Chemokines as Therapeutic Targets to Improve Healing Efficiency of Chronic Wounds

Latha Satish[1,2,*]

[1]Department of Plastic Surgery and [2]McGowan Institute of Regenerative Medicine, University of Pittsburgh, Pittsburgh, Pennsylvania.

Latha Satish, MSc, MPhil, PhD

Submitted for publication September 5, 2014.
Accepted in revised form November 4, 2014.

*Correspondence: Department of Plastic Surgery, University of Pittsburgh, 3550, Terrace St., Scaife Hall, 6th floor, Pittsburgh, PA 15261 (e-mail: las238@pitt.edu).

Significance: Impaired wound healing leading to chronic wounds is an important clinical problem that needs immediate attention to develop new effective therapies. Members of the chemokine family seem to be attractive and amenable to stimulate the healing process in chronic wounds. Targeting specific chemokines and/or their receptors has the potential to modify chronic inflammation to acute inflammation, which will hasten the healing process.

Recent Advances: Over the years, expression levels of various chemokines and their receptors have been identified as key players in the inflammatory phase of wound healing. In addition, they contribute to regulating other phases of wound healing making them key targets for novel therapies. Understanding the signaling pathways of these chemokines will provide valuable clues for modulating their function to enhance the wound healing process.

Critical Issues: Inflammation, an important first-stage process in wound healing, is dysregulated in chronic wounds; emerging studies show that chemokines play a crucial role in regulating inflammation. The knowledge gained so far is still limited in understanding the enormous complexity of the chemokine network during inflammation not just in chronic wounds but also in acute (normal) wounds. A much better understanding of the individual chemokines will pave the way for better targets and therapies to improve the healing efficiency of chronic wounds.

Future Directions: Effective understanding of the interaction of chemokines and their receptors during chronic wound healing would facilitate the design of novel therapeutic drugs. Development of chemokine-based drugs targeting specific inflammatory cells will be invaluable in the treatment of chronic wounds, in which inflammation plays a major role.

SCOPE AND SIGNIFICANCE

Chronic wounds are difficult-to-heal wounds and contribute to significant healthcare expenditures. Unlike normal wounds, chronic wounds usually do not follow the conventional stages of healing and may remain open for many years. Several events that occur in a coordinated manner in healing normal wounds do not happen in nonhealing chronic wounds. For instance, inflammation appears to be prolonged for an extended period leading to distraught production of pro- and anti-inflammatory cytokines. This results in an impaired angiogenic response, a decreased granulation tissue formation, and a reduced keratinocyte and fibroblast migration and proliferation.[1,2] Over the years, several clinically approved evidence-based approaches have been utilized as treatment strategies to heal chronic wounds utilizing growth factors and biological dressings, but

DOI: 10.1089/wound.2014.0602

with limited success.[3,4] Chemokines, a family of small chemotactic cytokines seem to be attractive candidates to target chronic wounds as the members of the chemokine family, tightly regulate the process of inflammation and angiogenesis. This review will provide a brief overview on the structure and classification of chemokines and its function in acute wound healing. Furthermore, an in-depth analysis on the chemokines that could be used as targets to improve healing efficiency of chronic wounds will be discussed.

TRANSLATIONAL RELEVANCE

To overcome the current failure of healing chronic wounds using the available strategies targeting chemokines seems conceivable as major adjuvants to stimulate wound healing. Increasing or decreasing the expression of chemokines in a chronic wound seems attainable, which can modulate the hostile nonhealing environment for successful closure of wounds.

CLINICAL RELEVANCE

It has been estimated that between 3 and 6 million people in the United States suffer from one of the three main types of chronic wounds—diabetic, venous, and pressure ulcers (PUs);[5] these are responsible for significant healthcare expenditures. Although there have been extensive investigations into the mechanisms responsible for chronic wound formation especially in diabetics, effective clinical therapies are yet to be developed. Chemokines that belong to a small family of cytokines have been the focus for many years now to understand the functional significance in wound healing. Over the years, a greater understanding on the function of chemokines has been achieved through *in vitro* studies and preclinical models. In the clinical realm, targeting chemokines holds a great promise as chemokines can be easily manipulated to tailor the needs of the patients to improve healing.

