

The Effect of Mesenchymal Stem Cells on Leukocyte Allergen-Specific Reactions in the Case of Atopic Hypersensitivity

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Abstract—Buffy coat samples containing lymphocytes, monocytes, and granulocytes were obtained from the peripheral blood of 16 donors who had clinical manifestations of atopic hypersensitivity in their medical background. After ex vivo incubation with donor-specific allergens, the percentage of B- and T-lymphocytes and natural killers remained unchanged. Incubation of buffy coat cells with allergens induced the production of IgE and IL-4 in all assayed samples. In 13 out of 16 cases, the reaction to contact with an allergen was also evident in the increasing of T-activated lymphocytes (CD3+, HLA-DR⁺) subpopulation. Cocultivation with mesenchymal stromal cells (MSCs) from bone marrow, adipose tissue and umbilical cord resulted in blocking of allergen-induced IgE and IL-4 secretion and increase in the subpopulation of HLA-DR+ T-lymphocytes. There was no significant difference in the effect of MSCs isolated from three different sources on allergen-specific responses of leukocytes. Coculturing of leukocytes with MSCs from all three sources increased the content of regulatory T-lymphocytes by approximately 30%. Thus, the immunomodulatory activity of MSCs in vitro results in blocking of the effector phase of allergic reactions.

Keywords: mesenchymal stem cells, hypersensitivity reaction, cocultivation

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INTRODUCTION

Mesenchymal stem cells (MSCs) exhibit immunomodulatory activity in experiments¹ in vitro and in vivo. The most convincing evidence concerns the suppressive effect of MSCs on T-lymphocytes (Glennie et al., 2005) and dendritic cells (Chen et al., 2007) functions. It was shown that MSC transplantation to experimental animals had a positive effect on the clinical course of a number of inflammatory autoimmune diseases (Chen et al., 2006; Nauta et al., 2007; Nasef et al., 2008). The effect of MSC on allergic reactions remains poorly understood. In a limited number of clinical trials, weakened reactions to food and inhalation allergens after transplantation of autologous bone marrow MSC has been described (Granov et al., 2005). Intravenous injection of syngeneic bone marrow MSCs (Nemeth et al., 2010) and allogenic embryonic or induced pluripotent stem cells (iPSCs) reduced the intensity of the disease symptoms (Ogular et al., 2014) in a mouse model of bronchial asthma. Similar positive effect was observed in the xenogeneic system after transplantation of MSCs derived from human-induced pluripotent cells into sensitized mice

(Sun et al., 2012). The aim of this work was to study the immunomodulatory effect of MSCs isolated from various human tissues on allergen-specific reactions of cultured leukocytes from donors sensitized with particular allergens.

MATERIALS AND METHODS

The study was performed on samples of venous blood from 16 volunteer donors having clinical manifestations of hypersensitivity (allergic rhinitis, atopic dermatitis, acute urticaria, gastroenterological disorders, atopic asthma, anaphylaxis) in their medical history. Four patients developed the reaction after food ingestion, three patients after drug administration, and the others after encountering household and pollen allergens (four and five patients, respectively). All donors at the time of blood sample collection were in remission and had no clinical manifestations of allergic reactions. Heparinized blood was layered on ficoll gradient and centrifuged. Leukocyte fraction was collected, washed, suspended in RPMI medium with 10% fetal bovine serum (HyClone, New Zealand), and adjusted to a concentration of 5×10^6 cells/mL. The cell composition of the leukocyte fraction was

¹ MSC—mesenchymal stromal cell, IgE—immunoglobulin E.

