Defective Adhesion and Homing of Hemopoietic Precursors from the Bone Marrow of Patients with Myelodysplasia to Stromal Cells of Normal Bone Marrow Cultures

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Hemopoietic precursors from the bone marrow of patients with myelodysplastic syndrome were characterized by lower adhesion to normal stromal sublayer compared to bone marrow precursors from healthy donors, while adhesion to fibroblast monolayer and fibronectin was similar in bone marrow cells from patients and donors. *In vitro* experiments showed that the percentage of adherent hemopoietic precursors from the bone marrow of patients with myelodysplastic syndrome in normal stromal sublayer and fibroblasts was lower compared to healthy donors. The decrease in adhesive activity of hemopoietic precursors from the bone marrow of patients with myelodysplastic syndrome probably contributes to impairment of cell-cell interactions in the bone marrow of these patients.

Key Words: *hemopoietic precursors; adhesion; homing; stroma; myelodysplastic syndrome*

Proliferation and differentiation of hemopoietic precursors (HP) tightly depends on their contact with the hemopoietic microenvironment consisting of stromal cells (fibroblasts, osteoblasts, endotheliocytes, adipocytes, and macrophages) and extracellular matrix. The interaction between HP and hemopoietic microenvironment is mediated by surface adhesion receptors of the integrin and immunoglobulin superfamilies [6]. Homing of hemopoietic cells (HC) in the bone marrow and their mobilization are a complex multistage process involving the interaction between cytokines, chemokines, proteases, selectins, and cell-specific receptors [4,5]. The term "homing" means that HC introduced into the vascular bed specifically migrate in the bone marrow, settle in this organ, and undergo proliferation and differentiation. This process is determined by adhesive interaction between HC and stroma.

Myelodysplastic syndromes (MDS), or myelodysplasia, constitute a group of hemopoietic disorders in humans characterized by clonal abnormality of stem HC with a defect in mature and developing cells (*e.g.*, changes in surface cell antigens). Little is known about disturbances in the bone marrow stromal microenvironment of these patients [2,3].

Here we compared *in vitro* adhesion of HC and HP from the bone marrow of patients with MDS and healthy donors to normal stromal microenvironment (model of homing).

MATERIALS AND METHODS

Bone marrow samples from 8 patients with MDS and 4 healthy donors were used in the experiments. Mononuclear cells were isolated from the bone marrow aspirate in a Ficoll density gradient (1.077 g/cm^3). The

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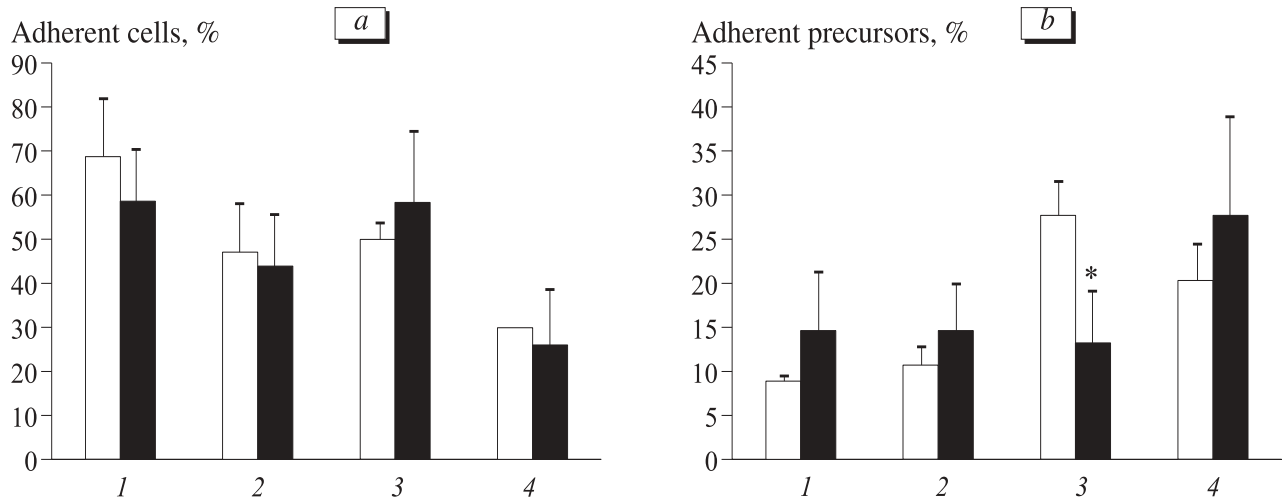

