**ASSIGNMENT TITLE: WOUND HEALING  
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**Questions:**

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

Cytokine biology is now recognized as a fundamental component of immunology and the actions of cytokines are understood to be essential mechanisms underlying host defense, immunoregulation, and autoimmunity (O'Shea *et al.,* 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. 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. Moreover, cytokines themselves and cytokine antagonists have become some of the most successful new drugs. On a more basic level, the biochemistry of cytokine action has become a paradigm for understanding rapid, evolutionarily conserved membrane-to-nucleus signal transduction, offering remarkable opportunities for understanding how extracellular cues are sensed and translated into the control of gene expression. As we approach the 20th anniversary of the discovery of the Jak-STAT pathway, it is useful to reconsider the pivotal insights that led to these discoveries, to briefly comment on the present status of this field, and consider future challenges (O'Shea *et al.,* 2011).

Members of the Type I/II cytokine receptor super family like erythropoietin, growth hormone, prolactin, and IFN were first purified more than 50 years ago. Colony stimulating factors began to be studied in the 1960s and ‘70s, and the discovery of the first lymphokines and interleukins followed quickly there after (O'Shea *et al.,* 2011). Thus, knowledge of the criticality of cytokines is by no means new. 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. Less obvious was any notion that these factors were all structurally related and used common elements in mediating their biological actions. With molecular cloning and structural analysis, it began to become clear that the 4-α helical family of cytokines comprises a rather large group of secreted factors with diverse functions (Boulay *et al.,* 2003).

During the late 1980s, the Darnell and Stark labs began to tackle this question by identifying rapidly inducible IFN-stimulated genes (ISGs)( Friedman *et al.,* 1984  Larner *et al.,* 1984). With the isolation of genomic clones of these inducible genes, it was appreciated that they shared promoter elements that were responsible for IFN-mediated induction. Two types of elements were identified: IFN-stimulated response elements (ISRE) and IFN-γ-activated sites (GAS elements). Jim Darnell, David Levy, Thomas Decker, and colleagues began to identify nuclear complexes induced by IFNs that bound to ISREs and soon recognized that the ISRE-bound complex ISGF3 comprised multiple subunits (Fu *et al.,* 1990, Kessler *et al.,* 1990). The cloning of these constituents led to the identification of the first two signal transducer and activator of transcription (STAT) proteins, STAT1 (Schindler *et al.,* 1992) and STAT2 (Fu *et al.,* 1992). The third component of the complex was a member of the IFN response factor (IRF) family, IRF-9(Veals *et al.,* 1992). Complexes bound to GAS (GAFs) also turned out to be STATs. Independent work from other labs, interested in prolactin (Wakao *et al.,* 1992) and IL-6 signaling (Wegenka *et al.,* 1993), identified similar complexes, the cloning of which also demonstrated the existence of new family members, STAT5 and STAT3, respectively (Darnell *et al.,* 1994, Schindler *et al.,* 1992, Greenlund *et al.,* 1994).

Many of the aforementioned discoveries represent groundbreaking work. However, the exciting finding we chose to highlight was the discovery that these new factors not only bound DNA, but they were also tyrosine phosphorylated, making it clear that this new transcription factor family might be directly linked to a signaling pathway. Schindler et al. (Schindler *et al.,* 1992) analyzed the covalent modifications and trafficking of the constituents of the ISGF3 complex. Another equally striking feature of the STATs was the presence of an SH2 domain, a recognition motif for phosphotyrosine, which was further evidence of linkage to the action of tyrosine kinases. A key subsequent finding was that STATs bound cytokine receptors (Greenlund *et al.,* 1994). This put STATs in the position of being receptor-to-nucleus shuttles, directly connecting events from the extracellular milieu to de novo transcription (Schindler *et al.,* 1995).

The importance of tyrosine phosphorylation as a mechanism of signal transduction became widely appreciated with the discovery of various oncogenes that were themselves tyrosine kinases (PTK) and the cloning of receptor tyrosine kinases like the insulin receptor and the epidermal growth factor receptor. The race was on to identify other tyrosine kinases, and investigators used PCR-based approaches or low stringency screening to identify new members of this family. Out of such screens came tyrosine kinase 2 (Tyk2) (Krolewski *et al.,* 1990, Firmbach-Kraft *et al.,* 1990), Janus kinase (Jak) 1 and Jak2 (Wilks *et al.,* 1991), which were recognized to represent a new class of PTK but at this stage lacked a clearly assigned physiological function.

In the meantime, George Stark and Sandra Pellegrini were engineering mutant cells that were defective in IFN-α/β and IFN-γ signaling. This somatic cell mutagenesis approach yielded several classes of mutant lines (McKendry *et al.,* 1991), which were then used to identify a component that restores signaling. The approach led to an explosion of papers that established the criticality of various Jaks and STATs in signaling via different cytokines (Schindler *et al.,* 1995). The study by Velazquez et al. (Velazquez *et al.,* 1992) was the first report showing that defective IFN-α signaling was complemented by a clone encoding a Jak, in this case Tyk2. The report linked the Jaks with a function for the first time – and it was an important one. Not only were Jaks involved in cytokine signaling, they were absolutely essential elements. 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–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 has accumulated to indicate that most cytokines transmit their signals via a distinct family of tyrosine kinases termed Janus kinases or JAKs. The approach of Pellegrini and colleagues involved the use of drug-sensitive cell lines mutagenized to select for insensitivity to IFN-α. Revertants were then isolated and genomic clones that conferred the IFN-sensitive phenotype were identified by construction of a cosmid library and transfection back into IFN-resistant cells. The cosmid responsible for phenotype conversion contained the gene coding for the tyrosine kinase Tyk2 which, at that point, had unknown function but exhibited homology to Jak1. This study provided unambiguous genetic evidence of the essential function of a Jak in IFN signaling. The schematic model provided in the paper inserts Tyk2 as the receptor-associated proximal factor responsible for phosphorylation of ISGF3 (O'Shea *et al.,* 2011).

