Future uses of mesenchymal stem cells in spine surgery

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Stem cells are currently being studied for use in numerous clinical applications, ranging from neurodegenerative diseases to cardiac insufficiency. The use of mesenchymal stem cells (MSCs) in spine surgery is also compelling, especially with the increasing age of the general population. In this review the authors discuss the use of MSCs for intervertebral disc repair and regeneration and for use in spinal arthrodesis procedures. Clearly, the routine use of cellular therapies by spine surgeons to improve outcome after a variety of surgical procedures is rapidly approaching.

KEY WORDS • spinal fusion • disc repair • mesenchymal stem cell • gene therapy • immune response

Numerous research groups are currently focusing on the purification and expansion of pluripotent stem cells for the treatment of a variety of disorders, including Parkinson disease, stroke, cardiomyopathy, hepatic failure, and renal disease.¹ Mesenchymal stem cells have also been identified and are currently being developed for bone, cartilage, muscle, tendon, and ligament repair and regeneration.^{3,7,9,21,37} These MSCs are typically harvested, isolated, and expanded from bone marrow or adipose tissue, and they have been isolated from rodents, dogs, and humans.^{8,10,12,38} Interestingly, these cells can undergo extensive subcultivation in vitro without differentiation, magnifying their potential clinical use.⁸ Human MSCs can be directed toward osteoblastic differentiation by adding dexamethasone, ascorbic acid, and β -glycerophosphate to the tissue culture media. This osteoblastic commitment and differentiation can be clearly documented by analyzing alkaline phosphatase activity, the expression of bone matrix proteins, and the mineralization of the extracellular matrix.⁶

OVERVIEW OF MSCS

Human MSCs express a variety of different cell surface proteins, including numerous integrins ($\alpha 1$, $\alpha 2$, $\alpha 3$, $\alpha 5$, $\alpha 6$, αV , $\beta 1$, $\beta 3$, and $\beta 4$), growth factor receptors (basic fibroblast growth factor receptor, platelet-derived growth factor receptor, interleukin-1 receptor, TGF β_1 receptor, and TGF β_2 receptor), and cell adhesion molecules (intercellular

adhesion molecule-1, vascular cell adhesion molecule, activated leukocyte cell adhesion molecule, and L-selectin).^{2,8,13} These MSCs should therefore be highly responsive to osteogenic growth factors produced using gene therapy techniques, as well as osteoconductive matrices used as cell delivery vehicles. Human MSCs delivered on porous hydroxyapatite/tricalcium phosphate ceramics have been shown to heal critical-size femoral defects in athymic nude rats.¹¹ Although therapies using MSCs in humans are theoretically attractive, the development of allogeneic grafts has several potential problems, including graft rejection, transmissible diseases, and bacterial and fungal contamination. If these issues are overcome, human MSCs may be ideal for ex vivo BMP growth factor gene therapy, because the expressed proteins will not only stimulate mesenchymal differentiation cascades by the grafted stem cells, but also by local host progenitor cells.

There have been several reported clinical trials of MSCs for the treatment of disease in humans. Horwitz, et al.,²² demonstrated that mesenchymal cells derived from allogeneic bone marrow and transplanted into three children with osteogenesis imperfecta significantly improved bone deposition in trabecular bone. The patients had a mean increase of 28 g in their bone mineral content, compared with predicted values of 0 to 4 g. Bruder, et al.,¹¹ placed autologous canine MSCs obtained from bone marrow onto porous cylinders composed of hydroxyapatite and tricalcium phosphate. The implant was subsequently grafted into critical-size femoral defects. Implants without MSCs produced atrophic nonunions in all treated animals. Implants containing the MSCs produced lamellar and woven bone within the carrier and resulted in a solid union at the site of the defect. Bruder, et al.,¹⁰ also successfully achieved bone induction by using human MSCs on a ceramic carrier in

Abbreviations used in this paper: BMP = bone morphogenetic protein; HLA = human leukocyte antigen; IVD = intervertebral disc; mHC = minor histocompatibility complex; MHC = major histocompatibility complex; MSC = mesenchymal stem cell; TGF = transforming growth factor.

athymic nude rats. Based on radiographic studies, biomechanical testing, and histological analysis, the human MSCs were found to be capable of healing critical-size femoral defects. Quarto, et al.,²⁸ studied the use of autologous bone marrow stromal cells delivered on a macroporous hydroxyapatite carrier for the healing of large bone defects (> 4 cm) in a small human clinical series. The implant was placed within the defect, and the fracture was stabilized with external fixation. In each instance, the composite implant fostered successful union of the bone defect, thus producing the first direct evidence that tissue engineering of bone can be successfully applied to humans in a clinical setting. These studies demonstrate the feasibility of using culture-expanded MSCs for the induction of osteogenesis in bone disorders.

