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**Question 1: Write on cytokine signaling and its role in wound healing.**

The word cytokine is derived from the Greek words “cyto”, meaning cell, and “kinos” meaning movement. Cytokines are a large group of cells signaling molecules that are responsible for cell to cell communication, or signaling, in order to generate an immune response (Mandal, 2019).

Cytokine signaling is controlled via the actions of the transcription factors transducer and activator of transcription (Stat). These connect the cytokine receptors via members of the Janus kinase (Jak) family with specific gene activation in the nucleus (Beata and Marc, 2011). 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, while other 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 and 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 in Wound Healing:**

One of the most well-known cytokines involved in the healing process is the proinflammatory 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 observed within twelve hours after injury, and TNF-α is primarily released by local macrophages, where it induces neutrophil recruitment and maturation (Rumalla and Borah, 2001; Feiken *et al.,* 1995; Kunkel *et al.,* 1989; Michie *et al.,* 1988). Wound fluid TNF-α levels peak at three days after dermal injury and are responsible for the increased vascular permeability and proliferation, as well as the increased homeostasis (Rumalla and Borah, 2001; Feiken *et al.,* 1995). Again, 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 will cause increased tissue disruption by the overactivation of immune cells and their protease products (Strieter *et al.,* 1990; Tracey *et al.,* 1988). Specifically, the murine TNF p55 plays a role in promoting leukocyte infiltration at the wound site and negatively affects wound healing by reducing angiogenesis and collagen accumulation (Mori *et al.,* 2002). Continued expression of TNF-α seven days after injury can decrease collagen synthesis and reduce granulation tissue (Rapala *et al.,* 1991; Rapala *et al.,* 1997; Rapala, 1996).

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 cytotoxicity (Rumalla and Borah, 2001); 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 (Loopnow *et al.,* 1998; Fong *et al.,* 1989; Trengove *et al.,* 2000; Barone *et al.,* 1998). These properties have made the administration of IFN-γ a possible treatment for hypertrophic scars. However, there is evidence that IFN-γ plays an enhancing role in postburn active hypertrophic scars, acting as a T-cell chemoattractant and a growth factor. In addition, IFN-γ production reduces re-epithelization and wound strength, and thus unopposed IFN-γ expression can be impaired (Rumalla and Borah, 2001; Miles *et al.,* 1994; Fu *et al.,* 1995).

IL-1 exists in two forms, IL-1α and IL-1β. IL-1β, released principally by monocytes, is an early proinflammatory cytokine with many properties similar to TNF-α. However, IL-1 is also released in keratinocytes in wound healing (primarily IL-1α). Although IL-1α expression in keratinocytes is generally considered to be constitutive, increased IL-1 activity is noticed in the wound surrounding within twenty-four hours of injury and peaks in concentration between twenty-four and seventy-two hours (Sauder *et al.,* 1990; Goretsky *et al.,* 1996). Similar 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 pathogenic and deleterious.

IL-8 represents one protein in a very large and diverse family of chemokines. It is one of the protein responsible for activation and recruitment of neutrophils in acute dermal wounds. Secreted by macrophages and fibroblasts, IL-8 is mostly detectable in the first twenty-four 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 (Liechty *et al.,* 1998; Engelhardt *et al.,* 1998; Nanncy *et al.,* 1995; 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 (Yu *et al.,* 1996). IL-8 is overexpressed in psoriasis and like IL-6, IL-8 is found in very low concentrations in fetal tissue (Konstantinova *et al.,* 1996; Bennett and Shultz, 1993).

Another cytokine often found in wound healing micro surrounding is IL-6. It has defiled classification as either a proinflammatory or anti-inflammatory cytokine, appearing to have both properties. IL-6 has both local and systemic effects on wound healing and plays a central role in the systemic response to injury as a primary transducer 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 cell types in the wound environment (Mateo *et al.,* 1994; Saba *et al.,* 1996; Goodman and Stein, 1994). This includes fibroblasts, monocytes, and polymorphonuclear cells, whose infiltration into the acute wound is similar to the increased rise in IL-6 concentrations in the local environment (Mateo *et al.,* 1994; Saba *et al.,* 1996; Goodman and Stein, 1994; Pajulo *et al.,* 1999). Detectable within the first twelve hours of injury, elevated quantities of IL-6 may remain in the fluid of the wound for more than seven days (Mateo *et al.,* 1994; Goretsky *et al.,* 1996). 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 elderly people (Mateo *et al.,* 1994; Saba *et al.,* 1996; Goodman and Stein, 1994), and its administration to fetal wounds increases scarring (Liechty *et al.,* 2000; Goodman and Stein, 1994).

