Human mesenchymal stem cells as a tool for joint repair in rheumatoid arthritis

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ABSTRACT

Rheumatoid arthritis (RA) is characterised with chronic inflammatory synovitis and progressive joint. Because damaged and/or deformed joints cannot be repaired, a novel treatment strategy aimed at both anti-inflammation and bone regeneration is a prerequisite. Mesenchymal stem cells (MSCs) can be easily isolated from various organs and possess multipotent capacity and exhibit immunoregulatory properties. Using human MSC derived from bone marrow and adipose tissue, we have clarified the following novel findings in vitro. 1) MSCs differentiated into osteoblasts or osteocytes under osteoblast-conditioned medium including the inflammatory stimuli such as IL-1. 2) The combination of IL-6 and soluble IL-6 receptor induced differentiation of MSCs to chondrocyte. 3) MSCs produced osteoprotegerin and inhibited osteoclastogenesis. Furthermore, we developed a local delivery system of MSCs by using nano-fibre scaffold. MSCs seeded on nano-fibre scaffold suppressed arthritis and joint destruction by inhibiting systemic inflammatory reaction and immune response through the induction of regulatory T cells and subsequent reduction in the production of anti-type II collagen antibody in vivo. Thus, our data may serve as a new strategy for MSC-based therapy in inflammatory diseases and an alternative delivery method for the treatment of damaged joints in RA.

Introduction

Rheumatoid arthritis (RA) is a systemic autoimmune disease characterised with chronic inflammatory synovitis and progressive joint destruction that causes severe morbidity and mortality. Auto-reactive T cells and inflammatory cytokines such as TNF play a pivotal role in the pathogenesis of synovial inflammation and irreversible join destruction that was induced by the production of matrix metalloproteinase as well as the maturation and activation of osteoclasts. (1-3) The combined use of methotrexate, a synthetic disease-modifying anti-rheumatic drug (DMARD), and biologic DMARD targeting TNF has revolutionised treatment of RA. Clinical remission has recently become an achievable goal in many patients and rapid and appropriate induction of remission is prerequisite to halt joint destruction (1-5). However, once a joint is damaged and/or deformed, joints cannot be repaired. Therefore, a novel treatment strategy aimed at both anti-inflammation and bone regeneration is a prerequisite.

Mesenchymal stem cells (MSCs) can be easily isolated from various organs and possess multipotent capacity and exhibit immunoregulatory properties. Using human MSC derived from bone marrow, our *in vivo* and *in vitro* experiments have clarified the importance of MSC in suppressing inflammation and subsequent protecting articular cartilage and bone. In this review, a new strategy for MSC-based therapy and an alternative delivery method for the treatment of damaged joints in RA will be discussed mainly based on our findings.

Mesenchymal stem cells for rheumatoid arthritis

MSCs reside in the bone marrow, peripheral blood, adipose tissue, synovium and other mesodermal tissues and are easily isolated from these organs. Furthermore, MSCs are capable of differentiating into various mesenchymal lineages, including osteoblasts, chondrocytes and adipocytes (6). MSCs are also involved in self-renewal and self-repair processes in damaged/injured tissues and organs. Both short-term and long-term exposure to inflammatory stimuli such as cytokines often results in tissue damage. However,

such damage is often repaired mainly by cells of mesenchymal lineages that differentiate from MSCs, which either migrate from the bone marrow or are resident in various organs (7). In fact, several preclinical and clinical studies in diseases such as osteogenesis imperfecta and cartilage defect have already demonstrated the therapeutic potential of these cells (8, 9).

MSCs also possess multipotent capacity and exhibit anti-inflammatory properties, suggesting their usefulness in both organ transplantation and treatment of autoimmune diseases. The use of MSC has been reported to be safe and efficacious in graft-*versus*-host disease (GvHD), systemic lupus erythematosus (SLE), multiple sclerosis and many (10, 11). Because MSCs are also known to induce regulatory T cells (Treg), the dual function of immune regulation as well as tissue repair prompted us to consider MSC as a new treatment tool for RA.

Differentiation of mesenchymal stem cells to osteoblasts

Inflammation is involved in the processes of tissue repair. In fact, although several inflammatory cytokines enhance osteoclast differentiation, resulting in bone resorption followed by bone damage, the same cytokines also cause chronic enthesitis and excess abnormal bone formation, as seen in ankylosing spondylitis (12). The precise molecular effects of inflammatory cytokines in the induction of cells of mesenchymal lineages remain unclear. However, Augello *et al*. reported that a single intravenous injection of MSCs prevented the occurrence of severe and irreversible damage to bone and cartilage in experimental collagen-induced arthritis in mice (13). To clarify the molecular mechanism of differentiation of human MSCs into osteoblasts in inflamed tissue in RA, we assessed the molecular mechanism of differentiation of human MSCs into osteoblasts in the presence of inflammatory cytokines by focusing on Wnt molecules that play important roles during osteoblast differentiation (14).

