VCAM-1 Expression on Bone Marrow Stromal Cells from Patients with Myelodysplastic Syndromes

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We studied the expression of VCAM-1 adhesion molecules on stromal cells from the bone marrow of patients with myelodysplastic syndromes, healthy donors, and patients with chronic myeloproliferative diseases and acute leukemias. Expression of adhesion molecule on mesenchymal stromal cells from the bone marrow of patients and healthy donors was evaluated after 2-4 passages by the methods of immunoprecipitation and electrophoresis. VCAM-1 expression in the majority of patients with myelodysplastic syndromes was lower than in healthy donors. At the same time, VCAM-1 expression was not identified on mesenchymal cells from acute leukemia patients. VCAM-1 expression on cells from patients with chronic myeloproliferative diseases did not differ from that in healthy donors. We conclude that VCAM-1 synthesis in bone marrow stromal cells is impaired in patients with myelodysplastic syndromes and acute leukemias. These changes can be followed by the loss of relationships between hemopoietic cells and stromal microenvironment in bone marrow niches. Hemopoietic cells gain the ability for uncontrolled growth, which results in progression of the disease.

Key Words: *adhesion molecules; VCAM-1; bone marrow mesenchymal cells; stroma; myelodysplastic syndromes*

Adhesion molecules play an important role in the regulation of normal hemopoiesis. VCAM-1 (vascular cell adhesion molecule-1) belongs to immunoglobulin-like transmembrane adhesion molecules that are involved in a variety of cell functions [8]. VCAM-1 is expressed on stromal/endothelial cells of the bone marrow and some hemopoietic cells (B cells, follicular dendritic cells, and macrophages) [7]. α4β1 integrins (VLA-4, very late antigen-4) serve as a ligand for VCAM-1. They are mainly expressed on hemopoietic cells. VLA-4/VCAM-1 complex mediates adhesion of hemopoietic cells to the stroma and extracellular matrix components and plays an important role in homing of stem cells [14]. Stromal microenvironment is a complex system of cells (fibroblasts, endothelial cells, adipocytes, and macrophages) and extracellular matrix. The interaction between hemopoietic precursor cells and stromal microenvironment plays the major role in further transformations of the cell (proliferation, differentiation, or apoptosis). The bone marrow from patients with myelodysplastic syndromes (MDS) serves as a model for *in vitro* studies of the stroma. This heterogeneous group of diseases is characterized by clonal injury to the stem hemopoietic cell and high risk for transformation into acute myeloleukemia [9].This diseases manifests *in vitro* by qualitative and functional changes in the hemopoietic microenvironment [2,3,11-13]. Most studies were conducted on non-fractionated stromal microenvironment, therefore it was difficult to characterize abnormalities in individual types of cells. Here we studied the expression of adhesion molecules (α5β1, VCAM-1, and CD44) on mesenchymal stromal cells (MSC) from the bone marrow of MDS patients using the methods of im-

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munoprecipitation and electrophoresis. For comparative analysis, the expression of adhesion molecules on bone marrow mesenchymal cells was evaluated in patients with chronic myeloproliferative diseases (CMPD) and healthy donors.

