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Lack of anti-inflammatory and anti-catabolic effects on basal inflamed osteoarthritic chondrocytes or synoviocytes by adipose stem cell-conditioned medium

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SUMMARY

Objective: To define whether good manufacturing practice (GMP)-clinical grade adipose stem cell (ASC)-derived conditioned medium (CM) is as effective as GMP-ASC in modulating inflammatory and catabolic factors released by both osteoarthritis (OA) chondrocytes or synoviocytes.
Methods: OA chondrocytes and synoviocytes were treated with ASC-CM or co-cultured with ASC. Inflammatory factors (IL6, CXCL1/GROα,CXCL8/IL8, CCL2/MCP-1, CCL3/MIP-1α and CCL5/RANTES) and proteinases, such as metalloproteinase (MMP13), a disintegrin and metalloproteinase with thrombospondin motifs (ADAMTS4, ADAMTS5) and their tissue metalloproteinase inhibitors (TIMP1, TIMP3) were evaluated by qRT-PCR or immunoassays. The involvement of prostaglandin E2 (PGE2) was also analyzed.
Results: Most ASC-CM ratios tested did not decrease IL6, CCL2/MCP-1, CCL3/MIP1-α, CCL5/RANTES on basal inflamed chondrocytes or synoviocytes in contrast to what we found using ASC in co-culture. CXCL8/IL8 and CXCL1/GROα were not decreased by ASC-CM on synoviocytes but were only partially reduced on chondrocytes. Moreover, ASC-CM was less efficient both on basal inflamed OA chondrocytes and synoviocytes in reducing proteinases, such as MMP13, ADAMTS4, ADAMTS5 and increasing TIMP1 and TIMP3 compared to ASC in co-culture. The different ratios of ASC-CM contain lower amounts of PGE2 which were not sufficient to reduce inflammatory factors.
Conclusions: These data show that ASC-CM has a limited ability to decrease inflammatory and proteinases factors produced by OA chondrocytes or synoviocytes. ASC-CM is not sufficient to recapitulate the beneficial effect demonstrated using ASC in co-culture with inflamed OA chondrocytes and synoviocytes and shows that their use in clinical trials is fundamental to counteract OA progression.

Introduction

Mesenchymal stem cell (MSC)-based therapy is a new emerging clinical strategy that holds great promise for treating immune, hematological disorders, cardiovascular diseases, and cancer and for regenerative medicine[1–4].

MSC from adult donors obtained from different sources (bone marrow, adipose tissue etc.)[5,6] are considered to be among the most promising candidate cell types in regenerative medicine applied to rheumatic diseases such as osteoarthritis (OA)[7–9]. In particular, adipose stem cell (ASC) as well as MSC shows the minimal criteria

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provided by the International Society for cellular therapy for defining MSC, by displaying an immunophenotype positive for CD73, CD90, CD105, and negative for CD3, CD14, CD45, CD31[10]. Moreover, in contrast to MSC, ASC express CD34, which is lost during the early phase of culture[11]. They exert their function using different pathways, not yet completely understood, that contribute to augmenting tissue regeneration. Their effects are mainly due, at least in part, to the "trophic" function of ASCs, characterized by the production of a large amount of secreted factors, such as chemokines, cytokines and growth factors, which are able to influence the behavior of the other cells[12,13]. In particular, it has been shown that ASC-secreted factors exert immunomodulatory, anti-inflammatory, anti-apoptotic, pro-angiogenic, proliferative, or chemoattractive effects[13–16]. Early studies using MSCs injected into animal models of OA well documented that they were able to orchestrate the differentiation process together with differentiated or undifferentiated resident cells for functional tissue restoration[17–19]. Moreover, in osteoarticular diseases, it has been shown that the evolution of OA in animal models might be prevented by intra-articular injections of bone marrow MSC or their delivery on a degradable hyaluronan scaffold[20–22]. Recent studies have confirmed that ASC injected intra-articularly in OA mice or rabbit models also showed anti-inflammatory and chondroprotective effects[23,24].

A body of evidence suggests that pro-inflammatory cytokines (IL1β, TNFα, IL6), chemokines (CXCL1/GROα, CXCL8/IL8, CCL2/MCP-1, CCL5/RANTES), metalloproteinases (MMP1, 3, 13) disintegrins and metalloproteinase with thrombospondin motifs (ADAMTS4, ADAMTS5) produced by different cell types of the joint tissues, are released in the synovial fluids of OA patients and contribute to the disruption of the balance between anabolism and catabolism[25–27], thereby causing progressive destruction of articular cartilage, changes to the synovium, subchondral bone, degeneration of ligaments, and menisci, and hypertrophy of the joint capsule[28].

