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In Vitro Comparison of Immunological Properties of Cultured Human Mesenchymal Cells from Various Sources

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Cell-mediated cytotoxicity was studied *in vitro* during the interaction of bone marrow mesenchymal stem cells, fibroblast-like cells from newborn umbilical cord, and skin fibroblasts of an adult donor with peripheral blood mononuclear cells. Independently on the origin, mesenchymal cells were not lysed with allogeneic natural killer cells and cytotoxic lymphocytes. Mixed cultures of mesenchymal cells had no cytotoxic effect on peripheral blood mononuclear cells and did not activate proliferation of T and B lymphocytes, natural killer cells, and $CD14^+$ lymphocytes. *In vitro* experiments showed that mesenchymal cells of different origin and allogeneic immunocompetent cells are tolerant to each other.

Key Words: *bone marrow mesenchymal stem cells; umbilical fibroblast-like cells; skin fibroblasts*

Bone marrow mesenchymal stem cells (MSC) *in vivo* induce specific immune tolerance [3,7] and *in vitro* inhibit proliferation of T and B lymphocytes in response to activation and maturation of dendrite cells [4-6].

We previously isolated adherent cultures of human skin fibroblasts (SF), human umbilical fibroblast-like cells (UF) after normal delivery at 38-40 weeks' gestation, and human bone marrow MSC. The expression of cytoplasmic (nestin, type 1 and 2 collagen, and von Willebrand factor) and surface proteins (CD13, CD44, CD49b, CD54, CD90, CD105, CD106, and CD117) in cultures of SF, UF, and bone marrow MSC was compared by immunocytochemical reaction and flow cytofluorometry. The culture of bone marrow MSC was practically homogeneous by surface protein expression of multipotent stromal cells and did not express cytoplasmic proteins of differentiated cells. The culture of UF was heterogeneous and consisted of at least 2 populations. Population 1 cells expressed only surface protein markers of multipotent stromal cells. Population 2 cells coexpressed surface protein markers of multipotent stromal cells and cytoplasmic proteins of differentiated cells. SF expressed only several surface protein markers of MSC and progenitor cells and cytoplasmic proteins of differentiated cells [2].

The ability of cultured SF, UF, and bone marrow MSC to differentiate into adipocytes, osteoblasts, and chondrocytes was compared [1]. Bone marrow MSC differentiate into adipose, cartilaginous, and bone tissue and serve as multipotent stromal cells. UF differentiate into adipocytes and chondrocytes. Only a small number of UF can dif-

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ferentiate into osteoblasts. Therefore, only a part of cultured UF serves as multipotent stromal cells. The majority of UF have properties of bipotent stem cells. SF differentiate only into adipose tissue.

Here we compared *in vitro* the immunological properties of bone marrow MSC and fibroblast-like cells from various sources.

Studying *in vitro* interaction of cultured cells with peripheral blood mononuclear cells may help us to predict the *in vivo* interaction of recipient immunocompetent cells with transplanted cells.

MATERIALS AND METHODS

Adult human SF, human bone marrow MSC, and UF after normal delivery at 38-40 weeks gestation were isolated and cultured as described elsewhere [2].

Cell-mediated cytotoxicity was studied during the interaction of cultured cells with peripheral blood mononuclear cells using Cyto Tox 96 kit (Promega) for the evaluation of functional activity of natural killer cells/lymphokine-inactivated killer cells. This method is based on quantitative study of lactate dehydrogenase. This cytosolic enzyme is released during membrane damage and target cell lysis. The lactate dehydrogenase-catalyzed formation of red formazan from iodine nitrotetrazolium salts in the substrate mixture serves as a marker reaction.

Peripheral blood mononuclear cells were isolated from healthy donors in a Ficoll gradient and served as effector cells. Cultured SF, UF, and bone marrow MSC served as the target cells. The suspension culture of B-lymphoma P3HR1 served as the positive control. The monolayer culture of SF, UF, and bone marrow MSC was transferred into the suspension with a mixture of Versene and 0.25% trypsin (1:1). The optimal concentration of target cells was estimated from the maximum release of lactate dehydrogenase by cells. Tween 20 (0.1%) was added to wells with serial binary dilutions of target cells. Serial binary dilutions of peripheral blood mononuclear cells were prepared in a 96-well plate. Target cells at the optimal concentration were added in such a manner that the effector/target cell ratio varied from 250:1 to 2:1. The mixed culture was incubated at 37°C for 4 h. The supernatant was removed. A substrate mixture for lactate dehydrogenase was added to wells and incubated at room temperature for 30 min. Optical density was measured at 492 nm. The relative content of damaged cells was estimated from the ratio of optical density of the solution in wells with a mixture of target and effector cells to optical density of the solution characterized by the maximum release of lactate dehydrogenase from target cells.

