Growth Factors as Therapeutic Agents*†

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An Instructional Course Lecture, The American Academy of Orthopaedic Surgeons

Many of the diseases treated by orthopaedic surgeons reflect a failure of the function of specific populations of cells. Skeletal deformities often stem directly or indirectly from the abnormal function of the cells of the growth plate and preosseous cartilage. Impaired healing after an injury is a failure of resident and immigrant cells to restore the tissue at the site of injury. Degenerative osteoarthrosis results from a diminished ability of the cells to balance the repair and degradation of articular cartilage. Osteoporosis is a failure of bone cells to maintain normal bone mass and architecture. Thus, an understanding of the regulation of cell behavior may provide important insight into the cause of orthopaedic diseases, and the ability to control cell behavior would be a powerful tool in the treatment of these diseases. Recent advances in cell and molecular biology have brought us to the point that manipulation of cell behavior is both plausible and feasible.

One of the principal candidates for altering cell behavior is the class of molecules known as growth factors. These are small proteins that serve as signaling agents for cells. Despite being present in plasma or tissue at concentrations that are generally measured in billionths of a gram, growth factors are the principal effectors of such critical functions as cell division, matrix synthesis, and tissue differentiation in virtually every organ system.

The role of growth factors in orthopaedic disease has been the subject of considerable interest and investigation recently. This lecture will focus on four specific, relatively common musculoskeletal processes or disorders in which growth factors play an important role: skeletal growth, fracture-healing, repair of articular cartilage, and osteoporosis.

Growth Factors

To our knowledge, no comprehensive taxonomy has been developed for the factors that regulate cells. General terms such as hormone, cytokine, and growth factor are principally of historical interest. Specific terms, such as insulin-like growth factor, fibroblast growth factor, and platelet-derived growth factor, were derived from early descriptions of a factor's action or source, and the names given to these substances are best viewed not as meaningful descriptors of their function but rather as identifiers accepted by tradition. Some of the principal regulators of the skeleton are the insulin-like growth factors (IGFs), fibroblast growth factors (FGFs), transforming growth factor-β (TGF-β), platelet-derived growth factor (PDGF), and bone morphogenetic proteins (BMPs) (Table I).

Two classes of IGF, IGF-I and IGF-II, have been identified to date. IGF-I mediates many of the stimulatory effects of growth hormone on the skeleton, while IGF-II helps to regulate fetal growth[122]. IGF-I stimulates cell division and matrix synthesis by cartilage[122], bone[12,110], tendon[1], and muscle[25] cells.

Although originally identified as a product of platelets that is released at sites of injury, PDGF has been discovered to be a potent regulator of bone cells both on its own[46,93] and in concert with other factors[71,93].

The FGFs are a group of heparin-binding polypeptides that were originally found to have mitogenic effects on fibroblasts[32]. Members of this family regulate cell functions as diverse as mitogenesis, differentiation, protease production, and receptor modulation[123]. FGF-1 and FGF-2 (also called acidic FGF and basic FGF, respectively) have both been implicated in the regulation of cartilage and bone cells[26].

The TGF-β family of polypeptides is composed of at least five molecules and is itself a member of a super-

*Printed with permission of The American Academy of Orthopaedic Surgeons. This article will appear in *Instructional Course Lectures, Volume 46,* The American Academy of Orthopaedic Surgeons, Rosemont, Illinois, March 1997.

†One or more of the authors has received or will receive benefits for personal or professional use from a commercial party related directly or indirectly to the subject of this article. In addition, benefits have been or will be directed to a research fund, foundation, educational institution, or other non-profit organization with which one or more of the authors is associated. Funds were received in total or partial support of the research or clinical study presented in this article. The funding source was National Institutes of Health Grant AR31068.

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family that includes the BMPs, activins, inhibins, Müllerian inhibiting substance, Drosophila decapentaplegic gene complex, growth and differentiation factors, and other cell regulatory polypeptides. Although originally discovered because of their ability to promote cellular transformation from a normal to a neoplastic growth pattern, the members of this family are now recognized as multifunctional signaling molecules that can act both as inhibitors and as stimulators of cell replication[115].

The BMPs, close cousins of TGF-β, comprise a family of at least fifteen growth factors that were originally identified for their ability to stimulate *de novo* formation of bone[97,127]. When implanted into soft tissues, most BMPs are capable of stimulating a cascade of cellular events that closely mimics the process of endochondral ossification as seen in the growth plate and in normal fracture-healing. Precursor cells are recruited and differentiate into chondrocytes that manufacture cartilage matrix. The cartilage is then gradually replaced by bone as osteoblasts populate the site. Eventually, marrow elements fill the newly formed intertrabecular spaces. In the absence of additional stimuli, as when the BMP is placed in subcutaneous tissue, the new bone is resorbed. In osseous defects, such as calvarial drill-holes[48] or segmental resection defects of the femur[29], the newly formed bone is incorporated into the structure of the host bone and subsequently remodels. In addition, members of this family of growth factors have been reported to regulate other cell types, including chondrocytes[97], limb-bud cells[97], and bone-marrow stromal cells[136].

TABLE I

SELECTED GROWTH FACTORS THAT REGULATE SKELETAL TISSUE

Growth Factor	Principal Actions
IGFs (*somatomedins*)	
IGF-I*, somatomedin-C (Sm-C)	Mitogenic, anabolic, mediates some growth hormone actions
IGF-II*, multiplication stimulating activity (MSA), skeletal growth factor (SGF)	Mitogenic, anabolic, generally less potent than IGF-I
FGFs	
FGF-1† (acidic fibroblast growth factor)	Mitogenic, angiogenic, regulates cell differentiation
FGF-2† (basic fibroblast growth factor)	Mitogenic, angiogenic, regulates cell differentiation
TGF-β‡	Context-dependent, multifunctional
BMPs‡	Prototypes induce bone formation
PDGF§	Mitogenic

*IGF-I and IGF-II are structurally and biologically related to insulin.

†FGF-1 and FGF-2 are the prototypes of the fibroblast growth factor family, of which there are at least nine members.

‡The TGF-βs and BMP families are representatives of a large superfamily that includes at least five TGF-βs, fifteen BMPs, the activins, the inhibins, and other factors involved in morphogenesis.

§PDGF occurs as a dimer of subunits, termed A and B, and may therefore take three forms: AA, AB, or BB.

