Effect of Parathyroid Hormone PTH (1-34) on Hemopoietic and Stromal Stem Cells

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Long-term administration of parathyroid hormone causing activation and proliferation of osteoblasts to mice increases the concentration of primitive hemopoietic precursor cells (cobblestone area-forming cells) in long-living bone marrow culture after 28-35 days. The concentrations of later precursors forming colonies in the spleen and the concentration of cobblestone-area forming cells in long-living bone marrow culture after 7 days decrease, while the concentration of more differentiated cells forming colonies in the culture does not change. Transplantation of the bone marrow from mice treated with parathyroid hormone under the renal capsule of syngeneic recipients results in the formation of a focus of ectopic hemopoiesis not differing by size from the control. Injection of parathyroid hormone to mice during the growth of the ectopic focus did not modulate its size. These foci tolerate re-transplantation procedure similarly as controls. Hence, parathyroid hormone has no effect on mesenchymal stem cells responsible for transfer of the stromal microenvironment. Therefore, the number of stem hemopoietic cells in the body is regulated by not stromal stem cells, but their better differentiated descendants.

Key Words: *parathyroid hormone; hemopoietic stem cells; mesenchymal stem cells; ectopic hemopoiesis focus*

Parathyroid hormone (PTH) regulating Ca metabolism and osteogenesis is used for stimulation of bone growth in patients with osteoporosis [5]. It activates and stimulates proliferation of osteoblasts in stromal cultures [3]. Bone marrow osteoblasts are important components of the hemopoietic "niche" (the site of hemopoietic microenvironment maintaining hemopoietic stem cells (HSC) [7]. It was shown that PTH indirectly stimulates HSC growth *ex vivo* [2]. The mechanism of this interaction is as follows: PTH-activated osteoblasts produce Jagged 1 surface protein in great amounts; this protein serves as the ligand for Notch 1 protein located on the surface of primitive HSC. The Notch—Jagged interaction stimulates HSC division, which leads to an increase in their number. This interaction is experimentally proven by the fact that enhanced proliferation of HSC can be blocked by with γ-secretase (enzyme inhibiting Notch 1 activation).

Production of full-value HSC in high amounts is important for their transplantation, particularly when the cells are taken from umbilical blood: in this case the number of HSC is insufficient for transplantation to adult patients. Therefore the possibility of stimulating the "niche" cells or molecules mediating the interaction of HSC with the microenvironment for stimulation of their growth and simultaneous blocking of their differentiation is an important problem.

Studies of stromal and hemopoietic precursor cells under conditions of long-term treatment with PTH are perspective. We studied the effect of PTH on mesenchymal stem cells (hemopoietic stromal precursors) capable of transfering hemopoietic microenvironment and on hemopoietic precursor cells from poly- and oligopotent HSC populations.

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MATERIALS AND METHODS

The study was carried out on 9-26-week-old C57Bl/6 female mice. For evaluation of the concentrations of colony-forming cells in the spleen (CFU-S) the recipients were exposed to ^{137}Cs on an IPK device (Hematology Research Center, Russian Academy of Medical Sciences) in a total dose of 10 Gy (two equal doses at 3-h interval). One-two hours after the exposure the recipients intravenously received 5×10^4 bone marrow cells. Colonies in the spleen were counted using binocular lens on days 9 and 13. Proliferative potential (self-maintenance) of 13-day CFU-S was evaluated by the number of daughter CFU-S per splenic colony as described previously [4]. Cobblestone area-forming cells (CAFC) were detected in the long-living culture as described elsewhere [6], colonies in semisolid media (CFU-C) were studied as described previously [1]. Rat synthetic PTH (1-34) (Bachem) was injected intraperitoneally in a dose increasing the count of osteoblasts (80 μg/kg 5 times a week over 4 weeks). Two weeks after the last injection the bone marrow from the femoral bones of control and experimental mice was implanted under the renal capsules of syngeneic mice [1] and half of recipients were injected with PTH according to the same protocol during the formation of ectopic hemopoietic focus. After 1.5 months the size of the new focus was evaluated by the number of hemopoietic cells and osteogenic activity of stromal precursors was evaluated by the weight of newly formed bone. In order to evaluate self-maintenance capacity of cells capable of transferring hemopoietic microenvironment in the initial bone marrow of mice treated and not treated with PTH, some new foci were retransplanted to second recipients. The size of these new foci was evaluated 1.5 months after retransplantation.