Other complementation studies quickly filled in the gaps, placing different Jaks and STATs with different cytokines (Muller *et al.,* 1992, Silvennoinen *et al.,* 1993, Watling *et al.,* 1993, Shuai *et al.,* 1993, Witthuhn *et al.,* 1993, Argetsinger *et al.,* 1993). Related studies showed that Jaks physically associated with cytokine receptors. The next phase in Jak/STAT biology was to assess whether data generated in a single mutant cell line had in vivo relevance. The answer was an unequivocal yes. Strikingly, in vivo evidence of the importance of the Jak/STAT pathway came from a human primary immunodeficiency, Jak3-SCID (Macchi *et al.,* 1995, Russell *et al.,* 1995). Within two months, the same phenotype was revealed in Jak3-knockout mice (Nosaka *et al.,* 1995, Thomis *et al.,* 1995), and a few months later the phenotype of STAT1-knockout mice was reported (Meraz *et al.,* 1996, Durbin *et al.,* 1996). STAT2, STAT3, STAT4, STAT5a, STAT5b, and STAT6 knockouts were soon generated and the message was clear: the Jak/STAT pathway was fundamentally important for the development and differentiation of diverse cell types (Leonard *et al.,* 1998). Knocking out Jaks and STATs had profound effects on immune cells, host defense, and immunoregulation. Thus, it became clear very quickly that George Stark’s mutant cell lines really did predict the essential functions of the Jaks in signaling by Type I/II cytokines. However, the impact of Jak-STAT signaling was far more extensive than just IFN signaling.

The attempt to elucidate IFN-inducible genes was accelerated by microarray technology, which showed that hundreds of genes were induced by these cytokines; however, this technology did not allow one to discriminate direct vs. indirect actions of STATs. Which of the genes were true STAT target genes? Newer technologies for mapping genome-wide transcription factor binding include ChIP-on-chip and ChIP-seq technology, and these technologies have quickly expanded our understanding of STAT action(O’Shea *et al.,* 2011). Coupling ChIP-seq data with expression data (either microarray or more recently RNA-seq data), now readily discriminates direct and indirect effects of STATs. Currently, genome-wide binding of all STATs have been profiled by ChIP–seq, and the original datasets are publicly available through the Gene Expression Omnibus (GEO) repository. Moreover, this technology permits one to examine the impact of STATs, not only on transcription but also on epigenetic changes in differentiating cells (Wei *et al.,* 2010).

During the last decade, more and more evidence for direct relevance of the Jak-STAT pathway in humans is emerging. We now know that gain-of-function *JAK2* mutations result in the myelofibrosis spectrum of disorders (Kralovics *et al.,* 2005), and many malignancies are associated with constitutive activation of the Jak-STAT pathway. Loss-of-function *STAT1* mutations are associated with impaired cellular responses to IFN-γ and susceptibility to viral and mycobacterial infections (Dupuis *et al.,* 2001, Dupuis *et al.,* 2003), but conversely, gain-of-function *STAT1* mutations underlie a disorder termed chronic mucocutaneous candidiasis (Liu *et al.,* 2011). These *STAT1* mutations result in enhanced IFN signaling and suppression of IL-17 production. Dominant-negative mutations of *STAT3* in humans also have profound effects on Th17 cell generation. Such *STAT3* mutations result in a disorder known as hyper-IgE syndrome (HIES; also known as Job’s syndrome), a classic primary immunodeficiency (Minegishi *et al.,* 2007, Holland *et al.,* 2007). Homozygous missense mutations of *STAT5b* are linked to a growth hormone insensitivity phenotype associated with autoimmunity and impaired Treg cell function (Cohen *et al.,* 2006). 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 carboxyl-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. 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). The advent of large-scale genome-wide association studies has also implicated cytokines, Jaks, and STATs in more common complex autoimmune diseases. For example, polymorphisms of *IL-23R*, *JAK2* and *STAT3* are linked to susceptibility to inflammatory bowel disease and ankylosing spondylitis (Danoy *et al.,* 2010). Similarly, a variant allele of *STAT4* has been found to be associated with rheumatoid arthritis, systemic lupus erythematosus (SLE) (Cohen *et al.,* 2006, Remmers *et al.,* 2007), Sjögren’s syndrome (Korman *et al.,* 2008), and inflammatory bowel disease (Glas *et al.,* 2010). SLE is associated with an “interferon-signature” and STAT4, like STAT1, is activated by type I IFNs (Cho *et al.,* 1996). Consistent with this idea, polymorphisms of *TYK2* may also be associated with SLE (Sigurdsson *et al.,* 2005).

