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Pretreatment of 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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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)						✔	✔	✔	✔			