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Growth Factors and Wound Healing: Part II. Role in Normal and Chronic Wound Healing

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Wound healing is a complex biologic process that involves the integration of inflammation, mitosis, angiogenesis, synthesis, and remodeling of the extracellular matrix. Part II of this two-part series reviews the results of experiments that indicate that growth factors and their receptors regulate key aspects of soft and hard tissue repair. Results of clinical studies are also reviewed that demonstrate that growth factor treatment accelerates healing of normal tissues and promotes healing of impaired wounds.

Wound healing is a complex biologic process that is well characterized at the microscopic level, but its regulation is poorly understood at the molecular level. Data now indicate that peptide growth factors and their receptors regulate key processes of wound healing. Full-thickness skin incisions have been studied extensively as a general model of wound healing, and the general principles of wound healing that have been learned from that model can be used to formulate hypotheses about the roles of growth factors and their receptors in wound healing.

Healing of skin incisions progresses through three general stages: (1) an inflammatory stage, (2) a proliferative and repair phase, and (3) a remodeling stage. There is considerable temporal overlap of these stages of healing, and the entire process can last many months. The inflammatory stage lasts several days and is initiated by the processes of blood clotting and platelet degranulation. Contained within the α-granules of platelets are several protein growth factors, including platelet-derived growth factor (PDGF), insulin-like growth factor-I (IGF-I), epidermal growth factor (EGF), and transforming growth factor-β (TGF-β), which are potent chemotactic factors for inflammatory cells [*1*]. The burst of growth factors released from platelets quickly diffuses from the wound, and neutrophils rapidly enter the wound, followed by macrophages. Macrophages in the wound begin to synthesize and secrete additional growth factors including TGF-β, TGF-α, macrophage-derived growth factor (MDGF, a PDGF-like protein), basic fibroblast growth factor (bFGF), and heparin-binding epidermal growth factor (HB-EGF). The growth factors secreted by macrophages stimulate migration of fibroblasts, epithelial cells, and vascular endothelial cells into the wound [*2*].

As the fibroblasts and other cells migrate into the site of injury, they begin to proliferate, and the cellularity of the wound increases. This begins the proliferative and repair phase, which often lasts several weeks. As the number of inflammatory cells in the wound begins to decrease, other cells in the wound, such as fibroblasts, endothelial cells, and keratinocytes, continue to synthesize growth factors. Fibroblasts secrete IGF-I, bFGF, TGF-β, PDGF, and keratinocyte growth factor (KGF). Endothelial cells produce bFGF and PDGF. Keratinocytes synthesize TGF-β, TGF-α, and keratinocyte-derived autocrine factor (KAF). These growth factors continue to stimulate proliferation, the synthesis of extracellular matrix proteins, and new capillary formation.

After the initial scar forms, proliferation and neovascularization cease, and the wound enters the remodeling phase, which usually lasts several months. During this

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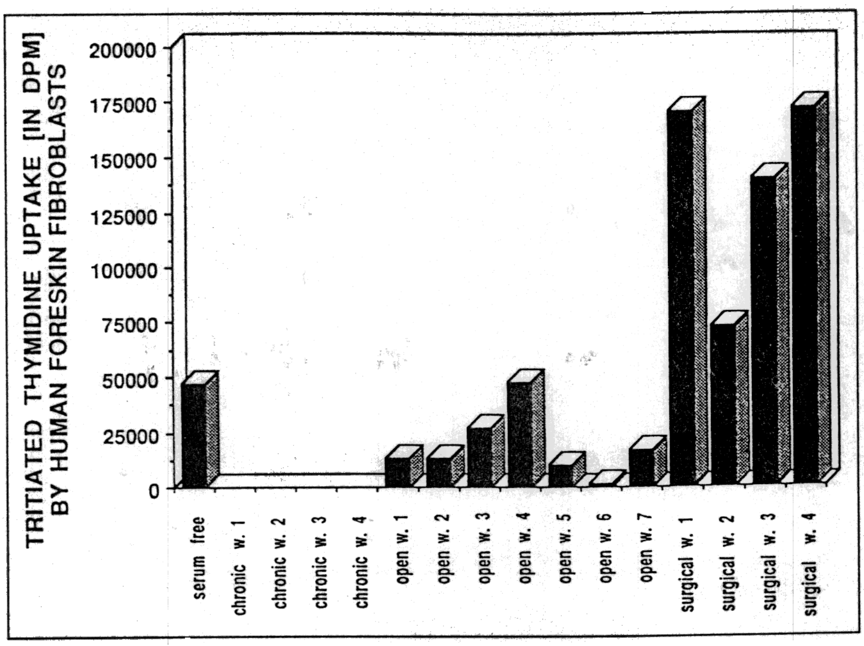

