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**MATRIC NUMBER: 16/MHS03/016**

**COURSE TITLE: INTRODUCTION TO HISTOPATHOLOGY**

**COURSE CODE: ANA 404**

**ASSIGNMENT TITLE: WOUND HEALING**

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**QUESTION**

1. Write on cytokine signaling and its role in wound healing.
2. When is wound healing referred to as impaired? And why?
3. Examine the role of oxidative stress in the development and progression of impaired wound healing.

**CYTOKINE SIGNALING AND ITS ROLE IN WOUND HEALING**

**Cytokine signaling**

Cytokines are key modulators of inflammation, participating in acute and chronic inflammation via a complex and sometimes seemingly contradictory network of interactions. Better understanding of how these pathways are regulated helps facilitate more accurate identification of agents mediating inflammation and the treatment of inflammatory diseases. It is possible to classify cytokines based on the nature of the immune response (Table 1), with individual cytokines also performing specific roles dependent upon cell type and location (Table 2). Key pro-inflammatory cytokines include interleukin-1 (IL-1), IL-6 and tumor necrosis factor (TNFα), all of which signal via the type I cytokine receptors that are structurally divergent from other cytokine receptor types. By contrast, the critical pro-inflammatory chemokine, IL-8, signals via G protein-coupled receptors (GPCRs).The major pro-inflammatory cytokines, their receptor-mediated signaling pathways, and their contribution to human disease are discussed below.

Table 1. Classification of cytokines by immune response. Cytokines can be broadly grouped based on whether they act on cells of the adaptive immune response, or promote or inhibit inflammation. Further, they can be classified based on the receptors used for signaling.

| **Family** | **Members** |
| --- | --- |
| Adaptive immunity | Common γ chain receptor ligands | IL-2, IL-4, IL-7, IL-9, IL-15, IL-21 |
| Common β chain (CD131) receptor ligands | IL-3, IL-5, GM-CSF |
| Shared IL-2β chain (CD122) | IL-2, IL-15 |
| Shared receptors | IL-13 (IL-13R–IL-4R complex) |
| TSLP (TSLPR–IL-7R complex) |
| Pro-inflammatory signaling | IL-1 | IL-1α, IL-1β, IL-1ra, IL-18, IL-33, IL-36α, IL-36β, IL-36γ, IL-36Ra, IL-37 and IL-1Hy2 |
| IL-6 | IL-6, IL-11, IL-31, CNTF, CT-1, LIF, OPN, OSM |
| TNFα | TNFα, TNFβ, BAFF, APRIL |
| IL-17 | IL-17A-F, IL-25 (IL-17E) |
| Type I IFN | IFNα, IFNβ, IFNω, IFNκ, Limitin |
| Type II IFN | IFNγ |
| Type III IFN | IFNλ1 (IL-29), IFNλ2 (IL-28A), IFNλ3 (IL-28B) |
| Anti-inflammatory signaling | IL-12 | IL-12, IL-23, IL-27, IL-35 |
| IL-10 | IL-10, IL-19, IL-20, IL-22, IL-24, IL-26, IL-28, IL-29 |

Abbreviations: CNTF, ciliary neurotrophic factor; CT-1, cardiotrophin-1; GM-CSF, granulocyte macrophage-colony stimulating factor; IFN, interferon; LIF, leukaemia inhibitory factor; OPN, osteopontin; OSM, oncostatin M; TNFα, tumour necrosis factor α; TSLP, thymic stromal lymphopoietin.

Table 2. Functions of cytokines. Cytokine action, defined by target cell and primary function.

| **Cytokine** | **Main sources** | **Target cell** | **Major function** |
| --- | --- | --- | --- |
| **Interleukins** | **IL-1** | Macrophages, B cells, DCs | B cells, NK cells, T-cells | Pyrogenic, pro-inflammatory, proliferation and differentiation, BM cell proliferation |
|  | **IL-2** | T cells | Activated T and B cells, NK cells | Proliferation and activation |
|  | **IL-3** | T cells, NK cells | Stem cells | Hematopoietic precursor proliferation and differentiation |
|  | **IL-4** | Th cells | B cells, T cells, macrophages | Proliferation of B and cytotoxic T cells, enhances MHC class II expression, stimulates IgG and IgE production |
|  | **IL-5** | Th cells | Eosinophils, B-cells | Proliferation and maturation, stimulates IgA and IgM production |
|  | **IL-6** | Th cells, macrophages, fibroblasts | Activated B-cells, plasma cells | Differentiation into plasma cells, IgG production |
|  | **IL-7** | BM stromal cells, epithelial cells | Stem cells | B and T cell growth factor |
|  | **IL-8** | Macrophages | Neutrophils | Chemotaxis, pro-inflammatory |
|  | **IL-9** | T cell | T cell | Growth and proliferation |
|  | **IL-10** | T cell | B cells, macrophages | Inhibits cytokine production and mononuclear cell function, anti-inflammatory |
|  | **IL-11** | BM stromal cells | B cells | Differentiation, induces acute phase proteins |
|  | **IL-12** | T cells | NK cells | Activates NK cells |
| **Tumour necrosis factors** | **TNF-α** | Macrophages | Macrophages | Phagocyte cell activation, endotoxic shock |
|  |  | Monocytes | Tumour cells | Tumour cytotoxicity, cachexia |
|  | **TNF-β** | T-cells | Phagocytes, tumour cells | Chemotactic, phagocytosis, oncostatic, induces other cytokines |
| **Interferons** | **IFN-α** | Leukocytes | Various | Anti-viral |
|  | **IFN-β** | Fibroblasts | Various | Anti-viral, anti-proliferative |
|  | **IFN-γ** | T-cells | Various | Anti-viral, macrophage activation, increases neutrophil and monocyte function, MHC-I and -II expression on cells |
| **Colony stimulating factors** | **G-CSF** | Fibroblasts, endothelium | Stem cells in BM | Granulocyte production |
|  | **GM-CSF** | T cells, macrophages, fibroblasts | Stem cells | Granulocyte, monocyte, eosinophil production |
|  | **M-CSF** | Fibroblast, endothelium | Stem cells | Monocyte production and activation |
|  | **Erythropoietin** | Endothelium | Stem cells | Red blood cell production |
| **Others** | **TGF-β** | T cells and B cells | Activated T and B cells | Inhibit T and B cell proliferation, inhibit haematopoiesis, promote wound healing |

**Abbreviations:** BM, bone marrow; DCs, dendritic cells; G-CSF, granulocyte-colony stimulating factors; M-CSF, macrophage colony stimulating factor; Th, T helper cells.