Fig. 1. Adhesion of hemopoietic cells (*a*) and hemopoietic precursors (*b*) from bone marrow of healthy donors (light bars) and patients with myelodysplastic syndrome (dark bars) to substrates after 2-h incubation. Here and in Figs. 2 and 3: plastic surface (*1*), fibroblast monolayer (*2*), stromal sublayer from long-term bone marrow cultures (*3*), and fibronectin (*4*). *$p<0.05$ compared to healthy donors.

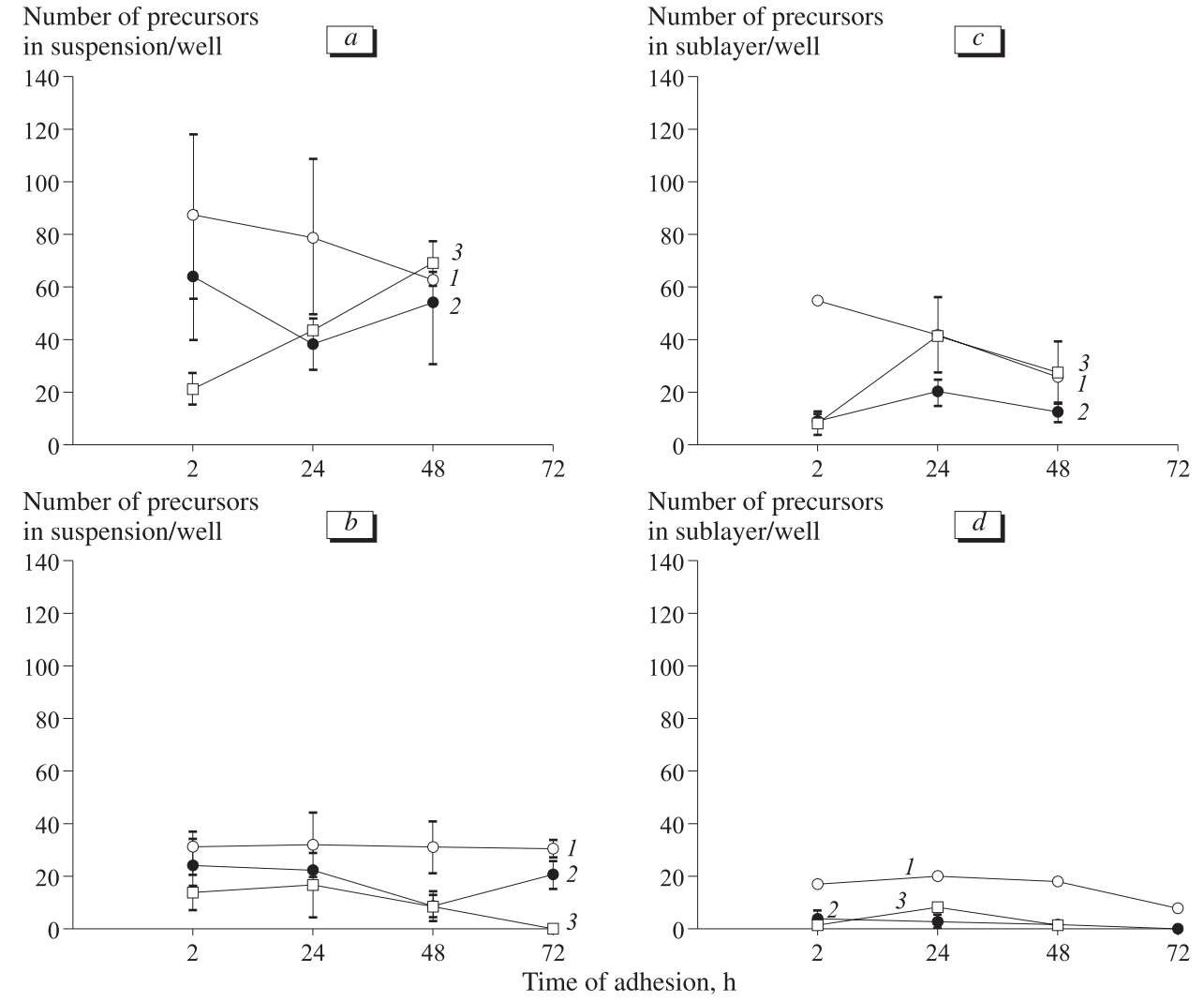

Fig. 2. Number of hemopoietic precursors in the suspension (*a*, *b*) and sublayer (*c*, *d*) from bone marrow cultures of healthy donors (*a*, *c*) and patients with myelodysplastic syndrome (*b*, *d*) during culturing for 2-72 h.

stromal sublayer from long-term cultures of normal bone marrow, monolayer of normal fibroblasts, plastic, and fibronectin-coated plastic (element of the extracellular matrix in the hemopoietic microenvironment) served as the adhesive substrate. Adhesion of HC and HP to the stromal sublayer, bone marrow fibroblasts from healthy donors, fibronectin, and plastic was assayed after 2-h incubation in a serum-free medium. Bone marrow mononuclear cells from patients with MDS and healthy donors were suspended in the medium (2×10^6 cells/ml) and transferred to the test substrate. The stromal sublayer was obtained by long-term culturing of the bone marrow from healthy donors [2]. The cells were treated with trypsin after 3-5 weeks in culture, transferred to 24-well plates (10^5 cells/well), and cultured in complete nutrient medium for 24 h. Fibroblast monolayer was obtained by repeated passaging of bone marrow cells from healthy donors (2-4 passages). Fibronectin in a concentration of 10 μg/ml was placed on the surface of a 24-well plate at 37°C for 1 h. The plastic was treated with 0.5% bovine serum albumin. Adherent cells were removed from the substrate with 0.25% trypsin. The count of viable cells per well was estimated using trypan blue.

HP (granulocyte-macrophage and erythroid cells) were studied in the methylcellulose culture [1] either 2 (adhesion) or 24-48-72 h (homing) after cell transfer to the corresponding adhesive substrate. Adhesion and homing were estimated as the percentage of HC or HP adhering to the substrate: number of adherent HC (HP)/number of adherent HC (HP)+number of non-adherent HC (HP)×100%.

The results were analyzed by Student's *t* test.

