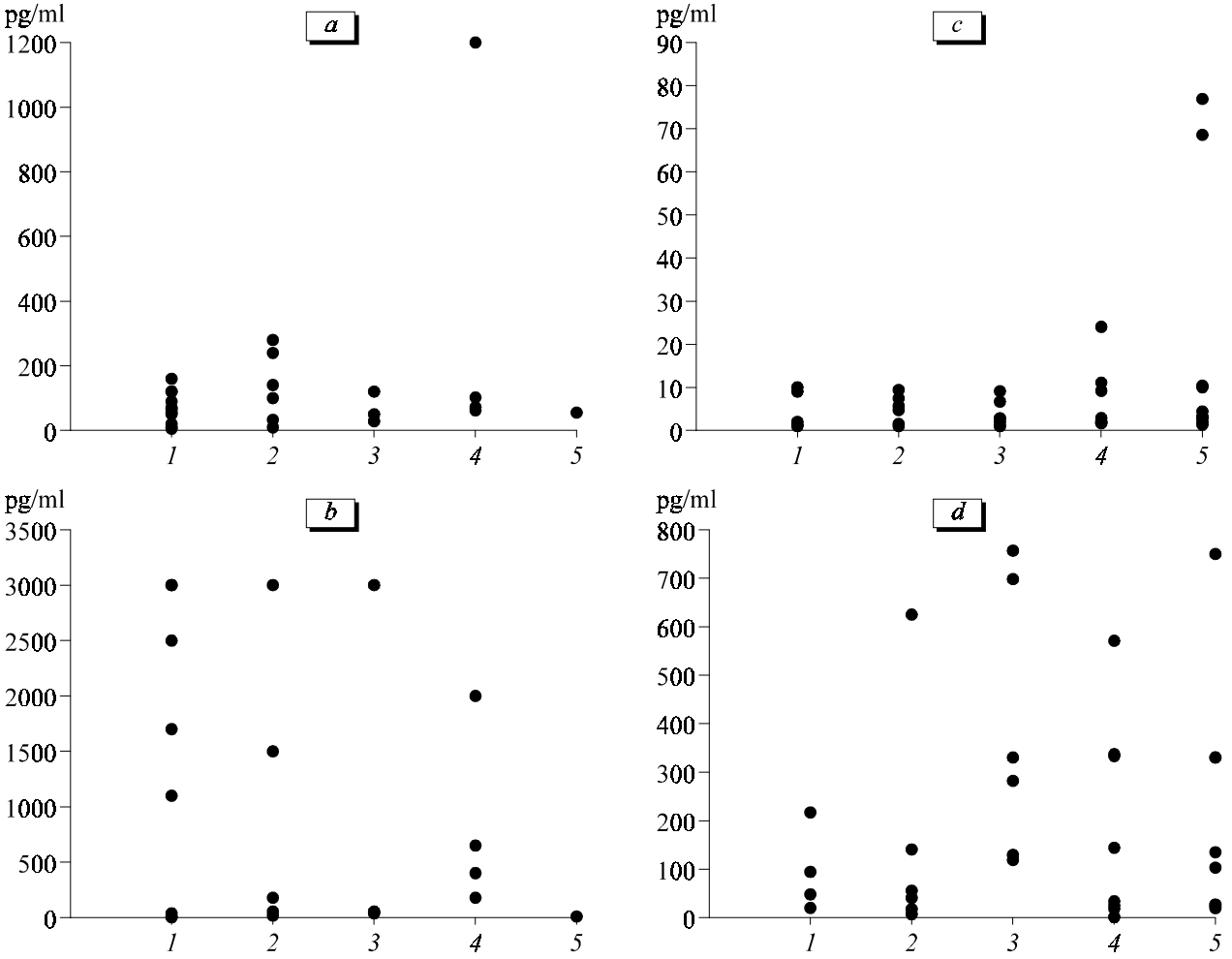

Fig. 1. Contents of tumor necrosis factor-α (*a*, *c*) and interleukin-6 (*b*, *d*) in media conditioned by stromal cells (*a*, *b*) and macrophages (*c*, *d*) from patients with various forms of myelodysplastic syndrome. Healthy donors (*1*), refractory anemia (RA, *2*), refractory sideroblast anemia (*3*), RA with excess blasts in transformation (*4*), and chronic myelomonocytic leukemia (*5*).