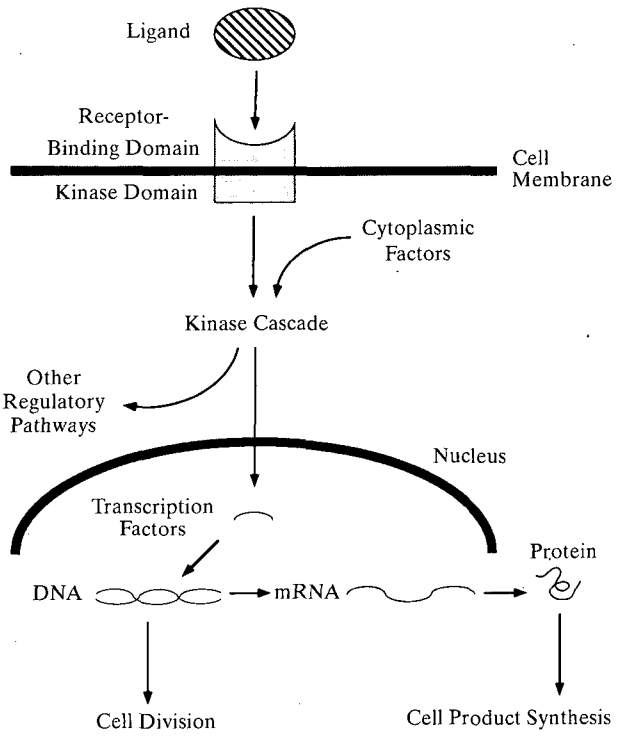

FIG. 1.

Schematic of the mechanism by which growth factors regulate cell behavior. A cell-signaling molecule (ligand) in the extracellular environment binds to the extracellular domain of its receptor. This ligand-receptor interaction activates the intracellular domain of the receptor. For many growth factor receptors, this kinase domain possesses the enzymatic ability to transfer phosphate groups to proteins (kinase activity), an important signaling step in intracellular communication. The activated receptor in turn activates a series of additional phosphorylation steps (kinase cascade) in association with other regulatory factors in the cytoplasm. This cascade culminates in the nucleus with the binding of transcription factors (proteins that bind to specific regulatory sequences of DNA) to activate gene transcription into messenger RNA (mRNA). The mRNA is then transcribed into protein to be used within the cell or exported for matrix production or other tissue functions.

Receptors

Growth factors elicit their cellular actions by binding to specific, large, transmembrane receptor molecules on their target cells. These receptors serve as information transducers, converting information carried by a growth factor into a form that is usable by the cell. The presence or absence of the receptor defines whether or not a cell can respond to the information in its external environment. In addition, receptors may add to the content of information carried by the signal molecule by integrating the message with information from the intracellular environment.

Growth factor receptors are linked by a cascade of chemical reactions in the cytoplasm to various genes in the nucleus[59] (Fig. 1). Often this cascade activates several genes at once. As a result, when use of a growth factor is considered to treat a specific cell defect, one must be aware that the factor may generate multiple effects, even within a single cell type. While these results may be advantageous (as when both cell division and

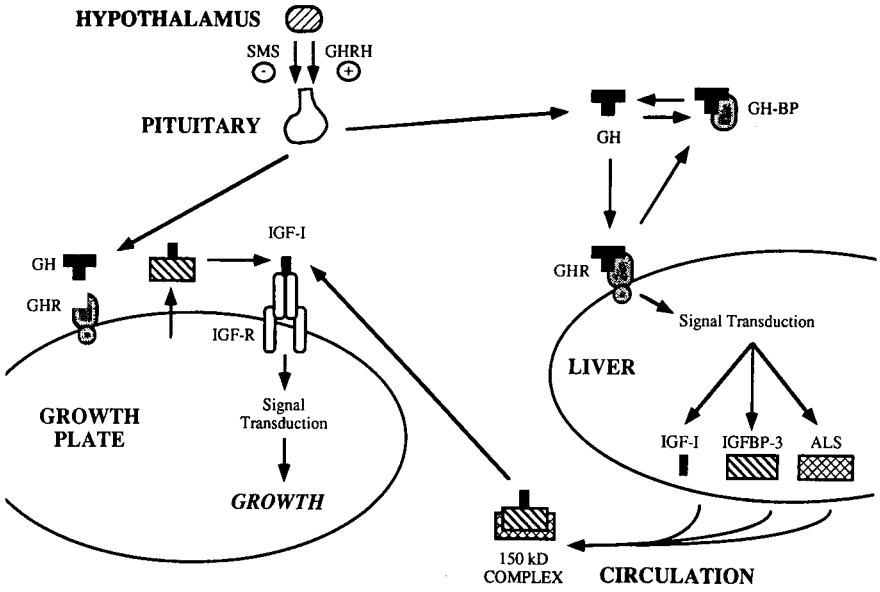

FIG. 2

Schematic of the regulation of bone growth by the growth hormone-IGF-I axis. The hypothalamus produces growth hormone-releasing hormone (GHRH) or somatostatin (SMS). Growth hormone-releasing hormone stimulates while somatostatin inhibits the release of growth hormone (GH) by the anterior pituitary. Growth hormone enters the systemic circulation in equilibrium with a binding protein (GH-BP) derived from the growth hormone receptor (GHR). Growth hormone binds to its receptors on liver cells, activating the synthesis of IGF-I and its predominant carrier protein, IGF-binding protein-3 (IGFBP-3). A second carrier protein, the acid labile subunit (ALS), combines with IGF-I and IGF-binding protein-3 to form a tripartite circulating complex (150 kD complex) that conveys IGF-I to its target tissues, including the growth plate. There, IGF-I is transferred to IGF receptors (IGF-R) on the chondrocyte membrane, initiating a cascade of intracellular reactions that regulate cell division and maturation. Growth hormone may also bind to growth hormone receptors on growth plate chondrocytes, stimulating IGF-I production by these cells for local regulation.

matrix synthesis are desired for a repair response), it is a theoretical disadvantage if so-called mismatched effects (for example, cell division and matrix degradation) are stimulated simultaneously.

Each family of growth factors has its own corresponding family of receptors. Despite marked differences in structure among receptor families, many of the key links in the gene-activating chain of reactions are shared by these families. Thus, binding of different growth factors to their respective receptors may lead to the same cellular response (such as cell division). Much more impressive than the similarities among post-receptor pathways, and much less well understood, are the differences. Many growth factors display pleiotropic activity, eliciting a variety of effects in different cell types or even in the same cell type at different stages of development. Although it is not yet clear how this remarkable versatility is achieved, these specific mechanisms probably will be important in the design of growth factor therapies that are capable of activating only certain genes and not others. Knowledge about receptors is crucial to the successful application of growth factors as therapeutic agents. Clearly, treatment with a growth factor will not help a problem caused by an abnormality in the receptor for that factor.

