**A TERM PAPER**

**ON**

**WOUND HEALING**

**BY**

**EBENEZER, ENO-ABASI ISRAEL**

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**QUESTIONS**

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.

**What is Cytokine Signaling?**

Cytokines are a broad and loose category of small proteins (~5–20 kDa) important in cell signaling. Cytokines are peptides and cannot cross the lipid bilayer of cells to enter the cytoplasm. Cytokines have been shown to be involved in autocrine, paracrine and endocrine signaling as immunomodulating agents. Their definite distinction from hormones is still part of ongoing research. Cytokines include chemokines, interferons, interleukins, lymphokines, and tumor necrosis factors, but generally not hormones or growth factors (Wolters *et al.,* 2006).

Cytokines are produced by a broad range of cells, including immune cells like macrophages, B lymphocytes, T lymphocytes and mast cells, as well as endothelial cells, fibroblasts, and various stromal cells. A given cytokine may be produced by more than one type of cell. They act through cell surface receptors and are especially important in the immune system (Wolters *et al.,* 2006).

Cytokines modulates the balance between humoral and cell based immune responses, and they regulate the maturation, growth, and responsiveness of particular cell populations. Cytokines also play a role in anti-inflammatory pathways and are a possible therapeutic treatment for pathological pain from inflammation or peripheral nerve injury. There are both pro-inflammatory and anti-inflammatory cytokines that regulate this pathway (Zhang & An, 2007).

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 (proper internal equilibrium).

**Cytokine Signaling in Wound Healing**

Cytokines are crucial for fighting off infections and in other immune responses (Dinarello, 2000). However, they can become dysregulated and pathological in inflammation, trauma, sepsis, and hemorrhagic stroke (Zhu *et al.,* 2019).

The fact that cytokines themselves trigger the release of other cytokines (Chokkalingam *et al.,* 2013) and also lead to increased oxidative stress makes them important in chronic inflammation, as well as other immune responses. Several inflammatory cytokines are induced by oxidative stress (David *et al.,* 2007).

The response to injury is a phylogenetically primitive, yet essential innate host immune response for restoration of tissue integrity. Tissue disruption in higher vertebrates, unlike lower vertebrates, results not in tissue regeneration, but in a rapid repair process leading to a fibrotic scar. Wound healing, whether initiated by trauma, microbes or foreign materials is a complex process and proceedes via a number of overlapping phases, including coagulation, inflammation, epithelialization, formation of granulation tissue, angiogenesis, matrix deposition and tissue remodeling. The process of repair is mediated in large part by interacting molecular signals, primarily cytokines, that motivate and orchestrate the manifold cellular activities which underscore inflammation and healing (Singer & Clark, 1999).

Response to injury is frequently modeled in the skin, but parallel coordinated and temporally regulated patterns of mediators and cellular events occur in most tissues subsequent to injury. The initial injury triggers coagulation and an acute local inflammatory response followed by mesenchymal cell recruitment, proliferation and matrix synthesis. Failure to resolve the inflammation can lead to chronic nonhealing wounds, whereas uncontrolled matrix accumulation, often involving aberrant cytokine pathways, leads to excess scarring and fibrotic sequelae. Continuing progress in deciphering the essential and complex role of cytokines in wound healing provides opportunities to explore pathways to inhibit/enhance appropriate cytokines to control or modulate pathologic healing. Cytokines play an important role in the evolution of granulation tissue through recruitment of inflammatory leukocytes and stimulation of fibroblasts and epithelial cells (Singer & Clark, 1999).

**Platelet Activation and Cytokine Release**

Most types of injury damage blood vessels, and coagulation is a rapid-fire response to initiate hemostasis and protect the host from excessive blood loss. With the adhesion, aggregation and degranulation of circulating platelets within the forming fibrin clot, a plethora of mediators and cytokines are released, including transforming growth factor beta (TGF-beta), platelet derived growth factor (PDGF), and vascular endothelial growth factor (VEGF), that influence tissue edema and initiate inflammation.

VEGF, a vascular permeability factor, influences the extravasation of plasma proteins to create a temporary support structure upon which not only activated endothelial cells, but also leukocytes and epithelial cells subsequently migrate. Angiopoietin-1 (Ang-1), the ligand for Tie-2 receptors, is a natural antagonist for VEGF’s effects on permeability, a key regulatory checkpoint to avoid excessive plasma leakage.

