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REVIEW

Stem cells for repair of cartilage and bone: the next challenge in osteoarthritis and rheumatoid arthritis

C Jorgensen, D Noel, F Apparailly, J Sany

Service d'Immuno-Rhumatologie, Hôpital Lapeyronie, 34295 Montpellier cedex 5, France
C Jorgensen
J Sany

INSERM U475, 99 rue Puech Villa, 34197 Montpellier cedex 5, France
C Jorgensen
D Noel
F Apparailly
J Sany

Correspondence to:
Dr Jorgensen, Service d'Immuno-Rhumatologie, CHU Lapeyronie, 371 avenue du Doyen Gaston Giraud, 34295 Montpellier cedex 5, France
jorgens@montp.inserm.fr

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Over the last few years, immunotherapy targeting proinflammatory cytokines has been the main goal of research into rheumatoid arthritis (RA), and recently the new anti-(tumour necrosis factor) blocking agents have dramatically improved the course of the disease by stabilising the symptoms.[1] However, this treatment has no effect on regeneration of articular cartilage damaged during the inflammatory process. The next challenge is thus to ensure cartilage repair through cell therapy and tissue engineering (fig 1). Tissue in the body is replaced by two main mechanisms. One is self repair by fully differentiated cells (healing), and the second is replacement with newly differentiated cells derived from stem cells. Recently, the regenerative potential of mesenchymal stem cells (MSCs) has been under intense investigation because of their ability for self renewal and differentiation to reconstitute muscle, cartilage, or bone.[2]

Articular cartilage is a complex tissue consisting of cartilage matrix and the chondrocytes that have produced the matrix during development. The matrix is composed of collagens and proteoglycan aggregates (aggrecan) which provide the architectural structure and biomechanical strength of the tissue. During RA pathogenesis, proinflammatory cytokines present in the rheumatoid synovial fluid activate metalloproteinase (MMP) secretion by chondrocytes and increase pericellular matrix degradation of these cells. Some of them (interleukin 1β and 6 (IL1β and IL6)) even display the synergistic ability to decrease aggrecan chondral synthesis and hyaluronan/aggrecan ratio in the extracellular matrix.[3 4] However, proteoglycan depletion may be partially inhibited by IL1 receptor antagonist.[5] Cartilage and bone degeneration in RA is also the result of the action of MMPs secreted into the synovial fluid by the synoviocytes. MMPs constitute a family of 18 endopeptidases, the secretion of which is stimulated by cytokines of the IL6 family (IL11, IL6, oncostatin M) through a protein called the extracellular matrix metalloproteinase inducer. This 58 kDa glycoprotein is expressed by activated monocytes and initiates the MMP cascade.[6] Simultaneously, serine proteases are released by polymorphonuclear cells present in the inflamed joints. Joint destruction in RA thus results from an imbalance between the activity of proteases and their inhibitors which are also released: tissue inhibitors of metalloproteinase for MMPs, and secretory leucocyte proteinase inhibitor for the serine protease.[7] Cartilage
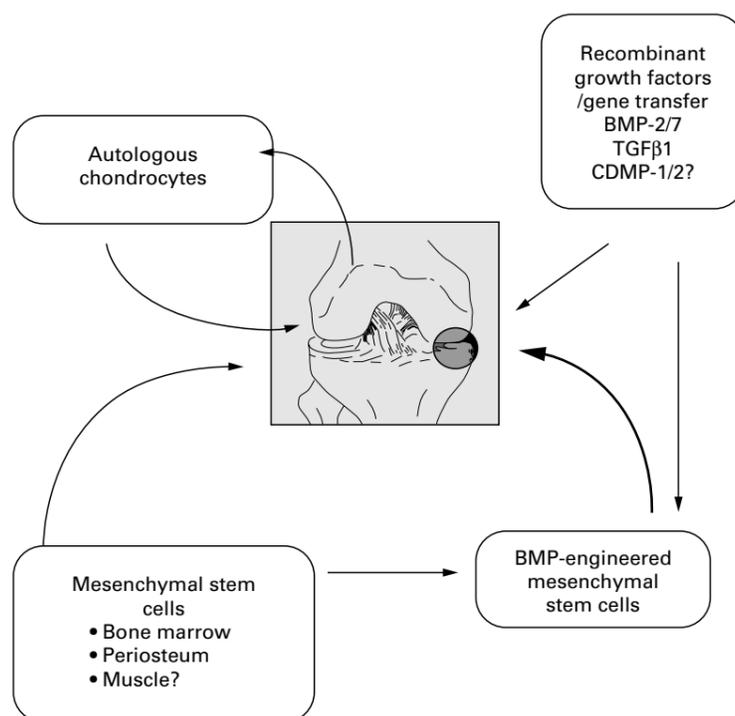

Figure 1   Different strategies for cartilage repair.

degradation is also inhibited by other factors: either anti-inflammatory cytokines such as IL10, which act indirectly by counteracting the effects of proinflammatory cytokines, or growth factors of the transforming growth factor β (TGFβ) family (TGFβ1 and bone morphogenetic protein (BMP)-2), which stimulate synthesis of collagen type II and proteoglycan by chondral cells.[8] These chondrocyte stimulating factors, in particular BMP-2, are secreted by synovial cells in the presence of IL1 in a dose dependent manner.[9] Thus some of the factors of cartilage healing are present in arthritic joints, but probably not in sufficient amount to inhibit the catabolic activities of proinflammatory cytokines.

