Multiplex Analysis of Cytokines, Chemokines, Growth Factors, MMP-9 and TIMP-1 Produced by Human Bone Marrow, Adipose Tissue, and Placental Mesenchymal Stromal Cells

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Comparative analysis of mesenchymal stromal cells isolated from human BM, adipose tissue, and placenta was carried out. The cells were compared by the levels of constitutive, spontaneous, and LPS-induced production of Th1/proinflammatory (IFN-γ, IL-2, IL-1β, TNF-α, IL-12, IL-17) and Th2/anti-inflammatory cytokines (IL-4, IL-5, IL-6, IL-10, IL-13), chemokines (IL-8, MCP-1, MIP-1β), growth factors (IL-7, granulocytic CSF, granulocytic macrophageal CSF, erythropoietin, VEGF, EGF, IGF-1, main FGF), matrix metalloproteinase-9, and tissue inhibitor of metalloproteinase-1. Mesenchymal stromal cells originating from different tissues were characterized by functional potential for hemopoiesis support (through production of granulocytic CSF, granulocytic macrophage CSF, erythropoietin), immunomodulation (through production of IFN-γ, IL-2, IL-6, IL-1β, TNF-α and chemokines IL-8, MCP-1, MIP-1β), and stimulation of reparative processes (through production of VEGF, FGF, IGF-1, IL-6, tissue inhibitor of metalloproteinase-1, and matrix metalloproteinase-9). By the type and levels of spontaneous (basal) production of cytokines, the adipose tissue mesenchymal stromal cells more distinctly demonstrated the proinflammatory (IL-1β, TNF-α), immunoregulatory (IFN-γ, IL-2, IL-4, IL-6, IL-8, MCP-1, MIP-1β), and hemopoiesis-stimulating (granulocytic CSF, granulocytic macrophage CSF) phenotype and at the same time were characterized by lower sensitivity to lipopolysaccharide stimulation than BM and placental mesenchymal cells.

Key Words: *mesenchymal stromal cells; bone marrow; adipose tissue; placenta; comparative analysis*

Reparative process of any kind includes certain stages: chemotaxis, mitosis, neovascularization, synthesis and organization of the extracellular matrix components [5]. The majority of these events are regulated through soluble mediators (growth factors, cytokines/chemokines, matrix metalloproteinases, *etc.*), which participate in the creation of a microenvironment favorable for cell protection and regeneration.

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An important biological feature of mesenchymal stromal cells (MSC) belonging to the somatic multipotent SC class is their low immunogenic activity, capacity to migrate to the focus of injury/inflammation, and hemopoiesis-stimulating and immunomodulating activities. Due to this MSC are regarded as potential inductors/regulators of reparative processes [2,7, 15,25,27]. These cells effectively stimulated hemopoiesis recovery and prevented the graft-versus-host reaction (GVHR) after transplantation of stem hemopoietic cells to patients with hematological malignancies after

high-dose chemotherapy [6,19,21], restored the reparative osteogenesis in patients with its disorders of local and systemic type [1,16,29].

MSC are usually isolated from BM where they have been identified for the first time [12,13]. However, aspiration of BM from the iliac crest is an invasive traumatic procedure. In addition, with age the count, differentiation potential, and viability of bone marrow mesenchymal cells in humans decrease [31]. Hence, intense search for alternative easier available sources of MSC, comparable by their biological characteristics with the bone marrow mesenchymal cells, is in progress. One of these sources is adipose tissue [34], another is human placenta [14,18]. However, functional characteristics of adipose and placental MSC, including their capacity to produce bioactive mediators, are not sufficiently well studied.

We compared secretory activities of human BM, adipose tissue, and placental MSC. We evaluated the production of Th1/proinflammatory (IFN-γ, IL-2, IL-1β, TNF-α, IL-12, IL-17) and Th2/anti-inflammatory cytokines (IL-4, IL-5, IL-6, IL-10, IL-13), chemokines (IL-8, MCP-1, MIP-1β), growth factors (IL-7, granulocytic CSF (G-CSF), granulocytic macrophage CSF (GM-CSF), erythropoietin, vascular endothelium growth factor (VEGF), epidermal growth factor (EGF), insulin-like growth factor-1 (IGF-1), basal FGF (bFGF), matrix metalloproteinase-9 (MMP-9), and tissue inhibitor of metalloproteinase-1 (TIMP-1).