Wound healing is a complex process involving several overlapping stages that include inflammation, formation of granulation tissue, reepithelialization, matrix formation and remodeling. Upon injury to the skin, the epidermal barrier is disrupted and keratinocytes release prestored interleukin-1 (IL-1). IL-1 is the first signal that alerts surrounding cells to barrier damage.1–11 In addition, blood components are released into the wound site activating the clotting cascade. The resulting clot induces hemostasis and provides a matrix for the influx of inflammatory cells. Platelets degranulate releasing alpha granules, which secrete growth factors such as: epidermal growth factor (EGF), platelet-derived growth factor (PDGF) and transforming growth factor-beta (TGF-b). PDGF, along with proinflammatory cytokines like IL-1, are important in attracting neutrophils to the wound site to remove contaminating bacteria (reviewed in Hantash et al.).12 With the help of TGF-b, monocytes are converted to macrophages which play an important role in augmenting the inflammatory response and tissue debridement. Macrophages initiate the development of granulation tissue and release a variety of proinflammatory cytokines (IL-1 and IL-6) and growth factors (fibroblast growth factor [FGF], EGF, TGF-b, and PDGF). With the assistance of platelet released vascular endothelial growth factor (VEGF) and FGF, endothelial cells proliferate and angiogenesis ensues. This process is essential for the synthesis, deposition, and organization of a new extracellular matrix (ECM). FGF, TGF-b, and PDGF then permit fibroblast infiltration. TGF-b and PDGF also initiate phenotypic changes in these cells converting fibroblasts into myofibroblasts which align themselves along the borders of the ECM to generate a constrictive force, facilitating wound closure (reviewed in Hantash et al.).12 Within hours of injury, reepithelialization is initiated and the release of EGF, TGF-a, and FGF act to stimulate epithelial cell migration and proliferation. This process begins with the dissolution of cell–cell and cell–substratum contacts followed by polarization and migration of keratinocytes over the provisional ECM. Once wound closure (100% epithelialization) is achieved, keratinocytes undergo stratification and differentiation to restore the barrier (reviewed in13,14). Matrix formation requires the removal of granulation tissue with revascularization. A framework of collagen and elastic fibers replaces the granulation tissue. This framework is then saturated with proteoglycans and glycoproteins. This is followed by tissue remodeling involving the synthesis of new collagen mediated by TGF-b, and the breakdown of old collagen by PDGF. The final product of this process is scar tissue. The success of the wound healing process depends on growth factors, cytokines, and. These agents are biologically active polypeptides that act to alter the growth, differentiation and metabolism of a target cell. They can act by paracrine, autocrine, juxtacrine, or endocrine mechanisms, and effect cell behavior as a consequence of their binding to specific cell surface receptors or ECM proteins. Binding to these receptors triggers a cascade of molecular events. The endpoint of this signaling is the binding of transcription factors to gene promoters that regulate the transcription of proteins controlling the cell cycle, motility, or differentiation patterns.13 This review will summarize the major growth factors and cytokines involved in wound healing with particular focus on the EGF family, TGF-b family, FGF family, VEGF, granulocyte macrophage colony stimulating factor (GM-CSF), PDGF-BB, CTGF, IL family, and tumor necrosis factor (TNF)-a family.

In adult humans, optimal wound healing involves rapid hemostasis, appropriate inflammation, mesenchymal cell differentiation, proliferation, and migration to the wound site, suitable angiogenesis, prompt re-epithelialization (re-growth of epithelial tissue over the wound surface); and proper synthesis, cross-linking, and alignment of collagen to provide strength to the healing tissue (Gosain and DiPietro et al., 2004, Mathieu et al., 2006). The first phase of hemostasis begins immediately after wounding, with vascular constriction and fibrin clot formation. The clot and surrounding wound tissue release pro-inflammatory cytokines and growth factors such as transforming growth factor (TGF)-β, platelet-derived growth factor (PDGF), fibroblast growth factor (FGF), and epidermal growth factor (EGF). Once bleeding is controlled, inflammatory cells migrate into the wound (chemotaxis) and promote the inflammatory phase, which is characterized by the sequential infiltration of neutrophils, macrophages, and lymphocytes (Gosain and DiPietro et al., 2004, Broughton et al., 2006; Campos et al., 2008). A critical function of neutrophils is the clearance of invading microbes and cellular debris in the wound area, although these cells also produce substances such as proteases and reactive oxygen species (ROS), which cause some additional bystander damage.

Macrophages play multiple roles in wound healing. In the early wound, macrophages release cytokines that promote the inflammatory response by recruiting and activating additional leukocytes. Macrophages are also responsible for inducing and clearing apoptotic cells (including neutrophils), thus paving the way for the resolution of inflammation. As macrophages clear these apoptotic cells, they undergo a phenotypic transition to a reparative state that stimulates keratinocytes, fibroblasts, and angiogenesis to promote tissue regeneration (Meszaros et al., 2000, Mosser and Edwards et al., 2008). In this way, macrophages promote the transition to the proliferative phase of healing. Inflammation is a normal part of the wound-healing process, and is important to the removal of contaminating micro-organisms. In the absence of effective decontamination, however, inflammation may be prolonged, since microbial clearance is incomplete. Both bacteria and endotoxins can lead to the prolonged elevation of pro-inflammatory cytokines such as interleukin-1 (IL-1) and TNF-α and elongate the inflammatory phase. If this continues, the wound may enter a chronic state and fail to heal. This prolonged inflammation also leads to an increased level of matrix metalloproteases (MMPs), a family of proteases that can degrade the ECM. In tandem with the increased protease content, a decreased level of the naturally occurring protease inhibitors occurs. This shift in protease balance can cause growth factors that appears in chronic wounds to be rapidly degraded (Edwards and Harding et al., 2004, Menke et al., 2007). Similar to other infective processes, the bacteria in infected wounds occur in the form of biofilms, which are complex communities of aggregated bacteria embedded in a self-secreted extracellular polysaccharide matrix (EPS; Edwards and Harding et al., 2004). Mature biofilms develop protected microenvironments and are more resistant to conventional antibiotic treatment. Staphylococcus aureus (S. aureus), Pseudomonas aeruginosa (P. aeruginosa), and β-hemolytic streptococci are common bacteria in infected and clinically non-infected wounds (Edwards and Harding et al., 2004 Davis et al., 2008).

P. aeruginosa and Staphylococcus appear to play an important role in bacterial infection in wounds. Many chronic ulcers probably do not heal because of the presence of biofilms containing P. aeruginosa, thus shielding the bacteria from the phagocytic activity of invading polymorphonuclear neutrophils (PMNs). This mechanism may explain the failure of antibiotics as a remedy for chronic wounds (Bjarnsholt et al., 2008).

#### 2. When is wound healing referred to as 'impaired'? And why?

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

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 transcutaneously in chronic wounds from 5 to 20 mm Hg, in contrast to control tissue values of 30 to 50 mm Hg (Tandara and Mustoe et al., 2004).