**Interleukin-1 (IL-1)**

The IL-1 family now includes 11 members: IL-1α, IL-1β, the IL-1 receptor antagonist [IL-1Ra], IL-18, IL-33, IL-36α, IL-36β, IL-36γ, IL-36Ra, IL-37 and IL-1Hy2 (Boraschi et al., 2011, pp. 127-147). These cytokines are expressed by numerous cell types, including macrophages and monocytes, and comprise both pro- and anti-inflammatory members (Boraschi et al., 2011, pp. 127-147) of these, IL-1β is a potent pro-inflammatory cytokine that was originally identified as an endogenous pyrogen. Further, IL-1β and has been found to have a stimulatory effect on CD4 + T cells and to promote differentiation into the T helper cell lineages, particularly T helper (Th17) cells and a non-classically derived Th1 cell lineage (Santarlasci et al., 2013, p. 182). Given its central role in mediating a wide array of inflammatory signaling responses, we will mainly discuss the actions of the pro-inflammatory cytokine, IL-1β, in this section.

IL-1α and IL-1β are synthesized by multiple cell types including monocytes, macrophages, neutrophils, hepatocytes, and tissue macrophages throughout the body (Arend, Palmer & Gabay 2008, pp. 20-38). Whereas the expression of IL-1α is constitutive in many cell types, by contrast IL-1β expression is induced mainly in response to microbial molecules, although it can also stimulate its own expression (Dinarello, 2009, pp. 519-550). Stimulation of pattern recognition receptors (PRRs) including Toll-like receptors (TLRs) and NOD-like receptors (NLLs) can be induced both by viral and microbial molecules. This can then lead to the induction of IL-1β expression (Kawai & Akira, 2011, pp. 637-650). Members of the IL-1 family, including IL-1β, are typically synthesised as precursors without a secretory sequence. IL-1β must therefore be cleaved in order to generate active cytokine (Casanova, Abel & Quintana-Murci 2011, pp. 447-491) and this is performed by the IL-1-converting enzyme (ICE), or caspase-1, which is contained within a specialised intracellular complex termed the inflammasome (Dinarello, 2009, pp. 519-550). In contrast, IL-1α is active in both its precursor and mature forms but typically remains in the nucleus, the cytoplasm, or on the cell membrane (Casanova, Abel & Quintana-Murci 2011, pp. 447-491). Despite these differences, IL-1α and IL-1β have similar affinities for the two IL-1 receptors, IL-1R1 and IL-1R2. The heterodimeric complex of IL-1R1 and the IL-1R accessory protein (IL-1RAcP) constitutes the functional receptor for IL-1 (O'Neill, 2008, pp. 10-18). Normally, IL-1β binds first to IL-1R1 on the surface of target cells and then the IL-1RAcP is recruited, thus forming a trimolecular signaling complex. IL-1R belongs to the IL-1R/TLR superfamily due to the presence a conserved cytoplasmic Toll/IL-1R (TIR) domain (O'Neill, 2008, pp. 10-18). Following ligand binding the adaptor molecule, MyD88, interacts with IL-1R1 via its TIR domain (O'Neill, 2008, pp. 10-18). Signal transduction leads to activation of both mitogen-activated protein kinases (MAPKs) and the transcription factor NF-κB, thereby resulting in pro-inflammatory cytokine expression. In addition to this classical signaling pathway, there is also evidence that regulated intramembrane proteolysis of IL-1R1 generates both a soluble ectodomain and an intracellular domain, with the latter directly modulating MAPK activation (Elzinger et al., 2009, pp. 1394-1409). Thus, proteolytic processing of cytosolic receptors could contribute to signaling by a non-canonical mechanism.

The IL-1 family also includes members that have an inhibitory role, enabling them to suppress signaling and thereby limit inflammatory response. Firstly, IL-1R2 has a minimal intracellular domain that renders it inactive, and thus signaling is not induced following ligand binding. IL-1R2 is therefore thought to act as a decoy receptor, sequestering IL-1 in an anti-inflammatory mechanism (Dinarello, 2009, pp. 519-550). Secondly, the IL-1R antagonist (IL-1Ra) is able to bind to the IL-1R1 and, in doing so, prevents the recruitment of IL-1RAcP (Boraschi et al., 2011, pp. 127-147), thereby inhibiting signal transduction. IL-1Ra is expressed as one of four different isoforms, generated by alternative splicing, including one secreted form (sIL-1Ra) and three cytosolic forms (Arend & Guthridge, 2000, pp. i60-i64). It is secreted by a number of cell types, including neutrophils, macrophages, monocytes and hepatocytes (Arend & Guthridge, 2000, pp. i60-i64). The sIL-1Ra is one of the acute phase proteins secreted by the liver in response to inflammatory stimuli (Arend & Guthridge, 2000, pp. i60-i64). Its absence is deleterious, with IL-1Ra-deficient mice being prone to the development of various inflammatory disorders and more susceptible to the effects of experimental immune challenges (Planck, 2012, pp. 753-760). Despite this finding, the efficacy of IL-1Ra is thought to be much lower relative to that of agonist, resulting in the need for an up to 1000-fold excess IL-1RA in order to inhibit IL-1 signaling (Gabay, Lamacchia & Palmer 2010, pp. 232-241). This is potentially due to the high level of IL-1R1 expression on most cells to which IL-1Ra may bind, while Il-1 itself need only bind a few receptors to trigger signal transduction (Gabay, Lamacchia & Palmer 2010, pp. 232-241). Given that three of the isoforms of IL-1Ra are intracellular, it has been hypothesized that the critical site of IL-1Ra could be a cytosolic location where it could block IL-1 expression or signaling by altering mRNA stability (Arend & Guthridge, 2000, pp. i60-i64). Thus, while IL-1Ra acts as a natural suppressor of inflammation, on its own it might be insufficient to quell IL-1 signaling and its function could be more complex, involving intracellular modulation of IL-1.

**Tumor necrosis factor (TNFα)**

The term tumor necrosis factor was initially conferred to two molecules, TNFα a monocyte-derived tumor necrosis factor, and TNFβ a lymphocyte-derived tumor necrosis factor, Tumor necrosis factor (TNFα) was initially identified in the 1970s as an endotoxin-induced serum factor responsible for the necrosis of certain tumors in vivo and in vitro (Carswell et al., 1975, pp. 3666-3670). Subsequently TNFα was isolated (Aggarwal et al., 1985, pp. 2345-2354) and its gene cloned (Pennica et al., 1984, pp. 724-729). It is a potent inflammatory mediator that is central to the inflammatory action of the innate immune system, including induction of cytokine production, activation or expression of adhesion molecules, and growth stimulation (Tartaglia, 1992, pp. 151-153). It stimulates the proliferation of normal cells, exerts cytolytic or cytostatic activity against tumor cells, and causes inflammatory, antiviral, and immunoregulatory effects (Gupta, 2002, pp. 293-299). TNFα has also been shown to perform a number of additional functions linked with lipid metabolism, coagulation, insulin resistance, and endothelial function. Indeed, it has been shown to be one of the most important and pleiotropic cytokines mediating inflammatory and immune responses.

