CELL TECHNOLOGIES IN BIOLOGY AND MEDICINE

Immunoregulatory Properties of Human Stem Cells of Mesenchymal and Ectodermal Origin after Their Transplantation to BALB/c Mice

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We studied immunoregulatory properties of cultured human stem cells of mesenchymal and ectodermal origins after their administration to mice. Xenotransplantation of mesenchymal stem cells from human placenta reduced the number of $CD11c^+$ dendritic cells in mouse spleens, but did not affect activation of dendritic cells from mouse spleen in culture. It was also shown that splenocytes isolated from animals 10 days after transplantation of mesenchymal stem cells more actively proliferated in response to the polyclonal stimulation. At the same time, transplantation of neither mesenchymal nor neural stem cells affected the ratio of $CD4^+/CD8^+$ T cells and their total content in the peripheral blood in comparison with the corresponding parameters in the control groups.

Key Words: *mesenchymal stem cells; neural stem cells; dendritic cells; xenogenic transplantation*

Stem cell studies opened new prospects in regenerative medicine. Mesenchymal stem cells (MSC) can be used for physiological, reparative, and therapeutic regeneration of various organs and tissues [4]. Moreover, it was recently found that BM MSC can suppress pathological immune reactions [5]. However, MSC isolation from patient's tissues is a laborious procedure and the obtained preparations practically cannot be standardized. Moreover, BM biopsy is a painful procedure for the patient. An alternative approach is transplantation of allogeneic SC characterized by low immunogenicity.

Transplantation of neural SC (NSC) is a promising trend in the therapy of neurodegenerative pathologies, inflammatory processes in CNS, and spinal and brain injuries [9]. Fetal NSC of ectodermal origin are characterized by a wide spectrum of differentiation lineages and therefore can be used for replacement therapy and neurotrophic stimulation [6].

MSC and NSC are now the most feasible candidates for medical use. At the same time, the problems of immunological compatibility of the allogeneic material remain unsolved. MSC are known to exhibit potent immunomodulating/immunosuppressive properties *in vitro* [10]. The immunoregulatory effect of

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MSC in animal models of autoimmune diseases, *e.g.* multiple sclerosis or rheumatoid arthritis, was demonstrated in numerous studies [7,12]. However, the mechanisms underlying the immunoregulatory effects of mesenchymal and ectodermal SC require further investigation. The results of these studies will help to elaborate principles of clinical use of allogeneic cell material on the basis of SC.

Here we compared the capacity of human MSC and NSC to modulate functional elements of the cellular immunity in BALB/c mice after xenotransplantation.

MATERIALS AND METHODS

Cryopreserved MSC from human placenta were defrosted and cultured for 1-2 passages in DMEM:F12 (Gibco) containing 10% embryonic calf serum (ECS, HyClone), 1 mM sodium pyruvate, and 50 μg/ml gentamicin (PanEko) at 37°C and 5% CO_2. NSC cultures were derived from cultured brain autopsy specimens at the Laboratory of Clinical Immunology, V. I. Kulakov Research Center of Obstetrics, Gynecology, and Perinatology. Neurospheres were cultured in DMEM/F12 (1:1 mixture; Gibco) supplemented with 20 ng/ml EGF (Calbiochem), 20 ng/ml FGF-2 (basic fibroblast growth factor, Calbiochem), 5 mM HEPES, 2 mM L-glutamine, N2-supplement (Gibco), and 8 mg/ml heparin (Sigma). For obtaining adherent NSC cultures, the neurospheres were disaggregated with 0.25% trypsin (PanEko) and cultured in DMEM/F12 medium containing 10% ECS.

In experiments with xenotransplantation of human cells, 2-3-month-old male BALB/c mice were used. For evaluation of changes in the parameters of cell immunity in the blood and spleen in response to xenotransplantation, 10^6 MSC or NSC in 100 μl PBS (PanEko) were injected into the caudal vein. For evaluation of cellular immunity in regional lymph nodes, 5×10^5 SC were injected into both hind paw pads. Each experimental group included at least 3 mice. Controls received no injections of xenogeneic cells.

