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ANATOMY

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INTRODUCTION TO HISTOPATHOLOGY

400 level

 ASSIGNMENT

1. WRITE ON CYTOKINE SIGNALING AND ITS ROLE IN WOUND HEALING.

2. WHEN IS WOUND HEALING REFERRED TO AS ‘IMPAIRED’ AND WHY?

3. EXAMINE THE ROLE OF OXIDATIVE STRESS IN THE DEVELOPMENT AND PROGRESSION OF IMPAIRED WOUND HEALING.

**1. CYTOKINE SIGNALING**

Cytokine signaling is an important part of the human body regulation. Most cytokines are cell-secreted proteins from glial cells in the nervous system and are necessary for intracellular signaling. Most cytokines are local regulators that alert and activate lymphocytes. Some cytokine-signaling pathways involve hormones such as growth hormones and leptin, the hormone that controls fat storage.

The immune system depends on cytokine signaling to keep the human body healthy. Macrophages and dendritic cells engulf foreign particles and send a cytokine signal to nearby dormant lymphocytes. The receptors on the lymphocytes recognize the signal and activate. Those cells are specialized to recognize certain antigens. The combination of the macrophages and activation of lymphocytes through cytokine signaling help keep the body in homeostasis or the proper internal equilibrium.

Some cytokine signals are not local but rather travel a long distance throughout the body. These cytokines are sometimes classified as hormones. This classification is changing, however, because cytokines are not secreted from glands. Instead, they are secreted from glial cells of the nervous system. These growth hormones are essential for embryonic development. Cytokines bind to receptors on target cells and activate a cascade of intercellular signals. The most common of these pathways is the protein kinase transduction cascade. After the cytokine binds to the receptor embedded in the membrane of the cell, inactive protein kinases are activated by a process known as phosphorylation.

