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CLINICAL APPLICATIONS OF RECOMBINANT GENE TECHNOLOGY: BONE AND CARTILAGE REPAIR

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Abstract

Over the past decade, the field of molecular biology has given rise to the development of the applied discipline of molecular medicine. Based on recent developments using recombinant gene technology, genetic mapping analysis and other investigational tools, the physician and surgeon is now ready to begin using those instruments in the diagnosis and treatment of musculoskeletal disease. This article reviews the history of scientific exploration in recombinant gene technology as it applies to bone and cartilage repair. Clinical cases are presented to show how the fruits of scientific knowledge may be brought to bear on some of the more challenging musculoskeletal problems. Although some of these examples may prove to be accurate representations of how molecular engineering will be used in specific clinical settings, the purpose of this review is to provide an orientation and philosophical approach to the applications of recombinant gene technology in traumatic and reconstructive surgery of the skeleton.

Key Words: Osteogenesis, Chondrogenesis, Growth Factor, Colony Stimulating Factor, Osteoporosis, Fractures, Non-union, Osteonecrosis, Joint Replacement, Bone Induction.

Introduction

The growth of scientific information appears to develop in a logarithmic-like fashion as opposed to a linear one. Each new piece of data acquired and each new technique developed leads to advances in scientific knowledge which accelerate technology by orders of magnitude. This growth of knowledge in the areas of bone and cartilage repair will soon be realized in our clinics and operating rooms. This article will briefly review the history of scientific developments which have lead up to the present state of technology in this field, and provide a vision of how this technology may be applied clinically.

To begin to understand how new scientific advances might be used in patients, it is important to identify the incidences and associated costs of the most commonly occurring conditions which require medical and surgical intervention. According to recent studies, it is estimated that approximately 200,000 bone graft operations[3] and 265,000 total joint procedures[4] are performed in the United States each year. In addition, 5.6 million traumatic fractures result in a 5-10% non-union rate[3] and about 1.5 million pathologic fractures are sustained in osteoporotic patients annually[16]. The cost to the American economy of osteoporosis alone is in excess of ten billion dollars[16].

Since cost has become a major factor in the allocation of medical services, scientists, universities, and biotechnology companies are now finding that cost plays an important role in directing research and development. It is important to recognize, however, that cost is measured in many ways. Work loss, early retirement, and monetary loss are among some of the costs incurred by a medical condition. It is therefore necessary to consider all costs when allocating expenditures to the study and treatment of specific diseases. It can be anticipated that the results of ongoing federally-driven outcome studies will determine which technologies are most cost-effective[17]. These findings will almost certainly lead to decisions on how research dollars are allocated in the future[17].

Table 1: Milestones in Induced Osteogenesis

1889	Senn[21]	First therapeutic use of decalcified bone (in canines)
1917	Neuhof[14]	Induced osteogenesis by urinary epithelium
1965	Urist[25]	Induced osteogenesis by demineralized bone matrix (DBM)
1968	Urist[26]	First successful use of decalcified bone in patients
1972	Reddi and Huggins[15]	Induced osteogenesis as model of cellular differentiation
1981	Sampath and Reddi[20]	Extraction and reconstitution of the bone inductive property from DBM
1988	Wozney *et al.*[30]	Recombinant production of BMPs

Historical Perspectives

The progress of research on bone and cartilage repair has been punctuated by the important contributions of several key investigators (Table 1). One of the first pioneers in this area was Senn, a late 19th century surgeon. Using decalcified bone from the ox tibia as a delivery system for an antiseptic (iodoform), Senn attempted to treat osteomyelitis in the skulls of dogs[21]. Although his main purpose was to eradicate the infections, he reported that this treatment led to the osseous healing of the bone cavities[21]. Later, in 1917, Neuhof described the formation of bone in a visceral site in the urinary tract site under the influence of implanted fascia[14]. Subsequent studies by Huggins[5] confirmed these findings.

In 1965, Marshall R. Urist demonstrated the induction of ectopic new bone in laboratory animals by the implantation of demineralized bone matrix[25]. Urist followed this report in 1968 by describing the first successful clinical use of demineralized bone in the treatment of ten patients[26]. These findings stimulated the interest of other scientists such as Reddi and Huggins[15] who showed that the phenomenon of induced osteogenesis was essentially a model of cellular differentiation. By the early 1980's, clinical investigators had begun using demineralized human allogeneic bone in the treatment of several osseous defects. The use of this demineralized bone matrix was applied to the reconstruction of maxillocraniofacial deformities[2, 11, 13], phalangeal cavities in the hand[24, 29] and in the augmentation of spinal fusions[27]. Today, this bone graft substitute material (DBM) is commercially available from several companies.

Using highly efficient protein purification technologies Sampath and Reddi[20] were the first to extract a specific bone inductive property from demineralized bone matrix. Urist succeeded in extracting a similar factor and he named this substance "bone morphogenetic protein" (BMP)[28]. By the end of the 1980's, orthopaedic trauma surgeons at UCLA Medical Center, under Urist's direction, implanted partially purified human BMP into phalangeal cavities[29], non-union defects in the tibia[9] and femur[8] and eventually fresh traumatic defects in the tibia[10]. Some of these operations were combined with local and free-flap soft tissue procedures to optimize the biological environment of the host bed. The early results demonstrated healing capacities similar to (and in some cases, better than) autogenous bone graft procedures. In most instances, the need to make an additional incision in order to harvest autogenous bone was obviated and, as a result, operative morbidity was reduced significantly.

