Cellular-Based Therapy for Osteonecrosis

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Nontraumatic osteonecrosis (ON) of the femoral head is a painful disorder of the hip most commonly associated with corticosteroid therapy and alcohol abuse, which often leads in its final stage to femoral head collapse and subsequent total hip arthroplasty.\textsuperscript{1} Various efforts have been made to enhance the healing of osteonecrotic sequesrum before collapse occurs because total hip arthroplasty is a less than optimal option in young patients. Although different pathophysiologic mechanisms leading to ischemia have been postulated for this disease, none can explain the insufficient bone repair following the occurrence of the lesion and its evolution to bone collapse. Hernigou and colleagues\textsuperscript{2} were among the first to suggest that ON might also be a disease of bone cells or mesenchymal stem cells. The levels of activity and the number of mesenchymal stem cells in the hematopoietic and the stromal compartments of the bone marrow have been shown to be depressed in patients with ON of the femoral head,\textsuperscript{3} as well as the capacity of proliferation of osteoblastic cells.\textsuperscript{4} These findings raise the possibility of a pathophysiologic approach to ON treatment by implantation into the necrotic lesion of stem cells from mesenchymal tissues including bone obtained after autologous bone marrow concentration.\textsuperscript{5,6}

This review article describes bone remodeling in the context of ON as a bone disease, the use of stem cells in bone and vascular diseases, and cellular therapy in ON.

BONE REMODELING

The skeleton is an extremely dynamic tissue at the microscopic level in being able to sustain the tremendous loads placed on it in everyday life which depends on, among other factors, being able to remodel and repair the constant microcracks or lesions that develop within the bone. The fundamental mechanisms of bone remodeling might be similar in cancellous and cortical bone, occurring in what has been termed the basic multicellular unit (BMU), which comprises the osteoclasts, osteoblasts, and osteocytes within the bone-remodeling cavity. Hauge and colleagues\textsuperscript{7} demonstrated that cells in the BMU in both bone compartments are not directly contiguous to the bone marrow but are covered by bone-lining cells that seem to be connected to the bone-lining cells of the quiescent bone surface. In turn, these bone-lining cells on the quiescent bone surface are in communication with osteocytes embedded within the bone matrix.\textsuperscript{7} Penetrating the canopy of bone-lining cells and presumably serving as a conduit for the cells needed in the BMU are capillaries.\textsuperscript{7} The BMU (consisting of osteoclasts, osteoblasts, and osteocytes) is placed within the bone-remodeling compartment which comprises the BMU, the canopy of bone-lining cells, and the associated capillaries.\textsuperscript{7}

Given this structure, it becomes easier to understand the key role that osteocytes have in controlling bone remodeling despite being imprisoned in the bone matrix. It is clear that osteocytes can sense microcracks and mechanical strain and be responsive to changes in the hormonal milieu of the bone (eg, glucocorticoids), essentially triggering bone remodeling, perhaps by communicating with bone-lining cells.\textsuperscript{8,9} By analogy with remodeling in cortical bone, which is clearly associated with growth of a blood vessel into the remodeling compartment.
site,\textsuperscript{10} the presumed ingrowth of a capillary into the bone-remodeling compartment provides the vascular supply for the cells in the BMU of cancellous bone and might also provide the necessary osteoclasts and, subsequently, the osteoblasts that are needed for bone remodeling in both cancellous and cortical bone. Within the BMU, preosteoblastic cells, which express RANKL,\textsuperscript{11} probably control the differentiation of osteoclasts from hematopoietic progenitors. In turn, completion of the bone resorption phase is followed by a wave of bone formation driven, in part, by factors produced by the osteoclast that stimulate osteoblast differentiation and activity, perhaps via direct cell–cell contact between the osteoclast and osteoblast. In the context of this orchestrated activity in the BMU, a number of cells are targeted by the ON disease—the osteoblast, the osteocyte, and possibly the endothelial cell in the capillaries.