MATERIALS AND METHODS

MSC were isolated from human BM (n=14), adipose tissue (n=18), and placenta (n=7). Previously described methods [3] were used for isolation and culturing of MSC, evaluation of the initial fibroblast CFU (CFU-F), of MSC phenotype with FITC- or PE-labeled monoclonal antiCD3, -CD14, -CD16, -CD20, -CD34, -CD73, -CD90, -CD105, -HLA-DR antibodies, and of MSC proliferative potential.

TABLE 1. Production of Cytokines, Chemokines, and Growth Factors in Unfractionated MSC Cultures during Confluent Growth (n=29)

Cytokines, pg/ml		*M*±*SE*	Median
Th1/proinflammatory	IFN-γ	562±59	539
	IL-2	147±28	115
	IL-1β	65.5±5.4	67
	TNF-α	67±15	51
	IL-12	4.8±1.1	2
	IL-17	54±8	52
Th2/anti-inflammatory	IL-4	37±7	20
	IL-5	3.2±0.3	2.9
	IL-10	3.2±0.4	2
	IL-13	53±10	25
	IL-6	14,083±1396	13,400
Growth factors	IL-7	15.0±2.3	9.8
	G-CSF	1058±390	277
	GM-CSF	505±75	376
	Erythropoietin, mU/ml	5.2±2.2	2.7
	VEGF	4125±426	4980
	IGF-1	17,060±2612	19,900
	bFGF	94±15	74
	EGF	<30 (<OOR)	<30 (<OOR)
Chemokines	IL-8	8258±1563	4500
	MCP-1	2588±364	2180
	MIP-1β	240±40	187

Note. Here and further on: <OOR: below the threshold lower level of the diagnostic test system's sensitivity.

The osteogenic and adipogenic differentiation potential of MSC was evaluated as follows. MSC after one passage were inoculated in 6-well flat-bottom plates (NUNC) at a density of 3000 cell/cm^2. After 24-48 h, the standard culture medium was completely replaced with induction medium αMEM+10% FCS (BioloT) with 10 mmol/liter β-glycerophosphate, 100 nmol/liter dexamethasone, and 0.2 mmol/liter ascorbic acid-2-phosphate for osteogenic differentiation or 1 μmol/liter dexamethasone, 0.5 mmol/liter 3-isobutyl-1-methylxanthine, and 1 mmol/liter indomethacin for adipogenic differentiation (all reagents from Sigma-Aldrich). Osteogenic and adipogenic induction medium was replaced twice weekly. The efficiency of osteogenic differentiation of MSC was evaluated by von Koss method after 18 days. The mineralization (formation in the extracellular matrix of calcium depositions colored black with silver citrate) was evaluated visually by light microscopy [3].

The efficiency of adipogenic differentiation of MSC was evaluated after 18 days by Oil Red staining (Sigma-Aldrich) by the number of stained cells with vacuoles containing neutral lipids. The cells were fixed for 10 min in 10% formaldehyde, washed in distilled water and 60% isopropanol, and stained for 15 min in 0.3% Oil Red solution in isopropanol, after which washed again in isopropanol. Lipid vacuoles in the cytoplasm were thus stained red.

The spectra and concentrations of released bioactive mediators were studied in supernatants of unfractionated MSC cultures (passage 2) and in MSC supernatants obtained by culturing under standard conditions (5×10^4 MSC/well, 48 h). The levels of spontaneous and stimulated production of cytokines (except

TABLE 2. Production of Cytokines, Growth Factors, and Chemokines in Unfractionated MSC Cultures Isolated from Different Tissues

Cytokines, pg/ml		BM (*n*=11)		Placenta (*n*=4)		Adipose tissue (*n*=14)	
		M±*SE*	median	*M*±*SE*	median	*M*±*SE*	median
Th1/proinflammatory	IFN-γ	445±104	501	476±191	493	678±69	746
	IL-2	89±36	26	119±45	113	200±47	134*
	IL-1β	56±9	62	58±20	59	75±6	80
	TNF-α	77±40	39	47±21	46	64±8	72
	IL-12	3.4±1.7	2	4.5±1.4	4	6.1±1.9	2
	IL-17	28±10	9	102±21	91*	61±11	60
Th2/anti-inflammatory	IL-4	26±14	10	68±12	67*	35±8	37
	IL-5	2.5±0.4	2	3.5±0.6	3.6	3.6±0.4	3.3
	IL-10	3.1±0.3	3.8	2.7±0.7	2	3.5±0.9	2
	IL-13	21.0±8.6	5	64±17	64*	74±18	68*
	IL-6	13,160±2023	13,404	11,255±5585	9720	15,615±1937	13,104
Growth factors	IL-7	12.5±2.4	9.2	5.2±2.14	20±4	24	
	G-CSF	341±85	234	1948±940	1918	1366±749	279
	GM-CSF	336±109	242	417±215	347	662±106	614*
	Erythropoietin	1.7±0.4	1.3	2.3±1.4	0.5	10.2±5.0	7.3*
	VEGF	4816±206	4920	2993±937	3129	4476±594	5112$^+$
	IGF-1	21,75±996	22,25	7110±2880	8730*	24,65±1997	25,80$^+$
	bFGF	97±24	73	105±36	94	81±18	74
	EGF	<30	<OOR	<30	<OOR	<30l	<OOR
Chemokines	IL-8	8160±2400	4500	13,84±7360	1165	6685±1630	5430
	MCP-1	2800±944	1720	3684±1165	3070	2156±257	2176
	MIP-1β	313±117	192	153±30	143	227±39	192