Because it has been recognized that the yield of BMP from cadaveric allogeneic bank bone is limited, and because it has been learned that this inductive protein may work in a more optimal fashion when it is implanted along with other specific substances contained in DBM (e.g., transforming growth factor-beta) efforts have been initiated to develop recombinant technologies for the production of sufficient quantities of certain cytokines and growth factors. The scientific contribution of Wozney and co-workers[30] was an important step in that direction. Using highly technical cloning and DNA sequencing techniques, these investigators began to express these factors in large quantities for potential clinical use. Presently, experiments are being conducted to investigate the biological activities of specific factors and how they may work when used in combination with each other.

Research in the field of growth factor and cytokine function is one of the most active areas of scientific exploration to date. Although it would be ideal to be able to provide a list of the current known factors and how they are expected to function biologically, this information is not sufficiently clear at this time. Nevertheless, based on what is known presently, it can be anticipated that certain key factors will achieve a

therapeutic role in clinical medicine. BMP, for example, is one of a group of related peptides which are part of the TGF-β superfamily. Peptides in this family may have far reaching effects ranging from the regulation of cell replication and differentiation to the control of bone resorption. As noted above, BMP has already been shown to have a role in inducing osteogenesis in patients. Other factors such as the interleukins and hematopoietic growth factors have been shown to regulate specific cellular activities in certain types of diseases. By extrapolating these findings to other clinical settings, it is possible to envision a broad role for these factors in the treatment of musculoskeletal disease.

Clinical Applications

Based on the early clinical results of induced osteogenesis, clinicians can now envision a variety of applications of bone morphogenetic proteins and other biological factors. To imagine how these factors may be applied, it is necessary to identify the clinical conditions which would benefit most from their use. In addition, these applications must be considered in relation to the alternatives which are presently available. Table 2 shows a partial list of those conditions for which the application of biological factors may become important. Since an approach to improving upon the treatments of these conditions must be guided by specific goals, it is necessary to identify what it is that the clinician requires in order to achieve these objectives.

The factors whose applications are previewed in this article are specific polypeptide products of cells that can function as bone and/or cartilage growth factors, hematopoietic colony stimulating factors, attachment-promoting proteins, or other biologic response modifiers which induce specific target cells or affect the response of these cells to other stimuli. However, in any given clinical setting, the responses which the clinician would require these factors to modulate are not necessarily of equal importance. Therefore, it is necessary to prioritize these responses by first identifying clinical "needs" and following this by the identification of specific clinical "wants".

In terms of "needs", the clinician and/or scientist must begin with the premise that a wound will not heal and a bone will not grow unless there exists an optimal systemic environment, an optimal systemic response, an optimal local environment, and an optimal local response. It would therefore be desirable to have both locally and systemically injectable[6], or implantable[23], materials which could ensure that these conditions will be present (e.g., such as normalizing the systemic and local response in a diabetic patient who has a soft tissue wound of the foot). If technology could accomplish this goal and provide for these "needs", it might become apparent that the human organism already has the necessary biological mechanisms for healing many conditions.

Table 2: Clinical Applications of Biological Factors

Condition	Present Alternatives
Fracture Healing	Autograft/Allograft Ceramics Distraction Osteogenesis
Cartilage Repair	None (Continuous Passive Motion?)
Osteoporosis	Antiresorptive Drugs
Implant Stabilization	Autograft/Allograft Ceramics
Osteonecrosis	Core Decompression Vascularized Autograft Electrical Stimulation
Tumor Reconstruction	Allograft Metal Implant Distraction Osteogenesis Vascularized Autograft
Joint Reconstruction	Allograft Metal Implant
Osteopetrosis	Bone Marrow Transplantation
Wound Healing in Debilitated Patients	Debridement/Skin Grafts

Beyond the question of "needs" of course, is the question of "wants". Here the clinician is dealing with a situation whereby the biologic response desired is beyond the capacity of the human system even under the most optimal set of conditions (e.g., such as regenerating a dead femoral head or replacing a large hyaline cartilage defect in a joint). Factors which the clinician would "want" to have in order to accomplish these goals could include chemotactic, attachment, competence, progression, and inductive factors, as well as conductive surfaces, delivery systems and possibly pluripotent stem cells.

Chemotactic factors could be extremely valuable for the purpose of attracting the migration or ingrowth of progenitor cells to the area of a wound. Factors which may possess this activity include TGF-β and platelet derived growth factor (PDGF). Attachment factors could function not only to maintain these newly attracted cells in this wound repair environment but also to influence their proliferative capacity and optimize their phenotypic expression. Examples include fibronectin, osteopontin

and thrombospondin. Competence factors function to prepare a cell to undergo a change in its cell cycle and thus may favorably alter its response to exogenous signals. Insulin and PDGF may possibly function in this manner. Progression factors stimulate cell division and proliferation and result in the production of sufficient numbers of cells of a specific type to ultimately produce new tissue. A variety of factors may be capable of this activity. These would include TGF-β, PDGF, insulin-like growth factors (IGF-I and IGF-II), fibroblastic growth factors (αFGF and βFGF) and Interleukin-1. Inductive factors stimulate differentiation and differentiated function and could be of major importance in mediating wound healing and matrix production. Most members of the TFG-β superfamily, including BMP, are presumed to possess this ability.

