Growth Factors in Pathologic Wound Repair in Horses*

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ABSTRACT: Dermal scarring is an important clinical problem, often leading to adverse effects on function as well as an undesirable cosmetic appearance. It may be related to an imbalance in the cytokine/growth factor profile of the wound environment. For example, it has recently been shown that overexpression of the fibrogenic growth factors transforming growth factor (TGF)-β1 and TGF-β2 is related to the excessive accumulation of collagen evident in keloids and hypertrophic scars, common forms of pathologic overhealing of dermal wounds in humans. Proud flesh in horses resembles human keloids and hypertrophic scarring and may thus share a similar pathogenesis.

Two processes are involved in the healing of most wounds: regeneration and repair. Regeneration is the replacement of destroyed tissue with normal functioning cells of the type lost and is only possible in tissues that have a sustained population of cells capable of undergoing mitotic division. Conversely, repair is a stopgap reaction designed to reestablish the continuity of interrupted tissues. Tissue forms between the severed parts, without differentiating totally new elements, and this ultimately results in a nonfunctional tissue (i.e., scar).

Scarring is an important clinical problem, often leading to adverse effects on function and growth as well as an undesirable cosmetic appearance. Numerous pathologic lesions manifest themselves as a result of impaired function caused by scar tissue (e.g., dermal fibrosis, hepatic cirrhosis, glomerulonephritis, pulmonary fibrosis, intraabdominal adhesions, impaired vision from scarring of the cornea).

Dermal scarring may be strongly related to inflammation. In adults, cutaneous wound repair is a highly inflammatory process regulated by a carefully orchestrated cascade of cytokines and growth factors. When injury occurs, growth factor production and secretion are induced largely within platelets and macrophages from the wound border, and the processes of inflammation and healing are initiated. These processes ultimately lead to the formation of well-developed scar tissue. Fetal wound healing, however, differs in that successful healing largely occurs in the absence of inflammation and the final outcome is scarless. Fetal wounds

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Specifically, basic fibroblast growth factor (bFGF) and transforming growth factor (TGF–β), and TGF–β3, growth factors with known fibrogenic effects, are absent at all times in fetal wounds examined immunocytologically, whereas TGF–β1, a protein with antifibrogenic effects, is present immunohistochemically in quantities equal to those found in adult wounds.

A direct consequence of the low level of some inflammatory growth factors at the fetal wound site may be the absence of scar tissue formation. Indeed, the significance to dermal scarring and fibrosis of elevated levels of TGF–β1 and TGF–β2 in particular has recently been abundantly documented. For example, Shah and colleagues mimicked the fetal wound situation in adult rat incisions by neutralizing TGF–β1 and TGF–β2 and noted decreased inflammation and subsequently reduced scarring by 42 days after injury in the antibody-treated incisions. The exogenous addition of the antifibrogenic isoform TGF–β3 had similar consequences and probably acts by downregulating TGF–β1 and TGF–β2. The effectiveness of this approach has been demonstrated clinically in such fibrotic conditions as glomerulonephritis, pulmonary fibrosis, and arthritis. In summary, the relative lack of TGF–β1 and TGF–β2, growth factors known to favor fibrosis, may be an important reason the fetus heals by regeneration rather than by scarring. Consequently, manipulation of the early cytokine/growth factor profile to more closely resemble that of the fetus may be a rational therapeutic objective to markedly ameliorate the quality of wound repair.

**PATHOLOGIC OVERHEALING AND DERMAL SCARRING**

Hypertrophic scars and keloids are the most common outcome of overexuberant dermal wound repair mechanisms in humans. The typical clinical picture is one of a tender, red, elevated, and often unsightly scar. Most lesions remain until treated and show a marked tendency to recur after surgical excision. Under normal circumstances, the balance between synthesis and degradation of dermal collagen is tightly controlled, but this equilibrium is transiently disrupted during wound repair. The distinct pathogenic mechanism underlying both hypertrophic and keloid scarring is one of accumulation of excessive collagen and extracellular matrix (ECM) within the wound. Indeed, it has recently been shown both in vitro and in vivo that collagen synthesis is augmented in keloid and hypertrophic scars compared with normal scars. It is likely that an abnormal cytokine/growth factor profile is related to this excessive accumulation of collagen/ECM evident in keloids and hypertrophic scars via an erratic regulation of cellular function.