Note. Here and in Table 4: p_U<0.05 compared to: *BM MSC, $^+$placental MSC (Mann–Whitney' *U* test).

MMP-9 and TIMP-1) in response to endotoxin (*E. coli* lipopolysaccharide, 10 μg/ml; Sigma-Aldrich) were thus evaluated. Aliquots of collected culture supernatants were frozen and stored at -80°C before analysis.

Cytokine/chemokine concentrations were measured on a double beam laser automated analyzer Bio-Plex Protein Assay System (BioRad) using Human Cytokine 17-Plex Panel (IL-1β, IL-2, IL-4, IL-5, IL-6, IL-7, IL-8, IL-10, IL-12 (p70), IL-13, IL-17, G-CSF, GM-CSF, IFN-γ, MCP-1 (MCAF), MIP-1β, TNF-α) according to the instruction [4].

The levels of erythropoietin and EGF (both from Protein Contour), insulin-like growth factor-1 (IGF-1; Diagnostic System Labs), bFGF (Biosource), VEGF (human VEGF, Invitrogen), MMP-9 (human MMP-9, R&D Systems), and TIMP-1 (human TIMP-1, Biosource) were measured by EIA. The intensity of color enzymatic immune reaction was evaluated on a Multiscan Ascent multichannel photometer (Thermo Electron Corp.) at a wavelength recommended by the manufacturer.

The results were processed using Statistica 6.0 software.

RESULTS

The results of preliminary studies showed that fibroblast-like cells isolated from BM, adipose and placental tissue corresponded to the "minimum" criteria for MSC approved by the International Society for Cell Therapy [11]. Irrespective of the source, the isolated cells were characterized by adhesion to plastic and quantitative expansion *in vitro*, resulting in the formation of a sufficiently homogenous population of cells expressing MSC-specific markers (CD73, CD90, CD105), and were capable of bilinear (osteoadipogenic) differentiation. These characteristics in BM, adipose, and placental tissue fibroblast-like cells confirm their appurtenance to the multipotent MSC class.

Constitutive production of cytokines. On the whole, MSC actively produced cytokines, chemokines, and growth factors at the stage of confluent growth: cytokines IFN-γ (median 539 pg/ml), IL-2 (115 pg/ml), IL-6 (13,400 pg/ml); chemokines IL-8 (4500 pg/ml), MCP-1 (2180 pg/ml), and MIP-1β (187 pg/ml); and growth factors erythropoietin (2.7 mU/ml), G-CSF (277 pg/ml), GM-CSF (376 pg/ml), VEGF (4980 pg/ml), and IGF-1 (19,900 pg/ml; Table 1). Median concentrations of other growth factors (bFGF) and cytokines (IL-4, IL-13), including the proinflammatory mediators (IL-1β, TNF-α, IL-17) in conditioned media varied from 20-25 to 50-70 pg/ml. The levels of other cytokines (IL-10, IL-5, IL-12, EGF) were at the lower boundary of method sensitivity threshold or did not exceed 10 pg/ml (IL-7).

Comparative analysis of MSC of different origin (Table 2) showed that placental MSC (in contrast to BM cells) were characterized by higher level of production of IL-4 (68±12 *vs.* 26±14 pg/ml; $p_U<0.05$), IL-13 (64±17 *vs.* 21±8.6 pg/ml; $p_U<0.05$), and IL-17 (102±21 *vs.* 28±10 pg/ml; $p_U<0.05$). In addition, placental MSC less effectively than BM and adipose tissue MSC produced IGF-1 and VEGF (8730 and 3129 pg/ml, respectively, *vs.* 22,825 and 4920 pg/ml for BM MSC and 25,980 and 5112 pg/ml for adipose tissue MSC). On the other hand, the intensity of G-CSF secretion in placental cell cultures was the highest (median 1918 pg/ml *vs.* 234 and 279 pg/ml for BM MSC and adipose tissue MSC, respectively), though these differences were statistically insignificant because of low number of observations.