To use these factors in patients, it will be necessary to have a delivery system which will bring the factors into contact with the target cells, protect them from immediate degradation in the host, and control the kinetics of their release such that their timing of appearance, local concentration, and inhibitory and stimulatory functions are properly coordinated. Finally, all of these factors could potentially lead to more rapid and predictable healing if, specifically responsive, immunologically controlled, pluripotent stem cells were available for local implantation.

With these considerations in mind, the following clinical cases are presented to show how molecular engineering may be applied to musculoskeletal disease:

Case Number 1 - Tibial Non-Union

A forty-one year old healthy white female slipped on an icy sidewalk and sustained a comminuted fracture of her right distal tibia and fibula (Figure 1). Standard conservative orthopaedic management in a cast led to delayed union after nine months. Pulsed electromagnetic stimulation for an additional three months failed to result in healing. The fracture was ultimately treated with open reduction and internal fixation. Healing occurred four months later. However, as a result of the long period (approximately 16 month) during which time she was unable to put weight on her leg, significant disuse osteoporosis developed throughout the tibia (Figure 2). The patient required an additional twelve months of intensive physical therapy in order to regain normal motion, bone mass and ambulatory function. As a result of her being out of work during most of this period of time, the patient lost her job and has not returned to the work force.

Comment

Prevention of non-union is perhaps the most obvious indication for the use of an osteogenic substance. If such a substance were to have been injected into the original fracture in the above case (and this could be done with a percutaneous needle and radiographic real-time visualization), perhaps the fracture would have healed within the expected time frame of three to four months. The patient would have been back to her normal activities within four or five month's time.

Currently, the Food and Drug Administration appears to be focusing on patients with existing non-unions as prime subjects for inclusion in studies to test the effects of osteogenic substances. While it is understandable why these patients are appropriate subjects for clinical trials of this nature (they already have a problem as opposed to being at risk for potentially developing one), it must be recognized that the response of a non-union to an implanted osteogenic substance may be entirely different from that of a fresh fracture. In the former, the host bed consists of an admixture of fibrous tissue and cartilage while in the latter, the host bed consists of a fresh hematoma in the midst of an acute inflammatory response. These differences could play significant and important roles in determining the types of cells present and the nature of their responses to injectable or implantable substances. Based on a knowledge of which types of fractures are at greatest risk for developing problems with healing, it should be possible to identify those fractures at the time of injury and intervene in a prophylactic manner using the appropriate inductive therapy.

Case Number 2 - Articular Cartilage Defect

A twenty-four year old healthy white female fell down a flight of steps and sustained a direct blunt contusion to her right knee. Eighteen months later, she continued to experience significant medial joint pain. An arthroscopic examination showed a 2.5 cm diameter area of cartilage degeneration (chondromalacia) without any evidence of healing. Treatment consisted of arthroscopic debridement and drilling of the subchondral bone in a weak attempt to stimulate a reparative response which could potentially lead to fibrocartilage production. Postoperative management consisted of a continuous passive motion protocol and intensive physical therapy. Currently, the patient is mildly improved but still experiences pain in the area of the original injury.

Comment

Most studies have shown that hyaline cartilage has very limited potential for tissue repair and almost no ability to heal large defects[18]. When articular cartilage injuries extend through the entire thickness of the cartilage and into subchondral bone, any healing response which is seen is usually initiated by the subchondral osseous tissue. Some reports suggest that motion of an injured joint in a continuous passive manner results in the formation of fibrocartilage which can provide articular

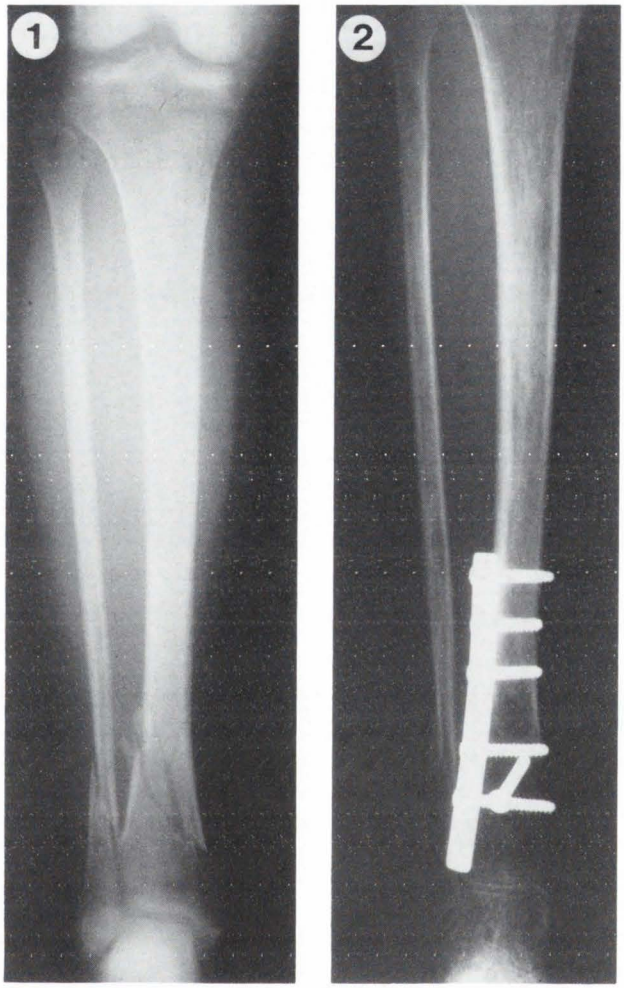

Figure 1 (at left). Anterior-posterior radiograph of the right lower leg taken immediately after ankle injury in a forty-one year old female. Note the presence of a mildly displaced, severely comminuted fracture of the distal tibia and fibula.