TNFα is the prototypic member of the TNF superfamily of type II transmembrance proteins that includes 30 receptors and 19 associated ligands with diverse functions in cell differentiation, inflammation, immunity and apoptosis (Lobito, Gabriel, Medema & Kimberley 2011, pp. 494-505). It is primarily secreted from activated macrophages, although it may also be secreted by other cell types including monocytes, T cells, mast cells, NK cells, keratinocytes, fibroblasts and neurons (Tracey et al., 2008, pp. 244-279). TNFα is synthesized as a transmembrane precursor protein (mTNFα) with a molecular mass of 26 kDa (Tracey et al., 2008, pp. 244-279), after which it is transported via the rough endoplasmic reticulum (RER), Golgi complex and the recycling endosome to the cell surface (Stow, Low, Offenhäuser & Sangermani 2009, pp. 601-612). The monomers of TNFα associate at the plasma membrane as non-covalent trimmers (MacEwan, 2002, pp. 477-492) prior to being cleaved by the metalloprotease, TNFα converting enzyme (TACE or ADAM17) (Black et al., 1997, pp. 729-733). Cleavage by TACE results in the production of 17 kDa soluble TNFα (sTNFα) ectodomain and it is trimers of sTNFα that constitute the potent ligand that activates TNF receptors (Tracey et al., 2008, pp. 244-279). Following TACE cleavage, the membrane stub is proteolytically processed by the signal peptide peptidases (SPPLs) SPPL2a and SPPL2b (Friedmann et al., 2006, pp. 843-848). This cleavage produces an intracellular domain (ICD) that translocates to the nucleus and induces pro-inflammatory cytokine signaling, particularly the expression of IL-12 (Friedmann et al., 2006, pp. 843-848). Thus, the precursor TNFα molecule is subjected to multiple cleavage events to release potent modulators of inflammation.

The molecular actions of extracellular sTNFα and mTNFα typically occur through binding to one of two receptors: TNFR1 (TNFRSF1A, p55TNFR1, p60, CD120a) and TNFR2 (TNFRSF1B, p75TNFR, p80 or CD120b) (Tracey et al., 2008, pp. 244-279). The receptors are expressed on different cell types, with TNFR1 being widely expressed, while TNFR2 is expressed predominantly on leukocytes and endothelial cells (Bradley, 2008, pp. 149-160). The two TNFRs have been reported to mediate distinct biological effects. Knockout mice for TNFRSF1A (TNFR1) are resistant to endotoxic shock, but they are much more susceptible than either TNFRSF1B (TNFR2) knockouts or wild type controls to challenge with Listeria monocytogenes (Peschon et al., 1998, pp. 943-952). Thus, pro-inflammatory effects of TNFα appear to be mediated predominantly through TNFR1.

Both TNFR1 and TNFR2 are single transmembrane glycoproteins with 28% homology in their extracellular domains (MacEwan, 2002, pp. 477-492). Common features in this region include four cysteine-rich domains (CRDs), each of which comprises three cysteine-cysteine disulphide bonds, and a pre-ligand binding assembly domain (PLAD) involved in trimerisation of the receptor (Chan et al., 2000, pp. 2351-2354). Importantly, the receptors differ by the presence of an intracellular death domain (DD) at the carboxyl-end of TNFR1 that is able to drive either apoptosis or inflammation through interaction with associated adaptor molecules. Recruitment of TRADD to TNFR1 is required for both signaling pathways (Pobezinskaya et al., 2008, pp. 1047-1054). Subsequently, one of two complexes is formed, either at the cell surface (complex I) or following internalization (complex II) (Micheau & Tschopp, 2003, pp. 181-190). The formation of complex I requires TNFR-associated factor 2 (TRAF2) and receptor-interacting protein (RIP), leading to kinase cascades that trigger pro-inflammatory gene expression. Alternatively, should the first complex fail to signal, Complex II is formed to induce apoptosis (Micheau & Tschopp, 2003, pp. 181-190). In Complex II, proteolysis and internalization of the receptor results in the recruitment of FADD and pro-caspase-8 to form the death-inducing signaling complex (Micheau & Tschopp, 2003, pp. 181-190).

Unlike TNFR1, TNFR2 lacks a DD. However it does possess an intracellular TRAF-binding motif, by which it too has the potential to modulate inflammatory status. The distinct cytoplasmic domains could account for the differential signaling of the receptors by sTNFα and mTNFα. It was found that mTNFα was a more potent activator of TNFR2 than sTNFα and induced distinct biological outcomes (Grell et al., 1995, pp. 793-802). Further, activation of TNFR1 was found to stimulate NF-κB expression to a significantly greater extent than TNFR2 (MacFarlane et al., 2002, pp. 119-126). Finally, Scatchard analysis of ligand binding to TNFR1 and TNFR2 found that the former had a higher affinity for TNFα (Moosmayer, 1994, pp. 295-301). Thus, TNFR1 is considered to be the more important of the two receptors for the activation of pro-inflammatory signaling pathways.

Importantly, several cytokine receptors have been found to undergo ectodomain shedding by membrane-localized proteolytic enzymes. This process releases a soluble form of the receptor that can have a biological role such as limiting cytokine availability to other cells, and akin to that discussed above for IL-1. Both TNFR1 and TNFR2 are cleaved by TACE following ligand binding, terminating the signal by decreasing availability of plasma membrane receptors as well as releasing soluble TNFR to sequester free TNFα (Müllberg, Althoff, Jostock & Rose-John 2000, pp. 27-38). The production of soluble TNFR1 has been described in inflammatory conditions (Sakimoto, Yamada & Sawa 2009, pp. 4618-4621). As mentioned above, TNFR1 forms a trimer prior to ligand binding, which could lead to activation of signal transduction by bringing together the DD of the receptors. This can be suppressed by the association of the silencer of death domains (SODD) with the TNFR DD (Jiang, Woronicz, Liu & Goeddel 1999, pp. 543-546). Following TNFα binding, SODD disassociates from TNFR, allowing adaptor molecules to be recruited (Jiang, Woronicz, Liu & Goeddel 1999, pp. 543-546). Thus, there are instrinsic mechanisms to limit TNFR signaling in the absence of ligand, as well as to curtail signaling following ligand binding.