Figure 1. Effect of wound fluids on DNA synthesis of normal human fibroblasts in culture. Fluids collected from closed surgical wounds, open wounds, or chronic wounds were added to cultures of human fibroblasts, and DNA synthesis was measured by incorporation of tritiated thymidine. Mastectomy fluids stimulated high levels of DNA synthesis, open wound fluids stimulated lower levels of DNA synthesis, and chronic wound fluids were inhibitory.

last phase, a balance is reached between synthesis of new components of the scar matrix and their degradation by proteases such as collagenase.

Assuming that this model for the role of growth factors in wound healing is correct, several hypotheses can be proposed and tested. First, protein growth factors should directly regulate many of the processes crucial for normal wound healing, including chemotactic migration of inflammatory cells, mitosis of fibroblasts, keratinocytes, and vascular endothelial cells, neovascularization, and synthesis and degradation of extracellular matrix components. Second, the environment of healing wounds should contain protein growth factors, and their levels should change during the course of wound healing. Also, wound healing should be impaired if a growth factor is prevented from acting. Third, the environment of chronic wounds should have low levels of growth factor activity. Fourth, treatment of chronic wounds with growth factors should promote healing.

BIOLOGIC EFFECTS OF GROWTH FACTORS

Proteins from each of the five major growth factor families (EGF, PDGF, IGF, TGF-β, and fibroblast growth factor [FGF]) have significant chemotactic activity *in vitro* for wound cells. Interestingly, growth factors often have some degree of selectivity for the type of cell that they attract. For example, TGF-β is an extremely potent chemotactic factor for human peripheral blood monocytes, with an optimal concentration of 0.5 pg/mL [*3*]. PDGF is chemotactic for fibroblasts but not for monocytes. bFGF and IGF-I are chemotactic for vascular endothelial cells [*4*], and EGF stimulates chemotactic migration of epithelial cells [*5*].

All growth factors stimulate mitosis of one or more cells involved in wound healing. However, each growth factor exhibits some degree of selectivity. EGF and TGF-α are very effective for epithelial cells. bFGF is an effective mitogen for fibroblasts and vascular endothelial cells. IGF-I and PDGF primarily stimulate mitosis of mesodermal cells, such as fibroblasts and smooth muscle cells. TGF-β is a bifunctional regulator of cell division, stimulating mitosis of cells derived from mesoderm, such as fibroblasts, but inhibiting mitosis of cells derived from ectoderm, such as keratinocytes (for a review of growth factor actions, see Part I of this review series).

Synthesis of extracellular matrix components is increased substantially by several growth factors. EGF stimulates synthesis of fibronectin [*6*]. TGF-β stimulates synthesis of collagen, elastin, and tissue inhibitors of metalloproteinases while decreasing production of collagenase [*7*]. Neovascularization is also induced by several growth factors including bFGF, EGF, TGF-α, and TGF-β [*8*]. These experimental data demonstrate that growth factors regulate many of the processes crucial for normal wound healing including chemotactic migration of inflammatory cells, mitosis of wound cells, neovascularization, and synthesis of extracellular matrix components.

Several models have been used to determine if growth factors are present in the environment of healing wounds and whether their levels change during the course of healing. TGF-β and IGF-I levels were measured in wound chambers implanted under the skin of rats. Average levels of TGF-β were low 2 days after implantation, then peaked at 7 days, and decreased by 16 days [*9*]. A substantial amount of IGF-I was present on day 7 after implantation (0.4 μg/mL) [*10*]. In humans, mastectomy drain fluids were assayed for PDGF and MDGF [*11*]. PDGF-AA was highest in the immediate postoperative period and was absent by 36 hours. No PDGF-BB form was found. MDGF levels remained elevated during the 36 hours of the evaluation. Levels of immunoreactive TGF-α in mastectomy drain fluid peak on day 3 after surgery (unpublished data). Thus, growth factors are found in the environment of spontaneously healing

