Cytotherapy, 2017; 19: 181–193

International Society for Cellular Therapy
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Pretreatment with IL-1β enhances proliferation and chondrogenic potential of synovium-derived mesenchymal stem cells

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Abstract

Background aims. Synovial mesenchymal stem cells (MSCs) are an attractive cell source for cartilage regeneration because of their high proliferative ability and chondrogenic potential. We have performed clinical trials using synovial MSCs to regenerate articular cartilage. To achieve good clinical outcomes for cell transplantation therapy, it is important to control both quantity (cell number) and quality (pluripotency or chondrogenic potential) of the cells for transplantation. Interleukin (IL)-1β is a pro-inflammatory cytokine with significant pro-proliferative potential for mesenchymal cells. However, the effects of IL-1β on synovial MSCs remain unknown. We investigated the effects of pretreatment with IL-1β on synovial MSCs. *Methods.* Human synovial tissue was harvested during total knee arthroplasty. Nucleated cells were plated and cultured in the absence or presence of IL-1β at 10^{-13}, 10^{-12}, 10^{-11}, 10^{-10}, 10^{-9} or 10^{-8} g/mL for 14 days. *Results.* The number of synovial MSCs increased in a concentration-dependent manner. When cultured for 21 days in chondrogenic medium after pretreatment with 10^{-8} g/mL IL-1β, pellet aggregation was observed, whereas pretreatment with 10^{-12}, 10^{-11} or 10^{-10} g/mL IL-1β significantly increased the weight of cartilage pellets ($P < 0.01$). Surface markers for adhesion ability and pluripotency were reduced with high concentrations of IL-1β. IL-6 and IL-8 expression increased, but no changes in the expression level of growth factors were indicated by cytokine array. *Conclusions.* We have demonstrated that pretreatment of IL-1β increased the proliferation and chondrogenic potential of synovial MSCs, which may promote the regenerative potential of synovial MSCs.

Key Words: *cell proliferation, cell transplantation therapy, chondrogenesis, interleukin-1beta (IL-1β), synovial mesenchymal stem cells (MSCs)*

Introduction

Many clinical trials using mesenchymal stem cells (MSCs) have been performed to treat patients with traumatic cartilage injuries and osteoarthritis [1–3]. As a cell source with such regenerative potential, MSCs derived from bone marrow, synovium, adipose tissue and skeletal muscle have been studied and utilized in clinical cases [1]. Among such tissue-derived MSCs, synovium-derived MSCs are an attractive cell source to treat articular cartilage disorders because of their high chondrogenic potential [4] and superior proliferative features when using autologous serum [5]. We have performed clinical trials using synovial MSCs to regenerate articular cartilage and to enhance repaired meniscus healing. This simple and minimally invasive technique has delivered promising preliminary outcomes [6,7]. However, to further enhance the regenerative potential of synovial MSCs, a key factor is to obtain a higher number of cells with increased chondrogenic potential during a limited period of time.

Interleukin (IL)-1β centrally works as a pro-inflammatory cytokine to initiate inflammation, to cause pain and to introduce autoimmune reaction [8]. IL-1β increases the content of IL-6, IL-8 and RANTES (regulated on activation normal T cell expressed and secreted) in chondrocytes, which are reportedly increased in the joint fluid of patients with osteoarthritis [9–12]. IL-1β is a well-known aggravating factor with high potential to promote inflammatory reactions and proliferation of synovial fibroblasts [13,14]. IL-1β has been shown to trigger pathological processes in rheumatoid arthritis (RA) and osteoarthritis (OA).

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(Received 24 July 2016; accepted 3 November 2016)

ISSN 1465-3249
http://dx.doi.org/10.1016/j.jcyt.2016.11.004

Researches focused on reproducing pathologic disease course of RA and OA have been performed by adding IL-1 to chondrocytes [15] and synovial cells [16]. Additional 100 pg/mL IL-1β reduced the expression of aggrecan in human osteoarthritic chondrocytes in culture [17]. IL-1β of 10 ng/mL inhibited the production of newly synthesized collagens in proliferating rabbit articular chondrocytes. The effect was accompanied by a decrease in the steady-state levels of type II collagen messenger RNA [18]. Human articular chondrocytes pretreated with 10 ng/mL IL-1β demonstrated early degenerative changes by transmission electron microscopy and the suppression of collagen type II synthesis [19]. Increase the levels of articular cartilage matrix degrading enzymes such as MMP1 (interstitial metalloproteinase), MMP3 (stromelysin), MMP13 (collagenase 3) and ADAMTS-4 (aggrecanase) are reported in IL-1β-stimulated human cartilage explants culture [20]. These may suggest that IL-1β has both anti-anabolic and catabolic roles in cartilage.