Figure 2 (at right). Anterior-posterior radiograph of the same lower leg shown in Figure 1 taken 16 months after the initial injury. Note the presence of plates and screws which were used to fix this fracture 12 months after injury. Although fracture healing is evident, there is now significant disuse osteoporosis.

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cartilage function[19]. How well tissue forms in large defects and how well it functions in the long-term remains an unanswered question.

With the advent of advanced arthroscopic surgical techniques and the development of new instrumentation, orthopaedic surgeons are now capable of doing many operations in joints using relatively atraumatic approaches. If a substance were available which could stimulate chondrogenesis, it should be possible to introduce it into the injury site in a joint with minimal surgical trauma. In a case such as the one presented above, one could envision a scenario whereby the patient undergoes an arthroscopic procedure in which the host bed is prepared by surgical debridement, followed by the coating of the exposed bone surface with a chondrogenic substance. Alternatively, an attachment-promoting material could be implanted (e.g., RGD-containing), and this could serve to anchor cultured chondrocytes embedded in a chondrogenic delivery system. Both of these techniques could be followed by rehabilitation protocols using continuous passive motion and other physical modalities to enhance the healing and functional recovery.

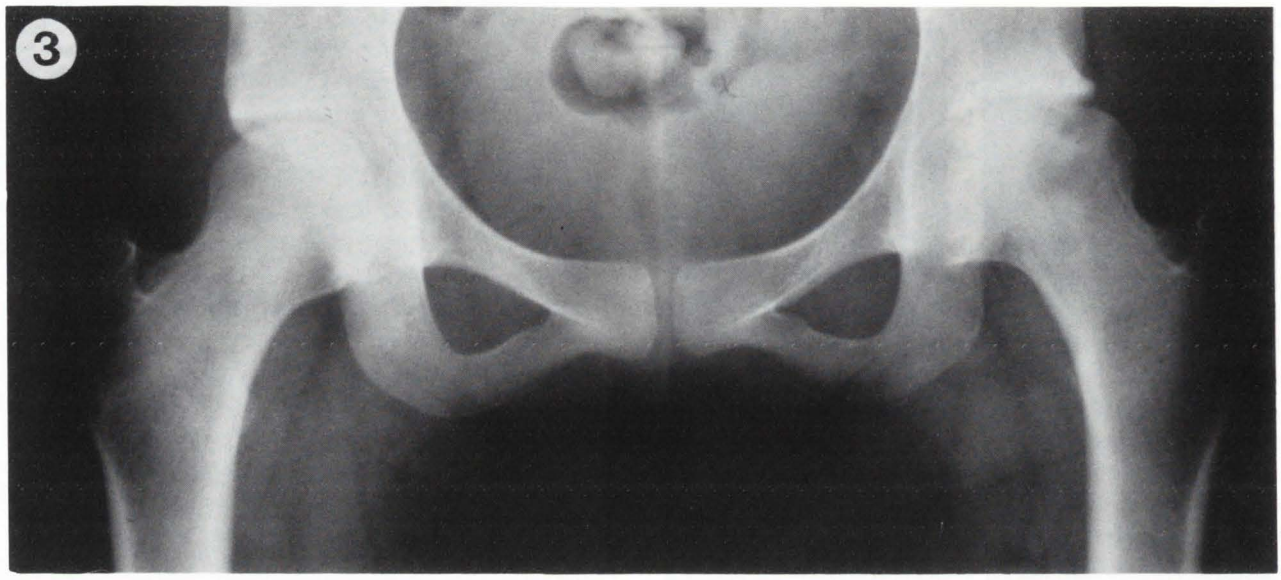
Case Number 3 - Total Hip Arthroplasty

A thirty year old black male developed spontaneous osteonecrosis of the hip. He presents with hip pain of one year's duration and an X-ray showing Stage IV osteonecrosis of the left femoral head (Figure 3). All attempts at conservative management have failed and no surgical procedure which would leave his own femoral head intact would appear to be effective. A total hip replacement was performed with a non-cemented, porous-coated prosthesis. The post operative X-ray (Figure 4) shows distal intimate contact between the stem and the endosteal surface of the femoral cortex. Proximally, the degree of bone-implant contact is less optimal.

Comment

This case describes an all-too-commonly occurring situation in which the only reasonable therapeutic option for a young patient with a diseased hip joint is an artificial joint replacement. While total hip replacement arthroplasty has been an extremely successful operation in the treatment of elderly patients with osteoarthrosis, its use in young patients has been much less successful. This has been attributed mainly to the fact that the long lasting durability of cemented implants is usually limited to not more than ten to fifteen years. While the new age of porous-coated hip implant technology has offered the hope that these implants will perform in a superior manner over time, the long term results are not yet available. It is presently thought that two factors which may influence these results will be, 1) the ability of bone to grow into the surface of the prosthesis, and 2) the way this ingrowth affects the stress environment and remodeling response of the skeleton. Biologically, one can only expect bone to grow into a prosthesis when the contact between the two is sufficiently intimate.