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 (Rumalla and Borah, 2001). Although IL-2 is considered to be predominantly a T-cell growth factor, the protein is pleiotropic and has a number of associated properties. IL-2 can increase fibroblast metabolism in-vitro. In vivo, IL-2 administration increases rodent wound-breaking strength in immunocompromised hosts, although it does not have the same effect on its noncompromised controls (DeCunzo *et al.,* 1990). Therefore, it is investigated for possible benefits in the immunocompromised wound. Evidence shows that chronic wound with delayed healing properties are often locked in the proinflammatory phases, with elevated levels of both early proinflammatory cytokines 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. Its previous function is often disregarded when compared with their abilities to suppress proinflammatory cytokine production. One of such cytokines 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 includes suppressing the expression of proinflammatory cytokines, as well as promoting B cell proliferation and mediating IgE production (Banchereau, 1995). Although excessive production has been implicated in the fibrotic wound healing seen in scleroderma, IL-4 plays an important role in wound healing, promoting fibroblast proliferation, proteoglycan synthesis by wound fibroblasts, and collagen production (Trautmann *et al.,* 2000; Postlethwaite *et al.,* 1992; Wegrowski *et al.,* 1995). In addition, IL-4 up-regulates arginase activity in normal wound fibroblast as well as macrophages, smooth muscle and endothelial cells (Witte *et al.,* 2002; Louis *et al.,* 1998; Boutard *et al.,* 1995; Corraliza *et al.,* 1995; Wei *et al.,* 2000; Durante *et al.,* 2001). Because arginase activity is known to play an important role in wound healing, this may be one additional mechanism through which IL-4 enhances the repair response (Witte *et al.,* 2002).

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 (Rumalla and Borah, 2001; de Waal *et al.,* 1991; Sato *et al.,* 1999). Detectable within twenty-four hours of injury, IL-10 is measurable for up to ten days from the initiation of wound healing (Sato *et al.,* 1999). Although it has counter regulation role, IL-10, like most cytokines, has injurious effect with excessive expression, including possibly causing the failed closure of chronic venous insufficient ulcers (Lundberg *et al.,* 1998).

**Question 2: When is wound healing referred to as “impaired”? And why?**

Wound healing is referred to as impaired “when there is an interference in one or more phases of the normal stages of wound healing”. As a result, the healing process is delayed, incomplete, and does not proceed in a coordinated manner, subsequently resulting in poor anatomical and functional outcome (Lazarus *et al.,* 1994).

**Why wound healing is impaired:**

Wound healing is impaired due to multiple factors affecting one or more phases of the healing process and these factors can be classified into the following categories: co-morbidities, medications, oncology interventions, and life style habits.

* **Co-morbidities**
1. **Diabetes:**

One complication of diabetes is ulceration of the foot secondary to neuropathic involvement (Vairamon *et al.,* 2009; Vuorisalo *et al.,* 2009; Tchaikovski *et al.,* 2009; Tiaka *et al.,* 2012). Peripheral neuropathy leads to decreased protective sensation and foot deformities (Vairamon *et al.,* 2009). The deformities then lead to a redistribution of pressure during gait and can result in ulceration at high pressure areas (Vairamon *et al.,* 2009; Vuorisalo *et al.,* 2009). Further, autonomic neuropathy results in trophic changes to the skin which can leave it vulnerable to cracking and breakdown, thus increasing the risk of infection (Vairamon *et al.,* 2009; Vuorisalo *et al.,* 2009).

For patients with wounds of other etiologies (such as; surgical incisions, pressure ulcers, or infected wounds) diabetes with poorly-controlled blood sugars results in cellular dysfunction that impedes all phases of wound healing. During hemostasis, there is decreased platelet-derived growth factor (PDGF) receptor expression on endothelial and epithelial cells, resulting in delayed transition to inflammation (Singer and Clarke 1999). An increase in the number of wound-activated macrophages (WAMs) causes increased and prolonged expression of inflammatory cytokines, thereby prolonging the inflammatory phase (Genco *et al.,* 2005; Wetzler *et al.,* 2000).