Treatment of Disorders of Skeletal Growth

Many disorders of skeletal growth may be viewed as disorders of the function of cells in the developing skeleton. Growth factors play an important role in both normal and abnormal skeletal growth and development. Indeed, some of the most dramatic successes in pediatric orthopaedics have resulted from the identification of deficiencies in cell-signaling molecules that are crucial to skeletal development, such as vitamin D in rickets, thyroid hormone in cretinism, and growth hormone in hypopituitarism. The importance of growth factors in skeletal development is emphasized further by the recent discoveries that the skeletal deformities of achondroplasia[104,113], Apert syndrome[134], Crouzon syndrome[96], Pfeiffer syndrome[82], and Jackson-Weiss syndrome[55] are all caused by mutations in receptors for the FGF family of signaling molecules.

The Growth Hormone-IGF-I Axis

Perhaps the best understood of the hormonal and growth factor systems that regulate skeletal growth is the growth hormone-IGF-I axis (Fig. 2). The hypothalamus elaborates growth hormone-releasing hormone, which stimulates the secretion of growth hormone by the cells of the anterior pituitary[102]. Growth hormone released into the systemic circulation is then carried to such target tissues as the liver and the growth plate. Binding of growth hormone to specific cell-surface receptors stimulates these cells to produce IGF-I. IGF-I may then enter the circulation to be carried to its target cells (endocrine action), may act locally by binding to cell-surface receptors on the same cells that produced it

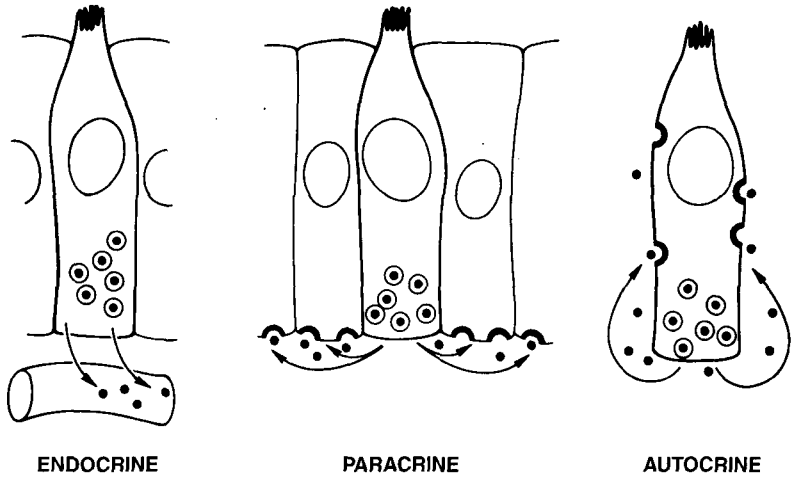

FIG. 3

Diagrammatic representation of endocrine, paracrine, and autocrine cell regulation. In the endocrine pathway, the cell-signaling molecules are released by the secreting cell into the circulation to act on distant target cells. In the paracrine pathway, the cell-signaling molecules are secreted locally to act on neighboring cells. The autocrine pathway is characterized by cellular self-activation. Cell-signaling molecules are represented by dots and their receptors, by thickened semicircles in the cell membrane. (Reprinted, with permission, from: Sporn, M. B., and Todaro, G. J.: Autocrine secretion and malignant transformation of cells. New England J. Med., 303: 879, 1980.)

(autocrine action), or may act on neighboring cells (paracrine action)[116,122] (Fig. 3). IGF-I helps to regulate its own synthesis by negative feedback on both the pituitary and the hypothalamus[6].

In the circulation and in tissue fluids, most IGF-I is bound to carrier proteins, termed IGF-binding proteins. The family of IGF-binding proteins consists of at least six structurally related proteins that transport IGF-I and modulate its actions on target tissues. Normally, IGF-binding protein-3 is the major binding protein in plasma. Like IGF-I, the production of IGF-binding protein-3 is dependent on growth hormone[17,67].

Disruption at any step of this growth-regulating system may be expected to disrupt normal skeletal development. If the specific site of abnormality can be identified, treatment can be initiated. For example, classic hypopituitary dwarfism is caused by deficient production of growth hormone by the cells of the anterior pituitary, and this disorder is readily treated with growth hormone replacement.

Growth Hormone Resistance

The cause of growth failure in individuals who do not have a growth hormone deficiency has, until recently, been less clear. In 1966, Laron et al. identified a family who had many features that are associated with a decrease in growth hormone but who, paradoxically, were found to have elevated circulating levels of growth hormone[68]. In its classic form, this syndrome, known as Laron dwarfism[101], is characterized by severe growth failure, normal or elevated secretion of growth hormone, and markedly reduced serum concentrations of both IGF-I and IGF-binding protein-3. The defect was eventually traced to the growth hormone receptor itself by demonstrating that cells from these patients were unable to bind growth hormone[24].

With the determination of the specific sequence of the human growth hormone-receptor gene, several abnormalities of the growth hormone-receptor gene that cause Laron dwarfism have been identified. Some patients have large deletions of the extracellular hormone-binding domain of this receptor. Most have only a point mutation, in which one of the many nucleotide bases spanned by the receptor gene is substituted by another nucleotide. More than twenty such point mutations have been identified to date[99]. Each of these mutations alters the amino acid sequence of the receptor, presumably interfering with its ability to recognize or bind to growth hormone and preventing growth hormone from initiating the signal-transducing function of the receptor. In some cases, the mutation appears to disrupt the dimerization, or pairing, of growth hormone receptors that is required for signal transduction[63].

Not all children who have mutations of the growth hormone-receptor gene manifest the features of complete growth hormone insensitivity. Recent studies have revealed that heterozygous mutations in the growth hormone receptor, in which only one of the two gene copies is abnormal, may produce a deficiency in skeletal growth. Clinically, these patients appear to have a partial growth-hormone insensitivity. Thus, there is a continuum of growth hormone responsiveness ranging from complete insensitivity, as in Laron dwarfism, to partial insensitivity, as in short stature[31].