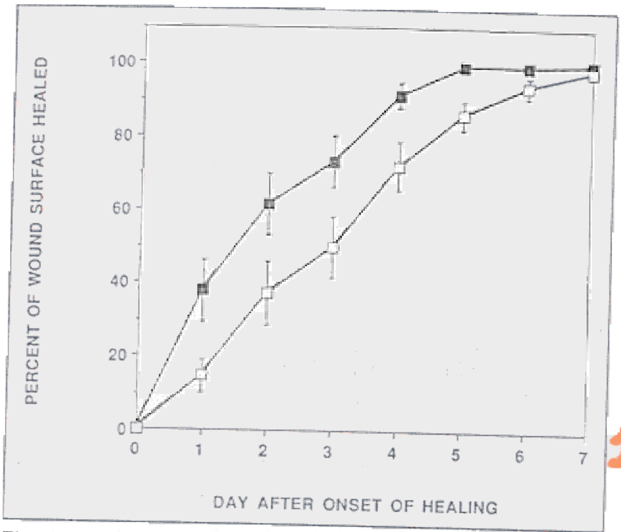

Figure 2. Effect of epidermal growth factor (EGF) on healing of partial-thickness dermatome injuries. Paired dermatome injuries were created in 12 patients and treated twice daily with EGF (■) or vehicle (□). Healing is expressed as the percentage of the original wound area that was epithelialized. Significant increases in healing were seen in the EGF-treated wounds on days 1 to 5.

wounds, and their levels change during the course of wound healing.

A few studies have reported attempts to inhibit the actions of growth factors in wounds. Treatment of rat incisions with neutralizing antibodies to TGF-β caused fewer macrophages and blood vessels, and lower collagen and fibronectin contents than incisions treated with vehicle [*12*]. Tensile strengths of incisions treated with TGF-β neutralizing antibody or with vehicle were not significantly different.

Analysis of the molecular environment of chronic wounds is just beginning. We found that fluids collected from chronic wounds consistently failed to stimulate DNA synthesis of human foreskin fibroblasts (**Figure 1**). In contrast, fluids from spontaneously healing mastectomy wounds increased DNA synthesis two- to threefold above the level of cells cultured in serum-free medium. One explanation for this difference could be that the fluid from healing wounds contains higher levels of growth factors than the fluid from chronic wounds. Our preliminary analysis indicates that growth factor levels are often decreased in fluids from chronic wounds. However, the levels do not appear to be low enough to account for the lack of mitogenic activity of the fluids.

A more likely explanation for the lack of mitogenic activity in fluids from chronic wounds may involve the levels of protease activity. Our results indicate that levels of protease activity in chronic wound fluids were an average of 125-fold higher than levels measured in mastectomy drain fluids (range: 4- to 500-fold higher). Furthermore, fluids from chronic wounds readily degraded EGF and the EGF receptor, whereas mastectomy fluids did not. Although much remains to be studied in this area, these results suggest that the molecular environment of chronic wounds may impair the ability of endogenous growth factors to stimulate healing.

SKIN WOUNDS

The ability of peptide growth factors to stimulate migration, mitosis, and synthesis of extracellular matrix proteins by fibroblasts and epithelial cells *in vitro* prompted the evaluation of several growth factors in animal models of wound healing. Franklin and Lynch [*13*] were among the first to show that repeated topical application of EGF accelerated closure of full-thickness wounds of rabbit ears. Topical application of EGF [*14,15*], TGF-α [*16*], FGF [*17*], and the combination of PDGF plus IGF-I [*18*] have all been reported to stimulate the rate of epidermal regeneration of partial-thickness dermatome injuries or partial-thickness burn injuries in pigs. Similar results have been reported in humans. 1 Brown and colleagues [*19*] reported that topical treatment of skin-graft donor sites with EGF accelerated the rate of epithelial regeneration in all patients by an average of 1.5 days (**Figure 2**).

Results of these experiments demonstrated some important concepts. First, the rate of spontaneous healing was not the maximum rate that could be achieved. The molecular environment of spontaneously healing skin wounds may not have the optimal level of peptide growth factors. Second, the acceleration of epidermal regeneration in these studies was approximately 20% to 30% faster than vehicle controls. In a small wound, the clinical significance of such an increase in healing would be negligible, but, in a large injury, increasing the rate of epithelialization by 30% might have clinical benefit.

