Autocrine Interleukin-6 Drives Skin-Derived Mesenchymal Stem Cell Trafficking via Regulating Voltage-Gated Ca\(^{2+}\) Channels

FANG Ke, a LINGYUN ZHANG, a ZHAOYUAN LIU, a JINLIN LIU, a SHA YAN, a ZHENYAO XU, a JING BAI, a HUYYUAN ZHU, a FANGZHOU LOU, a HONG WANG, a YUFANG SHI, b,c YONG JIANG, d BING SU, a,e HONGLIN WANG a,f

Key Words. Calcium flux • Immunosuppression • Mesenchymal stem cells • siRNA • T cells • Stem cell transplantation • gp130 • Experimental models

ABSTRACT

Mesenchymal stem cells (MSCs) have demonstrated promising therapeutic potential for a variety of diseases including autoimmune disorders. A fundamental requirement for MSC-mediated in vivo immunosuppression is their effective trafficking. However, the mechanism underlying MSC trafficking remains elusive. Here we report that skin-derived MSCs (S-MSCs) secrete high levels of interleukin-6 (IL-6) in inflammatory conditions. Disruption of the IL6 or its signaling transducer gp130 blocks voltage-gated calcium (Ca\(^{2+}\)) channels (VGCC) critically required for cell contraction involved in the sequential adhesion and de-adhesion events during S-MSC migration. Deletion of Il6 gene leads to a severe defect in S-MSC’s trafficking and immunosuppressive function in vivo. Thus, this unexpected requirement of autocrine IL-6 for activating Ca\(^{2+}\) channels uncovers a previously unrecognized link between the IL-6 signaling and the VGCC and provides novel mechanistic insights for the trafficking and immunomodulatory activities of S-MSCs.

STEM CELLS 2014;32:2799–2810

INTRODUCTION

Mesenchymal stem cells (MSCs), alternatively termed as multipotent mesenchymal stromal cells, are a heterogeneous population of cells with fibroblast-like morphology originally observed in bone marrow that proliferate in vitro as plastic-adherent cells [1]. Besides bone marrow, skin as the largest lymphoid organ, provides an easily accessible and ideal source of tissue for the isolation of stem cells including MSCs [2]. Upon activation by proinflammatory cytokines, MSCs can be driven to secret high levels of interleukin-6 (IL-6), which was thought to be involved in MSC-mediated suppression of T-cell proliferation and dendritic cell (DC) differentiation and function [3]. While IL-6 action on MSCs via autocrine or paracrine manner has been shown to enhance MSC proliferation, protect MSCs from apoptosis, and maintain the stemness of MSCs [4], its role in MSC trafficking to target tissues has not yet been characterized.

Irrespective of the type of stem/progenitor cells used in clinical applications, the fundamental requirement for a functional benefit of cell-based therapy is effective trafficking and tissue integration of the infused cells [5]. Cell trafficking/migration is a sequential and highly interrelated multistep process involving the formation of lamellipodia protrusion at the front edge, adhesion and de-adhesion cycles, cell body contraction, and tail retraction [6]. Adhesion, protrusion, and actin organization regulated by cell contraction are central features of migrating cells [7]. The major function of cell contraction may help break adhesive interactions by direct application of physical stress in migrating cells [8]. Of note, during cell migration calcium (Ca\(^{2+}\)) concentration is the highest at the rear end and the lowest at the front leading edge [9]. Ca\(^{2+}\) is a highly versatile intracellular signal for a wide range of physiological processes [10]. Increase of cellular Ca\(^{2+}\) levels is caused by either Ca\(^{2+}\) release from intracellular stores or Ca\(^{2+}\) influx through plasma membrane Ca\(^{2+}\) or other cation channels [10]. Among the membrane channels are the voltage-gated Ca\(^{2+}\) channels (VGCC) mediating Ca\(^{2+}\) entry into cells in response to membrane depolarization [11]. The VGCC selectively allow the influx of Ca\(^{2+}\) ions down an electrochemical gradient, from high concentration outside the cell to a low concentration inside the cell, to increase cytosolic Ca\(^{2+}\) levels [11]. On the basis of electrophysiological and pharmacological properties, the VGCC are divided into three subfamilies:
(a) L-type high voltage-activated Ca\(^{2+}\) channel family comprising the Ca\(_{v}\)1.1, 1.2, 1.3, and 1.4 channels that are inhibited by dihydropyridines [11, 12]; (b) non-L-type high voltage-activated Ca\(^{2+}\) channels Ca\(_{v}\)2.1 (P/Q-type), 2.2 (N-type), and 2.3 (R-type) that are very sensitive to ω-agatoxin IVA, and ω-conotoxin GVIA and SNX482, respectively [11, 13]; and (c) the low voltage-activated T-type Ca\(^{2+}\) channel family (Ca\(_{v}\)3.1, 3.2, and 3.3) that differs electrophysiologically from the high voltage-activated Ca\(^{2+}\) channels in opening transiently already upon modest depolarization [14, 15]. The VGCC regulate the intracellular Ca\(^{2+}\) concentration and contribute thereby to Ca\(^{2+}\) signaling in numerous cell types [16]. Ca\(^{2+}\) flow through these channels serves as the second messenger of electrical signaling, initiating intracellular events such as contraction, secretion, synaptic transmission, fertilization, and gene expression [10]. Mice with the inducible knockout of Ca\(_{v}\)1.2, the gene encodes L-type Ca\(^{2+}\) channel, die before day 14.5 postcoitum, and displayed a profound decrease in myocardial contractility [17]. Although it is becoming increasingly clear that Ca\(^{2+}\) signaling plays a fundamental role in the migration of a variety of cell types, such as neutrophils [18], eosinophils [9], neurons [19], fibroblast [20], and smooth muscle cells [21], to our knowledge no study has reported the role of VGCC regulated by IL-6 signaling in MSC trafficking.

Experimental autoimmune encephalomyelitis (EAE), a mouse model for multiple sclerosis, is widely accepted as an autoimmune inflammatory disease model in which Th1 and Th17 cells play a critical role [22, 23]. Given the importance of MSCs in the treatment of autoimmune disorders including EAE, we have revealed an important regulatory axis for their trafficking and immunomodulatory activities.

**Materials and Methods**

**Mice**

C57BL/6J mice (stock number: 000664) were purchased from The Jackson Laboratory (Bar Harbor, ME, http://www.jax.org). Mice were kept under specific pathogen-free conditions in compliance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals with the approval (SYXK-2003-0026) of the Scientific Investigation Board of Shanghai Jiao Tong University School of Medicine, Shanghai, China. To ameliorate any suffering of mice observed throughout these experimental studies, mice were euthanized by CO\(_2\) inhalation.