**OSTEONECROSIS, A BONE DISEASE**

Although numerous studies concerning the pathogenesis of ON have been presented, the pathophysiologic mechanisms that may be involved continue to be debated. Different mechanisms leading to ischemia have been postulated, including fat emboli,\textsuperscript{12} microvascular tamponade of the blood vessels of the femoral head by marrow fat,\textsuperscript{13} retrograde embolization of the marrow fat,\textsuperscript{14} and intravascular coagulation.\textsuperscript{15} Nevertheless, none of those mechanisms explore the necrotic lesion as a bone disease. In the early 1980s, the concept of accumulative cell stress was advanced, which is a theory that proposes that bone cells are exposed to multiple insults or stresses, the effects of which accumulate to the point that the cells cannot sustain themselves and die.\textsuperscript{16} Better understanding of bone biology and the risk factors for ON indicated that those mechanisms should be revisited. Indeed, ON is characterized by apoptosis of the osteocytes and cancellous bone-lining cells in the necrotic lesion but also at a distance of the lesion in the proximal femur.\textsuperscript{17} The replicative capacities of osteoblastic cells obtained from the intertrochanteric area of the femur are reduced in ON patients when compared with patients who have osteoarthritis.\textsuperscript{4} The number and the activity of fibroblast colony-forming units, reflecting the number of mesenchymal stem cells that could potentially give rise to mature osteoblasts, have been shown to be decreased in ON.\textsuperscript{2,3} Moreover, the function of the capillaries serving as a conduit for the cells needed in the BMU and providing blood supply within the bone-remodeling compartment could be altered by emboli or thrombosis.\textsuperscript{12,14} This altered bone remodeling can be responsible for three different events in the pathogenesis of ON: (1) the appearance of ON itself, (2) the bone repair that occurs after ON, and (3) its evolution to the subchondral fracture. First, glucocorticoids inhibit osteoblastogenesis and promote osteoblast and osteocyte apoptosis.\textsuperscript{18,19} The osteocyte apoptosis could disrupt the mechanosensory role of these cells and prevent the adaptation of bone to ischemia and the medullary changes seen in the early stages of ON.\textsuperscript{19–21} The decrease in osteoblast capacity to proliferate could reflect the disruption of the mechanosensory role of the osteocyte canalicular network and explain the evolution from marrow ischemia and edema to ON.\textsuperscript{4} Second, at a very early stage, a sufficient repair capability would make the lesion reversible. An insufficient repair mechanism related to a decrease in bone formation might explain the evolution to a further stage of ON and to the subchondral fracture. The rate of bone formation is indeed largely determined by the number of osteoblasts, which, in turn, is determined by the rate of replication of progenitors.\textsuperscript{22} Third, the altered capillary function enables the stem cells to travel from the bone marrow to the bone surface to meet the need in bone remodeling necessary to heal the necrotic lesion.

**ADULT STEM CELLS**

Adult stem cells are capable of maintaining, generating, and replacing terminally differentiated cells within their own specific tissue as a consequence of physiologic cell turnover or tissue damage due to injury.\textsuperscript{23} Stem and progenitor cell populations are the upstream components of continuous systems of cell renewal in virtually all human tissues. The most accessible adult stem cells are the hematopoietic ones which primarily reside in the bone marrow but can now be more easily collected in the blood through cytapheresis. The hematopoietic system has traditionally been seen as an organized, hierarchic system with multipotent, self-renewing stem cells at the top, committed progenitor cells in the middle, and lineage-restricted precursor cells which give rise to terminally differentiated cells at the bottom. The bone marrow contains hematopoietic stem cells that give rise to blood cells (red blood cells, leukocytes, and platelets) and that move between bone marrow and peripheral blood, and mesenchymal stem cells.

Mesenchymal stromal cells (MSCs) are nonehematopoietic stromal cells that were first isolated from the bone marrow and subsequently from other adult connective tissues. They exhibit a multilineage differentiation capacity and can develop
into diverse cells, including adipocytes, osteoblasts, chondrocytes, myocytes, tenocytes, and neural cells.\textsuperscript{24–28} They can contribute to the regeneration of mesenchymal tissues such as bone, cartilage, muscle, ligament, tendon, adipose, and stroma; however, this classic paradigm of stem cell differentiation restricted to its organ-specific lineage is being challenged by the suggestion that adult stem cells, including hematopoietic stem cells, retain a developmental plasticity that allows them to differentiate across boundaries of lineage and tissue.\textsuperscript{29,30} Stem cells derived from bone, bone marrow, and peritrabecular tissues in cancellous bone, periosteum, cartilage, muscle, fat, and vascular pericytes are capable of differentiation into multiple phenotypes, including bone, cartilage, tendon, ligament, fat, muscle, and nerve.\textsuperscript{31–34} This characteristic has important implications with regard to the design of tissue engineering strategies in that cells derived from one tissue might be useful in forming other tissue types.

