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PROSPECTS

Mesenchymal Stem Cells in Bone Development, Bone Repair, and Skeletal Regeneration Therapy

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Abstract Bone formation in the embryo, and during adult fracture repair and remodeling, involves the progeny of a small number of cells called mesenchymal stem cells (MSCs). These cells continuously replicate themselves, while a portion become committed to mesenchymal cell lineages such as bone, cartilage, tendon, ligament, and muscle. The differentiation of these cells, within each lineage, is a complex multistep pathway involving discrete cellular transitions much like that which occurs during hematopoiesis. Progression from one stage to the next depends on the presence of specific bioactive factors, nutrients, and other environmental cues whose exquisitely controlled contributions orchestrate the entire differentiation phenomenon. An understanding of the cellular and molecular events of osteogenic differentiation of MSCs provides the foundation for the emergence of a new therapeutic technology for cell therapy. The isolation and in vitro mitotic expansion of autologous human MSCs will support the development of novel protocols for the treatment of many clinically challenging conditions. For example, local bone defects can be repaired through site-directed delivery of MSCs in an appropriate carrier vehicle. Generalized conditions, such as osteoporosis, may be treatable by systemic administration of culture-expanded autologous MSCs or through biopharmaceutical regimens based on the discovery of critical regulatory molecules in the differentiation process. With this in mind, we can begin to explore therapeutic options that have never before been available.

Key words: differentiation, lineage, osteogenesis, chondrogenesis, bone marrow, osteoporosis, fracture repair, bioactive factors, monoclonal antibodies

The cellular and molecular events responsible for the formation of bone during embryogenesis, bone remodeling, and fracture repair are virtually identical. Although this perspective may initially appear to be an oversimplification of an extremely complex set of events, detailed studies of the individual steps responsible for these processes reveal striking similarities with respect to specific histologic, functional, and physiologic criteria. What emerges from such studies is that the cells responsible for these temporally and spatially diverse activities all arise from common progenitor cells, which we refer to as mesenchymal stem cells (MSCs) [Caplan, 1991]. It appears that these MSCs give rise not only to embryonic bone, but also to the continuous supply of osteogenic cells required for bone remodeling and fracture repair throughout adulthood. As is the case with hematopoietic stem cells (HSCs) [Ogawa et al., 1983], these marrow-derived MSCs are capable of differentiating along multiple cell lineages. However, MSCs are distinct from HSCs because they give rise to those cells that form mesenchymal tissues, including bone, cartilage, tendon, muscle, ligament, and marrow stroma (Fig. 1). We provide herein a brief discussion of our views of the bone cell biology of MSCs and, with this logic and data as a foundation, suggest several ways in which MSC technology can be used to effect repair of skeletal tissue.

The use of autologous MSCs for repair of bone defects is shown to contrast significantly with current treatment protocols and other clinical strategies that fail to add osteogenic precursor cells. While autograft bone is currently the clinician's gold standard, relatively small amounts of autograft are available, and its surgical harvest can be associated with significant morbidity

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[Younger and Chapman, 1989]. Historically, cadaveric allograft bone has been used widely; however, its unpredictable integration and the potential for transmission of lethal pathogens are serious drawbacks [Buck et al., 1989]. Bone substitutes composed of various ceramic, hydroxyapatite, and titanium materials have also been used [Holmes et al., 1987; Wolff et al., 1994]. Demineralized bone powder [Głowacki and Mulliken, 1985] or purified bone morphogenetic protein (BMP) [Wozney et al., 1988] has been implanted to induce local differentiation of resident MSCs. Recently, studies designed to test the effectiveness of human recombinant BMP-2 in the repair of segmental long bone defects demonstrate that bone formation improves when marrow-derived MSCs are combined with the BMP and delivery vehicle at implantation [Lane et al., 1994]. Thus, the presence of necessary target cells capable of responding to bioactive molecules, in this case BMP-2, is a key component in the repair process. One theme which emerges from a critical review of this body of literature is that bone substitutes, osteoconductive materials, and osteoinductive factors all achieve better results when combined with a source of MSCs, such as bone marrow.

EMBRYONIC BONE DEVELOPMENT

Bone formation in the embryonic chick tibia has been studied in detail [Fell, 1925; Pechak et al., 1986a,b]; these investigations provide the basis for understanding many of the cellular and molecular events of chondrogenic and osteogenic cell differentiation. In addition, mammalian long-bone formation has been compared and contrasted with that of the embryonic chick. A detailed exposition of these cellular and molecular events has already been presented [Caplan and Pechak, 1987] and provides the basis for our conceptual model of the events

