Multipotent mesenchymal stromal cells (MSCs) were first described by Friedenstein in 1976. He discovered that the bone marrow contains, along with hematopoietic stem cells, mesenchymal cells capable of producing cells of connective tissue: adipocytes, chondrocytes, osteocytes, and fibroblasts [1]. Later, cells with similar phenotype and expression of surface antigens were found in the stroma of adipose tissue [2–4]. In 1964, Rodbell et al. [5] extracted two populations of cells by proteolysis and centrifugation: mature adipocytes and the stromal-vascular fraction from adipose tissue. Later, this fraction was found to contain not only endothelial and smooth muscle cells, fibroblasts and macrophages, but also fibroblast-like cells that carried the bone marrow progenitor cell antigens CD90, CD105, CD73, CD146, and STRO-1 [2–4]. To date, MSCs have been found in many organs and tissues: the umbilical cord, endometrium, spleen, liver, thymus, and others. According to the current views, MSCs are able to differentiate into tissues of mesodermal origin. Recently, more studies on their ability to acquire the phenotypes of myoblasts, cardiomyocytes, endotheliocytes, hepatocytes, and neutral cells have been published [6, 7]. Presently, MSCs of adipose tissue (AT MSCs) are considered to be one of the most suitable sources of cells for regenerative and restorative medicine [3, 8]. This can be explained by the availability of adipose tissue samples with minimal traumatic influence on a patient, and further in vitro expansion of MSC to obtain a sufficient amount of cells. Many studies demonstrate their potential therapeutic effect on the regeneration of adipose, bone, and cartilaginous tissues and on regeneration of the liver, pancreas, and nervous tissue [6, 7].

Modern views on the mechanisms of regeneration of tissues are based on functional features of MSCs, such as adhesion, proliferation, differentiation, homing, synthesis and secretion of growth factors, and regulation of immune processes. Paracrine effects that depend on the secretory activity of MSCs determine their therapeutic effect to a considerable degree. Many studies on the expression and secretory profiles of MSCs have demonstrated that these cells secrete a wide range of growth and antiapoptotic factors. In our and other authors’ studies, AT MSCs have been demonstrated to produce a considerable amount of angiogenic factors, such as vascular endothelial growth factor; hepatocyte growth factor; placental growth factor; fibroblast growth factor; transforming growth factor β; angiopoietin-1; angiogenin; interleukins 6, 8, and 17; granulocyte colony-stimulating factor; nerve growth factor; and tissue inhibitor of matrix proteinases 1 and 2 [3, 8, 9].

Mesenchymal stromal cells form a heterogeneous population both in the body and in culture. These cells have different morphological and molecular-genetic features and different proliferation and differentiation capacities. Apparently, this population is organized into a certain hierarchy depending on their differentiation, from primary naive cell to stem cells of differentiated tissues. In applied medicine, the problem of “aging” of MSCs and possible impairment of their functions due to various disorders is important for the therapeutic use of MSCs. For example, cardiac ischemia is accompanied by changes in the levels of many metabolites in blood and tissues (glucose, lipids, pro- and antioxidants, etc.). Long-term risk factors include low-density lipoproteins (LDLs). During chronic circulation in the bloodstream, they undergo modifications due to release of free radicals and products of lipid peroxidation from cells and due to an increase in the concentrations of some enzymes and metabolites in blood and intercellular fluid. For example, glucose was found to initiate atherogenic oxidative modification of LDLs in vitro and in patients with type
2 diabetes mellitus. Maintaining the normal blood glucose level in these patients with hypoglycemic therapy is followed by a significant decrease in the LDL oxidation level [10]. Oxidized LDLs are involved in storage of cholesterol and other lipids in the walls of blood vessels and blood cells, inducing mosaic vascular damage. The level of oxidized LDL circulating in the blood was found to be an independent predictor of cardiac ischemia. The concentration of circulating oxidized LDLs from 6 to 14 μg/ml is a marker of subclinic atherosclerosis; concentrations from 13 to 75 μg/ml were discovered in patients with severe atherosclerosis and cardiac ischemia [11]. The concentration of oxidized LDLs in tissues and vascular walls remains unknown. Although the relationship between the level of lipoproteins in blood plasma and atherosclerosis has long since been discovered, the mechanisms of influence of oxidized LDLs on atherogenesis are still unclear. Lately, additional evidence that atherogenesis is an active process rather than a passive deposition of lipids in vascular walls was found. This process involves several types of resident cells and cells migrating from blood that are connected via paracrine regulation (endothelial cells, pericytes, fibroblasts, smooth muscle cells, monocytes, trombocytes, and erythrocytes) [12]. Nevertheless, the evidences of the effect of oxidized LDLs on progenitor cells are scarce and ambiguous. The effect of oxidized LDLs on AT MSCs was not studied before.

We studied the effect of native and oxidized LDLs on MSCs to discover a new target of risk factors of atherosclerosis.