The animals were sacrificed on days 5 and 10 after MSC or NSC injection, the peripheral blood, spleen, or inguinal lymph nodes were taken for isolation and evaluation of the percent content of lymphocyte fractions. All cell isolation procedures were performed on ice under sterile conditions. An aliquot of splenocyte suspension was cultured overnight in RPMI-1640 medium (Gibco) containing 10% inactivated ECS in the presence of mouse granulocyte-macrophage CSF (GM-CSF, Prospec) in a final concentration of 10 ng/ml. GM-CSF was added as a stimulator of activation of splenic dendritic cells (DC).

Immunocytochemical staining was performed using monoclonal antibodies to surface mouse leukocyte antigens conjugated with FITC, PE, or APC fluorescent dye (Becton Dickinson) according to manufacturer's instruction. Immediately before staining, the isolated cells were treated with Mouse Fc Block reagent (Becton Dickinson) for blocking surface immunoglobulin receptors to exclude non-specific binding. Analysis was performed on FACSAria cytofuorometer sorter (Becton Dickinson).

For evaluation of mouse splenocyte proliferation in response to transplantation of xenogeneic MSC and NSC, splenic cells (2×10^4 per well) were cultured for 48 h in 96-well plates with different concentrations of polyclonal stimulator phytohemagglutinin (PHA, Sigma). Proliferation was assessed using MTT test. Light absorption was measured at λ=540 nm (Multiscan, Titertek).

RESULTS

MSC cultures were prepared earlier from placenta fragments obtained after natural term delivery (gestation week 38-40, Fig. 1, *a*). The cells exhibited multilineage differentiation potential *in vitro* [2] and had a cytophenotype typical of mesenchymal lineage cells including expression of CD56 and CD106 and low expression of type-1 major histocompatibility complex molecules [3].

At the initial stage of culturing in the presence of EGF and FGF-2 human NSC formed floating neurospheres (Fig. 1, *b*). After neurosphere disaggregation, the cells were placed in complete nutrient medium

TABLE 1. Subpopulation Composition of T cells in Mouse Peripheral Blood and Spleen after Immunization with MSC or NSC (*M*±*m*; %)

T lymphocyte subpopulation	Peripheral blood			Spleen		
	control	MSC	NSC	control	MSC	NSC
$CD3^+$	35.3±7.4	28.1±6.0	29.2±5.8	44.1±2.9	54.2±3.1	46.7±1.6
$CD4^+$	19.2±3.7	18.9±4.3	17.9±4.6	29.8±4.1	33.4±2.8	32.3±3.0
$CD8^+$	23.5±2.6	23.7±3.5	18.7±2.0	16.7±3.9	19.0 ±1.7	15.1±5.5