Cell composition of leukocyte fractions isolated from donor blood sensitized with specific allergens

Donor number	Allergen	Lymphocytes, %	Monocytes, %	Granulocytes, %
1	Birch pollen	69.8 ± 2	4 ± 2	25.7 ± 2
2	Wool and cat epithelium	75 ± 4	8.9 ± 2	16 ± 3
3	Fish	71.4 ± 1	9 ± 0.5	19.6 ± 1
4	Shrimp	79 ± 4	10 ± 3	11 ± 3
5	Fish	85.7 ± 5	6 ± 0.5	8.3 ± 4
6	Shrimp	87.2 ± 8	5.7 ± 2	7.1 ± 5.5
7	Ambrosia artemisiaefolia	85.6 ± 4	8.1 ± 5	6.3 ± 1
8	Cat hair	78 ± 4.5	9 ± 2	13 ± 3
9	Citrus	77.9 ± 3	9.6 ± 2	12.5 ± 3
10	Penicillin	75.8 ± 7	9.3 ± 4	14.9 ± 3
11	Lidocaine	73 ± 2	8 ± 2	19 ± 3
12	Birch pollen	80 ± 4	8.9 ± 2	11 ± 3
13	Cat epithelium	69.5 ± 5	7.7 ± 1	23 ± 6
14	Poplar pollen	81 ± 0.7	10 ± 0.5	9 ± 0.5
15	Mustard	77.2 ± 1	6.8 ± 2	16 ± 3
16	Penicillin	67.3 ± 6	11.7 ± 2	21 ± 3

assessed with the use of Beckman-Coulter ACT Diff2 hematology analyzer. Sensitization of donors to allergens indicated by anamnesis was verified by incubation of leukocyte fraction cells with corresponding allergens followed by determination of immunoglobulin E (IgE) in the growth medium by immuno-enzyme assay (ELISA) using an IgE-total kit (Vektor-Best, Russia) according to the manufacturer's instructions.

In addition, leukocyte reaction on allergen *in vitro* was assessed by IL-4 content in the culture medium determined with ELISA using an IFA-IL-4 kit (Vektor-Best, Russia). Leukocyte cultures lacking allergens served as controls. Preliminary experiments showed that the optimal time of cell incubation with allergens was 72 h.

Samples of donor bone marrow, adipose tissue, and umbilical cord were obtained after informed consent had been received. MSC isolation, cultivation, and phenotypic properties have been reported previously (Ayzenshtadt et al., 2014). MSCs at the third to fourth passages in the logarithmic growth phase (30–60% confluence) were used in experiments on coculturing with leukocytes, in which 30×10^3 cells/cm² were seeded in a six-well plate and 24 h later the same wells were inoculated with an equal number of leukocyte fraction cells. Mixed cultures had allergens added to them and were cultivated in RPMI medium with 10% fetal bovine serum (HyClone, New Zealand) for 72 h. Leukocyte fraction cells and culture medium were then collected separately. The medium was used for IgE and IL-4 determination. The cell fraction was analyzed by flow cytometry. Populations of B-lymphocytes (CD19⁺), natural killers (CD3⁺, CD16⁺, CD56⁺), and activated T-lymphocytes (CD3⁺,

HLA-DR⁺), as well as regulatory T-cells (CD3⁺, CD4⁺, CD127^{low}), were assayed. After MSC coculturing, the medium was removed to determine IgE and IL-4 concentrations. Leukocytes were detached mechanically and then with 0.02% EDTA. Washes were combined to prepare cell suspension in 500 µL of PBS to assay lymphocyte subpopulations. For flow cytometry analysis, cells were stained with fluorochrome-labeled antibodies in the dark at room temperature using Beckman Coulter (United States) reagents. Analysis was performed on an FC500 flow cytometer and CXP software in accordance with the manufacturer's instructions (Beckman Coulter, United States). All experiments were repeated four times. The results were statistically analyzed using Microsoft Excel software. The errors of mean values were determined by the formula $\pm \sigma/\sqrt{n}$. Intergroup differences were evaluated with the Mann-Whitney *U*-test.

Reagents: RPMI medium, fetal bovine serum, AdvanceSTEM mesenchymal stem cell media, Mesenchymal Stem Cell Growth Supplement serum replacement, penicillin–streptomycin solution, phosphate saline buffer (HyClone, New Zealand), ficoll solution, EDTA solution (Biolot, Russia), IgE-total kit, IFA-IL-4 kit, allergen solutions (Vektor-Best, Russia), monoclonal antibodies CD3-FITC/CD19-PE, CD3-FITC/HLA-DR-PE, CD3-FITC/CD56+16-PE, CD45-PC5, CD45-PC7, CD25-PC5, CD127-PE, CD4-FITC (Beckman Coulter, United States).