As examples of how a lack of bone ingrowth could potentially lead to problems, consider the radiographs of the two cases shown in Figures 4 and 5. As indicated above, Figure 4 shows grossly evident contact between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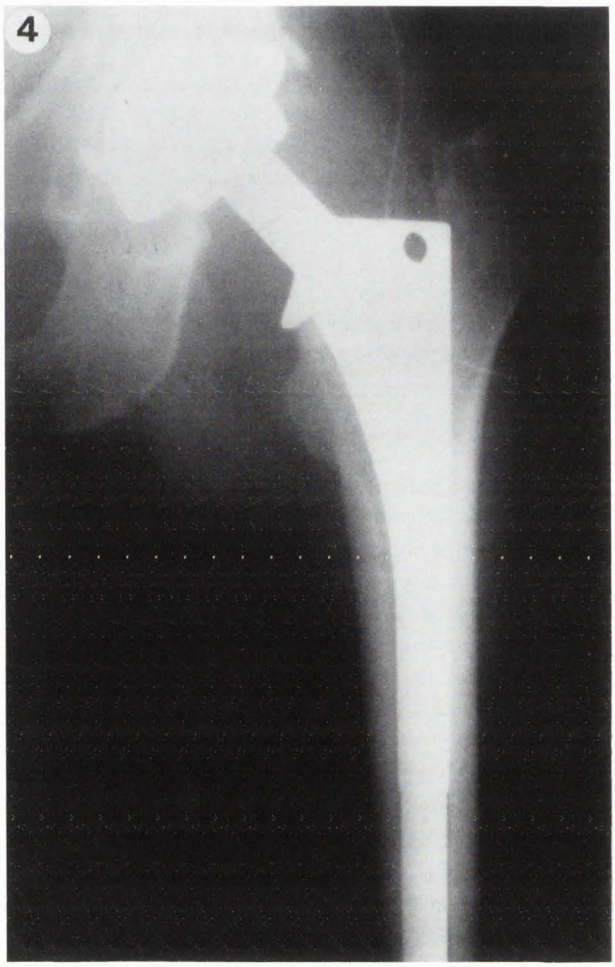
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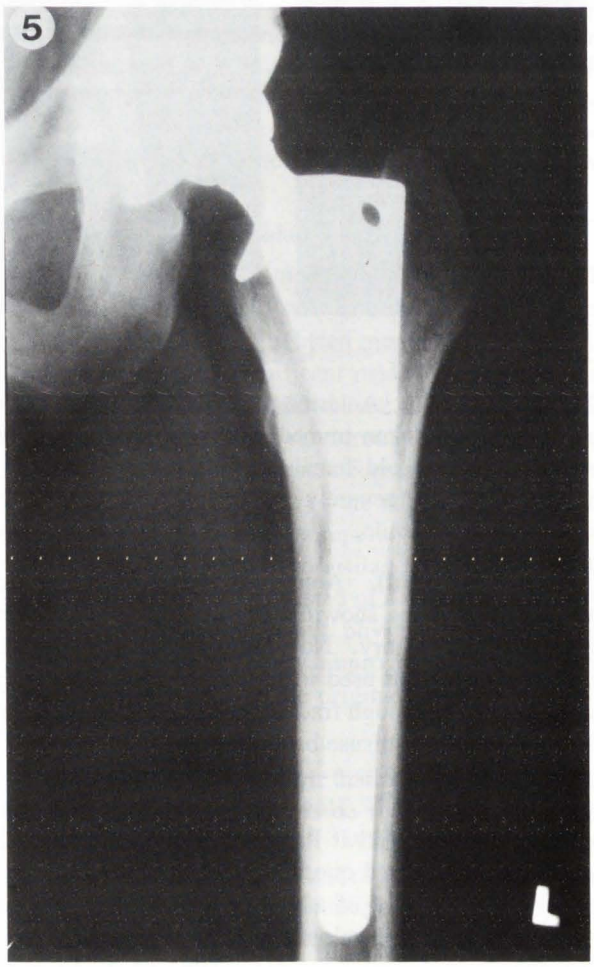
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Figure 3 (facing page at top). Anterior-posterior radiograph of the pelvis of a thirty-year old black male. Note the presence of Stage IV osteonecrosis of the left femoral head.

Figure 4 (facing page, bottom left). Anterior-posterior radiograph of the hip taken immediately after implantation of a non-cemented, porous-coated total hip system in the patient whose X-rays are shown in Figure 3. Note excellent distal bone-implant contact of the femoral component. Proximally, femoral bone implant contact is questionable.

Figure 5 (facing page, bottom right). Anterior-posterior radiograph of the same type of non-cemented, porous-coated total hip system shown in Figure 4. Note that in this patient, there is a complete line of radiolucency surrounding the entire femoral component suggesting poor contact between the implant and the bone.

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the distal part of the stem and the intramedullary canal of the femur. However, proximally, contact is questionable. The long term results of bone remodeling could potentially lead to bone hypertrophy distally with disuse osteoporosis proximally. This could result in a biomechanical cantilever system whereby proximal motion and distal fixation leads to fatigue fracture of the metal implant. In Figure 5, another young patient underwent a similar operation in which very little contact between the prosthesis and the bone existed anywhere. This implant remained unstable and required revision surgery within one year of the original operation.

