CHAPTER 15

Exuberant Granulation Tissue

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Summary

The formation of exuberant granulation tissue (EGT) is a frequent complication of wounds healing by second intention on the limbs of horses. Among the large number of contributing factors, chronic inflammation is foremost and often goes unrecognized because of the mild signs it elicits. The stimulus for formation of EGT is reduced when prevention and treatment of chronic inflammation are combined with excision of the protruding granulation tissue. This approach allows a smooth transition from fibroplasia to wound contraction and epithelialization and usually obviates the recurrence of EGT.

The topical application of a corticosteroid, used in a precise and controlled manner, and the use of silicone sheet dressings, as well as skin grafting, are valuable in preventing the formation of EGT. In cases where EGT is already present, excision of the protruding granulation tissue is, currently, the treatment of choice.

Introduction

The development of exuberant granulation tissue (EGT) in the horse has long been an enigma. Several studies, performed during the past two decades, have focused on equine EGT with the aim to elucidate the mechanisms underlying this phenomenon and thereby develop targeted therapies. The findings and interpretations of these investigations have been united in this chapter. They complement one another and have shed light on the pathophysiology of one of the most common and frustrating complications disturbing the repair of limb wounds of horses. The etiology of EGT appears to be multifactorial, involving

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environmental, biochemical, immunologic, and genetic factors. Insights into the physiology/pathology, predisposing factors, prevention, and treatment of EGT are described and discussed herein.

Physiology and pathology

Fibroplasia and the development of exuberant granulation tissue

Fibroplasia, or the formation of granulation tissue, is an essential component of wound healing. Apart from the nuisance of becoming exuberant, granulation tissue has many important functions that change continuously during healing. It fills in the wound gap, forms a barrier against external contaminants, provides myofibroblasts for wound contraction, and forms the bed over which epithelial cells migrate.

Granulation tissue provides several types of cells with important functions during healing. *Endothelial cells* form capillaries and other blood vessels through which oxygen and nutrients are transported to sustain cellular metabolism and through which leukocytes can migrate into the wound. *Leukocytes* clear the wound of contaminating agents and debris. Furthermore, they recruit additional inflammatory and mesenchymal cells and initiate healing. *Fibroblasts* form the extracellular matrix (ECM) needed to support cellular division, growth, and migration. The composition of ECM gradually changes as it is remodeled through the simultaneous synthesis and degradation of its components. Ideally, granulation tissue ceases to grow as soon as the gap in the wound has filled, allowing contraction and epithelialization to ensue. In many wounds on the limbs of horses, however, granulation tissue continues to grow for an indefinite period, resulting in the formation of EGT.

EGT is typically irregular and unhealthy in appearance, with many grooves and clefts, and protrudes over the margin of the wound (Figure 15.1). Although seen often in limb wounds, it is seen rarely in body wounds. EGT is characterized by chronic inflammation and the remains of fibrin deposits that have not been cleared by the acute inflammatory response. Microscopically, the tissue has an immature, chaotic appearance due to its disorganized cellular population (Figure 15.2).[2,3] In wounds suffering from EGT, cellular proliferation remains active, wound contraction is delayed, and the protruding granulation tissue may physically impede epithelial migration and/or may inhibit the growth of keratinocytes.[2,4]

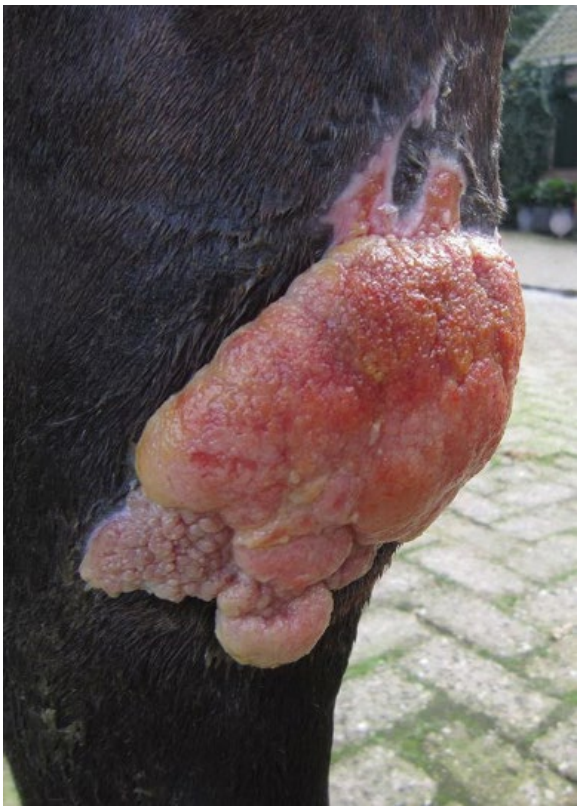
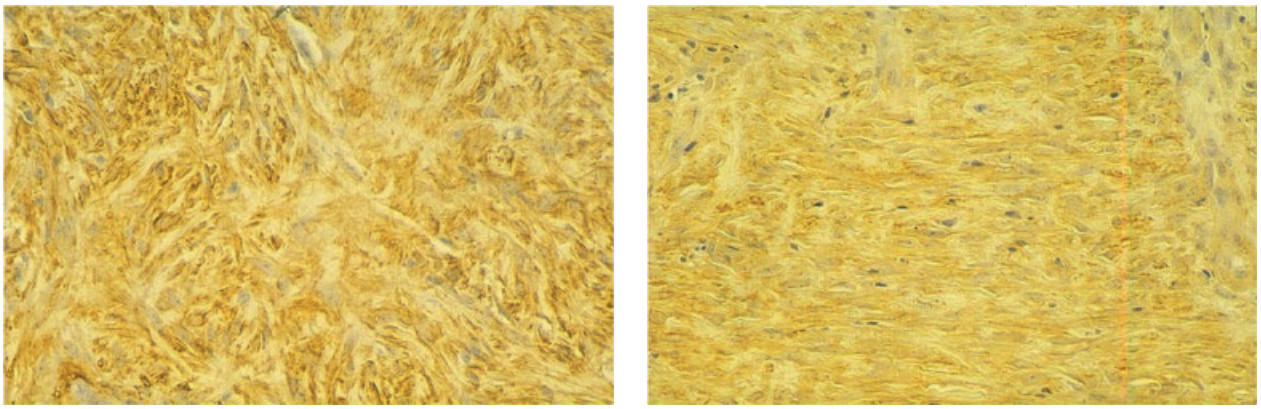


Figure 15.1 A wound on the dorsal surface of the tarsus showing the typical features of EGT: irregular surface riddled with grooves and clefts, protrusion over the wound margins, and purulent exudate.

Phenotype and function of fibroblasts

Phenotype and function of fibroblasts in normally healing wounds

The fibroblast, the major type of cell in granulation tissue and in EGT, changes its phenotype during healing. The phenotype and function of fibroblasts are closely related. When healing is uncomplicated, phenotypes reflect the various needs of the wound as healing progresses, and phenotypes succeed one another as the wound matures. Initially, fibroblasts have a migratory phenotype, allowing them to move from the surrounding tissues into the wound. Migration depends on chemoattractive agents released by platelets and macrophages at the wound's border in response to injury.[5] Once at its ultimate destination, the fibroblast changes its phenotype into a proliferative and synthesizing form. Consequently, the number of fibroblasts increases, and ECM is produced. The synthesis of ECM is stimulated by several cytokines, such as transforming growth factor beta (TGF-β), released either by inflammatory cells or by the fibroblasts themselves.[6,7] Thereafter, fibroblasts can differentiate into myofibroblasts, a phenotype that contains smooth muscle actin filaments and can pull the margin of the wound centripetally via the contractile force exerted by these filaments.[8] Differentiation of fibroblasts into myofibroblasts and the generation of contractile forces are also stimulated by TGF-β, but both are inhibited by many other mediators produced in a chronically inflamed environment. After contraction ceases, fibroblasts and myofibroblasts disappear from the wound by apoptosis (i.e. cellular fragmentation followed by phagocytosis by macrophages and activated fibroblasts), and the cellularity of the repair tissue diminishes.[9,10] Apoptosis, a form of non-inflammatory programmed cellular death, is thus critical to the transition from one phase of repair (proliferation and contrac-



(a) (b)

Figure 15.2 **(a)** EGT, when viewed under a microscope, shows a high number of chaotically arranged cells and capillaries and looks very immature in contrast to **(b)** the regularly arranged cells and parallel capillaries of more differentiated and contracting granulation tissue. Smooth muscle actin staining. Source: Wilmink and van Weeren 2004.[1] Reprinted with permission of Elsevier.

tion) to the next (remodeling). Myofibroblasts may represent the terminal differentiation state of fibroblasts, after which apoptosis can occur.