Unexpectedly, treatment of synovial MSCs with 3 ng/mL of IL-1β significantly increased cell yield during two-dimensional culture for 14 days in our pilot study. This effect seemed likely due to the enhancement of cell proliferation by IL-1β [21]. Moreover, the *in vitro* chondrogenic potential of MSCs was also significantly up-regulated by pretreatment with 5 ng/mL of IL-1β. These data indicate the biphasic potential of IL-1β on the physiology of MSCs, that is, IL-1β induces expression of catabolic factors in mesenchymal cells during the early stage of inflammation, whereas IL-1β enhances anabolic processes in the later stage of inflammation during repair. Our data suggest a novel physiological function of IL-1β and indicates that using IL-1β-stimulated synovial MSCs in a clinical setting may achieve better tissue regeneration quality.

We hypothesized that pretreatment of MSCs with IL-1β has the potential to yield a higher number of cells, improve colony formation, and enhance chondrogenic potential. This study aimed to investigate the effects of pretreatment of IL-1β on cell proliferation and chondrogenic potential and to find the optimal concentration of IL-1β to achieve these effects using synovial tissue.

Methods

Collection of synovial MSCs

This study was approved by the Ethical Committee of Medical Hospital, Tokyo Medical and Dental University, and written informed consent was obtained from all study subjects (Approval Number: 1030, 1431 and 2121). Human synovial tissue was harvested from the supra-patella synovial membrane of patients with knee osteoarthritis during total knee arthroplasty [5]. The average age of 12 female donors was 64 years (Table I). Human synovial tissue was digested in a 3 mg/mL collagenase solution (Roche Diagnostics) in a modified α-minimal essential medium (αMEM; Invitrogen) at 37°C for 3 h. Digested cells were filtered through a 70-μm-pore nylon filter (Becton Dickinson), and the remaining tissues were discarded. The digested cells were plated in a 150 cm^2 culture dish (Nalgene Nunc International) in complete culture medium: αMEM containing 10% fetal bovine serum (Invitrogen), 100 units/mL penicillin (Invitrogen), 100 μg/mL streptomycin (Invitrogen) and 250 ng/mL amphotericin B (Invitrogen). Cells were incubated at 37°C with 5% humidified CO_2.

Cell proliferation ability

The medium was changed to remove non-adherent cells 1 day after harvest. The remaining cells were cultured for 14 days as passage 0. Adherent nucleated cells were then plated at 10^4 cells per 60 cm^2 dish, and cultured for 14 days in the absence or presence of IL-1β at 10^{-13}, 10^{-12}, 10^{-11}, 10^{-10}, 10^{-9} or 10^{-8} g/mL [22] as passage 1 (Figure 1A). After 14 days, four dishes were stained with 0.5% crystal violet (Wako) in 4% paraformaldehyde for 10 min and observed with a microscope. The cells in the remaining six dishes were

Table I. Patient characteristics.

Donor number.	1	2	3	4	5	6	7	8	9	10	11	12
Cell proliferation and cartilage differentiation potential of synovial MSCs pretreated with IL-1β.												
7 groups proliferation (Figure 1)	✔	✔	✔									
7 groups Chondrogenesis (Figure 2)	✔			✔	✔							
Examination of the optimal concentration with IL-1β using four donors.												
3 groups proliferation (Figure 3)						✔	✔	✔	✔			
3 groups Chondrogenesis (Figure 3)						✔	✔	✔	✔			
3 groups FACS (Figure 4)						✔				✔	✔	✔
Why is pretreatment with IL-1β affects synovial MSCs?												
3 groups Cytokine array (Figure 5)						✔	✔	✔	✔			