The proliferative phase is affected by impaired fibroblast signaling resulting in poor granulation tissue formation (Werner *et al.,* 2007; Werner *et al.,* 1994), fibrotic extracellular matrix resulting in stalled keratinocyte migration and delayed re-epithelialization (Loots *et al.,* 1998), elevated metallomatrix proteinases and reactive oxygen species (ROS) resulting in extracellular matrix instability (Haines *et al.,* 2013) and altered sensitivity to VEGF resulting in decreased angiogenesis and poor vascularization (Tchaikovski *et al.,* 2009).

**2. Obesity:**

A major concern of obesity is the increased workload of the heart to supply oxygenated blood to body tissues. If the heart is unable to perfuse these tissues, ischemia can occur and thus contribute to necrosis and impaired wound healing (Wilson and Clark, 2003). An obese person has a tendency to hyperventilate because the diaphragm is unable to fully descend due to the large amount of adipose tissue. Hyperventilation and decreased chest expansion then result in decreased vital capacity and decreased oxygenation of blood, thereby negatively impacting tissue oxygenation (Goldman, 2009). If tissue near a wound is not adequately oxygenated, fibroblasts cannot form collagen and oxygen-dependent cellular repair processes cannot occur (Goldman, 2009; Shipman and Millington, 2011).

Once an obese patient develops a wound, the risk for infection is higher partly due to the avascularity of the surrounding adipose tissue (Goldman, 2009; Kranke *et al.,* 2012). Avascularity decreases the body's ability to defend against infection because the lack of oxygen prevents neutrophils from effectively phagocytizing bacteria, thus increasing the bacterial load of the wound (Goldman, 2009). Decreased blood supply to the wound prevents the necessary cells, e.g. neutrophils and macrophages, from reaching the wound site to protect against infection. On a cellular level, researchers have shown that obesity can impair wound closure via the effects on circulating blood cells.

**3. Protein Energy Malnutrition:**

Protein is one of the most important nutrient factors affecting wound healing. A deficiency of protein can impair capillary formation, fibroblast proliferation, proteoglycan synthesis, collagen synthesis, and wound remodeling. A deficiency of protein also affects the immune system, with resultant decreased leukocyte phagocytosis and increased susceptibility to infection (Gogia, 1995). Collagen is the major protein component of connective tissue and is composed primarily of glycine, proline, and hydroxyproline. Collagen synthesis requires hydroxylation of lysine and proline, and co-factors such as ferrous iron and vitamin C. Impaired wound healing results from deficiencies in any of these co-factors (Campos *et al.,* 2008).

* **Medications**

**1.Non-steroidal Anti-inflammatory Drugs (NSAIDs):**

Non-steroidal anti-inflammatory drugs (NSAIDs) have been shown to have a depressant effect on wound healing while simultaneously decreasing the granulocytic inflammatory reaction (Kaushal *et al.,* 2006; Su *et al.,* 2010). NSAIDs inhibit the production of PGE2, an inflammatory mediating prostaglandin, and can thereby reduce pain (Guo *et al.,* 2010). The suppression of PGE2 also occurs with excessive wound scarring and therefore NSAIDs may increase scar formation, especially if they are used during the proliferative phase of healing (Guo *et al.,* 2010). NSAIDs have an anti-proliferative effect on blood vessels and skin, thereby delaying healing rate (Krischak *et al.,* 2007). NSAIDs may be prescribed post soft-tissue injury or post-surgery to assist with pain control management and to diminish inflammation; however, due to their negative effects on wound healing, their use is controversial.

Platelets, inflammatory cells, fibroblasts and epithelial cells produce nitric oxide (NO), partially in response to inflammatory cytokines that are released with injury. Subsequently nitric oxide assists in angiogenesis and inflammation mediation. If the nitric oxide enzymes necessary for this cascade are inhibited by either medication or disease, wound healing is impaired (Schweniker *et al.,* 2002).

