Cytofluorometric Analysis of Phenotypes of Human Bone Marrow and Umbilical Fibroblast-Like Cells

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Comparative analysis of the expression of some surface markers of human bone marrow mesenchymal stem cells, umbilical fibroblast-like cells, and skin fibroblasts was carried out by the flow cytofluorometry method. Mesenchymal stem cells and umbilical fibroblast-like cells were similar by the levels of expression of the main histocompatibility complex antigens, adhesion molecules, and some growth factor receptors. The profile of skin fibroblast surface antigens was characterized by higher expression of the markers typical of differentiated cells. The results prove the possibility of using umbilical fibroblast-like cells as an alternative source of mesenchymal stem cells for cell replacement therapy.

Key Words: *mesenchymal stem cells; umbilical cells; fibroblasts; surface markers; flow cytofluorometry*

The use of cell preparations for replacement therapy poses a series of problems: cell material should be easily available, cause no serious immune reactions, and conform to ethical and legal standards. An obligatory condition for wide use of cell technologies in clinical medicine is the possibility of standardization of cell preparations.

Mesenchymal stem cells (MSC) are regarded as the most perspective material for cells transplantation due to a variety of their differentiation trends and the possibility of culturing. MSC isolated from the bone marrow are characterized by fibroblast-like morphology and, as pluripotent cells, possess a potential of differentiation into cells of different tissues, including bone, cartilage, fatty, muscle, and their tissues [1,13]. The unique surface antigens specific of MSC have not heretofore been described, and therefore search for these markers remains an important task.

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Transplantation of autologous MSC isolated from the bone marrow rules out untoward immunological reactions. However, the number of MSC in the bone marrow decreases with age and their proliferative and differentiation potentials also decrease [8]. The umbilical cord is regarded as one of the alternative sources of MSC [9]. In this case MSC are isolated from Wharton's jelly (primitive connective tissue of the umbilical cord). These fibroblast-like cells differentiate into cells of different types, including the neuron-like cells, expressing enolase specific of the nerve cells [11].

We carried out a comparative analysis of surface antigens of cells with fibroblast-like morphology: bone marrow MSC and umbilical fibroblast-like cells.

MATERIALS AND METHODS

Bone marrow MSC, umbilical fibroblast-like cells, and skin fibroblasts were isolated from primary cultures at Laboratory of Cellular Medical Technologies, Department of Biomedical Technologies of Russian State Medical University. All cells were

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cultured in a 1:1 mixture of DMEM and F12 (Gibco) at 37°C and 5% CO_2. The medium contained 10% fetal calf serum (FCS; HyClone), 100 U/ml penicillin, 100 U/ml streptomycin, and 10 ng/ml fibroblast growth factor (bFGF; Sigma). Cells passed no more than 10 passages and reaching 50% confluence were used in cytophenotyping experiments.

Immunocytochemical staining was carried out using fluorochrome-conjugated monoclonal antibodies to human surface antigens. The following fluorochromes were used: allophycocyanine (APC), FITC, or combined phycoerythrincyanine 5 (PeCy5; Becton Dickinson). FITC-conjugated rabbit antibodies (Molecular Probes) served as second antibodies for staining for CD105. Isotypical antibodies labeled with respective fluorescent stains of the same company served as the negative control. In order to determine the phenotype, the cells were removed from flasks with Versene and washed twice in staining buffer (PBS, 1% FCS, and 0.1% sodium azide). Labeled antibodies to surface markers (15 µl to each marker) were added to the cells (10^6) and the volume of cell suspension was brought to 100 µl. The samples containing the corresponding isotypical antibodies served as negative controls. Cell suspension was incubated at 4°C for 1 h. After incubation the cells were washed twice in 1 ml staining buffer and fixed in 0.5 ml 2% paraformaldehyde. The resultant cell suspension was filtered through a filter with 30-µ pores in order to eliminate cell aggregations.

Fluorescence intensity was analyzed on a FACSAria flow cytofluorometer/sorter (Becton Dickinson). The cell population was detected by direct and lateral light scattering. The expression of the marker was evaluated by fluorescence intensity histogram. To rule out recording of objects of size lesser than the cells, a registration threshold by direct light scattering equal to 20,000 was established. WinMDI 2.8 software was used for more demonstrative imaging and statistical processing of the results. The fluorescence intensity was evaluated by the geometrical mean and standard deviation.