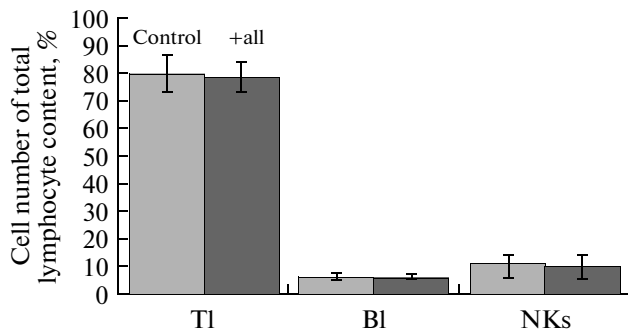


Fig. 1. Number of B-lymphocyte (Bl), T-lymphocytes (Tl), and natural killers (NKs) in the leukocyte fractions after their cultivation in the growth medium (control) and in the medium with allergens (+all.).

$n = 4$, mean $\pm$ SEM.

RESULTS

Analysis of cell populations in a buffy coat isolated from blood samples of donors sensitized with allergens showed that the ficoll density gradient enriched the cell population with lymphocytes and monocytes (table). Granulocytic cell number decreased by three to five fold, but remained within the range of 6–26%. Cultivation of leukocyte fraction cells with allergens did not affect the B-lymphocyte, T-lymphocyte, and natural killer composition in the population (Fig. 1). The presence of allergen in the culture medium for all 16 samples of leukocyte fractions significantly increased the IgE (Fig. 2) and IL-4 (Fig. 3) concentrations. Thirteen of the sixteen samples exhibited 1.5- to 3.0-fold augmented content of activated (HLA-DR-positive) T-lymphocytes after incubation with allergens (Fig. 4). Cocultivation of leukocyte fractions with MSCs inhibited allergen-induced manifestations of hypersensitivity: increased IgE and IL-4 concentration and enhanced content of activated T-lymphocytes were not observed in the presence of MSCs (Figs. 2, 3, 4). MSCs from bone marrow, adipose tissue, and umbilical cord equally suppressed both the T-lymphocyte number and IgE and IL-4 production. Allergen stimulation of regulatory T-lymphocyte was not observed in any blood samples (Fig. 5). Cocultivation of leukocyte fractions with MSCs from bone marrow, adipose tissue, or umbilical cord resulted in a statistically significant increase in fraction of T-regulatory cells ($CD4+CD25+CD127^{low}$). In cocultures with MSCs, the relative content of T-regulatory cells increased by an average of 34% (minimum 13%, maximum 59%). In experiments with MSCs from bone marrow, the increase was 31% (minimum 13%, maximum 53%), while with MSCs from adipose tissue it was 30% (minimum 14%, maximum 48%). No significant difference in content of regulatory T cells was observed with MSCs from different sources.