Immunological Aspects of MSC-Based Tissue Repair and Regeneration

As mentioned previously, the use of autologous MSCs that have been genetically modified to secrete BMPs has significant clinical potential for the repair of musculoskeletal tissues. Their use may be financially impractical, however, because it would necessitate the harvesting, isolation, and expansion of cells from every patient requiring treatment. The development of allogeneic or xenogeneic MSC-based gene delivery systems may therefore prove to be more feasible for widespread clinical use, yet the complex immune responses leading to allogeneic or xenogeneic MSC rejection need to be characterized and attenuated before these approaches can be applied clinically. The transplantation of allogeneic organs typically results in graft rejection unless the host immune responses are decreased using long-term immunosuppressive therapies. The immunological issues related to long-term MSC survival are similar to those for routine organ transplants, including allelic differences between the graft and host at the polymorphic loci that encode histocompatibility antigens (Fig. 1).⁵ The three classes of antigens that need to be dealt with in tissue transplantation are the ABO blood group, the HLA/MHC, and the mHC.

The ABO Blood Group Antigens. It is well known that ABO blood group antigens are expressed by circulating red blood cells; however, they are also expressed by almost all cell types in every organ in the body. Patients who do not express A or B alloantigens develop antibodies directed against the nonexpressed antigens. Hyperacute organ rejection is typically induced by anti-A and/or anti-B antibodies in the host binding to highly expressed antigens on the graft endothelium. Interestingly, results of previous studies have shown that ABO-induced rejection is also seen in avascular tissue transplants, such as pancreatic islet and corneal allografts. Although it is not clear whether MSCs express ABO blood group alloantigens, it can be hypothesized that after differentiation their cell products will, especially because they will include vascular endothelial cells. Therefore, ABO compatibility will certainly need to be addressed when developing allogeneic MSC-based therapies for human use.

The HLA/MHC Antigens. The HLAs are typically grouped into Class I proteins (HLA-A, HLA-B, and HLA-C) and Class II proteins (HLA-DR, HLA-DQ, and HLA-DP). The Class I molecules have been shown to be expressed in all

Fig. 1. Diagram of interactions between allograft or xenograft MSC grafts and the host immune system. Issues involving stimulation of the immune system need to be fully addressed before MSCs can be routinely used in the clinical setting. APC = antigen presenting cell; DTH = delayed type hypersensitivity reaction; Ig = immunoglobulin; TCR = T-cell receptor.

cell types, whereas Class II antigens are expressed in several immune system components only. Human embryonic stem cells and adult MSCs express HLA Class I antigens. Expression of these proteins is upregulated by interferon gamma, an inflammatory cytokine that is typically present in cell implantation sites. The Class II antigens have not been detected in differentiated MSCs. Clearly, HLA alloantigenic responses need to be carefully considered prior to performing MSC transplantation procedures.

The mHC Antigens. Human MSC-derived tissues will most likely express mHC antigens because they are derived from donor mitochondrial and *H-Y* gene products. Although mHC antigens are typically not as antigenic as MHC molecules, they can certainly lead to major immune responses in organ allografts. This response can be amplified when several mHC antigens act synergistically.

Immune Recognition of MSC-Derived Tissues. The incompatibility of ABO blood group, HLA, and mHC antigens

seen in tissues derived from MSCs will induce significant T-cell activation, which will subsequently induce alloimmune responses and ultimately graft rejection in immune-competent animals. Tissues derived from MSCs will not contain donor dendritic cells, however, which would strongly activate host T lymphocytes through direct allorecognition pathways. This factor may be important in the clinical situation, because allografts lacking donor dendritic cells have significantly longer survival periods. Therefore, cellular rejection of MSC-derived tissues will most likely occur through stimulation of the CD4-positive T cell-dependent pathway, which is extremely important because it can activate many other aspects of the immune system, including alloantibody production, cytotoxic T-cell (CD8⁺) activation, and delay-type hypersensitivity.