**2. Steroids:**

Steroids are used in diagnoses such as asthma, cancer, or autoimmune disorders. An example of a commonly used steroid is dexamethasone, an anti-inflammatory drug and immunosuppressant glucocorticoid. Despite the beneficial effects of glucocorticoids in rheumatoid arthritis and bronchospasms, the anti-inflammatory and immunosuppressant actions of these steroids can result in delayed healing (Stojadinovic *et al.,* 2007; Feeser *et al.,* 2009).

Glucocorticoids are known to have dermal effects that can impact wound healing, including inhibition of fibroblast proliferation and decreased collagen production. Glucocorticoids also inhibit production of hypoxia-inducible factor-1 (HIF-1), a key transcriptional factor in healing wounds (Wagner *et al.,* 2008). Beyond effects on repair itself, systemic corticosteroids may increase the risk of wound infection. Cortisol, another glucocorticoid, is released during the stress response and can increase blood glucose, inhibit the immune system, decrease bone formation and impede wound healing.

* **Oncology Interventions**

**1.Radiation:**

Ionizing radiation does not solely target the cancerous tissue it was meant to irradiate. The radiation beam also affects the surrounding tissues (such as the epithelium that it passes through in order to reach the malignant cells) by destroying the DNA and preventing cell replication needed for tissue injury. Actively dividing cells, such as tumor or epithelial cells, are the most prone to radiation damage. As a consequence, radiation-induced damage to the epithelium can result in skin breakdown, lower tensile strength, atypical fibroblasts and delayed healing rates (Payne *et al.,* 2008). Tissue damage can occur more than six months after radiation therapy is completed and can result in erythema, swelling, moist or dry desquamation and ulceration. Further delayed effects can include fibrosis, capillary bed telangiectasia, and skin necrosis (Krischak *et al.,* 2007).

Radiation therapy can occur before or after tumor excision, but either situation has been shown to result in wound complications.

**2. Chemotherapy:**

Chemotherapy, like radiation therapy, targets rapidly dividing cells and results in impaired tumor growth; however, it also impairs wound healing. Many chemotherapeutic agents can be utilized in cancer treatment, including Adriamycin and bevacizumab. Chemotherapeutic drugs delay cell migration into the wound, decrease early wound matrix formation, lower collagen production, impair proliferation of fibroblasts, and inhibit contraction of wounds (Franz *et al.,* 2007). In addition, these agents weaken the immune functions of the patients, and thereby impede the inflammatory phase of healing and increase the risk of wound infection. Chemotherapy induces neutropenia, anemia, and thrombocytopenia, thus leaving wounds vulnerable to infection, causing less oxygen delivery to the wound, and also making patients vulnerable to excessive bleeding at the wound site.

Impaired wound healing due to chemotherapeutic drugs such as Adriamycin is most common when the drugs are administered pre-operatively or within 3 weeks post-operatively (Lawrence *et al.,* 1986). Additionally, low post-operative albumin levels, low post-operative hemoglobin, advanced stage of disease, and electrocautery use have all been reported as risk factors for the development of wound complications (Kolb *et al.,* 1992). Another chemotherapeutic drug, bevacizumab, targets VEGF and impairs angiogenesis to slow the progression of metastatic breast cancer, colon cancer, and non-small cell lung cancer (Gordon *et al.,* 2009). However, bevacizumab has negative effects on wound healing secondary to its anti-angiogenic properties which decrease its ability to carry nutrients, oxygen and important cells to the wound site. These effects can result in surgical complications such as dehiscence, surgical site bleeding or infection.

* **Life-style Habits:**

**1.Smoking:**

Smoking is most associated with its effects on lung tissue and the increased risk of cancer; however, smoking also has a detrimental influence on wound healing. Nicotine, an alkaloid poisonous substance present in all tobacco products, reduces cutaneous blood flow by vasoconstriction, stimulates release of proteases that may accelerate tissue destruction, suppresses the immune response and leads to an increased risk of infection. Tobacco altogether slows collagen production, weakens scar tissue, and leaves healed tissues more susceptible to risk of recurrent injury (Pignataro *et al.,* 2012) These effects can alter all phases of wound healing, thereby resulting in inefficient and slower closure of wounds.