Bone marrow aspirates and trabecular bone have both been identified as sources of MSCs, although the quantity obtained from aspirates is less than 0.01\%.\textsuperscript{35,36} In the laboratory, MSCs from bone marrow can be isolated and expanded using relatively simple protocols based on culture expansion of adherent cells. Expanded MSCs can be guided along specific differentiation pathways in culture by using specific media that contain growth factors or other substances such as dexamethasone (Fig. 1).\textsuperscript{24,34}

In bone, the continuous remodeling requires the formation of many new osteoblasts. Osteoblasts, in turn, are continuously derived from a much smaller number of preosteoblasts and upstream progenitor cells. The number of true stem cells needed to support this process may be very small (on the average, less than 1 in 20,000 nucleated cells in native marrow).\textsuperscript{35} MSCs are thought to be reservoirs of reparative cells, which lack specific tissue characteristics and are ready under different signals to mobilize and differentiate into cells of a connective tissue lineage. The activation of stem cells and the proliferation of progenitor cells to form new osteoblasts are vastly accelerated as a result of trauma, fracture, inflammation, necrosis, and tumors.\textsuperscript{37} The mobilization and differentiation of MSCs can be influenced by chemotaxis and interactions with the extracellular matrix through transmembrane proteins such as integrins;\textsuperscript{38,39} however, in many cases, MSCs appear to differentiate toward the local cell population under the influence of the microenvironment.

**STEM CELLS IN BONE REPAIR**

Bone marrow cells contribute to bone repair after systemic or local transplantation in animals and humans. The feasibility of allogeneic bone marrow transplantation to treat a systemic bone disease...
was demonstrated in children with severe osteogenesis imperfecta.\textsuperscript{40} In that study, functional marrow-derived mesenchymal cells engrafted and contributed to the formation of new dense bone. The percentage of grafted osteoblasts could not be improved after the transplantation of mesenchymal stem cells only (plastic adherent marrow stromal cells).\textsuperscript{41} The interpretation of these observations was that cells other than those in the adherent population, where mesenchymal cells are thought to reside, are potent transplantable progenitors of osteoblasts, consistent with laboratory studies showing that nonadherent cells can give rise to bone. It was demonstrated in the mouse that transplantation of adherent cells would allow an engraftment of transplanted adherent cells representing 1.5\% of osteocytes and osteoblasts. Transplantation of nonadherent cells in contrast yielded clusters of donor cells that accounted for 18\% of such bone cells. These data showed that the nonadherent marrow cells have a more robust bone repopulating activity than do adherent cells after systemic infusion and that there are two presumably distinct populations of marrow cells with the capacity to generate osteoprogenitors.

For local bone disease, several experimental approaches in animal models have been used to elicit bone formation in segmental bone defects, including implantation of bone marrow,\textsuperscript{42} of MSCs,\textsuperscript{43} of osteoconductive extracellular matrix scaffolds,\textsuperscript{44,45} and of bone morphogenetic proteins (BMPs) in various matrix.\textsuperscript{46,47}

In humans, only a few studies have evaluated the efficacy of bone marrow implantation in bone disorders such as nonunion, spinal fusion, or ON (Table 1). Goel colleagues\textsuperscript{48} evaluated the effect of percutaneous bone marrow grafting in patients with a tibial nonunion, resulting in union in most patients. Siwach colleagues\textsuperscript{49} treated 72 patients who had delayed or nonunion of a fracture or poor regeneration in segmental bone transportation or limb lengthening with a percutaneous injection of autologous bone marrow. They achieved union in 68 of 72 patients. Outcomes in these procedures seem to be influenced by the number of MSCs injected into a nonunion. It has been reported that 20 mL of bone marrow is needed to generate 3 mL of new bone.\textsuperscript{50,51}