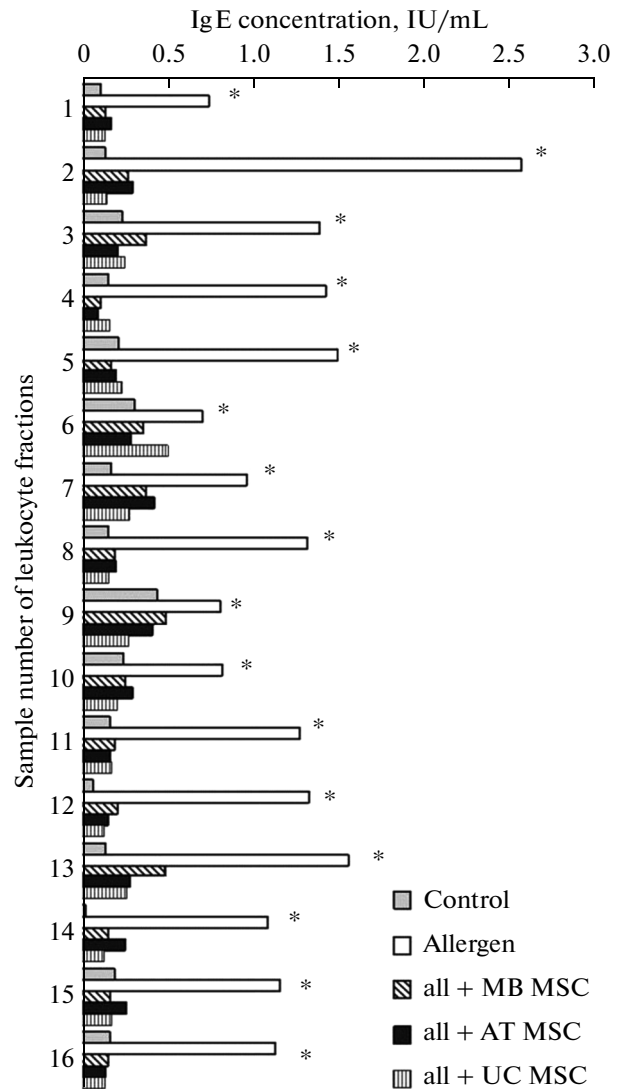


Fig. 2. IgE concentration in cultures of leukocyte fraction cells cultivated in the growth medium (control), or in medium with allergen (allergen), or after cocultivation with MSCs from various sources in the presence of allergens.

Cells from leukocyte fractions were cultivated in the presence of allergen together with MSCs from bone marrow (all + BM MSC), adipose tissue (All + AT MSC), or umbilical cord (All + UC MSC). Asterisks designate significant difference from control ($p < 0.05$).

DISCUSSION

Cells isolated from a sensitized organism and cultivated with the respective allergen represented the model of effector phase of allergic reactions. The allergen binding is ensured by specific IgE class antibodies incorporated in B-lymphocyte membranes by the B-cell IgE receptor (Wu et al., 2014) or immobilized on the surface of mast cells, basophils and eosinophils by high-affinity Fc-receptor (Stone et al., 2010). B-lymphocytes response to an allergen binding by IgE production whereas granulocytes produce IL-4 which in

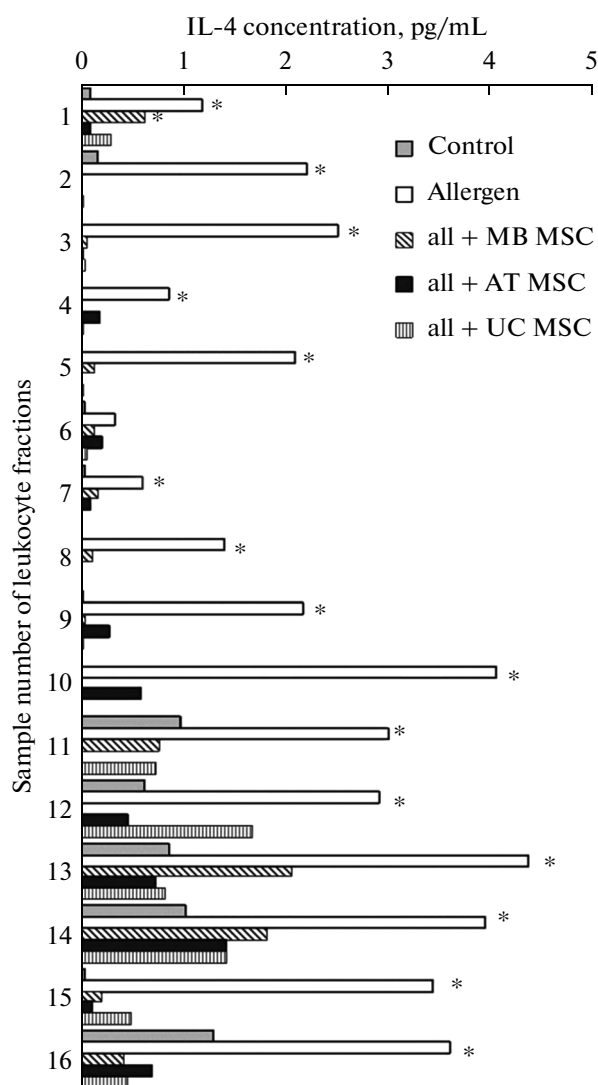


Fig. 3. IL-4 content in cultures of leukocyte fraction cells cultivated in the growth medium (control) and in medium with allergen or cocultivated with MSCs from various sources in the presence of allergens.

