Effect of Cytokines (G-CSF and SCF) on Stromal Precursor Cells

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Repeated injections of hemopoietic cytokines (granulocyte colony-stimulating factor and stem-cell factor) to normal mice increase the content of bone marrow stromal precursors, which are capable of transferring hemopoietic microenvironment; cytokines have no effect on osteogenic potencies of stromal precursors. In contrast, injection of granulocyte colony-stimulating factor during organization of a hemopoietic microenvironment considerably decreases the number of stromal precursors in the ectopic focus. The role of these stromal effects of cytokines in cytokine-induced mobilization of stem cells from the bone marrow into circulation remains unclear.

Key Words: *stem hemopoietic cell; cytokines; stromal precursor cells; ectopic hemopoietic focus*

Peripheral blood cells have long attracted interest as an alternative source of hemopoietic stem cells (HSC) for transplantation. In steady-state hemopoiesis only minor population of HSC belonging to a more differentiated pool compared with the bone marrow is presented in the circulation [2,7]. It was found that regeneration of hemopoietic tissues in patients after chemotherapy is accompanied by a considerable increase in blood content of HSC. Studies with hemopoietic growth factors showed that cytokines not only potentiate the release of HSC into circulation after chemotherapy, but also induce an appreciable mobilization of precursors without chemotherapy: maximum concentration of precursors in the blood attained or surpassed that in the bone marrow [4,5,8]. This allowed for the use of mobilized precursors from peripheral blood as an additional or alternative source of HSC for transplantation. At present, the number of autotransplantations of peripheral blood cells surpassed that of transplantation of autologous bone marrow [3]. Since cytokines are less dangerous than chemotherapy, practical studies on cytokine-induced mobilization of HSC in healthy donors as a source of peripheral blood precursor cells are recommended and have been initiated [6].

The mechanisms of the mobilizing effect of cytokines remains unclear. This activity may be due to changes in hemopoietic microenvironment, in particular, weak adhesion of HSC to the stroma, and effect on HSC, for instance, blockade of adhesion molecules and enhanced release of HSC into circulation.

Here we report on the effects of the widely used cytokines granulocyte colony-stimulating factor (G-CSF) and stem-cell factor (SCF) on the number of stromal precursors, which are capable of transferring hemopoietic microenvironment [1], and on their activity during organization of this microenvironment.

MATERIALS AND METHODS

Experiments were carried out 12-25-week-old female and male CBF_1 $(CBA/Lac \times C57Bl/6)F_1$ rats. Cytokines, recombinant rat SCF (Amgen) and recombinant human G-CSF (Neupogen 48, Amgen) were dissolved in physiological saline containing 0.1% bovine serum albumin and injected subcutaneously in doses 250 μg/kg (G-CSF) and 34 μg/kg (SCF)

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for 6, 10, or 17 days. Control mice received the vehicle. Twenty hours and one month after the last injection, bone marrow from the femurs of control and experimental mice was implanted to syngeneic mice under the kidney capsule [1]. The size of the ectopic islet (by number of hemopoietic cells) and osteogenic activity of stromal precursors (by the mass of newly formed bone tissue) were assessed 1.5 months after implantation. In one group the effect of G-CSF on the formation of ectopic hemopoietic islet was evaluated. To this end implantation of syngeneic bone marrow was performed 1 day prior to the first cytokine injection; G-CSF was injected for 10 and 17 days. The size of ectopic hemopoietic islet was determined 1.5 months after transplantation. To determine the content of cells in the initial bone marrow, which are able to transfer hemopoietic microenvironment, some newly formed islets in the bone marrow were retransplanted to secondary recipients, in whom the size of hemopoietic foci was measured 1.5 months after transplantation. The data were processed statistically using the Student *t* test.

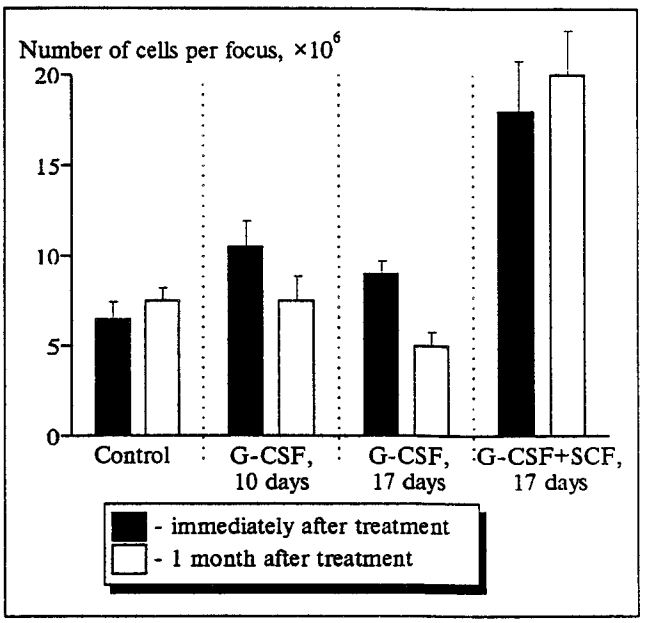

Fig. 1. Size of ectopic hemopoietic focus from mouse bone marrow 1 day and 1 month after injection of cytokines.

RESULTS

For evaluation of the effect of cytokines on stromal precursors the method of ectopic hemopoietic islets was used. Ectopic hemopoietic islet is formed 1-2 months after transplantation of syngeneic bone marrow under the kidney capsule. Stroma in this islet is derived from donor, while hemopoiesis is provided by host HSC. The number of cells in this focus reflects the amount of stromal precursors in it [1].