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

Diabetes affects hundreds of millions of people worldwide. Diabetic individuals exhibit a documented impairment in the healing of acute wounds. Moreover, this population is prone to develop chronic non-healing diabetic foot ulcers (DFUs), which are estimated to occur in 15% of all persons with diabetes. DFUs are a serious complication of diabetes, and precede 84% of all diabetes-related lower leg amputations (Brem and Tomic-Canic et al., 2007). The impaired healing of both DFUs and acute cutaneous wounds in persons with diabetes involves multiple complex pathophysiological mechanisms. DFUs, like venous stasis disease and pressure-related chronic non-healing wounds, are always accompanied by hypoxia (Tandara and Mustoe et al., 2004). A situation of prolonged hypoxia, which may be derived from both insufficient perfusion and insufficient angiogenesis, is detrimental for wound healing. Hypoxia can amplify the early inflammatory response, thereby prolonging injury by increasing the levels of oxygen radicals (Mathieu et al., 2006, Woo et al., 2007). Hyperglycemia can also add to the oxidative stress when the production of ROS exceeds the anti-oxidant capacity (Vincent et al., 2004). The formation of advanced glycation end-products (AGEs) under hyperglycemia and the interaction with their receptors (RAGE) are associated with impaired wound healing in diabetic mice as well (Huijberts et al., 2008). High levels of metalloproteases are a feature of diabetic foot ulcers, and the MMP levels in chronic wound fluid are almost 60 times higher than those in acute wounds. This increased protease activity supports tissue destruction and inhibits normal repair processes (Woo et al., 2007, Sibbald and Woo et al., 2008).

Several dysregulated cellular functions are involved in diabetic wounds, such as defective T-cell immunity, defects in leukocyte chemotaxis, phagocytosis, and bactericidal capacity, and dysfunctions of fibroblasts and epidermal cells. These defects are responsible for inadequate bacterial clearance and delayed or impaired repair in individuals with diabetes (Loots et al., 1998 Sibbald and Woo et al., 2008). As mentioned above, hypoxia contributes to the compromised healing of DFUs, and diabetic wounds exhibit inadequate angiogenesis. Several studies that have investigated the mechanisms behind the decreased restoration of vasculature in diabetic wounds have implied that EPC mobilization and homing are impaired, and that the level of VEGF, the primary pro-angiogenic factor in wounds, is decreased in the diabetic state (Brem and Tomic-Canic et al., 2007, Gallagher et al., 2007, Quattrini et al., 2008). Stem-cell-based therapies aimed at inducing EPCs or BM-MSCs have shown a promising outcome in diabetic non-healing wounds, both in animals and in clinical trials (Wu et al., 2007, Liu and Velazquez et al., 2008,  Rea et al., 2009). In animal studies, therapeutic restoration of VEGF has been shown to improve repair outcomes significantly (Kirchner et al., 2003, Galiano et al., 2004).

The neuropathy that occurs in diabetic individuals probably also contributes to impaired wound healing. Neuropeptides such as nerve growth factor, substance P, and calcitonin gene-related peptide are relevant to wound healing, because they promote cell chemotaxis, induce growth factor production, and stimulate the proliferation of cells. A decrease in neuropeptides has been associated with DFU formation. In addition, sensory nerves play a role in modulating immune defense mechanisms, with denervated skin exhibiting reduced leukocyte infiltration (Galkowska et al., 2006, Sibbald and Woo et al., 2008).

SUMMARY

* Oxidative stress was a condition which was 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 played an important role in the development of many kinds of diseases.
* Free radical relatively increased during oxidative stress. Normally free radical was necessary for defense of organism and there was a balance between its produce and scavenge.
* Oxidative stress was closely associated with reactive oxygen species. Reactive oxygen species could play an important role in physiology in some extent, also it led to damage of tissue or cells when organism could not defend excessive reactive oxygen species.
* Excessive reactive oxygen species and its degradation product generated during the healing of cutaneous wound. Oxidation increased in acute and chronic wound. After wound oxidative stress generates, antioxidation increased in chronic wound, which indirectly reflected the increasing of oxidative stress and compensation and defense of tissue to oxidative stress.
* The generation of oxidative stress in wound maybe closely relate 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.