Adipose tissue MSC more actively than BM cells produced not only IL-13 and IL-17, but also IFN-γ (median 746 *vs.* 501 pg/ml; $p_U<0.05$), IL-2 (134 *vs.* 26 pg/ml; $p_U<0.05$), IL-1β (80 *vs.* 62 pg/ml; $p_U<0.08$), GM-CSF (614 *vs.* 242 pg/ml; $p_U<0.05$), and erythropoietin (7.3 *vs.* 1.3 mU/ml, $p_U<0.05$).

On the other hand, MSC isolated from different tissues were comparable by the production of CXC (IL-8) and CC (MCP-1, MIP-1β) chemokines.

In addition to production of cytokines, evaluation of constitutive secretory activity of MSC included analysis of production of matrix proteinases (for example, MMP-9) and their inhibitors (for example, TIMP-1) playing an important role in the morphogenesis and reparation of tissues and in cell adhesion, homing, proliferation, and apoptosis [23,24,33].

On the whole, MSC isolated from the studied tissues produced TIMP-1 at the level of 440 ng/ml during their confluent growth (Fig. 1). In addition, MSC secreted MMP-9, but its level was significantly lower (7.4 ng/ml), about 50 times lower than the production of tissue inhibitor. Bone marrow and adipose tissue MSC were comparable by the production of MMP-9 and TIMP-1. Placental MSC were characterized by more than 2-fold lower production of TIMP-1 (240 *vs.* 545 and 494 ng/ml; $p_U<0.05$) and the TIMP-1/MMP-9 index (21.6 *vs.* 58.6 and 55.6 estimated units; $p_U<0.05$).

A pronounced shift of TIMP-1/MMP-9 balance towards TIMP-1 seemed to reflect the biological mechanism maintaining the conditions essential for MSC adhesion/confluent growth *in vitro* and presumably for cell homing in appropriate tissue niches (in BM stroma and perivascular space of the adipose and placental tissue) [9,10,20].

Our data reflect the overall production of mediators in MSC cultures by the moment of confluence. Heterogeneity of the parameters within the groups and specific features of MSC from different tissues could

be partially explained by differences in MSC cultures in the counts of producer cells or by cell population growth (proliferation rate, number of cell doubling, subconfluence terms) [3].

For more detailed analysis of the spectra of cytokines produced by MSC of different tissue origin we carried out an additional series of experiments in which the studied populations were standardized by the counts of producer cells (5×10^4 MSC/well) and by the duration of culturing (48 h).

Spontaneous and LPS-induced production of cytokines. Forty-eight hours before culturing MSC spontaneously produced cytokines: IFN-γ (median 372 pg/ml), IL-6 (8460 pg/ml), growth factors G-CSF (287 pg/ml), VEGF (3125 pg/ml), IGF-1 (19,350 pg/ml), bFGF (233 pg/ml), and chemokines IL-8 (7970 pg/ml), MCP-1 (954 pg/ml), MIP-1β (81 pg/ml) at a sufficiently high level (Table 3). The levels of other cytokines (IL-2, IL-1β, TNF-α, IL-17, IL-13) in supernatants of unstimulated MSC cultures varied (22-37 pg/ml), were at the lower threshold level of the sensitivity of the diagnostic test systems (IL-12, IL-4, IL-5, IL-10, IL-7, GM-CSF) or beyond their sensitivity (erythropoietin, EGF). Obviously, elevation of IL-2 (at the level of 115 pg/ml), GM-CSF (376 pg/ml), and erythropoietin (2.7 mU/ml) detected in unfractionated MSC cultures (Table 1) resulted from later (>48 h) triggering of the synthesis these cytokines or their gradual accumulation in the conditioned medium reaching the maximum values by MSC subconfluence.