Patients who have growth hormone insensitivity are, unfortunately, unresponsive or only marginally responsive to growth hormone therapy[31]. However, because the stimulatory effects of growth hormone on skeletal growth are thought to be mediated by IGF-I and because individuals who have abnormalities of the growth hormone receptors have low serum concentrations of IGF-I, these patients are candidates for

treatment with IGF-I, and several clinical trials of such treatment are now under way. To our knowledge, these trials represent the first reported use of a growth factor to correct an abnormality in skeletal development.

The largest study of IGF-I therapy reported to date involved thirty patients, three to twenty-three years old, who were treated for six to twelve months[135]. All but the oldest patients (who were twenty and twenty-three years old) responded with growth rates that were an average of two centimeters a year greater than the rates before treatment. Although a few of these patients responded to the relatively low dose of forty micrograms of IGF-I per kilogram of body weight twice daily, most needed as much as 120 micrograms of IGF-I per kilogram of body weight twice daily to achieve this degree of growth acceleration.

One randomized, prospective, double-blind placebo-controlled study has been reported thus far[39]. Children who had a deficiency of the growth hormone receptors were managed with twice-daily subcutaneous injections of IGF-I (120 micrograms per kilogram of body weight) for twelve months or with a placebo for six months and then IGF-I for six months. Treatment with the growth factor alone produced a significant ($p < 0.05$) increase in the rate of growth from an average of 2.9 centimeters a year before treatment to an average of 8.6 centimeters a year after treatment. This response was sustained over the one-year course of therapy. IGF-I therapy also resulted in noticeable increases in the size of the feet and the length of the lower segment compared with the measurements after the placebo therapy. No changes were detected in the circumference of the head, the brachial circumference, the arm span, or the weight of the patient. Interestingly, the mean cumulative increase in bone age for the subjects who had received IGF-I therapy for the full twelve months was only nine months. This suggests that this regimen of IGF-I treatment accelerates skeletal maturation, resulting in premature termination of growth and hence an ultimate shortening of stature. Currently, however, the effect of IGF-I treatment on ultimate stature remains unknown. These studies on IGF-I therapy are still in the early stages, and information about the optimum dose and frequency of administration is just being obtained.

Complications Related to IGF-I Therapy

Side effects and toxicity can limit the usefulness of any novel therapy. IGF-I has been associated with a variety of side effects and complications. Most, but not all, have been minor and include electrolyte shifts, changes in serum concentrations of insulin and growth hormone, hypoglycemia (sometimes severe enough to cause convulsions), hypercalciuria, pseudotumor cerebri, convulsions, papilledema, facial nerve palsy, parotid swelling, and tachycardia[131,135]. In the only available placebo-controlled trial that we know of, the subjects managed with IGF-I therapy had higher prevalences of upper respiratory infections, transiently abnormal liver-function tests, anti-IGF-I antibodies, and transient papilledema than those who were managed with a placebo[39]. Curiously, the most common complication of IGF-I therapy was hair loss. This occurred in six of seven patients, and it was followed by the regrowth of thick, curly hair that was coarser in quality than the original hair[39]. Although the administration of any mitogen (IGF-I is mitogenic for multiple cell types) raises the concern of tumorigenicity, currently this does not appear to be a side effect of IGF-I therapy.

The growth response to exogenous IGF-I incidentally clarifies the mechanism of its action on the cells responsible for skeletal growth. The clinical data discredit the previously popular dual-effector theory of growth regulation[36]. This theory postulated that precursor cells in the growth plate could respond to the mitogenic action of IGF-I only after they had been induced to differentiate by a previous exposure to growth hormone. The current data suggest that IGF-I is fully capable of stimulating skeletal growth by itself. However, a role for growth hormone itself has not been excluded. At the very least, growth hormone stimulates the production of IGF-binding protein-3 and a protein called the acid labile subunit, which combine with IGF-I during transport. It is also of note that in most studies of the treatment of growth hormone insensitivity with IGF-I the growth response was not quite equal to that observed following treatment of previously untreated growth hormone-deficient children with growth hormone. This may be because of the failure of IGF-I therapy to increase serum concentrations of IGF-binding protein-3 and acid labile subunit, creating a short half-life for serum IGF polypeptides.

Although the preliminary results of studies[39,135] of the use of IGF-I to regulate skeletal growth and development have been encouraging, it remains to be established whether this growth response is sustained over the full course of normal skeletal development, leading to a substantial increase in adult stature.

Role of FGFs

Progress in the application of other growth factors to the treatment of skeletal disorders is much less advanced. This is, in part, because the role of growth factors in the etiology of specific disorders has been recognized only relatively recently. A case in point are the FGFs. This family of polypeptides was named for its ability to stimulate fibroblasts but subsequently was found to influence multiple cell types, including cartilage. The chondrocytes of the growth plate have been known for some time to be under the mitogenic influence of FGF[61,126]. However, it has been only in the last few years that FGF receptors on these cells have been identified[53] and characterized[124] or that the mammalian FGF-receptor genes have been cloned[58]. Thus, it was not until 1994 that mutations in the receptors for the FGF

family were discovered to be the cause of an expanding group of chondrodysplasias, including achondroplasia, the most common genetic form of dwarfism[55,82,96,104,113,134].

Unlike the growth hormone-IGF-I axis, the actions of the FGF family do not appear to involve a cascade of events involving multiple tissues and mediators. There is no currently available means of bypassing the FGF-receptor defect in achondroplastic dwarfism in a fashion analogous to the use of IGF-I to circumvent the growth hormone-receptor defect in Laron dwarfism.

Ethics of Intervention

The availability of therapeutic agents that enhance skeletal growth raises several ethical questions. Who is to be considered "too short"? In an era of increasing focus on the cost-effectiveness of all therapies, what is the value of a centimeter of stature? Of ten centimeters? If growth factor therapy proves to be capable of increasing normal growth to supranormal levels, should this therapy be used to augment height that is not abnormal but that would become "desirably abnormal" with treatment? Clearly, progress in the application of growth factors to disorders of skeletal development requires advances in ethics and socioeconomics as well as in the necessary science and technology.