2 The ability of exogenous peptide growth factors to accelerate healing of skin wounds in normal animals justified investigations using animal models of impaired healing. Treatment of full-thickness skin wounds in genetically diabetic mice with recombinant PDGF-BB or bFGF significantly decreased the time to wound closure and increased the number of fibroblasts and capillaries in the wound bed [*20*]. Injection of TGF-β into wound chambers of doxorubicin-treated rats reversed some of the healing impairment, and injection of TGF-β with EGF and PDGF restored wound collagen deposition to 86% of the collagen deposition seen in normal rats [*21*]. Daily injections of EGF into subcutaneous wound chambers of rats reversed the inhibition of granulation tissue formation produced by methylprednisolone treatment [*22*]. In guinea pigs radiation-impaired healing of skin incisions wounds was partially reversed by low doses of TGF-β [*23*].

3 Clinical trials have also assessed the effects of growth factor treatment on [redacted] mans. One of the earliest studies utilized an extract of autologous platelets to treat 41 patients with chronic, nonhealing cutaneous ulcers in a nonrandomized phase I trial. Knighton and colleagues [*24*] reported successful epithelialization in 93% of the chronic wounds, with an average time to complete epithelialization of 7.5 weeks. In a subsequent prospective, randomized, double-blind, cross-over, placebo-controlled clinical trial of platelet extract, 17 of 21 wounds (81%) treated in the positive arm achieved complete epithelialization in a average of 8.6 weeks of treatment. In the placebo arm, 2 of 13 wounds

(15%) healed during the initial 8 weeks of placebo treatment. The 11 wounds that failed to heal with placebo treatment were crossed over to treatment with platelet extract, and all 11 wounds had complete epithelialization in an average of 7.1 weeks [*25*]. Although the autologous platelet extract has not been completely characterized biochemically, platelets have been shown to contain multiple peptide growth factors including PDGF, TGF-β, IGF-I, and EGF. These studies demonstrated that chronic wounds may benefit from treatment with combinations of growth factors.

More recent clinical studies assessed the effects of individual peptide growth factors on chronic wounds. In a phase-I cross-over study, eight of nine patients with chronic wounds achieved complete epithelialization after cross-over to EGF treatment [*26*]. Robson and colleagues [*27*] recently tested PDGF-BB in a phase I/II, randomized, double-blind, placebo-controlled study of 20 patients with chronic pressure ulcers. After 28 days of treatment, ulcers of patients treated with 100 mg/mL of PDGF-BB had significantly smaller volume than ulcers treated with vehicle (6% versus 22% of initial ulcer volume). Lower doses (1 or 10 mg/mL of PDGF) had little effect. Although these initial clinical results are encouraging, more research remains to be done to achieve the potential benefit of peptide growth factor treatment of chronic skin wounds.

SPECIAL CONSIDERATIONS FOR TREATMENT OF WOUNDS WITH GROWTH FACTORS

1 Several conditions appear to contribute to the effectiveness of growth factor treatment of wounds. One important consideration is that many growth factors require prolonged exposure to wound cells in order to stimulate a response. This requirement was demonstrated both *in vitro* and *in vivo*. Cultured fibroblasts require continuous exposure to EGF for 3 to 4 hours before they commit to cell division [*28*]. Lynch *et al* [*29*] noted that single topical applications of EGF, TGF-α, bFGF, IGF-I, and PDGF-BB in gels failed to accelerate the healing of partial-thickness dermatome wounds in pigs. Greaves [*30*] reported no effect on healing of suction bullae wounds in healthy adult volunteers when EGF was applied in saline for 5 minutes, after which the excess solution was wiped off. EGF also had no effect on healing of partial-thickness burns when applied once daily as a mist [*31*].

Prolonged exposure of cells to growth factors can be accomplished by formulating the growth factor in a vehicle that slowly releases it over an extended period of time. Brown and colleagues [*32*] illustrated this principle by demonstrating that a single application of EGF formulated in saline and placed into surgical incisions in rat skin failed to increase tensile strength. However, a single application of EGF formulated in multilamellar liposomes, which release EGF slowly over 5 days, significantly increased tensile strength 14 days after surgery. In a related study, Buckley *et al* [*33*] found that daily injections of EGF in saline into polyvinyl alcohol sponges implanted subcutaneously in rats produced only a modest increase in granulation tissue. However, placement of EGF into slow-release pellets prior to implantation into the sponges caused a dramatic increase in the extent and organization of granulation tissue.