RESULTS

The population of fibroblast-like cells was distinguished from other recorded events by direct and lateral light scattering parameters (Fig. 1). Human skin fibroblasts served as the control. The expression of phenotypical markers was evaluated by the intensity of fluorescence of cells of the studied population. The markers were tentatively divided into 4 groups, depending on their functional role: main histocompatibility complex (HLA) antigens, adhesion molecules, growth factor receptors, and markers characteristic of hemopoietic cells.

The relative level of class I HLA molecules (HLA-ABC) expression on bone marrow MSC and umbilical fibroblast-like cells was 11.81±5.71 and 13.17±5.40 arb. units of fluorescence, respectively (Fig. 2). The level of HLA-ABC expression on skin fibroblasts was significantly higher (289.53±34.74 arb. units). As was expected, class II HLA antigens (HLA-DR) were not detected on fibroblast-like cells or fibroblasts. These results are in line with the data on reduced immunogenicity of umbilical fibroblast-like cells [2], which suggests fibroblast-like cells as a perspective material for allogenic transplantation.

Adhesion molecules attract special interest as stem cell markers, because they can be involved in

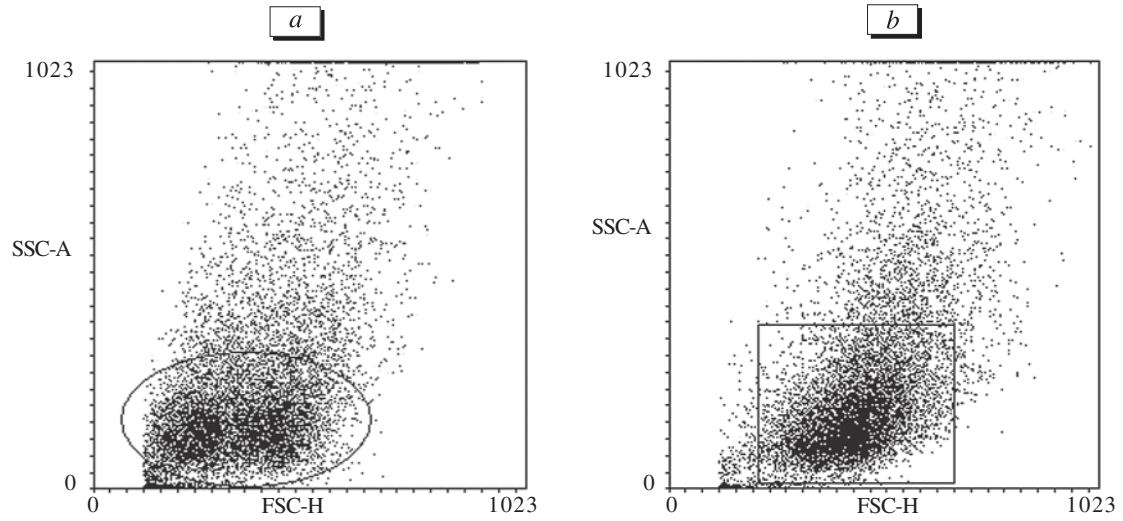

Fig. 1. Bone marrow MSC (*a*), umbilical fibroblast-like cells (*b*). Abscissa: direct light scattering; ordinate: lateral light scattering.

homing and migration processes. CD44 (hyaluronic acid receptor) is expressed on the majority of body cells and triggers the cascade of reactions regulating cell differentiation and proliferation [15]. The level of CD44 expression on fibroblast-like cells was significantly lower than on fibroblasts (Fig. 3). Umbilical cells were stained less intensely. This result can be explained by lack of strong connection between stem cells and intercellular matrix.

CD54 (intracellular adhesion molecule, ICAM-1) serves as the ligand for β_2-integrins present on the surface of blood cells (monocytes, basophils, lymphocytes) [13]. ICAM-1 plays an important role in tissue interactions with immune cells, for example, in inflammatory processes. In contrast to skin fibroblasts virtually not expressing CD54, the expression of this marker on umbilical and bone marrow MSC was significant.