Phenotype and function of fibroblasts in wounds with exuberant granulation tissue

The development of EGT in a wound coincides with a disordered succession of fibroblastic phenotypes. Specifically, the proliferative and synthesizing phenotypes predominate in EGT, while differentiation into contractile myofibroblasts is delayed.[3] This is consistent with the microscopic observation in limb wounds that persistent mitotic activity accompanies chaotically arranged myofibroblasts, a pattern not conducive to contraction,[2,3] as well as with the clinical observation that the presence of EGT is often coupled with poor contraction. Confirmation of this phenomenon was established *in vitro*, where the rate of proliferation of fibroblasts appeared inversely proportional to the capacity of fibroblasts for contraction because rapidly proliferating fibroblasts produced lower contractile forces (Figure 15.3).[11] Indeed, because myofibroblasts represent the terminal state of differentiation of fibroblasts, tardy progression to the contractile phenotype also implies that apoptosis will be impaired and high cellularity will be maintained, thereby favoring the development of EGT.

Because EGT occurs primarily in poorly contracting limb wounds, as opposed to efficiently contracting body wounds, investigators hypothesized that fibroblasts from diverse anatomical origins might possess different inherent characteristics accountable for the variable succession of phenotypes.[11,12] It was found, however, that the elevated mitotic activity in limb wounds is not based on innate differences in characteristics of growth between fibroblasts from limbs and those from the trunk because those of limb origin grow significantly more slowly than those of trunk origin when cultured *in vitro*.[11,12] Additionally, the inadequate contraction seen in limb wounds is not based on a weak inherent contractile capacity of fibroblasts

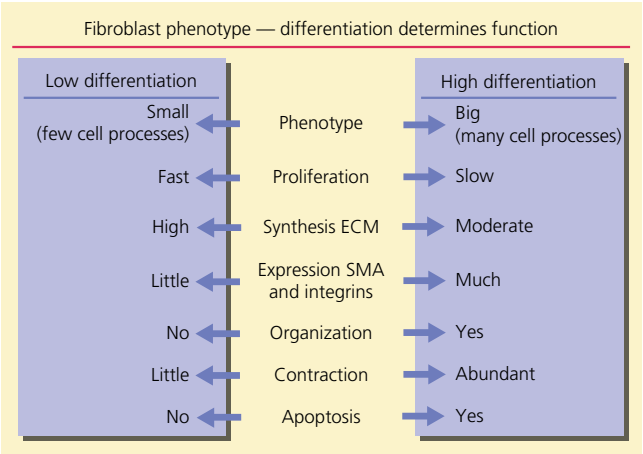


Figure 15.3 Fibroblast phenotype – differentiation determines function. Fibroblasts able to contract have different phenotypic features and are more differentiated than those exhibiting a proliferating phenotype.

from limbs; fibroblasts from limbs contract more than those from the trunk when cultured *in vitro*.[11]

In conclusion, the different phenotypes and functions attributed to fibroblasts from the limb or trunk, as well as the contrasting modes of repair characterizing limb and body wounds, are not based on distinct intrinsic cellular characteristics but, instead, must be the result of other factors. The extracellular environment's biochemical, molecular, and physical components govern the phenotype of the fibroblast, whereas the phenotype determines the fibroblast's response to environmental signals.[8]

Factors affecting the formation of exuberant granulation tissue

Although the exact causes of development of EGT during wound repair in the horse have yet to be established, research has revealed a number of factors that may contribute to this

condition. Some of these factors cannot be controlled or can be only partially controlled, whereas others can be prevented or eliminated.

Physiologic factors

Inflammatory response

An inefficient inflammatory response to injury may influence the phenotype and function of fibroblasts and thereby play an important role in the development of EGT in limb wounds of horses (Figure 15.3). After trauma, the acute inflammatory response in limb wounds of horses is weaker during the first 3 weeks than that of limb wounds of ponies, and the concentration of TGF-β in limb wounds of horses is lower during the first 10 days than that in limb wounds of ponies.[3,13,14] TGF-β1 not only stimulates production of ECM but also favors the differentiation of fibroblasts into myofibroblasts, thereby encouraging wound contraction. An inferior initial concentration of TGF-β may delay this differentiation, resulting in the presence of fewer myofibroblasts in favor of the rapidly proliferating and synthesizing fibroblast phenotypes. A reduced number of myofibroblasts means that contraction is delayed and inefficient, whereas proliferation of fibroblasts and synthesis of ECM continue unabated (Figure 15.3).

The weak acute inflammatory response seen in wounds of horses was shown to be followed by a persistent or chronic inflammatory response,[3] due in part to the continued presence of contaminants and non-viable tissue not resolved by the initial, feeble inflammatory response. Additionally, a delay in contraction means that the surface area of an open wound remains larger, thus perpetuating the inflammatory response because leukocytes disappear only after epithelium covers the surface of the wound. The substantial presence of leukocytes in exposed granulation tissue may explain up-regulated synthesis of cytokines in the absence of epithelium[15] and may lower oxygen tension in the wound as a result of the high oxygen consumption by these cells. Persistence of mediators, such as TGF-β, platelet-derived growth factor (PDGF), and fibroblast growth factor (FGF), induces fibrosis, whereas prostaglandin (PG)E_1, PGE_2, and interferon (IFN)γ inhibit contraction, while yet others, such as tumor necrosis factor (TNF)α, interleukin (IL)-1, and IL-6 do both (Figure 15.4).[16,17] Low oxygen tension additionally stimulates proliferation of fibroblasts and production of ECM.[18,19] The inflammation persisting in limb wounds of horses, therefore, likely enhances the formation of EGT and inhibits contraction, phenomena that are often seen simultaneously in the clinical setting. This hypothesis is substantiated by a recent study confirming that exacerbated and prolonged inflammation in healing wounds on the ears of rabbits favors the development of hypertrophic scarring.[20] Moreover, the observation that corticosteroids, potent anti-inflammatory drugs, control the formation of EGT in the wounds of horses adds further credence to this scenario.[21]

In summary, the combination of an inefficient, weak, acute inflammatory response and the ensuing chronic inflammation in limb wounds of horses delays the differentiation of fibroblasts into myofibroblasts, reducing wound contraction and favoring proliferation of fibroblasts and synthesis of proteins. This leads to a rapid increase in tissue volume by cellular proliferation, rather than a decrease in tissue volume by contraction (Figure 15.5). The chronic inflammation inherent to second-intention healing in limb wounds of horses, while often unrecognized clinically because of the mild accompanying signs, is no doubt a very important trigger for formation of EGT. The interaction between inflammation, subsequent formation of EGT, and lack of contraction establishes a vicious cycle because these physiologic phenomena stimulate one another.