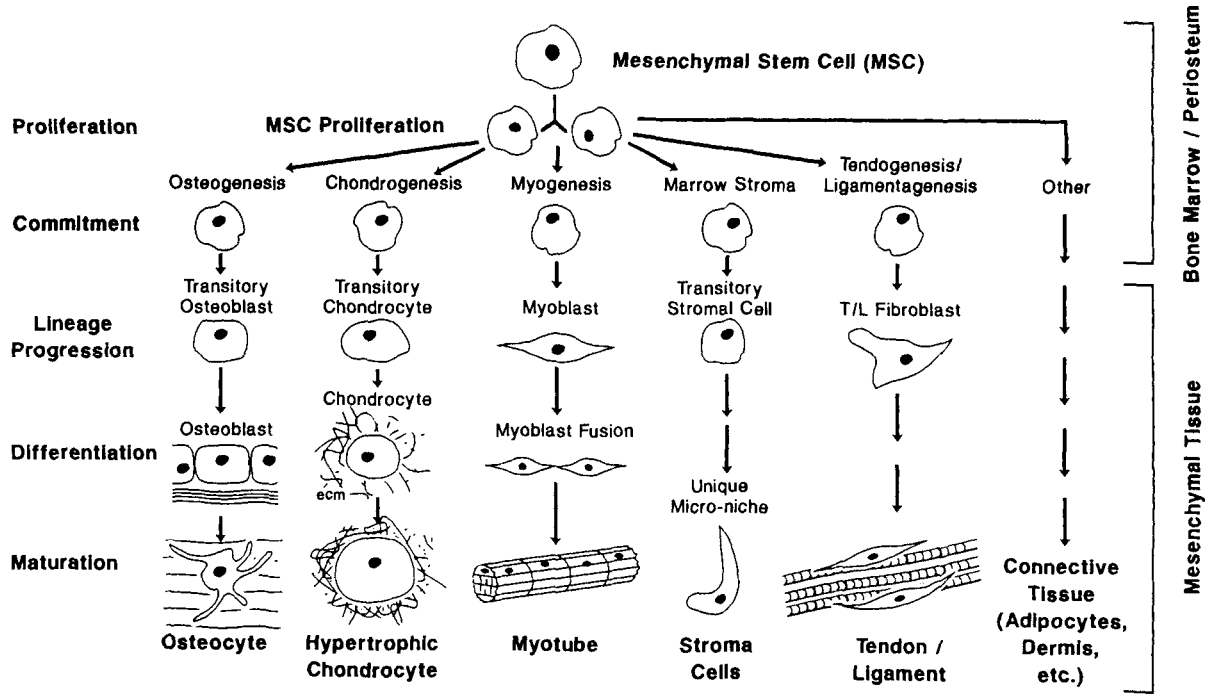

Fig. 1. The mesengenic process. MSCs have the potential to differentiate into a variety of mesenchymal tissues, such as bone, cartilage, tendon, muscle, marrow, fat, and dermis. Proliferating MSCs enter a lineage following their commitment to that particular pathway. The commitment event involves the action of specific growth factors and/or cytokines, as does the next phase in which the lineage-committed cells progress through a number of transitory stages in the lineage progression process. Terminal differentiation involves the cessation of proliferation and the massive biosynthesis of tissue-specific products. Lastly, these differentiated cells go through a maturation stage in which they acquire an ability to function in aspects of tissue homeostasis, as opposed to high levels of synthetic activity. All these end-stage differentiated cells have fixed half-lives and can be expected to expire; these cells are replaced by newly differentiated cells arising from the continuous transition down the lineage pathway. The lineages are arranged from left to right based on the relative information known about definitive lineage stages. (Reproduced from Caplan [1994], with permission of the W.B. Saunders Co.)

responsible for generating the complex tissue called bone.

During embryogenesis, mesenchymal tissue undergoes differentiation and condensation to form cartilaginous models in the anatomical areas and shapes of the future bones. While it has historically been thought that these cartilage models provide the scaffolding onto which bone is built, critical analysis of the dynamic events in the developing limb reveals that such is not the case. Rather, the cartilage model derives its boundaries from the concurrent differentiation of a unique outer-circumscribing collar of cells that provides the boundaries for differentiating cartilage and simultaneously establishes the location of the first bone differentiation [Caplan and Pechak, 1987]. In this sequence, cartilage is not replaced by bone, but is replaced by marrow, vascular, and resorptive tissue. The dimensions of the original cartilage model exactly define the boundaries of this initial marrow cavity [Pechak et al., 1986b]. As such, cartilage does not provide the scaffolding on which bone is formed, but rather acts as a boundary and morphologic guide for vasculature and marrow. Only much later in limb development is bone found at the site of the original cartilage model. The reasons for this observation will become clear as we develop the information that focuses on the role of the MSC during bone development.

The formation of the collar of osteoprogenitor cells either precedes or coincides with the emergence of the chondrogenic core of the limb early in development. These osteoprogenitors, also called Stacked Cells, likely serve as a physical boundary for the expansion of the core. As development proceeds, expansion of the cartilage core is physically restricted by the initial deposition of an osteoid matrix collar by a monolayer of differentiating osteoblasts arising from the Stacked Cell layer [Pechak et al., 1986a]. This rigid bone matrix collar, present initially at the mid-diaphysis, may also act as a nutrient or diffusion barrier for the cartilage core, since the nurturing vasculature resides exclusively outside of the core [Drushel et al., 1985]. These events must contribute to and/or signal the chondrocytes of the core to progress through the developmental stages commonly referred to as cartilage hypertrophy. Subsequent bone matrix synthesis and mineralization of the collar further limits nutrients to the core and contributes to the full expression of the hypertrophic chondrocytic phenotype. During this phase, the chondrocytes switch from the synthesis of anti-angiogenic factors to the synthesis of chemotactic factors for resorptive cells and vascular elements [Moses et al., 1990]. Eventually, at the mid-diaphyseal region, the first bony collar is resorbed, allowing penetration of vascular, resorptive and marrow elements into the space originally occupied by the cartilage core. The end result of this erosion process is that the cartilage core is replaced by marrow, not by bone. For these reasons, this process has recently been referred to as *endochondral myelopoiesis,* rather than the historical term of endochondral osteogenesis [Caplan, 1990]. The further positioning of the vasculature, first onto the initial bony collar and then subsequent layers, is directly responsible for the formation of bone in a directed manner wherein vasculature orients the secretory osteoblasts much like an epithelial tissue [Pechak et al., 1986a,b]. This and other observations lead to two fundamental rules of bone formation: vasculature is obligatory for osteogenic differentiation, and such differentiation is an oriented process resulting in predictable areas of bone matrix synthesis.