The ability to obtain intimate contact between a prosthesis and the bone, and to do it in the appropriate places, is beyond the capability of even the most skilled surgeon. The development of a material which could be applied to a prosthesis to promote bone ingrowth, even without close and intimate contact, would make the technology of non-cemented joint replacement surgery potentially much safer and reliable. Since bone remodels around such implants in response to mechanical forces, the ability of the surgeon to determine where bone ingrowth should occur and where it should not, may enhance his or her ability to control the stress environment and optimize the results. Presently available materials such as hydroxyapatite coatings and different types of surfaces are only passively conductive and may ultimately prove to be inadequate for achieving these goals.

Case Number 4 - Traumatic Osteonecrosis of the Hip

A thirty year old healthy white male suffered a traumatic dislocation of his right hip while playing softball. An attempted closed reduction in a local emergency room converted this injury to a fracture dislocation (Figure 6). Open reduction and internal fixation was performed however, three months later, there was no evidence of healing and a bone scan showed photopenia of the right hip consistent with ischemic necrosis (Figure 7). As a result, this young patient underwent hip arthroplasty with a bipolar prosthesis.

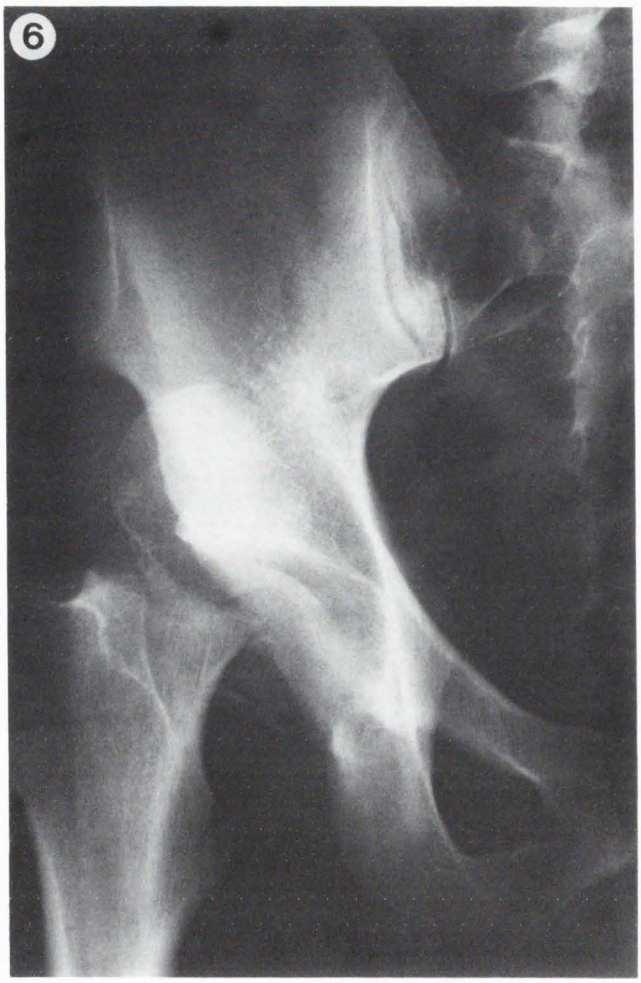
Comment

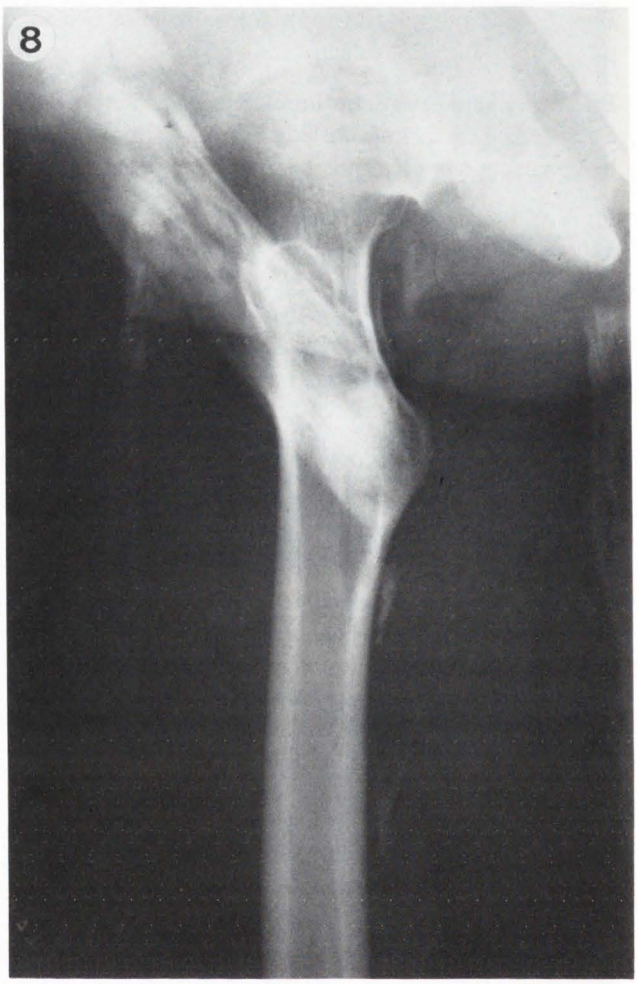
While the treatment of traumatic ischemic necrosis of the femoral head is still controversial, most femoral head-sparing procedures require that some significant portion of the femoral head remain alive. In this case, it is assumed that the entire femoral head was necrotic and thus not salvageable in its present form. Some have advocated using vascularized fibular autograft transplants or bone-muscle pedicle flaps from the lesser or greater trochanters but the successes of these procedures alone are unreliable. A more predictable and successful surgical outcome could potentially result if these vascularized transplants could be combined with growth factors and perhaps pluripotent stem cells. One could envision a procedure in which the necrotic femoral head is excavated such that it is hollow with the exception of a thin shell of subchondral bone and articular cartilage. Into this shell could be implanted a vascularized fibular autograft or bone muscle-pedicle flap. This graft could then be surrounded by a composite implant of inductive and cell attachment proteins incorporated in a delivery system to enhance osteogenesis.