Changes in the expression of major histocompatibility complex class I proteins (HLA-ABC) by SF, UF, and bone marrow MSC during coculturing with peripheral blood mononuclear cells were estimated by means of flow cytofluorometry. Mononuclear cells were added to the monolayer culture of SF, UF, and bone marrow MSC in the 1:50 ratio. The mixture was incubated at 37°C for 3 days. Suspension cells were removed. The adherent cells were transferred into the suspension with Versene. The cells were stained with phycoerythrin-labeled anti-HLA-ABC antibodies (Becton Dickinson) in phosphate-buffered saline containing 1% fetal serum and postfixed with 2% paraformaldehyde. The study was performed in a FACSAria flow cytofluorometer (Becton Dickinson). The results were analyzed using WinMDI software.

The fractional composition of peripheral blood mononuclear cells during coculturing with SF, UF, and bone marrow MSC was studied by means of flow cytofluorometry. Mononuclear cells were added to the monolayer culture of SF, UF, and bone marrow MSC in the 1:50 ratio and incubated at 37°C for 5 days. Suspension cells were taken after 1, 3, and 5 days, washed in phosphate buffered saline, and stained with FITC-labeled and phycoerythrin-labeled antibodies against CD4, CD8, CD14, CD19, and CD56.

RESULTS

At the peripheral blood mononuclear cell/target cell ratio of 250:1, the relative content of damaged P3HR1 cells, SF, UF, and bone marrow MSC was 64, 5.1, 5.8, and 1.3%, respectively. Mononuclear cells had

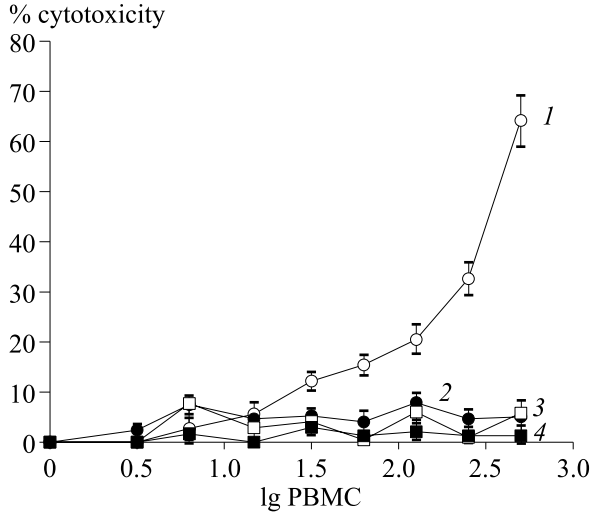

Fig. 1. Cytotoxic effect of peripheral blood mononuclear cells on cultures of B-lymphoma P3HR1 cells (*1*), SF (*2*), UF (*3*), and bone marrow MSC (*4*).