**ROLE OF CYTOKINE SIGNALING IN WOUND HEALING**

Wound healing is a complex process that depends upon the interactions among a large number of distinct cell populations whose regulation is tightly controlled (Harding *et al*., 2002). One of the most well-known cytokines involved in the healing process is the pro inflammatory molecule tumor necrosis factor-α (TNF- α). Although essential in the early phases of wound healing, continued expression of TNF- α in the repair response is considered to be deleterious. Increased expression is detectable within 12 hours after injury, and TNF- α is primarily released by local macrophages, where it induces neutrophil recruitment and maturation (Rumalla and Borah 2001) (Michie *et al*., 1988). Wound fluid TNF- α levels peak at 3 days after dermal injury and are responsible for the increased vascular permeability and proliferation, as well as the increased hemostasis (Rumalla and Borah 2001) (Feiken *et al*., 1995). In addition, continued expression in the maturing wound can increase collagen synthesis and wound-disruption strength (Mooney *et al*., 1990) (Fu *et al*., 1996). However, overproduction or prolonged expression of TNF- α at this point may cause increased tissue destruction by the over activation of immune cells and their protease products (Strieter *et al*., 1990) (Tracey *et al*., 1988). More specifically, the murine TNF p55 receptor plays a role in promoting leukocyte infiltration at the wound site and negatively affects wound healing by reducing angiogenesis and collagen accumulation. Continued expression of TNF- α 7 days after injury can decrease collagen synthesis and reduce granulation tissue (Rapala *et al*., 1997) (Rapala, 1996). This phenomenon is also seen in delayed wound healing states where TNF- α production is sustained. Administration of a TNF- α antagonist (TNF-binding protein) to septic rats can partially reverse the delayed healing process in skin and intestinal wounds, which is frequently seen (Cooney *et al*., 1997). Data in nonseptic rodents suggest that systemic TNF- α inhibition throughout healing leads to qualitative impairments in wound healing with a significant alteration in collagen deposition, although local TNF- α abrogation by a TNF- α antibody down-regulates collagen synthesis (Lee *et al*., 2000). In addition, administration of an IL-1 receptor antagonist and TNF- α to rats further abrogates the diminished wound healing observed in mice given TNF- α alone. Also essential to wound healing, the cytokine interferon- gamma (IFN- γ) is secreted predominantly by T lymphocytes. The primary effects of IFN- γ are not limited to polymorphonuclear leukocytes and macrophage activation and cytotoxicity10; IFN- γ induces tissue remodeling and directly reduces wound contraction. IFN- γ has this effect by increasing collagenase expression as well as by decreasing collagen production and lattice crosslinking (Tamai *et al*., 1995). These properties have made the administration of IFN- γ a possible treatment for hypertrophic scars. However, there is some evidence that IFN- γ plays an enhancing role in post burn active hypertrophic scars, acting as a T-cell chemoattractant and a growth factor (Castagnoli *et al*., 2002). In addition, IFN- γ production reduces re-epithelialization and wound strength, and thus unopposed IFN- γ expression can be detrimental. IL-1 exists in two forms, IL-1 α and IL-1β. IL-1 β, released principally by monocytes, is an early proinflammatory cytokine with many properties similar to TNF- α. However, IL-1 is also released in keratinocytes during wound healing (primarily IL-1 α). Although IL-1 α expression in keratinocytes is generally considered to be constitutive, increased IL-1 activity (composed of both IL-1 α and IL-1 β) is detectable in the wound environment within 24 hours of injury and peaks in concentration between 24 and 72 hours.52. In addition to activating neutrophils and promoting chemotaxis, IL-1 induces cells, such as endothelial cells, to express pro inflammatory cytokines. Analogous to TNF- α, initial IL-1 expression is required and beneficial to the wound healing process, increasing collagen synthesis as well as keratinocyte and fibroblast growth (Sauder *et al*., 1990). High levels of IL-1 after the first week of healing, however, appear to be deleterious and pathogenic. Dysregulation of IL-1 expression is thought to be partially responsible for the delayed wound healing that is observed during stress or with steroid administration (Mercado *et al*., 2002). Animal models have demonstrated the benefit of using IL-1 receptor antagonists in the synovial lining during arthritis or in reversing wound impairment induced by TNF- α administration. IL-8 represents one protein in a very large and diverse family of chemokines. IL-8, a prototypical member of the CXC family of chemokines, is one protein responsible for the activation and recruitment of neutrophils in acute dermal wounds. Secreted by macrophages and fibroblasts, IL-8 is mostly detectable in the first 24 hours of healing. IL-8 has numerous biological effects, including increased myeloid leukocyte chemotaxis, neutrophil activation, endothelial cell adhesion protein expression, and keratinocyte maturation and margination (Engelhardt *et al*., 1998) (Clark, 1993). Low-energy laser irradiation is thought to enhance wound healing through increased IL-8 expression. However, any excess expression of this cytokine can be detrimental to wound healing and can cause increased scarring. IL-8 is overexpressed in psoriasis and like IL-6, IL-8 is found in very low concentrations in fetal tissue. Mice do not express a true IL-8 homolog but rather a family of chemokines with overlapping biological activities, including proteins KC and GRO. These chemokines appear to play analogous roles in the murine healing process, and inappropriate production of these mediators, either excess or an absence, appears to alter the healing process. Another cytokine frequently found in the wound microenvironment is IL-6. IL-6 has defied classification as either a proinflammatory or anti-inflammatory cytokine, appearing to have properties of both. In addition, IL-6 has both local and systemic effects on wound healing. IL-6 plays a central role in the systemic response to injury (along with IL-1 and TNF- α) as a primary inducer of the hepatic and myeloid acute phase responses (Baumann *et al*., 1987). At the local wound level, IL-6 stimulates fibroblast proliferation and is secreted by many cells types in the wound environment. This includes fibroblasts, monocytes, and most importantly polymorphonuclear cells, whose infiltration into the acute wound parallels the increased rise in IL-6 concentrations in the local environment. Detectable within the first 12 hours of injury, elevated quantities of IL-6 may remain in the wound fluid for greater than seven days. IL-6 secretion is vital to endothelial protection from ischemic injury in the early wound (Gallo *et al*., 1997). In addition, impaired IL-6 secretion is thought to cause weakened healing in the elderly. However, like all of the inflammatory mediators described to date, inappropriate IL-6 expression can be unfavorable. As mentioned above, IL-6 is practically undetectable in fetal wounds, and its administration to fetal wounds increases scarring. Circulating IL-6 levels parallel wound IL-6 concentrations in burn wounds, and non survivors of burn injury have elevated IL-6 levels when compared with their surviving controls (Ueyama *et al*., 1992) (Frieling *et al*., 1995). Finally, IL-2 is produced primarily by T lymphocytes as a T-cell growth factor that supports the clonal expansion and activation of T cells.10 Although IL-2 is traditionally considered to be predominantly a T-cell growth factor, the protein is pleiotropic and has a number of associated inflammatory properties. IL-2 can increase fibroblast metabolism in vitro. In vivo, IL-2 administration increases rodent wound-breaking strength in immunocompromised (doxorubicin impaired) hosts, although it does not have the same effect on its non-compromised controls. Therefore, it is being investigated for its possible benefits in the immunocompromised wound. The response to dermal injury is characterized by an early proinflammatory response, followed temporally by the appearance of a number of anti-inflammatory molecules. This biphasic response has been characterized as an endogenous effort to limit both the magnitude and the duration of the proinflammatory response and allow the wound to migrate into a proliferative healing phase. Evidence has shown that chronic wounds with delayed healing properties are often locked into these early proinflammatory phases, with elevated levels of both early proinflammatory cytokines (such as TNF- α, IL-1, and chemokines) as well as many anti-inflammatory cytokines. Thus, anti-inflammatory cytokines also play key roles in the repair response, both directly as well as through the modulation of proinflammatory cytokine production. The former functions are often disregarded when compared with their abilities to suppress proinflammatory cytokine production. One such cytokine is IL-4, which is expressed by T lymphocytes, basophils, and mast cells (Chomarat and Banchereau 1997) (Brown and Hural 1997). The effects of IL-4 include suppressing the expression of proinflammatory cytokines, as well as promoting B cell proliferation and mediating IgE production. Although excessive production has been implicated in the fibrotic wound healing seen in scleroderma, IL-4 plays an important role in normal wound healing, promoting fibroblast proliferation, proteoglycan synthesis by wound fibroblasts, and collagen production (Wegrowski et al., 1995). In addition, IL-4 up-regulates arginase activity in normal and wound fibroblasts, as well as macrophages, smooth muscle, and endothelial cells. Because arginase activity is known to play an important role in wound healing (presumably through nitric oxide generation, although the exact mechanism remains to be determined) this may be one additional mechanism through which IL-4 enhances the repair response. IL-10 is also an anti-inflammatory cytokine secreted by T lymphocytes. In addition, dendritic cells and macrophages express IL-10, which inhibits the production of proinflammatory cytokines at the level of gene expression, as well as preventing neutrophil and macrophage infiltration into the wound. Detectable within 24 hours of injury, IL-10 is measurable for up 10 days from the initiation of wound healing (Sato et al., 1999). Although it has an important counter regulatory role, IL-10, like most cytokines, has injurious effects with excessive expression, including possibly causing the failed closure of chronic venous insufficiency ulcers (Lundberg *et al*., 1998).