Latent TGF-beta1, released in large quantities by degranulating platelets, is activated from its latent complex by proteolytic and non-proteolytic mechanisms (Khalil, 1999) to influence wound healing from the initial insult and clot formation to the final phase of matrix deposition and remodeling.

Autocrine expression of TGF- beta 1 by leukocytes and fibroblasts, in turn, induces these cells to generate additional cytokines including tumor necrosis factor alpha (TNF-a), interleukin 1 beta (IL-1 beta) and PDGF, as well as chemokines, as components of a cytokine cascade.(McCartney & Wahl, 2001) Such factors act to perpetuate the inflammatory cell response, mediating recruitment and activation of neutrophils and monocytes. In response to TGF- beta and other cytokines, which engage their respective cell surface receptors, intracellular signaling pathways are mobilized to drive phenotypic and functional responses in target cell populations.

**Inflammation**

Of the innumerable cytokines that have been investigated in terms of wound healing, TGF- beta 1 has undoubtedly the broadest effects. Despite the vast number of reports documenting the actions of TGF-beta in this context, both in vitro and in vivo, controversy remains as to its endogenous role. The paradoxical actions of TGF-beta are best appreciated in inflammation, where dependent upon the state of differentiation of the cell and the context of action, TGF-beta acts in a bi-directional manner. Moreover, this understanding of the nature of TGF-beta has led to the hypothesis that it may act as a therapeutic tool in some circumstances, but also a target for therapeutic intervention in others (Wahl, 1994).

Recent studies, in particular those utilizing genetically manipulated animal models, have highlighted the impact of TGF-beta on various aspects of wound healing, and surprisingly, not all of its effects are conducive to optimal healing. Intriguingly, mutations within the TGF-beta1 gene, or in the cell signaling intermediate Smad3, lead to normal or even accelerated cutaneous wound healing responses. An excess of TGF-beta may be a normal constituent of the response for rapid and optimal protection of the host. In the absence of infection, however, reduction of this overexuberant recruitment, inflammation and keratinocyte suppression may result in a more cosmetically acceptable scar (Wahl, 1994).

With the initial barrage of mediators, including TGF-beta, a chain reaction is set in motion, with recruitment, proliferation and activation of the cellular participants. Among the first cells to respond are the vascular endothelial cells, which not only respond to cytokines, but release them as well. Cytokine-induced enhancement of adhesion molecules (VCAM-1, ELAM-1, ICAM-1) on the endothelium provides the platform upon which circulating leukocytes expressing counter-adhesion molecules (integrins, selectins, Ig superfamily members) tether, slowing them down to sense the microenvironment and respond to chemotactic signals at the site of tissue injury (Sundy & Haynes, 2000).

Adhesion molecule interactions between blood leukocytes and endothelium enables transmigration from inside to outside the vessel wall in response to multiple chemotactic signals. In addition to the powerful chemotactic activity of TGF-beta1 for neutrophils and monocytes, multiple chemokines are released to entice leukocytes into the site of tissue injury (Wahl, 1994).

Chemokines are represented by several families of related molecules based on the spatial location of the cysteine residues. Deletion of genes for chemokines leads to specific alterations in wound healing, underlying their role in this process (Gillitzer & Goebeler, 2001).

Migrating through the provisional matrix (scaffolding) provided by the fibrin-enriched clot, leukocytes release proteases and engage in essential functions including phagocytosis of debris, microbes and degraded matrix components. Proteolytic activity is not constitutive, but transcriptionally driven by the cytokines, TGF-beta, IL-1beta and TNF-&alpha, released from multiple cellular sources. Neutrophil recruitment typically peaks around 24-48 hours post wounding, followed by an increasing representation of monocytes which are essential for optimal wound healing (Clarke, 1996).

Activation of these cells in the context of the wound microenvironment results in enhanced release of chemokines, recruitment of reinforcements, and amplification of the response, with the further release of cytokines, TNF-a, IL-1 and IL-6, that act as paracrine, autocrine and potentially, endocrine mediators of host defense. Antigen stimulation drives lymphocytic recruitment and activation, but at a delayed pace compared to the rapid acute response essential to maintain tissue integrity (McCartney & Wahl, 20001). Beyond the neutrophil, monocyte/macrophage and lymphocyte participants, mast cells have become increasingly recognized as active participants with increased numbers noted at sites of cutaneous injury. Once the inflammatory cells are activated, they become susceptible to TGF-beta1 mediated suppression to reverse the inflammatory process (Wahl, 1994).