observed high level of TNF-α in peripheral blood macrophages in some patients with RA and CMML. M. Mencobony *et al*. [10] reported low TNF-α production by bone marrow macrophages in patients with RA and RAEB and high production in patients with RSA and CMML. In our experiments, increased TNF-α content was found in media conditioned by stromal cells and macrophages from some patients with MDS. Less intensive production of TNF-α by macrophages compared to stromal cells suggests that this cytokine is mainly secreted by other hemopoietic cells.

The concentration of IL-6 in media conditioned by cells from patients with various forms of MDS did not differ from that in donors (Fig. 1, *b*, *d*). In patients with MDS and healthy donors, IL-6 content in media conditioned by stromal cells was higher than in macrophage-conditioned medium. However, IL-6 production in some patients with MDS surpassed that in healthy donors, which was associated with tumor progression or inflammatory processes. Previous studies demonstrated that IL-6 content increases in patients with RAEBT and rapidly progressing CMML [4].

Our experiments demonstrated impaired production of cytokines regulating hemopoiesis by cultured stromal cells and macrophages from patients with MDS. Production of factors stimulating the growth of normal erythroid and granulocytic precursors by cells of the stromal sublayer and macrophages from patients with RSA and RAEB is impaired compared to cells from healthy donors. Cultured stromal cells and macrophages produce TNF-α and IL-6, whose content in the medium conditioned by cells from some patients with MDS is higher than in cultures of donor cells. Therefore, cytokine production by hemopoietic microenvironmental cells is impaired in patients with various forms of MDS.

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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. T. E. Manakova, L. P. Gerasimova, N. V. Tsvetaeva, *et al*., *Ibid*., **130**, No. 9, 255-258 (2000).
3. S. Akira, T. Hirano, T. Taga, *et al*., *FASEB J*., **4**, 2860-2867 (1990).
4. M. Alexandrakis, S. Coulocheri, I. Xylouri, *et al*., *Haematologia (Budap.)*, **29**, 13-24 (1998).
5. M. Fontenay-Roupie and F. Dreyfus, *Pathol. Biol*., **45**, 569-572 (1997).
6. S. Gartner and H. S. Kaplan, *Proc. Natl. Acad. Sci. USA*, **39**, 1133-1136 (1980).
7. S. P. Hauser, O. Kajkenova, D. A. Lipschitz, *et al*., *Stem Cells*, **15**, 125-132 (1997).
8. Y. Hirayama, Y. Kogho, T. Matsunaga, *et al*., *Br. J. Haematol*., **85**, 676-682 (1993).
9. J. W. Janssen, M. Buschle, M. Layton, *et al*., *Blood*, **73**, 248-254 (1989).
10. M. Mencobony, G. Castello, R. Lerzak, *et al*., *Eur. J. Haematol*., **56**, 148-152 (1996).
11. L. Molnar, T. Berki, A. Hussain, *et al*., *Pathol. Oncol. Res*., **6**, 18-23 (2000).
12. P. Musto, R. Matera, M. M. Mivervini, *et al*., *Haematologica*, **79**, 265 (1994).
13. A. Raza, S. Mundle, V. Shetty, *et al*., *Int. J. Hematol*., **63**, 265-278 (1996).
14. V. Shetty, S. Mundle, S. Alvis, *et al*., *Leuk. Res*., **20**, 891-900 (1996).
15. R. Stasi, M. Brunetti, S. Bussa, *et al*., *Clin. Lab. Haematol*., **19**, 197-201 (1997).
16. G. B. Tennant, V. Walsh, L. N. Truran, *et al*., *Br. J. Haematol*., **111**, 853-862 (2000).
17. M. Wetzler, R. Kurzrock, Z. Estrov, *et al*., *Leuk. Res*., **19**, 23-34 (1995).