Human MSCs were cultured in commercialised osteogenic induction medium with inflammatory cytokines for up to 10 days. Among the various cytokines tested, IL-1 induced differentiation of human MSCs into osteoblasts, which was confirmed by alkaline phosphatase (ALP) activity, expression of RUNX2 mRNA, and strong alizarin red S staining, indicating synthesis of bone matrix. Among various molecules of the Wnt family, Wnt-5a and receptor tyrosine kinase-like orphan receptor 2 (Ror2), a major receptor of Wnt-5a, were significantly induced in human MSCs by the stimulation with IL-1. Silencing of either Wnt-5a or Ror2 by small interfering RNA with 2 different sequences reduced ALP activity, RUNX2 expression, and alizarin red S staining of human MSCs induced by IL-1 (Fig. 1).

Thus, IL-1 effectively and rapidly induced human MSC differentiation into osteoblasts and mineralisation, mainly through the Wnt-5a/Ror2 pathway, suggesting a potential benefits of IL-1–treated human MSCs for the treatment of damaged bone as well as in the induction of self-renewal or self-repair of damaged tissue, including bone (14). We also found that adipose-derived stem cells (ADSCs), known for their multipotency, possess not only powerful angiogenic potential but also potential of differentiation into osteoblast-like cells by stimulation with IL-6 and soluble IL-6 receptor, involved in pathological processes in abnormal tissue calcification (15).

Inhibition of osteoclastogensis by mesenchymal stem cells

MSCs are known for their strong immunosuppressive functions *in vitro* by producing soluble mediators such as anti-inflammatory cytokines and might be useful as a therapeutic application in autoimmune diseases (16). Although the suppressive effects of MSCs on T cells, dendritic cells and NK cells are well known, the effect of MSCs on differentiation and function of osteoclasts, which play an important role in bone resorption leading to joint destruction in RA, remains unclear. We found that MSCs inhibited osteoclastogenesis by constitutive production of osteoprotegerin (OPG), a decoy receptor for RANKL, which blocks RANKL–RANK interaction essential for differentiation and activation of osteoclasts (17).

Human MSCs and peripheral blood mononuclear cells were cultured under cell-cell contact-free conditions in osteoclast induction medium. The number of osteoclast-like cells was decreased and expression of cathepsin K and NF-ATc1, specific markers for osteoclasts, was downregulated by the addition of either MSCs or a conditioned medium obtained from MSCs. OPG was constitutively produced by MSCs and inhibited osteoclastogenesis. Osteoclast differentiation was partially recovered by the treatment with either anti-OPG antibody or OPG small interfering RNA. Moreover, bone-resorbing activity of osteoclast-like cells was partially recovered by addition of anti-OPG antibody into the conditioned medium. Therefore, we conclude that human MSCs inhibit osteoclastogenesis without cell-cell contact, partly due to secretion of OPG and that MSCs producing OPG and other inhibitory factors are also beneficial for the inhibition of bone erosion in RA.

Differentiation of mesenchymal stem cells to chondrocytes

Articular cartilage is a structurally unique tissue, lacking blood, lymph vessels, and nerves, and it is considered to be in a low-nutrient, low-oxygen environment. Also, an inflammatory milieu breaks down the cartilage matrix and induces chondrocyte apoptosis, which leads to irreversible defects in the cartilage and currently difficult to repair in cartilage degenerative diseases, including RA and osteoarthritis (OA). MSCs are expected to be a new tool for cartilage repair because they are present in the cartilage and can differentiate into chondrocytes. Although clinical trials using MSCs that can differentiate into chondrocytes for patients with cartilage defects have already begun, its efficacy and repair mechanisms remain unknown. However, recent studies revealed that cartilage contains chondrogenic progenitor cells with MSC-like character-