**Interleukin-6 (IL-6)**

The interleukin-6 (IL-6) family are pleiotropic cytokines that include the members IL-6 itself and IL-11 (Peters, Müller & Rose-John 1998, pp. 3495-3504). IL-6 is expressed by an array of cells, including mononuclear phagocytes, T cells, B cells, fibroblasts, endothelial cells, keratinocytes, hepatocytes, and bone marrow cells (Jücker et al., 1991, pp. 2413-2418). IL-6 is involved in haematopoiesis, and is critical in the final maturation of B-cells into antibody-producing plasma cells (Kishimoto, 2010, pp. 347-352), T cell activation, differentiation and regulation of Th2 and Treg phenotypes (Smith & Maizels, 2014, pp. 150-161). It is also important in the secretion of acute phase proteins by the liver (Gauldie et al., 1989, pp. 46-58), which it does in cooperation with IL-1 (Dinarello, 2009, pp. 519-550). Previous names for IL-6 illustrate some of these biological activities, including hepatocyte-stimulating factor (HSF), B-cell stimulatory factor 2 (BSF-2), and B cell differentiation factor (BCDF). The discovery and cloning of BSF-2 in 1986 (Hirano et al., 1986, pp. 73-76) was followed by recognition of the fact that these diverse activities were due to a single cytokine, renamed IL-6 (Kishimoto, 2010, pp. 347-352). The gene encoding IL-6 was mapped to chromosome 7 in humans and it encodes a glycoprotein that ranges in mass from 21 to 28 kDa, depending on the extent of post-translational modification (Parekh et al., 1992, pp. 135-141).

IL-6 signals through a ligand-binding IL-6 receptor (IL-6R) α chain (gp80, CD126) and the signal-transducing component gp130 (CD130) (Peters, Müller & Rose-John 1988, pp. 3495-3504). CD130 is the common signal transducer for several cytokines in the IL-6 family and is ubiquitously expressed (Taga & Kishimoto 1997, pp. 797-819), whereas the IL-6R subunit is typically restricted to lymphocytes and hepatocytes (Goette, 2010, pp. 868-876). Following IL-6 binding, the signal is transduced by the gp130 chains to activate JAK-STAT signaling. This signaling pathway leads to phosphorylation of STAT3, a member of the wider STAT family of transcription factors which are associated with cytokine signaling, and results in STAT3 nuclear translocation and IL-6-responsive gene expression. In addition to the membrane-bound receptor, a soluble form of IL-6R (sIL-6R) is generated by TACE-mediated cleavage and can capture circulating IL-6 and make it available to bind and activate gp130 via a process of trans-signaling (Peters, Müller & Rose-John 1988, pp. 3495-3504). In contrast, a soluble form of gp130 functions as an anti-inflammatory decoy receptor, blocking sIL-6R trans-signaling (Jostock et al., 200, pp. 160-167). More recently, the caspase-mediated cleavage of gp130 has been found to produce a small, 18 kDa C-terminal fragment and to thereby terminate IL-6 signal transduction, possibly leading to apoptosis (Graf et al., 2008, pp. 330-338). Thus, a common theme of cytokine signaling involves the proteolytic cleavage of the receptor to modulate signaling or produce a soluble ectodomain to bind to circulating ligands.

**Interleukin-2 in wounds**

IL-2 is produced throughout the body and by multiple cells types found at wounds. Thus, it has the potential to act both globally and locally at the wound site. The timing and location of IL-2 production and signaling may be critically important to its impact on wound healing. Elucidating the temporal and spatial contribution of IL-2 to wound healing will both allow characterization of the complex wound healing process and promote the development of wound-specific therapeutics targeting this pathway.

**Systemic effects of IL-2 on wound healing**

It is not just signals originating within the wound site that impact wound healing, signals from elsewhere in the body can impact wound healing in response to the initiation of the wound. One example of this is the pain and stress caused by the injury. Stress leads to increased glucocorticoid hormones levels, which are identified to impact the immune system (Woo, 2012). Systemic signals have also been revealed to add to the attraction of the immune and skin cells required for wound closure (Broughton, Janis & Attinger 2006). Also, systemic IL-2 may also act as a growth factor or could impact growth factor secretion (Cozzolino et al., 1993). Finally, IL-2 levels in the body in general have the potential to impact the local wound environment. Thus, more understanding of the levels and impact of systemic IL-2 during wound healing is likely important in understanding the role of IL-2 in this process.

Wounds have been shown to alter systemic IL-2 levels. One study of adult burn patients proved that blood IL-2 levels are improved on both day 1 and day 5 following burn injury and that the levels of IL-2 correlate positively with the percentage of the body burned (Kowal-vern et al., 1994). Another study of burns in children proves that IL-2 levels at the wound site are lower than they are in the peripheral blood, which works as evidence for IL-2 acting systemically rather than locally (Mikhal'chik et al., 2009). On the other hand, these data may indicate that at some points during the healing process, low levels of IL-2 are favorable (Mikhal'chik et al., 2009). The high levels of IL-2 in the blood and high white blood cell counts may also mean that the role of IL-2 in wound healing involves systemic immune activation, rather than local immune cell activation at a wound. Thus, more research is needed to fully understand the timing and locations of IL-2’s contribution to wound healing.

To further understand global and local effectors of wound healing, one group has engrossed their study of healing following long bone fractures on the spatial and temporal expression of cytokines (Currie et al., 2014). This group examined IL-2 signaling within the muscle and blood vessels at the fracture site, 1 cm away from the fracture site, and in the opposite, unbroken limb at 0, 6, 24, and 168 h after the fracture. The data comparing IL-2 levels and the phosphorylation states of downstream signaling molecules are consistent with the possibility that systemic IL-2 contributes to wound healing. While this study did not involve skin wounds directly, some of its findings are relevant to cutaneous wound healing, especially given the spatial and temporal characterization of the behavior of IL-2 and several downstream signaling pathways.

Consistent with the studies of burn patients, the IL-2 level in the vessel was lower at the fracture location than in the opposing limb 6 h after fracture, which may show high systemic IL-2 signaling at this time point (Currie et al., 2014). Additionally, the IL-2 level in the muscle was higher at the fracture site than 1 cm away from it immediately following the fracture, where it could be playing a role in the initiation of healing. To further clarify the timing and location of IL-2 signaling, this group went on to perform a further study, which included not only IL-2 levels but also phosphorylation states of some downstream signaling pathways for the IL-2Rs. Immediately following fracture, phosphorylation of both STAT3 and ERK1/2 was higher in the muscle of the opposing limb than at the fracture site or 1 cm away from the fracture, indicating higher activity of these factors. These effects could be mediated by IL-2 signaling via the MAPK pathways. Another potential downstream mediator of IL-2 signaling, Akt, which signals in the Pi3K pathway, also indicated bigger phosphorylation at distant sites early in wound healing (Han et al., 2016). These constant patterns of MAPK and Pi3K signaling implicate IL-2, which signals via both pathways (Lan, Selmi & Gershwin 2008). These patterns may be consistent with a decrease in IL-2 signaling at the site of injury immediately or a relative increase in IL-2 signaling systemically that is not experienced at the wound site. These studies could hint that systemic IL-2 signaling helps motivate cells, especially immune cells, to migrate to wounds or could contribute to growth factor production. Thus, systemic production of IL-2 may be very important in orchestrating the wound healing process.