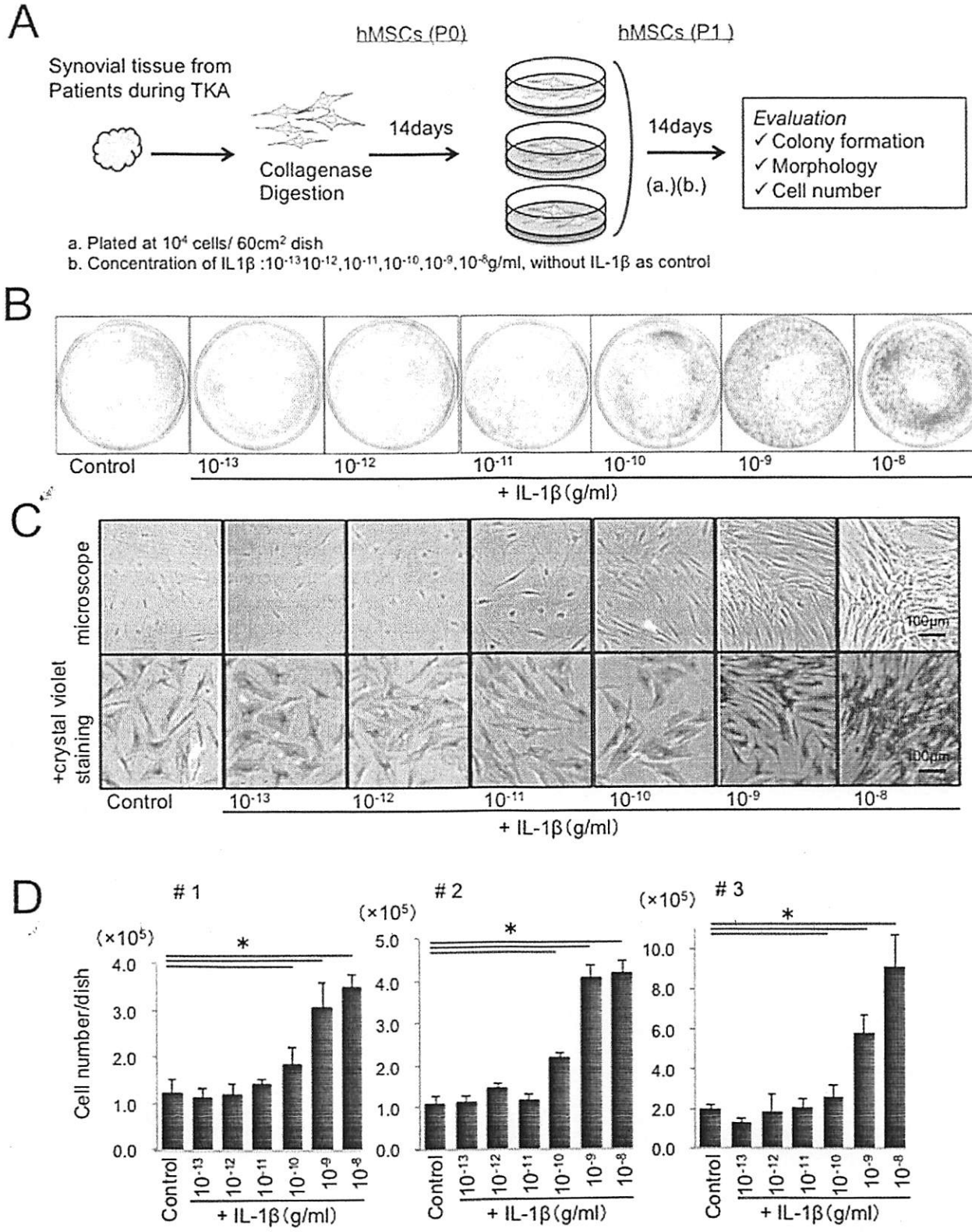

Figure 1. Cell proliferation of synovial human MSCs (hMSCs) pretreated with IL-1β. (A) MSCs were divided into seven groups: one untreated control group and six groups pretreated with varying concentrations of IL-1β. (B) Representative cell colonies stained with crystal violet. Staining of the colonies was enhanced with the IL-1β pretreatment in a dose-dependent manner. (C) Microscopic observation. The cell morphology appeared more elongated and less spindle shaped with increased IL-1β dosage. (D) Graph of the mean value of the number of cells per dish in seven groups. Bar shows the mean ± SD (three donors, n = 6, $^*P < 0.01$).

harvested with 0.25% trypsin and 1 mmol/L ethylenediaminetetraacetic acid (EDTA; Invitrogen), and the number of isolated cells was counted manually by a hemocytometer. To secure the reproducibility of the manual counting of the cells, the number of the cells was counted in quadruplicate and average number was employed for all the experiments in this study. Number of cells was compared on the mean value of each group.