Treatment of Fractures

The response of bone to structural damage is nearly unique in biology. Most injured tissues heal with a fibrous scar, the cells and structure of which are unable to assume the function of that tissue. In contrast, bone and a few other tissues (such as the cornea and, to a limited extent, the liver) are capable of true cellular, morphological, and functional restoration. The initial phase of fracture-healing is characterized by an inflammatory response and the consolidation of a hematoma within the fracture site. This is followed by the proliferation of periosteal, endosteal, and marrow stromal cells adjacent to the fracture as well as the recruitment of undifferentiated mesenchymal cells from nearby soft tissues. These cells and their progeny differentiate to become chondroblasts, chondrocytes, osteoblasts, and osteocytes. Cartilage is formed and is eventually replaced by woven bone that is then remodeled to a more mature lamellar bone[40].

Although 90 to 95 per cent of the estimated 5.6 million fractures that occur annually in the United States heal satisfactorily, the remaining 5 to 10 per cent progress to delayed union or non-union[95]. In these adverse circumstances, agents with the ability to ensure or to accelerate the repair process could be of great benefit. Growth factors are, by virtue of their ability to regulate cell behavior, excellent candidates to serve as such agents.

Several growth-promoting substances have been identified at the sites of healing fractures and hence are believed to play a role in the healing process[7,20]. Among these are the TGF-β family, BMPs, FGFs, IGFs, and PDGFs.

TGF-β

The broad range of cellular activities regulated by TGF-β include the proliferation and expression of the differentiated phenotype of many of the cell populations that make up the skeleton: chondrocytes, osteoblasts, and osteoclasts[114]. *In vivo* studies have shown that, during endochondral ossification, chondrocytes and osteoblasts synthesize TGF-β that accumulates in the extracellular matrix[60]. Indeed, the largest source of TGF-β in the body is the extracellular matrix of bone[15].

An *in vitro* study performed with fracture-callus organ cultures demonstrated TGF-β messenger RNA (mRNA) in the soft callus (which is mostly cartilage) approximately fourteen days after a fracture and in the hard callus (which is mostly bone) on the fifth and fifteenth days[11]. A recent *in situ* hybridization study of normal human fracture-healing *in vivo* localized TGF-β mRNA to areas of proliferating mesenchymal tissue, cartilage, and bone[2]. Lower levels of mRNA were found in cells in the fracture hematoma and in hypertrophic chondrocytes[2]. Because the level of mRNA generally reflects gene activity and because mRNA is the template from which protein is synthesized, these data suggest that a fracture activates the genes for TGF-β, increasing the levels of TGF-β mRNA and thus the synthesis and release of TGF-β itself.

Several studies have tested the hypothesis that exogenous administration of TGF-β can stimulate the repair of bone defects[4,69,85]. Exogenously applied recombinant human TGF-β stimulated the recruitment and proliferation of osteoblasts in defects in the skulls of rabbits[4]. In the presence of TGF-β, there was rapid deposition of bone matrix and normal remodeling whereas, in its absence, the defects failed to heal[4]. In another study, native human TGF-β was found to augment the healing of tibial fractures in rats in a dose-dependent manner[85]. In a mid-diaphyseal tibial osteotomy model fixed with a plate in adult rabbits, the continuous administration of TGF-β in doses of one or ten micrograms a day for six weeks through an implanted osmotic pump increased maximum bending strength and callus formation[69].

BMPs

In 1965, Urist demonstrated that the demineralized matrix of bone graft was capable of inducing *de novo* bone formation[127]. Purification of the active component of the demineralized bone graft led to the discovery of a family of regulatory molecules called bone morphogenetic proteins. Although the BMPs were originally studied in their crude form (as demineralized bone matrix)[8,23], recombinant human BMPs are now available and have been used in more recent *in vivo* studies of bone repair. The clinical utility of the BMPs has been explored in animal models by several investigators and

is presently under investigation in human trials.

Recombinant human BMP-2 in combination with an inactive demineralized bone-matrix carrier induced formation of endochondral bone in a dose-dependent manner in five-millimeter segmental defects in the femora of rats[138]. Similar results were obtained in a sheep model with use of a 2.5-centimeter non-healing osteoperiosteal segmental defect of the femur stabilized with a plate and screws[29]. On radiographic examination and on mechanical testing, the healing of the defects treated with BMP was comparable with the healing of defects treated with autogenous bone graft[29]. Similar results have been obtained with the use of a closely related protein, osteogenic protein-I (OP-1 or BMP-7), to treat a 1.5-centimeter ulnar defect in rabbits[19].

Recently, BMP-1, another polypeptide isolated from the osteogenetic fractions of bone, was identified not as a growth factor but as the procollagen C-proteinase that cleaves the carboxy termini of procollagens I, II, and III to yield the major fibrous components of bone and cartilage matrix[64]. Thus, the BMPs are involved not only in the support of cellular activities but also in the chemical modification of the extracellular matrix[64].

Curiously, there are few reports describing the effects of BMPs on the healing of fresh fractures (as opposed to segmental defects) or of non-unions, despite the fact that it is in these two human situations that these molecules would be expected to have their greatest clinical utility. An early exception is, however, encouraging. Heckman et al. demonstrated that a bone extract enriched on canine BMP significantly ($p < 0.03$) increased new-bone formation in a radial non-union model in dogs[44].

FGFs

Members of the FGF family, like those of the TGF-β and BMP families, are both present in and act on bone. The local injection of FGF-1 (acidic FGF) in a rat fracture model produced an enlargement of the fracture callus in the cartilage-formation stage of repair[56]. This apparent stimulation occurred despite an inhibition of expression of the cartilage matrix gene (type-II procollagen and proteoglycan core protein mRNA)[56].

FGF-2 (basic FGF) has been evaluated with the use of an operatively created fibular fracture model both in normal rats and in streptozocin-induced diabetic rats, a model in which fracture-healing is impaired[62]. Immunohistochemical staining for FGF-2 showed that endogenous FGF-2 was widely distributed around the fracture site in the normal rats one and three weeks after the fracture but was reduced in the diabetic rats. One application of FGF-2 in fibrin gel, immediately after the fracture and before closure of the wound, facilitated the repair process in the normal rats and restored the impaired healing process in the diabetic rats. Specifically, treatment with FGF-2 increased the volume and the mineral content of the callus in both the normal and the diabetic rats and stimulated callus formation in the diabetic rats to levels that were similar to those in non-treated normal rats. The action of FGF-2 on bone appears to be complex, as illustrated by the observation that a low dose (fifteen nanograms) of FGF-2 stimulated the induction of bone when injected into the marrow cavity of bone implants in rats while a high dose (1900 nanograms) had a profoundly inhibitory effect[132].