**2. WHEN IS WOUND HEALING REFERRED TO AS ‘IMPAIRED’ AND WHY?**

The wound-healing process consists of four highly integrated and overlapping phases: hemostasis, inflammation, proliferation, and tissue remodeling or resolution (Gosain and DiPietro, 2004). There are many factors that can affect wound healing which interfere with one or more phases in this process, thus causing improper or impaired tissue repair. Wounds that exhibit impaired healing, including delayed acute wounds and chronic wounds, generally have failed to progress through the normal stages of healing. Such wounds frequently enter a state of pathologic inflammation due to a postponed, incomplete, or uncoordinated healing process. Most chronic wounds are ulcers that are associated with ischemia, diabetes mellitus, venous stasis disease, or pressure.

In wounds where oxygenation is not restored, healing is impaired. Temporary hypoxia after injury triggers wound healing, but prolonged or chronic hypoxia delays wound healing (Bishop, 2008) (Rodriguez *et al*., 2008). In acute wounds, hypoxia serves as a signal that stimulates many aspects of the wound-healing process. Hypoxia can induce cytokine and growth factor production from macrophages, keratinocytes, and fibroblasts. Cytokines that are produced in response to hypoxia include PDGF, TGF-β, VEGF, tumor necrosis factor-α (TNF-α), and endothelin-1, and are crucial promoters of cell proliferation, migration and chemotaxis, and angiogenesis in wound healing (Rodriguez et al., 2008).

**3. ROLE OF OXIDATIVE STRESS IN THE DEVELOPMENT AND PROGRESSION OF IMPAIRED WOUND HEALING**

The wound healing process is regulated by a large variety of different growth factors, cytokines and hormones. In addition, a series of recent studies revealed that nitric oxide as well ROS (reactive oxygen species) are crucial regulators of this process (Wlaschek and Scharffetter-Kochanek 2005). ROS are required for the defense against invading pathogens, and low levels of ROS are also essential mediators of intracellular signaling (D’Autreaux and Toledano 2007). For example, a recent study revealed that low levels of hydrogen peroxide are important for efficient wound angiogenesis (Roy *et al*., 2006). However, excessive amounts of ROS are deleterious due to their high reactivity. In this review, we will first summarize the evidence for the presence of oxidative stress in skin wounds, in particular in chronic non-healing wounds. Subsequently, we will report on the presence of low molecular weight antioxidants in the wound tissue and their function in the repair process. Finally, we will summarize recent results on the expression and function of ROS-detoxifying enzymes in the wound healing process.

Due to the short half-life of ROS, their concentrations in vivo are difficult to determine. Nevertheless, H2O2 levels could recently be determined in wound fluid from acute murine excisional wounds using a real-time electrochemical H2O2 measurement (Roy et al., 2006). These studies revealed that low concentrations (100–250M) of H2O2 are present at the wound site. Higher levels were found during the early inflammatory phase (day 2 after injury) compared to the later phase, when new tissue formation occurs (day 5 after injury). In addition to H2O2, the presence of superoxide at the wound edge was detected by staining of frozen sections with the redox-sensitive dye dihydroethidium (Roy et al., 2006). The same group recently confirmed these results using an electron paramagnetic resonance spectroscopy-based approach, where the metabolism of topically applied nitroxide 15N-perdeuterated tempone was measured noninvasively. These studies revealed that superoxide levels peak at around day 2 after injury in full-thickness excisional mouse wounds (Ojha et al., 2008). Superoxide production was impaired in mice lacking Rac2, one of the essential subunits of NADPH oxidase, and this correlated with impaired wound healing in these mice (Ojha *et al*., 2008). These results suggest that the low levels of ROS that are produced in normal wounds are important for the repair process. It will be interesting in the future to use these technologies for the analysis of ROS levels in chronic, non-healing wounds.

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