**Isolation of Skin-Derived MSCs**

Neonatal mice (1–3 days old) were euthanized by high concentration of CO\(_2\), and subsequently mice were disinfected by submerging into 75% alcohol for 2 minutes. Skin from back and abdomen of mice was carefully dissected free of other tissues, cut into 2–3 mm\(^3\) pieces, washed three times in Hanks Balanced Salt Solution. Skin pieces were transferred to a new tube containing 100 \(\mu\)L dispase solution (BD) and incubated overnight at 4°C. Epidermis from each piece of skin was peeled off, and the remainder of the skin was transferred into a tube containing 2 mg/mL collagenase I (Gibco, Grand Island, NY, http://www.invitrogen.com) solution. Tissue pieces were completely immersed with the collagenase I solution, and then digested at 37°C in water bath for 45 minutes, followed by 200 U/mL DNase I (Sigma) for another 15 minutes at 37°C in water bath. To stop the enzymatic digestion, complete medium (low glucose Dulbecco’s modified Eagle’s medium [DMEM], supplemented with 10% fetal bovine serum (FBS, Gibco), 2 mM L-glutamine, 100 U/mL penicillin, and 100 \(\mu\)g/mL streptomycin) was added. Tissue pieces were washed twice with HBSS. Skin pieces were then mechanically dissociated in complete medium, and the cell suspension poured through a 70 \(\mu\)m cell strainer (Falcon). Dissociated cells were collected by centrifugation at 300g for 5 minutes. Cells were resuspended in complete medium and then cultured in 100-mm tissue culture dish (Corning Life Sciences, Acton, MA, http://www.corning.com/lifesciences) in a 37°C, 5% CO\(_2\) culture incubator for 6 hours. To maintain the culture, medium was changed with the fresh complete medium. After 2 days, the cells were subcultured and re-seeded. The cells were used for the 4th to 10th passages.

**Differentiation of S-MSCs**

For osteogenic differentiation, skin-derived MSCs (S-MSCs) were plated in complete medium at a density of 3 \(\times\) 10\(^4\) cells per cm\(^2\) in six-well tissue plates. After 24 hours, the cells were stimulated for 21 days with complete medium supplemented with 0.2 mM ascorbate acid, 10 mM β-glycerophosphate, and 100 nM dexamethasone and then stained with Alizarin red. For adipogenic differentiation, S-MSCs were plated in complete medium at a density of 2 \(\times\) 10\(^4\) cells per cm\(^2\) in six-well tissue plates and re-fed every 3 days until they were 100% confluent or postconfluent. The cells were stimulated for 21 days with complete medium supplemented with 0.5 mM 3-isobutyl-1-methylxanthine, 100 mM indomethacin, 10 \(\mu\)g/mL insulin, 100 nM rosiglitazone, and 1 \(\mu\)M dexamethasone and then stained with oil red O. For chondrogenic differentiation, 0.5 mL (2.5 \(\times\) 10\(^5\) cells) of the S-MSCs suspension was aliquoted into 15 mL polystyrene culture tubes, and the cells were centrifuged at 150g for 5 minutes at room temperature. The cell pellets were stimulated for 21 days with complete medium supplemented with 0.3 mM ascorbate acid, 40 \(\mu\)g/mL L-proline, 100 \(\mu\)g/mL sodium pyruvate, 1% insulin-transferrin-selenium plus culture supplement, 10 ng/mL transforming growth factor-β3 (TGF-β3), and 100 nM dexamethasone. The pellets were formalin fixed and paraffin embedded for Alcian Blue staining. Reagents were purchased from Sigma-Aldrich (St. Louis, MO, http://www.sigmaaldrich.com). Expression of markers specific for differentiated cells was assessed by reverse transcriptase quantitative polymerase chain reaction (qPCR).

**Flow Cytometry and Intracellular Cytokine Staining**

The following antibodies were used for flow cytometry: allophycocyanin (APC) rat anti-mouse CD4 (GK1.5, Bioscience); fluorescein isothiocyanate (FITC) rat anti-mouse interferon γ (IFN-γ) (XMG1.2, Bioscience); phycoerythrin (PE) rat anti-mouse IL-17A (TC11–1810, BD Bioscience, San Diego, CA, http://www.bdbio.com); rat anti-mouse CD19 (45–0193, BioLegend), CD34 (HM34, BioLegend), CD45 (30-F11, BioLegend), HMCII (MS/114.15.2, BioLegend), CD44 (IM7, BioLegend), CD90 (OX-7, BioLegend), CD73 (TY/11.8, BioLegend), and CD105 (M1/78, BioLegend); APC goat anti-rat IgG2b, FITC goat anti-rat IgG1, and PE goat anti-rat IgG1 (all from BD Bioscience) were used as isotype controls. Intracellular staining of splenocytes or T-cell subsets for IFN-γ and IL-
17A was performed after stimulation with phorbol 12-myristate 13-acetate (50 ng/mL, Sigma) and ionomycin (1 µg/mL, Sigma) for 5 hours in the presence of Brefeldin A (eBioscience, San Diego, CA, USA, http://www.ebioscience.com) and incubated with APC-conjugated anti-CD4 antibody. After fixation and permeabilization, cells were stained with anti-IFN-γ and anti-IL-17A.

**ELISA**
Quantitative analysis of IL-6 in supernatants was performed by enzyme-linked immunosorbent assay (ELISA) according to the manufacturer’s instructions (Invitrogen, Carlsbad, CA, http://www.invitrogen.com).

**Protein Preparation and Protein Array**
The mouse protein membrane array (Ray Biotech) simultaneously profiles 39 proteins that were in duplicate, and supernatants derived from six mice were pooled together for the measurement. Experiments were performed in accordance with the manufacturer’s instructions. In brief, mouse cytokine array membranes were incubated for 30 minutes in 2 mL of blocking buffer and afterward for 2 hours in 2 mL of culture supernatants from either splenocytes alone, splenocytes with addition of myelin oligodendrocyte glycoprotein (MOG), S-MSCs with MOG or splenocytes with addition of MOG and S-MSCs at 20°C. After being washed, the membranes were incubated with biotin-conjugated antibodies (1:250 dilution, 1 mL per array membrane) at room temperature for 2 hours and washed again. A solution containing horseradish peroxidase-conjugated streptavidin (Invitrogen). RNA isolation was performed according to the manufacturer’s instructions. After being washed, the membranes were incubated with biotin-conjugated streptavidin (1:250 dilution, 1 mL per array membrane) and incubated with APC-conjugated anti-CD4 antibody. After 30 minutes, and 4 minutes. Array images were acquired at a resolution of 300 dpi on a computer photo scanner.