**STEM CELLS IN VASCULAR REPAIR**

Endothelial progenitor cells have been proposed to circulate in adult organisms and to be recruited and incorporated into sites of physiologic and

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pathologic neovascularization.\textsuperscript{52,53} New therapeutic approaches to promote angiogenesis evolved when it was suggested that the infusion of circulating bone marrow–derived stem or endothelial progenitor cells may improve blood flow recovery in various ischemic models.\textsuperscript{52,54} Thus far, one cannot conclude whether these effects can be attributed to the incorporation of stem cells into the wall of new vessels or to cytokines released by chemotracted bone marrow cells inducing the proliferation of resident endothelial and smooth muscle cells. Recently, Kinnaird colleagues\textsuperscript{55} indicated that cultured human bone-derived stromal cells promote arteriogenesis through paracrine mechanisms. They demonstrated that the expression of genes encoding for cytokines related to arteriogenesis (vascular endothelial growth factor [VEGF], fibroblast growth factor-2 [FGF-2], interleukin-6, placental growth factor) was up-regulated. Moreover, in a murine hind limb ischemia model, intramuscular injection of the cultured medium of those bone-derived stromal cells improved collateral blood flow recovery and limb function. It was concluded that paracrine signaling and not cell incorporation may be an important mediator of bone marrow cell therapy in tissue ischemia. Other studies using different approaches had the same conclusions that stem cells promote vasculature growth by their paracrine effects and not by incorporation into the wall of growing vessels.\textsuperscript{56,57}

Similarly, a controlled and randomized trial of therapeutic angiogenesis for patients with limb ischemia by autologous transplantation of bone marrow cells explained their therapeutic effect by the injection of endothelial stem cells and by the release of angiogenic factors (VEGF, FGF-2) and angiopoietin-2, which are known to have important functions in maturation and maintenance of the vascular system.\textsuperscript{58} Furthermore, in the context of bone disease, Wang colleagues\textsuperscript{59} showed that increased VEGF production by osteoblastic cells has a marked anabolic effect on bone, apparently due to increased angiogenesis and subsequent influx of osteoblasts onto bone surface.

The potential of bone marrow mononuclear cell implantation for angiogenesis stimulation has also been demonstrated in patients sustaining myocardial infarction. Infarcted myocardium was reported as repairable by intramyocardial and intracoronary bone marrow cell transplantation.\textsuperscript{60–62}

**CELLULAR THERAPY IN OSTEONECROSIS OF THE FEMORAL HEAD**

Studies on the treatment of ON using cellular therapy from the first report in 1994 to the present are reviewed herein. Autologous bone marrow transplantation was reported for the first time in a patient sustaining ON of the humeral head due to sickle cell anemia. Hernigou colleagues\textsuperscript{63,64} reported the case of a 13-year-old patient who had ON of the humeral head secondary to sickle cell disease.\textsuperscript{64} The transplantation by marrow intravenous infusion was performed after administration of chemotherapy and total lymphoid irradiation to suppress the immune response. The donor was an HLA-identical sibling who was heterozygous for sickle cell anemia. Three months after the transplantation, radiographs showed rapid reconstruction of the left proximal humerus epiphysis, and T1-weighted MR images demonstrated a tendency toward normalization of the marrow signal in this region. In addition, pain and range of motion considerably improved. Thereafter, Hernigou and colleagues\textsuperscript{5,6} and Gangji and colleagues studied the efficacy of bone marrow implantation into the necrotic lesion of ON of the femoral head. A total of 400 mL of bone marrow was aspirated from the anterior iliac crest with the patient under general anesthesia and then transferred into a bone marrow collection kit. The rest of the bone marrow preparation was performed in a sterile room in the cellular and molecular therapy unit. In the mean time, the second step of the procedure, the core decompression, was accomplished. Under fluoroscopic control, a 3-mm trephine was inserted manually through the trochanter, the neck, and the head of the femur to the necrotic lesion. The tip of the trephine was placed at a distance of 2 to 3 mm from the articular cartilage (Fig. 2). In the cellular and molecular therapy unit, the bone marrow was filtered to eliminate bone spicules, fat, and cellular debris. Mononuclear cells were then isolated on a cell separator and concentrated to a final volume of 50 mL. This bone marrow was injected through the trephine placed into the necrotic lesion.