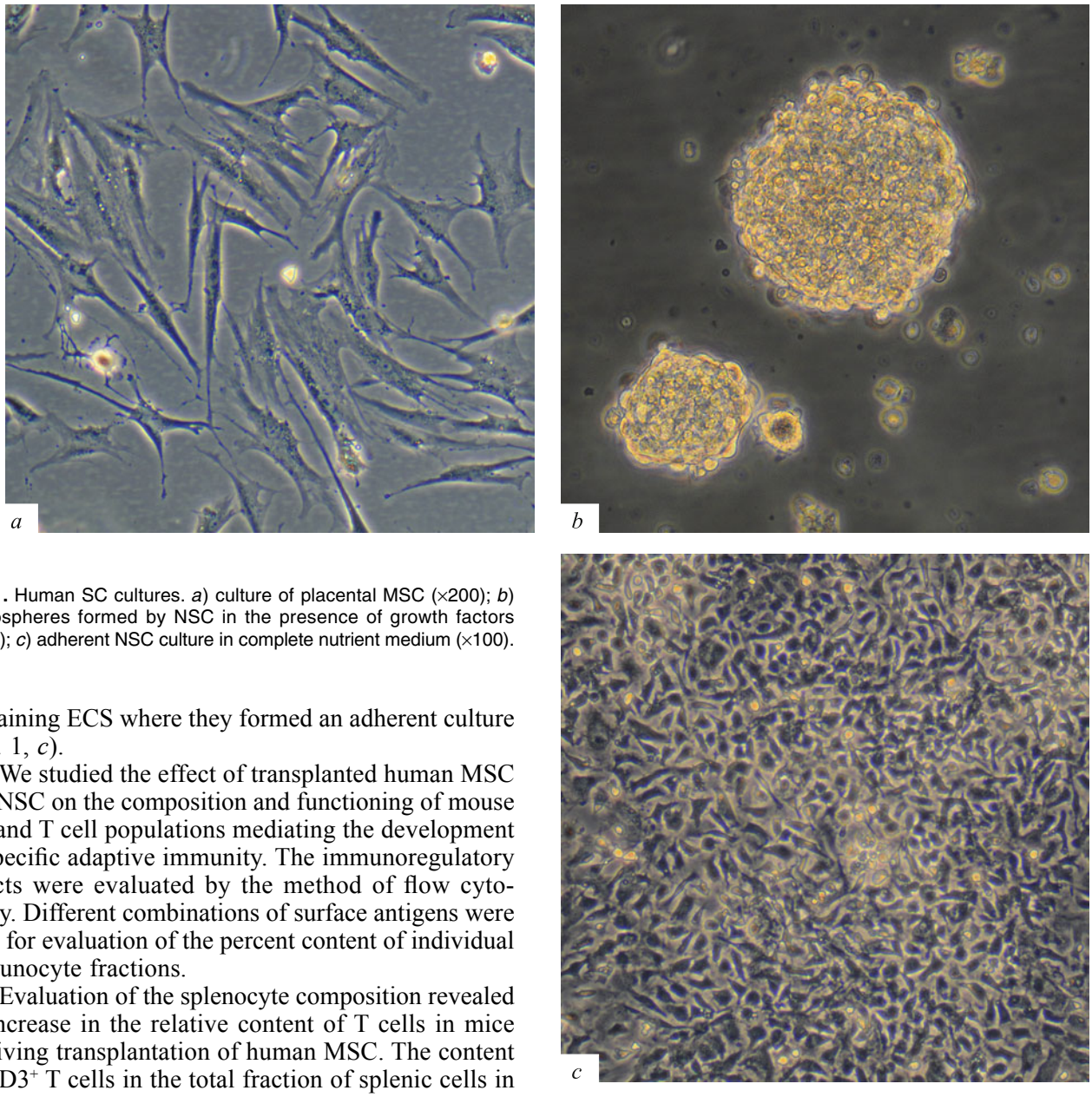

Fig. 1. Human SC cultures. *a*) culture of placental MSC (×200); *b*) neurospheres formed by NSC in the presence of growth factors (×100); *c*) adherent NSC culture in complete nutrient medium (×100).

containing ECS where they formed an adherent culture (Fig. 1, *c*).

We studied the effect of transplanted human MSC and NSC on the composition and functioning of mouse DC and T cell populations mediating the development of specific adaptive immunity. The immunoregulatory effects were evaluated by the method of flow cytometry. Different combinations of surface antigens were used for evaluation of the percent content of individual immunocyte fractions.

Evaluation of the splenocyte composition revealed an increase in the relative content of T cells in mice receiving transplantation of human MSC. The content of $CD3^+$ T cells in the total fraction of splenic cells in experimental animals increased by on average 10.5% in comparison with the control, the $CD4^+/CD8^+$ T cell ratio being unchanged. At the same time, no significant changes in the content and composition of T cell population in the peripheral blood were detected. Transplantation of human NSC, in turn, induced no considerable shifts in T-cell compartment of the immune system in mice (Table 1).

MSC can induce reversible suppression of T cell proliferation in a mixed culture [8]. It is believed that the absence of co-stimulating molecules on the surface of MSC can lead to lymphocyte anergy. Since the interaction is antigen-specific, anergy involves only certain T cell subpopulation. To find out whether the transplanted SC can induce total anergy of immune system cells, we evaluated splenocyte proliferation capacity in response to polyclonal stimulatior PHA. Splenocytes of immunized animal demonstrated more potent proliferative response to polyclonal stimulation than cells from control mice (Fig. 2, *a*). This result not only indicates the absence of anergy, but also attests to activation of the immune system in response to MSC xenotranaplantation. Similarly, proliferative response of splenocytes to PHA after immunization with NSC was evaluated (Fig. 2, *b*). Our findings suggest that transplantation of neural cells does not affect splenocyte response to polyclonal stimulator.