Growth factors for cartilage repair

The TGFβ superfamily consists of more than 40 polypeptide growth factors which share high homology with seven conserved cysteine residues in their C-terminal region.[10] These peptides bind to a cell surface receptor complex that activates the Smad proteins. The Smads then assemble multisubunit complexes in the nucleus, where they act as transcription factors.[11] TGFβ related molecules or BMPs regulate the morphogenetic events during embryonic development and are important signalling molecules during the development of the skeleton in vertebrates.[12] BMP is also involved in bone healing as well as in cartilage metabolism in adults. After bone fracture, healing proceeds through endogenous cartilage formation followed by blood vessel invasion delivering to the wound both chondroclasts and osteoblasts, which are responsible for resorption of cartilage and its replacement by bone (endochondral ossification). Therefore the use of growth factors that are able to stimulate regeneration of depleted cartilage could be of therapeutic value.

MORPHOGENETIC PROTEINS FOR BONE REPAIR
Bone healing may be achieved through local implantation of a biodegradable matrix impregnated with recombinant human BMP-2 (rhBMP-2), as shown in a 5 mm femoral defect created in Lewis rats.[13] By week 12, 80% of the union was achieved with good mechanical properties compared with 38% when a bone graft was used or 47% when bone marrow alone was used. This formation of new bone is due to the recruitment of bone marrow MSCs induced by the chemoattractant properties of BMP-2. In addition, BMP-2 has proliferative and osteocyte differentiating properties.[14] Pluripotent stem cells may also be recruited from the muscle, as suggested by endochondral bone formation after intramuscular injection of an adenoviral vector expressing human BMP-2 in nude rats.[15] In these experiments, histological observations disclosed the presence of chondrocytes in the muscle by day 9, which then secreted a cartilaginous matrix on day 12. Four days later, osteoblasts could be observed, and mineralisation of the matrix occurred. Bone had undergone remodelling three months later, and showed evidence of a mature stroma heavily populated with cells.

MORPHOGENETIC PROTEINS FOR CARTILAGE REPAIR
In the joint, BMP-2 induces synthesis of extracellular matrix by chondrocytes and therefore cartilage growth. Injection of rhBMP-2 into the joint induced proteoglycan synthesis in a dose dependent manner, up to three times the initial value.[16] Compared with TGFβ1, human BMP-2 stimulated proteoglycan synthesis more efficiently, but its biological effect was more time restricted. In the same way, implantation of collagen sponges impregnated with rhBMP-2 into osteochondral defects of rabbit knees enabled regeneration of the impaired tissue after four weeks.[17] Safranin O and collagen type II staining showed the presence of histologically normal cartilage at 24 weeks. However, four of the 12 defects showed a persistent gap at the margins of the surrounding host cartilage, and the regenerated tissue remained 70% thinner and irregular at its surface. These studies did not evaluate the mechanical properties of the treated joints, and long term follow up (after 24 weeks) results were not available. Finally, cartilage repair in induced arthritis in rabbit was not evaluated. Thus the importance of inflammation has been shown in experiments with arthritic mice by rhBMP-2 injection into joints unable to induce proteoglycan synthesis. This study shows that stimulation of proteoglycan synthesis with rhBMP-2 did not inhibit IL1α induced proteoglycan depletion.[18]

OTHER CARTILAGE MORPHOGENIC FACTORS
In contrast with BMP-2, BMP-7 or osteogenic protein (OP-1) was effective in restoring proteoglycan synthesis by chondrocytes in culture despite the presence of IL1β.[19] It should, however, be noted that persistent stimulation with BMP-2 or OP-1 could induce bone formation. Other members of the TGFβ family, such as CDMP-1 or CDMP-2 (GDF-5 and GDF-6 respectively) seem to be more specific differentiation factors of cartilaginous tissue and could be useful for cartilage repair without inducing further ossification of the regenerative tissue.[20] Several additional factors involved in chondrogenesis during embryogenesis have also been identified. Of these, Indian Hedgehog, parathyroid hormone related protein, fibroblast growth factor, insulin-like growth factor, and Noggin regulate chondrogenesis. The nuclear factor of activated T cells (NFATp) is critical for cartilage morphogenesis in the adult animal and controls differentiation of MSCs into cartilage as shown in NFATp−/− mice, which develop ectopic formations of cartilage and show extensive destruction of peripheral joints after 6 months of age.[21] However, to obtain effective cartilage repair, chondral specific factors have to be combined with cell therapy.