Designations are the same as in Fig. 2.

turn stimulates generation of activated T-lymphocytes.

It is generally believed that separation of peripheral blood cells by the Ficoll method results in accumulation of lymphocytes and monocytes in the upper gradient layer, whereas granulocytes and erythrocytes occur in the lower fraction (Noble et al., 1968). A cell population assay of the buffy coat obtained in this study showed that, along with lymphocytes, they contained monocytes and granulocytes, i.e., all cell types responsible for the changes observed in cell cultures with allergens.

Three samples (6, 7, and 10) exhibited IgE and IL-4 production by leukocytes in the culture medium

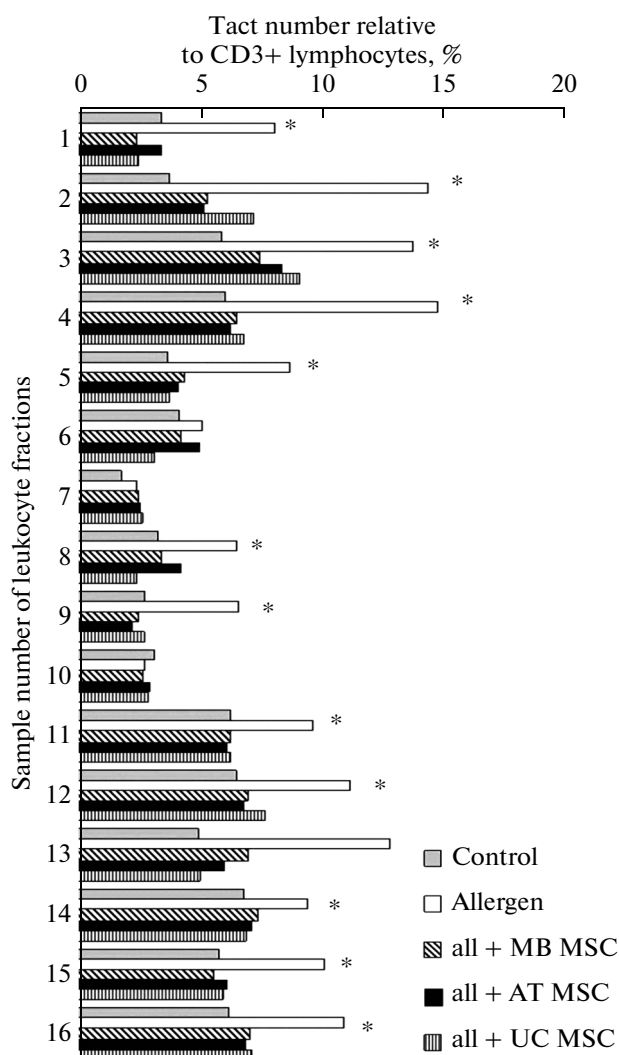


Fig. 4. The number of activated CD3+ and HLA-DR+ T-lymphocytes (Tact) relative to CD3+ lymphocytes in cultures of leukocyte fraction cells cultivated in growth medium (control) and medium with allergen or cocultivated with MSCs from various sources in the presence of allergens.

Designations are the same as in Fig. 2.

after allergen exposure, but no increase in the number of activated T-lymphocytes was observed. It is likely that in these samples the differentiation was delayed and no cell accumulation was accomplished by the termination of cultivation.

Notably the donor group was heterogeneous with respect to the sensitizing allergen and type and severity of clinical manifestations of atopic hypersensitivity. Nevertheless, as a whole, the cell reactions of the leukocyte fraction upon contact with allergen were virtually uniform: in all cases, contact with allergen resulted in IgE and IL-4 production, in most cases the population of activated T-lymphocytes was increased.