DC as specialized antigen-presenting cells DC are an important element in induction of the primary immune response [11]. Here we studied the capacity of DC population, the key element of adaptive immunity

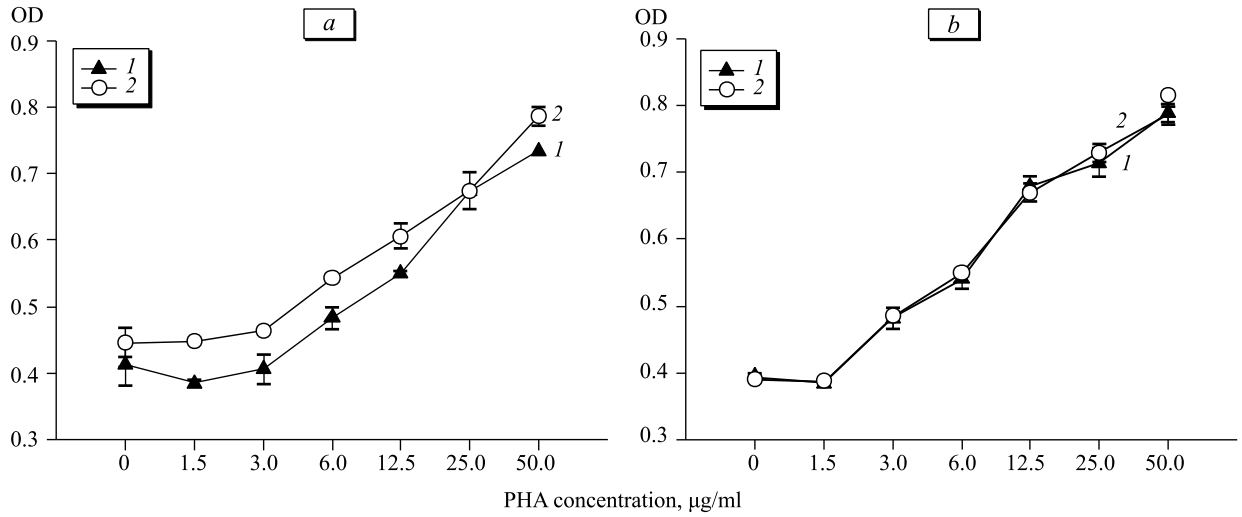

Fig. 2. Mouse splenocyte proliferation intensity as a function of PHA concentration. a) day 10 after immunization with human MSC; b) day 10 after immunization with human NSC. OD: optical density at λ=540 nm. *1*) control, *2*) experiment.