During the initial phase of hemostasis, the clot chemical composition is altered in smokers in regard to cytokines, chemo-attractants, and growth factors (Sørensen, 2012); however, smoking also enhances the formation of a clot via platelet activation and increases release of blood fibrinogen. This increased concentration of fibrinogen could be attributed to damage from oxidative processes on vascular endothelial cells (Bennett *et al.,* 2012). Although there is faster clot formation, the inflammatory phase of healing is delayed and neutrophil cell count is increased (Bennett *et al.,* 2012; Sørensen *et al.,* 2010). As a result, there is a decreased chemotactic responsiveness and migratory capacity of cells, as well as an increased release of proteolytic enzymes. These enzymes, in combination with reduced protease inhibition, can lead to connective tissue degradation (Bennett *et al.,* 2012).

The proliferative and remodeling phases of healing are also affected by smoking. In the proliferative phase, a decrease in collagen synthesis and deposition may impair wound angiogenesis (Bennett *et al.,* 2012). Also, while the effects of smoking on the inflammatory phase can be reversed by smoking cessation, the effects on the proliferative phase cannot be reversed (Gulcelik *et al.,* 2006). Not only may angiogenesis be affected by smoking, the formation of new epithelium and epidermis is affected as a result of reactive oxygen species and toxins in tobacco smoke that can cause vascular endothelial injury and impaired migration of neutrophils and monocytes (Bennett *et al.,* 2012). Tobacco smoke also contains carbon monoxide which preferentially binds to hemoglobin over oxygen and thereby impedes oxygen delivery to healing tissue (Bennett *et al.,* 2012).

**2. Alcohol Intake:**

Patients who have a history of alcohol abuse have higher hospital acquired infection rates and increased incidence of dehisced infection surgical incisions (deWit *et al.,* 2012).

The mechanisms of alcohol intake that impair wound healing include increased insulin resistance and higher blood sugar levels (Markuson *et al.,* 2009). In addition, alcohol abusers tend to have poor eating habits with higher risk of protein energy malnutrition. The results include decreased inflammatory and immune responses to tissue injury, decreased fibroblast migration and angiogenesis, and decreased Type I collagen production and weaker scar tissue during remodeling. Thus, there is slower healing and increased risk for recurrence with any mechanical force.

**Question 3: Examine the role of oxidative stress in the development and progression of impaired wound healing.**

A delicate balance between the positive role of reactive oxygen species (ROS) and their deleterious effects is important for proper wound healing. Whereas production of reactive oxygen species is essential to initiate wound repair, excessive amount of ROS generation is deleterious in wound healing. Ongoing oxidative stress, associated with lipid peroxidation, protein modification and DNA damage has been shown to impair wound healing processes via increased cell apoptosis and senescence (Sen and Roy, 2008; Schafer and Werner, 2008; Dunnill *et al.,* 2017; Sen, 2009; Bryan *et al.,* 2012). Excessive oxidative processes can disrupt healing processes, by direct tissue damage and over-stimulation of inflammatory responses. For example, excessive stimulation of NADPH oxidase by cytokines can lead to over expression of cell adhesion molecules (Munro, 1993). H2O2 overproduction caused over expression of matrix metalloprotease 1 in cultured human fibroblasts (Wenk *et al.,* 1999). In physiological conditions, low levels of ROS production by nitrogen oxides activation in neutrophils and macrophages are responsible for respiratory bursts during phagocytosis of the inflammatory phase (Hoffmann and Griffiths, 2018; Jiang *et al.,* 2011; Levigne *et al.,* 2016). In contrast, as chronic inflammation develops in pathological conditions, nitrogen oxide activation is exacerbated, which may lead to excessive production of ROS production, further accelerating inflammation and oxidative stress cellular damage. Clinical studies suggest that non-healing wounds are maintained in highly oxidizing environment, which lead to impaired wound repair, and clinical conditions such as tissue hypoxia and hyperglycemia are typically associated with highly oxidizing environments.

Excessive ROS production or impaired detoxification of the aggressive molecules can induce oxidative stress, which has been identified as an important feature in the pathogenesis of chronic, non-healing wounds. Excessive ROS levels lead to the oxidative modifications and biomolecular damage, altering lipid, protein, DNA structure and functions, inducing the irreversible oxidation of reactive protein thiol groups, which is a hallmark of oxidative stress, and the dysregulation of cell-signaling pathways, triggering downstream signaling cascades leading to altered cytokine release and exacerbation of inflammatory skin diseases.

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