Effect of Leukemia Inhibitory Factor on Hemopoietic and Stromal Precursor Cells in Prolonged Culture of Mouse Bone Marrow

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Treatment of prolonged bone marrow cultures with leukemia inhibitory factor during the first 2 weeks after explantation has no appreciable effect on the production of precursors and mature hemopoietic cells during 4 weeks of culturing. However, the proliferative potential of polypotent hemopoietic precursors in these cultures increases substantially. The addition of exogenous cytokine has a pronounced effect on the hemopoietic stroma, specifically, on the content of osteogenic precursors and cells transporting the hemopoietic microenvironment to prolonged bone marrow cultures treated by leukemia inhibitory factor. This effect is confirmed by formation of ectopic hemopoietic foci *in vivo*, being 2-3 times higher than in the control.

Key Words: *prolonged bone marrow culture; leukemia inhibitory factor; CFUs; CFU-GM; focus of ectopic hemopoiesis*

Leukemia inhibitory factor (LIF) is a polyfunctional regulator performing important functions at the early stages of embryogenesis. In adult life, LIF modulates platelet formation, the function of osteoblasts, osteoclasts, and neurons and is involved in the regulation of calcium and lipid metabolism as well as in the production of the acute inflammation phase proteins [6,7,14]. In addition, LIF participates in bone resorption by osteoclasts and, together with other osteotropic factors, modifies differentiation of osteoblasts. Presumably, LIF is produced locally in different tissues. Intraosseous osteoblasts are known to possess the receptors for LIF [2]. The data on the effects of LIF in a cell culture are contradictory. At a concentration of 200 U/ml, LIF elicits no effect on hemopoietic precursors in a prolonged culture of human bone marrow (PCBM) [10]. On the other hand, it was reported that LIF stimulates early hemopoietic precursors in a culture [13]. To our knowledge, no data on the stromal effects of LIF in PCBM have been published.

This study demonstrates for the first time that LIF stimulates stromal precursors with osteogenic potential *in vitro*.

MATERIALS AND METHODS

Recombinant Hilda/LIF cytokine was purified from culture medium conditioned by SHO (Syrian hamster ovaries) cells transfected with cDNA coding for Hilda/LIF. Biological activity of LIF was evaluated from its effect on DA1 cells [9].

Prolonged bone marrow culture was obtained by the method of Dexter [5] with some modifications [3]. Leukemia inhibitory factor was added to final concentrations of 100, 500, 2500, and 5000 units/ml every day five times a week during the first two weeks after explantation. CFU-GM were determined by the standard method [1]. A mixture of media conditioned by L929 and WEHI 3B cells (2:1) was used as a stimulator. Female CBF1 (C57Bl/6×CBA)

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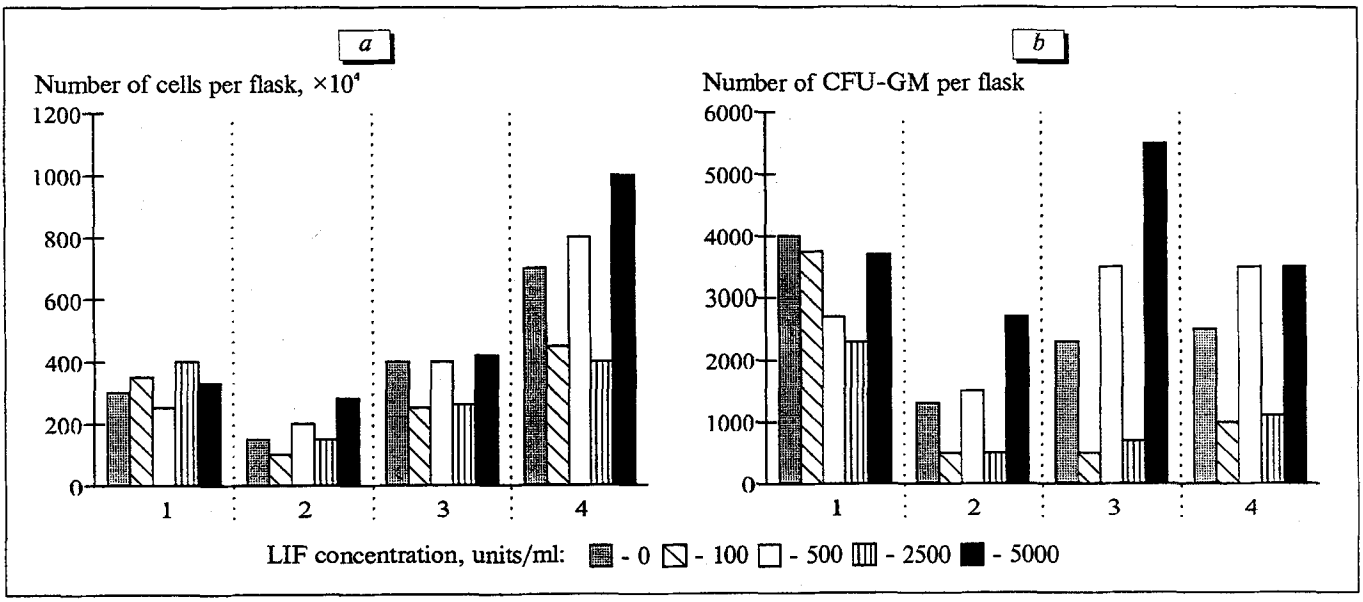