Cells for cartilage repair

To obtain cartilage repair in arthritic joints, both downregulation of inflammatory cytokines and introduction of cartilage-producing cells must be achieved. In contrast, cartilage injuries caused by trauma can be

managed by allogenic cartilage transplantation or implantation of differentiated chondrocytes into the cartilage defect.

AUTOLOGOUS CHONDROCYTES

The first approach uses osteochondral shell allograft resurfacing of the knee, with 76% good outcome with a 75 month follow up.[22] The second approach, used in a study by Brittberg *et al*,[23] is autologous chondrocyte transplantation into the articular surface of injured knee joints. A clinical benefit was obtained in 14 out of 16 patients, and normal cartilage was observed after a two year follow up. However, in this clinical trial, the patients were less than 50 years of age with presumably healthy chondrocytes. Moreover, in the therapeutic procedure, a periosteal flap was sutured over the cartilage defect. As this tissue is rich in osteoprogenitor cells, participation of stem cells in the repair pathway cannot be excluded. This method will probably not be applicable in chronic inflammatory diseases such as RA, in which injured chondrocytes are often observed in the cartilage. Chondrocytes present in inflamed joints lose their differentiation, and up to 3% of the cells undergo apoptosis.[24]

BONE MARROW

Another strategy uses autologous cells that can differentiate into chondrocytes. These cartilage potent stem cells may arise from synovial tissue or migrate from the bone marrow. In fact, formation of new cartilage was observed in synovial explants cultured with TGFβ1 in either agarose or aggregate culture.[25] Without TGFβ1, chondrocyte differentiation was not observed, suggesting the need for continuous TGFβ1 secretion. Total bone marrow transplantation has also been used. Patients with osteogenesis imperfecta (a genetic disorder caused by a mutation in the type I collagen gene and characterised by generalised osteopenia) have been treated by systemic injection of allogeneic bone marrow. The treatment resulted in osteoblast engraftment (1.5–2% of donor cells) at three months and an increase in total body bone mineral content and skeletal growth.[26] Total bone marrow has also been used to fill cartilage defects experimentally created in the distal femoral condyle of New Zealand White rabbits. In these experiments, six weeks after transplantation, cartilaginous cells were observed in the defect, which were then rapidly replaced by bone and covered by a fibrous layer at the top of the defect.[27] By 12 weeks, bone had completely filled the defect, and there was no cartilage left. These results prompted a search for autologous pluripotent stem cells in bone marrow with the ability to differentiate into chondrocytes and their possible use for regeneration of tissue of mesenchymal origin.

MSCS FOR BONE REPAIR

MSCs are pluripotent cells present in the bone marrow in low quantity (1 out of 10^4–10^5 mononuclear cells), which are capable of differentiating into chondrocytes, osteocytes, myocytes, and adipocytes.[28] These stem cells support the growth of haematopoietic progenitors by secreting a number of haematopoietic cytokines (GM-C SF, IL6, IL7, IL8, IL11) and stem cell factors such as SDF-1. They are distinct from haematopoietic stem cells as they are not progenitors for the haematopoietic lineages and they do not express CD34, CD45, CD14 or T or B cell markers.[29]

MSCs can be used in two different therapeutic strategies. The first uses MSCs as a cell factory for protein delivery by introducing ex vivo the gene coding for a secreted protein and reimplanting the cells back into the animal. In experimental SCID/hu models, MSCs engineered to produce IL3 were seeded into ceramic cubes which were then subcutaneously implanted into mice. MSC differentiation inside the cubes resulted in bone formation, and human IL3 was detected in the systemic circulation for at least 12 weeks.[30] Similarly, implantation of MSCs genetically engineered to express soluble tumour necrosis factor receptor II into NOD/SCID mice resulted in detection of the cytokine in the serum, and its functionality was shown by decreased serum levels of mouse tumour necrosis factor α.[31]