**MATERIALS AND METHODS**

**Extraction and culturing of AT MSCs.** AT MSCs were extracted from adipose tissue samples from healthy donors obtained during surgery. Tissue was mechanically homogenized and treated with type I collagenase solution (Worthington Biochemical, United States). The suspension was filtered, and then erythrocytes were lysed. The cells were resuspended in Advance STEM Mesenchymal Stemcell Basal Medium (HyClone, United Kingdom) containing 10% stem cell growth supplement (HyClone, United Kingdom), 100 U/ml penicillin, and 100 U/ml streptomycin (GIBCO BRL). After the extraction, the cells were cultured in subconfluent layers in plastic dishes at 37°C and 5% CO2 until the second passage.

To perform the experiment, AT MSCs received from three healthy donors were, after the first passage, plated at a concentration of 10⁵ cells/ml on six-well plates and then cultured until subconfluent layers were obtained. Various concentrations of native and oxidized LDLs were added into the growth medium under the conditions of MSC deprivation or without deprivation. Cell deprivation was maintained by
replacing the medium with 0% stem cell growth supplement for 24 h. The viability of MSCs was estimated by the number of living cells found by flow cytometry 24 h after the addition of lipoproteins.

**Extraction of lipoproteins.** Extraction of lipoproteins was performed as described by Havel et al. [13]. LDLs ($d = 1.019–1.063 \text{ g/ml}$) were extracted from plasma of healthy donors at the age of 20–40 years by serial ultracentrifugation in a NaBr gradient and in the presence of 1 mg/ml EDTA. Before the analysis, lipoproteins were dialyzed with 0.01 M phosphate buffer solution (PBS), pH 7.4. Evaluation of the protein concentration was performed by the method of Lowry [14].

**Preparation of oxidized LDLs.** Freshly extracted LDLs were dialyzed with 0.01 M PBS and oxidized by autooxidation in the air at $37^\circ\text{C}$ overnight. The oxidation level of LDLs was estimated by spectrophotometry with absorption at 234 nm and formation of conjugated dienes.

**Cytofluorometry.** To perform the analysis of surface antigens, AT MSCs were removed from dishes with 2 mM/l EDTA in PBS (PAN EKO, Russia). The cell concentration of $0.5–1.0 \times 10^5$ in 100 $\mu$l of PBS with 1% bovine serum albumin was achieved; then, 2 $\mu$l of one of the following mouse monoclonal antibodies were added: CD73-PE, CD90-CyChrome, PDGFR-$\beta$-PE, CD34-APC, CD45-PE-Cy7 (BD Biosciences, Bedford, United States), and CD105-FITC (Serotec, Oxford, United Kingdom). The equivalent amount of isotypic antibodies with the corresponding fluorescent marker was used as a control. Cells were incubated at room temperature in the dark for 45 min. Before the analysis, the cells were washed with PBS once.

The viability of AT MSCs was estimated using the 7-AAD pigment (Invitrogen, United States). The number of 7-AAD-negative (7-AAD-) cells was counted. Expression profile of surface antigens and viability of AT MSCs were estimated using a flow FACS Canto II cytometer (BD Bioscience, United States).

**Statistical analysis.** The analysis of the results was performed using the Microsoft Excel 2007 software.

**RESULTS AND DISCUSSION**

All experiments were performed on stromal cells after the second passaging. MSCs were identified by the presence of the surface markers CD105, CD73, and CD90 and the absence of CD45 and CD34. The cytofluorometric analysis showed that the majority of cells were characterized by the expression of CD105, CD73, and CD90 and did not express CD34 or CD45; i.e., they were multipotent mesenchymal cells (Fig. 1). These cells express platelet-derived growth factor receptor (PDGFR), which is the marker of pericytes. The presence of common markers in AT MSCs and pericytes was also observed by other authors [15]. We suppose that this is evidence of close positions of these cell types in the differentiation hierarchy.

The experiments were performed both without MSC deprivation (in the presence of 10% stem cell growth supplement), when the in vivo conditions of native and oxidized LDLs were reproduced in vitro (Fig. 2a), and with deprivation (0% stem cell growth supplement) (Fig. 2b).

Native LDLs did not affect the viability of MSCs without deprivation, and oxidized LDLs decreased the number of living cells in the culture by 30–35% (Fig. 2a). The decrease in MSC viability was possibly determined by the cytotoxic effect of oxidized LDLs, which was also demonstrated in other cell types [11, 12]. When MSCs were cultured in the medium with 0% stem cell growth supplement, the effect of native and oxidized LDLs on the number of living cells was also different (Fig. 2b). Native LDLs were found to increase the viability of the cells. This effect is called...
“nutritious.” Oxidized LDLs had a similar effect; however, considerably larger concentrations of LDLs were necessary. We used autooxidized (i.e., partly oxidized) LDLs; hence, a double effect could appear: the effect of native LDLs and the effect of oxidized LDLs.

Our results demonstrate that AT MSCs can be a novel target of the damaging effect of oxidized LDLs. The increased concentration of oxidized LDL in patients with hyperlipidemia may decrease the number of MSC in tissues and vascular walls, reducing their ability to regenerate. This effect is possibly another mechanism of atherosclerotic alterations in vascular walls, which is typical of cardiac ischemia associated with metabolic abnormalities.

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