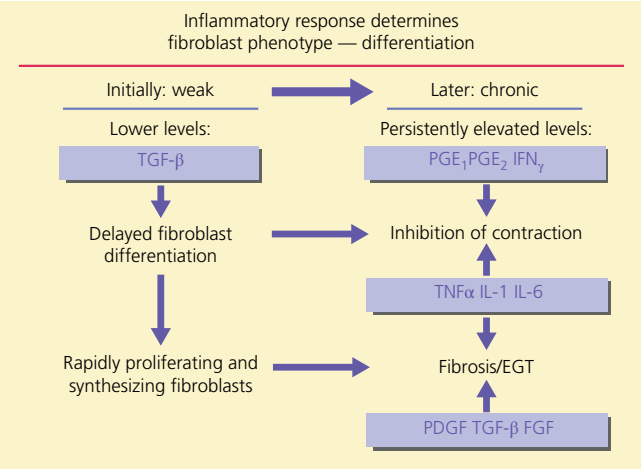


Figure 15.4 Fibroblast phenotype and differentiation is influenced by several cytokines present during the acute and chronic inflammatory response to wounding. Both the inefficient, weak acute inflammatory response and the ensuing chronic inflammation seen in limb wounds of horses delay the differentiation of fibroblasts into myofibroblasts, ultimately reducing wound contraction and favoring fibroblast proliferation.

Local cytokine profile

The aforementioned development of chronic inflammation in limb wounds of horses substantiates several studies documenting a fibrogenic-rich, local cytokine profile in limb wounds.[13,22–25] One of these cytokines, TGF-β1, stimulates migration and proliferation of fibroblasts and their production of ECM proteins, such as fibronectin and collagen,[6] while inhibiting the degradation of ECM.[25,26] It is thus noteworthy that the expression of TGF-β1 persists in limb wounds throughout the proliferative phase of repair, whereas it quickly returns to baseline values in body wounds after the initial inflammatory phase of healing.[13,22] Persistent production of TGF-β1 in limb wounds may partially be the work of the fibroblasts within the wound that also express more TGF-β receptors;[27,28] the signaling components are thus in place to stimulate cellular proliferation and encourage accumulation of components of ECM.

The persistent expression of TGF-β1 can be explained, at least in part, by various characteristics of limb wounds in horses, such as absence of epithelium, presence of tightly fixed

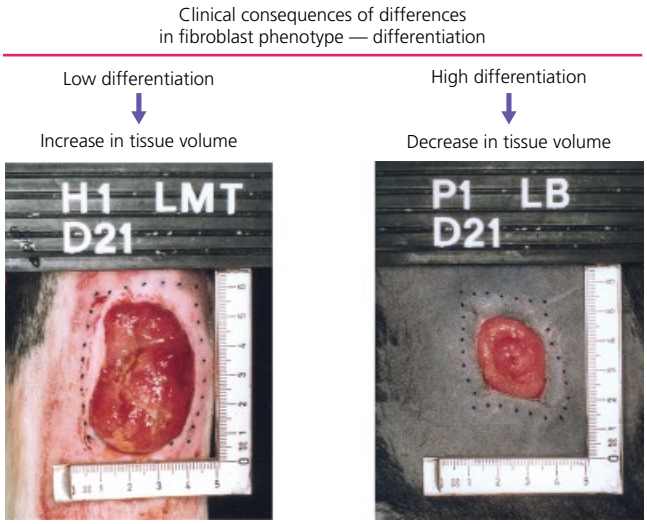


Figure 15.5 Experimental wounds initially of the same size, 21 days after creation on the limb of a horse (left) or on the buttock of a pony (right), which show the clinical consequences of differences in fibroblast phenotype and differentiation. Delayed differentiation of fibroblasts in limb wounds favors their proliferation but inhibits wound contraction, leading to an increase in tissue volume. In contrast, the faster differentiation of fibroblasts into myofibroblasts in buttock wounds of ponies, and the ensuing contraction, reduce tissue volume.

surrounding skin, as well as hypoxia of local tissue. Indeed, the synthesis of fibrogenic cytokines is up-regulated in the absence of an epithelial cover.[15] Persistent mechanical tension in a wound also plays a role, because mechanical unloading of fibroblasts is required to desensitize cytokine receptors, abrogating cytokine responsiveness and thereby favoring apoptosis.[29,30] Consequently, mechanical stress must be relieved for a wound to progress from granulation tissue to scar, by apoptosis.[29] Additionally, the secretion of TGF-β1 by fibroblasts is strongly stimulated by low oxygen tension.[19,31]

Collagen synthesis, deposition, and lysis

Investigators have assumed for some time that aberrant metabolism of collagen plays a key role in the formation of EGT. Theoretically, either abundant synthesis or impaired lysis may lead to excessive accumulation of collagen. The horse forms collagen speedily in response to wounding, indicating a prompt and excessive connective tissue response compared to the response of other species.[32] As described earlier, protracted expression of TGF-β1 in limb wounds may give rise to excessive formation of collagen and other proteins of the ECM.[13,23] Moreover, superior concentrations of type-I collagen and tissue inhibitors of metalloproteinase (TIMP)-1 mRNA have been measured in limb wounds compared to body wounds of horses at 1 and 4 weeks of healing.[24] Because TIMP-1 inhibits lysis of collagen, high concentrations present 4 weeks post wounding might favor accumulation of ECM. An imbalance between synthesis and degradation of collagen is likely correlated to the development of EGT, indicating that agents inhibiting collagen synthesis or stimulating the activity of MMPs, in particular collagenase, may represent therapeutic options. Indeed, the Food and Drug Administration approved intralesional collagenase for the treatment of Dupuytren's disease, a proliferative connective tissue disorder in humans;[33] moreover, this approach is currently under investigation for the management of keloid scarring in humans, a dermal fibroproliferative condition that resembles equine EGT.[34] Because an imbalance between synthesis and degradation of a single component of the ECM is unlikely to be the sole basis of formation of EGT, however, the effect of influencing only the metabolism of collagen might be limited.

Angiogenesis and wound oxygenation

Because low oxygen concentrations have been shown to stimulate the proliferation of fibroblasts and their synthesis of components of the ECM, it was postulated that local hypoxia within the granulation tissue of limb wounds of horses might contribute to the development of EGT. This hypothesis was verified via a series of experiments, the first of which mapped the expression of several genes and their corresponding proteins, selected from a larger pool[35–41] because of their known contribution to angiogenesis during wound healing.[42,43] Healing limb wounds of horses were found to be deficient in anti-angiogenic molecules, compared to body wounds, suggesting that the "control switch" to limit angiogenesis is defective in wounds on the limbs of horses. This coincides with the exacerbated angiogenesis observed clinically in limb wounds,[44] an important feature of EGT. Although the granulation tissue of limb wounds of horses is characterized by marked vascular regeneration, the lumens of these new microvessels are occluded significantly more than those of microvessels found in body wounds,[44] owing to hypertrophy of the lining endothelial cells.[45] This hypertrophy is also observed in keloid scars of humans,[46] which share numerous clinical and histopathologic features with equine EGT.[47,48]

Given this occlusion, the function of new blood vessels in healing wounds of horses was assessed by monitoring derivatives of cutaneous blood flow, namely the temperature of skin and wound[49] as well as transcutaneous oxygen saturation levels.[50] Cutaneous wound temperature, and by extension blood flow, was found to be significantly inferior in limb wounds compared to body wounds and even lower in limb wounds predisposed to the formation of EGT.[49] This data was corroborated using laser Doppler flowmetry.[51] Concomitantly, the degree of oxygen saturation in limb wounds of horses was found to be significantly inferior to that of body wounds during the early period of healing, indicating a temporary, relative state of hypoxia during the inflammatory phase of repair.[50] Likewise, metabolic disturbances were found, via microdialysis, confirming an inadequate supply of oxygen during healing of equine limb wounds that developed EGT.[51]

Because oxygen is required for bactericidal efficiency of leukocytes,[52,53] the relative hypoxia present acutely in limb wounds of horses may explain the feeble yet prolonged inflammatory response

in these wounds thought to contribute to the development of EGT.[3] Moreover, hypoxia itself has been shown to up-regulate angiogenic and fibrogenic mediators,[54] thereby encouraging angiogenesis as well as the proliferation of fibroblasts and their synthesis of components of ECM, substantiating the relationship between the (low) oxygen saturation levels in a wound and the wound's propensity to become fibro-proliferative. These findings were confirmed in a study that showed that subjecting cultured equine dermal fibroblasts to hypoxia stimulated the fibroblasts to proliferate and to synthesize ECM while decreasing the turnover of ECM.[19] Interestingly, no behavioral differences in response to hypoxia were observed between fibroblasts originating from the body or the limb, implying that development of EGT does not depend on intrinsic properties of limb fibroblasts but rather on the local environment of the wound.