The second strategy uses MSCs as pluripotent cells which will differentiate in vivo to repair non-functional tissue or generate new tissue.[32] This approach was successfully used in a breast cancer phase I trial for patients receiving high dose chemotherapy. Rapid haematopoietic recovery was obtained after co-infusion of autologous expended human MSCs and peripheral blood progenitor cells.[33] This strategy should be particularly useful for the repair of bone and cartilage in articular pathologies and fracture healing. Primary bone marrow cells were not able to heal femoral defects after two months whereas engineered cells expressing BMP-2 induced complete bone fusion in 22 out of 24 treated animals.[34] Moreover, BMP-producing cells induced robust trabecular bone, compared with defective bone when injected with recombinant BMP-2. In Lewis rats, implantation of bone marrow in polylactic acid/polyglycolic acid carrier resulted in less than 50% of bone fusion by 12 weeks, whereas rhBMP-2 and bone marrow together achieved 100% union as early as six weeks.[13] In rabbit, Boden *et al*[35] did not obtain bone fusion using bone marrow seeded in ceramic without BMP stimulation for the four weeks of follow up. However, when bone marrow and osteoinductive rhBMP-2 were combined in an inert polylactic acid carrier, a synergistic effect leading to bone formation was observed. All femur defects in the rabbit were filled and united at six weeks. It should be noted that, in these experiments, rhBMP-2 in the absence of bone marrow achieved 80% union by week 12, probably through recruitment of local stem cells. Thus, a composite bone graft using osteoprogenitor cells, osteoinductive factors, and a biodegradable matrix showed synergy and superiority over a simple bone graft.

To enhance the quality of bone repair further, several groups have developed MSCs originating from the bone marrow. C3H/10T1/2 is a murine mesenchymal stem cell line

which has often been used both in vitro and in vivo. Engineered C3H/10T1/2 cells expressing human BMP-2 spontaneously differentiate in vitro into osteogenic cells and induce segmental defect repair by four to eight weeks after transplantation in syngeneic mice.[36] In this case, cell mediated treatment offers the advantages of recruiting stem cells from the recipient (paracrine effect) and inducing new endochondral bone formation (autocrine effect). Adenovirus mediated transfer of human BMP-2 gene into C3H/10T1/2 cells resulted in bone formation after intramuscular injection in nude mice.[15] In these experiments, human BMP-2 induced both cell proliferation and differentiation in vitro.

MSCS FOR CARTILAGE REPAIR

Urist *et al*[37 38] reported many years ago that MSCs may be used as chondrocyte progenitor cells for cartilage healing after stimulation by cell-cell contact and soluble factors such as TGFβ1 and human BMP-2. As early as day 5 a cellular matrix containing type II collagen was present.[39] Caplan *et al*[27] showed that, after implantation into the femoral condyle, rabbit MSCs enclosed in a highly hydrated type I collagen matrix filled the cartilage defects after four weeks. The quality of the regenerated cartilage at six months was impressive, but a discontinuity between the host tissue and the new one was observed and 20–100% of the edges were not physically integrated to the cartilage. This could be partially solved by a short exposure of the cartilage surface to trypsin, which alters the type II collagen scaffolding and favours infiltration of host cartilage by the expanding new tissue. MSCs arising from bone marrow or periosteum were compared with respect to their capacity for repair of articular cartilage in rabbit.[40] In these experiments, cartilage defects were filled with 10^6 cells included in a collagen matrix, and, as early as two weeks, MSCs were differentiated into chondrocytes which were subsequently replaced by bone. At 12 weeks, the subchondral bone was completely repaired without any loss of articular cartilage at the surface. This repair process was limited by progressive thinning of the repaired tissue, which was clearly discernible at 24 weeks. Moreover, with the periosteal derived MSCs, the repaired surface tended to become fibrillate, whereas, with the bone marrow derived MSCs, the articular surface remained smooth. In fact, despite normal histological appearance, the mechanical properties of the repaired tissue did not return to normal (average compliance 5.2 compared with 11 in normal cartilage) and a gap between it and the adjacent cartilage appeared in most of the treated condyles. Thus MSCs from bone marrow have useful applications for cartilage repair, but the methodology requires improvement in the area of both cartilage thickness and mechanical properties. Engineered MSCs may improve the quality of the repair through continuous secretion of a chondral differentiating factor.

Conclusion

Regeneration of damaged cartilage in different pathological situations is a major goal which could be achieved through cell and/or gene therapy. Although autologous chondrocytes have been successfully used in traumatic pathologies, they do not have the qualities required for cartilage repair in cases of chronic inflammation. In the same way, transplantation of total bone marrow is limited by the low number of progenitor cells present in bone marrow aspirates. MSCs seem to be the best candidates for cell therapy for regeneration of injured tissue.[2] They are easily isolated from bone marrow and can be rapidly amplified up to 2000-fold over about 10 days.[41] When these cells are implanted into cartilage defects, they appear to be able to proliferate and differentiate into chondrocytes. This procedure is of considerable relevance for the regeneration of large areas of cartilage lesions. However, complete healing is difficult to obtain and requires integration of newly regenerated tissue with the surrounding host tissue and true differentiation through pathways involved in embryonic development. This goal may be achieved through the combination of MSC mediated therapy and gene transfer of selective differentiating cytokines.

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