OSTEOGENIC LINEAGE

With the above observations in mind, we set out to describe the precise transitions, or lineage stages, through which osteogenic cells progress as a function of their differentiation state. A compilation of studies by Bruder and Caplan [1989, 1990a,b,c] and their collaborators [Bruder et al., 1990] has led to the generation of the lineage scheme presented in Figure 2. These studies primarily reflect analyses of the MSC-derived collar of osteoprogenitor cells present during embryonic development. The generation of monoclonal antibodies against unique surface antigens on differentiating osteogenic cells provides the evidence for defining each lineage stage. The inference is that, like the complex multistep lineage pathway of hematopoiesis, specific, as-yet-unknown cytokines or growth factors induce the transition from one stage to another (e.g., from transitory 2 to secretory osteoblast). An important consequence of this lineage scheme is that the number of secretory osteoblasts involved in fabricating bone is directly proportional to the number of committed osteoprogenitor cells that enter or commit to the pathway. As discussed below, this conclusion implies that the number of MSCs in a particular locus determines the bone formation capacity at that loca-

tion. If the biosynthetic activity of Secretory Osteoblasts is at or near the optimal rate, drugs that have these cells as their target may have little or no effect on the rate or extent of bone formation in clinical conditions of inadequate bone formation, such as osteoporosis. By this logic, the only way to increase the rate and extent of bone formation is to direct more MSCs into the osteogenic lineage.

The cytokine/growth factor environment controlling cells of the osteoblastic lineage is likely to be provided both by the cells of the lineage themselves and by neighboring cells and tissues. Vascular elements are obligatory to all bone formation, since they contribute to regulation of oxygen tension, nutrient accessibility, and other complex parameters. Recent experimental evidence suggests that vascular endothelial cells may produce powerful cytokines that affect the osteoblastic cells directly [Triffitt, 1994]. In attempting to understand this process, the use of monoclonal antibodies will be essential to obtain purified cell populations and identify specific bioactive factors. In addition, questions relating, for example, to the reversibility of individual steps within the lineage pathway and to the capacity of cells to "skip" steps must be answered experimentally.

The concept that osteogenic cells derived from MSCs proceed through various substages prior to achieving their overt bone-forming capacity is further strengthened by experiments in which bone marrow was incubated in diffusion chambers in athymic mice. Here again, the monoclonal antibodies provided evidence for the stepwise sequential lineage progression during bone formation [Bruder et al., 1990]. The rules regarding vascular-dependent bone formation are con-