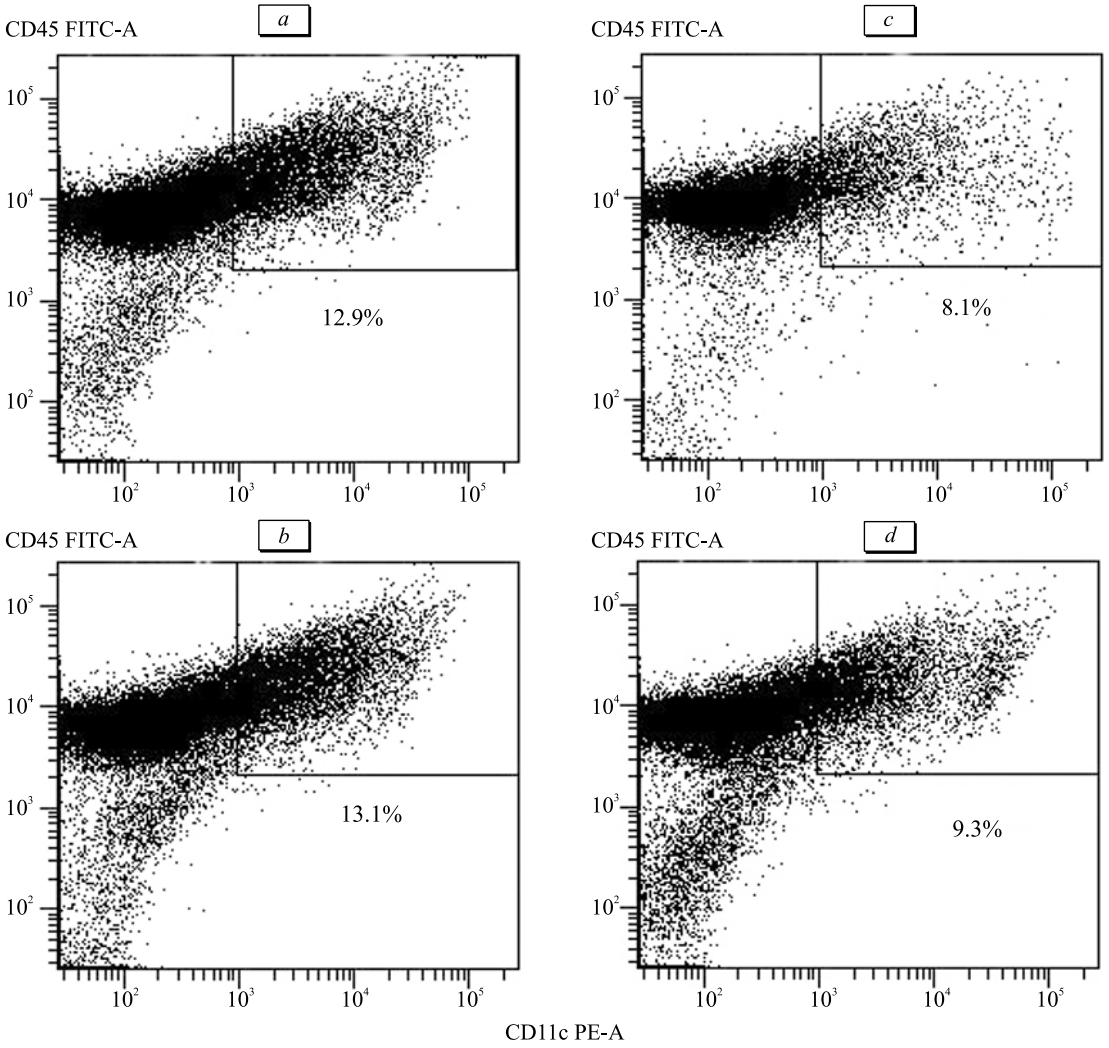

Fig. 3. Percentage of CD11c⁺ DC among mouse splenocytes. *a*) control group; *b*) day 10 after intravenous injection of NSC; *b*) day 5 after intravenous injection of placental MSC; *b*) day 10 after intravenous injection of placental MSC.

TABLE 2. Percentage of Mouse CD11c+ DC at Different Terms after Immunization with Human SC

Organ	Control	MSC		NSC	
		day 5	day 10	day 5	day 10
Blood	4.2±1.0	4.9±0.4	4.5±0.6	5.2±0.5	2.8±1.1
Lymph nodes	2.2±0.7	3.0±0.5	1.9±0.2	2.1±0.6	0.8±0.4
Spleen	12.9±1.2	8.4±0.9	9.8±0.3	11.2±0.7	13.6±0.1

to be a target of negative immunoregulation by transplanted SC. To this end we determined the number of DC in mouse peripheral blood and spleen after intravenous injection of human MSC or NSC and in regional lymph nodes after local injection of these cells.

During counting of DC, the percent of $CD11c^+$ cells among $CD45^+$ leukocytes was evaluated. In cell populations isolated from the blood and lymph nodes, no significant differences in DC content were revealed without and after immunization. At the same time, the relative content of DC among splenic cells somewhat decreased after injection of MSC from human placenta (Fig. 3). The results of DC analysis are presented in Table 2.

Our findings suggest that only injection of placental MSC (Fig. 3, *b*) somewhat reduced the content of $CD11c^+$ DC in the total mouse splenocyte population. In contrast to the spleen, no changes in DC content were found in the peripheral blood and inguinal lymph nodes (Table 2). The decrease in DC content in the spleen can result from blockade of their *in vivo* dif-

