New Era of Cell-Based Orthopedic Therapies

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There has long been a described relationship between mesenchymal stem cells (MSCs) and blood vessels in aspects of bone and other skeletal tissues with regard to their embryonic formation and their adult repair and regeneration dynamics. The use of exogenously added MSCs to supplement the naturally available progenitor cell stock has been a standard practice in several orthopedic surgeries by adding bone marrow to the repair constructs. This, coupled with the well-established need for vasculature to orient and drive bone formation, firmly established the functional relationship between MSCs, osteoprogenitors, and blood vessels. It is now apparent that MSCs are pericytes (cells that surround blood vessels) throughout the body. In addition, MSCs can function to secrete bioactive factors that are immunomodulatory, thus allowing allogeneic MSCs to be infused into patients requiring clinically relevant treatments. Such infused MSCs trophically establish microenvironments that support the regeneration of the injured tissue. These new functions usher in a new era of cell-based therapies.

Introduction

THERE ARE SEVERAL TRUISMS regarding the management of reparative therapies in orthopedic medicine. First, the presence of vasculature, bone marrow, or periosteum is a key feature in the reformation of structural bone at break sites. Indeed, marrow-rich autograft is often taken from the iliac crest to give a boost to fracture repair or spinal fusion surgeries. Second, we long ago identified blood vessels and their positional context as the orientors and drivers of bone formation in the long bones of chick, mouse, and human embryos.^{1–3} Age-related decrease of vascular density or localized ischemia has long been identified as a cause for poor bone repair and maintenance as well as for nonunions. Third, we identified unique cells in adult bone marrow, referred to as mesenchymal stem cells (MSCs), that readily form bone at both ectopic and orthotopic sites.^{4–9} As seen in Figure 1, these cells are capable of differentiating down a number of mesenchymal tissue lineage pathways, including the formation of bone, cartilage, muscle, fat, tendon/ligament, and other connective tissues. Together with site-specific scaffolds, MSCs have been used by our lab and others in distinctive tissue engineering repair schemes for skeletal tissues (Fig. 2). Further, the use of microfracture of the subchondral bone plate to cause a bleed has short-term positive healing benefit to cartilage defects.^{10,11}

Because differentiated cells are the biosynthetic units that fabricate specific tissues, the management of reparative progenitor cells is the key to repair/regeneration strategies in orthopedics in adults.⁸ This fact brings us into the new era of cell-based therapies.

Autologous Chondrocyte Implantation

As a current clinical example of this new era, surgeons repairing massive articular cartilage defects have used autologous chondrocyte implantation (ACI) for more than 20 years. First introduced by Dr. Lars Pedersen and his student/colleague Dr. Mats Brittberg, this technology involves obtaining a small biopsy of non-weight-bearing cartilage from which autologous chondrocytes are obtained, culture-expanded, and subsequently introduced into a chondral lesion and covered with a periosteal flap.^{12,13} This procedure has been made broadly available by Genzyme Corporation with over 20,000 patients receiving the ACI procedure with generally good outcomes, mainly seen as years of pain-free activity. Several variations on this cell-based approach have been developed and used clinically. For example, Fidia Advanced Biopolymers of Italy¹² and TiGenix of Belgium have conducted clinical trials using autologous,^{15,16} culture-expanded chondrocytes with Fidia Advanced Biopolymers recently reporting a 5-year follow-up. Key aspects of these cell-based therapies are the quality of the autologous chondrocytes and the properties of the engineered delivery vehicle.

MSCs

We have published studies using autologous MSCs in bone,¹⁷ cartilage,¹⁸ muscle,¹⁹ and tendon²⁰ tissue engineering strategies for repairing (regenerating) skeletal tissues. The logics for these studies focused on driving cells down specific lineage pathways by using unique inductive agents or scaffolds. In addition, one must manage the vascular availability that must be plentiful for bone and absent for cartilage. Thus,

FIG. 1. The mesengenic process. Adult mesenchymal stem cells (MSCs) are able to differentiate into bone, cartilage, muscle, marrow stroma, tendon/ligament, fat, and other connective tissues in a sequence of lineage transitions. The figure was first drawn to mirror the sequence of events observed in hematopoietic differentiation. The state of knowledge in the late 1980s and early 1990s provided the most information for the lineages on the left and the least information for the pathways on the right. This information is reviewed in Refs.⁴⁻⁹

there is an intimate relationship between success and failure of tissue-engineered skeletal tissues and the availability of both vasculature and progenitor cells. As hypothesized below, this natural relationship of MSCs to blood vessels is functionally quite specific.