IGFs

The pivotal role of IGF-I in the regulation of the endochondral ossification of the growth plate[125] suggests that it may also participate in endochondral ossification during fracture repair. *In vitro* studies have demonstrated that, in bone cells, IGF-I regulates both the formation of bone matrix and the replication of cells[47]. *In vivo*, systemic administration of IGF-I has been shown, both radiographically and histologically, to potentiate the healing of five-millimeter defects in the zygomatic arches of rats[120]. In another study, eight-millimeter full-thickness calvarial defects (which do not heal spontaneously) in rats were treated systemically with two milligrams of IGF-I by means of a subcutaneous osmotic infusion pump[119]. The defects in animals that received IGF-I for fourteen days healed by proliferation of cortical bone from the margins of the defect[119]. These data suggest that IGF-I may enhance the repair of intramembranous bone defects, but its therapeutic potential in endochondral fracture repair remains unknown.

The putative stimulatory effect of IGF-I on bone repair may also be invoked indirectly. Because IGF-I is growth-hormone dependent, IGF-I levels may be increased *in vivo* by the administration of growth hormones. Several investigators have tried to find out whether growth hormone augments fracture repair, with some discovering that it stimulates healing[65] and some, that it has no effect[87]. A recent study of the biomechanics of fracture-healing in a rabbit tibial model revealed that, with the numbers available, growth hormone treatment could not be shown to produce a significant effect on serial *in vivo* measurements of fracture stiffness or on *ex vivo* measurements of load to failure[13]. In addition, there was no association between the biomechanical properties of the healing fracture and the serum levels of IGF-I. In this rabbit model[13], as in some human fractures, there was a persistent nutritional deficit that may have accounted, at least in part, for the failure of the growth hormone to increase circulating levels of IGF-I. Taken together, these reports indicate that additional studies are required to establish a therapeutic role for either growth-hormone-elicited or direct IGF-I treatment of fractures.

IGF-II, a close relative of IGF-I, is among the most abundant growth factors in bone[27]. The biological actions of IGF-II are similar or identical to those of IGF-I, probably because they are transduced largely or exclusively by the same receptor on the cell surface. Although

IGF-II binds with a lower affinity than IGF-I to the type-I IGF receptor and is generally less potent than IGF-I in stimulating cells, it circulates at higher concentrations than does IGF-I. It is thus possible that IGF-II plays a role in fracture-healing. Of considerable interest in this regard is the recent observation that externally applied magnetic fields stimulate the production of IGF-II in both human osteoblast-like cell cultures and explanted rat fracture callus cultures[107].

PDGF

PDGF is another polypeptide-signaling molecule with the ability to regulate bone cells. In a rabbit tibial osteotomy model, a single injection of PDGF increased the density and volume of callus compared with those in controls[84]. In addition, non-quantitative histological analysis suggested that there may have been a more advanced stage of osteogenesis around the osteotomy sites treated with PDGF; however, mechanical testing showed no improvement in strength in response to this treatment[84].

Therapeutic Implications

The full therapeutic potential of these growth factors probably will be realized when they are combined appropriately with other growth factors. As a case in point, PDGF, IGF-I, and TGF-β have each been shown individually to generate a dose-dependent increase in bone matrix apposition in an *in vitro* fetal rat calvarial model[93]. In combination, however, the stimulatory effect of these factors was greater than that of any of the individual factors alone[93]. Similar results have been observed *in vivo*. The combination of PDGF and IGF-I delivered locally in a methocellulose gel to cortical defects in adult Yucatan miniature pigs significantly increased the area ($p < 0.05$), perimeter ($p < 0.01$), and percentage ($p < 0.05$) of mineralized tissue within the callus[71]. In contrast, no significant differences could be detected in the measured parameters of callus formation in defects treated with either PDGF or IGF-I alone[71].

The therapeutic application of growth factors to stimulate fracture-healing faces a variety of challenges. Not only must the growth factors lack immunogenicity, side effects, and toxicity, but they also must be available in a delivery system that is user-friendly both to the cells at the fracture site and to the orthopaedic surgeon treating the fracture. A second challenge is to demonstrate efficacy convincingly. For example, to establish that the prevalence of non-unions is reduced by treatment with growth factor requires large numbers of patients and carefully designed experimental protocols that standardize the many variables associated with the development of non-unions. Similarly, to demonstrate that treatment with growth factor accelerates the rate of healing of fresh fractures necessitates both large numbers of patients and meticulously objective and reproducible follow-up to document the progress of healing. Additionally, in this era of cost-consciousness in health care, the cost-effectiveness of any new treatment must be demonstrated.

Repair of Articular Cartilage

Articular cartilage provides the low-friction bearing surface that is essential for motion of a joint. Adult articular cartilage is hypocellular, avascular, alymphatic, and insensate, and it has a severely limited capacity to heal after damage caused by injury or disease. The considerable effort devoted to elucidating the response of articular cartilage to injury or disease has shown that this tissue retains the ability to mount a repair response and that the type of response depends on the type of injury[5,10,18,28,30,73,76,77,105,121].

Studies of growth factors in cartilage repair vary considerably with respect both to design and to the variables analyzed. Most *in vitro* studies of cartilage have been performed with cultured cells, a method that has well established limitations. For example, cells in the cartilage line very quickly lose the cartilage phenotype under standard passaged monolayer conditions and convert to a fibroblastic form. This loss of phenotype can be prevented by a variety of culture techniques, including use of three-dimensional or suspension medium, high cell density, primary culture, or short culture duration, or a combination of these. Although isolated cell systems are the most common method used to study chondrocytes, the question always remains whether the response seen truly represents *in vivo* cell behavior. Tissue or explant culture, wherein pieces of cartilage are maintained in a nurturing medium, is a more lifelike model for studies of chondrocytes, as the matrix interactions with the cells as well as the cartilage phenotype are preserved. Of course, the best studies are *in vivo* studies in which the cartilage cells function in their normal environment.

In addition, investigators obtain cartilage cells from a variety of anatomical sites, including the growth plate, the nasal septum, the ears, and the cartilaginous portion of the ribs. Studies are performed on cells from subjects of all ages; these cells are from embryonal limb buds, fetal cartilage, cartilage from skeletally immature animals, and cartilage from mature and aged animals. Add to this the fact that some growth factors produce different results when used alone as opposed to in combination with other factors, or the fact that the effect of a growth factor varies according to the maturational stage of the cell in culture, and one can appreciate the difficulties faced by investigators in sorting out the mysteries of growth factor and cell interactions.