**REFERENCES**

1. Gosain A, DiPietro LA. (2004). Aging and wound healing. *World J Surg 28*, pp 321-326
2. Broughton G, 2nd, Janis JE, Attinger CE. (2006). the basic science of wound healing (retraction of Witte M., Barbul A. In: Surg Clin North Am; 77:509-528.
3. Campos AC, Groth AK, Branco AB. (2008). Assessment and nutritional aspects of wound healing*. Curr Opin Clin Nutr Metab Care 11*:281-288.
4. Brem H, Tomic-Canic M. (2007). Cellular and molecular basis of wound healing in diabetes. *J Clin Invest 117*, pp 1219-1222.
5. Tandara AA, Mustoe TA. (2004). Oxygen in wound healing—more than a nutrient. *World J Surg 28*, pp 294-300.
6. Brem H, Tomic-Canic M. (2007). Cellular and molecular basis of wound healing in diabetes. *J Clin Invest 117*, pp 1219-1222.
7. Woo K, Ayello EA, and Sibbald RG. (2007).  The edge effect: current therapeutic options to advance the wound edge. *Adv Skin Wound Care 20*, pp 99-117.
8. Huijberts MS, Schaper NC, Schalkwijk CG. (2008). Advanced glycation end products and diabetic foot disease. *Diabetes Metab Res Rev 24(Suppl 1)*, pp S19-S24.
9. Sibbald RG, Woo KY. (2008). The biology of chronic foot ulcers in persons with diabetes. *Diabetes Metab Res Rev 24(Suppl 1*), pp 25-30.
10. Meszaros AJ, Reichner JS, Albina JE. (2000), Macrophage-induced neutrophil apoptosis*. J Immunol 165*, pp 435-441.
11. Mosser DM, Edwards JP. (2008). Exploring the full spectrum of macrophage activation. *Nat Rev Immunol 8*, pp 958-969.
12. Menke NB, Ward KR, Witten TM, Bonchev DG, Diegelmann RF. (2007). Impaired wound healing. *Clin Dermatol 25*, pp 19-25.
13. Edwards R, Harding KG. (2004). Bacteria and wound healing. *Curr Opin Infect Dis 17*, pp 91-96
14. Davis SC, Ricotti C, Cazzaniga A, Welsh E, Eaglstein WH, Mertz PM. (2008). Microscopic and physiologic evidence for biofilm-associated wound colonization in vivo. *Wound Repair Regen 16*, pp 23-29.
15. Bishop A. (2008). Role of oxygen in wound healing. *J Wound Care 17*, pp 399-402.
16. Rodriguez PG, Felix FN, Woodley DT, Shim EK. (2008). The role of oxygen in wound healing: a review of the literature*. Dermatol Surg 34*, pp 1159-1169.
17. Tandara AA, Mustoe TA. (2004). Oxygen in wound healing—more than a nutrient. *World J Surg 28*, pp 294-300
18. Bjarnsholt T, Kirketerp-Moller K, Jensen P, Kit M, Krogfelt K, Phipps R, et al. (2008). Why chronic wounds won’t heal: a novel hypothesis. *Wound Repair Regen 1*, pp 2-10
19. Boyapati L, Wang HL. (2007). The role of stress in periodontal disease and wound healing. *Periodontol  44*, pp 195-210
20. Friedberg IM, Tomic-Canic M, Komine M, Blumenberg M.(2000). Keratins and the keratinocyte activation cycle. *J Invest Dermatol* , pp 116: 633–640.
21. Kupper TS, Deitch EA, Baker CC, Wong WC. (1986). The human burn wound as a primary source of interleukin-1 activity. Surgery, pp 100 409–415.
22. Murphy GM, Dowd PM, Hudspith BN, Brostoff J, Greaves MW. (1989). Local increase in interleukin-1-like activity following UVB irradiation of human skin in vivo. *Photodermatol 6,* pp 268–274.
23. Bochner BS, Charlesworth EN, Lichtenstein LM, Derse CP, Gillis S, Dinarello CA, Schleimer RP.(1990). Interleukin-1 is released at sites of human cutaneous allergic reactions. *J Allergy Clin Immunol 86 (6 Pt 1)*: 830–839.
24. Mizutani H, Schechter N, Lazarus G, Black RA, Kupper TS.(1991). Rapid and specific conversion of precursor interleukin 1 beta (IL-1 beta) to an active IL-1 species by human mast cell chymase. *J Exp Med 174*, pp 821–825.
25. Chan LS, Hammerberg C, Kang K, Sabb P, Tavakkol A, Cooper KD.(1992). Human dermal fibroblast interleukin-1 receptor antagonist (IL-1ra) and interleukin-1 beta (IL-1 beta) mRNA and protein are co-stimulated by phorbol ester: implication for a homeostatic mechanism. *J Invest Dermatol* *99*: 315–322.
26. Wood LC, Elias PM, Calhoun C, Tsai JC, Grunfeld C, Feingold KR. (1996). Barrier disruption stimulates interleukin-1 alpha expression and release from a pre-formed pool in murine epidermis. *J Invest Dermatol 106*, pp 397–403.
27. Lundqvist EN, Egelrud T. (1997). Biologically active, alternatively processed interleukin-1 beta in psoriatic scales*. Eur J Immunol 27*, pp 2165–2171.
28. Zepter K, Haffner A, Soohoo LF, De Luca D, Tang HP, Fisher P, Chavinson J, Elmets CA. (1997). Induction of biologically active IL-1 beta-converting enzyme and mature IL-1 beta in human keratinocytes by inflammatory and immunologic stimuli. *J Immunol* 159, pp 6203–6208.
29. Murphy JE, Robert C, Kupper TS. (2000). Interleukin-1 and cutaneous inflammation: a crucial link between innate and acquired immunity. *J Invest Dermatol 114*, pp: 602–8.
30. Hantash BM, Zhao L, Knowles JA, Lorenz HP. (2008). Adult and fetal wound healing. *Front Biosci 13*, pp 51–61.
31. Raja, Sivamani K, Garcia MS, Isseroff RR. (2007). Wound reepithelialization: modulating keratinocyte migration in wound healing. *Front Biosci12*, pp 2849–2868.
