**ANA 404 ASSIGNMENTS**

**COURSE TITLE; INTRODUCTION TO HISTOPATHOLAGY**

**BY**

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**Write on cytokine signalling and its role in wound healing**

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. (Cytokine Signaling 2020).

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. (Cytokine Signaling 2020).

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. (Cytokine Signaling 2020).

Cytokine receptors contain one to three chains, one or more of which generally have limited similarity in the membrane-proximal region (often referred to as box1/box2 motifs). According to the nomenclature the ligand-binding subunit of a receptor is referred to as the alpha chain. Other signal transducing subunits are named beta chains, or gamma chains. All cytokine receptors are associated with one or more members of JAKs, which couple ligand binding to tyrosine phosphorylation of various signaling proteins (STATs) recruited to the receptor complex.

Molecular cloning of cytokine receptors and subsequent structure and function studies has revealed that unlike growth factor receptors, cytokine receptors are devoid of catalytic activity. Nevertheless, interaction of a cytokine with its receptor rapidly induces tyrosine phosphorylation of the receptor and a variety of cellular proteins, suggesting that these receptors transmit their signals through cellular tyrosine kinases. During the past 10–15 years, a large amount of experimental data have accumulated to indicate that most cytokines transmit their signals via a distinct family of tyrosine kinases termed Janus kinases or JAKs. (Cytokine Signaling 2020).

Cytokine receptors activate many signaling pathways generally by means of phosphotyrosine residues, which are recognized by SH2 domains on the signaling molecules. The STATs contain a carboxy-terminal SH2 domain, an SH3-like domain and several conserved amino-terminal regions, and a conserved region in the middle of the protein that binds DNA. Tyrosine phosphorylation of a carboxy-terminal site mediates homo- or heterodimerization through the SH2 domains, triggering movement to the nucleus and DNA binding. (Cytokine Signaling 2020).

A native un-liganded receptor in complex with a JAK is in a catalytically inactive latent state. Receptor dimerization/oligomerization due to ligand binding results in the juxtapositioning of the JAKs, which are in the vicinity through either homo- or heterodimeric interactions. The recruitment of JAKs appears to result in their phosphorylation, either via autophosphorylation and/or cross phosphorylation by other JAKs or via other families of tyrosine kinases. This activation is presumed to result in increased JAK activity. Activated JAKs then phosphorylate receptors on target tyrosine sites. The phosphotyrosine sites on the receptors can then serve as docking sites that allow the binding of other SH2-domain containing signaling molecules such as STATs, Src-kinases, protein phosphatases and other adaptor signaling proteins such as Shc, Grb2 and phosphatidylinositol 3-kinase (PI3K). (Cytokine Signaling 2020).

**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 itinduces neutrophil recruitment and maturation (Rumalla andBorah 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-αat 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 healingstates where TNF-α production is sustained. Administrationof a TNF-α antagonist (TNF-bindingprotein) to septic rats can partially reverse the delayedhealing 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-αantibody 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-γplays an enhancing role in postburn 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-γexpression can be detrimental.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 during wound healing (primarily IL-1α). Although IL-1α expression in keratinocytes is generally considered to be constitutive, increased IL-1 activity (composed of both IL-1α and IL-1β) 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 proinflammatory 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 woundmicroenvironment 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 woundlevel, 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 nonsurvivors 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 Hural1997). 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, proteoglycansynthesis 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).

**When is wound healing referred to as 'impaired'? And why?**

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.

The overlapping intricacy of the wound healing pathway serves to prevent a single primary factor from disrupting the process. As examples, local tissue ischemia and neuropathy can impair chemotaxis during the hemostasis and inflammatory stages, tissue necrosis and infection alter the balance of inflammation and compete for oxygen, and uncontrolled periwound edema and wound instability disrupt myofibroblast activity, collagen deposition, and cross-linking. Impaired wound healing often occurs in the setting of multiple, smaller contributing issues to stall the healing process; however, infection or ischemia alone can impair wound healing.

When the healing process is stalled, a chronic wound may develop, and this is more likely to occur in patients with underlying medical disorders. Chronic ulceration commonly affects the lower extremities with a prevalence that ranges between 0.18 and 1.3 percent in the adult population. The most common non healing wounds affecting the lower extremities are associated with chronic venous insufficiency, peripheral artery disease, and diabetes mellitus. (UpToDate, 2020).



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

A delicate balance between the positive role of ROS and their deleterious effects is important for proper wound healing. Whereas production of ROS 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, C.K., *et al* 2008)(Schafer, M., *et al* 2008)(Dunnill, C., *et al* 2017)(Bryan, N., *et al* 2012).

In physiological conditions, low levels of ROS production by NOX activation in neutrophils and macrophages are responsible for respiratory bursts during phagocytosis of the inflammatory phase (Jiang, F., *et al* 2011) (Hoffmann, M.H., *et al* 2018) (Levigne, D., *et al* 2016).

In contrast, as chronic inflammation develops in pathological conditions, NOX 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. Clinical conditions such as tissue hypoxia and hyperglycemia are typically associated with highly oxidizing environments. (Cano Sanchez., *et al* 2018)

**Hypoxia wound**

Whereas generation of ROS during the normal wound healing is related to NOX activation (Jiang, F., *et al* 2011) (Hoffmann, M.H., *et al* 2018) (Levigne, D., *et al* 2016).

The presence of hypoxia stimulates oxidant production by the electron transport chain (ETC) of the mitochondria mainly via complexes I and III [28]. This observation is paradoxical, in the sense that superoxide is a product of the one-electron reduction of O2, which is reduced in hypoxia. ETC-derived ROS are transferred across the inter-membrane space to reach the cytosol where they act as second messengers. During hypoxia, mitochondria augment the release of ROS in the cytosol, which appears counter intuitive as O2 tension is reduced in the mitochondrial compartment (Cano Sanchez., *et al* 2018).

Hypoxia-induced mitochondrial ROS release has been shown to activate cell protection signaling through transcriptional and post-translational mechanisms. In line, low oxygen levels leading to mitochondrial ROS production activate prolyl-4-hydroxylases. Prolyl-4-hydroxyases can induce hypoxia-inducible factor 1 (HIF-1) activation, which is involved in regeneration of lost or damaged tissue in mammals (Cano Sanchez., *et al* 2018).

In the microenvironment of early wounds, ischemia due to vascular disruption and high O2 consumption by immune competent cells can favor O2 depletion and hypoxia. Moreover, pathological conditions, such as diabetes, impair microvascular blood flow, thus aggravating tissue oxygenation, whereas temporary hypoxia after injury can be beneficial for wound healing, prolonged or chronic hypoxia delays wound healing. Impaired wound repair in hypoxic tissue has been related to the combination of mechanisms that increase ROS production and reduce antioxidant defences. (Cano Sanchez., *et al* 2018)

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