Several other wound healing studies also hint that systemic IL-2 might play a role in determining the quality of wound healing. In a rat model of wound healing, high doses of intraperitoneal IL-2 improved wound breaking strength as tested by a constant speed tensometer (Barbul, Knud-Hansen, Wasserkrug & Efron 1986). The IL-2-treated wounds had upper levels of hydroxyproline, which is indicative of collagen crosslinking and may elude to bigger ECM deposition or scarring in response to IL-2 (Sakakibara et al., 1973). The authors of this study interpret these results to mean that lymphocyte activation by IL-2, rather than IL-2 action on skin cells, mediates increased strength following IL-2 treatment, although this study does not directly test that hypothesis. However, this study does hint that the role of IL-2 in altering the course of wound healing may involve systemic actions. In order to successfully improve wound healing using IL-2-dependent mechanisms, the systemic role of IL-2 in wound healing would therefore need to be clarified.

**Local effects of IL-2 on wound healing**

Local IL-2 production may also alter the course of wound healing. As stated before, sources of IL-2 in wounds contain macrophages, lymphocytes, and keratinocytes (Broughton, Janis & Attinge 2006). These cells, as well as others found in wounds, are also capable of reacting to IL-2 (Broughton, Janis & Attinge 2006). Understanding how IL-2 interacts with cells within the wound environment will help clarify its potential roles within the wound site.

The role of T-cells and other lymphocytes at the wound site is a newly appreciated phenomenon that may be a mechanism by which IL-2 may impact wounds. One study has revealed that treatment with activating antibodies to Cluster of Differentiation 3 (CD3), a surface receptor that stimulates T-cell activation, along with fibroblast growth factor (FGF)-1 or -2, factors identified to be important for wound healing and angiogenesis, appear to stimulate T-cell IL-2 production (Byrd, Ballard, Miller & Thomas 1999). Because T-cells are present and activated at wound sites and wound sites contain growth factors, including FGFs, this response to combined IL-2 and FGF may represent a mechanism by which T-cells produce IL-2 in wounds. This locally produced IL-2 could impact the rate and quality of the closure by promoting immune and skin cell proliferation and differentiation at the wound site. These findings may also show that there is a feed-forward mechanism by which FGF in wounds increases IL-2 production by immune cells and that both of these signaling molecules act as growth factors at the wound site (Cozzolino et al., 1993). These results could mean that IL-2 treatment would increase scar formation through local action but that this type of treatment might be appropriate in patients in whom wound healing is delayed. Thus, more study of the response to IL-2 locally at the wound site is needed to understand how to best utilize it therapeutically.

In addition to immune cells, skin cells at the wound site also appear capable of directly responding to IL-2, which may alter their behavior and the behavior of cells around them. For example, IL-2 signaling may contribute directly to the activity of fibroblasts in wound sites. Several studies have revealed that fibroblasts express IL-2R, specifically the β and γ subunits that are capable of signaling (Ozawa et al., 2003). One of these studies, concerning fibroblast-like cells isolated from human joint fluid, demonstrated that IL-2 treatment could induce production of pro-inflammatory cytokines by these cells. Another study proved that fibroblast signaling through the IL-2Rγ-JAK3 pathway led to improved production of monocyte chemoattractant factors, including monocyte chemoattractant protein-1 (MCP-1) and intracellular adhesion molecule-1 (ICAM-1) (Ozawa et al., 2003). These observations may mean that local IL-2 signaling in skin cells at a wound may promote the recruitment and activation of immune cells at wound sites. IL-2 appears to act as a growth signal in fibroblasts in a mechanism including autophagy, or the digestion and reallocation of the materials of a cell’s organelles (Kang, Tang, Lotze & Zeh Iii 2013) Ultimately, all of these mechanisms by which IL-2 impacts fibroblasts may contribute to the local impact of IL-2 within wounds.

In addition to receiving signals from exogenous IL-2, studies have shown that human skin fibroblasts can produce IL-2 in some contexts. This IL-2 may act locally at the wound site. One study proved IL-2 production by fibroblasts upon high-dose exposure to advanced glycation end products (AGEs), which are sugar-conjugated proteins that happen in diabetes (Seban et al., 2016). Blocking either AGE receptors or TGF-β signaling led to decreased IL-2 production. The authors interpret this to mean that IL-2 production may be involved in scar formation. Fibroblast secretion of IL-2 may thus be pathologic and lead to poor wound healing or could also be a compensatory response to a lack of IL-2 signal usually provided by other sources. Because fibroblasts can be induced to make IL-2 by AGEs, they may also be able to produce IL-2 in other contexts, which could have implications for the rate and quality of wound healing. Thus, this phenomenon of IL-2 production by fibroblast warrants more study.

At the wound site, IL-2 may also contribute to the secretion of and reaction to growth-promoting factors and cytokines by a variety of cell types, both immune and skin. IL-2 promotes the release of IFN-γ and the development of IFN-γ-producing TH1 cells, which then leads to IL-1 making, which may stimulate wound healing (Zhu & Paul, 2008). Furthermore, IL-2 appears to synergize with IFN-α to promote local endothelial cell growth and angiogenesis, which is needed for revascularization of a wound site (Cozzolino et al., 1993). Combined treatment with IL-2 and IFN-α increases endothelial cell proliferation and IFN-α alone increases expression of and signaling via IL-2R. Thus, there may be crosstalk between IL-2 and IFN-α, such that IFN-α increases the capacity for a response to IL-2 and IL-2 promotes endothelial cell growth. IL-2 and IFN-α together also increase release of FGF, which likely contributes to the increased endothelial cell growth. Blocking FGF decreases cell proliferation in response to IL-2 and IFN-α, thereby demonstrating that IFN-α and IL-2 synergy may be FGF-mediated. These results are consistent with the formerly discussed study that hinted at a feed-forward mechanism by which IL-2 and FGFs synergize to stimulate growth (Byrd, Ballard, Miller & Thomas 1999). Growth promotion by IL-2 via FGF, and possibly other growth factors, may also be applicable to other cell types, including skin cells. Thus, IL-2 may be involved in the growth of both skin and blood vessel cells to close wounds.

Finally, IL-2 inhibition at the wound site may also be significant to adequate wound healing, perhaps by contributing to the resolution of inflammation (Broughton, Janis & Attinger 2006). Wound exudates collected 10 days after wounding have a specific inhibitor of THC proliferation that can be incompletely overcome by treating with IL-2 (Breslin et al., 1998). In contrast, cultured fibroblast cells proliferate in response to these same wound extracts. This study does not identify the inhibitor of IL-2-mediated THC proliferation, demonstrate the timing or source of the inhibitor, or determine the exact role of the inhibitor in wound healing. However, it is possible that the IL-2 inhibitor helps resolve inflammation, promote T-reg growth by favoring a low level of IL-2, or slow IL-2-mediated collagen fiber crosslinking without stopping cell proliferation, thus improving the quality of wound closure (Boyman & Sprent, 2012). The discovery of this inhibitor lends evidence that IL-2, either directly or indirectly, promotes immune activation of early, but may be toxic if it is not inhibited later in the wound healing process. On the other hand, this inhibitor could be preventing overly robust cell growing or ECM deposition or maturation, processes which IL-2 may influence (Broughton, Janis & Attinger 2006). Thus, more research is needed to determine the exact role of IL-2 and the timing and location of its action in wound healing before IL-2-related treatments can be designed.