Injection of G-CSF to donor mice 1.5-fold enlarged the size of ectopic islet formed by their bone marrow in syngeneic recipients (Table 1). Retransplantation of these foci showed that this enlargement is due to increased content of stromal precursors in the bone marrow of cytokine-treated mice. Injection of G-CSF/SCF combination had a stronger effect on the size of ectopic islets and the content of stromal precursors in it; the maximum effect was produced by long-term (17 days) treatment with cytokines, the size of ectopic islet and the number of stromal precursors being increased 3-4 fold (Table 1). Stromal precursors possess osteogenic potencies and form *de novo* bone tissue. The test cytokines had no significant effect on *de novo* bone formation (Table 1). The observed effect of cytokines can play a negative role in their clinical use in healthy donors for mobilization of HSC into circulation for allogenic transplantation. In light of this, we studied persistence of the stromal effect of test cytokines. As seen from Fig. 1, one

TABLE 1. Size of Ectopic Hemopoietic Focus Formed by Bone Marrow Cells from Cytokine-Treated Mice (*M±m*)

Group		Implantation of bone marrow from cytokine-treated mice			Reimplantation of ectopic focus to secondary recipients		
		number of foci	number of cells per focus, ×10⁶	bone weight, mg	number of foci	number of cells per focus, ×10⁶	bone weight, mg
Control		9	6.5±1.0	2.0±0.2	4	5.9±1.2	2.6±0.4
G-CSF	6 days	4	7.4±2.2	1.1±0.2			
	10 days	3	10.7±2.0	1.0±0.2	3	11.3±2.9	3.0±0.6
	17 days	4	9.2±1.0	3.3±0.7	3	13.3±2.8	3.5±0.7
G-CSF+SCF	6 days	4	6.4±1.6	2.2±0.5			
	10 days	4	12.8±1.5	2.4±0.6			
	17 days	4	18.4±3.9	1.7±0.5	5	19.5±4.2	3.2±0.6

TABLE 2. Effect of G-CSF on Ectopic Hemopoietic Focus Formed by Bone Marrow from Normal Mice (*M±m*)

Treatment with G-CSF	Implantation of bone marrow from normal mice			Reimplantation of ectopic focus to secondary recipients		
	number of foci	number of cells per focus, $\times 10^6$	bone weight, mg	number of foci	number of cells per focus, $\times 10^6$	bone weight, mg
Control	4	8.3±1.7	2.2±0.4	4	16.2±3.3	2.3±0.5
10 days	4	5.3±0.7	2.7±0.7	4	9.0±1.8	1.9±0.5
17 days	6	3.4±0.6	2.0±0.4	6	1.7±0.3	1.3±0.4

month after treatment with G-CSF the content of stromal precursors returned to normal regardless the duration of the treatment. Other results were obtained in long-term combined treatment with G-CSF and SCF. Effect of this combination did not decay over one month after completion of cytokine treatment. Hence, special attention in clinical studies should be paid not only to choice of cytokines, but also to duration of cytokine treatment for HSC mobilization.

We studied the effect of cytokines on the content of stromal precursor cells in the bone marrow. It could be assumed that cytokines modulate proliferation and differentiation of stromal precursors during formation of hemopoietic microenvironment, i.e., ectopic hemopoietic islet. We explored this possibility for G-CSF (Table 2). Experiments showed that G-CSF has an opposite effect on stromal precursors: it inhibits proliferation of transferred stromal precursors and sharply reduces both the size of ectopic islet (1.5-2.5-fold) and the content of stromal precursors in it (2-10-fold, Table 2). Cytokines had no appreciable effect on osteogenic potencies of the stromal precursors.

Thus, we observed two different stromal effects of hemopoietic cytokines:

— during the formation of ectopic hemopoietic islet characterized by proliferation and differentiation of stromal precursors [1], pharmacological concentrations of G-CSF suppress the formation of hemopoietic focus and reduce the content of newly formed stromal precursors;

— in the bone marrow of normal mice (differentiated non-proliferating system) cytokines increase the number of stromal precursors capable of transferring the hemopoietic microenvironment. The question whether cytokines are involved into HSC mobilization remains unanswered.

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REFERENCES

1. J. L. Chertkov and O. A. Gurevich, *Stem Hemopoietic Cell and Its Microenvironment* [in Russian], Moscow (1984).
2. J. L. Chertkov, O. A. Gurevich, and G. A. Udalov, *Exp. Hematol.*, **10**, 90-96 (1982).
3. J. I. O. Craig, M. L. Turner, and A. C. Parker, *Blood Rev.*, **6**, 59-67 (1992).
4. N. J. Drize, J. L. Chertkov, N. L. Samoylina, and A. R. Zander, *Exp. Hematol.*, **24**, 816-822 (1996).
5. N. J. Drize, J. L. Chertkov, and A. R. Zander, *Ibid.*, **23**, 1180-1186 (1995).
6. M. Korbling, D. Przepiorka, Y. O. Huh, *et al.*, *Blood*, **85**, 1659-1665 (1995).
7. H. S. Micklem, N. Anderson, and E. Ross, *Nature*, **256**, 41-44 (1975).
8. A. R. Zander, J. Lyding, and S. Bielach, *Blood Cells*, **17**, 301-319 (1991).