BACKGROUND

Chemokines and chemokine receptors

The chemokines or chemotactic cytokines are an important family of signaling molecules. Chemokines were first discovered as substances that assist in the recruitment of leukocytes to sites of injury or infection and, thereby, modulate immune and inflammatory responses.[6] However, now, it is very apparent that chemokines contribute to a wide variety of processes, such as hematopoiesis, angiogenesis, and tissue growth and repair. To date, ~50 human chemokines have been identified, and they are classified into four subgroups according to the polypeptide chain cysteine location: C, CC, CXC, and CX3C.[7] Most of the known chemokines belong to the CC and CXC families. Chemokines interact with cells through receptors belonging to the superfamily of rhodopsin-like, G protein-coupled seven-transmembrane receptors.[8] Some receptors bind with multiple chemokines, and various chemokines can interact with several different receptors.[9]

Most chemokines produced in the human body are inflammatory chemokines. The monocyte chemoattractant protein (MCP-1; CCL2), regulated on activation, normal T-cell expressed and secreted (RANTES) (CCL5), and eotaxin (CCL11) are representatives of this group. Inflammatory chemokines are produced in an inducible manner by various tissues and infiltrating leukocytes in response to bacterial toxins. This is followed by the activation of the key proinflammatory cytokines, such as interleukin-1 (IL-1), tumor necrosis factor (TNF), and interferons (IFNs). The primary task of inflammatory chemokines is to regulate leukocyte recruitment toward the site of inflammation or infection to provide the host defense. CXC chemokines primarily attract neutrophils and lymphocytes and are believed to orchestrate the early phases of wound healing.[7] CXC chemokines are further subdivided based on the presence or absence of a glutamic acid–leucine–arginine (ELR) motif immediately before the first cysteine residue. In most cases, the ELR+ chemokines promote angiogenesis and the ELR− chemokines are angiostatic. During normal wound healing, neutrophil infiltration induced by CXC chemokines is followed by mononuclear cell recruitment in response to CC chemokines. Thus, the coordinated expression of chemokines orchestrates a well-ordered pattern of inflammatory cell infiltration and aids in effective wound healing.[10,11]

It has been documented extensively in the literature that ligands for the chemokine receptors CCR1, CCR2, CCR5, and CXCR3 are ubiquitous in chronic inflammation, whereas in acute inflammation, CXCR1 and CXCR2 dominate. Activated neutrophils and T lymphocytes have high expression of CXCR1 and CXCR2, and the Th1 lymphocytes also express CXCR3. Monocytes, eosinophils, and basophils express CCR1 and CCR2, and monocytes also express CCR5.[12,13] For an extensive discussion on the role of chemokines and chemokine receptors in normal wound healing, refer to the review by Martins-Green *et al.*[14] Roy *et al.*[15] also provide a

detailed description on chemokines and chemokine receptors and explored the possibility of utilizing chemokines as a potential pharmaceutical target for various malignancies. The role of chemokines, in both acute and chronic wound healing, is growing, and several chemokines and their receptors are reported as significant players, as discussed below in detail.

Chronic nonhealing wounds

The body is naturally programmed for self-repair, and most wounds proceed toward healing in a timely manner. In chronic metabolic disease, diabetes in particular, the healing process is defective, leading to what is termed a "chronic wound."[5] Over 29.1 million people or 9.3% of the US population suffer from diabetes, costing 176 billion dollars in direct medical costs (American Diabetes Association, 2014). Diabetic complications plague macro- and microvascular structures causing stroke, atherosclerosis, impaired wound healing, retinopathy, neuropathy, and nephropathy.[16] Age-related decline in repair and regeneration potential in the skin might be a cause of nonhealing in wounds, such as chronic venous leg ulcers, PUs, and diabetic foot ulcers. Indeed, a steady increase in their incidence in individuals older than 65 years has been reported[17] with a longer hospital stay along with diminished quality of life.