**IL-2 in diseases involving wound healing**

A number of pathological methods involve IL-2, many of which occur as components of autoimmune diseases, highlighting the role of IL-2 in immune cell development and regulation (Boyman & Sprent 2012). Interestingly, several diseases that involve IL-2 signaling alterations are diseases that also involve skin or tissue damage.

**Sarcoidosis**

Another multifactorial inflammatory disease that links IL-2 with wound healing is sarcoidosis. Sarcoidosis is a compound and relatively rare disease that involves immune-mediated damage to multiple organs, including the lungs, kidneys, eyes, and skin (Iannuzzi, Rybicki & Teirstein 2007). Roughly 25–35% of patients experience skin symptoms, including plaque and ulcer formation, which can occur on normal skin or around scars and tattoos. The relationship to prior sites of injury is indicative of aberrant wound healing in patients suffering from this disease. Furthermore, the lung pathology seen in sarcoidosis includes excessive fibrosis, possibly implicating an overactive scarring response in the disease state, a process which may also impact cutaneous healing. Thus, impaired balance of wound healing versus scarring may warrant additional study in sarcoidosis.

Sarcoidosis encompasses significant immunopathologies, notably the development of granulomas, or collections of macrophages and lymphocytes (Iannuzzi, Rybicki & Teirstein 2007). The pathogenesis of these granulomas in sarcoidosis involves expansion and activation of T-cell subsets, many of which secrete IL-2. IL-2-secreting T-cells are more numerous in the granulomas of patients with active sarcoidosis compared to those with chronic sarcoidosis (Buechner, Winkelmann & Banks 1983). Furthermore, many of the therapies used for the cutaneous lesions of sarcoidosis decrease IL-2 levels, which may be a part of their mechanism of action (Badgwell & Rosen 2007). IL-2 secretion by T-cells has long been recognized to play a role in the pulmonary fibrosis found in sarcoidosis and patients with lively sarcoidosis have lung lymphocytes that spontaneously secrete IL-2 in the absence of activation (Saltini, Spurzem, Lee, Pinkston & Crystal 1986) This aberrant IL-2 secretion in the context of lung fibrosis could be a reaction to the fibrosis or could be involved in its pathogenesis. It is possible that spontaneous IL-2 production by immune cells also occurs in the skin and leads to some of the cutaneous pathologies associated with sarcoidosis. The IL-2 production by T-cells in sarcoidosis can be overcome by immunosuppression using systemic corticosteroids, as proven in a prospective clinical trial, which could be an attractive therapeutic for this illness (Pinkston, Saltin, Müller-Quernheim & Crystal 1987).

SIL-2R is also elevated in sarcoidosis and has been expansively studied in the disease (Su et al., 2013). Su et al. found that sIL-2R levels were elevated in sarcoidosis compared with healthy controls, although the sIL-2R levels did not correlate with lung disease severity (Su et al., 2013). The amount of sIL-2R does correlate, though, with eye inflammation in sarcoidosis patients (Gundlach, Hoffmann, Prasse, Heinzelmann & Ness 2016). Methotrexate therapy in sarcoidosis patients is associated with decreased serum sIL-2R, and this decrease associates with developed lung function (Vorselaars et al., 2015). These studies do not clarify whether sIL-2R is pathologic or is produced in response to excess IL-2. Furthermore, it is unclear what role sIL-2R may play in the cutaneous lesions accompanying this disease. However, IL-2 and IL-2R signaling likely play a role in the skin damage involved in sarcoidosis and warrant further study in the context of this disease, which may shed light on the role of IL-2 in wound healing in other contexts.

**Diabetes**

Another disease that includes both altered IL-2 and impaired wound healing is DM. DM is a growing epidemic within the United States and includes both Type I (T1DM), which is caused by autoimmune destruction of insulin-producing cells, and Type II (T2DM), which is a multifactorial disease including decreased tissue insulin sensitivity (Forbes & Cooper, 2013) Both T1DM and T2DM diabetes lead to, among other pathologies, abnormal wound healing (Forbes & Cooper, 2013). The nature of the impaired wound healing in DM is not totally characterized, but it appears to include a combination of neuropathy leading to decreased sensation of injury, vascular insufficiency triggering decreased blood delivery to the wound site, and excessive inflammation (Ackermann & Hart, 2013).

In the setting of T1DM, IL-2 and IL-2R have been extensively studied in humans. In T1DM, genome wide association studies (GWAS) have proved allelic variation in multiple IL-2 signaling cascade participants (Lowe et al., 2007). These include single nucleotide polymorphisms that occur within the IL-2Rα gene and PTPN2, ultimately conferring susceptibility in acquiring T1DM. Moreover, in healthy patients lacking an IL-2R polymorphism known to reduce T1DM risk, IL-2 signaling was attenuated in several T-cell subsets, containing memory T-cells and T-regs (Garg et al., 2012). Interestingly, decreased IL-2 signaling was observed in patients with T1DM as demonstrated by diminished phosphorylation of STAT5 in response to IL-2 treatment. Based on these data, it is unsurprising that there have been clinical trials involving the administration of IL-2 to T1DM patients, with an aim to prevent pathologies. IL-2 administration to these patients appears to rise T-reg frequency, although this IL-2 administration is also associated with an increase in adverse events (Long et al., 2012). Rapamycin combined with IL-2 therapy further increases T-reg frequency when compared to other T-cell subsets and improves T-reg signaling via the IL-2 pathway, but has a deleterious effect on insulin production by pancreatic β-cells. Additionally, expression of PTPN2 was improved in the T-regs of diabetic patients likened with healthy controls, which may have contributed to a lack of STAT5 signaling in response to IL-2 (Long et al., 2010). Thus, overactive and underactive IL-2 signaling appears relevant to diabetes pathogenesis. Altered IL-2 signaling in some T1DM patients may impair early immune cell engagement at wound sites, which could lead to an increased risk of wound infection and impairment of early inflammatory events necessary to close the wound. Conversely, other patients may experience excessive IL-2 signaling, either locally at wounds or systemically, which may worsen some diabetes-associated pathologies. Thus, in T1DM, more research is needed to clarify the role of IL-2 in diabetes pathogenesis and in its manifestations.