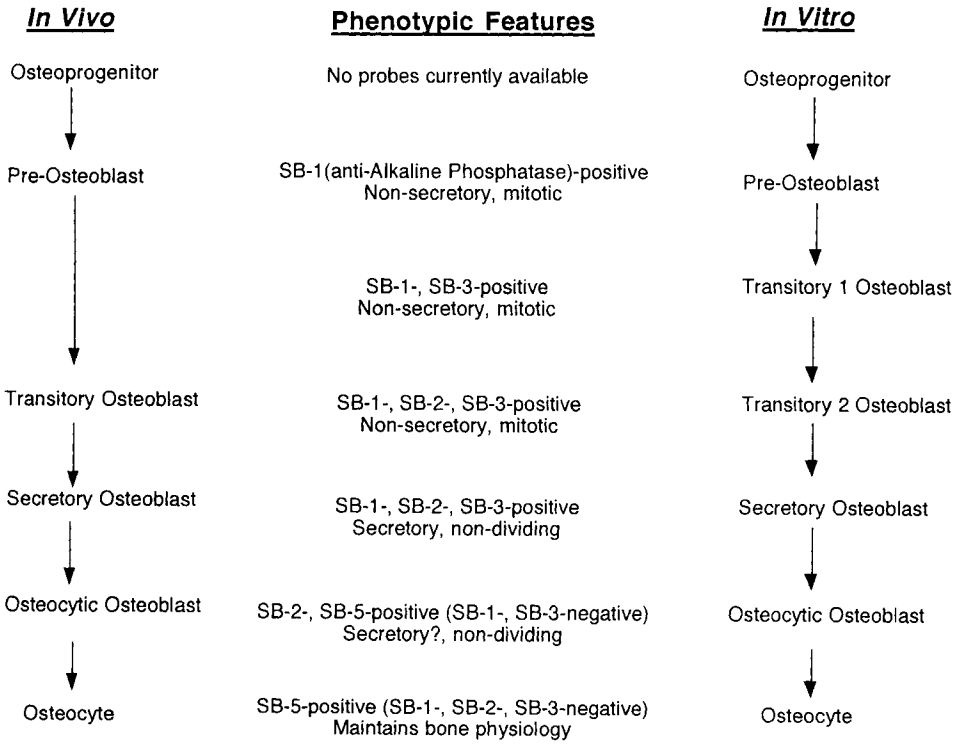

Fig. 2. The osteogenic lineage. MSCs differentiate into osteo-committed cells referred to as osteoprogenitors. These cells give rise pre-osteoblasts, which interact with a monoclonal antibody termed SB-1, that is directed against cell surface alkaline phosphatase. The next differentiation stage, referred to as transitory 1 osteoblast, is indicated by the reactivity to antibody SB-3, and the next stage, transitory 2 osteoblast, is marked by the acquisition of antibody SB-2 reactivity. These transitory stages are also identified by their relatively high mitotic activity. Eventually, these cells differentiate into secretory osteoblasts; these nondividing highly synthetic cells coordinately synthesize osteoid. A small percentage of these cells become encased in osteoid and acquire reactivity to antibody SB-5 as they mature into osteocytes. A transitory stage in this terminal process is marked by cells that are SB-2- and SB-5-positive, while being unreactive to SB-1 and SB-3; the cells of this stage are referred to as osteocytic osteoblasts. In vivo observations were made on sections of developing embryonic tibia [Bruder and Caplan, 1989, 1990b]; in vitro observations were made on cultures of folded periosteal explants [Bruder and Caplan, 1990c]. (Reproduced from Bruder and Caplan [1990c], with permission of the Academic Press.)

served in this experimental system as well, whereby proximity to vasculature determines the location of osteogenesis. Finally, studies with intramembranous bone-forming preparations of folded calvarial periosteum further support the concept of the lineage [Bruder and Caplan, 1990c] and demonstrate the equivalence between intramembranous bone formation and the sequence of events that occurs during long-bone formation [Bruder and Caplan, 1989, 1990b]. The last point is of particular importance because the embryonic cranium originates from neural crest while long bones originate from lateral plate and somitic mesenchyme.

CHONDROGENIC LINEAGE

Cartilage formation, like bone, results from a series of cellular activities which reflects a stepwise developmental program of undifferentiated MSCs. Like osteogenesis, this program involves a sequence of transitory stages that are uniquely defined by molecules synthesized by differentiating chondrocytes. In the developing limb, MSCs present in the loose mesenchyme give rise to the cartilage anlage through the series of steps depicted in Figure 3. Studies by Solursh and others describe the transient expression of type I collagen, followed by types IV and II [Solursh et al., 1978; Castagnola et al., 1988]. In specific regions, those which yield hypertrophic cartilage, type X collagen is eventually produced [Schmid and Linsenmayer, 1985]. Additional cartilage-specific extracellular matrix molecules, including various proteoglycans, help to delineate the distinct stages within this lineage paradigm [Franzen et al., 1987; Castagnola et al., 1991]. Further experimentation is required to prove the existence of these precise cellular stages; however, it is clear that cartilage formation occurs through a sequence of events wherein the fabricated tissue changes its molecular composition over time. In particular, whether the cells and resultant tissue become hypertrophic or remain as functional cartilage is likely to depend largely on the local cues available in the immediate microenvironment. The hypertrophic lineage step has further implications for subsequent osteogenesis, as will be discussed below. However, the recent observations that calcifying hypertrophic chondrocytes synthesize molecules previously referred to as "bone-specific" [McKee et al., 1992] clarifies the equivocal interpretation that chondrocytes can transdifferentiate into osteoblasts [Kahn and Simmons, 1977]. In the schema of cartilage development, like bone development, the delicate interplay between angiogenic, anti-angiogenic, and various other bioactive factors is likely to have dramatic effects on the ultimate fate of the cartilage core. Identification and characterization of these factors is critical for our further understanding of the molecular events that govern the regulation of chondrogenic lineage progression.

ENDOCHONDRAL SEQUENCE AND BONE REPAIR

During embryonic and adolescent development of long bones, growth plate cartilage in the metaphysis undergoes a complex series of cellular transitions resulting in cartilage hypertrophy [Grant et al., 1987]. The type X collagen-rich matrix becomes calcified and is subsequently resorbed by vascular and marrow elements which originate from the underlying marrow space. In this process, marrow-derived MSCs are brought to the resorbed site of the hypertrophic cartilage, where they differentiate along the osteogenic lineage to produce bone matrix. Spicules of new bone are formed on the surface of resorbing hypertrophic cartilage in an oriented manner which is obligatorily driven by the presence of vasculature. Eventually, through a rapid process that involves the complex coupling of resorptive and synthetic events, as well as mechanical forces acting on the structure, these bone spicules are remodeled into the mature weight-bearing bone [Brighton, 1984]. Like the previously discussed phenomena of osteogenic differentiation, these events are also highly regulated and multistepped. This series of events, in which cartilage is replaced by marrow elements, resulting eventually in new bone formation, has historically been termed "endochondral ossification." Figure 4 presents our interpretation of the endochondral sequence in diagrammatic form [Caplan and Boyan, 1994].