**Gene Expression Analysis**
Total RNA was extracted from cultured cells with TRIzol Reagent (Invitrogen). RNA isolation was performed according to the manufacturer’s instructions. Quantities of 1,000 ng of total RNA were reverse transcribed using Invitrogen Superscript II reverse transcriptase according to the manufacturer’s instructions (Invitrogen). First-strand complimentary DNA synthesis was performed using SuperScript II RT. qPCR was carried out using the SYBR Green PCR Master Mix (TaKaRa, Otsu, Japan, http://www.takara.co.jp) in the ABI Prism 7500 (Applied Biosystems, Foster City, CA, http://www.appliedbiosystems.com). Steps used to perform qPCR analysis included a hold step at 50°C for 2 minutes to activate uracil-DNA glycosylase, followed by another hold at 95°C for 10 minutes. Samples then underwent 40 cycles of 95°C for 15 seconds followed by 60°C for 1 minute. Subsequently, melt analysis was performed by increasing the temperature from 65 to 95°C. Relative expression levels were calculated using the ΔCT-method. ΔCT values represent the difference between two samples, transcript expression either as relative expression to β-actin mRNA by using the formula 2−ΔΔCT or as fold increase by using the formula 2−ΔCT. All gene expression results were normalized to expression of housekeeping gene β-actin. Primer sequences are listed in Supporting Information Table I.

**Histology and Immunofluorescence**
Cultured S-MSCs was fixed in 4% paraformaldehyde and was subsequently paraffin-embedded. Five micrometer sections were stained with Alcian blue. For immunofluorescence staining, the cultured S-MSCs were stained with anti-mouse IL-6 (MP5-20F3, R&D Systems, Minneapolis, MN, http://www.rndsystems.com), anti-mouse IL-6Rα (Clone 255821, R&D), and isotype control antibodies. Alexa488 or Alexa555-conjugated goat anti-Rat IgG1 or IgG2 was as secondary antibody. 4’,6-diamidino-2-phenylindole (DAPI) (Sigma) was used for nuclei staining.

**T-Cell Differentiation Assay**
CD4+CD25− T cells were isolated from spleens of 6- to 8-week-old C57BL/6j mice. After density gradient centrifugation with splenocyte/lymphocyte separation medium (Dakewe, China), the purified CD4+ T cells were collected in a first-step procedure where non-CD4+ T cells were depleted using a CD4+CD25+ Regulatory T Cell Isolation Kit (Milteny Biotec, Bergisch Gladbach, Germany, http://www.miltenyibiotec.com; Cat No: 130-091-041). Subsequently, the CD4+ T cells were stained with anti-CD25 FITC (Miltenyi Biotec) mAb and sorted into CD4+CD25− T cells and isolated soluble mAb (2 µg/mL) to CD28 (37.51, BD Biosciences). CD4+CD25− T cells were stimulated with coated mAb (5 µg/mL) to CD3 (145-2C11, BD Biosciences) and soluble mAb (2 µg/mL) to CD28 (37.51, BD Biosciences). To obtain Th1 cells, rmIL-12 (419-ML, R&D) 10 ng/mL and IL-4-specific antibody (504108, BioLegend) 10 µg/mL were added. To obtain Th17 cells, rmIL-6 (216-16, PeproTech, Rocky Hill, NJ, http://www.peprotech.com) 20 ng/mL, rmTGF-β (S2311L, Cell Signaling Technologies, Beverly, MA, http://www.cellsignal.com) 2 ng/mL, anti-IFN-γ (16–7311–85, ebioscience) 10 µg/mL, and anti-IL-4 mAb (504108, BioLegend) 10 µg/mL were added.

**Real-Time Cell Adhesion Assay**
The kinetics of cell adhesion was assayed using xCELLigence real-time cell analyzer (RTCA DP, Roche Diagnostics, Basel, Switzerland, http://www.roche-applied-science.com). Cells were seeded in special plates (E-plate) with microelectrodes covering well bottoms. The more cells attached on the electrodes, the larger the increases in electrode impedance. Electrode impedance which is displayed and recorded as cell index values reflects the biological statue of monitored cells. 10,000 cells were plated in each well in complete DMEM. Cells were allowed to settle for 30 minutes at room temperature before being placed in the RTCA DP in a humidified incubator at 37°C with 5% CO2. Readings were taken every 8 minutes for 6 hours and plotted curves represent the averages from three independent wells.

**Transwell Migration Assay**
Cell migration was assessed using transwell chambers with 8-µm pores (Corning Life Sciences, Acton, MA, http://www.corning.com/lifesciences). FBS was used as the chemoattractant, and 2×106 S-MSCs cells were resuspended in 100 µL serum-free DMEM medium and were placed into the upper chamber. After 8 hours, cells which had migrated through the membrane to the lower surface were fixed, stained, and counted.

**In Vivo Imaging Analysis**
Before mice were anesthetized with Forane (Abbott), an aqueous solution of luciferin (150 mg/kg) was intraperitoneally
injected 10 minutes prior to imaging. The animals were placed into the light-tight chamber of the CCD camera system (Xenogen), and the photons emitted from the luciferase-expressing cells within the animal were quantified for 2 minutes using the software program Living Image (Xenogen).

**Microarray Analysis**

RNA was isolated from IL-6−/− or IL-6+/+ S-MSCs (six individual subjects for each group) as described above, labeled using the Illumina Total Prep RNA amplification kit, and hybridized to Ref12v3 Arrays. Data were normalized and analyzed using Genome studio and Mayday software.

**Measurement of Intracellular Ca2+ Signaling**

Briefly, cultured S-MSCs grown on 8 × 8 mm glass coverslips were washed three times with phosphate buffer solution (PBS) and incubated with 4 μM fluo 3-AM (Dojindo, Kumamoto, Japan) in HBSS for 20 minutes at 37°C. Pluronic F-127 (20%, v/v) was added to facilitate the dye loading. S-MSCs were again washed for three times and then incubated in the standard bath solution of pH 7.4 for 20 minutes at 37°C. The coverslip was transferred to a perfusion chamber placed on a laser scanning confocal microscope (LSM-710, Zeiss), which is equipped with a ×40 (NA 0.5) UV fluor oil-immersion objective lens. In some experiments, KCl (50 mM) was added. Images were typically taken every 10 seconds and all acquired images were processed by Zeiss Zen software.