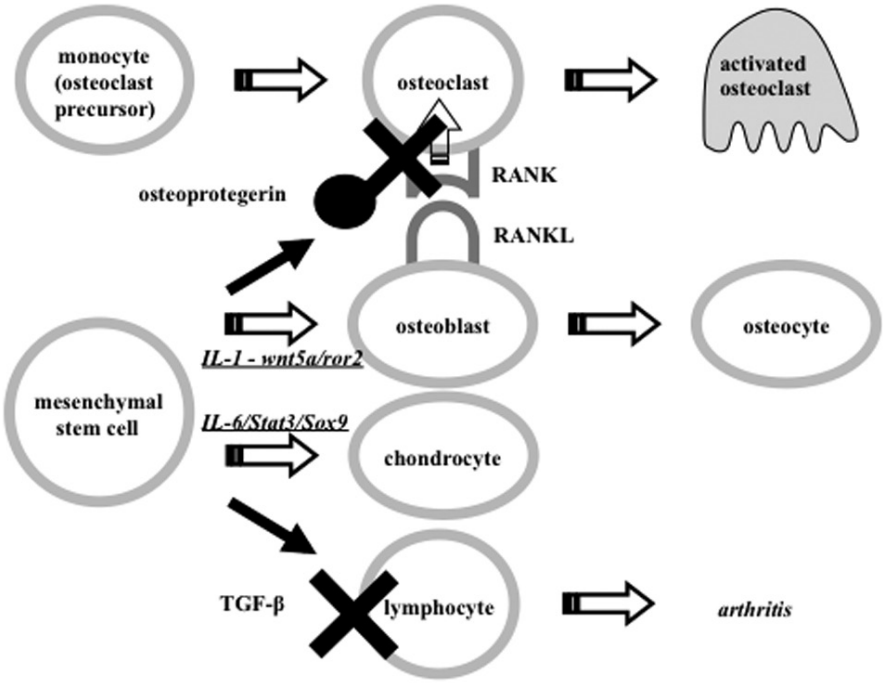

Fig. 1. Multipotent capacity of mesenchymal stem cells (MSCs).
MSCs differentiated into osteoblasts or osteocytes and chondrocyte, but MSCs inhibited osteoclastogenesis by producing osteoprotegerin.

istics, suggesting that the homeostasis of cartilage is maintained through differentiation from chondro-progenitor cells into chondrocytes, which allows the turnover of cartilage (18, 19, 20, 21). MSC produces multiple factors in a large amount, among them IL-6 is one of the major humeral factors from MSCs (22). We have investigated the involvement of IL-6-mediated signalling in MSC differentiation into chondrocytes (23).

Addition of IL-6 and soluble IL-6 receptor (sIL-6R) to human MSCs in chondrogenic culture medium resulted in increases in cartilage marker gene expression and cartilage matrix accumulation such as type II collagen, aggrecan and type X collagen. Phosphorylation of the master transcription factor SOX9 was enhanced upon the addition of IL-6 and sIL-6R to MSCs. The expression of the IL-6R was significantly increased, accompanied by increased phosphorylation and expression of STAT-3. However, STAT-3 knockdown suppressed chondrogenic differentiation. Thus, IL-6 and sIL-6R promote chondrogenic differentiation of MSCs through the phosphorylation of STAT-3/SOX9 signalling, during homeostasis and self-repair of cartilage in pathological processes.

Inhibition of joint destruction by mesenchymal stem cells in arthritis models

Clinical application of MSC for treatment of rheumatic animal models and patients remains conflicting and treatments usually require large cell number, which is processed through numerous subcultures that could enhance the appearance of various cytogenetic abnormalities. The route of delivery is another key to consider MSC as a therapeutic tool. MSCs originating from bone marrow lose their homing ability after a few hours *in vitro* culture (24). Thus, the migration and localisation of MSCs to target lesions after systemic injection such as intravenous (IV) or intraperitoneal (IP) remain unknown. Furthermore, unlike other promising results in patients with GvHD or SLE, previous studies reported the low efficacy of IV or intra-articular (IA) administration into arthritis model or patients with RA, indicating that the relative unique and complex structure of the joints may be a challenge for MSCs to migrate during arthritis (25, 26). To establish MSC as a realistic treatment tool for RA, we have developed a new delivery method that would force MSCs to reside at the implanted site maintaining their dual function.