CD49b is a collagen receptor binding types I and III collagens [4]. The molecule belongs to β_1-integrins. The expression of this marker was not high in comparison with other adhesion molecules and about the same for cultures of umbilical fibroblast-like cells and skin fibroblasts. This marker was not expressed on bone marrow MSC.

CD90 is present on epidermal cells, myoblasts, keratinocytes, fibroblasts, and hemopoietic stem cells isolated from different sources. The molecule belongs to the immunoglobulin-like receptor superfamily and seems to be essential for cell interactions with endotheliocytes [8]. High expression of this marker was observed in cell populations of all three cultures. CD90 expression was maximum on skin fibroblasts, intermediate on umbilical fibroblast-like cells, and minimum on bone marrow cells.

CD106 vascular cell adhesion molecule (VCAM-1) is present on the surface of activated endothelium of skin vessels, muscles, and lungs, is expressed on hemopoietic precursor cells, providing interactions with bone marrow stromal cells [12]. VCAM-1 promotes lymphocyte migration to the focus of inflammation in inflammatory reactions. Minor expression of this marker was detected only in bone marrow MSC culture.

CD105 (endoglin) is a growth factor receptor for activin A, transforming growth factor-β [10]. Endoglin triggers the cascade of reactions modifying the morphology and adhesive properties of cells. The level of endoglin expression is virtually the same for all three studied populations (Fig. 4). CD117 (*c-kit* gene product) is tyrosine kinase receptor for stem cell factor. This factor is involved in the maintenance and development of melanocyte and hemopoietic precursors and germinative stem cells. CD117 is not expressed on the surface of bone marrow MSC [7] or umbilical fibroblast-like cells (Fig. 4).

CD34 hemopoietic stem cell marker [3] and CD45 common leukocytic antigen [5] were not