New alternative therapies to counteract OA progression and restore joint tissue features are needed. The clinical use of these cells requires ASC production under good manufacturing practice (GMP) as well as the evaluation of their biosafety and purity[29,30]. We previously showed that GMP-clinical grade ASC *in vitro* were able to exert anti-inflammatory effects on chondrocytes and synoviocytes[31] and protect chondrocytes from degeneration[32], a typical feature associated with OA.

Cell-free-based therapy might overcome the limitations and risks associated with the cell-therapy approach. However, comparative studies are necessary to establish the potentiality of using conditioned medium (CM) from ASCs instead of ASC in OA cell-based treatment.

Knowing that ASC preparation is influenced by medium factors used[32], to gain new insight into this issue, we evaluated the effects of clinical grade GMP-ASC-CM on both OA chondrocytes and synoviocytes and compared these results with our previously published data. We focused our analysis on the main catabolic and inflammatory factors involved in the progression of OA. Our results clearly show that ASC-CM was less effective than ASC in reducing inflammatory and catabolic factors secretion by both on OA inflamed chondrocytes and synoviocytes.

Method

Specimens

Articular cartilage and synovia were harvested from 25 OA patients (14 women and 11 men; mean age: 69 ± 10.5 years; body mass index (BMI): 28 ± 4.04; disease duration: 5 ± 3.08 years, Kellgren–Lawrence: 3/4)[33] undergoing total knee replacement. Subcutaneous abdominal fat was obtained from 10 healthy patients undergoing liposuction. The study was approved by the local ethical committee and all patients provided their informed consent (Protocol number 15274).

Isolation of human ASC and ASC-CM preparation

Clinical grade ASCs were isolated from subcutaneous abdominal fat according to GMP[30] grown in αMEM supplemented with platelet lysate (PLP) and characterized for the CD markers CD14, CD34, CD45, CD73, CD90 (BD Pharmingen, San Jose, CA, USA) and CD13 (eBioscience, San Diego, CA, USA) as we previously described[30,31] (data not shown).

ASC-CM was prepared by collecting the supernatant, on day 2 and day 7, of ASC seeded at a concentration of 100,000/well in Transwells® (0.4 μm pore size, Corning, Toledo, OH, USA) in DMEM with ascorbic acid (0.17 mmol/L), proline (0.35 mol/L) and sodium pyruvate (1 mol/L) (complete DMEM), previously defined[31], and stored at 4°C before use.

Chondrocyte and synoviocyte cultures

Chondrocytes and synoviocytes were isolated following a standardized procedure previously described[34,35] and used for the experiments at the first passage. Chondrocytes and synoviocytes were seeded in the lower chamber of a 6-well plate and both treated for 7 days (medium was changed on day 2) with ASC-CM or co-cultured with ASC in Transwells® in complete DMEM using a defined cell ratio (1:8) as we previously reported[31]. ASC-CM (obtained by pooling the supernatant of day 2 and 7 to have all factors produced by ASC during the co-culture experiments) was mixed with DMEM at three different ratios (DMEM:CM 75:25, 50:50, 25:75) and added to chondrocytes or synoviocytes seeded in a 6-well plate. Control cells were mono-cultures of ASC, chondrocytes and synoviocytes. The cells were harvested on day 7 for RT-qPCR analysis and supernatant stored at −80°C. Co-cultures of chondrocytes in micromasses were also tested to confirm the results in monolayers. Briefly, chondrocytes in micromass culture were inserted in the lower chamber of a 6-well plate, maintaining the same 1:8 cell ratio and time point, and co-cultured with ASC as previously described[31].

Real time RT-qPCR analysis

Total RNA was extracted from human ASC, chondrocyte and synoviocyte mono- and co-cultures, using RNA PURE reagent (EuroClone S.p.A., Pero, Italy) according to the manufacturer's instructions, and then treated with DNase I (DNA-free Kit, Ambion, Austin, TX, USA). Reverse transcription was performed using SuperScript VILO (Life Technology) reverse transcriptase and random hexamers, following the manufacturer's protocol.

Forward and reverse oligonucleotides for PCR amplification of IL6, CXCL8/IL8, ADAMTS4, ADAMTS5, TIMP1, TIMP3 and MMP13 are described in Table I. Real-time PCR was run in a LightCycler Instrument (Roche Molecular Biochemicals, Mannheim, Germany) using the SYBR Premix Ex Taq (TaKaRa Biomedicals, Tokyo, Japan) with the following protocol: initial activation of HotStarTaq DNA polymerase at 95°C for 10 min, 45 cycles of 95°C for 5 s and 60°C for 20 s. Amplification efficiency (E) of each amplicon was determined using 10-fold serial dilutions of positive control cDNAs and calculated from the slopes of the log input amounts (from 20 ng to 2 pg of cDNA) plotted vs the crossing point values, according to the formula: $E = 10^{-1/\text{slope}}$. All primer efficiencies were confirmed to be high (>90%) and comparable (Table I). For each target gene, mRNA levels were calculated, normalized to RPS9 according to the formula