It is important to stress that the definition of an MSC is a culture-adherent, multipotent progenitor capable of differentiating into, at the least, bone, cartilage, and fat. Additionally, these mesenchymal progenitors have a distinctive set of cell surface markers as recently reviewed.²¹ We long ago identified SH2 and SH3,^{4,22} (endoglin [CD105] and ecto-5'-nucleotidase [CD73]) as markers for MSCs.^{23,24} These progenitor populations exist in every tissue in the body and have been isolated from bone marrow, fat, muscle, synovium, dermis, brain, liver, tendon, and so on. Although these progenitors have been isolated from all of these tissues and they can develop into bone, cartilage, or fat, these progenitors are distinctive and reflect aspects of their tissue of origin. For example, if pellets of adult human marrow MSCs in chemically defined medium are exposed to TGF- β , they become chondrocytes and make cartilage extracellular matrix.^{25,26} The mesenchymal progenitors from adult fat require both TGF- β and BMP-6 to make cartilage.²⁷

Lastly and now extremely important, we reported that human MSCs secrete substantial quantities of bioactive fac-

tors whether continuing in their mitotic expansion, in the osteogenic pathway or in the marrow stromal (hematopoietic support) pathway.²⁸ Indeed, the quantity and identity of these secreted bioactive factors is a signature of the MSCs in mitosis or their presence in a lineage pathway (Fig. 3) as we pointed out previously.²⁸ This last observation can now be understood in the context of the new era of MSC therapies.

New MSC Treatments

Recent clinical trials have been conducted by independent investigators²⁹ and by Osiris Therapeutics (see website www.osiris.com) of Maryland (see Disclosure). These clinical trials and the reports of preclinical models using allogeneic or xenogenic MSCs²⁸⁻³² usher us into a new, exciting era of cell-based therapies. Space does not permit a full exposition of all the properties and capacities of MSCs in this context. The two key functions of culture-expanded human MSCs are that they are strongly immunomodulatory and immunosuppressive especially toward T-cells (recognition, antigen presentation, and expansion), and, second, the MSCs are trophic³³ (Fig. 3). This trophic activity involves (1) anti-apoptosis, especially in ischemic zones, that limits the field of cell death and injury, (2) anti-scarring/anti-fibrosis, (3) angiogenesis, and (4) mitotic stimulation of tissue-intrinsic progenitors.

FIG. 2. Tissue engineering using MSCs. A bone marrow aspirate provides the source for MSCs that can be expanded in culture. These MSCs can be stored frozen and thawed without loss of potency. Using tissue-specific scaffolds, cartilage, bone, tendon, and fat can be tissue engineered to fill defects and contribute to the functional regeneration of these tissues.

The immunomodulatory capacity of MSCs^{33–37} is quite potent as documented by the results from completed phase I and II clinical trials (as well as pediatric compassionate use) for Graft versus Host Disease and Crohn's disease, which are quite striking. The results of these clinical trials involve completely inhibiting the graft versus host disease such that most of the treated patients are alive at 1 year posttreatment or for Crohn's disease most have vastly improved clinical

scores. These immunomodulatory effects allow the use of allogeneic MSCs with intravenous delivery, the preferred route of administration for clinical uses. Some evidence exists to indicate that MSCs function at the tissue target sites, but these observations also indicate that very few of the MSCs are in the target tissues; nonetheless, substantial clinical efficacy is observed in the limited number of patients thus far infused. The oversimplification that MSCs home to sites of tissue injury may be correct, but relatively few cells are observed to dock in these afflicted tissues.