**Electrophysiology**

Ca2+ currents were recorded from mouse S-MSCs at room temperature in whole-cell voltage clamp configuration with a HEKA EPC10 amplifier (HEKA, Germany). Patch electrodes with resistance of 3–5 MΩ were pulled and polished from borosilicate glass by a horizontal puller (Zeitz, Germany). The pipette solution was filled with following solution (in mM): CsCl 120, TEA-Cl 20, CaCl2 1, EGTA 11, Hepes 10, and Mg-ATP 5 (pH 7.30 with CsOH). The bath solution contained (in mM) NaCl 130, CaCl2 1, MgCl2 1, glucose 10, and HEPES 10 (pH 7.3 with NaOH). To elicit Ca2+ currents from cells with a holding potential of −95 mV, 400 millisecond step depolarizations were applied from −75 mV to +55 mV in 10 mV increments with pulse intervals at 2 seconds. Data acquisition and analysis were performed with Patchmaster and GraphPad Prism 5.1.

**Western Blotting**

Lysates of transfected S-MSCs were resolved by electrophoresis, transferred to a Polyvinylidene-Fluoride (PVDF) membrane, and probed with antibodies against gp130 (#3732, Cell Signaling Technologies), and phosphorylated MLC (#3671, Cell Signaling Technologies), Cav2.3 (ab81097, Abcam) or β-actin (#37005, Cell Signaling Technologies). Western blotting was performed as previously published [24].

**Statistical Analysis**

Data were presented as the mean ± SEM, and statistics were done using Student’s t test when two conditions were compared or by an ANOVA with Bonferroni or Newman-Keuls correction for multiple comparisons and for flow cytometry data, statistical analyses were performed by Mann-Whitney U test for unpaired values using GraphPad Prism 5. Statistical significance was considered at p < .05.

## Results

### Isolation and Characterization of S-MSCs From Mouse Dermis

MSCs are a heterogeneous subset of stromal cells that can be isolated from bone marrow, marrow aspirates, skeletal muscle, adipose tissue, synovium, and many other connective tissues [25, 26]. The recent discovery of nestin-positive multipotent stem cells from the dermis of juvenile and adult mice as well as human dermis [27, 28] motivated us to study the S-MSC modulation of T-cell-mediated immune responses. For this purpose, we isolated and propagated plastic adherent cells from the dissociated dermis of 1- to 3-day-old C57BL/6J mice. The S-MSCs were characterized by their ability to extensively proliferate in culture with an attached well spread fibroblast-like morphology (Fig. 1A). The S-MSCs cultured in vitro were negative for CD19, CD34, CD45, MHCII and were positive for CD44, CD90, CD73, and CD105 (Fig. 1B). In conditioned medium, S-MSCs consistently differentiated into adipocytes, osteocytes, and chondrocytes (Fig. 1C). Expression of three lineage-specific markers was also quantified by qPCR (Fig. 1D–1F). Together, these data suggest that skin is an easily accessible and ideal source of tissue for the isolation of MSCs.

### S-MSCs Secrete High Levels of IL-6 in Inflammatory Condition

MSCs inhibited the peripheral autoimmune response, decreased inflammatory cell infiltration, decreased the release of proinflammatory cytokines and chemokines, and enhanced local tissue repair [29]. We here focused on identifying the intrinsic factor(s) responsible for the therapeutic effects of S-MSCs in autoimmunity. We took advantage of protein array technology and scanned 39 proinflammatory and anti-inflammatory factors in the coculture of S-MSCs and myelin-sensitized splenocytes stimulated with MOG35-55. Notably, we found that IL-6 was the most pronounced cytokine induced by S-MSCs in cocultures (Fig. 2A, 2B). Subsequently, we measured the expression levels of IL-6 in the supernatant by ELISA. Our data demonstrated that increased IL-6 was mainly secreted from S-MSCs, but not splenocytes (Fig. 2C). As S-MSCs expressed relatively high levels of Toll-like receptor (TLR) 4 compared to other TLRs (Fig. 2D), we activated S-MSCs by lipopolysaccharide and confirmed that IL-6 and IL-6 receptor (IL-6R) were upregulated at mRNA levels (Fig. 2E) and protein levels (Fig. 2F) in S-MSCs. Thus, we suggest that S-MSCs are activated by effective stimuli and are capable of producing high amount of IL-6.

### IL-6 Signaling is Required for Immunosuppressive Function of S-MSCs In Vivo

We showed that the addition of S-MSCs led to a complete inhibition of differentiation of Th1 and Th17 cells from purified CD4+CD25− T cells in vitro (Supporting Information Fig. 1A, 1B). To elaborate the functionality of IL-6 in suppressive properties of S-MSCs, we administrated i.p. S-MSCs derived from IL-6 knockout (IL-6−/−) mice at day 8 after immunization. In contrast to IL-6 wild-type (IL-6+/+) S-MSCs, no
A therapeutic effect was observed in mice treated with either IL-6\(^{+/−}\) S-MSCs or PBS (Fig. 3A, 3B). Surprisingly, we did not detect a significant difference for the inhibition of the Th1- and Th17-cell induction between IL6\(^{+/−}\) and IL-6\(^{+/−}\) S-MSCs in vitro coculture (Fig. 3C–3E). This indicates that IL-6 is not required for inhibiting Th1- and Th17-cell induction in vitro. We thought that this phenomenon might be due to a compensatory role of exogenous IL-6 produced by MOG35–55-stimulated myelin-sensitized lymphocytes. Thus, to directly investigate the role of IL-6 signaling in the suppressive function of S-MSCs in vitro, we generated S-MSCs stably expressing IL-6 signal transducer, gp130-targeted small hairpin RNA (shRNA). shRNA-mediated knockdown strategy resulted in greater than 93% inhibition of gp130 expression at protein levels (Supporting Information Fig. 2A–2E). Similar to IL-6\(^{+/−}\) S-MSCs, knockdown of gp130 expression did not affect the S-MSC-mediated inhibition of IL-17 and IFN-γ in vitro (Fig. 3F). To further determine whether gp130-deficient (gp130\(^{−/−}\)) S-MSCs possess immunosuppressive properties in vivo, we injected i.p. gp130\(^{−/−}\) S-MSCs into the EAE mice. Contrary to the observation in vitro, the administration of gp130\(^{−/−}\) S-MSCs apparently failed to prevent the EAE development (Fig. 3G). Collectively, our data indicate that the in vivo, but not in vitro immunosuppressive properties of S-MSCs appear to be critically dependent on autocrine IL-6 and an intact IL-6 signaling pathway.