32. Boulay JL, O’Shea JJ, Paul WE. (2003). Molecular phylogeny within type I cytokines and their cognate receptors*. Immunity. 19*, pp 159–163.
33. Loots MA, Lamme EN, Zeegelaar J, Mekkes JR, Bos JD, Middelkoop E. (1998). Differences in cellular infiltrate and extracellular matrix of chronic diabetic and venous ulcers versus acute wounds. *J Invest Dermatol 111*, pp 850-857.
34. Galiano RD, Tepper OM, Pelo CR, Bhatt KA, Callaghan M, Bastidas N, (2004). Topical vascular endothelial growth factor accelerates diabetic wound healing through increased angiogenesis and by mobilizing and recruiting bone marrow-derived cells*. Am J Pathol 164*, pp 1935-1947.
35. Brem H, Tomic-Canic M. (2007). Cellular and molecular basis of wound healing in diabetes. *J Clin Invest 117*, pp 1219-1222.
36. Kirchner LM, Meerbaum SO, Gruber BS, Knoll AK, Bulgrin J, Taylor RA, et al. (2003). Effects of vascular endothelial growth factor on wound closure rates in the genetically diabetic mouse model. *Wound Repair Regen 11*, pp 127-131.
37. Gallagher KA, Liu ZJ, Xiao M, Chen H, Goldstein LJ, Buerk DG, (2007). Diabetic impairments in NO-mediated endothelial progenitor cell mobilization and homing are reversed by hyperoxia and SDF-1 alpha. *J Clin Invest 117*, pp 1249-1259.
38. Quattrini C, Jeziorska M, Boulton AJ, Malik RA. (2008). Reduced vascular endothelial growth factor expression and intra-epidermal nerve fiber loss in human diabetic neuropathy. *Diabetes Care 31*, pp 140-145.
39. Rea S, Giles NL, Webb S, Adcroft KF, Evill LM, Strickland DH, (2009). Bone marrow-derived cells in the healing burn wound—more than just inflammation*. Burns 35*, pp 356-364.
40. Liu ZJ, Velazquez OC. (2008). Hyperoxia, endothelial progenitor cell mobilization, and diabetic wound healing. *Antioxid Redox Signal 10*, pp 1869-1882.
41. Wu Y, Wang J, Scott PG, Tredget EE. (2007). Bone marrow-derived stem cells in wound healing: a review. *Wound Repair Regen 15(Suppl 1)*, pp S18-S26.
42. Galkowska H, Olszewski WL, Wojewodzka U, Rosinski G, Karnafel W. (2006). Neurogenic factors in the impaired healing of diabetic foot ulcers*. J Surg Res 134*, pp 252-258.
43. Friedman RL, Manly SP, McMahon M, Kerr IM, Stark GR. (1984). Transcriptional and posttranscriptional regulation of interferon-induced gene expression in human cells. *Cell.  38*, pp 745–55.
44. Larner AC, Jonak G, Cheng YS, Korant B, Knight E, Darnell JE., (1984). Transcriptional induction of two genes in human cells by beta interferon.*Proc Natl Acad Sci U S A. 81,* pp 6733–6737.
45. Larner AC, Chaudhuri A, Darnell JE. (1986). Transcriptional induction by interferon. New protein(s) determine the extent and length of the induction.*J Biol Chem. 261*, pp 453–459.
46. Kessler DS, Levy DE, Darnell JE., (1988). Two interferon-induced nuclear factors bind a single promoter element in interferon-stimulated genes.*Proc Natl Acad Sci U S A. 85*, pp 8521–8525.
47. Fu XY, Kessler DS, Veals SA, Levy DE, Darnell JE., (1990). ISGF3, the transcriptional activator induced by interferon alpha, consists of multiple interacting polypeptide chains.*Proc Natl Acad Sci U S A. 87*, pp 8555–8559.
48. Kessler DS, Veals SA, Fu XY, Levy DE. (1990). Interferon-alpha regulates nuclear translocation and DNA-binding affinity of ISGF3, a multimeric transcriptional activator.*Genes Dev. 4*, pp 1753–1765.
49. Schindler C, Fu XY, Improta T, Aebersold R, Darnell JE. (1992). Proteins of transcription factor ISGF-3: one gene encodes the 91-and 84-kDa ISGF-3 proteins that are activated by interferon alpha.*Proc Natl Acad Sci U S A.  89,* pp 7836–7839.
50. Fu XY, Schindler C, Improta T, Aebersold R, Darnell JE., (1992). The proteins of ISGF-3, the interferon alpha-induced transcriptional activator, define a gene family involved in signal transduction.*Proc Natl Acad Sci U S A.  89*, pp 7840–78403.
51. Veals SA, Schindler C, Leonard D, Fu XY, Aebersold R, Darnell JE, Jr, Levy DE. (1992). Subunit of an alpha-interferon-responsive transcription factor is related to interferon regulatory factor and Myb families of DNA-binding proteins. *Mol Cell Biol.  12*, pp 3315–3324.
52. Wakao H, Schmitt-Ney M, Groner B. (1992). Mammary gland-specific nuclear factor is present in lactating rodent and bovine mammary tissue and composed of a single polypeptide of 89 kDa.*J Biol Chem.  267*, pp 16365–16370.
53. Wegenka UM, Buschmann J, Lutticken C, Heinrich PC, Horn F. (1993). Acute-phase response factor, a nuclear factor binding to acute-phase response elements, is rapidly activated by interleukin-6 at the posttranslational level. *Mol Cell Biol. 13,* pp 276–288.
54. Darnell JE, Jr, Kerr IM, Stark GR. (1994). Jak-STAT pathways and transcriptional activation in response to IFNs and other extracellular signaling proteins. *Science.*, pp 1415–1421.