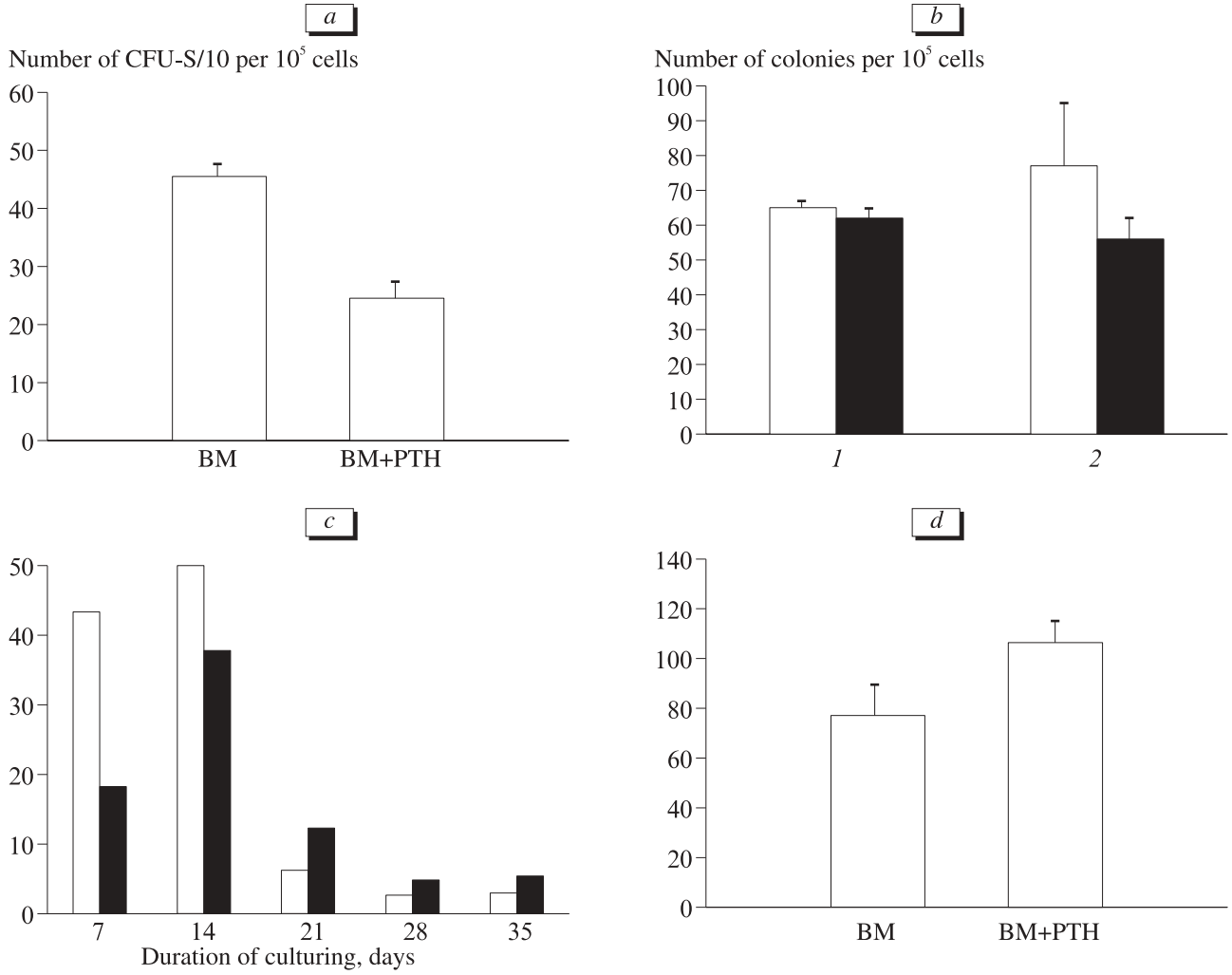

Fig. 1. Concentration of hemopoietic precursor cells in the bone marrow (BM) of mice after a course of parathyroid hormone (PTH) treatment. *a*) concentration of cells forming colonies in the spleen (CFU-S); *b*) concentration of cells forming colonies in culture (CFU-C). *1*) agar; *2*) methylcellulose. *c*) concentration of cobblestone area-forming cells (CAFC). Ordinate: their number per 10^5 cells. *d*) proliferative potential of CFU-S. Ordinate: number of daughter CFU-S in a 13-day colony.

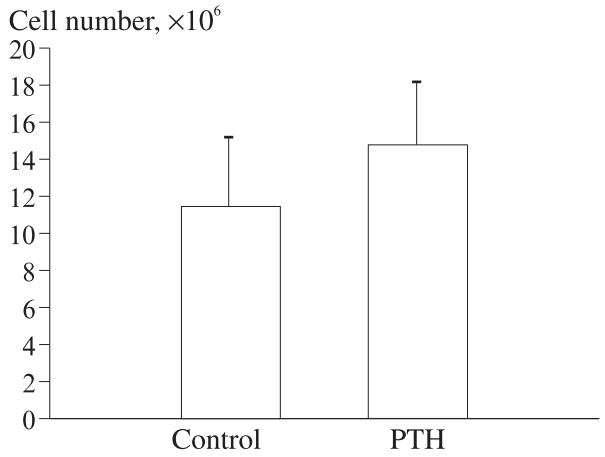

Fig. 2. Size of ectopic hemopoiesis focus formed from the bone marrow of mice after 4-week course of PTH.