S-MSC Trafficking is Driven by Autocrine IL-6

We have demonstrated that the disruption of IL-6 signaling did not affect immunosuppressive function of S-MSCs in vitro, which could not explain the molecular mechanism underlying the observed in vivo effects of IL-6 signaling in the immunomodulatory function of S-MSCs. We hypothesized that the IL-6 signaling might have a potential physiological function to influence the migration of S-MSCs [30]. To test this idea, we first measured cell adhesion of S-MSCs deficient for IL-6 signaling using the RTCA methodology that is a new label-free technology and can be based on impedance measurement.

Figure 1. Immunophenotypic and functional characterization of mouse S-MSCs. (A): S-MSCs were isolated from the dermis of neonatal mice and the cells were used for the 4th to 10th passages. The morphology of the S-MSCs was examined by light microscopy. (B): S-MSCs were characterized by cell surface marker expression using flow cytometry. The gray curves indicate the corresponding negative rat IgG1 or IgG2a control antibodies. (C): S-MSCs differentiated into adipocytes (oil red O), osteoblasts (Alizarin Red), and chondrocytes (Alcian Blue). The three lineage differentiation was confirmed by upregulation of specific markers measured by reverse transcriptase quantitative polymerase chain reaction. (D): PPAR-γ and Fabp4 for adipogenesis. (E): OC and AP for osteogenesis. (F): Col II and Agg for chondrogenesis. **, \(p < .01\); ***, \(p < .001\), two-tailed Student’s t test. Results are representative of three to four independent experiments. Abbreviations: Agg, aggrecan; AP, Alkaline phosphatase; Col II, collagen II; Fabp4, fatty acid binding protein 4; OC, osteocalcin; PPAR-γ, peroxysome proliferator-activated receptor; S-MSCs, skin-derived mesenchymal stem cells.
MSCs were seeded onto plates with microelectrodes, allowing for precise measurement of subtle changes in cell number, cell morphology, and substrate attachment [31]. We noted that both of IL-6^{−/−} and gp130^{−/−} S-MSCs revealed a marked increase in cell index compared to controls, suggesting that cytoskeletal structure and cellular contraction was defective due to formation of actin stress fibers (Fig. 4A, 4B) [32]. We next determined whether migratory properties of S-MSCs were affected by the disruption of IL-6 signaling using the Boyden chamber assay. Over a 6-hour period, we detected a dramatic decrease in the numbers of migrated IL-6^{−/−} S-MSCs compared to IL-6^{+/+} S-MSCs, and however the migration defect of IL-6^{−/−} S-MSCs was rescued after adding recombinant murine IL-6 (Fig. 4C, 4D). To substantiate that IL-6 signaling is truly required for S-MSC migration in vivo, we transfected IL-6^{−/−} or IL-6^{+/+} S-MSCs with luciferase reporter gene and performed in vivo migration assay. We transplanted i.p. the luciferase-labeled IL-6^{+/+} or IL-6^{−/−} S-MSCs into EAE mice. After 10 days, we tracked the transplanted cells and found a significant decrease in luciferase bioluminescence of IL-6^{−/−} S-MSCs in inflamed spleens of recipient mice compared with the IL-6^{+/+} S-MSCs, indicating that the disruption of autocrine IL-6 led to a defect in migration of S-MSCs in vivo (Fig. 4E, 4F). We further confirmed that IL-6^{+/+} S-MSCs indeed migrated to the inflamed spleen but not other intra-abdominal organs in EAE mice (Fig. 4G). In addition, the luciferase bioluminescence in inflamed spleens derived from IL-6^{−/−} S-MSCs injected mice was much weaker than those derived from IL-6^{+/+} S-MSCs injected mice (data not shown). Taken together, our findings established that autocrine IL-6 is critically required for S-MSC trafficking to inflamed organ.

MSCs were seeded onto plates with microelectrodes, allowing for precise measurement of subtle changes in cell number, cell morphology, and substrate attachment [31]. We noted that both of IL-6^{−/−} and gp130^{−/−} S-MSCs revealed a marked increase in cell index compared to controls, suggesting that cytoskeletal structure and cellular contraction was defective due to formation of actin stress fibers (Fig. 4A, 4B) [32]. We next determined whether migratory properties of S-MSCs were affected by the disruption of IL-6 signaling using the Boyden chamber assay. Over a 6-hour period, we detected a dramatic decrease in the numbers of migrated IL-6^{−/−} S-MSCs compared to IL-6^{+/+} S-MSCs, and however the migration defect of IL-6^{−/−} S-MSCs was rescued after adding recombinant murine IL-6 (Fig. 4C, 4D). To substantiate that IL-6 signaling is truly required for S-MSC migration in vivo, we transfected IL-6^{−/−} or IL-6^{+/+} S-MSCs with luciferase reporter gene and performed in vivo migration assay. We transplanted i.p. the luciferase-labeled IL-6^{+/+} or IL-6^{−/−} S-MSCs into EAE mice. After 10 days, we tracked the transplanted cells and found a significant decrease in luciferase bioluminescence of IL-6^{−/−} S-MSCs in inflamed spleens of recipient mice compared with the IL-6^{+/+} S-MSCs, indicating that the disruption of autocrine IL-6 led to a defect in migration of S-MSCs in vivo (Fig. 4E, 4F). We further confirmed that IL-6^{+/+} S-MSCs indeed migrated to the inflamed spleen but not other intra-abdominal organs in EAE mice (Fig. 4G). In addition, the luciferase bioluminescence in inflamed spleens derived from IL-6^{−/−} S-MSCs injected mice was much weaker than those derived from IL-6^{+/+} S-MSCs injected mice (data not shown). Taken together, our findings established that autocrine IL-6 is critically required for S-MSC trafficking to inflamed organ.