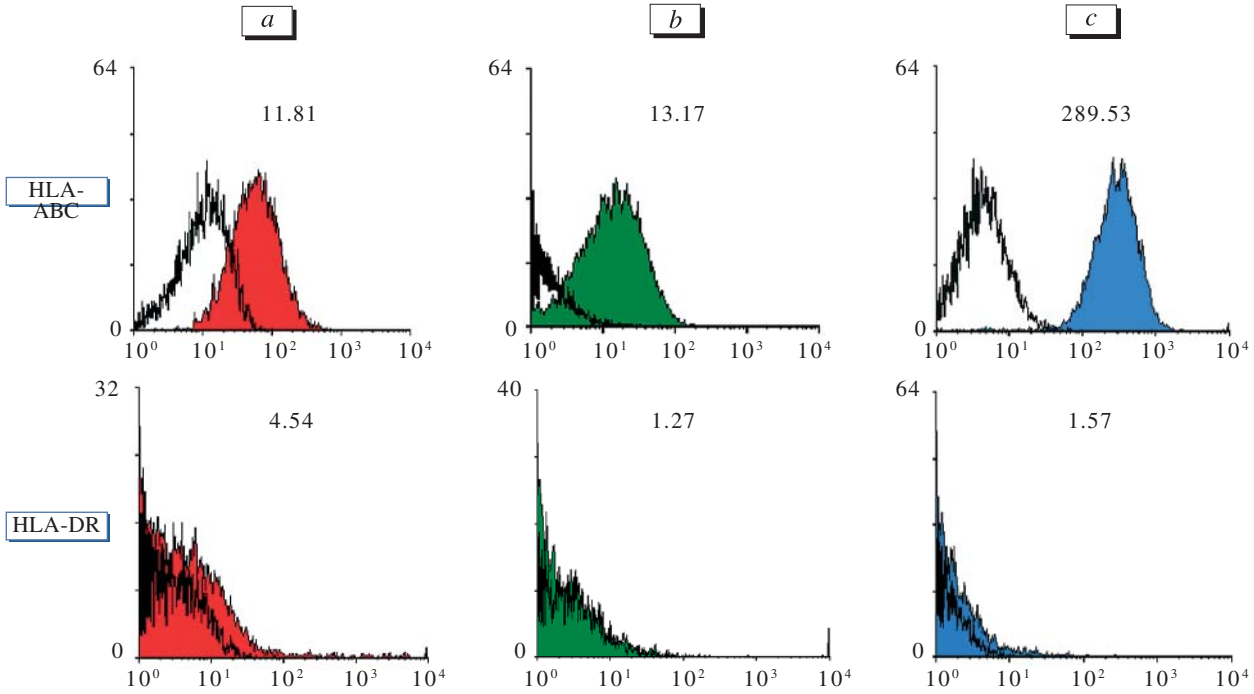

Fig. 2. HLA antigen expression on bone marrow MSC (*a*), umbilical fibroblast-like cells (*b*), and skin fibroblasts (*c*). APC-conjugated antibodies were used. Here and in Figs. 3-5: abscissa: fluorescence intensity; ordinate: number of events (cells). Dark line: isotypical control; figures show the geometrical mean of specific fluorescence intensity.

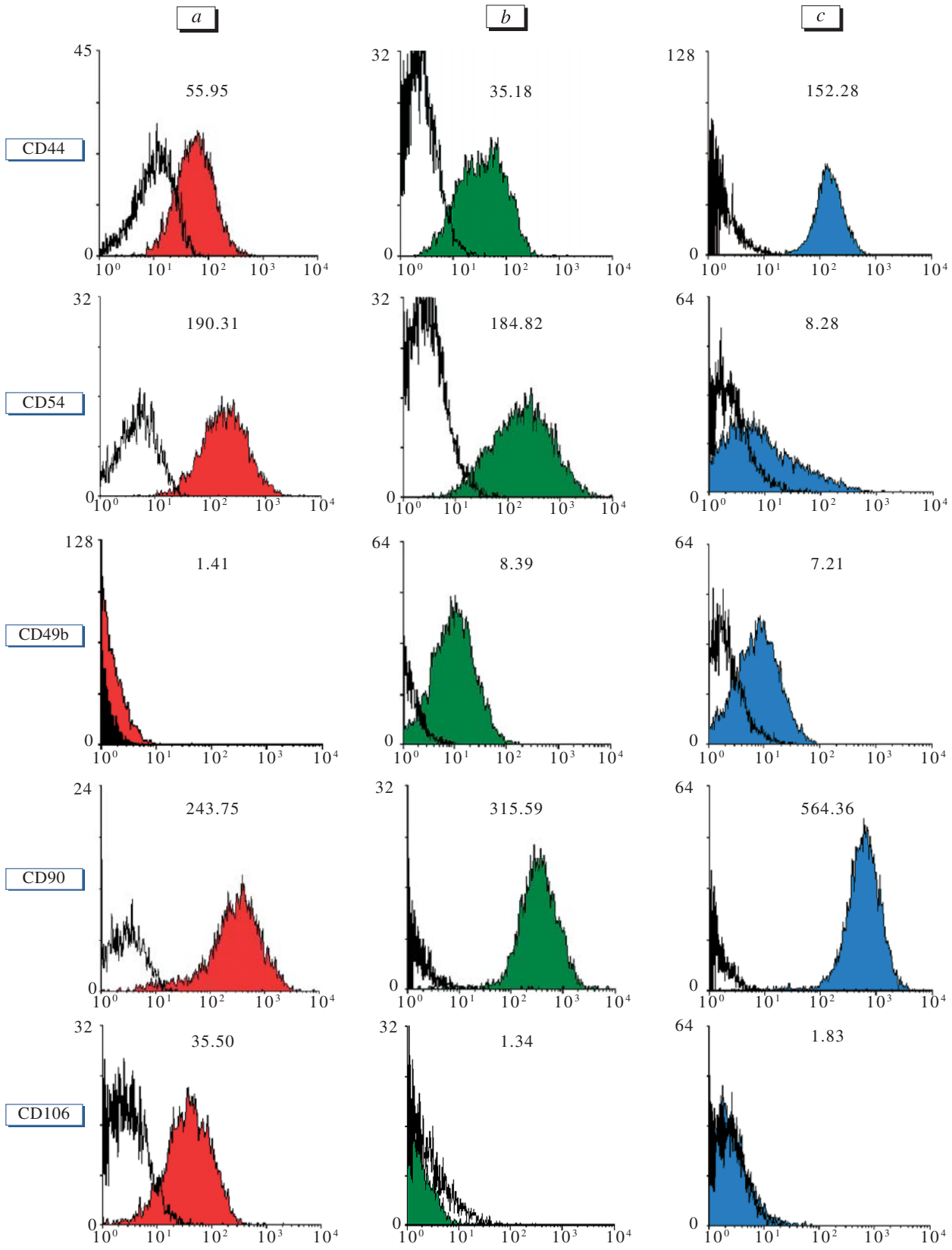

Fig. 3. Expression of cell adhesion molecules on bone marrow MSC (*a*), umbilical fibroblast-like cells (*b*), and skin fibroblasts (*c*). CD44, CD49b: FITC; CD54, CD90, CD106: APC.