MSC express toll-like receptors (TLR), including TLR-4 [17,26], and therefore, we also studied LPS-stimulated production of cytokines, growth factors, and chemokines. On the whole, MSC isolated from different tissues exhibited high LPS reactivity (Table 3). Even initially elevated basal production of IFN-γ,

TABLE 3. Concentrations of Cytokines, Growth Factors, and Chemokines in Supernatants of 48-h MSC Cultures (n=10)

Cytokines, pg/ml		Sontaneous production		LPS-stimulated production		IE_{LPS}	
		M±SE	median	*M±SE*	median	*M±SE*	median
Th1/proinflammatory	IFN-γ	392±73	372	823±76	792**	2.8±0.5	2.8
	IL-2	46.6±14.3	23	66.6±17.6	72	3.1±1.2	1.3
	IL-1β	33±11	31	85.2±10.6	91*	17.7±6.3	4.7
	TNF-α	34.7±6.8	28	87.1±12.5	80**	3.2±0.6	3.1
	IL-12	2.9±0.9	2.0	6.8±1.8	7.0	2.8±0.8	1.4
	IL-17	31±16	22	129±24	122**	26.9±13.5	6.4
Th2/anti-inflammatory	IL-4	11.8±7.4	2.0	63.7±14.1	75**	25±8	26
	IL-5	3.4±0.2	3.3	7.2±3.0	4.2	2.3±1.0	1.4
	IL-10	3.8±1.8	2.0	11.2±5.8	2.0	3.0±1.2	1.0
	IL-13	30.0±8.5	37	46.0±11.7	37	2.6±0.6	2.1
	IL-6	8505±2358	8460	19,935±2557	21,990**	4.8±1.3	3.2
Growth factors	IL-7	7.5±2.7	3.3	12.8±2.9	15**	2.2±0.4	1.9
	G-CSF	672±332	287	4420±1204	4345**	13.3±3.6	8.9
	GM-CSF	88±45	2.0	470±65	376**	129±51	121
	Erythropoietin	<1 mU/ml	<OOR	<1 mU/ml	<OOR		
	VEGF	3034±465	3125	3077±502	2910	1.00±0.01	1.0
	IGF-1	23,850±3360	19,350	23,490±3540	19,670	1.00±0.04	1.0
	bFGF	252±32	233	263±37	219	1.00±0.01	1.0
	EGF	<30	<OOR	<30	<OOR		
Chemokines	IL-8	8674±2382	7970	28,694±3424	28,380**	7.0±2.0	5.1
	MCP-1	1180±215	954	2040±110	1200**	2.2±0.4	1.9
	MIP-1β	136±48	81	1275±564	301**	32.5±16	5.1

Note. *p<0.05, **p<0.01 compared to spontaneous production (Wilcoxon's paired test).

IL-6, G-CSF, and chemokines (IL-8, MCP-1, MIP-1β) increased significantly in response to endotoxin (median index of LPS effect E_{LPS}=1.9-8.9 estimated units).

In addition, the production of proinflammatory cytokines increased significantly: IL-1β (IE_{LPS}=4.7), TNF-α (IE_{LPS}=3.1), and IL-17 (IE_{LPS}=6.4), as well as of some other cytokines, i.g. IL-4 (IE_{LPS}=26) and IL-7 (IE_{LPS}=1.9).

Secretion of IGF-1, bFGF, and VEGF seemed to be constitutive, because it did not increase in response to LPS stimulation (IE_{LPS}=1.0). On the other hand, the production of GM-CSF increased significantly in LPS-stimulated cultures (IE_{LPS}=121 estimated units), while 48-h production of erythropoietin and EGF remained equally low in spontaneous and LPS-stimulated MSC cultures.

We failed to detect the production of EGF (>30 πg/ml) by the Russian test systems in unfractionated MSC cultures during their confluent growth and during their culturing under standard conditions. Presumably, MSC originating from different tissues poorly produce this growth factor, though express the specific receptors (EGFR), binding to which induces the mobility and proliferation of MSC [32], this suggesting the use of exogenous EGF for quantitative expansion of MSC *in vitro* [28,32].

The results of comparative evaluation of spontaneous production of cytokines by different MSC types are presented in Fig. 2 (results of IL-5, IL-7, IL-10, IL-12, erythropoietin, and EGF measurements are not presented, because basal production of these cytokines was minimum and did not differ in MSC of different types). Bone marrow and placental MSC were generally similar by the levels and profiles of secreted Th1/pro- and Th2/anti-inflammatory cytokines, except IL-13 which was not produced by BM MSC and was clearly detected in placental MSC supernatants. The main differences between BM and placental MSC consisted in the levels of production of some growth factors and chemokines. The intensity of VEGF, IGF-1, and MIP-1β secretion was significantly lower, while the secretion of IL-8 was significantly higher in unstimulated umbilical MSC cultures than in BM cell cultures.