MATERIALS AND METHODS

Bone marrow samples were obtained from 8 patients with MDS, 2 patients with acute leukemia (AL), 5 healthy donors, and 12 patients with CMPD. Mononuclear cells were isolated from the bone marrow aspirate in a Ficoll density gradient (1.077 g/cm^3). Bone marrow cells from patients and healthy donors were cultured in α-MEM medium (HyClone) with 10% FBS (HyClone) to obtain the monolayer cultures. The concentration of bone marrow cells was 5-9×10^6 cells per flask (bottom area 25 cm^2). After attaining 80-100% confluence, the cells were subcultured 2-4 times and used in further experiments as MSC. Immunoprecipitation was performed with monoclonal antibodies to α5β1 (CD49d)), VCAM-1 (CD106), and CD44 (all antibodies were from Pharminger and Chemicon). Expression of adhesion molecules was evaluated by Western blotting on biotinylated MSC (after 2-4 passages). Incubation with biotin (Pierce) was conducted at 37°C for 40 min. The cell monolayer was scraped with a policeman and lysed in the presence of proteinase inhibitors. Monoclonal antibodies were added to the cell lysate. The mixture was agitated at 4°C for 1 h and treated with protein G-Sepharose (Pierce) at 20°C for 1 h. Protein G-Sepharose was washed out with a buffer solution. The samples for electrophoresis were prepared. Electrophoresis was performed in 10% SDS gel followed by transfer to nitrocellulose membranes. After incubation with avidin peroxidase, the membranes were treated with a chemiluminescent substrate (SuperSignal West Pico Chemiluminescent Substrate; Pierce). The imprints were put on a roentgen film and developed. Several immunoblots (2-4) were performed for each patient. Albumin served as the control for electrophoresis. Protein content was measured by the standard method. The results were analyzed by Student's *t* test.

RESULTS

Expression of adhesion molecules was studied on the monolayer of bone marrow MSC. The monolayer of control cultures and specimens from MDS patients consisted of fibroblast-like spindle-shaped cells. Hemopoietic cells were not identified. The characteristics of this monolayer differed in MDS patients and healthy donors. At the same number of explanted cells in a culture, the time of attaining confluence in MDS patients was longer: 45 days *vs.* 6-20 days in healthy donors (Table 1). Moreover, the monolayer of MSC from bone marrow cells could be obtained not in all patients with MDS. In some patients with MDS, the number of MSC passages did not exceed 5 (*vs.* 8 in healthy donors).

The expression of cell markers was studied on bone marrow MSC from 5 patients with MDS and 2 patients with AL. Bone marrow MSC from 4 healthy donors were used as the control. Comparative study was conducted on MSC from 4 patients with CMPD.

Figure 1 shows representative immunoblots from MSC cultures. VCAM-1 expression on cells from 4 patients with MDS (P1-P3 and P5) was less significant than in healthy donors (D1-D4; Fig. 1). The exception was 1 patient (P4). VCAM-1 expression in this patient was much higher than in healthy donors and other patients with MDS (Fig. 1, *d*). VCAM-1 expression on immunoblots was not identified in 2 patients with AL