55. Schindler C, Shuai K, Prezioso VR, Darnell JE., (1992). Jr Interferon-dependent tyrosine phosphorylation of a latent cytoplasmic transcription facto*r. Science.  257*, pp 809–813.
56. Greenlund AC, Farrar MA, Viviano BL, Schreiber RD. (1994). Ligand-induced IFN gamma receptor tyrosine phosphorylation couples the receptor to its signal transduction system *(p91) EMBO J.13*, pp 1591–1600.
57. Schindler C, Darnell JE., (1995). Jr Transcriptional responses to polypeptide ligands: the JAK-STAT pathway*. Annu Rev Biochem. 64*, pp 621–651.
58. Krolewski JJ, Lee R, Eddy R, Shows TB, Dalla-Favera R. (1990). Identification and chromosomal mapping of new human tyrosine kinase genes. *Oncogene.  5*, pp 277–82.
59. Firmbach-Kraft I, Byers M, Shows T, Dalla-Favera R, Krolewski JJ. (1990). tyk2, prototype of a novel class of non-receptor tyrosine kinase genes*. Oncogene.  5*, pp 1329–1336.
60. Wilks AF, Harpur AG, Kurban RR, Ralph SJ, Zurcher G, Ziemiecki A. (1991). Two novel protein-tyrosine kinases, each with a second phosphotransferase-related catalytic domain, define a new class of protein kinase.*Mol Cell Biol.  11*, pp 2057–2065.
61. McKendry R, John J, Flavell D, Muller M, Kerr IM, Stark GR. (1991). High-frequency mutagenesis of human cells and characterization of a mutant unresponsive to both alpha and gamma interferons.*Proc Natl Acad Sci U S A. 88*, pp 11455–11459
62. Velazquez L, Fellous M, Stark GR, Pellegrini S.(1992). A protein tyrosine kinase in the interferon alpha/beta signaling pathway.*Cell.  70*, pp 313–322.
63. Muller M, Briscoe J, Laxton C, Guschin D, Ziemiecki A, Silvennoinen O, Harpur AG, Barbieri G, Witthuhn BA, Schindler C,. (1993). The protein tyrosine kinase JAK1 complements defects in interferon-alpha/beta and -gamma signal transduction. *Nature.  366*, pp 129–135
64. Silvennoinen O, Ihle JN, Schlessinger J, Levy DE. (1993). Interferon-induced nuclear signaling by Jak protein tyrosine kinases. *Nature.366*, pp 583–585.
65. Watling D, Guschin D, Muller M, Silvennoinen O, Witthuhn BA, Quelle FW, Rogers NC, Schindler C, Stark GR, Ihle JN,(1993). Complementation by the protein tyrosine kinase JAK2 of a mutant cell line defective in the interferon-gamma signal transduction pathway. *Nature.  366*, pp 166–170.
66. Shuai K, Ziemiecki A, Wilks AF, Harpur AG, Sadowski HB, Gilman MZ, Darnell JE. (1993). Polypeptide signaling to the nucleus through tyrosine phosphorylation of Jak and Stat protein*s. Nature. 366*, pp 580–583.
67. Witthuhn BA, Quelle FW, Silvennoinen O, Yi T, Tang B, Miura O, Ihle JN. (1993). JAK2 associates with the erythropoietin receptor and is tyrosine phosphorylated and activated following stimulation with erythropoietin.*Cell.  74*, pp 227–236.
68. Argetsinger LS, Campbell GS, Yang X, Witthuhn BA, Silvennoinen O, Ihle JN, Carter-Su C. (1993). Identification of JAK2 as a growth hormone receptor-associated tyrosine kinase. *Cell.  74*, pp 237–244.
69. Macchi P, Villa A, Giliani S, Sacco MG, Frattini A, Porta F, Ugazio AG, Johnston JA, Candotti F, O’Shea JJ,. (1995). Mutations of Jak-3 gene in patients with autosomal severe combined immune deficiency (SCID). *Nature.  377*, pp 65–68.
70. Russell SM, Tayebi N, Nakajima H, Riedy MC, Roberts JL, Aman MJ, Migone TS, Noguchi M, Markert ML, Buckley RH, O’Shea JJ, Leonard WJ. (1995). Mutation of Jak3 in a patient with SCID: essential role of Jak3 in lymphoid development*. Science.  270*, pp 797–800.
71. Thomis DC, Gurniak CB, Tivol E, Sharpe AH, Berg LJ. (1995). Defects in B lymphocyte maturation and T lymphocyte activation in mice lacking Jak3. *Science. 270*, pp 794–797.
72. Nosaka T, van Deursen JM, Tripp RA, Thierfelder WE, Witthuhn BA, McMickle AP, Doherty PC, Grosveld GC, Ihle JN. (1995). Defective lymphoid development in mice lacking Jak3.*Science.  270,* pp 800–802.
73. Durbin JE, Hackenmiller R, Simon MC, Levy DE. Targeted disruption of the mouse Stat1 gene results in compromised innate immunity to viral disease*. Cell.  84*, pp 443–450.
74. Meraz MA, White JM, Sheehan KC, Bach EA, Rodig SJ, Dighe AS, Kaplan DH, Riley JK, Greenlund AC, Campbell D, Carver-Moore K, DuBois RN, Clark R, Aguet M, Schreiber RD. (1996). Targeted disruption of the Stat1 gene in mice reveals unexpected physiologic specificity in the JAK-STAT signaling pathway.*Cell.  84*, pp 431–442.
75. Leonard WJ, O’Shea JJ. (1998). Jaks and STATs: biological implications.*Annu Rev Immunol. 16*, pp 293–322.
76. O’Shea JJ, Lahesmaa R, Vahedi G, Laurence A, Kanno Y. (2011). Genomic views of STAT function in CD4+ T helper cell differentiation.*Nat Rev Immunol.11*, pp 239–250.
77. Wei L, Vahedi G, Sun HW, Watford WT, Takatori H, Ramos HL, Takahashi H, Liang J, Gutierrez-Cruz G, Zang C, Peng W, O’Shea JJ, Kanno Y. (2010). Discrete roles of STAT4 and STAT6 transcription factors in tuning epigenetic modifications and transcription during T helper cell differentiation.*Immunity.  32*, pp 840–851.