MSCs were seeded onto plates with microelectrodes, allowing for precise measurement of subtle changes in cell number, cell morphology, and substrate attachment [31]. We noted that both of IL-6^{−/−} and gp130^{−/−} S-MSCs revealed a marked increase in cell index compared to controls, suggesting that cytoskeletal structure and cellular contraction was defective due to formation of actin stress fibers (Fig. 4A, 4B) [32]. We next determined whether migratory properties of S-MSCs were affected by the disruption of IL-6 signaling using the Boyden chamber assay. Over a 6-hour period, we detected a dramatic decrease in the numbers of migrated IL-6^{−/−} S-MSCs compared to IL-6^{+/+} S-MSCs, and however the migration defect of IL-6^{−/−} S-MSCs was rescued after adding recombinant murine IL-6 (Fig. 4C, 4D). To substantiate that IL-6 signaling is truly required for S-MSC migration in vivo, we transfected IL-6^{−/−} or IL-6^{+/+} S-MSCs with luciferase reporter gene and performed in vivo migration assay. We transplanted i.p. the luciferase-labeled IL-6^{+/+} or IL-6^{−/−} S-MSCs into EAE mice. After 10 days, we tracked the transplanted cells and found a significant decrease in luciferase bioluminescence of IL-6^{−/−} S-MSCs in inflamed spleens of recipient mice compared with the IL-6^{+/+} S-MSCs, indicating that the disruption of autocrine IL-6 led to a defect in migration of S-MSCs in vivo (Fig. 4E, 4F). We further confirmed that IL-6^{+/+} S-MSCs indeed migrated to the inflamed spleen but not other intra-abdominal organs in EAE mice (Fig. 4G). In addition, the luciferase bioluminescence in inflamed spleens derived from IL-6^{−/−} S-MSCs injected mice was much weaker than those derived from IL-6^{+/+} S-MSCs injected mice (data not shown). Taken together, our findings established that autocrine IL-6 is critically required for S-MSC trafficking to inflamed organ.
The VGCC are Defective in the Absence of IL-6 in S-MSCs

To explore the molecular mechanism underlying the essential role of IL-6 signaling in the migration of S-MSCs, we compared gene expression between S-MSCs derived from IL-6+/- mice and IL-6-/- mice using the cDNA microarray analysis. The most significantly enriched pathway was the type II diabetes mellitus and the Ca^2+ signaling pathway (Fig. 5A). Intriguingly, among downregulated genes in both pathways the VGCC genes were distinctly focused (Supporting Information Fig. 3A, 3B, yellow marked nodes) (Microarray data at: http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE42914). Ca^2+ entry across the plasma membrane occurs via two distinct pathways, store-operated Ca^2+ channels and VGCC. To test which type of Ca^2+ channels might be affected in the absence of IL-6 in S-MSCs, S-MSCs derived from either IL-6+/- or IL-6-/- mice were cultured with 10 nM thapsigargin (TG), a noncompetitive inhibitor which raises cytosolic Ca^2+ concentration via blocking the ability of the cell to pump Ca^2+ into the sarcoplasmic and endoplasmic reticula, subsequently activate plasma membrane Ca^2+ channels [33]. Evidently, no significant difference of TG-induced intracellular Ca^2+ influx was detected between IL-6+/- and IL-6-/- S-MSCs (Fig. 5B–5D). We next used 20 μM ATP, a receptor ligand to stimulate IL-6+/- or IL-6-/- S-MSCs and found no significant difference of Ca^2+ influx between ATP stimulated IL-6+/- S-MSCs and ATP stimulated IL-6-/- S-MSCs with or without extracellular Ca^2+ (Supporting Information Fig. 4A–4F), suggesting that the release of intracellular Ca^2+ caused by ATP is not affected by IL-6. Together, our data suggest that knockout of IL-6 severely impairs the VGCC function in S-MSCs.
The VGCC Channels are Regulated by IL-6 signaling and Mediate S-MSC Contraction and Migration

Next, we intended to confirm the contribution of IL-6 to the VGCC after membrane depolarization using 50 mM KCl [34]. Again, using intracellular Ca\(^{2+}\) imaging analysis we found that the VGCC were significantly different between IL-6\(^{-/-}\) S-MSCs compared to IL-6\(^{+/+}\) S-MSCs (Fig. 6A). To further verify whether IL-6 signaling is involved with VGCC, we performed electrophysiology experiments to measure Ca\(^{2+}\) currents in IL-6\(^{-/-}\) and IL-6\(^{+/+}\) S-MSCs. We found IL-6\(^{-/-}\) S-MSCs produced much lower Ca\(^{2+}\) currents compared with IL-6\(^{+/+}\) S-MSCs (Fig. 6B). The VGCC comprise 10 subsets, CaV1.1, CaV1.2, CaV1.3, CaV1.4, CaV2.1, CaV2.2, CaV2.3, CaV3.1, CaV3.2, and CaV3.3 encoded by cacna1s, cacna1c, cacna1d, cacna1f, cacna1a, cacna1b, cacna1e, cacna1g, cacna1h, and cacna1i, respectively. To identify the subunit(s) of the VGCC regulated by IL-6 signaling, we measured mRNA levels of cacna1s, cacna1c, cacna1d, cacna1f, cacna1a, cacna1b, cacna1e, cacna1g, cacna1h, and cacna1i for IL-6\(^{+/+}\) S-MSCs and IL-6\(^{-/-}\) S-MSCs. Interestingly, we observed that, apart from cacna1a (encoding Ca\(_{v}\)2.1) and cacna1i (encoding Ca\(_{v}\)3.3), the expression levels of other VGCC channels were significantly decreased in IL-6\(^{-/-}\) S-MSCs compared with IL-6\(^{+/+}\) S-MSCs (Fig. 6C). We have demonstrated that IL-6 signaling regulates S-MSC contraction and migration (Fig. 6A–6G). We thus decided to investigate whether the impaired migration of IL-6\(^{-/-}\) or gp130\(^{-/-}\) S-MSCs was a consequence of defective Ca\(^{2+}\) channels. First, we found that chelating extracellular Ca\(^{2+}\) by EGTA resulted in a complete loss of adherence capacity of S-MSCs, suggesting that extracellular Ca\(^{2+}\) influx is essential for S-MSC adhesion. Ca\(^{2+}\) influx through the VGCC can be evoked using elevated extracellular KCl. Remarkably, opening the VGCC by 50 mM KCl increased S-MSC cell index (Fig. 6D). These data together suggest that calcium channels are essentially required for the contraction and migration of S-MSCs. We next sought to identify the signaling pathway involved in regulation of the VGCC by IL-6. We cultured IL-6\(^{-/-}\) S-MSCs stimulated with rIL-6 in the presence of inhibitors of phosphatidylinositol 3-kinase (PI3K), Signal transducer and activator of transcription 3 (STAT3), Janus kinase 3 (JAK3) and
extracellular regulated protein kinases1/2 (ERK). By measuring one of VGCC subunits Ca V2.3, we found that inhibition of JAK3/STAT3 signaling, not others remarkably decreased its expression at both mRNA and protein levels (Supporting Information Fig. 5A, 5B), suggesting that the JAK3/STAT3 signaling pathway was essential for activation of the VGCC. Previous findings suggested that myosin II is involved in the generation of the contraction for cell migration, and the myosin II activity is mainly controlled by its light chain (MLC) phosphorylation regulated by two classes of enzymes, MLC kinases and myosin phosphatase [35]. Western blotting experiments demonstrated that the phosphorylation of MLC in IL-6+/+ S-MSCs was apparently decreased in contrast to IL-6−/− controls (Fig. 6E).