**Re-epithelialization**

Clearance of debris, foreign agents, and/or infectious organisms promotes resolution of inflammation, apoptosis, and the ensuing repair response that encompasses overlapping events involved in granulation tissue, angiogenesis, and re-epithelialization. Within hours, epithelial cells begin to proliferate, migrate and cover the exposed area to restore the functional integrity of the tissue. Re-epithelialization is critical to optimal wound healing not only because of reformation of a cutaneous barrier, but because of its role in wound contraction.

Immature keratinocytes produce matrix metalloproteases (MMPs) and plasmin to dissociate from the basement membrane and facilitate their migration across the open wound bed in response to chemo-attractants. The migration of epithelial cells occurs independently of proliferation and depends upon a number of possible processes including growth factors, loss of contact with adjacent cells, and guidance by active contact. TGF-beta1 stimulates migration of keratinocytes in vitro, (Hebda, 1998) possibly by integrin regulation and/or provisional matrix deposition. Behind the motile epidermal cells, basal cell keratinocyte proliferation is mediated by the local release of growth factors, with a parallel up-regulation of growth factor receptors including TNF-a, heparin-binding epidermal growth factor (EGF) and keratinocyte growth factor (KGF or FGF-7) (Barrandon & Green, 1987).

Such growth factors are released not only by keratinocytes themselves, acting in an autocrine fashion, but also by mesenchymal cells and macrophages, as paracrine mediators. Once contact is established with opposing keratinocytes, mitosis and migration stop, and in the skin, the cells differentiate into a stratified squamous epithelium above a newly generated basement membrane. Other factors secreted by keratinocytes may exert paracrine effects on dermal fibroblasts and macrophages. One such factor is a keratinocyte-derived non-glycosylated protein termed secretory leukocyte protease inhibitor (SLPI), which inhibits elastase, mast cell chymase, NF-B and TGF-beta1 activation.

**Granulation Tissue and Angiogenesis**

Granulation tissue forms below the epithelium and is composed of inflammatory cells, fibroblasts and newly formed and forming vessels. This initial restructuring of the damaged tissue serves as a temporary barrier against the hostile external environment. Within granulation tissue, angiogenesis is potentiated by hypoxia, nitric oxide (NO), VEGF and fibroblast growth factor-2 (FGF-2) and by the chemokines, MCP-1 and macrophage inflammatory protein (MIP-1a) (Ferrara, 1999).

VEGF, released from wound epithelium and from the extracellular matrix by endothelial-derived proteases, stimulates endothelial cell proliferation and increases vascular permeability. VEGF may be transcriptionally upregulated in response to NO, which also influences vasodilatation, an early step in angiogenesis. In a cyclic fashion, VEGF also drives nitric oxide synthase (NOS) in endothelial cells. Endothelial cells express high affinity receptors for VEGF, VEGF R-1 (Flt-1) and VEGF R-2 (Flk-1) and represent a primary target of this angiogenic and vascular permeability factor (Ferrara, 1999).

Angiogenesis is a tightly controlled process. It is characterized not only by the presence of endogenous inducers, but also inhibitors which mediate a decline in the process as the granulation tissue, named for the granular appearance of the blood vessels in the wound, matures and scar remodeling continues.

**Matrix Formation and Scar Formation**

With the generation of new vasculature, matrix-generating cells move into the granulation tissue. These fibroblasts degrade the provisional matrix via MMPs and respond to cytokine/growth factors by proliferating and synthesizing new extracellular matrix (ECM) to replace the injured tissue with a connective tissue scar. Although the stage is being set for tissue repair from the beginning (provisional matrix, platelet release of PDGF and TGF-beta, cytokine reservoir), fibroblasts migrate into the wound and matrix synthesis begins in earnest within a couple of days, continuing for several weeks to months (Branton & Kopp, 1999).

TGF-beta contributes to the fibrotic process by recruiting fibroblasts and stimulating their synthesis of collagens I, III, and V, proteoglycans, fibronectin and other ECM components. TGF-beta concurrently inhibits proteases while enhancing protease inhibitors, favoring matrix accumulation (Branton & Kopp, 1999).

The progressive increase in TGF-beta (Khalil, 1999) over time and its association with scar less fetal healing have implicated this member of the TGF-beta family in the cessation of matrix deposition. Other members of the TGF-beta superfamily may also contribute to the wound healing response (Niesler & Ferguson, 2001).