78. Kralovics R, Passamonti F, Buser AS, Teo SS, Tiedt R, Passweg JR, Tichelli A, Cazzola M, Skoda RC.(2005). A gain-of-function mutation of JAK2 in myeloproliferative disorders. *N Engl J Med352*, pp 1779–1790.
79. Dupuis S, Dargemont C, Fieschi C, Thomassin N, Rosenzweig S, Harris J, Holland SM, Schreiber RD, Casanova JL.(2001). Impairment of mycobacterial but not viral immunity by a germline human STAT1 mutation.*Science.293*, pp 300–303.
80. Dupuis S, Jouanguy E, Al-Hajjar S, Fieschi C, Al-Mohsen IZ, Al-Jumaah S, Yang K, Chapgier A, Eidenschenk C, Eid P, Al Ghonaium A, Tufenkeji H, Frayha H, Al-Gazlan S, Al-Rayes H, Schreiber RD, Gresser I, Casanova JL. (2003). Impaired response to interferon-alpha/beta and lethal viral disease in human STAT1 deficiency. *Nat Genet. 33*, pp 388–91.
81. Liu L, Okada S, Kong XF, Kreins AY, Cypowyj S, Abhyankar A, Toubiana J, Itan Y, Audry M, Nitschke P, Masson C, Toth B, Flatot J, Migaud M, Chrabieh M, Kochetkov T, Bolze A, Borghesi A, Toulon A, Hiller J, Eyerich S, Eyerich K, Gulacsy V, Chernyshova L, Chernyshov V, Bondarenko A, Maria Cortes Grimaldo R, Blancas-Galicia L, Madrigal Beas IM, Roesler J, Magdorf K, Engelhard D, Thumerelle C, Burgel PR, Hoernes M, Drexel B, Seger R, Kusuma T, Jansson AF, Sawalle-Belohradsky J, Belohradsky B, Jouanguy E, Bustamante J, Bue M, Karin N, Wildbaum G, Bodemer C, Lortholary O, Fischer A, Blanche S, Al-Muhsen S, Reichenbach J, Kobayashi M, Rosales FE, Lozano CT, Kilic SS, Oleastro M, Etzioni A, Traidl-Hoffmann C, Renner ED, Abel L, Picard C, Marodi L, Boisson-Dupuis S, Puel A, Casanova JL.(2011). Gain-of-function human STAT1 mutations impair IL-17 immunity and underlie chronic mucocutaneous candidiasis.*J Exp Med. 208*, pp 1635–1648.
82. Holland SM, DeLeo FR, Elloumi HZ, Hsu AP, Uzel G, Brodsky N, Freeman AF, Demidowich A, Davis J, Turner ML, Anderson VL, Darnell DN, Welch PA, Kuhns DB, Frucht DM, Malech HL, Gallin JI, Kobayashi SD, Whitney AR, Voyich JM, Musser JM, Woellner C, Schaffer AA, Puck JM, Grimbacher B.(2007). STAT3 mutations in the hyper-IgE syndrome. *N Engl J Med*.*357*, pp 1608–1619.
83. Minegishi Y, Saito M, Tsuchiya S, Tsuge I, Takada H, Hara T, Kawamura N, Ariga T, Pasic S, Stojkovic O, Metin A, Karasuyama H.(2007). Dominant-negative mutations in the DNA-binding domain of STAT3 cause hyper-IgE syndrome.*Nature*.*448*, pp 1058–1062.
84. Cohen AC, Nadeau KC, Tu W, Hwa V, Dionis K, Bezrodnik L, Teper A, Gaillard M, Heinrich J, Krensky AM, Rosenfeld RG, Lewis DB.(2006). Cutting edge: Decreased accumulation and regulatory function of CD4+ CD25(high) T cells in human STAT5b deficiency.*J Immunol*.*177*, pp 2770–2774.
85. Remmers EF, Plenge RM, Lee AT, Graham RR, Hom G, Behrens TW, de Bakker PI, Le JM, Lee HS, Batliwalla F, Li W, Masters SL, Booty MG, Carulli JP, Padyukov L, Alfredsson L, Klareskog L, Chen WV, Amos CI, Criswell LA, Seldin MF, Kastner DL, Gregersen PK.(2007). STAT4 and the risk of rheumatoid arthritis and systemic lupus erythematosus.*N Engl J Med.357*, pp 977–86.
86. Korman BD, Alba MI, Le JM, Alevizos I, Smith JA, Nikolov NP, Kastner DL, Remmers EF, Illei GG.(2008). Variant form of STAT4 is associated with primary Sjogren’s syndrome.*Genes Immun*. 9:267–270.
87. Glas J, Seiderer J, Nagy M, Fries C, Beigel F, Weidinger M, Pfennig S, Klein W, Epplen JT, Lohse P, Folwaczny M, Goke B, Ochsenkuhn T, Diegelmann J, Muller-Myhsok B, Roeske D, Brand S.(2010). Evidence for STAT4 as a common autoimmune gene: rs7574865 is associated with colonic Crohn’s disease and early disease onset. PLoS One.5:e10373.
88. Cho SS, Bacon CM, Sudarshan C, Rees RC, Finbloom D, Pine R, O’Shea JJ. (1996).Activation of STAT4 by IL-12 and IFN-alpha: evidence for the involvement of ligand-induced tyrosine and serine phosphorylation.*J Immunol. 157*, pp 4781–4789.
89. Mathieu D, Linke J-C, Wattel F. (2006). Non-healing wounds. In: Handbook on hyperbaric medicine, Mathieu DE, editor. Netherlands: Springer, pp. 401-427.
90. Sigurdsson S, Nordmark G, Goring HH, Lindroos K, Wiman AC, Sturfelt G, Jonsen A, Rantapaa-Dahlqvist S, Moller B, Kere J, Koskenmies S, Widen E, Eloranta ML, Julkunen H, Kristjansdottir H, Steinsson K, Alm G, Ronnblom L, Syvanen AC. (2005).Polymorphisms in the tyrosine kinase 2 and interferon regulatory factor 5 genes are associated with systemic lupus erythematosus. *Am J Hum Genet.76*, pp 528–537.
91. Ghoreschi K, Laurence A, O’Shea JJ. Janus kinases in immune cell signaling. *Immunol Rev.228*, pp 273–287.
92. Verstovsek S, Kantarjian H, Mesa RA, Pardanani AD, Cortes-Franco J, Thomas DA, Estrov Z, Fridman JS, Bradley EC, Erickson-Viitanen S, Vaddi K, Levy R, Tefferi A. (2010).Safety and efficacy of INCB018424, a JAK1 and JAK2 inhibitor, in myelofibrosis.*N Engl J Med.363*, pp 1117–11127.
93. O'Shea, J. J., Gadina, M., & Kanno, Y. (2011). Cytokine signaling: birth of a pathway. *The Journal of Immunology*, *187*(11), pp 5475-5478.