Immunomodulatory Effects of MSCs

Although MSC-derived tissues express the expected alloantigens of donor origin prior to differentiation, MSCs exhibit immunosuppressive effects on T-lymphocyte responses to allogeneic cells.^{20,23,31} Di Nicola, et al.,¹⁷ demonstrated that both human CD4-positive and CD8⁺ T-cell proliferation could be inhibited by MSCs derived from human bone marrow, an effect that could be abolished by treatment with a cocktail of anti-recombinant human TGFβ₁ and anti-recombinant human hepatocyte growth factor monoclonal antibodies. Therefore, TGFβ₁ and hepatocyte growth factor-β secreted by the MSCs may mediate this effect. Djouad, et al.,¹⁸ subsequently demonstrated that systemic injections of murine MSCs could induce systemic immunosuppression and lead to allogeneic tumor tolerance. Interestingly, these investigators demonstrated that the MSCs needed to be “activated” by a coculture with stimulatory splenocytes. Bartholomew, et al.,⁴ also demonstrated that intravenous administration of allogeneic MSCs prolonged the survival of allogeneic skin transplants in a primate model. Noel, et al.,²⁶ demonstrated that allogeneic MSCs genetically modified with the human *BMP-2* gene could induce bone formation in immunocompetent mice. The genetic modification of MSCs with adenoviral BMP vectors will also elicit an immune response via host antigen presenting cells. Both donor and viral antigens in the genetically modified MSCs most likely stimulated the host immune system, but the immunoinhibitory nature of the donor MSCs may have increased cell survival and stimulated significant bone formation.

Spinal Fusions Using MSCs

Numerous research groups are currently studying the use of MSCs, with or without genetic modification, to enhance spinal fusions.²⁴ The use of bone marrow stromal cells, with or without clonal expansion of osteoprogenitor cells, has been evaluated in several animal models. Wang, et al.,³⁶ demonstrated that rat bone marrow cells genetically modified to secrete BMP-2 were able to induce consistent spinal fusion in a rat model. Dumont, et al.,¹⁹ demonstrated that human MSCs genetically modified to secrete BMP-9 can induce spinal fusions in athymic nude rats. Interestingly, these cells are capable of inducing significant bone formation in immunocompetent rodents as well. Riew's group^{14,29} demonstrated that *BMP-2* gene-transduced rabbit bone marrow-derived MSCs can induce spinal fusions follow-

ing implantation into the intertransverse process space. Cui, et al.,¹⁶ showed that mixed marrow cells and D1-BAG osteoprogenitor cells could induce solid spinal fusions in rodents at a rate of 50 and 100%, respectively. Muschler, et al.,²³ also demonstrated that concentrated marrow aspirates can improve the efficacy of spinal fusions in a canine model. Finally, Orii, et al.,²⁷ demonstrated that bone marrow-derived stromal cells loaded on a β-tricalcium phosphate matrix can lead to higher spinal fusion rates than autologous bone in a macaque posterolateral spinal fusion model. Clearly, the use of MSCs alone or to enhance autologous bone grafts has a bright future for use in spinal fusion procedures. Randomized prospective clinical studies now need to be conducted before these approaches can be routinely performed in the clinical setting.

Use of MSCs for Disc Repair

The use of MSCs for the development of novel strategies for treating degenerated discs is a challenge in the field of tissue engineering and cell-based therapeutic approaches. It is speculated that biological repair strategies that increase disc cellularity, and hence matrix synthesis, can be therapeutic. The normal aging process typically alters the volume, structure, shape, composition, and biomechanical properties of the IVD. The more relevant characteristics of disc degeneration are loss of cellularity and degradation of the extracellular matrix, resulting in morphological changes and alterations in biomechanical properties. The most consistent biochemical change observed with aging is the loss of proteoglycans and the concomitant loss of water and disc pressure. Secondary changes due to redistribution of tissue stress include fibrocartilage production, with disorganization of the annular architecture and increases in Type II collagen.

Crevensten, et al.,¹⁵ used an *in vivo* model to investigate the feasibility of exogenous cell delivery, retention, and survival in the pressurized disc space. Injection of MSCs into rat coccygeal discs was performed using 15% hyaluronan gel as a carrier. Seven and 14 days after injection, stem cells were still present within the disc, but their numbers were significantly decreased. At 28 days, a return to the initial number of injected cells was observed, and viability was 100%. An increased disc height suggested an increase in matrix synthesis. The results indicate that MSCs can maintain viability and proliferate within the rat IVD. Although hyaluronan gel may not be an ideal carrier for this application, the results of this study support the suggestion that MSCs can survive injection into the disc and can proliferate *in situ*.