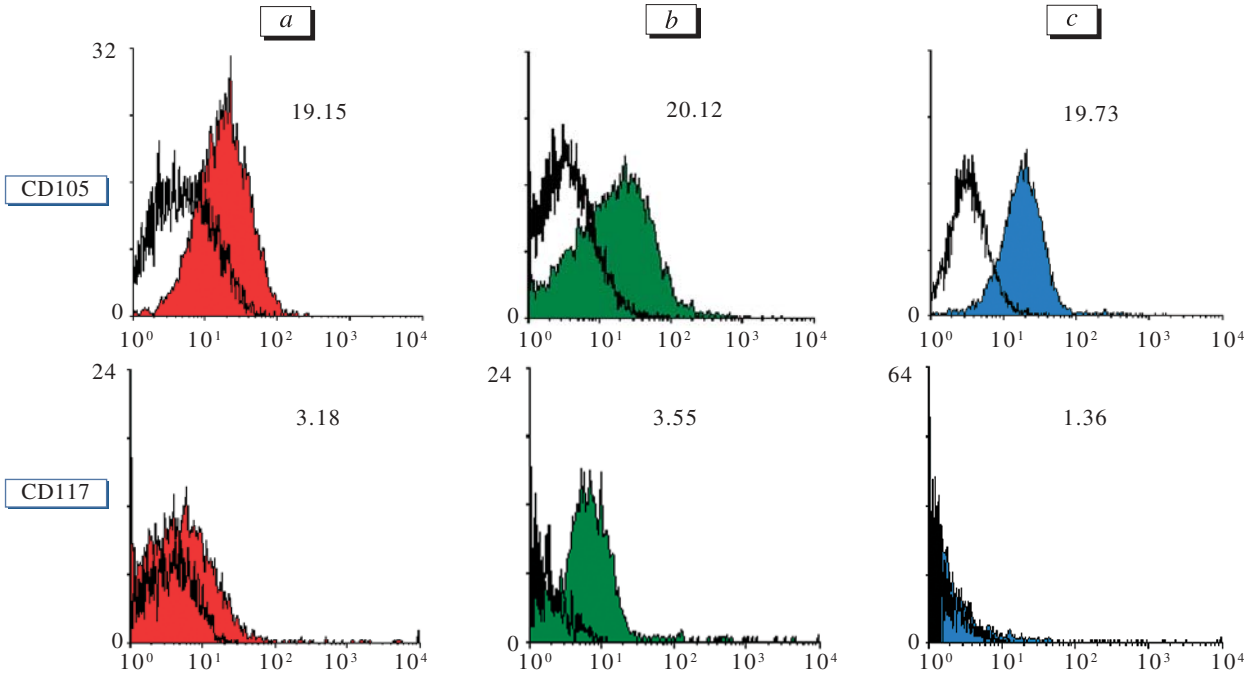

Fig. 4. Expression of growth factor receptors on bone marrow MSC (*a*), umbilical fibroblast-like cells (*b*), and skin fibroblasts (*c*). CD105: FITC; CD117: Pe-Cy5.