Under normal circumstances, cartilage, like bone, exists in a steady state of matrix degradation and rejuvenation. The collagen in cartilage turns over slowly, but at least some of the proteoglycans have a fairly rapid turnover[74]. The processes of matrix breakdown and re-

placement are mediated by catabolic and anabolic cell-signaling molecules. The anabolic side of the balance may be augmented by growth factors. IGF may be important in the maintenance of the cartilage matrix, and deficiencies in its amount or effectiveness may contribute to the development of osteoarthrosis[124].

FGF is a powerful mitogen in connective tissues, including cartilage in culture[89,108]. FGF-2 may also stimulate the synthesis of matrix constituents, including proteoglycans and collagen. However, there are some interesting variations in the response of cartilage to FGF-2 that depend, at least in part, on the age of the tissue donor. In one study, low doses of FGF-2 in young bovine explants stimulated anabolic processes, while higher doses stimulated catabolic processes[109]. In adult bovine cartilage, low concentrations of FGF-2 accelerated the release of proteoglycan, but with increasing concentrations these growth factors tended to normalize the synthesis of proteoglycan, protein, and collagen[109]. Thus, FGF-2 may be capable of stimulating both reparative and degradative behavior in articular cartilage. The functional importance of this seemingly paradoxical phenomenon remains to be determined.

TGF-β is produced by articular chondrocytes and resides in cartilage matrix, albeit in a latent form. It can be activated by decreased local pH or by proteases[9,81,98,117]. Various *in vitro* investigations into the role of TGF-β in cartilage revealed quite different cellular responses. In some studies, TGF-β increased the synthesis of collagen[45,52], proteoglycan[79,81,112], and inhibitors of matrix breakdown[66] and also stimulated cell proliferation[29,37,100]. In other studies, TGF-β decreased chondrocyte collagen[49,103] and proteoglycan[16] production and inhibited cell proliferation[117]. The apparent discrepancies among these reports probably depend on the cell type studied and on the experimental conditions, as TGF-β has been found to exert different and even opposite effects depending on its dose or on the stage of differentiation of the target cell[14,103,130]. In human chondrocytes[38], there is an age-related decrease in the mitotic response of chondrocytes to TGF-β. This decrease could play a role in the degeneration of cartilage. On the other hand, explants of human osteoarthrotic cartilage have been reported to produce more proteoglycan than explants of normal cartilage in response to TGF-β, suggesting an increased responsiveness in association with this disease[94]. Interestingly, TGF-β has been shown to promote cartilage phenotypic expression in explant cultures of periosteum[88]. In summary, the *in vitro* action of TGF-β on cartilage is variable. Depending on the culture conditions under which it is tested, it may either promote or impede repair processes. Because it exists in the cartilage matrix in latent form, TGF-β may, in theory, be activated when repair is needed. Although the normal mechanisms that regulate the transition between inactive and active TGF-β are unclear, they, like TGF-β itself, could hold therapeutic promise. Optimistically, it appears that in osteoarthrosis[94] (a circumstance calling for repair) the chondrocytes become more responsive to at least some of the anabolic actions of TGF-β.

Several growth factors have recently been tested in *in vivo* models of articular cartilage repair. Such studies have been limited in number and scope, but the results have been encouraging. Fibroblast growth factor has been reported to facilitate the healing of superficial lacerations in cartilage in *in vivo* rabbit models[21,133]. IGF-I administered intra-articularly in conjunction with intramuscularly administered sodium pentosan polysulfate (an inhibitor of matrix catabolism) reduced the severity of the disease in a canine model of osteoarthrosis in which the anterior cruciate ligament had been removed and maintained levels of neutral metalloproteinase, tissue inhibitor of metalloproteinase collagenase, uronate, and hydroxyproline at nearly normal levels[99]. The effect of TGF-β on articular cartilage *in vivo* is controversial. It has been reported not only to produce considerable loss of cartilage proteoglycan but also to stimulate proteoglycan synthesis and content in articular cartilage[129]. Recent *in vivo* studies have indicated that TGF-β may augment the repair of partial-thickness defects in articular cartilage through a mechanism that involves cell recruitment from the synovial membrane[57].

Treatment of Osteoporosis

Osteoporosis is characterized by progressive loss of bone mass and increased fragility of bone that predisposes to fracture. Osteoporotic fractures are a major source of morbidity and mortality among the elderly, and agents that can prevent such fractures are increasingly needed as the population ages.

Bone formation is a complex but highly ordered process that is most likely triggered as osteoclasts discontinue resorbing bone[83]. However, the signal that triggers bone formation is apparently brief and leads to sequential changes within the cells themselves, which produce subsequent cellular and molecular effects. As bone is resorbed, growth factors are released in active or locally activated form. These factors may have chemotactic and proliferative effects on osteoblast precursors. Once bone cells begin to differentiate, they express other growth factors that may produce later osteoblast differentiation. Primary cultures of fetal rat calvarial osteoblasts, for instance, express BMPs as the cells differentiate to form mineralized bone nodules[41,42]. Enhanced new-bone formation results from the exogenous addition of BMPs to these osteoblast cultures[83]. The orderly formation of bone that occurs under normal physiological conditions may be the result of endogenous expression of BMPs by differentiating bone cells.

Patients who have established osteoporosis need to be managed with agents that substantially increase the formation of bone, but no widely acceptable agent is currently available. The development of such agents will

likely come from an increased understanding of the factors that control normal bone formation. The complex and highly integrated process of bone formation in adult humans generally occurs at sites of previous osteoclastic bone resorption. However, endochondral bone formation occurs in growing long bones, and appositional bone formation may appear without previous local resorption (particularly on periosteal surfaces) during growth. Among the cellular events involved in the formation of bone are chemotaxis of osteoblast precursors; proliferation of committed osteoblast precursors; differentiation, including expression of growth regulatory factors and the structural proteins of bone such as osteocalcin, osteopontin, and type-I collagen; and, lastly, mineralization of the proteinaceous extracellular matrix formed by the osteoblasts. Tight regulatory control of these cellular events is essential. The events can be modulated by hormones such as calciotropic hormones, parathyroid hormone, 1,25-dihydroxyvitamin D, and other systemic hormones (for example, the pituitary and thyroid hormones and sex steroids), but the predominant regulators are likely to be local factors or cytokines generated in the bone-cell microenvironment.