In view of these findings, one would assume that delivery of supplemental oxygen during the acute inflammatory phase of healing might protect against the development of EGT in limb wounds of horses. Hyberbaric oxygen therapy (HBOT) involves the inhalation of 100% oxygen within a pressurized chamber, which leads to increased tissue oxygen tensions (for more information on HBOT, the reader is referred to Chapter 19).[55] Topical oxygen therapy (TOT) involves increasing the supply of oxygen directly to the wound by placing an oxygen-filled bag, boot or extremity chamber around the limb or affected area. HBOT is used to manage wounds in humans but as yet, evidence of its efficacy, based on high-quality research, is lacking.[56] Preliminary evidence suggests that HBOT is not indicated for use in horses after full-thickness skin grafting of uncompromised, fresh, granulating wounds,[57] but this modality has not been evaluated in the management of other types of wounds in horses. Likewise, a preliminary study on the use of TOT in the management of experimental dermal wounds on the limbs of healthy horses also showed little effect on healing.[58]

Apoptosis

The elevated and persistent mitotic activity characterizing the granulation tissue of limb wounds may relate to deficient apoptosis. This assumption has been partially substantiated by the observation that the balance of apoptotic signals is skewed against apoptosis in limb compared to body wounds of horses.[44] High concentrations of TGF-β1 persisting in limb wounds[23] may play a role because this particular cytokine appears to have an anti-apoptotic effect on fibroblasts.[59,60] Furthermore, in addition to encouraging the synthesis of fibrogenic cytokines, the absence of an epithelial cover, as is characteristic of EGT, debilitates apoptosis because many signals that favor elimination of fibroblasts by this process are normally released by the keratinocyte.[61] Signals that bring about apoptosis also participate in decreasing deposition of collagen, not only by reducing numbers of fibroblasts but also by activating collagenase.[61]

If the signal to down-regulate the activity of fibroblasts and myofibroblasts is delayed beyond a specific point in time, then apoptosis is permanently impaired.[61] This may explain why wounds chronically affected with EGT are unlikely to resolve spontaneously; indeed, the impairment of apoptosis limits the elimination of unwanted cells and subsequent transition to the next phase of repair.

General clinical factors

Location of the wound

The likelihood that a wound will develop EGT depends on its anatomic location. Wounds of the body heal quickly and without the formation of EGT in contrast to limb wounds, which heal slowly and are prone to excessive fibroplasia. The exact location of the wound on the limb further influences healing and formation of EGT; wounds over the dorsal surface of the metacarpo/metatarsophalangeal joint (fetlock) heal more slowly than similar wounds located over the dorsal surface of the metacarpus/metatarsus.[62,63] Additionally, limb wounds located on the extensor and flexor surfaces of joints and the heel bulbs appear prone to the development of EGT. These clinical differences are assumed to relate to movement that tears the granulation tissue, inciting more inflammation and cellular proliferation (Figure 15.6). The movement of partially lacerated or frayed tendons may exert a similar influence. Indeed, restricting movement by applying a cast or splint can prevent repeated damage and reduce the formation of EGT in these wounds, despite the fact that the local environment created by the cast would normally favor the formation of EGT.[64]

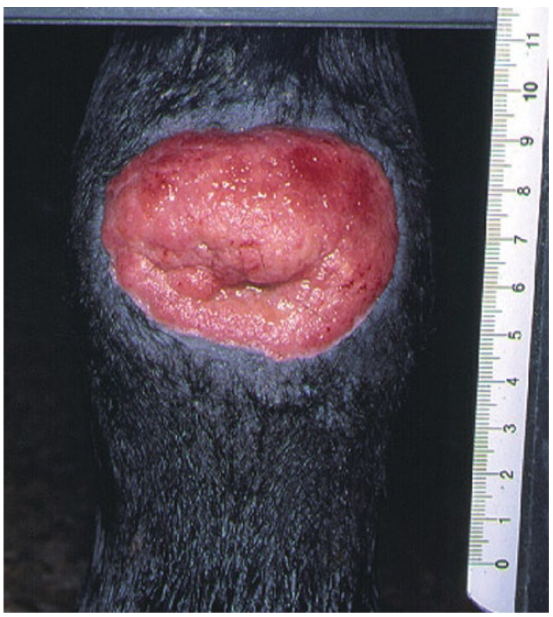


Figure 15.6 A 3-week-old wound at the dorsal surface of the fetlock joint. The groove in the granulation tissue is probably caused by tearing during flexion, which predisposes to EGT formation.

Another factor enhancing the formation of EGT in the distal aspect of the limb is the relative lack of tissue covering the underlying bone and, subsequently, a reduced vascular bed and relatively poor collateral circulation. Impairment of circulation from trauma results in lower oxygen tension in the healing wound, as addressed earlier, with the ensuing effects on proliferation of fibroblasts and synthesis of ECM.[31,54] Conversely, thick and well-vascularized musculature covers most structures of the trunk so that perfusion of a wound in this location is not usually substantially disturbed.

Breed

Formation of EGT is influenced by the breed, but not in an exclusive manner; both horses and ponies can develop EGT, although to differing extents.[2] Ponies form EGT with lesser frequency and quantity, and EGT of ponies tends to disappear spontaneously after the wound is left uncovered. In contrast, horses consistently form more EGT that does not disappear when left uncovered and must be excised. This variable breed predilection might be the confounding factor that explains the conflicting conclusions of past investigations of whether ponies heal with or without EGT.[21,62,64]

Factors related to inflammation and infection

Many factors in the wound can stimulate the overproduction of granulation tissue; most of them are related to inflammation and/or infection. A generalized wound infection may not lead to the formation of EGT but might arrest healing, resulting in an indolent wound. On the other hand, local infection related to the presence of bony sequestra, necrotic segments of tendons, ligaments or other tissue, and/or foreign bodies, triggers a chronic inflammatory response leading to the aforementioned cycle, especially when the condition is long-standing. Similarly, leukocytes are strongly attracted to wounds contaminated with dirt or bacteria, leading to chronic inflammation of the wound.

This emphasizes the importance of thoroughly examining a wound with EGT to identify or exclude factors related to inflammation and infection. If no specific causal factor is incriminated, the inherent chronic inflammatory response commonly occurring in limb wounds of horses may well be the trigger for the formation of EGT. This may, in fact, be the most common cause of EGT and often goes unrecognized because the signs of chronic inflammation may be limited to irregularity of the surface of the granulation bed accompanied by the presence of purulent exudate. These clinical signs, as well as culturing bacteria from the wound, do not indicate that a wound is infected. The wound usually improves dramatically, however, in response to reduction of the bioburden at the surface of the wound, in combination with a single local application of a corticosteroid, a fact substantiating the involvement of chronic inflammation in formation of EGT.

Specific clinical factors

Bandages and casts

Bandages used to cover wounds on the distal aspect of the limb are usually comprised of a primary layer (the dressing), a secondary layer that provides protection and support and can absorb excess exudate, and a tertiary layer that compresses and supports the other layers.[65] Full-thickness wounds on the distal aspect of the limb of horses have been shown to be more likely to develop EGT when covered with a bandage or cast than are similar wounds left unbandaged.[21,27,63–66] This effect is thought to result because bandaging or casting induce the following phenomena: (1) an increase in the oxygen gradient between deeper tissues and the surface of the wound, stimulating angiogenesis;[67] (2) a reduction in oxygen tension in the wound tissues, enhancing proliferation of fibroblasts;[18] and (3) the creation of a moist, warm, and acidic environment, favoring cellular migration and proliferation. Furthermore, some dressings can irritate the wound, evoking more inflammation, and can cause exudate to accumulate at the wound's surface. All these features prompt the formation of EGT.[62] In this respect, management practices can explain, at least partially, why EGT is more often seen in limb wounds because these wounds are usually bandaged whereas body wounds are not. Interestingly, body wounds, when bandaged, can also form EGT, albeit to a lesser degree. EGT would likely form more often in body wounds, if these wounds were routinely bandaged. In conclusion, management practices contribute to the effect of location on the formation of EGT.