The greatest differences in the spontaneous production of cytokines were detected for adipose tissue MSC. Similarly as placental MSC, they differed from BM MSC by high spontaneous production of

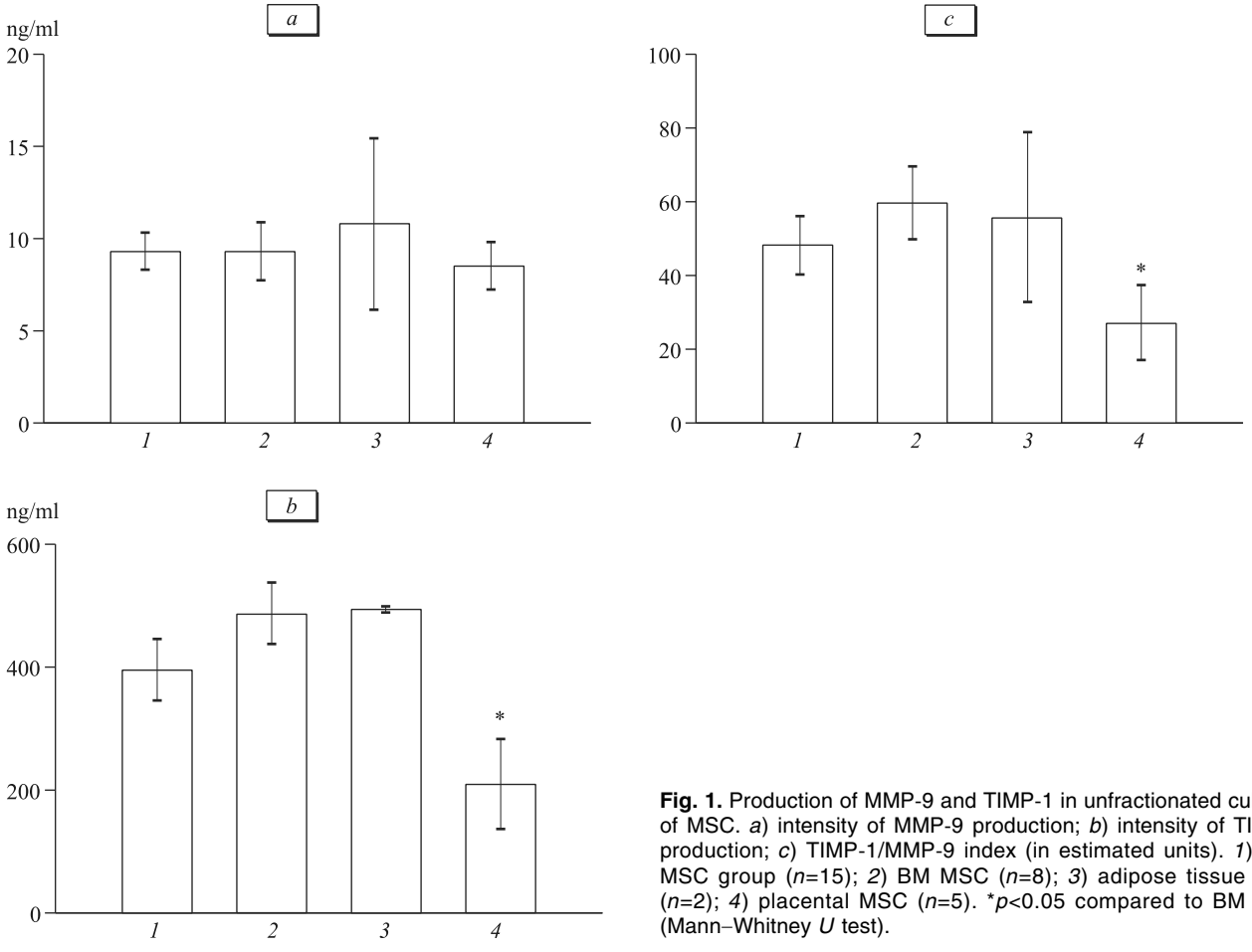

Fig. 1. Production of MMP-9 and TIMP-1 in unfractionated cultures of MSC. *a*) intensity of MMP-9 production; *b*) intensity of TIMP-1 production; *c*) TIMP-1/MMP-9 index (in estimated units). *1*) total MSC group (*n*=15); *2*) BM MSC (*n*=8); *3*) adipose tissue MSC (*n*=2); *4*) placental MSC (*n*=5). *p<0.05 compared to BM MSC (Mann–Whitney *U* test).