The well-established paradigm of coupled bone synthesis and resorption leads to the conclusion that bone is relatively rapidly turned over, when compared to other mesenchymal connective tissues. Epidermis, an ectodermal derivative, is constantly regenerating itself from a basal layer of progenitor cells. Thus bone, like epidermis, is maintained as a relatively "young" tissue, even during skeletal maturity. For this reason, the potential for successful bone repair and full implant integration should be higher than in the case of repair of mesenchymal tissues which do

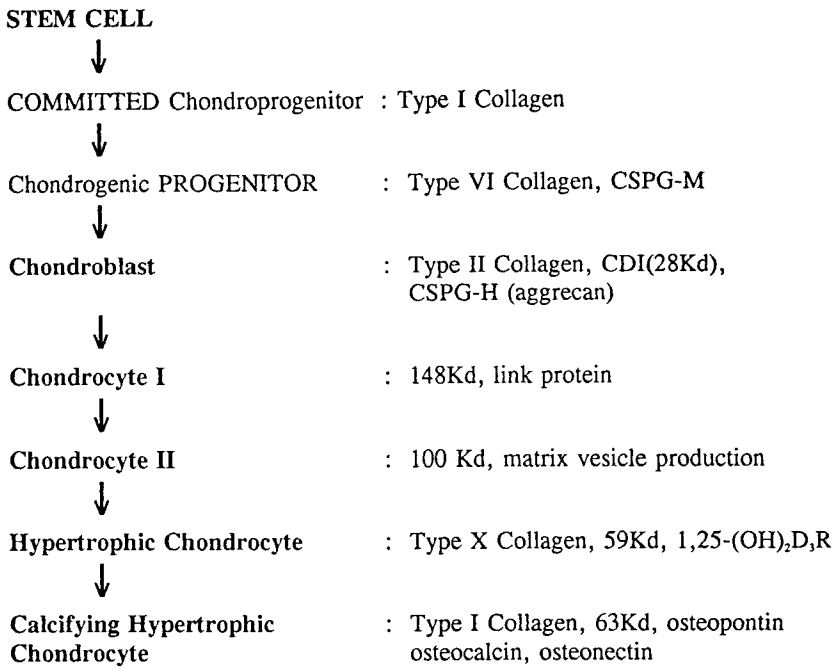

Fig. 3. The chondrogenic lineage. MSCs differentiate into committed chondroprogenitor cells that exhibit relatively high proliferative capacity. Each of the succeeding lineage stages or transitions is marked by the synthesis of molecules whose initial appearance identifies that particular lineage stage. For example, the chondroblast is defined as a cell that synthesizes type II collagen for the first time in combination with the chondroitin sulfate proteoglycan, CSPG-H (aggrecan). The terminal lineage stage is the calcifying hypertrophic chondrocyte, which actively synthesizes proteins that integrate into the calcifying cartilage; these proteins have previously been identified as osteoblast-specific biosynthetic products but are clearly synthesized by these terminal cells of the chondrogenic lineage. (Reproduced from Caplan [1991], with permission.)

not turn over, as in the case of mature articular cartilage or tendon.

This endochondral sequence is also observed in certain cases of fracture repair. When a bone break occurs, a complex series of events follows, modulated in part by the mechanics of the fracture site itself, by the presence of coordinately controlled local and systemic bioactive factors, and by the host's ability to mount an appropriate cellular response. Figure 5 illustrates the sequence of events which occurs during fracture repair under conditions of mechanical stability or instability. During the inflammatory reaction of the acute-phase injury, release of bone-derived bioactive factors, including BMPs, TGF-β, and other proteins, contributes to vigorous chemotaxis and aggregation of MSCs at the fracture site to form a continuous repair tissue [Caplan, 1988]. This "repair blastema" spans the fracture gap, and the MSCs subsequently differentiate into cartilage or bone, depending on the mechanical stability of the union. When the fracture site is unstable, this blastema gives rise to cartilage which spans the gap and provides flexible mechanical stability. Simultaneously, MSCs residing within the periosteum give rise to subperiosteal bone which forms an outer bridge between the fractured ends and thus, provides additional strength to the fracture site. Once firm mechanical stability is established, as a result of these early events, the cartilage undergoes hypertrophy in the same way as that which occurs in the growth plate. Vasculature begins to invade and bring resorptive elements to the hypertrophic and calcified cartilage. In this wave of myelopoiesis, new MSCs are delivered to the surfaces of hypertrophic and resorbing cartilage. Osteogenic differentiation of these MSCs is propelled and oriented by the presence of capillaries, and new bone spicules are laid down on, or in place of, the cartilage matrix. The cartilage callus is eventually replaced by woven marrow-filled bone, which undergoes significant remodeling to become weight-bearing bone in the same way as is observed in the growth plate [Brighton, 1984].