The precise effect of bandages on the development of EGT is especially dependent on the type of dressing used. In general, the more occlusive the dressing, the higher is the incidence of EGT. Synthetic, occlusive dressings were shown to significantly stimulate the formation of EGT, thereby increasing the need to trim granulation tissue, compared to other less occlusive dressings.[68] This effect may be explained by an excessive accumulation of exudate beneath the occlusive dressing, which in turn, favors bacterial proliferation and encourages inflammation. Some dressings that are not occlusive by nature can nevertheless "seal" the wound because they insufficiently absorb exudate, causing it to accumulates at the wound's surface, thereby creating a barrier to diffusion. Likewise, the surface of wounds dressed with a non-occlusive dressing, such as gauze, may become occluded when exudate passing through the gauze into the secondary layer of the bandage causes the latter to become occlusive. Several topical wound-care products applied to the surface of a wound, particularly those of a fatty nature, may also cause occlusion.

Conversely, semi-occlusive dressings with a high absorptive capacity, such as foams, provide an environment for the wound that is less likely to induce the formation of EGT because exudate and bacteria are wicked away from the wound (the reader is referred to Chapter 6 for more information on wound dressings).

A notable exception to the general rule, "the more occlusive the dressing, the higher the incidence of EGT," pertains to an occlusive silicone gel sheet dressing (CicaCare®, Smith & Nephew) that was better able to prevent the formation of EGT in limb wounds of horses than was a permeable dressing (Melolite®, Smith & Nephew).[69] Silicone gel sheets are currently the gold standard dressing for managing excessive scarring of the skin in humans.[70,71] Although the mode of action of the silicone membrane is not entirely understood, the dressing has been shown, in people suffering from hypertrophic and keloid scars, to increase tissue temperature[72] and hydration,[73] thereby enhancing the activity of collagenase, which favors remodeling of ECM.[74]

Although bandages and casts can promote the formation of EGT, this does not preclude their use in managing wounds. To the contrary, bandages exert many positive influences on healing. They keep the wound clean and prevent contamination and irritation by environmental factors, such as dirt and straw, which induce inflammation. They facilitate administration of local wound therapies, stimulate more rapid formation of granulation tissue, which is initially required for healing, accelerate epithelialization by creating a moist environment, encourage the development of a more cosmetic scar,[64] reduce the risk for sarcoid transformation of the wound, and protect the wound from additional trauma. Additionally, casts restrict movement in highly mobile regions, thus reducing disruption of the healing process. All these functions limit the development of EGT. Bandages and casts are, therefore, an important component in the management of wounds healing by second intention, because the aim to support the overall healing process largely surpasses the need to prevent EGT.

Iatrogenic factors

The way in which a wound is managed in the early stages has a dramatic effect on the time required for healing, as well as on the formation of EGT. Application of non-physiologic materials, including powders and chemicals (e.g., antiseptics), as well as some antibiotics, may adversely affect wound healing (Figure 15.7). Additionally, application of caustic substances, including copper sulfate, nitric acid, acetic/malic acid mixtures, silver nitrate, triple dye, supersaturated potassium permanganate, sodium hypochlorite (Dakin's solution), lye, and many other home remedies, in an effort to prevent or treat EGT, seriously delays repair. These caustic agents induce necrosis not only of the granulation tissue but also of the migrating and proliferating epithelium.[62,75] Cryogenic surgery applied to granulating wounds similarly delays healing.[64] The resultant necrosis encourages a chronic inflammatory response and the release of many mediators that inhibit wound contraction and overstimulate cellular proliferation, as previously outlined. Healing by epithelialization and contraction is arrested, and the stimulus for formation of granulation tissue accrued, leading to a recurrence of EGT. Cautery of any type, therefore, delays healing and promotes more EGT. Although wounds treated in this way eventually heal, they are frequently characterized by unacceptable scarring.[75] The aforementioned problem is unfortunately still encountered in some equine practices, because many owners attempt to manage a wound themselves prior to consulting a veterinarian.

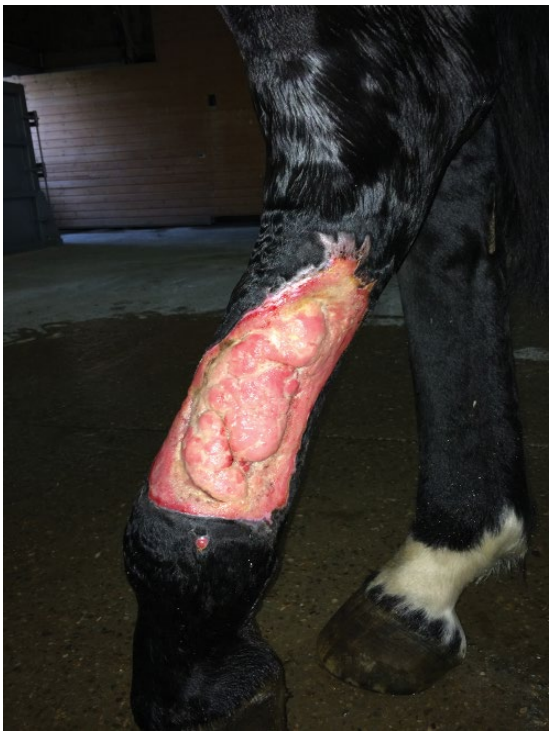


Figure 15.7 This wound, which initially exposed the metatarsal bone, was treated with disinfectant solutions (cleaned with Disifin solution – containing an N-chlorinated and N-deprotonated sulfonamide – followed by povidone–iodine spray 5%) for 2 months prior to the photo. The granulation tissue, which does not yet entirely fill the wound, is traversed by deep grooves and shows areas of necrotic tissue covered by purulent exudate. No bone sequestrum was visible radiographically. Although the disinfectants may have killed most bacteria in the wound, they are toxic to fibroblasts and could be responsible for the delayed formation of healthy granulation tissue.

Differential diagnoses

EGT can be confused with tumors, especially sarcoids (Figure 15.8). An equine wound can transform into a sarcoid, a serious cause of failure of healing of a cutaneous wound (for more information the reader is referred to Chapter 21). Sarcoid transformation can occur at any wound, but the type of sarcoid that develops is highly dependent on the anatomic location of the wound. A sarcoid that forms in a wound on the distal aspect of the limb is invariably fibroblastic in nature, which explains why it is often confused with EGT. Horses with a sarcoid appear to be particularly prone to sarcoid transformation of wounds, as are horses in close contact with other horses bearing sarcoids. Transformation is also more likely to occur if the wound is left uncovered, especially in the summer when flies are abundant.