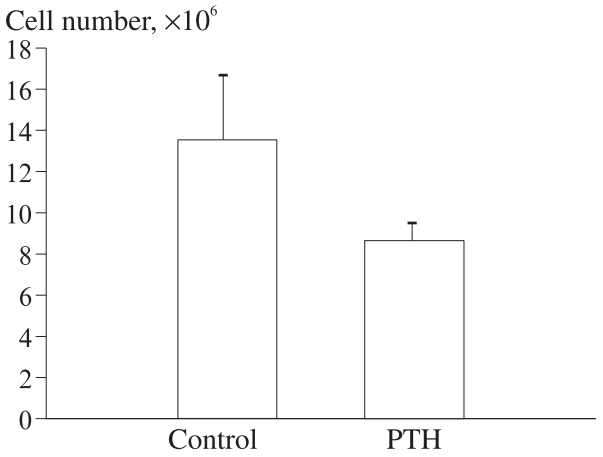

Fig. 3. Size of ectopic hemopoiesis focus in mice injected with PTH.

The data were statistically analyzed using Student's *t* test.

RESULTS

In order to characterize hemopoiesis after long course of PTH in mouse bone marrow, the concentration of CAFC was evaluated 7-35 days after the treatment, CFU-S count and their self-maintenance and the count of CFU-C were studied 2 weeks after the end of PTH course. The concentration of 9-day CFU-S was significantly lower ($p<0.001$) in the bone marrow of mice treated with PTH (Fig. 1, *a*). The proliferative potential of splenic colonies differed negligibly, but the colonies isolated from PTH-treated mice were characterized by higher self-maintenance capacity (Fig. 1, *d*). The concentration of later precursors producing colonies in semisolid media (CFU-C) was the same in experimental and control animals (Fig. 1, *b*). The concentration of CAFC in the bone marrow after 7 days (corresponding to CFU-C [6]) was appreciably lower in mice treated with PTH. After 14 days this difference was less significant for earlier precursors of CAFC, while after 21, 28, and 35 days the concentrations of CAFC in PTH-treated mice were higher than in controls. These data attest to expansion of early HSC after injections of PTH, which is in line with the previous data [2] (Fig. 1, *c*). Presumably, long treatment with PTH does not impair maturation of hemopoietic precursor cells.

The effect of PTH on mesenchymal stem cells capable of transferring the hemopoietic microenvironment was studied on a model of ectopic hemopoietic focus. The bone marrow of mice treated with PTH for 4 weeks was implanted under the renal capsules of syngeneic recipients 2 weeks after the end of PTH course. The size of the new focus of ectopic hemopoiesis and the weight of new bone (2.6 mg) virtually did not differ from those for the control bone marrow (1.75 mg; Fig. 2). The microenvironment was created anew during the formation of ectopic hemopoiesis focus. Stem cells transporting the hemopoietic microenvironment were insensitive to PTH. When PTH was injected to bone marrow recipients during the first 4 weeks after transplantation, the forming focus was smaller than in the control (bone weight in controls 1.7 mg *vs.* 1.9 mg in experimental animals; Fig. 3). The number of osteoblasts and the weight of new bone did not increase either. In order to test the self-maintenance capacity of mesenchymal stem cells, the foci formed in control and PTH-treated mice were retransplanted to second recipients under the right and left renal capsules, respectively. Some secondary recipients received PTH courses. Mesenchymal stem cells did not loose their capacity to self-maintenance and well tolerated additional transplantation procedure (Table 1).

Treatment with PTH after lethal irradiation and transplantation of the minimum doses of bone marrow improves survival of mice and increases cellularity of

TABLE 1. Sizes of Ectopic Hemopoiesis Foci after Retransplantation (*M*±*m*)

Group	Primary recipients	Secondary recipients	
		from mice not treated with PTH	from PTH-treated mice
Controls	13.5±3.1	6.9±1.3	8.7±1.4
PTH-treated	8.6±0.9	12.1±1.1	7.0±0.9

the bone marrow [2]. In the absence of stress-associated shortage of hemopoietic cells (under conditions of normal hemopoiesis) PTH had no effect on stromal precursor cells and their differentiated descendants (osteoblasts). Mesenchymal stem cells retained their priority functions (transfer of hemopoietic microenvironment, self-maintenance, and multilinear differentiation). Treatment with PTH increased the content of early hemopoietic precursors, which can hardly be attained by known methods, even *ex vivo*. Further studies of the therapeutic potentialities of PTH in this sphere seems to be promising.

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