The formation of the cartilage intermediate during fracture repair can be circumvented if adequate mechanical stability is provided early. A comprehensive review of animal studies detail-

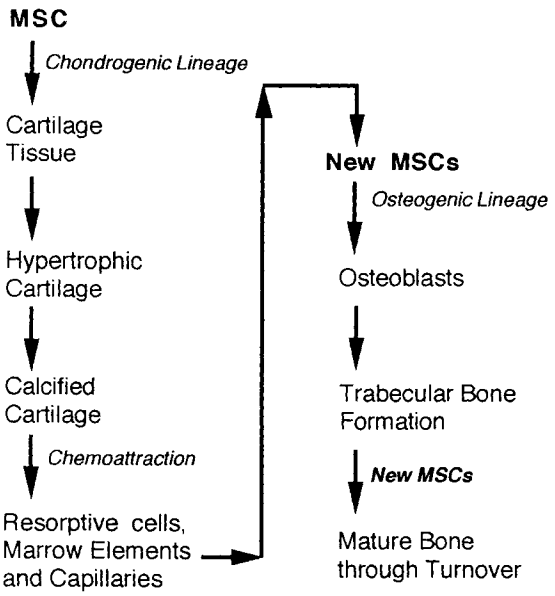

Fig. 4. Endochondral bone formation. Formation of bone through the endochondral sequence involves the initial differentiation of MSCs into chondrogenic phenotypes. Eventually, calcified cartilage is resorbed and a new wave of MSCs, in the presence of vascular tissue, enters the osteogenic lineage to form secretory osteoblasts that fabricate trabecular bone. Mature bone forms through a combination of resorption and the contribution of newly formed osteoblasts, again derived from MSCs. (Reproduced from Caplan and Boyan [1994], with permission of CRC Press, Inc.)

ing the cellular and molecular events of such repair [Ashhurst, 1991] indicates that the MSCs are brought to the fracture site, replicate in the repair blastema, and differentiate directly into osteoblasts without a cartilaginous intermediate. The important parameter in this phenomenon seems to be rapid, but mechanically stable, angiogenesis. Subperiosteal bone is formed by periosteal MSCs extracortically, and both woven and compact bone are formed in apposition to the fractured ends by blastema-derived MSCs. Again, vasculature is obligatory to this process, and we believe that the stability provided by fixation appliances fosters angiogenesis across the fracture gap. As in all the processes of bone formation discussed here, the remodeling phase significantly contributes to the final product.

MESENCHYMAL STEM CELLS

Based on the above analyses, it is clear that the cellular and molecular events of bone formation during the temporally and spatially disparate phases of embryonic development and bone repair are nearly identical. The rules outlined above that govern the behavior of cells and tissues are extraordinarily well conserved throughout development and adulthood. The cells responsible for these marvelous morphogenetic events have been termed Mesenchymal Stem Cells [Caplan, 1991]. As noted in Figure 1, we propose that these cells give rise to several tissue types; we have experimental evidence to show MSC differentiation into bone, cartilage, tendon, muscle, and marrow stroma [Caplan et al., 1993; Wakitani and Caplan, 1994; Wakitani et al., 1994; Haynesworth et al., unpublished]. These cells have been successfully isolated, expanded in culture, and experimentally tested from bone marrow and/or periosteal samples from chick, mouse, rat, rabbit, goat, dog, and human [Bruder et al., 1990; Goshima et al., 1991a,b; Nakahara et al., 1990a,b, 1992; Haynesworth et al., 1992a,b; Young et al., unpublished observations]. In some cases, we have sequentially subcultured purified MSC preparations for 20 passages with no loss in osteochondral potential when implanted subcutaneously in a porous ceramic vehicle [Goshima et al., 1991a].

The key to the use of MSCs for regeneration of bony tissue in the clinical setting lies in our ability to isolate, purify, and mitotically expand these cells from humans with no loss of their pluripotentiality. Haynesworth and colleagues have developed these techniques, as well as a number of monoclonal antibody probes which confirm the purity of human MSCs [Haynesworth et al., 1992a,b]. It is critical to note that MSCs derived from either newborn or elderly patients possess the same differentiative potential. It has been suggested however, that there is a diminution in the number of MSCs as a function of age [Haynesworth et al., 1994]. This postulate may help explain why elderly patients have a reduced capacity to heal and/or regenerate mesenchymal tissues such as bone. By this logic, when a fracture occurs in an elderly patient, the low number of responding MSCs in the repair blastema may not provide adequate chondrogenic or osteogenic capacity, even though sufficient inductive bioactive factors may be present locally. Without an appropriate number of cells to respond, the repair process will be effete and may result in clinical non-union. Such a reduction in MSC number may also underlie the cellular imbalance of osteoporosis. The paucity of osteogenic precursors, as a function of age and hormonal influence, probably allow osteoclastic resorptive activity to overpower osteoblastic synthetic activity, which results in a slow