Biologic Response Modifiers: Early Clinical Results

A variety of immunomodulatory proteins and hematopoietic colony stimulating factors have been used in the treatment of immunosuppressed patients. Several of these factors have undergone testing in phase I and phase II clinical trials and others have even achieved FDA approval for specific indications. Colony stimulating factors such as granulocyte-colony stimulating factor and granulocyte macrophage-colony stimulating factor have achieved FDA approval for the treatment of patients with failed bone marrow grafts and chemotherapy-induced leukopenia.

The preliminary clinical results from studies using these biologic response modifiers will pave the way for their potential applications in the treatment of musculoskeletal disease. Not only has it been shown that many of these factors are involved in bone and cartilage formation, turnover, and degradation, but specific metabolic bone diseases may be related to deficiencies of one or more of these factors. Recent evidence that a defect in the structural gene responsible for the production of macrophage-colony stimulating factor (M-CSF) is present in animals with a specific form of osteopetrosis[31], combined with evidence that treatment of these animals





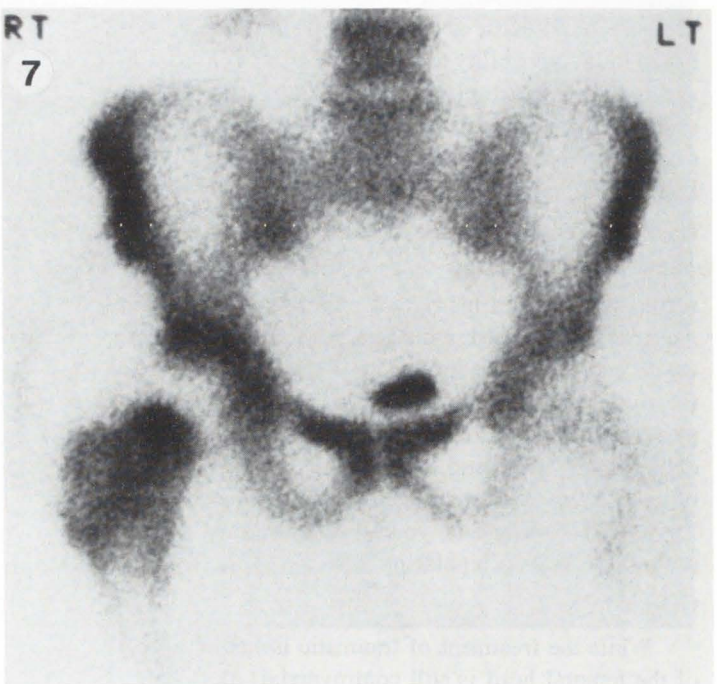


Figure 6. Anterior-posterior radiograph of the right hip in a thirty year old male who had just undergone attempted closed reduction for a hip dislocation. This X-ray now shows the presence of a complete fracture-dislocation of the femoral head and neck.

Figure 7. Technetium-99m methylene diphosphonate (MDP) bone scan of the pelvis of the patient shown in Figure 6. Note the increased isotopic activity in the acetabulum and trochanteric areas of the right hip with complete photopenia of the right femoral head. This study was interpreted as showing complete necrosis of the right femoral head.

Figure 8. Lateral radiograph of the hip in a patient with fibro-dysplasia ossificans progressiva. Note the presence of an ossified iliopsoas muscle originating in the pelvis, crossing the groin, and inserting on the lesser trochanter of the hip.

with M-CSF cures this condition[1], is an example of how a metabolic bone condition may respond to a hematopoietic colony stimulating factor. Further evidence that targeted disruption of the c-src proto-oncogene by homologous recombination leads to osteopetrosis in mice[22] may provide evidence to support the exploration of new genetic pathways of investigation of metabolic bone disorders.

Questions and Concerns

An important part of the development of any new medical technology is the careful and systematic evaluation of potential toxic effects. While some of these effects may be manifest as acute short-term problems, a much greater concern exists over the possibility of patients developing serious complications which may become obvious only after months or years of treatment. With regard to the clinical experiences using some of the biologic response modifiers, examples of these effects include fever, hypotension, hepatic and renal failure, myocardial infarction, capillary leak syndrome, and massive edema, to name a few.

Specificity is another important consideration in the development of a new therapeutic agent. The greater the specificity of a factor for its target cell, the greater control the clinician has over the patient's response. Furthermore, a biologic response, such as a wound healing process, may require the participation of several types of cells at different points in time. Therefore, the specificity of a factor may change at different times during the response period. The need to develop delivery systems which not only protect factors from degradation but also optimize their concentrations and kinetics of release may be critical to the success of any molecular product. Finding the optimal combinations of factors may also be critical to the success of this technology.

Although not yet observed, an ever present concern with regard to growth factors is their potential cancer producing effects. It would seem intuitive that any factor which has the ability to change a cell's behavior could possibly lose control over that cell and lead to the induction of a neoplastic process. One of the most important concerns with regard to the use of molecular engineering in treating diseases and developing new drugs will be the ability to interrupt an induced process once it has been initiated.