The growth factors present in bone may be released in active form during the process of bone resorption and stimulate bone formation *in vivo*, albeit acting at different sites in the osteoblast lineage. In addition to being stored in the bone matrix[43], all of these growth regulatory factors are also present at other sites in the bone microenvironment. Some are stored in the alpha granules of the platelets, some are expressed by the osteoclasts, and some are expressed by other types of bone cells or mesenchymal stromal cells.

TGF-β

TGF-β is released from matrix-binding proteins in the active form, making it capable of targeting cells for specific biological effects[90]. Its effects on osteoblasts are complex. The major effect of TGF-β is apparently stimulation of osteoblast proliferation, presumably increasing the pool of committed osteoblast precursors[42,75,86]. TGF-β has also been shown to cause osteoblast chemotaxis[92]. Histomorphometry studies have demonstrated that, when injected into the subcutaneous tissue over the calvarium of rodents or infused into the marrow cavity of rats, TGF-β causes a prominent increase in bone formation *in vivo*[72,75,86]. *In vitro*, TGF-β inhibits osteoblast differentiation, although it stimulates osteoblast proliferation[41,42]. Its stimulatory effects on bone formation *in vivo* are evidently due to its powerful mitogenic effects on osteoblast precursors. Its effects on osteoclasts are even more complex. TGF-β appears to stimulate some osteoclasts while inhibiting others. TGF-β inhibits osteoclast formation[91,118] and promotes osteoclast apoptosis[50]. However, it may actually increase osteoclastic activity under certain circumstances. For example, when injected locally over the calvarium, TGF-β stimulates osteoclastic bone resorption within the adjacent marrow cavity[75], possibly as a result of the stimulation of local prostaglandin synthesis[42,118].

BMPs

Although BMPs are members of the extended TGF-β family, their effects on bone cells differ markedly from those of TGF-β. *In vitro* studies have shown that BMPs actually enhance the differentiation of bone cells[41,42]. Additionally, they may play a role in directing cells along the osteoblast lineage. *In vivo* studies have revealed that BMPs also possess the unique property of stimulating ectopic bone formation[127,137]. On injection into the subcutaneous tissue of rats, BMPs produce local accumulation of ectopic bone, forming an ossicle complete with marrow cavity and marrow cell constituents. Such findings have spurred the current interest in developing BMPs as therapeutic agents for diseases in which enhanced bone formation is needed.

FGF

Like TGF-β, the FGFs enhance cell proliferation. They probably increase the pool of osteoblast precursors[54]. They do not share the effects of TGF-β on bone resorption. Once again, these agents effectively restore bone mass and stimulate new-bone formation. It has also been discovered that an N-terminal extended form of basic FGF stimulated bone growth-factor activity produced by a human tumor associated with bone formation *in vivo*[54].

PDGF

Another powerful bone-cell mitogen is PDGF. Although PDGF receptors are frequently expressed in bone cells[33-35], the reported actions of PDGF *in vivo* are variable. When delivered systemically in an osteoporotic rat model, PDGF increased the compressive strength of the vertebral bodies and the torsional stiffness of the femoral shaft[78]. When injected locally over the calvarium in mice, PDGF stimulated both new-bone formation and an increase in bone resorption. This dual effect may limit its therapeutic potential.

IGFs

In vitro studies[47] have demonstrated powerful bone formation in response to IGF-I. *In vivo* studies[3] have provided additional support to the idea that IGF-I has an anabolic effect on bone. In an ovariectomized rat model of osteoporosis, the systemic administration of recombinant human IGF-I for eight weeks promoted periosteal and endosteal bone formation, reduced the endosteal resorption induced by oophorectomy, and increased trabecular thickness[3]. IGF-I did not increase the number of trabeculae. Interestingly, the delivery of IGF-I complexed to its carrier protein, IGF-binding protein-3, may augment these actions.

The actions of IGF-I on human bone are beginning

to be elucidated. When delivered by subcutaneous injection to eighteen postmenopausal women for six days, IGF-I produced dose-dependent increases in serum concentrations of type-I procollagen carboxyl-terminal propeptide, an index of collagen synthesis, as well as in urinary excretion of deoxypyridinolone, an index of bone collagen breakdown[22]. These results demonstrate that short-term systemic administration of IGF-I increases bone turnover in normal women. These actions of IGF-I appear to be similar in osteoporotic men[57]. Although long-term studies are not available, six months of treatment with IGF-I in a patient who had osteoporosis associated with Werner syndrome produced an increase in the bone mineral density of the vertebral bodies, suggesting that IGF-I generated a net increase in bone formation[106].

IGF-II, like IGF-I, stimulates bone cells *in vitro*[111] and is abundant in bone matrix. Systemic hormones, such as parathyroid hormone, apparently regulate production of IGF-I by bone cells and may have similar effects[70] on the production of IGF-II. Currently, both agents are being used in patients in clinical trials in an attempt to increase bone mass. It is hoped that it will be demonstrated that factors such as IGF and the BMPs as well as other growth factors can produce beneficial effects without the limitations of toxicity and be a useful therapy for osteoporosis in the near future.

In conclusion, the armamentarium of the modern orthopaedic surgeon includes a remarkable array of techniques, instruments, implants, and devices with which to influence musculoskeletal tissues. In some instances (such as limb-lengthening), these methods manipulate the tissue biology. In others (such as stabilization of a fracture in an osteoporotic hip), they correct a mechanical problem without addressing the underlying tissue defect. In still others (such as total joint arthroplasty), they simply remove the diseased tissue altogether and insert a substitute. In short supply in this armamentarium are methods that are capable of restoring normal function to the cells whose malfunction is contributing to the disease. The ability to stimulate bone cells to restore normal bone mass and architecture in osteoporosis or to reverse the degradation and to repair the imbalance in the activities of articular chondrocytes in osteoarthrosis, in order to activate more effectively the cells responsible for fracture-healing, would mark major improvements in our ability to care for patients. Recent advances in cell and molecular biology have identified and begun to characterize the molecules responsible for regulating cell behavior. While, in the past few years, much has been learned about the effects of these factors on musculoskeletal tissues and a few notable therapeutic successes have been achieved, the understanding of their role in orthopaedic diseases and of their application in the treatment of these diseases remains rudimentary. With continued progress in the basic science and clinical investigation of these factors, it is probable that they will become the method of choice for the prevention and treatment of a variety of currently unsolved orthopaedic problems.

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