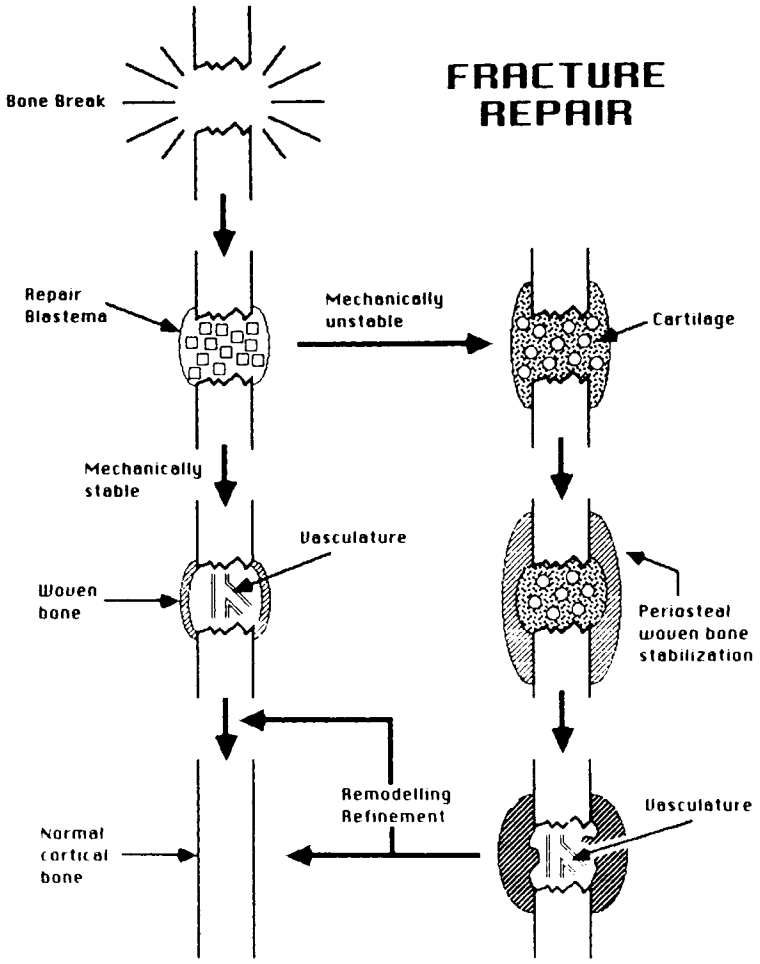

Fig. 5. Fracture repair. Following the fracture of a long bone, a group of cells containing MSCs congregates at the fracture site to form the repair blastema. If this fracture is mechanically unstable, these MSCs will, for the most part, differentiate into cartilage. Periosteal bone formation provides a bony bridge to establish stability, and vascularization ensues. Under these conditions, the cartilage hypertrophies and is replaced by marrow and eventually by bone. If the original fracture is mechanically stable, the repair blastema can be spanned by vasculature, and the MSCs differentiate directly into bone. (Reproduced from Caplan [1990], with permission of J.B. Lippincott Co.)

reduction of bone mass over many years. Other examples of impaired healing or regeneration of mesenchymal tissue may similarly reflect a decrease in endogenous MSC number. We propose to treat these and other clinical conditions by harvesting *autologous* MSCs, mitotically expanding them in tissue culture, and then providing these *autologous* cells into the host in a delivery vehicle appropriate for the desired clinical result. Since there is no loss in developmental potential of purified and culture-expanded MSCs, even from elderly patients [Haynesworth et al., 1992b, 1994], such therapeutic cell regimens should be broadly applicable. Our goal is to develop the optimal delivery vehicles, cell preparations, and/or bioactive factors for each therapeutic application.

SKELETAL REGENERATION THERAPY

Based on the above discussion, the manipulation of *autologous* marrow-derived MSCs will provide an effective approach to achieve osseous repair in clinically challenging scenarios such as fracture non-union, delayed union, segmental defect repair, spinal fusion, and osteoporosis, to name but a few indications. As previously noted, the low abundance of these MSCs in vivo will require ex vivo mitotic expansion, without lineage progression, prior to re-implantation. With this in mind, Haynesworth and colleagues

[1992a,b, 1994] have developed techniques and conditions for in vitro mitotic expansion, without differentiation, of human MSCs. In other settings, it may be desirable to stimulate osteogenic commitment and differentiation in vitro prior to re-implantation. Such treatments may serve to hasten the in vivo lineage progression and thereby lead to more rapid and consistent bone formation. In the case of osteoporosis, however, providing culture-expanded MSCs to the patient through an intravenous or intramedullary route may lead to a renewed source of endogenous MSCs. In this setting, in vitro induction of osteogenic lineage progression would be undesirable, since the commitment of these cells would not allow their perpetuation as MSCs following re-implantation or infusion into the host. By providing undifferentiated stem cells, the patient would also have a renewed cache of precursors available for other mesenchymal tissues that may benefit from an MSC storehouse. In this way, either the host's natural repertoire of bioactive factors or injected biopharmaceuticals will influence the differentiation of the newly increased repository of autologous MSCs. Pharmacologic manipulation of MSCs, either in vitro or in vivo, will require identification of the regulatory molecules discussed above. With one or more of these factors in hand, the opportunities for therapeutic implementations are apparent.

In order to effect osseous repair in a local defect, the cells must be delivered to the site in an appropriate carrier. We envision the ideal vehicle as biocompatible to minimize inflammation, osteoconductive to foster integration, resorbable to promote its own replacement, supportive of mesenchymal stem cell attachment, and porous to facilitate rapid vascularization. In many ways, this vehicle would functionally resemble hypertrophic cartilage of the growth plate or fracture callus. Since the behavior of cells depends largely on local microenvironmental cues, the ideal vehicle must also be compatible with the correct bioactive factor and matrix milieu. Since MSCs in normal bone repair are in direct contact with type X collagen or calcified cartilage, delivery vehicles with these components on their surface could be very effective. Alternatively, since osteoblasts normally function by secreting, residing on, being influenced by, and eventually mineralizing oriented fibrils of type I collagen [Nakamura and Caplan, 1994], one may argue that a vehicle that provides this as a substratum for cell attachment would be advantageous. This type of surface might be particularly useful for delivering cells that were first stimulated in vitro to become osteogenic. In this paradigm, lineage-progressed osteoblasts would be seeded upon a type I collagen matrix and, therefore, cued for immediate phenotypic expression once implanted into the host.