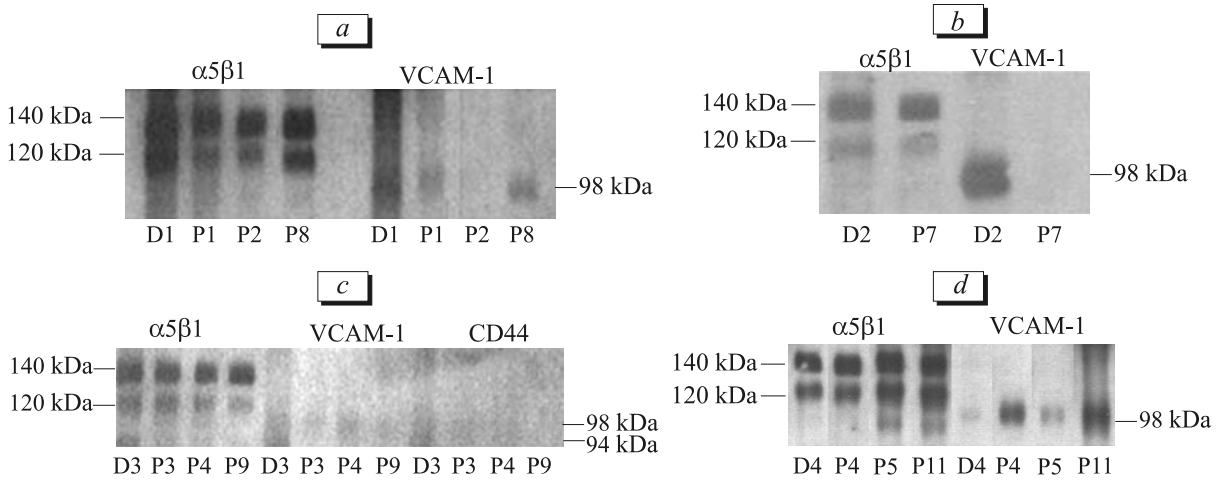

Fig. 1. Expression of α5β1, VCAM-1, and CD44 on mesenchymal cells from patients with MDS, CMPD, and AL (immunoblots) and healthy donors. D1-D4, healthy donors; P1-P5, MDS patients; P7, AL patient; P8-P11, CMPD patients.

TABLE 1. Characteristics of Bone Marrow MSC Cultures from Healthy Donors and MDS Patients

Diagnosis	Number of observations	Number of ceeded cells, $\times 10^6$	Confluence		Number of passages	Number of scrapped cells per flask, $\times 10^6$
			%	time to confluence, days		
Normal (donor)	5	7.8-8.5	100	6-20	>8	0.5-0.8
MDS	7	5.0-8.0	50-100	7-45	4-5	0.3-0.7

(P6 and P7; Fig. 1, *b*). VCAM-1 expression in CMPD patients practically did not differ from that in healthy donors (Fig. 1, *a*, *d*). No differences were found in the expression of α5β1 and α2β1 on MSC from patients and healthy donors. CD44 expression was not identified on cells from patients and healthy donors (Fig. 1, *c*).

These data indicate that VCAM-1 is expressed on bone marrow MSC from MDS patients and healthy donors. However, VCAM-1 expression in MDS patients was lower than in healthy donors. VCAM-1 expression was practically absent in 2 patients with AL. Taking into account the fact that MDS are "preleukemic disorders", the reduced expression of VCAM-1 on stromal cells from these patients can serve as a criterion of leukemia progression. The degree of VCAM-1 expression was very high only in 1 patient with MDS. This patient was classified to the individual group of MDS (5q syndrome) with a favorable course and good prognosis. These findings are consistent with published data and results of our previous experiments, which illustrate dysfunction of the stroma in MDS patients. For example, MDS patients are characterized by a reduced number of fibroblast precursors in the bone marrow [1] and low capacity of the hemopoietic microenvironment to maintain hemopoiesis in long-term cultures [1,2] and monolayer cultures of the bone marrow [6]. Recent cytogenetic studies revealed the presence of chromosomal aberrations in mesenchymal cells from patients with MDS and AL [4,5]. Variations in the production of cytokines by stromal cells were described previously [6]. However, no differences were found in the expression of CD14, CD34, and CD68 on hemopoietic cells, as well as in the expression of CD29, CD90, and CD105 on stromal cells from MDS patients and healthy donors. We failed to reveal the differences in functional activity of VCAM-1 adhesion molecules on MSC from CMPD patients and healthy donors. Our results are consistent with published reports on only insignificant changes in the hemopoietic microenvironment of CMPD patients [10].

We conclude that the expression of VCAM-1 genes is impaired in mesenchymal cells (one of the elements of the hemopoietic microenvironment) of MDS patients. Signal transduction between hemopoietic and stromal cells is realized via the VLA-4/VCAM-1 complex, which plays an important role in the regulation of cell adhesion. The observed changes in the expression of adhesion molecules on the surface of mesenchymal cells (*e.g.*, in preleukemic patients with MDS) can occur during the interaction between hemopoietic and stromal cells in bone marrow niches (stromal–osteoblast and sinusoidal–vascular niches). It results in the impairment of signal mechanisms, appearance of oncogene mutations, genomic instability, and loss of relationships between transformed hemopoietic cells and abnormal stromal microenvironment. Abnormal expression of VCAM-1 genes in mesenchymal cells (an element of the hemopoietic microenvironment) of MDS patients suggests that the stroma plays an important role in the pathogenesis of this disease.

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