It is important to note that not all peptide growth factors appear to require repeated or prolonged application to enhance wound healing. A single application of TGF-β in a collagen vehicle increased the tensile strength of surgical incisions in rats [*32*], and a single application of bFGF in saline increased the tensile strength of rat surgical incisions [*34*].

2 Other conditions may be important in optimizing the effect of growth factors on wound healing. Combinations of peptide growth factors may be more effective than individual growth factors. This concept was demonstrated by Lynch and colleagues [*18*] who reported that neither PDGF or IGF-I alone significantly increased the thickness of the regenerated epidermis or dermis of partial-thickness dermatome injuries on pig skin. However, the combination of PDGF plus IGF-I induced a significant increase in the thickness of new dermal tissue and epidermis in the wound. Using a different wound model, Sprugel and colleagues [*35*] found that a combination of PDGF plus bFGF increased the DNA content of wound chambers implanted in rats better than any single growth factor. These results suggest that growth factors in combination may act synergistically on wound cells to augment the healing process.

3 Another consideration is the potential enhancement of growth factor action that may be produced by simultaneous treatment of wounds with growth factors and protease inhibitors. Okamura and colleagues [*36*] reported that an ointment containing EGF and nafamostat, a serine protease inhibitor, increased the dry weight of granulation tissue in open wounds in rats threefold over control wounds treated with ointment alone. Other parameters of wound healing also increased, including the hyaluronic acid and hydroxyproline content. The degradation of ^{125}I-EGF by an extract of the wound tissue was also significantly decreased by the addition of nafamostat, suggesting that the stability of EGF in the wound site was increased. Protease inhibitors should also help prevent degradation of growth factor receptors on wound cells.

The concentration of an exogenous growth factor also influences the extent of healing. In general, the concentrations of exogenous growth factors that are applied to wounds are in the range of 10 to 1,000 mg/mL, which is about 1,000 times higher than the concentrations of growth factors that are needed to stimulate DNA synthesis or migration of wound cells *in vitro*. The reason why higher concentrations are needed *in vivo* is not known but it may be a consequence of rapid diffusion of the growth factor from the wound site or proteolytic destruction of the growth factor.

CORNEA

The cornea is another tissue that has been investigated extensively for the effects of growth factors on wound healing. The cornea consists of three distinct layers of tissue: the epithelium, the stroma, and the endothelium.

Because each of these layers has a different complement of cell types, they heal by different mechanisms. Nonetheless, peptide growth factors appear to have key roles in the healing wounds to each layer.

Corneal epithelial cells were one of the first cells to be recognized as targets for growth factor action. In 1972, Frati *et al* [*37*] injected ^{125}I-EGF into the peritoneal cavity of rats and found that high concentrations of radioactivity accumulated in the corneal epithelium. Soon afterward, Savage and Cohen [*38*] reported that EGF stimulated the proliferation of epithelial cells of embryonic human and chicken corneas during organ culture. These results demonstrated that corneal epithelial cells expressed EGF receptors and proliferated in response to EGF *in vitro*. It was reasonable to test whether exogenous EGF might stimulate healing of corneal epithelial wounds. Numerous studies in animals have indeed demonstrated that the topical application of EGF eye drops accelerated healing of epithelial wounds created by various methods, including scraping, organic solvents, or mild chemical burns [*39*]. In these animal models, the rate of closure of epithelial wounds was increased 15% to 30% compared with vehicle-treated corneas. EGF is not the only growth factor that has been shown to enhance healing of corneal epithelial wounds. bFGF increased corneal epithelial cell proliferation *in vitro* and increased the rate of epithelial wound healing *in vivo* [*40*]. Thus, several peptide growth factors promote corneal epithelial wound healing in animal models.

These experiments were the basis for several initial clinical trials utilizing EGF eye drops to treat corneal epithelial wounds. Daniele *et al* [*41*] treated 104 patients with various categories of epithelial defects and stromal ulcers with eye drops of mouse EGF. Treatment with EGF improved the rate of epithelial regeneration, particularly in patients with traumatic epithelial loss or thermal burns. EGF was not effective in patients with metaherpetic ulcers or stromal keratitis. The investigators noted that the effectiveness of EGF decreased as the extent of stromal erosion increased. A multicenter, double-blind trial of recombinant human EGF treatment of 104 patients with traumatic epithelial defects found that EGF eye drops accelerated healing by approximately 30% compared with eyes treated with vehicle [*42*]. In another study, mouse EGF eye drops were reported to not accelerate epithelial regeneration in 35 patients following corneal transplantation [*39*]. Stromal keratocytes express receptors for EGF and respond mitogenically to EGF in culture [*43*]. Several studies reported that [redacted] even when EGF treatment was combined with steroid treatment [*39*]. The potential usefulness for growth factors in stromal healing may be greatest in situations such as corneal transplantation in which healing is greatly delayed due to the use of postoperative steroids, which reduce inflammation and graft rejection.