**DISCUSSION**

During autoimmune responses, IL-6 production arises from a large number of cell types that include infiltrating leukocytes and tissue-resident cells [36]. IL-6 highly expressed in an inflammatory microenvironment is critically required for autoantigen-reactive CD4+ T-cell proliferation during EAE [37]. A cytokine protein array-based analysis helped us identify IL-6 as the most abundant mediator released by S-MSCs when exposed to an inflammatory microenvironment. Although a study reported that the inhibition of T-cell proliferation by MSCs was partially mediated by IL-6 in vitro [3], we showed that neither IL-6−/− nor gp130−/− S-MSCs exhibited defective suppressive properties in the inhibition of the Th1- and Th17-cell differentiation in vitro setting. Nonetheless, using IL-6−/− or gp130−/− S-MSCs we demonstrated that IL-6 signaling could work in an autocrine manner to regulate the functional and to promote the immunosuppressive efficacy of S-MSCs in vivo. We were intrigued by the apparent discrepancy between in vitro and in vivo studies. As trafficking and tissue integration are critically required for high efficiency and engraftment of MSC-based cell therapy [5], we focused on the migratory ability of S-MSCs in the absence of IL-6 or gp130. Cell migration is characterized by a cycle of four interrelated steps, namely polarization, adhesion, protrusion, and contraction [6, 38]. Using the RTCA, we showed that disruption of the il6 or silencing gp130 gene led to increased adhesion of S-MSCs, indicating that the contraction of S-MSCs was defective when IL-6 signaling was absent. Consistently, the in vitro Boyden chamber assay revealed that the migratory ability of S-MSCs was dependent on IL-6 signaling that evidently regulated cell contraction during migration.

Interestingly, EAE could be passively induced in sublethally irradiated nontreated C57BL/6J mice by adoptively transferring either splenocytes or CD4+ T cells derived from inflamed spleens of EAE mice [39]. These data indicated that once mice developed EAE, spleen harbors MOG antigen-specific T cells

---

**Figure 5.** Defective voltage-gated calcium (Ca2+) channels in the absence of IL-6 in skin derived mesenchymal stem cells (S-MSCs). cDNA microarray analysis of IL-6+/+ and IL-6−/− S-MSCs was performed. (A): Signaling pathways of different genes between IL-6+/+ and IL-6−/− S-MSCs. The p value denotes the significance of the pathway correlated to the indicated conditions. Lower the p value, more significant in the pathway (p value cut-off is .05). (B, C): Fluo 3-AM responses to cytosolic Ca2+ in IL-6+/+ S-MSCs and IL-6−/− S-MSCs with addition of TG (10 nM). Arrow indicates the time point for addition of TG. (D): Statistical analysis for the Ca2+ peak of the fluo 3-AM experiments. ns, not significant. Data (B, C) are representative of three independent experiments. Abbreviations: FI, fluorescence intensity; IL, interleukin; TG, Thapsigargin.
that are able to directly cause the disease. Therefore, targeting CD4+ T-cell polarization in spleen by S-MSCs should represent an effective therapeutic approach for the EAE treatment. Despite that the IL-6-dependent Prostaglandin E2 (PGE2) secretion of MSCs effectively reduced T cell proliferation in vitro and in vivo in collagen-induced arthritic mouse model [40], our in vivo imaging study confirmed that IL-6
2/
2 S-MSCs exhibited impaired migration to inflamed spleens in EAE mice, suggesting that the IL-6-mediated trafficking of S-MSCs likely accounts for the observed in vivo immunosuppressive functionality. Possibly, during the migration of MSCs, IL-6 regulates the cell contraction and adhesion, and once MSCs reach the target tissue the IL-6 controls the release of PGE2 for immuosuppression.

Ca2+ plays critical roles in a diverse range of cell functions, including adhesion, motility, gene expression, and proliferation [34]. Our cDNA microarray analysis revealed that the disruption of the il6 gene affected two important pathways, the type II diabetes mellitus and the Ca2+ signaling pathway. Very clearly, among downregulated genes in both pathways in IL-6
2/
2 S-MSCs, the VGCC genes were distinctly impacted, suggesting a major involvement of Ca2+ signaling. Ca2+ influx through the VGCC controls the trailing tail contraction in growth factor-induced fibroblast cell migration [20]. We further proved our findings by measuring the MLC phosphorylation of IL-6
1/
1 and IL-6
2/
2 S-MSCs. Our findings for the first time unveiled that IL-6 regulates cell contraction through controlling Ca2+ channels, providing the molecular mechanism underlying the immunosuppressive properties of S-MSCs in autoimmunity.

CONCLUSION

In summary, this study uncovers a link between the IL-6 signaling and the VGCC and provides a novel mechanism for the
trafficking of S-MSCs to the inflammatory sites and their in vivo immunomodulatory properties. This novel function identified for IL-6 could be applied to not only for understanding of MSC immunosuppression but also other critical biological processes such as cancer cell metastasis.

ACKNOWLEDGMENTS

This work was supported by grants from 973 program and National Natural Science Foundation of China (Nos.: 2012CB917100, 2014CB541905, 2010CB529704, 91029730, and 31330026), by the Program for Professor of Special Appointment (Eastern Scholar) at Shanghai Institutions of Higher Learning, by the Science and Technology Commission of Shanghai Municipality (No: 09140902600), by the Leading Academic Discipline Project of the Shanghai Municipal Educa-