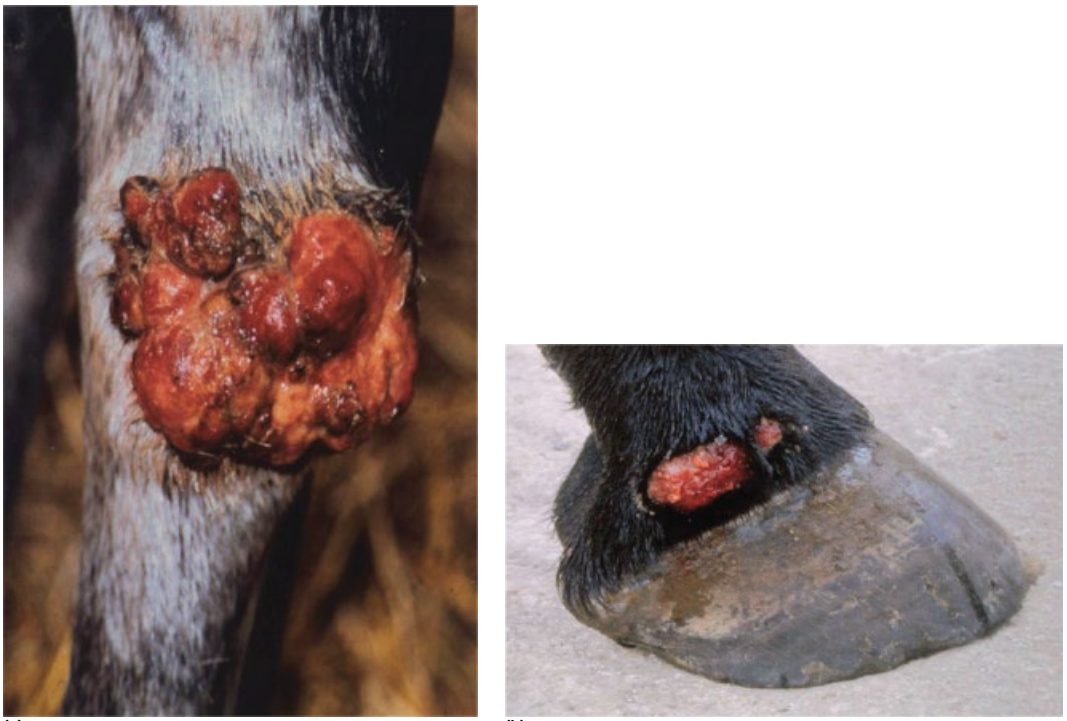


Figure 15.8 **(a)** A wound caused by a wire cut on the dorsal surface of the tarsus of a 5-year-old mare, which failed to heal. A harvested tissue sample allowed confirmation of pure sarcoid in the upper area and a mixture of sarcoid and EGT in the lower area of the wound. **(b)** Five-week-old wound in the pastern region of a gelding. The horse was admitted because the granulation tissue remained exuberant. Excised granulation tissue was examined histologically, enabling diagnosis of a pure fibroblastic sarcoid. Source: Wilmink and van Weeren 2004.[1] Reprinted with permission of Elsevier.

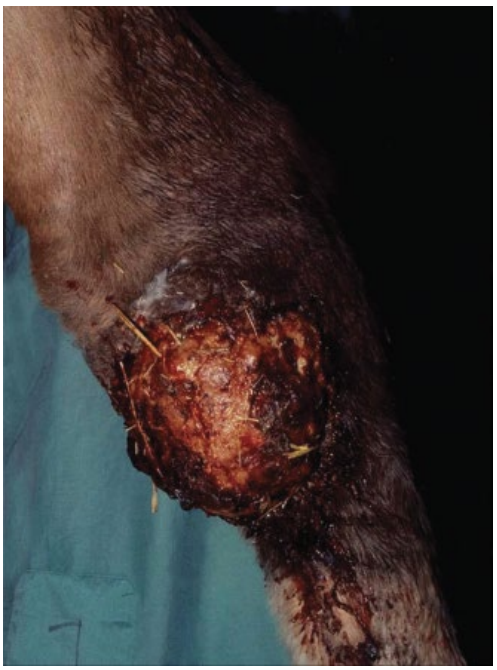


Figure 15.9 Wound on the dorsal surface of the tarsus that was treated for 6 months without resolution. Histologic examination of a tissue sample confirmed transformation to a squamous cell carcinoma. Courtesy of Dr. Ted Stashak.

Although uncommon, chronic granulating wounds can also transform into a squamous cell carcinoma (Figure 15.9). Conversely, some tumors, such as sarcoids, some hemangiomas, and squamous cell carcinomas, can develop independently of an apparent wound and adopt the appearance of granulation tissue.

If healing of a wound does not progress and tissue that appears to be EGT reappears regularly after being trimmed, excised tissue or a deep biopsy sample from the wound should be examined histologically, or a swab of the tissue should be examined using polymerase chain reaction (PCR),[76] to obtain a definitive diagnosis of sarcoid or other tumors. This is critical because the best practice for managing EGT is contraindicated in the management of sarcoids or other tumors.[75] For more information on the management of sarcoid transformation at wound sites, the reader is referred to Chapter 21.

Prevention of exuberant granulation tissue

Exclusion of factors related to inflammation and infection

The likelihood of a wound developing EGT can be reduced by excluding causal factors, particularly those that are related to inflammation and infection.

What to do

- Examine the wound for bony sequestra, necrotic segments of tendon or ligament, and foreign bodies, and eliminate these causes of inflammation.

What to avoid

- Avoid caustic compounds or other irritating substances.

Tip

- Use bandages (and splints or casts, where appropriate) to prevent additional contamination and trauma, protect exposed bone and tendons from desiccation and contamination, and reduce motion.

What to do

- Use bandages to prevent contamination and dessication of tissue.

What to avoid

- Avoid occlusion: i.e., occlusive dressings or topical products (except for silicone sheet dressings), collection of exudate on the wound due to infrequent bandage changes, wet padding (secondary layer of the bandage), and occlusive/plastic tapes as the tertiary layer of the bandage.

Tip

- Use a foam dressing as a primary layer, to absorb exudate and bacteria and thereby prevent occlusion.

Use of bandages

Using pressure bandages to restrain excessive fibroplasia is counterproductive. These bandages may effectively suppress the swelling of young edematous granulation tissue but generally do not impair its formation.

The fact that bandaging limb wounds favors the development of EGT which, if not resected, delays healing, has led to the practice of controlling formation of EGT by omitting bandages after a wound has filled with granulation tissue. This approach was partly inspired by evidence that unbandaged wounds created experimentally in ponies healed faster and without EGT, although with more scarring, than did bandaged wounds in which healing was delayed by EGT.[64] Subsequent studies have determined that this approach may not be so straightforward. Unbandaged and bandaged experimental wounds on the limbs of horses healed in a similar amount of time when EGT that developed in the bandaged limbs was excised.[65,66]

Importantly, the magnitude of many clinically encountered wounds makes bandaging compulsory, in contrast to relatively harmless wounds created for experimental purposes that can be left unbandaged. In some situations, leaving a wound unbandaged after it is filled with granulation tissue is reasonable, for example when costs must be limited, when the horse is difficult to treat, or when the cosmetic outcome is unimportant. Leaving a limb wound unbandaged, however, may lead to increased swelling of the limb and arrested healing because of contamination and dessication.

In conclusion, the fact that bandages favor the formation of EGT, which can subsequently delay healing, must not be taken to mean that healing can be stimulated by omitting bandages. When managed correctly, EGT does not delay healing, and bandages provide more advantages than disadvantages. Moreover, the formation of EGT can be limited by selecting an appropriate wound dressing.

Skin grafts

Skin grafts exert a significant inhibitory effect on the formation of EGT by controlling proliferation of endothelial cells and fibroblasts and by reducing the synthesis of components of ECM by fibroblasts. The inhibitory effect of grafts on fibroblasts may be regulated by a soluble epithelial-derived product that potentiates apoptosis of underlying cells.[61,77] A vascularized skin flap also favors apoptosis of fibroblasts and endothelial cells and rapid remodeling of the underlying granulation tissue.[78] This is likely the result of reduced expression of TGF-β1 along with increased degradation of the ECM due to an altered balance between MMPs and their inhibitor TIMP-1, as well as increased expression of inducible nitric oxide synthase (iNOS) generating free radicals that arrest the cell cycle and promote apoptosis.[78] Additionally, the ability of the graft to reduce the surface area of the wound, thereby attenuating inflammation, helps to control of the development of EGT. The reader is referred to Chapter 18 for more information on skin grafting in the horse.