Human corneal endothelial cells perform the essential task of pumping ions from the stroma to maintain the proper level of stromal hydration necessary for corneal clarity. Human corneal endothelial cells, however, rarely divide *in vivo*, and endothelial wounds heal predominately by migration and enlargement of surviving cells. There is considerable interest in evaluating whether peptide growth factors can stimulate mitosis in human corneal endothelial cells and, thereby, enhance endothelial wound healing. [redacted], which suggests that intraocular growth factor treatment may augment the healing of endothelial injuries [*44*].

GASTROINTESTINAL TRACT

The clinical observation that pregnant women have a low incidence of peptic ulcers led to the isolation of a peptide called urogastrone from urine. This peptide inhibited gastric acid secretion and had a beneficial effect on ulcers in dogs [*45*]. The structure of urogastrone was subsequently determined to be identical to that of human EGF. In addition to its ability to affect gastric acid secretion, EGF has been shown to stimulate gut epithelial proliferation [*46*]. Moreover, EGF has been found in human saliva [*47,48*], gastric juices [*48,49*], and duodenal secretions [*48*], leading researchers to postulate that EGF may have a role in healing ulcers of the gastrointestinal tract. Studies in rats using cysteamine [*50*], acetic acid [*51*], stress via water immersion [*52*], aspirin plus hydrochloric acid [*53*], or acidified ethanol [*54*] to induce ulcers demonstrated that EGF accelerated the healing of ulcers or decreased the extent of ulceration when administered prophylactically.

The mechanism by which EGF exerts its cytoprotective effects is unknown but involves more than just the inhibition of gastric acid secretion. Several experimental protocols that used EGF in a manner that did not affect gastric acid secretion still found EGF to be protective. EGF stimulates DNA synthesis of gastric and duodenal mucosa. Inhibition of DNA synthesis by the administration of difluoromethylornithine abolished the protective effect of parenteral EGF in models of water immersion and acetic acid administration, suggesting that cell proliferation is an integral part of the cytoprotective mechanism of EGF. However, another study showed that parenteral EGF administered just 10 to 30 minutes before the initiation of gastric injury with acidified ethanol decreased the extent of mucosal injury [*54*]. In this model, the time from EGF administration to injury was too brief for mitosis to occur; therefore, other cytoprotective mechanisms were probably employed.

Sucralfate is a medication routinely used for the treatment of peptic ulcers. Many mechanisms of cytoprotection by sucralfate have been proposed including the binding of EGF. Sucralfate has a large capacity to bind EGF at low pH and, thus, can act as a reservoir to deliver EGF to the ulcer bed [*55*]. Furthermore, animal experiments found that the healing ability of sucralfate was significantly diminished in rats whose salivary glands had been surgically removed, but when the combination of sucralfate and EGF was used, the healing ability of sucralfate was restored [*56*]. This may be partly due to the fact that rat salivary glands secrete large amounts of EGF.

The effect of EGF on the healing of gastrointestinal anastomosis has also been evaluated [57]. The tensile strength of linear incisions of stomach, ileum, and colon in pigs was measured 5 days after injury. In the animals given a constant intraperitoneal infusion of EGF, a significant increase in wound strength was found for each type of incision.

bFGF has also been reported to enhance the healing of gastrointestinal injuries in an animal model. In a recent review, Folkman *et al* [58] investigated the role of bFGF in healing rat duodenal ulcers induced with oral cysteamine-hydrochloride. Since bFGF is denatured at low pH, an acid-stable mutant bFGF protein (mutein) was created. Rats were treated with mutein, cimetidine, wild-type bFGF, or vehicle alone. After 21 days of treatment, only 40% of rats treated with mutein still had ulcers, as opposed to more than 85% of rats in the other groups.