MSCs unequivocally displayed immunomodulatory activity to leukocytes from allergic donors. Allo-

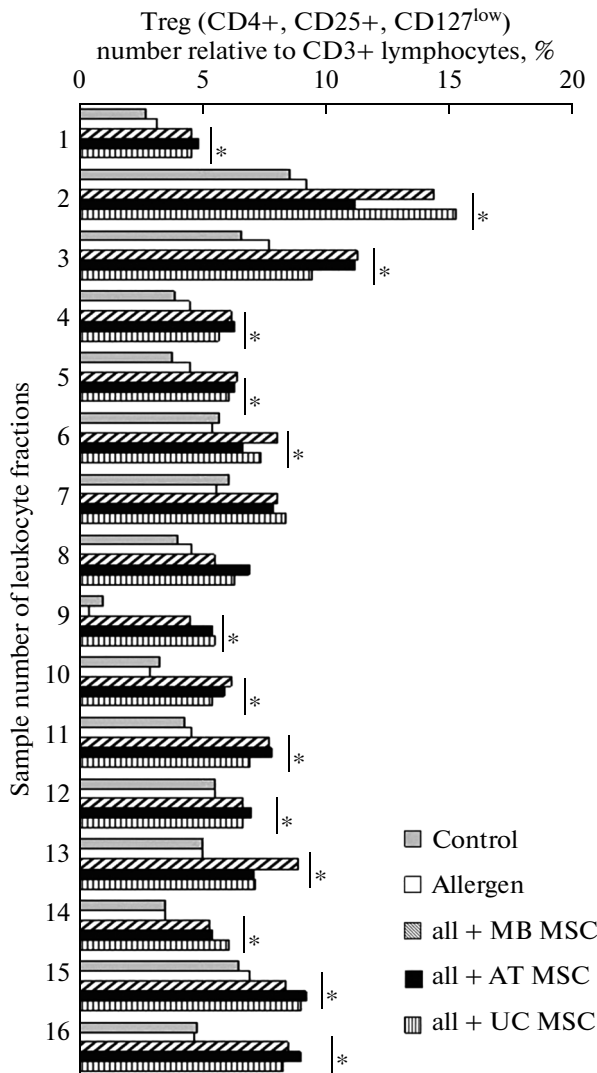


Fig. 5. Number of regulatory CD4+, CD25+, and CD127^{low} T-lymphocytes (Treg) in cultures of leukocyte fraction cells cultivated in growth medium (control) and medium with allergen or cocultivated with MSCs from various sources in the presence of allergens.

Asterisks designate significant difference from control ($p < 0.05$).

genic MSCs blocked allergen-induced IgE and IL-4 production, as well as an increase in content of T-lymphocytes with the late activation marker HLA-DR. These findings are consistent with the data on the immunomodulatory activity of MSCs manifested as a block to T-lymphocyte activation (Le Blanc et al., 2004; Cappellesso-Fleury et al., 2010; Kronsteiner et al., 2011). Enhanced expression of lymphocyte activation markers (CD25, CD71, HLA-DR) is considered as a symptom of the exacerbation of acute atopic disease. The level of HLA-DR expression is frequently correlated with severity of disease (Antúnez et al., 2006; Gemou-Engesaeth et al., 2002).

Cocultivation of leukocyte fraction cells with MSCs from various sources increased the number of regulatory T-lymphocytes with CD4+CD25+CD127^{low} phenotype. The presence of allergen in leukocyte cultures did not affect their number. MSC-induced generation of regulatory T-lymphocyte population has been described in a number of papers. According to the current paradigm, reduction in the number and/or functional activity of T-regulatory cells can influence the development of allergic pathology. Regulatory T-cells participate in the maintenance of balance between Th1 and Th2 response. Reduced functional activity of T-regulatory cells may be compensated by an increased quantity thereof (Zhang et al., 2014).

The properties of MSCs from various sources, including their immunomodulatory activity, may differ (Wagner et al., 2005; Ayzenshtadt et al., 2014). We did not find differences in MSCs derived from bone marrow, adipose tissue, and umbilical cord as regards their capacity to inhibit the manifestation of allergen-specific reactions in vitro.

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