Several studies have demonstrated enhanced expression of TGF–β1 protein or mRNA in both keloid and hypertrophic scar cells and tissues. Lee and colleagues characterized expression of the different TGF–β proteins in keloids and found that fibrogenic TGF–β1 and TGF–β2 are elevated in keloid fibroblast cultures compared with normal human dermal fibroblasts, whereas levels of antifibrogenic TGF–β3 are comparable. Susceptibility to the formation of hypertrophic and keloid scars is multifactorial and certainly under genetic control. A familial predisposition and an autosomal dominant pattern of inheritance of the keloid tendency have been described among blacks. Furthermore, Polo and colleagues demonstrated that TGF–β2 levels are elevated in the peripheral blood mononuclear cell fractions of patients with proliferative scarring. In summary, abundant evidence supports the role of an excessive fibrogenic growth factor profile in the development of pathologic overhealing of the dermis.

**DERMAL WOUND REPAIR IN HORSES**

Skin is a complex organ that cannot regenerate and therefore must be repaired after injury. Under normal circumstances, the healing process is achieved by wound debridement, protection of the wound bed with highly vascular and resistant granulation tissue, reduction of wound size by contraction, and permanent surface protection by epithelial migration. However, this is not always the case in horses because the early stages of wound healing are often excessive and may lead to abnormal resolution.

**Second-Intention Healing**

Second-intention wound healing, as opposed to primary closure, is often necessary for the management of soft tissue injuries in horses, particularly those involving the distal portions of the limb. Excessive skin tension, extreme wound contamination, and massive tissue loss in these areas frequently preclude management by primary closure. Second-intention healing in horses is subject to numerous species-specific and interrelated complications such as the formation of exuberant granulation tissue (proud flesh) and subsequent retardation of contraction and closure as well as delayed formation of a fragile neoe epithelium susceptible to reinjury, especially when wounds are located on the distal aspect of the limb (Figure 1). Indeed, compared with wounds of the trunk, limb wounds show greater retraction, slower rates and earlier cessation of contraction, and slower rates of epithelialization. Wilminck and colleagues found that the mitotic activity of granulation tissue fibroblasts from the body is initially high and declines.
rapidly from 1 week onward. However, the mitotic activity of granulation tissue fibroblasts in equine metatarsal wounds remains significantly elevated. When granulation tissue protrudes maximally, the mitotic activity of epithelial cells is markedly reduced, suggesting that proud flesh may inhibit epithelial cell mitosis in addition to physically impeding migration of the cells onto the wound bed. In vitro growth of fibroblasts from tissues isolated from the horse limb is similar to or significantly less than growth from tissues of the horse trunk. However, growth characteristics of fibroblasts may differ considerably between in vitro and in vivo environments due to the absence of a variety of cellular growth mediators in the former, particularly those released after injury and during inflammation. Also, even if limb fibroblasts have normal growth characteristics, their synthetic activity may be high, as has been reported for keloid fibroblasts.

An early statement by Meager and Adams described proud flesh as a form of keloid. In fact, exuberant granulation tissue differs from both hypertrophic scars and keloids in that it lacks an epithelial cover and forms earlier in the repair process. However, these three lesions share an imbalance between collagen synthesis and lysis that ultimately leads to dermal fibrosis. Indeed, Chvapil and colleagues have noted that horses activate wound collagen formation to a greater extent and earlier than do rats. Numerous mechanisms may be involved in the production of exuberant granulation tissue in horses, including infectious or foreign body responses; excessive motion or tension on the surrounding skin; minimized blood supply with resultant hypoxia; and an imbalance of collagen synthesis, deposition, and lysis. These could result in or be attributable to a growth factor profile that differs from that of body wounds. For example, bandaging maintains a slightly hypoxic environment and retains inflammatory cells at the wound surface, both of which stimulate neovascularization and fibroplasia. Indeed, Barber has shown that the application of a bandage to leg wounds increases the amount of granulation tissue produced. Hypoxia may stimulate TGF-β synthesis and may increase the number of TGF-β receptors in a particular subset of fibroblasts, both of which would enhance collagen production by fibroblasts.

**Literature Reports**

In the equine literature, many authors describe treatments to promote wound healing that often attempt to control the excess fibrosis that appears in response to injury. Aside from corticosteroids, none of these treatments has consistently eliminated the formation of exuberant granulation tissue on wounds of the distal limb in horses. This is not surprising as the specific cause for the development of proud flesh remains unknown.

It has become increasingly apparent that some autocrine-acting and paracrine-acting cytokines and growth factors are important in cutaneous biology, although until recently this fact had not been verified in horses. Theoret and colleagues undertook several studies to investigate the role of certain growth factors in the cellular biology of equine wound repair. Since wound healing in equine limbs can be problematic, the authors were specifically interested in comparing the expression kinetics of fibrogenic TGF-β and bFGF and antifibrogenic TGF-β in healing leg and thoracic wounds. They successfully mapped the temporal expression of these growth factors using an ELISA technique during normal equine wound repair. They also demonstrated that expression of fibrogenic TGF-β persists throughout the proliferative phase of healing in wounds located on the distal aspect of the limb, while in thoracic wounds it quickly returns to baseline with the culmination of the inflammatory phase. This finding lends credence to the hypothesis that TGF-β is associated with fibrosis in horses as well since, in this species, limb but not body wounds seem predisposed to the development of proud flesh. Using immunohistochemistry, Theoret and colleagues further identified that macrophages and fibroblasts were the cells responsible for this augmented TGF-β expression. Based on these results, they then compared growth factor expression in normally healing limb wounds and those developing exuberant granulation tissue in order to more clearly elucidate the pathogenesis of proud flesh.