Gangji colleagues\textsuperscript{5} studied 13 patients (18 hips) with stage I or II (without subchondral fracture) ON of the femoral head according to the system of the Association Research Circulation Osseous in a controlled double-blind trial. The associated risk factors were corticosteroid therapy and alcohol abuse in all but two patients. The hips were allocated to a program of core decompression only (control group) or core decompression and implantation of bone marrow mononuclear cells (bone marrow graft group). The outcomes were safety, clinical symptoms, and disease progression. After 24 months of follow-up, there was a significant reduction in pain and joint symptoms within the bone marrow graft group ($P = .021$). At 24 months, five of the eight hips in the control
group had deteriorated to stage III (subchondral fracture), whereas only one of the ten hips in the bone marrow graft group had progressed to this stage \((P = .016)\). Survival analysis showed a significant difference in the time to collapse between the two groups. In addition, in the bone marrow graft group, the volume of the necrotic lesion decreased by 35% after 24 months.

Similarly, Hernigou and Beaujean\(^6\) reported the results of a prospective study of 189 hips in 116 patients treated with core decompression and bone marrow grafting in 2002. The patients were followed up from 5 to 11 years with a mean of 7 years. The associated risk factors were corticosteroids for 16% of the hips, alcohol abuse for 30%, sickle cell disease for 34%, organ transplantation for 11%, idiopathic for 5%, and miscellaneous causes for 4%. The outcomes were changes in clinical symptoms, progression in radiographic stages, and the need for hip replacement. When patients were treated before collapse (stage I and II), hip replacement was done in 9 of the 145 hips. Total hip replacement was necessary in 25 hips among the 44 hips operated after collapse (stage III and IV). The number of MSCs implanted was significantly lower in ON attributable to corticosteroid therapy, alcohol abuse, or organ transplantation than in patients with sickle cell disease. The different number of transplanted progenitor cells might have had an influence on the outcome.

Altogether, the two studies showed great improvement in femoral head preservation after stem cell implantation in early stage ON of the femoral head.

**HYPOTHESIS FOR THE EFFICACY OF MARROW CELL IMPLANTATION INTO THE OSTEONECROTIC LESION**

Recent advances in the understanding of the pathophysiology of ON suggest that a decrease in the mesenchymal stem cell pool of the proximal femur and in the osteoblastic cell proliferation rate might not provide enough osteoblasts to meet the needs of bone remodeling in the early stages of the disease. An insufficiency of osteogenic cells could explain the inadequate repair mechanism that is postulated, leading to femoral head collapse. The effectiveness of bone marrow cells may be related to the availability of stem, mesenchymal, and endothelial stem cells endowed with osteogenic and angiogenic properties, arising from an increase in the supply of such cells to the femoral head via bone marrow implantation. Indeed, in the early stages of ON, creating sufficient repair capacity through the implantation of osteogenic cells could make the lesions reversible. In a study performed by the authors, the volume of the necrotic lesion decreased by 35% in bone marrow–grafted patients, whereas it increased by 23% in the control group. This finding also suggests that necrotic lesions might be reversible. The efficacy of BMPs like BMP-2 or BMP-7 in treating bone diseases such as nonunions\(^{65}\) or ON\(^{66}\) could be explained by the same mechanisms, because they have the ability to initiate new bone formation by recruiting mesenchymal stem cells and stimulating their differentiation into osteoprogenitor cells. Another possible explanation for the therapeutic effect of bone marrow implantation is that injected marrow cells supply skeletal and angiogenic factors resulting in increased osteogenesis and angiogenesis.\(^{59}\) FGF-2,\(^{67,68}\) transforming growth factor-\(\beta\),\(^{69}\) platelet-derived growth factor, and VEGF contained in the bone marrow may also serve as a therapeutic substrate to enhance bone formation. They have indeed demonstrated their ability to increase bone formation in fracture repair\(^{67,69}\) and ON.\(^{58}\) Nevertheless, larger trials and the use of other techniques are needed to fully understand the results obtained with cellular therapy in ON, because it is not possible thus far to localize the bone marrow cells after the injection or to demonstrate that the bone repair originated from the injected bone marrow cells.
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