In T2DM, IL-2 signaling is also likely changed, although this association has not been well-studied. One group, (Lagman et al., 2015) proved a robust reduction of IL-2 in the plasma of T2DM patients compared with healthy controls (Lagman et al., 2015). Interestingly, other groups have proved an rise in blood sIL-2R in T2DM patients (Cai et al., 2013). One of these studies also demonstrated that increased sIL-2R levels were associated with decreased T-reg frequencies, increased T-helper cell frequencies, and higher percentages of IL-2Rα+ non-T-reg T-cell subsets. These findings implicate IL-2Rα-possessing T-cells in the mechanism by which excessive sIL-2Rα is produced in T2DM patients. Overall, the investigations of IL-2 and T2DM have generally only measured differences in IL-2 and sIL-2R in peripheral blood and have not related these measurements back to severity of T2DM-associated pathologies such as impaired wound healing. However, based upon the evidenced discussed here, it can be hypothesized that the wound healing impairment in T2DM may be due to a decrease in IL-2 signaling early during the skin repair process. These low IL-2 levels, either at the wound site or systemically, may fail to attract relevant inflammatory cells to the wound site or may permit microbial colonization of the injury, ultimately leading to failed wound healing. To further clarify how IL-2 impacts wounds in T2DM patients, however, more study is needed.

Animal studies have been fundamental to further elucidating the contribution of IL-2 to pathologies in DM. Numerous studies in a mouse model of T1DM have proved that IL-2 treatment may prevent or improve T1DM. IL-2 stimulation through a diversity of approaches including extrinsic IL-2 treatment, viral vectors containing IL-2, and a combination treatment with an anti-IL-2 antibody and IL-2 have been successful in demonstrating that increasing IL-2 signaling is a potential therapeutic avenue (Grinberg-Bleyer et al., 2010). IL-2 induction likely acts by expanding T-regs, which avoid the destruction of insulin-producing cells (Grinberg-Bleyer et al., 2010). However, the protective nature of IL-2 treatment is dose dependent. Low doses of IL-2 or IL-2-containing virus prevent DM by keeping pancreatic insulin-producing cells, whereas high-dose IL-2 has a harmful effect on DM prevention (Grinberg-Bleyer et al., 2010). Furthermore, in order to avoid loss of the insulin-producing cells of the pancreas, the β-cells, the treatment must be administered prior to the loss of these β-cells, indicating that IL-2 is attenuating an immune-mediated destruction of the β-cells rather than avoiding downstream diabetes problems (Diaz-de-Durana et al., 2013). In spite of these difficulties in using IL-2 to prevent T1DM initiation, IL-2-related treatments may still prove useful in treating wound complications. DM animal models could serve as a means to test IL-2 for this indication.

Currently there is no direct evidence that IL-2 signaling is disrupted in diabetic wound healing. However, the contribution of the immune system in the wound healing impairment in DM is clear (Ackermann & Hart, 2013). Thus, the well-established link between diabetes, especially its immunologic derangements, and impaired wound healing, coupled with the role of IL-2 in diabetes pathogenesis, provides an opportunity to explore the role of IL-2 in wound healing. Such studies might yield attractive therapeutic strategies or a better understanding of the contribution of IL-2 to wound healing, both in DM and in general.

**Q2)**

The wound-healing process comprises of four highly integrated and overlapping phases: hemostasis, inflammation, proliferation, and tissue remodeling or resolution (Gosain and DiPietro, 2004). These phases and their bio physiological functions must happen in the proper sequence, at a specific time, and continue for a specific period at an optimal intensity (Mathieu et al., 2006). There are many factors that can affect wound healing which interfere with one or more phases in this process, thus causing improper or impaired tissue repair.

Wounds that exhibit impaired healing, including delayed acute wounds and chronic wounds, generally have failed to progress through the normal stages of healing. Such wounds frequently enter a state of pathologic inflammation due to a postponed, incomplete, or uncoordinated healing process. Most chronic wounds are ulcers that are associated with ischemia, diabetes mellitus, venous stasis disease, or pressure. Non-healing wounds affect about 3 to 6 million people in the United States, with persons 65 years and older accounting for 85% of these events. Non-healing wounds result in huge health care expenditures, with the total cost estimated at more than $3 billion per year (Mathieu et al., 2006; Menke et al., 2007).

**Q3)**

**Definition of Chronic Wound**

Some pathologic conditions result in an incomplete and prolonged healing process (Guo & Dipietro, 2010). Pathogenesis of chronic wounds is difficult to study due to many factors including complexity of the wound repair process and heterogeneity of chronic wounds. Local ischemia and reperfusion injury, type 2 diabetes, chronic inflammation, aging and senescence are the most significant factors that may cause chronic wound states (Guo & Dipietro, 2010).

Among these factors, the chronic state of inflammation is present in the common of cases (Kasuya & Tokura, 2014). Inflammation is associated with persistent macrophages which limit and delay proliferation. Chronic inflammation also induces cell senescence, which is now considered as a critical pathophysiological process in the growth of chronic wounds (Kasuya & Tokura, 2014). A chronic wound environment is characterized by raised levels of proteases such as matrix metalloproteinase (MMPs), reduced levels of protease inhibitors such as tissue inhibitors of mMMP (TIMPs), and an abundance of inflammatory cells discharging excessive amounts of proinflammatory cytokines, proteolytic enzymes, and ROS (Smith, Waypa & Schumacker 2017). A combination of these factors elicits accelerated degradation of extracellular matrix and growth factors, deregulation of the inflammatory response, inhibition of cellular proliferation, inadequate vascularization, and accumulation of necrotic tissue due to ischemia. In turn, these effects encourage bacterial colonization and can perpetuate the inflammatory response, inhibiting wound repair.

**Oxidative Stress in Chronic Wound**

As discussed above, 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 amplified cell apoptosis and senescence (Sen & Roy, 2008). In physiological conditions, low levels of ROS production by NOX activation in neutrophils and macrophages are in charge for respiratory bursts during phagocytosis of the inflammatory phase (Hoffmann & Griffiths, 2018). 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.

**Hypoxic Wound**

Whereas generation of ROS during the normal wound healing is connected to NOX activation (Hoffmann & Griffiths, 2018), the presence of hypoxia stimulates oxidant making by the electron transport chain (ETC) of the mitochondria mainly through complexes I and III (Waypa, Smith & Schumacker 2016). 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 enhance the release of ROS in the cytosol, which appears counter intuitive as O2 tension is reduced in the mitochondrial compartment (Waypa, Smith & Schumacker 2016). Hypoxia-induced mitochondrial ROS release has been revealed to activate cell guard signaling through transcriptional and post-translational mechanisms (Waypa, Smith & Schumacker 2016).

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 (Fuhrmann & Brüne, 2017). 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 (Guo & Dipietro, 2010). Moreover, pathological conditions, such as diabetes, impair microvascular blood flow, thus aggravating tissue oxygenation (Guo & Dipietro, 2010), 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 defenses (Sen, 2009).