**Figure 1**—Pathologic overhealing of a full-thickness wound in a horse. Proud flesh has developed on the distal aspect of the limb.
Wounds healing with exuberant granulation tissue showed trends toward higher concentrations of fibrogenic TGF-β1 and lower concentrations of antifibrogenic TGF-β3; however, these differences were not statistically significant. Thus, additional studies are needed to clarify the relationships between growth factors and proud flesh in horses.

A few equine studies have evaluated the effects of exogenous application of growth factor preparations on the different stages of wound repair. Cell extracts and supernatant fluids from human and pig epidermal cells grown in tissue culture were topically applied to chronic granulomas on the distal portion of the limbs of horses. Excessive proliferation of granulation tissue was clearly suppressed by the treatment, and epithelialization was accelerated. The investigators did not determine the active ingredient of these extracts/fluids but termed them collectively as epidermis-derived factors. A macrophage supernatant was used by Wilson and colleagues on wounds of the distal aspect of the limb and found to slightly encourage epithelialization but not affect the rate of healing. Similarly, Steel and coworkers have shown that application of recombinant human TGF-β1 to full-thickness excisional limb wounds has no significant effect on total wound granulation tissue or epithelialization areas or on histologic or subjective clinical assessments of sequential wound biopsies. Finally, Ohnemus and colleagues demonstrated that antifibrogenic TGF-β, applied to full-thickness excisional wounds on the distal aspect of the limb results in healthier granulation tissue, although these findings were not statistically significant.

CONCLUSION

The acceleration of wound repair may not prove as simple as applying a single growth factor to the wound. Indeed, further knowledge of the roles of endogenous growth factors in the various stages of repair is needed before reasoned therapeutic approaches using recombinant growth factors can be implemented in an attempt to prevent or cure situations of impaired healing in horses.

REFERENCES


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1. Which of the following cells involved in wound repair are the most important for growth factor production and secretion?
   a. endothelial cells
   b. neutrophils
   c. macrophages
   d. c and d
   e. platelets

2. Which wound-healing complications are commonly encountered in horses?
   a. excessive fibroplasia, delayed contraction, delayed reepithelialization
   b. excessive fibroplasia, contracture, delayed reepithelialization
   c. excessive fibroplasia, delayed contraction, accelerated reepithelialization
   d. inadequate fibroplasia, contracture, delayed reepithelialization
   e. inadequate fibroplasia, delayed contraction, delayed reepithelialization

3. The distinct pathogenesis underlying hypertrophic and keloid scarring involves
   a. accumulation of excessive collagen and ECM
   b. excessive degradation of collagen
   c. deficient cross-linking of newly formed collagen fibrils
   d. deficient reepithelialization
   e. deficient angiogenesis

4. Which of the following growth factors is/are known to favor fibrosis?
   a. TGF-β
   b. TGF-β
   c. TGF-β

5. Fetal and adult wounds have similar levels of which of the following growth factors?
   a. TGF-β
   b. TGF-β
   c. TGF-β

6. Which of the following are possible mechanisms involved in the production of exuberant granulation tissue in equine wounds?
   a. hypoxia
   b. infection
   c. altered growth factor profile
d. imbalance in collagen synthesis
e. all of the above

7. How does equine proud flesh differ from other forms of pathologic overhealing common to humans, such as keloid and hypertrophic scarring?
a. It involves an imbalance between collagen synthesis and lysis.
b. It involves deficient angiogenesis.
c. It lacks an epithelial cover.
d. It forms later in the repair process.
e. It results from contracture of the wound.

8. The formation of exuberant granulation tissue (proud flesh) in equine wounds is particularly prevalent in the
a. head and neck.
b. distal aspect of the limb.
c. thorax.
d. abdomen.
e. proximal aspect of the limb.

9. Which growth factor has been detected in high concentrations in the peripheral blood of patients with proliferative scarring?
a. bFGF
d. TGF-β₂
b. PDGF
e. TGF-β₃
c. TGF-β₁

10. Fetal wound healing differs from adult wound repair in that
a. it occurs in the absence of inflammation.
b. the outcome is scarless.
c. high levels of growth factors are detectable at the wound site.
d. a and b
e. a and c