It is of interest to review the historic basis for using MSCs as immunomodulatory agents because this pathway does not appear in Figure 1 nor is it intuitively obvious why marrow MSCs might so function. Originally, we reasoned that marrow MSCs must be capable of differentiating into bone marrow stroma that is able to support *in vitro* and *in vivo* hematopoiesis. Indeed, there are clear experimental data to support this assertion: simply, MSCs maintained in culture under Dexter conditions with hydrocortisone and horse serum form a fibroblastic connective tissue layer that supports hematopoietic expansion as if it were bone marrow stroma.³⁸ Likewise, the first clinical use of culture-expanded MSCs was as an additive to bone marrow transplantation³⁵; this assumed that MSCs homed to bone marrow, engrafted, and re-fabricated the bone marrow stroma. Because allogeneic bone marrow transplantation has proven useful to cure certain genetic diseases involving mesenchymal tissues or their functions, it was a natural extension of such procedures to consider the delivery of allogeneic MSCs alone.^{39–42} To ensure that such delivery of allo-MSCs would not cause an immunologic event, MSCs were tested in the standard mixed lymphocyte reactions and found to be immunosuppressive. A number of studies have been conducted to delineate the mechanism of this immunomodulation, and this is far from being completed and is quite multi-factorial and complex.^{43,44} The bioactive molecules secreted by MSCs clearly act to decrease TNF- α and IFN- γ levels with a prostaglandin-mediated increase in IL-10. In addition to suppressing T-cell replication, there is also an inhibition of antigen presentation ability and possibly a B-cell component that is affected. The cumulative result is that any T-cell or immunosurveillance cell that comes into the range of MSCs will be suppressed. As discussed below, this has a profound effect *in vivo* for intrinsic MSCs.

The trophic effects produced by the secretion of bioactive factors by MSCs have been seen in preclinical models of myocardial infarcts,⁴⁵ stroke,^{30,31} spinal cord (cuts and contusions⁴⁶), tendons,²⁰ acute renal failure,⁴⁷ and meniscal regeneration.⁴⁸ A phase I study for acute myocardial infarct (AMI) has been completed by Osiris with rather substantial clinical success. The allogeneic MSCs were introduced intravenously within 48 h after the AMI with a fourfold improvement in arrhythmia and premature ventricular contractions and a prompt return of heart rate after exercise at all time points (Osiris website www.osiris.com). Importantly, a threefold improvement of lung function also suggested that the infused allogeneic MSCs positively affected damaged tissue in the lungs of some AMI patients.

Without reviewing all of the details of the clinical trials or preclinical models, I would propose that the MSCs protected cells that would have expired (apoptosis) as a result of ischemia or other tissue injuries and that this serves to limit the

	MSC		
	Dex	IL-1 α	
	Osteogenesis	Growth	Stromagenesis
G-CSF	+	+	+++
GM-CSF	O	O	+++
M-CSF	+	+	+++
LIF	-	+	+++
IL-6	-	+	+++
IL-11	-	+	+++
SCF	+	+	+
IL-3	O	O	O
TGF β 2	O	O	O
OSM	O	O	O

FIG. 3. Bioactive factor secretion by MSCs. Culture-expanded MSCs were put into osteogenic, growth, or stromagenic (i.e., hematopoietic support conditions comparable to Dexter) conditions, and the 24h media were assayed by ELISA for specific bioactive molecules as listed. MSCs from six donors were separately analyzed. The relative (to growth conditions) quantities are represented by "+" or "-" signs (+, increases; -, decreases); the "O" means none detected. Although the absolute quantities varied greatly from donor to donor, the percent change in osteogenic or stromagenic conditions relatively tightly clustered for all donors.²⁸

scope and extent of the field of injury.⁴⁹ The MSCs also reduce or eliminate scarring either by inhibiting the entry of circulating or tissue-intrinsic cells capable of forming scar or inhibiting their scar-forming biosynthetic capacity. In all cases, there is an acceleration of angiogenesis and also a mitosis of tissue-intrinsic progenitors that results in the regeneration of cells and tissues that are damaged. I summarily refer to these multiple effects as “trophic” in keeping with the use of this term to describe the role of nerves on the formation of the regeneration blastema in amphibian limbs and the maintenance of innervated tissues and organs.^{49,50}