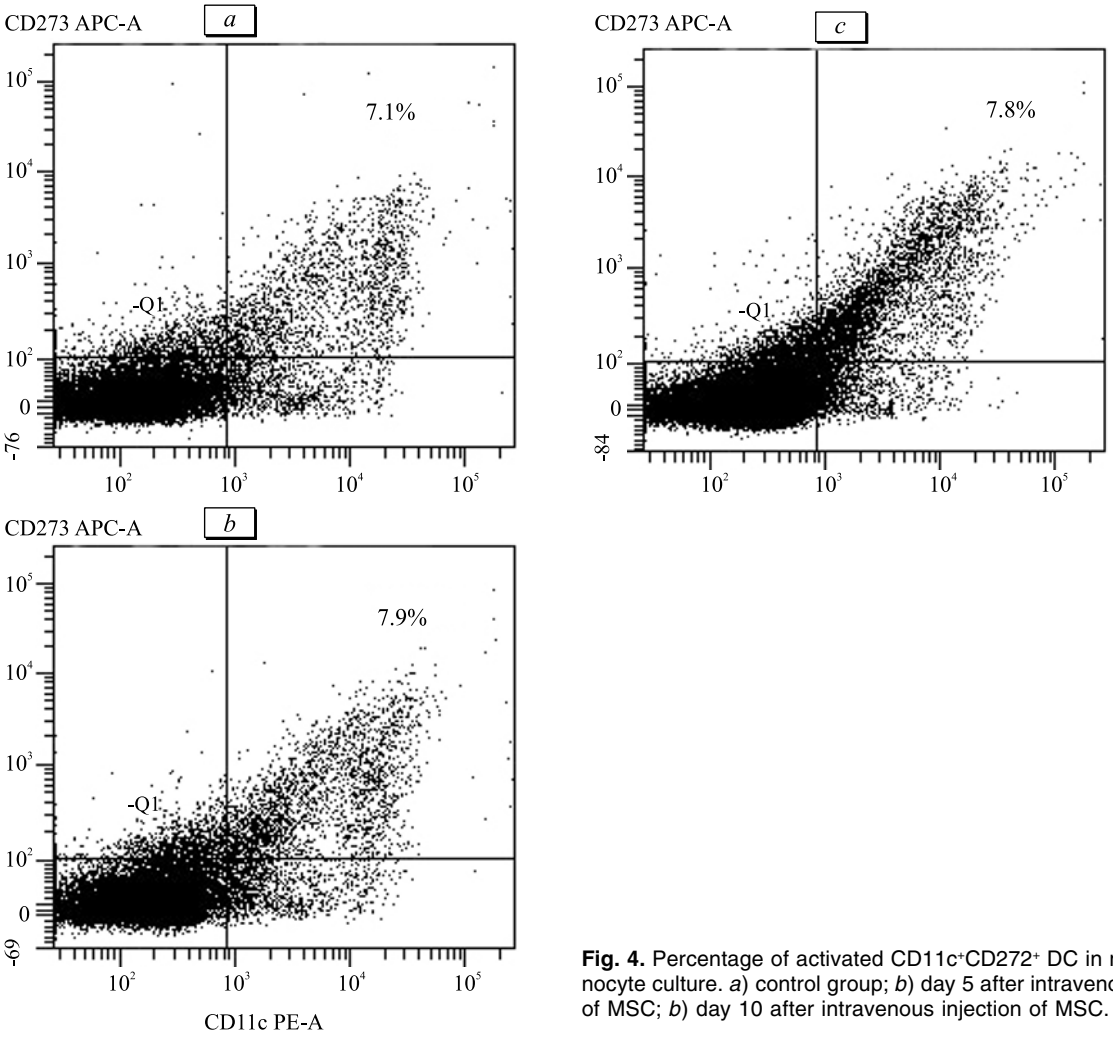

Fig. 4. Percentage of activated $CD11c^+CD272^+$ DC in mouse splenocyte culture. *a*) control group; *b*) day 5 after intravenous injection of MSC; *b*) day 10 after intravenous injection of MSC.

ferentiation by MSC. We previously showed that MSC practically completely suppressed DC differentiation in culture, but did not affect their maturation [1].

At the next stage we studied the effect of MSC on responsiveness of splenic DC to activation stimuli. It is known that addition of some cytokines, *e.g.* GM-CSF, IL-4, and IFN-γ, stimulate the expression of surface marker CD273 (or B7-DC) on DC. This marker is a ligand of programmed cell death receptor-1 (PD-1), which after appearance on the surface of DC participates in negative regulation of activated T and B cells [13]. As expected, 18-h culturing of mouse splenocyte in the presence of GM-CSF increased the number of activated $CD11c^+CD273^+$ DC. At the same time, activation capacity of DC isolated from immunized or control animals did not significantly differ (Fig. 4). The number of activated DC was on average 7.5±0.5%. Thus, MSC apparently prevented DC differentiation in the spleen, but did not affect activation potential of differentiated DC.

Our findings suggest that transplantation of human MSC induced various shifts in the parameters of cell immunity, in particular, a decrease in DC content and activation of cell proliferation in the spleen. At the same time, ectodermal cells had no significant effect on the studied cell populations of mouse immune system.

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