Tip

- Skin grafts should be harvested from a site that normally heals well and in which contraction is prominent (e.g., the lateral cervical, abdominal, and pectoral regions).[75]

Treatment of exuberant granulation tissue

The treatment of EGT depends, to a certain extent, on the age of the wound and the nature of the granulation tissue. This section describes the situations most often encountered clinically.

Protruding young edematous granulation tissue

Young, edematous granulation tissue bulging just above the margin of the wound generally does not require special treatment. Swelling can usually be limited by moderate pressure exerted by

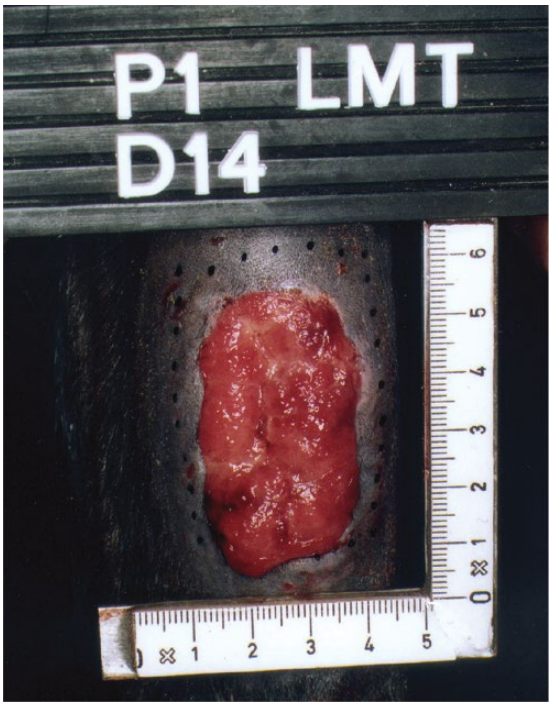


Figure 15.10 Example of a 2-week-old experimental wound created on the limb of a pony, showing edematous, protruding granulation tissue that does not yet qualify as EGT. Such increase of the swelling can be seen upon bandage change. Source: Wilmink and van Weeren 2004.[1] Reprinted with permission of Elsevier.

a bandage. Protrusion of the granulation tissue is noted when the bandage is removed, and increases when the wound is left uncovered for a short time (Figure 15.10). Frequently, the edematous swelling disappears when wound contraction begins. The tissue is classified as EGT when the protrusion feels firm and takes on a granular appearance. Firm tissue protruding over the margin of the wound should be treated.

Exuberant granulation tissue in general

Most limb wounds form some degree of EGT during healing. It is important to determine, by carefully examining the wound, if the periosteum was injured thereby exposing cortical bone or if a tendon or ligament was partially or completely severed. Bone sequestra, necrotic parts of tendons or ligaments, foreign bodies, or dirt can induce and perpetuate chronic inflammation that favors the development of EGT; these conditions, therefore, should be resolved.

What to do

- Probe any clefts that have formed within the new granulation tissue using flexible and rigid sterile probes to identify draining tracts. Complementary diagnostic modalities, such as radiographic or ultrasonographic examination, may be required.
- Radiographically examine underlying bone to rule out the possibility of a bone sequestrum when the injury has exposed cortical bone.

When the wound is located near or over a joint, movement is likely the reason EGT has formed. Immobilizing the wound, by applying a cast, can be helpful when the granulation tissue is freshly exuberant and appears healthy. When the wound is close to a synovial structure or when EGT is due to chronic inflammation that requires repeated treatment, a bandage splint is preferred to a cast (the reader is referred to Chapter 7 for more information on techniques of bandaging, splinting, and casting).

When no underlying inciting cause can be found, the most probable culprit for the formation of EGT is the inherent chronic inflammatory response characteristic of limb wounds of horses. Reducing surface contamination by thorough wound debridement and irrigation, followed by one topical application of corticosteroid, halts the aforementioned vicious cycle, allowing healing to ensue. The effect of a topically applied non-steroidal anti-inflammatory drug (NSAID) on chronic equine wounds is unknown, but topical application of a non-steroidal non-selective cyclooxygenase (COX) 1-2 inhibitor to experimental wounds of rabbits suffering chronic inflammation did not affect the number of leukocytes in the wounds and the NSAID delayed wound closure.[20]

In most cases, treatment of EGT is straightforward, and excision appears to be the best choice of treatment. Granulation tissue should be excised as soon as it protrudes above the wound's margin.[3] Excision can be performed with the horse standing, and desensitizing the wound is not necessary because granulation tissue is not innervated. The excess granulation tissue is excised as close to the adjacent skin level as possible while taking care to preserve the migrating epithelium at the wound's periphery. Excision should commence at the distal-most aspect of the wound and progress proximally so that hemorrhage does not obscure the surgical field. While a tourniquet may be applied above the wound in a sedated horse, it is rarely required to achieve the desired result. The goal of excision is to remove excess and non-viable tissue, as well as gross contaminants, which consequently, also eliminates a large number of leukocytes present in the superficial layer of the granulation tissue, thereby diminishing the stimulus for chronic inflammation. Dramatic improvement of the health of the wound's surface is usually achieved when excision is preceded by aseptic preparation of the skin around the wound and followed by sterile bandaging and a short period of topical antibacterial therapy to further reduce contamination (Figure 15.11). Indeed, repair gets a new impulse – contraction is "jump-started," and epithelialization proceeds. This said, repeated excisions may be required.

The use of silicone sheet dressings to prevent recurrence of EGT in horses has been validated.[69] The dressing should be applied after bleeding arising from excising EGT is controlled. The silicone sheet dressing should be maintained on the wound until contraction and epithelialization are underway, after which it may be replaced by a light foam dressing or maintained until healing is complete. The silicone dressing is reusable, which offsets its initial high cost.

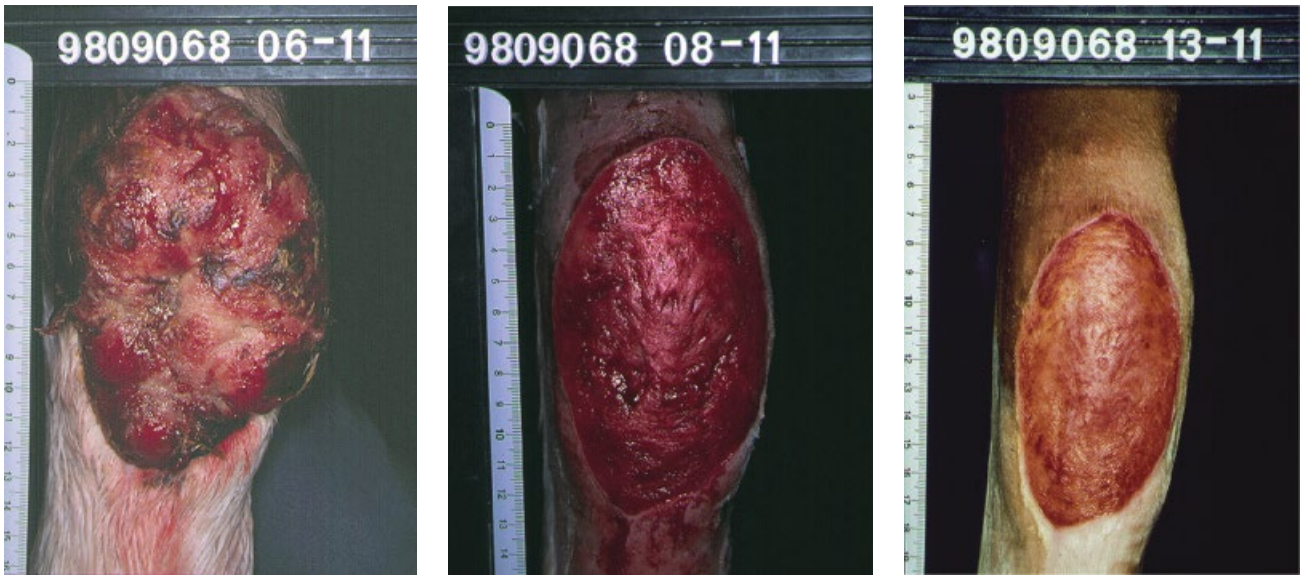


Figure 15.11 **(a)** Wound on the plantar surface of the metatarsus of a 3-year-old mare. EGT had been present for a couple of weeks and healing had ceased. **(b)** Two days after excision of EGT, dramatic improvement of the wound bed can be observed. **(c)** Excision was followed by a short-term course of topical antibacterial treatment supplemented with one topical application of corticosteroids the day before the picture was taken. Wound contraction has occurred and epithelialization has begun, thus diminishing the risk for recurrence of EGT. Source: Wilmink and van Weeren 2004.[1] Reprinted with permission of Elsevier.