Sakai, et al.,³³ hypothesized that maintenance of proteoglycan content in the disc, achieved by avoiding the depletion of nucleus pulposus cells and preserving the structure of the annulus, is a primary factor in decelerating disc degeneration. To evaluate the possible potential of MSCs in disc cell research and treatment of degenerative disc disease, autologous MSCs embedded in atelocollagen gel were transplanted into the discs of rabbits that had undergone a procedure proven to induce degeneration. Results from this study provided initial evidence for the potential of MSCs to differentiate into IVD cells, and constitute new information for MSC research (although further long-term studies with detailed analysis of the tissue newly formed by transplanted

MSCs will be needed). Transplantation of MSCs was effective in preserving the anular structure by the use of MSC/atelocollagen conjugates. These conjugates served to fill depleted nucleus pulposus and to prevent proteoglycan decrease by increasing production from the differentiated cells of transplanted MSCs.

Still, the capacity of MSCs to differentiate toward IVD-like cells is unknown. Recently, Steck, et al.,³⁵ compared the molecular phenotype of human IVD cells and articular chondrocytes and analyzed whether MSCs can differentiate toward both cell types after TGF β -mediated induction in vitro. After TGF β -mediated differentiation, bone marrow-derived MSCs in spheroid cultures showed positive staining for collagen Type II and expressed a large panel of genes characteristic for chondrocytes, including aggrecan, decorin, fibromodulin, and cartilage oligomeric matrix protein, although at levels closer to that of IVD tissue than to hyaline articular cartilage. Like IVD tissue, the spheroids were strongly positive for collagen Type I and osteopontin; they also expressed more differentiation markers at higher levels than did culture-expanded IVD cells and chondrocytes, both of which dedifferentiated in monolayer culture. In the final analysis, MSCs adopted a gene expression profile that resembled native IVD tissue more closely than it resembled native joint cartilage.

The same question was recently investigated by Sakai, et al.,³² who used an in vivo approach, in which autologous MSCs from bone marrow were transplanted into a rabbit model of disc degeneration to determine if stem cells could repair degenerated IVDs. These investigators transplanted LacZ-expressing MSCs into rabbit L2–3, L3–4, and L4–5 IVDs 2 weeks after induction of degeneration. The study showed that 24 weeks after MSC transplantation, degenerated discs of animals in the MSC-transplanted group regained a disc height of approximately 91% and demonstrated a signal intensity on magnetic resonance imaging of approximately 81% compared with control discs. On the other hand, discs in the sham-operated group demonstrated a height of approximately 67% and a magnetic resonance imaging signal intensity of approximately 60%. Macroscopic and histological evaluations confirmed relatively preserved nuclei with circular anulus structures in MSC-transplanted discs compared with indistinct structures seen in the untreated discs. The restoration of proteoglycan accumulation in MSC-transplanted discs was suggested based on immunohistochemical and gene expression analysis. These data indicate that transplantation of MSCs effectively led to regeneration of IVDs in a rabbit model of disc degeneration.

Once the MSCs are transplanted or induced to differentiate into the nucleus pulposus phenotype, tissue hypoxia becomes a critical factor. It was hypothesized that the low oxygen environment serves to drive commitment of MSCs to the nucleus pulposus-like phenotype. In early work by Semenza, et al.,³⁴ hypoxia increases the expression of the *hypoxia-inducing factor* gene, a transcription factor that serves to regulate glycolytic activity. Hypoxia drives matrix metalloproteinase 2 and glucose transporter 1 expression in MSCs along with aggrecan and Type II collagen, classic markers of the nucleus pulposus phenotype.³⁰ The possibility exists that synergizing the effects of TGF β and low oxygen tension primes MSCs for differentiation along a nucleus pulposus-like lineage and supports viability (I Shapiro, personal communication, 2005). By using specific

growth factors, it may also be possible to recruit or activate residual host cells to promote the regeneration process. Development of delivery systems to transport genes of interest without using viral vectors may also enhance the function of transplanted MSCs or residual stem cell populations. As mentioned previously, one obvious advantage in the use of MSCs is that both human and animal cells possess immunomodulatory properties and may be immune privileged prior to differentiation.⁴ Although the molecular mechanisms governing this process have not been entirely elucidated, these studies indicate the likelihood of the establishment of a pool of universal donor cells for cell-based tissue-engineering procedures.

CONCLUSIONS

Cell-based therapies for use in spinal procedures are clearly in their infancy; however, numerous preclinical studies in a variety of animal models support the belief that MSCs have a bright future, especially in the area of disc repair and spinal fusions. In addition, stem cells that have been differentiated into a neural phenotype may also be used for repair and regeneration of the spinal cord, although this is a much more difficult application than musculoskeletal tissue repair. Cell viability and cellular rejection remain significant hurdles before these techniques will be successful in the human population, although it can be predicted that cell-based therapies are clearly in the future of spine surgery.

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