PDGF, released at the outset by degranulating platelets, represents a family of cytokines consisting of two polypeptide chains (A and B) which form the dimers PDGF-AA, AB and B. In addition to platelets, PDGF is released by activated macrophages, endothelial cells, fibroblasts and smooth muscle cells and is a major player in regulating fibroblast and smooth muscle cell recruitment and proliferation through PDGF specific receptor-ligand interaction. Beyond its role in fibroblast migration and matrix deposition, PDGF-A and -B also up-regulate protease production, in contrast to the anti-protease activity of TGF-beta (Claesson-Welsh, 1996)

PDGF represents the only FDA approved cytokine/growth factor for the clinical enhancement of delayed wound healing. As repair progresses, fibroblasts display increased expression levels of adhesion molecules and assume a myofibroblast phenotype, mediated in part by TGF-beta and PDGF-A and -B, to facilitate wound contraction (Grinnell, 1994).

**Remodeling Phase**

The remodeling phase (i.e. re-epithelialization and neovascularization) of wound healing during which collage is synthesized, degraded and dramatically reorganized is also cytokine mediated. Degradation of fibrillar collagen and other matrix proteins is driven by serine proteases and MMPs under the control of the cytokine network.

Although repaired tissue seldom achieves its original strength, it provides an acceptable alternative. Degradation of fibrillar collagen and other matrix proteins is driven by serine proteases and MMPs under the control of the cytokine network. MMPs not only degrade matrix components, matrix and adhesion molecules to generate biologically active fragments. TIMPs provide a natural counterbalance to the MMPs and disruption of this orderly balance can lead to excess or insufficient matrix degradation and ensuing tissue pathology (Birkedal-Hansen, 1995).

Similarly, there exists a naturally occurring inhibitor of elastase and other serine proteases (i.e. SLPI). The coordinated regulation of enzymes and their inhibitors ensures tight control of local proteolytic activity. In physiologic circumstances, these molecular brakes limit tissue degradation and facilitate accumulation of matrix and repair.

**2. Why is wound healing referred to as impaired?**

Oxygen is important for cell metabolism, especially energy production by means of ATP, and is critical for nearly all wound-healing processes. It prevents wounds from infection, induces angiogenesis, increases keratinocyte differentiation, migration, and re-epithelialization, enhances fibroblast proliferation and collagen synthesis, and promotes wound contraction (Bishop, 2008).

In addition, the level of superoxide production (a key factor for oxidative killing pathogens) by polymorphonuclear leukocytes is critically dependent on oxygen levels (Rodriguez *et al.,* 2008).

Due to vascular disruption and high oxygen consumption by metabolically active cells, the microenvironment of the early wound is depleted of oxygen and is quite hypoxic. Several systemic conditions, including advancing age and diabetes, can create impaired vascular flow, thus setting the stage for poor tissue oxygenation. In the context of healing, this overlay of poor perfusion creates a hypoxic wound. Chronic wounds are notably hypoxic; tissue oxygen tensions have been measured trans-cutaneously in chronic wounds from 5 to 20 mm Hg, in contrast to control tissue values of 30 to 50 mm Hg. In wounds where oxygenation is not restored, healing is impaired (Tandara & Mustoe 2004).

1. **The role of oxidative stress in the development and progression of impaired wound healing.**

Oxidative stress plays an important role in the development of all kinds of diseases. Oxidative stress is a condition which is the imbalance of prooxidant and antioxidants, abnormally high levels of free radicals and/or the decline of antioxidant defense mechanisms. Excessive oxidative stress could lead to damage of tissue, which plays an important role in the development of many kinds of diseases (Yang *et al.,* 2007).

Free radical relatively increases during oxidative stress. Normally free radical is necessary for the defense of organism and there is a balance between its produce and scavenge. Oxidative stress was is closely associated with reactive oxygen species. Reactive oxygen species could play an important role in physiology in some extent, also it leads to damage of tissue or cells when organism can’t defend excessive reactive oxygen species. Excessive reactive oxygen species and its degradation product is generated during the healing of cutaneous wound (Yang *et al.,* 2007).

Oxidation increases in acute and chronic wound. After wound, oxidative stress generates, antioxidation increases in chronic wound, which indirectly reflects the increasing of oxidative stress and compensation and defense of tissue to oxidative stress. The generation of oxidative stress in wound maybe closely related to inflammatory reaction. In the inflammatory stage of wound healing, oxidative stress induced the damage of tissue because of the imbalance of prooxidant and antioxidant (Yang *et al.,* 2007).

Oxidative stress should be considered in the inflammatory processes of wound healing and treatment of chronic wound. The treatment of antioxidation is a good strategy. If it is used in wound healing in time, it can be good to wound healing.

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