REFERENCES

1 Friedenstein AJ, Challakyan RK, Latsnik NV et al. Stromal cells responsible for transferring the microenvironment of the hemopoietic tissues. Cloning in vitro and retransplantation in vivo. Transplantation 1974;17:331–340.
2 Bartsch G, Yoo JJ, De Coppi P et al. Propagation, expansion, and multilineage differentiation of human somatic stem cells from dermal progenitors. Stem Cells Dev 2005;14:337–348.
3 Djouad F, Charbonnier LM, Bouffi C et al. Mesenchymal stem cells inhibit the differentiation of dendritic cells through an interleukin-6-dependent mechanism. Stem Cells 2007;25:2025–2032.
4 Piccola KL, Kuhn NZ, Haleem-Smith H et al. Interleukin-6 maintains bone marrow-derived mesenchymal stem cell stemness by an ERK1/2-dependent mechanism. J Cell Biochem 2009;108:577–588.
5 Karp JM, Leng Teo GS. Mesenchymal stem cell homing: The devil is in the details. Cell Stem Cell 2009;4:206–216.
6 De Coppi P, Schwartz MA, Burridge K et al. Cell migration: Integrating signals from front to back. Science 2003;302:1704–1709.
7 Lauffenburger DA, Horwitz AF. Cell migration: A physically integrated molecular process. Cell 1996;84:359–369.
8 Jay PY, Pham PA, Wong SA et al. A mechanical function of myosin II in cell motility. J Cell Sci 1995;108 (Pt 1):387–393.
9 Brundage RA, Fogarty KE, Tuft RA et al. Calcium gradients underlying polarization and chemotaxis of eosinophils. Science 1991;254:703–706.
10 Berridge MJ, Bootman MD, Roderick HL. Calcium signalling: Dynamics, homeostasis and remodelling. Nat Rev Mol Cell Biol 2003;4:517–529.
11 Catterall WA. Structure and function of neuronal Ca2+ channels and their role in neurotransmitter release. Cell Calcium 1998;24:307–323.
12 Dolphin AC. L-type calcium channel modulation. Adv Second Messenger Phosphoprotein Res 1999;33:153–177.
13 Reid CA, Bekkers JM, Clements JD. Pre-synaptic Ca2+ channels: A functional patch-work. Trends Neurosci 2003;26:683–687.
14 Heady TN, Gomora JC, Macdonald TL et al. Molecular pharmacology of T-type Ca2+ channels. Jpn J Pharmacol 2001;85:339–350.
15 Perez-Reyes E, Van Deusen AL, Vitko I. Molecular pharmacology of human Cav3.2 T-type Ca2+ channels: Block by antihypertensives, antiarrhythmics, and their analogs. J Pharmacol Exp Ther 2009;328:621–627.
16 MacVicar BA. Voltage-dependent calcium channels in glial cells. Science 1984;226:1345–1347.
17 Rosati B, Yan Q, Lee MS et al. Robust T-type calcium current expression following heterozygous knockout of the Cav1.2 gene in adult mouse heart. J Physiol 2011;589:3275–3288.
18 Marks PW, Maxfield FR. Transient increases in cytosolic free calcium appear to be required for the migration of adherent human neutrophils. J Cell Biol 1990;110:43–52.
19 Komuro H, Rakic P. Modulation of neuronal migration by NMDA receptors. Science 1993;260:95–97.
20 Yang S, Huang XY. Ca2+ influx through L-type Ca2+ channels controls the trailing tail contraction in growth factor-induced fibroblast cell migration. J Biol Chem 2005;280:27130–27137.
21 Hollenbeck ST, Nelson PR, Yamamura S et al. Intracellular calcium transients are necessary for platelet-derived growth factor but not extracellular matrix protein-induced vascular smooth muscle cell migration. J Vasc Surg 2004;40:351–358.
22 Langrish CL, Chen Y, Blumenschein WM et al. IL-23 drives a pathogenic T cell population that induces autoimmune inflammation. J Exp Med 2005;201:233–240.
23 Park H, Li Z, Yang XO et al. A distinct line-eage of CD4 T cells regulates tissue inflammation by producing interleukin 17. Nat Immunol 2005;6:1133–1141.
24 Li Q, Laumontier Y, Syrovets T et al. Plasmin triggers cytokine induction in human monocyte-derived macrophages. Arterioscler Thromb Vasc Biol 2007;27:1383–1389.
25 Crisan M, Yap S, Casteilla L et al. A perivascular origin for mesenchymal stem cells in multiple human organs. Cell Stem Cell 2008;3:301–313.
26 da Silva Meirelles L, Chagastelles PC, Nardi NB. Mesenchymal stem cells reside in virtually all post-natal organs and tissues. J Cell Sci 2006;119:2204–2213.
27 Toma JG, Akhavan M, Fernandes KJ et al. Isolation of multipotent adult stem cells from the dermis of mammalian skin. Nat Cell Biol 2001;3:778–784.
28 Toma JG, McKenzie IA, Bagli D et al. Isolation and characterization of multipotent skin-derived precursors from human skin. Stem Cells 2003;21:727–737.
29 Caplan AI. Adult mesenchymal stem cells for tissue engineering versus regenerative medicine. J Cell Physiol 2007;213:341–347.
30 Rattigan Y, Hsu JM, Mishra PJ et al. Interleukin 6 mediated recruitment of mesenchymal stem cells to the hypoxic tumor milieu. Exp Cell Res 2010;316:3417–3424.
31 Salinas F, Kodake T. Phage T4 homologous strand exchange: A DNA helicase, not the strand transferase, drives polar branch migration. Cell 1995;82:111–119.
32 Guo L, Degenstein L, Dowling J et al. Gene targeting of BPAG1: Abnormalities in mechanical strength and cell migration in stratified epithelia and neurologic degenera-
33 Thastrup O, Cullen PJ, Drobak BK et al. Thapsigargin, a tumor promoter, discharges intracellular Ca2+ stores by specific inhibition of the endoplasmic reticulum Ca2+ (1-ATPase. Proc Natl Acad Sci USA 1990;87:2466–2470.
34 Dolmetsch RE, Pavjani U, Fife K et al. Signaling to the nucleus by an L-type calcium channel-calmodulin complex through the MAP kinase pathway. Science 2001;294:333–339.
35 Riento K, Guasch RM, Garg R et al. RhoE binds to ROCK I and inhibits downstream signaling. Mol Cell Biol 2003;23:4219–4229.
36 Ishihara K, Hirano T. IL-6 in autoimmune disease and chronic inflammatory proliferative disease. Cytokine Growth Factor Rev 2002;13:357–368.
37 Samoilova EB, Horton JL, Hilliard B et al. IL-6-deficient mice are resistant to experimental autoimmune encephalomyelitis: Roles of IL-6 in the activation and differentiation of...
autoreactive T cells. J Immunol 1998;161:6480–6486.
38 Selmani Z, Naji A, Zidi I et al. Human leukocyte antigen-G5 secretion by human mesenchymal stem cells is required to suppress T lymphocyte and natural killer function and to induce CD4+CD25highFOXP3+ regulatory T cells. Stem Cells 2008;26:212–222.
39 Stromnes IM, Goverman JM. Passive induction of experimental allergic encephalomyelitis. Nat Protoc 2006;1:1952–1960.
40 Bouffi C, Bony C, Courties G et al. IL-6-dependent PGE2 secretion by mesenchymal stem cells inhibits local inflammation in experimental arthritis. PLoS One 2010;5:e14247.

See www.StemCells.com for supporting information available online.