Chondrogenesis

In a manner similar to the colony-forming cell culture of synovial cells, nucleated cells were plated at 3×10^4 cells/ 150 cm^2 dish (Figure 2A) and cultured for 14 days in the absence or presence of IL-1β at 10^{-13}, 10^{-12}, 10^{-11}, 10^{-10}, 10^{-9} and 10^{-8} g/mL for 14 days as passage 1. Two and a half million synovial MSCs isolated with 0.25% trypsin and 1 mmol/L EDTA (Invitrogen) were placed in a 15-mL polypropylene tube (Becton Dickinson) and then centrifuged at 1500 rpm for 10 min. The pellet formation culture was performed at 37°C with 5% CO_2 in 400 μL chondrogenesis medium that contained 1000 ng/mL bone morphogenetic protein (BMP)-7 (StrykerBiotech) in high-glucose Dulbecco's Modified Eagle's Medium (Invitrogen) supplemented with 10 ng/mL transforming growth factor-β 3 (R&D Systems, Minneapolis), 100 nmol/L dexamethasone, 50 ng/mL ascorbate-2-phosphate, 40 mg/mL proline, 100 μg/mL pyruvate (Sigma-Aldrich) and 50 mg/mL ITS + Premix (Becton Dickinson) excluding IL-1β (Figure 2A). The medium was replaced every 3–4 days for 21 days. For microscopy, the pellets were embedded in paraffin, cut into 5-μm sections and stained with toluidine blue and safranin O.

Assay for sulfated glycosaminoglycan content of pellets

The glycosaminoglycan (GAG) contents in each pellet were measured by the Blyscan assay kit (Biocolor) according to manufacturer's instructions. Briefly, after 21 days of culture, each pellet was digested for 24 h at 60°C in papain buffer (200 mg/mL papain; Sigma-Aldrich) in 50 mmol/L phosphate buffer containing 1 mol/L NaCl, 5 mmol/L cysteine HCl and 1 mmol/L EDTA), and sulfated GAG (sGAG) concentration in the solution was measured using a spectrophotometer (620 nm) [23].

Adipogenesis

Nucleated cells were plated at 10^4 cells per 60 cm^2 dish and cultured for 14 days in the absence or presence of IL-1β at 10^{-10} or 10^{-9} g/mL as passage 1. Synovial MSCs dispersed by using 0.25% trypsin and 1 mmol/L EDTA (Invitrogen) were placed at 10^2 cells per six-well plate in αMEM. After 24 h, the medium was washed with phosphate-buffered saline (PBS) and then switched to an adipogenesis medium that consisted of complete medium supplemented with 100 nmol/L dexamethasone, 0.5 mmol/L isobutylmethylxanthine (Sigma-Aldrich) and 50 nmol/L indomethacin (Wako). The cells were cultured for an additional 21 days. The dishes were then fixed in 10% formalin and stained with fresh oil red O (Sigma-Aldrich) solution for adipogenesis evaluation [24,25]. After taking the pictures, oil red O dye was eluted in 60% isopropanol solution, and the absorbance at 510 nm was measured using a spectrometer [26].

Calcification

As with the adipogenesis studies, synovial MSCs at passage 1 were cultured at 10^2 cells per six-well plate. After 24 h, the cells were washed with PBS and then cultured in a calcification medium consisting of complete medium supplemented with 1 nmol/L dexamethasone (Sigma-Aldrich), 20 mmol/L b-glycerol phosphate (Wako) and 50 mg/mL ascorbate-2-phosphate. The cells were cultured for an additional 21 days. The dishes were then fixed in 10% formalin and stained with 40 mmol/L alizarin red solution (pH 4.1; Sigma-Aldrich) for the calcification evaluation [24,25]. After taking pictures, alizarin red dye was eluted in 10% formic acid solution, and the absorbance at 409 nm was measured using a spectrometer [27].

Epitope profile

Nucleated cells were plated at 10^4 cells per 60 cm^2 dish and cultured for 14 days in the absence or presence of IL-1β at 10^{-10} or 10^{-9} g/mL as passage 1. Ten million cells were suspended in 50 μL fluorescence-activated cell sorting (FACS) staining buffer (0.2% fetal bovine serum and 5 mmol/L EDTA in PBS). After incubation for 30 min with antibodies against surface antigens at 4°C, the cells were washed with PBS and resuspended in 0.7 mL FACS staining buffer for flow cytometric analysis. Fluorescein isothiocyanate (FITC), phycoerythrin (PE), peridinin chlorophyll protein Cy5.5 (PerCP-Cy5.5) or Alexa Fluor 647-coupled antibodies against CD105, CD73, CD90, CD44, CD45, CD34, CD140b, SSEA3, CD271, CD31, CD140a and CD146 (Becton Dickinson) were used for the flow cytometric analysis. For isotype controls, FITC-, PE-, PerCP-Cy5.5- or Alexa Fluor 647-coupled nonspecific rat immunoglobulin G (IgG; Becton Dickinson) was substituted for the primary antibody. Cell fluorescence was evaluated using a FACSVerse instrument (BD Biosciences). The data were analyzed using FACSuite software (BD Biosciences).