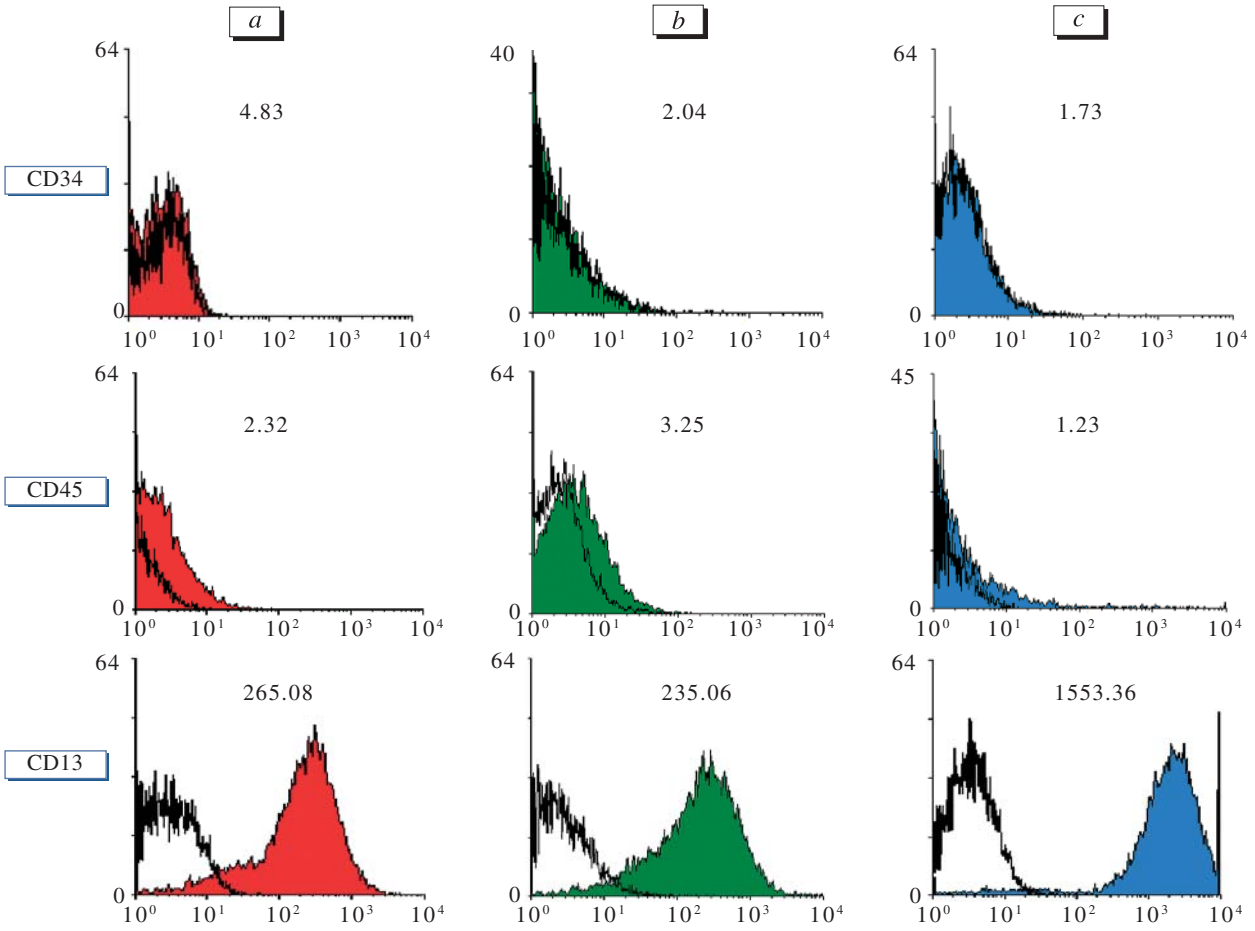

Fig. 5. Expression of hemopoietic markers on bone marrow MSC (*a*), umbilical fibroblast-like cells (*b*), and skin fibroblasts (*c*). CD34, CD13: APC; CD45: FITC.

detected in the studied cells (Fig. 5), which rules out the appurtenance of these cultures to hemopoietic cells. Myeloid membrane glycoprotein (CD13) is expressed not only on blood cell surface, but also on fibroblasts, neurons, bone marrow stromal cells [6]. The content of CD13 in cultures of umbilical and bone marrow cells was 6-fold lower than on fibroblasts (Fig. 5).

The study revealed similarity of phenotypical profiles of bone marrow MSC and umbilical fibroblast-like cells by the expression of HLA antigens, adhesion molecules, myeloid membrane glycoprotein, and growth factor receptors. Hence, new proofs of the possibility of using umbilical fibroblast-like cells as an alternative source of MSC were obtained. Due to low level of HLA expression on both cell types, positive results of allogenic transplantation in clinical practice can be expected.

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