Fig. 1. Effect of leukemia inhibitory factor (LIF) on hemopoiesis in a prolonged bone marrow culture. *a*) number of cells; *b*) number of CFU-GM in culture. Here and in Fig. 2: abscissa: period of culturing, weeks.

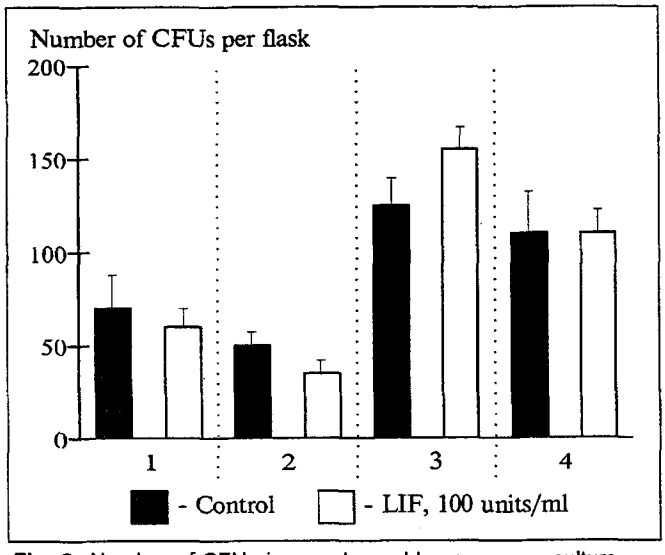

Fig. 2. Number of CFUs in a prolonged bone marrow culture.

F_1 mice 12 to 25 weeks old were used. The recipients received a total irradiation dose of 12 Gy. In order to determine the number of splenic colony-forming units (CFUs) [12] irradiated mice were injected with 1.5-2×10^5 nonadhering cells from PCBM. The proliferative potential of CFUs (PPC) was expressed as the count of daughter CFUs in 11-day-old splenic colony [8]. The layer of adhering cells (LAC) from 4-week-old cultures was implanted under the renal capsule of a syngeneic mouse [3]. After 1.5 months, the ectopic focus was characterized by the number of nuclear cells in it, and the newly formed bone was weighed. For reimplantation, the entire 1.5-2-month-old foci were removed from the kidney and implanted under the renal capsule into a secondary recipient.

RESULTS

It was demonstrated that the stromal precursors proliferate and form LAC within a 2-week period after explantation of bone marrow [4]. Treatment of the cultures with LIF during the first 2 weeks (10 times) caused no morphological changes in the culture and in the production of mature cells, CFU-GM (Fig. 1, *a*, *b*), and CFUs (Fig. 2), during the subsequent 4 weeks. Stimulation of CFUs was the only detected hemopoietic effect of LIF (Table 1). However, LIF had a potent effect on stromal precursors. In ectopic foci formed by adherent cells from LIF-treated cultures, the weight of bone concha increased 2- to 3-fold in some cases (Fig. 3, *a*), and the size of ectopic foci increased 2 to 4 times. Generally, foci of high ossification contained fewer cells than those of low ossification (Fig. 3, *b*). A similar inverse relationship between the weight of bone concha and the number of cells in a focus was observed after implantation of fresh bone marrow [1]. Retransplantation experiments showed that LAC from LIF-treated cultures contains stromal precursors capable of transferring the hemopoietic microenvironment *in vivo* and of

TABLE 1. Number of CFUs and their Proliferative Potential in LIF-Treated 4-Week PCBM (*M*±*m*)

LIF concentration, units/ml	Number of CFUs per flask	Number of daughter CFUs per 11-day colony
0	35.5±15.2	9.0±1.5
500	24.7±14.8	17.0±2.5
2500	15.8±6.3	25.5±2.5

TABLE 2. Effect of LIF on the Formation of Ectopic Hemopoiesis Focus from a Layer of Adhering PCBM Cells (*M±m*)

LIF concentration, units/ml	Implantation of LAC to 4-week PCBM			Transfer of ectopic hemopoietic focus to secondary recipients		
	number of foci	number of cells per focus, $\times 10^6$	weight of bone concha, mg	number of foci	number of cells per focus, $\times 10^6$	weight of bone concha, mg
0	5	6.9±1.2	2.3±0.5	4	3.4±0.7	4.0±0.8
100	5	9.4±1.9	4.8±0.9	4	2.7±0.5	6.0±1.2

self-maintaining both in a culture and *in vivo*, which is confirmed by formation of ectopic foci in secondary recipients (Table 2).