IL-13 and IL-8, but their production of MIP-1β and VEGF was not reduced. In addition, adipose tissue MSC, in contrast to BM and placental cells, more actively released IFN-γ, IL-2, IL-1β, TNF-α, IL-4, IL-6, G-CSF, GM-CSF, and all chemokines. Adipose tissue MSC spontaneously produced GM-CSF (median 161 pg/ml), while in the BM and placental MSC supernatants the content of this growth factor was at the lower threshold level of the method sensitivity (Bio-Plex Assay, 2 pg/ml). Hence, by the type and level of basal production of cytokines MSC derived from adipose tissue more distinctly exhibited the proinflammatory, immunoregulatory, and hemopoiesis-stimulating phenotype.

Bone marrow and placental MSC were characterized by high LPS reactivity, because in mitogen-stimulated cultures they actively produced ($IE_{LPS} \geq 3.0$) Th1/proinflammatory (IFN-γ, IL-1β, TNF-α, IL-17) and Th2 cytokines (IL-4, IL-6), all chemokines (IL-8, MCP-1, MIP-1β), and hemoimmunopoiesis growth factors (G-CSF and GM-CSF; Table 4). Previously detected differences between spontaneous production of mediators by the BM and placental MSC manifested in LPS stimulated cultures as well. The intensity of LPS-induced secretion of VEGF, IGF-1, and MIP-1β in placental MSC cultures was lower, while secretion of IL-8 and IL-13 significantly higher than in BM cell cultures.

Adipose tissue MSC, characterized by high level of spontaneous (basal) production of the majority of the studied cytokines, differed from the BM and placental MSC by a lesser sensitivity to LPS stimulation. This manifested by significantly lower mean levels of IE_{LPS} for IFN-γ, IL-1β, TNF-α, IL-17, IL-4, IL-6, GM-CSF, IL-8, MCP-1, and MIP-1β. However, similarly as in BM and placental MSC cultures, the intensity of these cytokines production by the cells derived from adipose tissue increased in response to LPS, but less markedly.

The level of 3 cytokines produced by MSC virtually did not change in the presence of endotoxin (IE_{LPS}=1.0): IGF-1, bFGF, and VEGF.

On the whole, our results were in good agreement with previous data obtained by semiquantitative sandwich method for protein evaluation on membranes with immobilized anticytokine antibodies [8]. Those studies have shown low (+) content of EGF in supernatants of human MSC cultures and high (++ — ++++) expression of IGF-1, VEGF, FGF, IL-6, IL-8, MCP-1, MIP-1β, and TIMP-1, as well of some other mediators (angiopoietin, thrombopoietin, neurotrophic factors (BDNF), hepatocyte growth factor (GDNF),

TABLE 4. Production of Cytokines, Chemokines, and Growth Factors, Stimulated by LPS in 48-h Supernatants of MSC Cultures Derived from Different Tissues (*M*±*SE*)

Cytokines, pg/ml		BM MSC (*n*=3)		Placental MSC (*n*=3)		Adipose tissue MSC (*n*=4)	
		IE_{LPS}	LPS	IE_{LPS}	LPS	IE_{LPS}	LPS
Th1/proinflammatory	IFN-γ	803±69	3.0±0.2	890±248	4.4±0.6	782±41	1.4±0.2*+
	IL-2	47.5±24.5	7.3±4.2	46.7±34.7	2.0±1.5	91±28	1.8±1.1
	IL-1β	73±3	36.5±1.5	99±24	27.1±11.9	81±17	1.36±0.30*+
	TNF-α	74.5±5.5	4.2±0.4	102±38	4.8±0.9	82.5±11.0	1.6±0.2*+
	IL-17	166±79	83±39	139±28	20.6±15.3	103±39	3.5±1.1*+
Th2/anti-inflammatory	IL-4	49±37	24.5±18.5	89±25	44.7±12.5	52±20	10.5±9.0+
	IL-13	8.0±1.0	4.0±0.8	58.0±11.8*	2.6±1.1	57±21*	2.0±0.9
	IL-6	25,630±3590	7.0±3.8	13,890±3590	7.5±2.1	21,620±600	1.7±0.3*+
Growth factors	G-CSF	839±312	8.8±0.1	7844±2150*	21.0±8.7	3645±1050	9.8±4.2
	GM-CSF	376±10	188±10	516±210	258±105	483±38	4.0±1.5*+
	VEGF	4868±480	1.10±0.03	1815±547*	1.1±0.1	3127±626	0.90±0.06
	IGF-1	32,120±6800	1.00±0.13	14,370±1230	1.00±0.04	26,130±6450	0.95±0.06
	bFGF	171±12	1.00±0.12	272±81	1.14±0.09	323±53	1.03±53.00
Chemokines	IL-8	16,560±2220	14.6±4.4	36,430±5090*	8.3±1.7	28,955±4130	2.1±0.5*+
	MCP-1	1900±96	2.90±0.01	1920±112	3.0±0.6	2196±223	1.2±0.1*+
	MIP-1β	4000±938	38.5±10.9	197±59*	68±43	723±364*	2.8±0.6*+