The MSC Niche

Given this new information regarding the immunosuppressive and trophic activities of MSCs, the question arises how these capabilities reflect the *in vivo* functioning of MSCs and the unanswered question where MSCs reside in various tissues (i.e., the MSC niche). In addition, MSC-like cells have been isolated from almost every tissue of the body, including umbilical cord and placenta. Last, several investigators have suggested that isolated pericytes^{49,51–54} are capable of exhibiting some of the cell types pictured in Figure 1. Pericytes are mesenchymal cells that reside on blood vessels (large and small) on the tissue side of the vessel.^{51–53} Although studied by several groups, a definitive study has recently been completed that shows that human adult MSC markers are found on pericytes and vice versa; these observations were made for both *in vitro* and *in situ* situations.⁵⁵ Given all of the above, I would propose that all MSCs are pericytes. (The reverse is not correct that all pericytes are MSCs.) When a focal injury occurs, blood vessels are disrupted and the pericytes are dislodged from their attachment domains on endothelial tubes. These dislodged pericytes divide and, in this mode, secrete massive amounts of bioactive factors and are, thus, identical to the MSCs that we culture as adherent cells on Petri dishes. The dividing and secretory MSC pericytes inhibit immunosurveillance of the damaged tissue and, thereby, prevent autoimmunity from developing.⁴⁹ These MSCs also prevent scar-forming cells from entering the field of injury while secreting VEGF to bring in endothelial cells to reform vascular tubes. The MSCs reform pericytes as they physically and chemically stabilize the reforming vasculature.⁵⁶ Some MSCs or their progeny may serve as a progenitor cell for the regeneration of the damaged tissue or might stimulate the mitosis of tissue-intrinsic progenitors that replace the damaged tissue.

In the special case of bone marrow, these MSCs must be particularly vigilant in preventing T-cell-mediated immune events^{57,58} as a safeguard to the hematopoietic stem cell that could be damaged should such T-cell events take place in the marrow. Thus, my view is that the MSC niche is to sit as a functioning pericyte on vasculature in every tissue of the body (except avascular cartilage) to interact with vascular tissue to ensure homeostatic nutrient and cellular transport. In situations of tissue stress or damage, such as ischemia, the pericytes are detached, and they migrate to focused sites of tissue injury where they divide and serve to trophically³¹ establish a regenerative microenvironment while standing guard against self immunosurveillance. In cases of severe or extensive tissue damage in adults, the local MSCs are insufficient to handle these demands. In some cases, the MSCs

call up sentinels from other deposits⁵⁵ or we systemically supply additional contributors. Allogeneic MSCs can serve this purpose because they are immunomodulatory. Indeed, in some cases, it may be that allo-MSCs are more effective than autologous MSCs. In this regard, it maybe that deficiencies in autologous MSCs may account for some autoimmune diseases or immunodestructive conditions. Several assays can now be envisioned to determine if certain clinical conditions arise⁵⁹ or are maintained because of defects in specific tissue MSCs/pericytes.

The Future

Increased use of both autologous and allogeneic MSCs for tissue engineering or their trophic capacities can be safely contemplated. To date, no adverse events have been reported in the over 1000 patients treated by physicians at our institution, independent investigators elsewhere, or by Osiris Therapeutics in their clinical trials. To date, defects in MSCs or MSC-mediated functions have not been reported. All of this will change as the use of MSCs increases for cell-based therapies; indeed, one of the biggest hurdles will be to establish potency assays that correlate MSC functions with clinical outcomes. Although we started working on MSCs in the late 1980s and started Osiris Therapeutics on December 23, 1992, as a bio-orthopedic company, the future is already here and we still have a long way to go to know all there is to know about this interesting family of perivascular stem cells and their use in cell-based therapies and regenerative medicine.

Acknowledgments

Supported in part by grants from NIH and funds from the David and Virginia Baldwin Fund. My thanks to my colleagues, who continue to inspire me by their clever and insightful experimentation. In particular, I dedicate this manuscript to Carl Brighten, who long ago saw the pericyte as a mesenchymal progenitor cell, and to Marshall Urist, for bringing orthopedics into the molecular arena; both individuals were talented orthopedic healthcare practitioners and inspirational scientists.

Disclosure Statement

I am a founder of Osiris, and I had stock in the company and, thus, could be considered to be conflicted although I have had no impact on or involvement with their current clinical trials.

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Received: September 16, 2008

Accepted: February 2, 2009

Online Publication Date: April 10, 2009