LIF had no effect on the number of cells and production of CFU-GM in a culture of mouse bone marrow, which agrees with results obtained in experiments on human hemopoietic cells [10]. However, LIF did not alter proliferation and differentiation of human cells but significantly ($p<0.001$) increased the proliferative potential of murine CFUs in a dose-dependent manner. It is known that LIF stimulates proliferation of human early hemopoietic precursors (HLA-DR-CD34$^+$) in a culture [13]. Consequently, it can stimulate proliferation of early but not of mature precursors, which is consistent with its capacity to inhibit differentiation [11,15]. Presumably, there is a direct relationship between the inhibition of differentiation and an increase in proliferative potential of early precursors. The effect of LIF on hemopoietic precursors may be mediated by stromal cells creating a hemopoietic microenvironment in the culture. In fact, our findings indicate that LIF affects stromal precursors by stimulating bone formation. Stromal precursors form a simple hemopoietic microenvironment in the culture, which is capable of maintaining the proliferation and differentiation of CFUs for a long time. On the other hand, they are also maintained in the culture, as evidenced by the presence of cells capable of transferring the microenvironment during retransplantation into the body. Although neither osteo- nor chondrogenesis occur in the culture, active osteogenesis proceeds in a developing ectopic focus after implantation into the body, indicating that stromal precursors have been maintained. Treatment of cells with LIF during the development of LAC stimulates multiplication of osteogenic precursors. A dose-dependent stimulation of osteogenesis with LIF in ectopic foci and an increase in the number of osteogenic precursors detected by transplantation of the foci into secondary recipients (Table 2) suggest that LIF plays an active role in osteogenesis and maintenance of osteogenic precursors. Thus, that LIF may be useful for local stimulation of osteogenesis, for example, at the sites of bone fracture. This hypothesis is under investigation.

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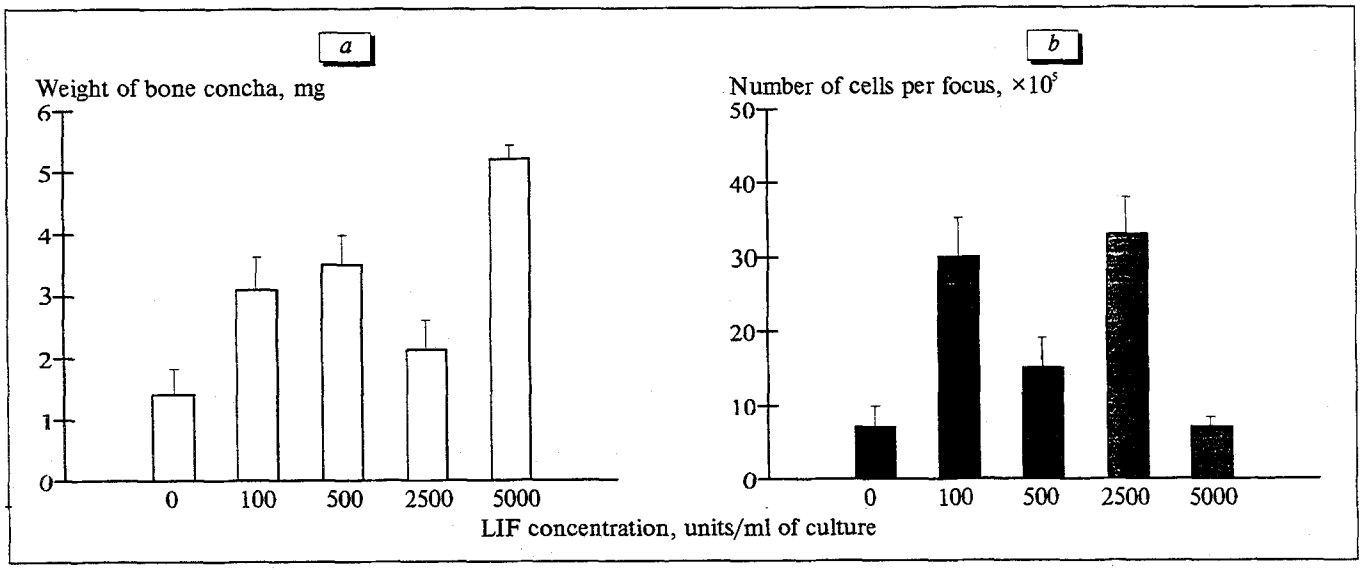

Fig. 3. Characteristics of ectopic foci formed by the layers of adhering cells from 4-week-old bone marrow cultures. *a*) weight of bone in an ectopic focus; *b*) number of cells in an ectopic focus.

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