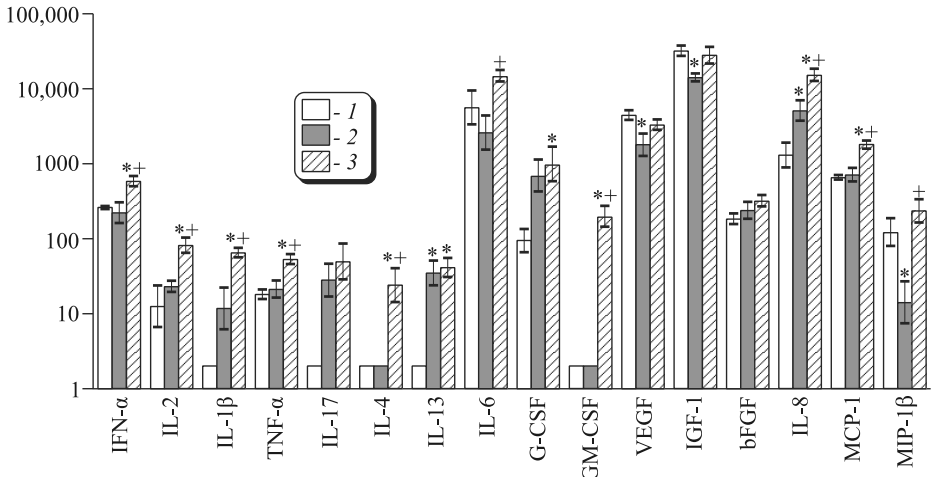

Fig. 2. Spontaneous production of cytokines, chemokines, and growth factors in 48-h cultures of MSC derived from the BM (n=3), adipose tissue (n=4), and placenta (n=3). Abscissa: mean levels of spontaneous production of cytokines (in pg/ml). $p<0.05$ compared to *BM MSC, +placental MSC (Mann–Whitney U test).

placental growth factor, leukemia inhibitory factor, CX3CL1 chemokine (fractalkine), TIMP-2) [8].

Hence, multiplex analysis of constitutive production of bioactive mediators has shown that MSC originating from different tissues possess a functional potential for stimulation of hemopoiesis (through production of G-CSF, GM-CSF, erythropoietin), immunomodulation (by producing IFN-γ, IL-2, IL-6, IL-1β, TNF-α, and chemokines IL-8, MCP-1, and MIP-1β), stimulation of angiogenesis (through production of VEGF, bFGF) and neuroregeneration (through production of IGF-1, bFGF, IL-6, erythropoietin, VEGF) [22,23], regulation of glucose metabolism in tissue microenvironment (through production of IGF-1) and of extracellular matrix remodeling (through production of MMP-9, TIMP-1). By the type and levels of spontaneous (basal) production of cytokines the adipose tissue MSC have more definitely exhibited the proinflammatory (IL-1β, TNF-α), immunoregulatory (IFN-γ, IL-2, IL-4, IL-6, IL-8, MCP-1, MIP-1β), and hemopoiesis-stimulating (G-CSF, GM-CSF) phenotype and are at the same time characterized by lesser sensitivity to LPS stimulation than BM and placental MSC.

It has been assumed for a long time that TLR are intrinsic for exclusively immune system cells reacting to potentially hazardous antigens of viral, bacterial, or fungal nature. However, expression of TLR on MSC has been described [17,26]. Our results agree with these data. We have found that the ligand (endotoxin) interactions with the receptor (TLR4) on MSC generally potentiate their secretory activity, though different MSC types may differ by the levels of LPS reactivity. Our data indicate that the functional potential of MSC, realized through production of numerous bioactive mediators, can play an important role in regulation of reparative processes not only under physiological conditions, but in destructive exposure of different kinds, for example, within the framework of immune response to bacterial infections.

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