Tip

- Washing a silicone sheet dressing gently under tap water with mild liquid detergent, as recommended by the manufacturer (Smith & Nephew), preserves the dressing's exceptional adherence to the wound's surface and allows it to be reused.

Topical application of a corticosteroid to arrest the formation of EGT remains controversial. Corticosteroids counter inflammation, and thus may be useful to control the chronic inflammatory response present in limb wounds of horses. Moreover, some corticosteroids may selectively attenuate the release of fibrogenic TGF-β1 and -β2 from monocytes and macrophages, counteracting proliferation of fibroblasts and formation of ECM.[79] This rationalizes the use of a corticosteroid in the treatment of newly formed EGT. Corticosteroids have been shown, however, to exert a negative influence on angiogenesis, contraction, and epithelialization thereby delaying wound healing.[80,81] A corticosteroid, therefore, if used, should be applied at the first signs of excessive fibroplasia but not repeatedly, so as to limit the negative influence exerted by the corticosteroid on healing.

A caustic agent or cryogenic surgery should not be used to treat EGT because these induce necrosis, stimulate chronic inflammation, damage the new epithelial border, and ultimately inhibit healing by promoting proliferation of the granulation tissue.

Recurrent exuberant granulation tissue

In some cases, in the absence of an apparent cause, EGT may recur in spite of repeat excisions and topical treatment to control chronic inflammation. This is seen more often when owners change the bandages. In these cases, the bandaging protocol should be critically assessed.

What to do

- Proceed with aseptic preparation of the skin surrounding the wound. Clean the wound using sterile isotonic saline solution and swabs, debride the wound using sterile instruments, apply antimicrobial dressings for 1–2 weeks to reduce surface contamination, and thereafter topically apply a single dose of a short-acting corticosteroid.

Tip

- Obtain a tissue sample for histologic examination in cases where tumor transformation of the wound is suspected.

Some horses mount a very strong and chronic inflammatory response at the site of a wound, often accompanied by periosteal new bone formation when the wound initially exposed bone. In such a case, resorting to repeated applications of a longer-acting corticosteroid, such as triamcinolone, may be appropriate to break the vicious cycle of "inflammation–proliferation."

Rarely, the only escape from recurrent EGT is to temporarily remove bandages in an effort to diminish the stimulus for fibroplasia. This option, however, may be considered unappealing from an esthetic perspective because such wounds are often large and particularly unattractive. Resuming bandaging of a large wound, after the wound is flat and contracting, is advantageous because bandaging provides a moist environment that encourages epithelialization. If exuberant-appearing granulation tissue recurs despite the aforementioned approach, the clinician should suspect tumor transformation of the wound.

What to do

- Obtain a tissue sample for histologic examination in cases where tumor transformation of the wound is suspected.

Exuberant granulation tissue after skin grafting

EGT may occur after skin grafting. In this case, excision is inadvisable because of the risk of damage to the newly grafted tissue. As soon as granulation tissue protrudes above the grafts, further development of EGT can be limited by intermittent topical application of a corticosteroid. Generally, after applying the corticosteroid topically only once or twice, epithelialization can be seen to progress from the grafts. The corticosteroid should not be applied too often because epithelialization from the graft margins will be disturbed. As soon as epithelialization progresses from the grafts, the development of EGT is naturally controlled.

Chronic exuberant granulation tissue

Horses having large lumps of chronic EGT are, unfortunately, known to most practitioners (Figure 15.12). Chronic EGT is usually very fibrous, is nourished by large blood vessels, and, in some cases, may be partially innervated. The impetus for healing of these wounds has disappeared and is unlikely to be recovered without intervention. Excising the EGT, followed by application of a skin graft is the best approach to get these wounds to heal at an optimum rate and with an acceptable outcome.[62] The wound can be debulked with the horse standing and sedated, but often debulking the wound with the horse anesthetized is more prudent because of the violent reaction that may be displayed when the EGT is excised, possibly because the tissue is partially reinnervated. Debulking with the horse anesthetized also allows for better control of hemorrhage and its possible systemic consequences. After excising the exuberant fibrous tissue, a pressure bandage is applied to control hemorrhage. Although excision can be followed immediately by skin grafting, in most cases, it is preferable to leave the wound ungrafted and bandaged for a few days until hemorrhage has abated and a new bed of granulation has begun to form (for more information on skin grafting, the reader is referred to Chapter 18).

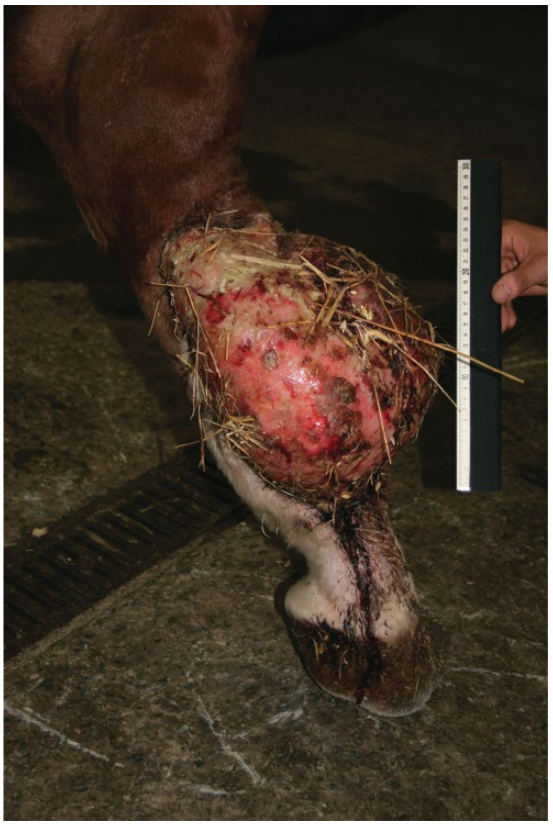


Figure 15.12 A wound with marked and chronic EGT. Treatment of such a wound is challenging. Excision of the EGT, followed by skin grafting, is the best approach.

Conclusion

The formation of EGT is a frequent complication in wounds healing by second intention on the limbs of horses. Among the large number of factors contributing to EGT, chronic inflammation is foremost and often goes unrecognized because of the mild accompanying signs. The stimulus for formation of EGT is reduced when prevention and treatment of chronic inflammation are combined with excision of the protruding granulation tissue. Transition from the fibroblastic phase of healing to the phases of contraction and epithelialization then occurs smoothly and usually obviates the recurrence of EGT.

Many approaches have been used to treat EGT, but the best therapy, at this time, remains excision of the protruding tissue. The limited and appropriate application of a corticosteroid and the use of silicone sheet dressings or skin grafting are useful to prevent EGT or its recurrence.

New methods of preventing the development of EGT are being sought through fundamental research in equine wound healing. Innovative, targeted therapies consisting of specialized interactive dressings combined with engineered reconstitution of tissues using cell-based therapy, scaffold-based therapy, and/or bioactive molecule-based therapy will likely someday be available (the reader is referred to Chapter 22 for more information on this topic).

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