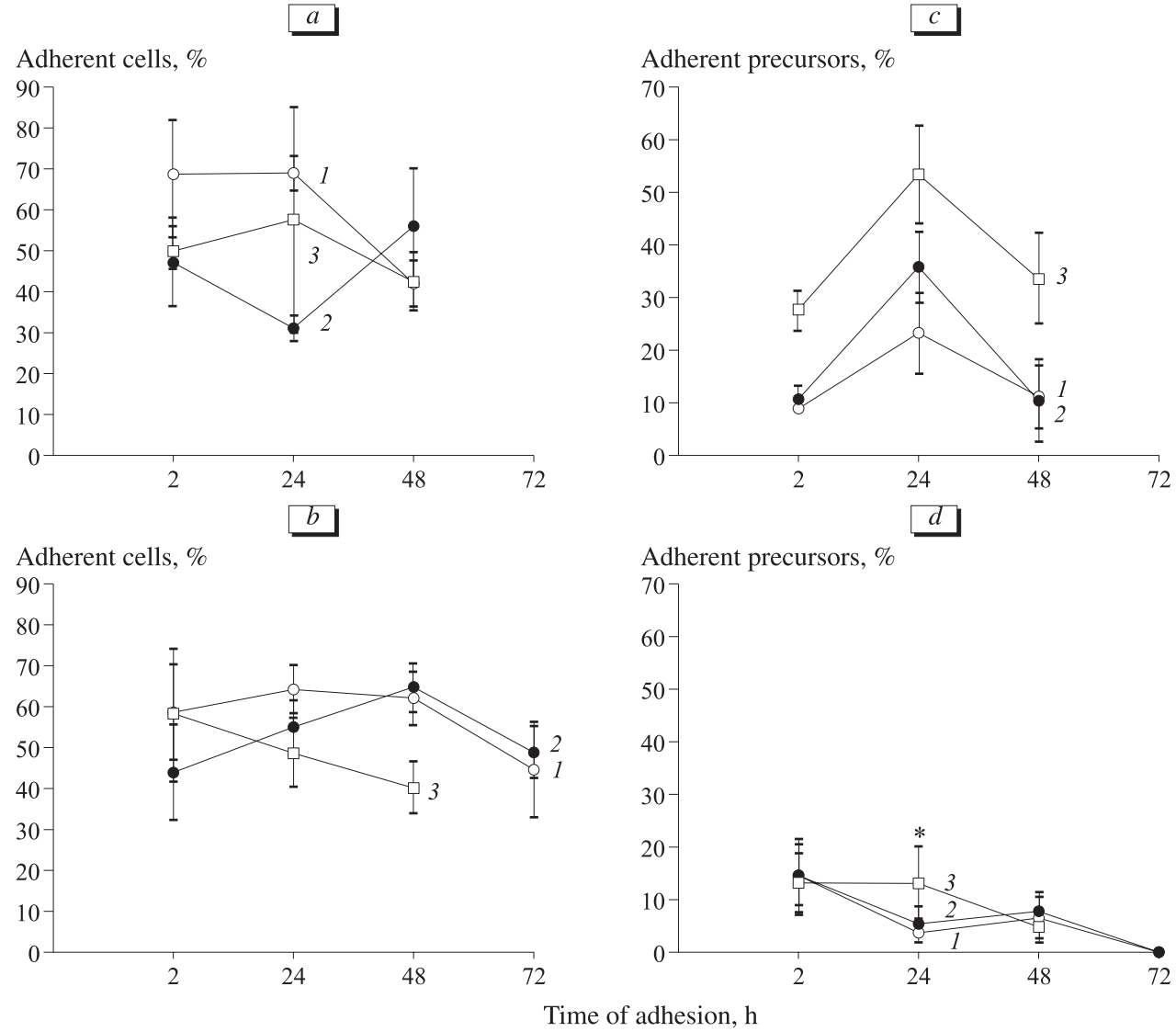

Fig. 3. Percent of hemopoietic cells (*a*, *b*) and hemopoietic precursors (*c*, *d*) isolated from the bone marrow of healthy donors (*a*, *c*) and patients with myelodysplastic syndrome (*b*, *d*) and adhering to the substrate during culturing for 2-72 h. *$p<0.05$ compared to healthy donors.

RESULTS

No differences were revealed in the number of HC from the bone marrow of MDS patients and healthy donors adhering to different substrates (Fig. 1, *a*). However, in patients with MDS the ratio of bone marrow HP adhering to normal stromal sublayer was 2-fold lower compared to healthy donors (13.2±5.9 and 27.7±3.7%, respectively, Fig. 1, *b*). Adhesion of HP to fibroblast monolayer did not differ in MDS patients and healthy donors (Fig. 1, *b*).

The number of HC in the suspension and the number of cells adhering to the stromal sublayer and fibroblasts during 2-72-h culturing (homing) decreased insignificantly and did not differ in patients with MDS and healthy donors. It can be hypothesized that nearly all cells from patients and healthy donors adhered over the first 2 h of culturing. The decrease in the number of cells by the 3rd day in culture was probably related to their natural death *in vitro*. It should be noted that in MDS patients (Fig. 2, *b*, *d*) the number of HP in the suspension and the number of cells adhering to the stromal sublayer and fibroblasts was lower than in healthy donors (Fig. 2, *a*, *c*). The ratio of bone marrow HC adhering to normal stromal sublayer and fibroblasts did not differ in patients with MDA and healthy donors. However, in MDS patients the ratio of HP adhering to various substrates over 3-day culturing was lower than in healthy donors (Fig. 3).

Our results show that more than 50% HC and 10% HP adhere to the substrate over the first hours of culturing. In patients with MDS the absolute and relative numbers of colony-forming units adhering to various substrates were lower than in healthy donors. The decrease in adhesive activity of HP from the bone marrow of patients with MDS probably contributes to the impairment of cell-cell interactions in the bone marrow of these patients and attests to changes in signaling mechanisms mediated by adhesion molecules.

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