Although there is no single unifying theory as to why chronic wounds fail to heal, chronic inflammation[18,19] and bacterial infection[20,21] may be the two major factors contributing to the persistence of the wound chronicity. In addition, three cell populations that are key players in wound healing, namely fibroblasts, keratinocytes, and endothelial cells, are also found to be functionally compromised in chronic wounds. Decreased proliferative efficiency has been described for fibroblasts isolated from the chronic wound site compared with normal nonwound skin fibroblasts.[22–24] Several studies also point toward decreased migration ability of fibroblasts derived from chronic wounds in comparison to healthy fibroblasts. It has been shown that increased alpha-smooth muscle actin expression tends to slow the process of migration.[25–27] Keratinocytes at the nonhealing edge of the chronic wounds have a very different phenotype and biological function when compared with a healing acute wound. Various signaling molecules and activation pathways have been identified for nonmigratory hyperproliferative epidermis of chronic wounds that fail to reepithelialize and restore the epidermal barrier for successful healing (see review by Pastar *et al.*).[28] It has been well established in chronic wounds associated with diabetes that the process of angiogenesis is impaired.[2]

Growth factors secreted by all these cell populations contribute extensively toward the final outcome of wound healing. Growth factors have been extensively studied due to their influence in various phases of healing. *In vitro*, growth factors, such as epidermal growth factor (EGF), vascular endothelial growth factor (VEGF), insulin growth factor, nerve-derived growth factor, and platelet-derived growth factor (PDGF), have been shown to promote cell migration, proliferation, and synthesis of extracellular matrix (ECM) proteins.[29] Cytokines are important in regulating the intensity and duration of the inflammatory response. In general, the balance between pro- and anti-inflammatory cytokines is lost in chronic wounds, which leads to an extended inflammatory response leading to nonhealing wounds. In particular, the levels of interleukin-6 (IL-6), interleukin-8 (IL-8), interleukin-1 (IL-1), and TNF-α are high, which might be the leading cause for persistent inflammation seen in chronic wounds.[30] Interleukin-10 (IL-10), a potential anti-inflammatory protein has been researched extensively and has been shown that elevated levels of IL-10 in wounds recapitulated fetal-like scarless wound healing in postnatal tissues (refer to a review article by King *et al.*).[31] The significance of increased expression of IL-10 seen in chronic venous insufficiency ulcers needs to be studied.[32] In general, more studies are needed to explore the functional importance of IL-10 in chronic wounds. PDGF is the only recombinant growth factor approved by the U.S. Food and Drug Administration to promote wound closure through topical application. Recent conflicting reports detailing the efficacy of PDGF for the treatment of chronic wounds have been reported from within the wound healing community (refer to Park *et al.* for more references on this topic).[33] A recent study by Barrientos *et al.*[34] discusses the literature-based evidence on effective growth factors and cytokines in the management of nonhealing chronic wounds. The author concludes that PDGF, VEGF, basic fibroblast growth factor, and cytokine granulocyte–macrophage colony-stimulating factor show great promises in enhancing the healing of chronic wounds in small randomized control trials, and long-term controlled trials are required to deliver long-term outcomes.

There is still a pressing need to develop new therapeutic regimens to heal chronic wounds for which chemokines seem to be the best option as their small size makes them ideal candidates for treating the wounds exogenously. By targeting

chemokines through genetic and molecular biology tools, one might alter signaling pathways that directly affect cellular migration/proliferation, angiogenesis, and epithelialization; this may serve as a useful approach in healing chronic wounds. Characteristic differences between acute and chronic wounds are summarized in Table 1.