Expectations for the Future

How well the human organism will respond to cells, growth factors and other biological materials remains to be examined. While the responses in lower mammals have been encouraging, and while significant clinical effects have been observed in patients who have been treated with recombinant biologic response modifiers, the application of recombinant osteogenic or chondrogenic factors in patients has not been tested extensively.

Teleologically, it is recognized that bone regeneration in humans is possible. This is exemplified by the phenomenon of normal fracture healing by endochondral ossification. In this process, new mature bone actually regenerates in response to an injury. An extension of this phenomena which has been exploited in patients is the bone which is regenerated during distraction osteogenesis using the methods originally developed by Ilizarov[7]. In these cases, significant gaps and segments of the skeleton have been restored by surgical and mechanical manipulations.

One of the most impressive, and for the purposes of this discussion, interesting pathological conditions to affect the musculoskeletal system is fibrodysplasia (myositis) ossificans progressiva (FOP). This is a rare autosomal dominant disorder characterized by symmetrical congenital malformations of the blastemal anlage of the hands and feet and by the progressive heterotopic chondrogenesis and ossification of soft connective tissues[12]. It is unknown what triggers this mechanism (although it has been suggested to be related to an injury and repair process) and no treatment is known. It is an extremely disabling disorder particularly because the ossified soft tissues generally take the form of fascial planes associated with specific muscle groups. In many cases, this leads to the partial or complete immobilization of the involved joints. Figure 8 shows an ossified iliopsoas muscle crossing the groin of a twenty-four year old woman with FOP. This patient is completely unable to flex or extend her hip joint.

A recent review provided strong evidence to suggest that FOP is a genetic disorder characterized by a disturbed developmental expression of the endochondral program and represents a mutation resulting in a dominant gain of function[12]. It was shown that an array of developmental gradients (characteristic patterns of disease expression) similar to developmental anomalies induced by pleiotropic mutations of the decapentaplegic (dpp) locus in Drosophila melanogaster may be related to a 75% sequence homology between the protein encoded by the dpp locus in Drosophila and the C-terminal region of two recently cloned human bone morphogenetic proteins (BMP-2A, BMP-2B), members of the TGFβ superfamily. It was therefore suggested that the genetic predisposition to develop this phenotype in humans may be related to bone morphogenetic protein expression[12]. This case provides evidence that it may be possible to obtain, in humans, a significant and even massive osteogenic response to a bone morphogenetic protein-like factor. It also suggests that an array of responses in

humans may be possible by the proper molecular engineering of other factors in the TGFβ superfamily.

Much has been learned over the past ten years concerning the technologies discussed in this review. The need to develop these advances into safe and effective medical treatments is the challenge to today's investigator. The ability to provide these technologies to all patients rich and poor will require the cooperation of scientists, physicians, economists and government officials. As we enter the age of molecular medicine, the future looks bright for the treatment of musculoskeletal injury and disease.

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Discussion with Reviewers

J.M. Lane: The readers would benefit from a table of the growth factors and their proposed function.
Author: I fully agree with you. However, a careful and comprehensive review of the literature on growth factors shows that no such table has ever been developed. The most probable reason to account for this is the fact that the reported functions of these growth factors may be highly dependent on the conditions under which they were investigated. The same growth factor may have opposite effects in *in vitro* culture systems when compared to an *in vivo* setting. Moreover, there may be a dose dependency of growth factor function such that at low concentrations it may have one set of functions while at high concentrations it has another. In several places in the text, I have attempted to indicate how a specific factor may function based upon what has been shown. However, it would be misleading to provide a table which would simplify the information on this subject since the information unfortunately cannot be simplified in this manner.

J. Glowacki: With regard to prevention of non-unions by treating fresh fractures with bone-promoting substances, what is the frequency of non-unions in various bones and what are the predisposing features?
Author: The most accurate data on the frequency of occurrence of musculoskeletal injures is documented by a variety of subcommittees of the American Academy of Orthopaedic Surgeons. Presently, it is estimated that there are 5.6 million fractures sustained in the United States each year of which 5 to 10% result in non-union. The most common bones affected are the tibia, humerus, femoral neck and carpal schapoid however, case reports have shown that nearly every bone in the body is capable of developing a non-union under certain conditions. The features which generally predispose to non-union are infection, excessive comminution and bone loss, extensive soft tissue injury, poor blood supply, poor systemic nutrition, inappropriate surgical management, and failure of the patient to comply with the surgeon's instructions. The use of a bone-promoting substance at the time of initial fracture treatment may be effective in preventing a large number of these cases.

M.E. Bolander: It may be important to point out that there are several bone morphogenetic proteins, and that these proteins are related to a family of growth factors called the TGF-β superfamily.
Author: Originally, the bone inductive substances were individually studied and names such as bone morphogenetic protein, osteogenin, and osteogenic protein-1 (OP-1) were used. While these names still exist, it has now become evident that they are all members of a larger group of peptides called the TGF-β superfamily. This family of peptides now includes several BMPs, all of the TGF-β's, as well as other substances such as activins, inhibins, and the decapentaplegic gene expressed in Drosophila. Undoubtedly, as time goes on, other peptides will be identified within this family and the functions of each peptide will become more clear. What is important to understand is that there is significant homology and overlap between the various substances identified and the potential clinical applications of each of these substances may possibly be enhanced by their combined use.