Many of the above criteria for an ideal delivery vehicle are met by porous calcium phosphate ceramics. Our collective experience with these materials and marrow-derived MSCs has led to an optimization of the conditions for cell adhesion, cell retention, and initial loading density [Dennis et al., 1992; Dennis and Caplan, 1993]. Following subcutaneous implantation of ceramic cubes, loaded with marrow or purified MSCs, into syngeneic animals or athymic mice, bone and cartilage are observed within the pores of the cube [Ohgushi et al., 1989a, 1990; Goshima et al., 1991a,b; Nakahara et al., 1992; Haynesworth et al., 1992b]. Bone formation consistently occurs on the walls of the pores as the synthetic product of monolayered sheets of secretory osteoblasts. Vasculature is always seen immediately behind this layer of osteogenic cells; this vasculature provides the nutrient source for this phenomenon. Cartilage is observed only in dead-end pores which are devoid of blood vessels. Therefore, this system again highlights the obligatory role which angiogenesis plays in the support of osteogenesis. If made of calcium carbonate, this ceramic cube would be quickly resorbed by host osteoclasts [Damien and Parsons, 1991] and replaced by host-derived bone and marrow, so that eventually no trace of the original delivery vehicle would remain.

In a companion series of experiments, porous ceramic vehicles loaded with marrow MSCs were used to repair mechanically stabilized large segmental defects in weight-bearing bones [Ohgushi et al., 1989b]. In 1–2 months, the MSCs differentiated into bone and effected a repair of the large gap. In regions where vasculature could not penetrate, for example, at dead-end pores or in regions of very high cell density, stem cells differentiated into chondrocytes and formed cartilage. Again, for the repair of local defects, it should be possible to expose MSCs in vitro to powerful bioactive factors, which can activate or stimulate osteogenic differentiation, and then use these cells in appropriate delivery vehicles as noted above. Not only can this mode of treat-

ment be accomplished in a very precise manner with small amounts of purified material, but the in vitro treatment focuses only on the responding MSCs; deleterious side effects of systemic administration on other cells or tissues are avoided.

Recent studies [Wakitani et al., 1994] of cellular implants in full-thickness osteochondral defects in rabbit femoral condyles revealed that committed chondrocytes could not be driven by local cues to become hypertrophic. Consequently, chondrocytes in the subchondral element of the implant failed to be resorbed and replaced by trabecular bone. By contrast, MSCs implanted in parallel studies differentiated into the chondrogenic lineage throughout the defect site. Those cells in the vicinity of the articular cartilage progressed through the lineage enough to become functional chondrocytes. However, those MSCs in the region of the subchondral bone transited through the chondrogenic lineage to become hypertrophic chondrocytes, which were replaced by marrow and trabecular bone in less than one month.

SUMMARY

The realization that the differentiation of a variety of tissues, including bone, involves the progression of cells from progenitors through complex multistep lineage pathways stimulated the search for MSCs. This search has resulted in several "rules" that appear to govern all processes involving MSC-mediated bone formation, repair, or regeneration:

1. Synthesis, repair, and regeneration of all mesenchymal tissues are dependent on an adequate supply of MSCs.
2. MSCs can be directed into the osteogenic, chondrogenic, or other lineages, depending on site of implantation, cell density, and specific molecular cues.
3. Vascularization of the MSC-rich site or MSC-loaded implant vehicle leads to, and is obligatory for, bone formation; lack of vascularization promotes cartilage formation.
4. The transition from chondrocyte to hypertrophic chondrocyte is necessary before active resorption can occur.
5. Turnover of surrounding mature tissue may be necessary to effect complete integration of MSC-matrix implants; for this reason, bone may be easier to regenerate than cartilage.
6. Cell implant vehicles must be fully and relatively rapidly resorbable to effect complete bone repair, including the normal remodeling process.

Our challenge for the future, as basic and clinical scientists, will be aimed at characterizing the relationship between as-yet-undiscovered bioactive factors and MSCs during lineage progression. Other areas requiring investigation include the role that extracellular matrix has on lineage progression, as well as the means by which mechanically driven forces influence MSC differentiation and bone formation in general. The molecular nature of hypertrophic cartilage resorption, leading to new bone formation, also needs further clarification. Potential interactions between drugs, local pathology, systemic disease and MSC behavior in health and in cell therapy represent extensive areas for future research. As our understanding of the control of these lineage steps becomes even more sophisticated, and we discover the critical regulatory cytokines and growth factors, we will be able to exert a profound influence on the behavior of MSCs, both in the laboratory and in patients. By combining MSCs with appropriately designed delivery vehicles, restoration and repair of damaged or absent tissue will be possible. We predict that an era of *autologous* cell therapy will evolve from these efforts, ushering in new technologies for the regeneration of skeletal and other mesenchymal tissues previously not capable of self-repair.

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