LIVER

Following a two-thirds hepatectomy, total regeneration of rodent liver occurs in 10 to 14 days. Substantial data indicate that TGF-α, TGF-β, acidic FGF (aFGF), and EGF regulate hepatic regeneration. EGF, TGF-α, and aFGF all stimulate DNA synthesis of hepatocytes *in vitro* [59,60] and *in vivo* [61]. Levels of mRNA for TGF-α and aFGF in regenerating liver also correspond temporally to the peak of DNA synthesis that occurs 48 hours after partial hepatectomy. It appears that TGF-α or aFGF produced by hepatocytes may be stimulating hepatocyte proliferation in an autocrine manner. In addition, reduction of serum EGF levels by removal of the salivary glands retards hepatic regeneration in rats, and treatment with exogenous EGF returns the rate to normal [62].

[redacted]or DNA synthesis in the liver. TGF-β is not expressed by hepatocytes but is secreted by nonparenchymal cells, such as endothelial cells. The increase in hepatocyte DNA synthesis normally caused by EGF or TGF-α was blocked by exogenously administered TGF-β both *in vitro* [59] and *in vivo* [63]. Following partial hepatectomy in rats, TGF-β mRNA levels peak at eightfold over normal at the time when hepatocyte DNA synthesis starts to fall [59].

BONE

Bone matrix is a rich source of growth factors, and cultures of bone cells produce IGF-I, IGF-II, PDGF, FGF, and TGF-β [64]. The addition of IGF-I, PDGF, EGF, or TGF-β to cultures of chondrocytes or bone cells stimulates cell growth and matrix synthesis [65].

In vivo experiments have demonstrated that IGF-I stimulates bone formation and mediates the effects of growth hormone on bone [66]. PDGF and bFGF may also benefit bone formation. Both PDGF and bFGF are mitogenic for osteoblasts [67] and stimulate matrix protein synthesis [68]. PDGF has also been found to stimulate heterotopic bone growth in rats [69]. TGF-β appears to be the most important growth factor for promoting osteogenesis and bone repair. TGF-β mRNA and protein were detected around bone particles implanted into subcutaneous tissues of rats during the period of cartilage and endochondral bone formation [70]. Closed femoral fractures in rats were found to contain TGF-β mRNA and protein at a time corresponding to the period of intramembranous and endochondral bone formation [71].

TGF-β injections also promoted bone and cartilage formation *in vivo*. The injection of TGF-β under the femoral periosteum of newborn rats induced chondrogenesis followed by endochondral bone formation at the injection site [71], and the injection of TGF-β over the calvarial bones of mice induced the formation of woven bone followed by formation of lamellar bone [72]. Treatment of bone fractures with local injections of TGF-β is under active investigation, and preliminary results appear promising. Coating implant devices with TGF-β to enhance ingrowth of bone is another area of interest.

NEURAL TISSUE

The most recent area of investigation for growth factors and wound healing is nerve regeneration. Although little data are available, the results are encouraging. Many growth factors are found in neurons, including IGF-I and bFGF, and injury to neurons or adjacent cells appears to stimulate release of the factors [73]. Most experiments have examined the ability of growth factors to increase neuron survival or enhance regeneration of peripheral nerves. bFGF prolonged the survival of cells cultured from the hippocampus, cortex, septum, thalamus, and spinal cord, and stimulated replication in Schwann cells, oligodendrocytes, astrocytes, and neuroblasts [74]. bFGF also prevented death of lesioned cholinergic neurons *in vivo* [75]. Several studies indicated that bFGF may promote regeneration of peripheral nerves. Experiments using the transected rat sciatic nerve model showed that bFGF treatment improved the rate of nerve regeneration, although long-term complications developed from scarring [76–78].

IGF-I and IGF-II have been found in the central and peripheral nervous systems [79], and immunohistochemical analysis of transected sciatic nerves revealed an increase in IGF-I in the region of the injury [73]. *In vivo* experiments of crush-injured or freeze-injured rat sciatic nerve demonstrated an increase in the rate of regeneration with IGF-I administration [80,81]. TGF-α and EGF are both present in brain. Recently, EGF was reported to induce proliferation of cells isolated from the adult mouse striatum [82]. Proliferation could not be induced with PDGF, bFGF, or nerve growth factor. The proliferating cells subsequently developed the morphology and antigenic characteristics of neurons and astrocytes. These results indicate that the central nervous system, at least in mice, may contain stem cells that may be stimulated to undergo neurogenesis.

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