**Diabetic Chronic Wound**

ROS production by several ROS-generating enzymes is higher in diabetic wounds (Golebiewska & Poole, 2015). Expression and activity of NOX, the major source of ROS in many cell types, are increased in response to hyperglycemia through activation of the receptor for advanced glycation end products (RAGE) (Shah MS, Brownlee 2016). NOX activity is also improved downstream of hyperglycemia-induced protein kinase C (PKC) activation in smooth muscle and endothelial cells (Schramm, Matusik, Osmenda & Guzik 2012). Similarly, hyperglycemia-induced angiotensin II type 1 receptor AT1 activation rises expression of p47phox and enhances ROS production by NADPH oxidase (Kurosaka et al., 2009). AT1 is conveyed by several cell types in the wound, including myofibroblasts and keratinocytes (Fernandez, Stupar, Croll, Leavesley & Upton 2018). Expression and activity of H2O2-producing enzyme xanthine oxidase (XO) is also bigger in diabetic mouse wounds and in response to high glucose levels (Forrester, Kikuchi, Hernandes, Xu & Griendling 2018).

One of the most significant sources of ROS in diabetes is the mitochondrial electron transport chain (Schramm, Matusik, Osmenda & Guzik 2012). In line, hyperglycemia increases superoxide production by increasing the quantity of pyruvate oxidation in the TCA cycle and as a result the availability of electron donors NADH and FADH2 (Zinkevich & Gutterman, 2011). Increased electron flux then rises the proton gradient across the inner mitochondrial membrane, which at a critical threshold interrupts electron transport through complex III (Zinkevich & Gutterman, 2011). Electron transport is then largely facilitated by coenzyme Q, which transfers only one electron to oxygen, producing excess superoxide (Zinkevich & Gutterman, 2011). Excessive mitochondrial superoxide production further impacts ROS levels by altering the flux through several intracellular pathways. For example, ROS leads to GAPDH inhibition by poly (ADP-ribose) modification, which rises levels of glycolysis intermediates upstream of GAPDH (Zinkevich & Gutterman, 2011). This provides improved substrate levels for the polyol, protein kinase C, and hexosamine pathways (Zinkevich & Gutterman, 2011). Activation and interaction of these pathways ultimately alters gene expression, depletes antioxidant resources, and favors the production of further ROS and advanced glycation end products. In addition, multiple lines of evidence have emerged showing that intracellular locations of ROS production are functionally connected. So-called ROS-induced ROS release cross talk signifies a common mechanism for ROS amplification and regional ROS generation (Zorov, Juhaszova & Sollott 2014). A large number of mitochondrial pores (mPTP, inner membrane anion channel (IMAC), voltage reliant on anion channels VDAC) has been identified as facilitating superoxide escape to the cytosol (Zorov, Juhaszova & Sollott 2014).

Hyperglycemia, mitochondrial ROS generation, and oxidative stress are involved in the pathogenesis of several diabetic complications. Deleterious effects of ROS on cellular homeostasis are also related to the reduction in antioxidant defenses, which intensifies the redox imbalance. Analysis of blood collected from diabetes patients presented reduced SOD, CAT, and glutathione peroxidase activity, and a total decrease in antioxidant status (David, Rifkin, Rabbani & Ceradini 2017). Of note, signaling via the transcription factor nuclear factor erythroid 2-related factor 2 (Nrf2), a master regulator of antioxidant gene expression, is impaired in diabetes (Bitar & Al-Mulla, 2011). Expression and nuclear translocation of Nrf2 are decreased in diabetic dermal fibroblasts. In response to oxidative stress, Nrf2 activity decrease was associated with reductions in expression of CAT, NADPH dehydrogenase quinone 1 (NOQ1), glutathione reductase, and glutathione S-transferase (Soares et al., 2016). In fibroblasts open to high glucose concentrations, Nrf2 is retained in the cytoplasm by its regulator Keap1, and transcription of MnSOD and NOQ1 is reduced (Ambrozova, Ulrichova & Galandakova 2017). Activation of ATF-3 and NF-κB is involved in antioxidant enzyme regulation is also changed in response to foot ulceration in diabetic patients (Wan et al., 2017).

**Targeting Oxidative Stress in the Treatment of Chronic Wound**

In diabetic patients, typical wound care practices include surgical debridement, antibiotic treatment, moisture dressing, and pressure off-loading (Guo & Diepietro, 2010). Recent advances have focused on specific defects in the diabetic wound environment, including topical application of growth factors, introduction of bone marrow-derived endothelial and epithelial cells, and collagen-based tissue-engineered grafts. As a different concept, strict control of ROS levels through antioxidants and antioxidative enzyme systems may decrease oxidative stress-induced cellular damage (Ziellins et al., 2015). In line, studies using gene-modified animals and pathological models have shown beneficial effects of antioxidative enzyme upregulation in normal wound healing. For example, deficiency of SOD1, heme oxygenase (HO)-1 can delays wound healing procedures in mice (Iuchi et al., 2010). Reducing excessive ROS by the means of growth factor treatment, antioxidant N-acetyl cysteine or dietary antioxidants generation has been shown beneficial in experimental models of chronic wound. For example, decreasing activity of XO by topical application of siRNA targeting its precursor, xanthine dehydrogenase, meaningfully advances healing in db/db diabetic mice (Weinstein, Lalezarzadeh, Soares, Saadeh & Ceradini 2015). Similarly, genetic deletion of the H2O2-generating enzyme p66Shc elicited reduction of nitrosative oxidative stress and enhanced healing rate in diabetic mice (Fadini et al., 2010). Increasing antioxidant capacity via in vivo MnSOD transfer expression has also proven to be active in diabetic mice (Luo, Wang, Fu, Wu & Chen 2004). Activation of Nrf2-mediated antioxidant defenses has been clearly related in the recent literature with protection against diabetic wound healing in mice (Bitar & Al-Mulla, 2011). In human cells, inducers of Nrf2 have been recommended as a promising pharmacological strategy for skin photoprotection (Tao, Justiniano, Zhang, Wondrak 2013).

The next step in the evaluation of antioxidant-based therapy of chronic wound in humans may be clinical use of strategies targeting mitochondria ROS production. Mitochondria-targeted antioxidants have been initially designed to deliver treatments to mitochondria in cardiovascular and neurodegenerative diseases in order to decrease ROS and mitochondrial dysfunction (Kim & Han, 2017). Mitochondria-targeted antioxidants contain triphenylphosphonium lipophilic cation-based molecules such as MitoQ Mito-αlipoic acid and 10-(6′-plastoquinonyl) SkQ1, small-cell permeable tetra peptide molecules such as elamipretide choline esters of glutathione and N-acetylL-cysteine (Kim & Han, 2017). As mentioned above, 10-(6′-plastoquinonyl) SkQ1 antioxidant may increase dermal wound healing via better resolution of inflammation in genetically diabetic mice (Demyanenko, 2017). Mitochondrial-targeted antioxidant elamipretide can bind to cardiolipin, reduce ROS production and stabilize mitochondrial function. Human studies have already made known that elamipretide increases heart failure and mitochondrial myopathy (Karaa et al., 2018). It is likely that elamipretide would also improve chronic wounds, thanks to effects of this compound on mitochondrial ROS production, NLRP3 inflammasome activity as well as NF-κB and Nrf2-ARE signaling pathways.

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