For appropriate delivery of MSC into

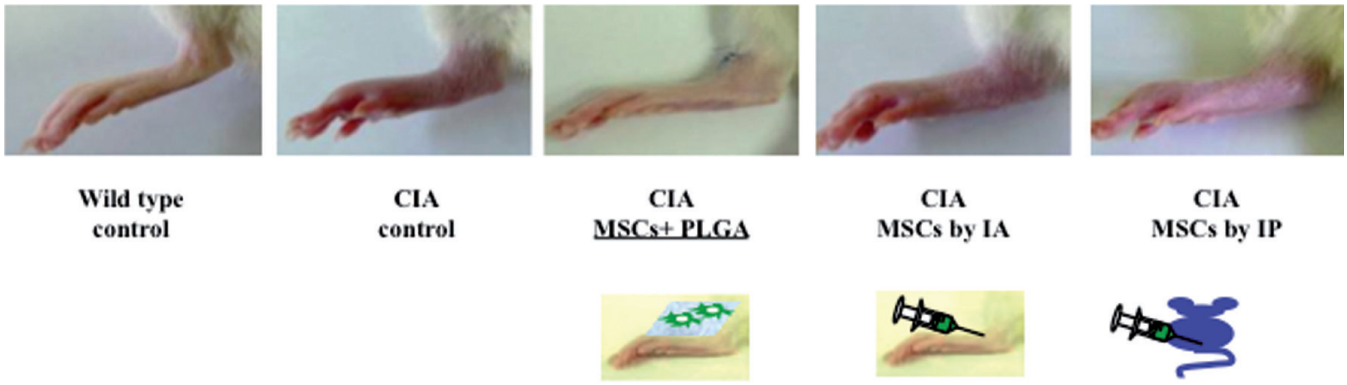

Fig. 2. Implantation of MSCs with nano-fibre sheet suppressed hind paw swelling in CIA.
MSCs seeded on nano-fibre scaffold, but not intra-articular or intraperitoneal administration, suppressed arthritis and joint destruction by inhibiting systemic inflammatory reaction and immune response *in vivo*.

the inflamed lesion, we developed a local delivery system of MSC using nano-fibre poly-lactic-co-glycolic acid (PLGA) (nano-fibre) as a scaffold (Fig. 2). PLGA possesses the following advantages; controlled biodegradability and low immunogenicity, efficacy as a carrier in drug delivery system, usefulness as a scaffold for regeneration of bone defect and supporting effects of cell residence and cell differentiation (27, 28, 29). For the treatment of murine autoimmune models, MSCs are administered systemically with at least 5×10^6 cells, leading to limitation in clinical usage. However, we observed that a single inoculation of a small number of MSC (1×10^5 cells) seeded on nano-fibre scaffold (nano-hMSC) into ankles of collagen-induced arthritis (CIA) rats significantly suppressed arthritis and bone destruction (30). Interestingly, nano-hMSC implantation into the ankles prevented arthritis in the front paws as well as ankles, although hMSC were detectable on nano-fibre only within the initial three days *in vitro*. Furthermore, our *in vivo* tracing studies after inoculation of MSC transfected with GFP plasmid DNA showed that MSCs remained within the scaffold and did not migrate to other organs.

Inhibition of immunological abnormalities by msenchymal stem cells in arthritis models

MSCs have strong immunosuppressive functions caused by soluble mediators such as anti-inflammatory cytokines, and a therapeutic application in GvHD and SLE has been expected (10, 11). We have proposed that the effects of MSCs on scaffold were due to suppression of the systemic inflammatory reaction and immune response. *Ex vivo* experiments showed that nano-hMSC implantation in CIA rats significant suppressed not only proliferation and cytokines production of T cells but also serum levels of anti-type II collagen (CII) IgG (30). Conditioned medium of *in vitro* culture of MSCs on the scaffold contained high levels of TGF-β1 and *in vitro* co-culture of MSC with $CD4^+$ T cells isolated from the RA patients induced regulatory T cells (Treg). It was also reported that *in vivo* administration of MSC increased inducible Treg (iTreg) in the knee joints and draining lymph nodes (26). Taken together, the following cascade of events can be proposed; nano-hMSC locally produced TGF-β1, TGF-β1 induced iTreg in lymph nodes, iTreg inhibited T cell proliferation, cytokine production and anti-CII IgG production from B cells, which suppressed the initial phase of CIA resulting in prevention of arthritis and joint destruction in the front paws as well as ankles.

Conclusion

Taken together, regeneration of the damaged joints of patients with RA could potentially be induced by MSCs, based on the following findings. First, MSCs can differentiate into osteoblasts and chondrocytes even in the presence of inflammatory cytokines such as IL-1 and IL-6, which are abundantly present in the inflamed synovium. Second, MSCs produce high amounts of OPG, which efficiently suppresses RANKL/RANK pathway–mediated osteoclastogenesis. Third, human MSCs have a strong immunosuppressive potential by inducing TGF-β1 and Treg, suppressing T cell proliferation and reducing anti-CII antibody production *in vivo*. Furthermore, we developed a local delivery system of MSCs by using nano-fibre scaffold in CIA rats. Both the *in vivo* and *in vitro* experiments suggest the importance of MSC seeded on nano-fibre scaffold residing at the local site of inflammation in the suppressing of inflammation and subsequent protecting of articular cartilage and bone. Our data may serve as a new and promising strategy for MSC-based therapy in inflammatory diseases and an alternative delivery method for the treatment of joint destruction, especially shown in patients with RA. However, further studies are necessary to establish the best-case scenario to ascertain the concept of MSCs use in RA treatment.

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