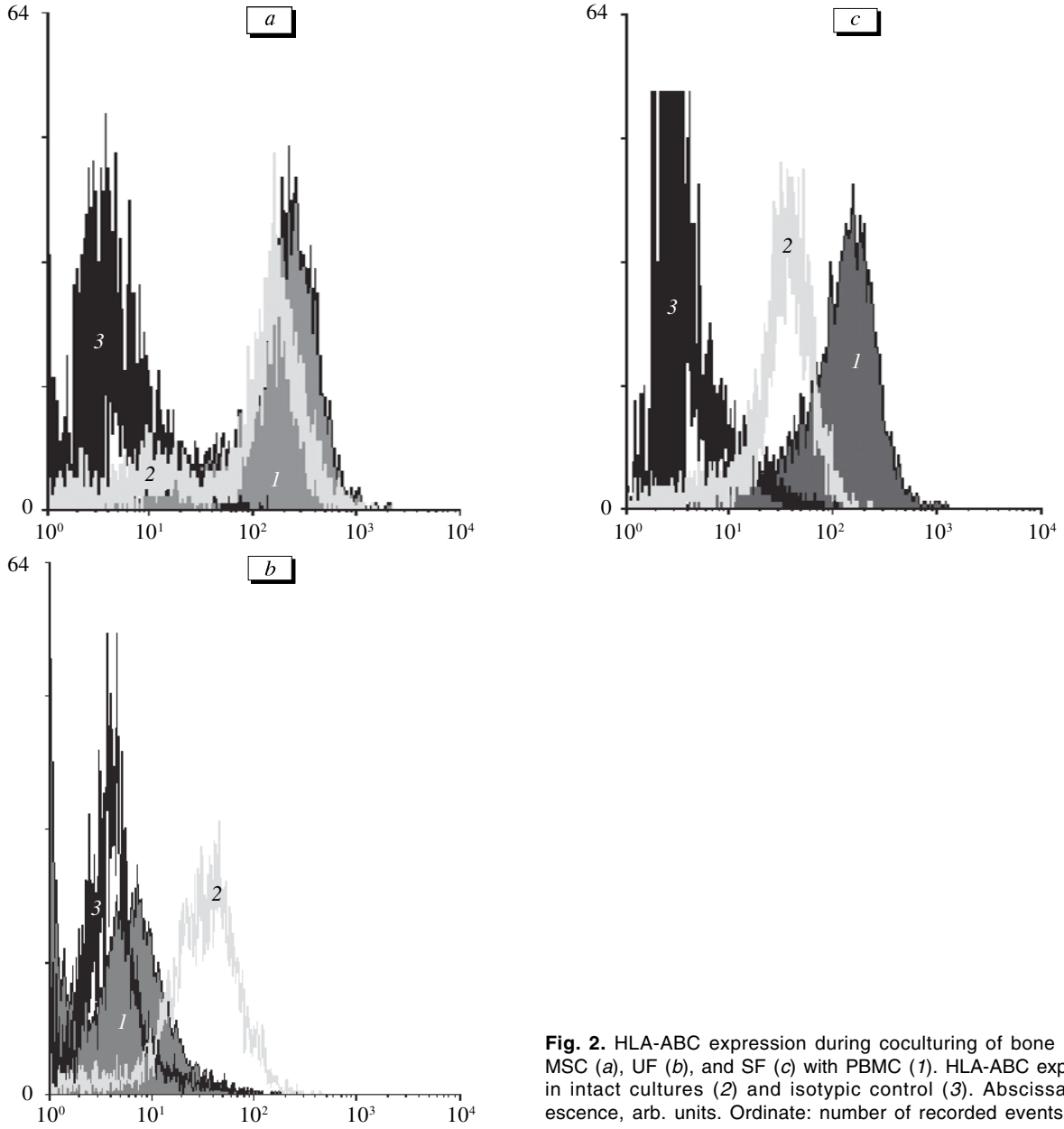

Fig. 2. HLA-ABC expression during coculturing of bone marrow MSC (*a*), UF (*b*), and SF (*c*) with PBMC (*1*). HLA-ABC expression in intact cultures (*2*) and isotypic control (*3*). Abscissa: fluorescence, arb. units. Ordinate: number of recorded events (cells).

TABLE 1. Fractional Composition of Peripheral Blood Mononuclear Cells during Coculturing with SF, UF, and Bone Marrow MSC

Antigen	Initial content	Time of coculturing, days							
		1				3			
		PBMC	PBMC+ SF	PBMC+ UF	PBMC+ MSC	PBMC	PBMC+ SF	PBMC+ UF	PBMC+ MSC
CD4	32%	28%	33.5%	38%	31.5%	34.5%	36%	43.6%	33.2%
CD8	19%	16.2%	17.5%	19.8%	24%	16.4%	23.8%	22%	18.4%
CD14	11%	9%	3%	4.8%	4.9%	11.1%	4.5%	7.2%	6.7%
CD19	6%	4.3%	3%	4%	2.2%	2.9%	7.1%	10.4%	8.4%
CD56	12%	12.3%	10.2%	11.4%	9.1%	8.7%	13.4%	13.2%	13.7%
CD4/CD8	1.7	1.75	1.9	1.9	1.3	2.1	1.5	2.0	1.8

Note. PBMC, peripheral blood mononuclear cells.

the cytotoxic effect on P3HR1 cells at the ratio of more than 2:1. Mononuclear cells induced a slight cytotoxic effect on cultures of SF, UF, and bone marrow MSC, which did not depend on the effector/target cell ratio (Fig. 1). Increasing the time of incubation in mixed cultures to 72 h did not modify the relative content of damaged SF, UF, and bone marrow MSC (data not shown).

Our previous experiments showed that cultures of these cells differ in the expression of HLA-ABC. HLA-ABC expression was highest in cultured SF, but intermediate in the culture of bone marrow MSC. The culture of SF was heterogeneous. This culture included small cells with intermediate expression of HLA-ABC and large spread cells with basal expression of proteins. Cultured cells did not express HLA-DR (major histocompatibility complex class II proteins) [2]. Coculturing of cells with peripheral blood mononuclear cells in the 1:50 ratio for 1 day had no effect on HLA-ABC expression in the culture of bone marrow MSC (Fig. 2, *a*). Coculturing of UF with peripheral blood mononuclear cells under the same conditions was followed by a significant decrease in HLA-ABC expression in the culture of UF (Fig. 2, *b*). The cells with high expression of HLA-ABC probably disappear from the culture of UF under the influence of immunocompetent cells. Probably, HLA-ABC expression slightly increased in SF after coculturing with mononuclear cells (Fig. 2, *c*). These changes are probably related to the fact that immunocompetent cells induce the disappearance of cells with lower expression of HLA-ABC (as compared to the majority of cultured cells) from the culture of SF.

We studied the effect of mesenchymal cells on peripheral blood mononuclear cells after *in vitro* coculturing for 3 days. The total number of mononuclear cells slightly decreased, which was probably associated with cell death (data not shown). The ratio of $CD4^+$, $CD8^+$, $CD56^+$, $CD19^+$, and $CD14^+$ mononuclear cells remained unchanged during this period (compared to the control, Table 1).

Independently on the origin and expression of HLA-ABC, mesenchymal cells were not lysed in mixed cultures with allogeneic peripheral blood mononuclear cells. These cells had no effect on proliferation and relative content of T and B lymphocytes, natural killer cells, and $CD14^+$ lymphocytes and, therefore, were tolerant to each other *in vitro*.

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