DISCUSSION OF FINDINGS AND LITERATURE

Chemokines identified in normal wound healing as targets to treat chronic wounds

Inflammatory phase

Following hemostasis, inflammation, the first stage of wound healing, is initiated by platelet granule release. It begins within minutes to hours of wounding and typically lasts for 4 days. The inflammatory process sets in with the infiltration of polymorphonuclear neutrophils followed by the invasion of monocytes and macrophages into the wound that are key players during this phase.

Table 1. *Characteristic differences observed between acute and chronic wounds*

Stages in Wound Healing	*Acute Skin Wounds*	*Chronic Skin Wounds*
Inflammation[2]	Robust	Prolonged
Neutrophils[2]	Recruited immediately and disappear by apoptosis after macrophage recruitment	Neutrophils stay around for a longer period of time than required
Macrophages[2]	Recruited once neutrophils disappear	Recruitment delayed
Growth factor/cytokine expression		
EGF[57]	Increased	Low to none
PDGF[2]	Increased	Decreased
TGF-β1[58]	Increased	Increased
VEGF[2]	Increased	Decreased
bFGF[2]	Increased	Decreased
GM-CSF[34]	Increased	Decreased
IFN-γ[58]	Expressed	Increased
MCP-1[37]	Expressed	Increased
IL-1[59]	Expressed	400-fold increase
IL-6[60]	Increased	Increased
IL-8[37]	Increased	20-fold increase
IL-10[32]	Expressed	5-fold increase
Formation of granulation tissue[2]	Yes	No
Fibroblast morphology[25]	Compact and spindle-shaped	Larger and polygonal
Angiogenesis[61]	Good	Poor
Re-epithelialization[62]	Yes	No
MMP activity[63]	Low	Very high

bFGF, basic fibroblast growth factor; EGF, epidermal growth factor; GM-CSF, granulocyte–macrophage colony-stimulating factor; IFN, interferon; IL, interleukin; MCP-1, monocyte chemoattractant protein-1; MMP, matrix metalloproteinase; PDGF, platelet-derived growth factor; VEGF, vascular endothelial growth factor.

Inflammatory cells. Chemokines produced at the injured site promote the directional migration of leukocytes (neutrophils and monocytes/macrophages). In wounds that have demonstrated delayed or decreased repair capacity as seen in chronic wounds, the main cause is attributed to impairment of inflammatory cell infiltration and activation in the early stages of wound healing. Multiple chemoattractants regulate neutrophil trafficking that includes CXCL1, 4, 5, 6, 7, and 8. In humans, CXCL8 is a key potent chemoattractant for neutrophils, although both the CXCR1 and CXCR2 receptors and newly arrived neutrophils also secrete CXCL8, increasing the neutrophil numbers.[35] A recent study by Hasegawa *et al.*[36] showed that by topically applying recombinant human dermokine-β (shown to be abundant in stratified epithelia and differentiating keratinocytes) to excisional wounds in mice reduced the expression of CXCL1 and CXCL5; both of which are chemoattractants for neutrophils into wounds. This resulted in reduced influx of neutrophils and macrophages, inhibition of angiogenesis, decrease in the number of myofibroblasts into the wounds, and eventually leading to delay in early skin wound healing. Patreaca *et al.*[37] showed that deletion of TNF superfamily member 14 gene in mice leads to impaired wounds (mirrors human chronic wound); these mice showed excessive production of chemokines CXCL8, CXCL10, and CCL2 very early after wounding, which may have led to abnormal initiation and resolution of inflammation. In contrast, a diabetic rabbit ear wound model showed an increase in baseline gene expression of IL-6, IL-8, CXCR1, and CXCR2 postinjury and the expression was significantly less in diabetic wounds.[38] The differences observed between the two studies could be argued due to the use of different animal models and need a much better understanding of CXCL8 and its receptors CXCR1 and CXCR2 in chronic wounds.

A previous study by Devalaraja *et al.*[39] has shown that CXCR2 knockout mice showed delayed healing as the wounds exhibited defective neutrophil recruitment, altered pattern of monocyte recruitment, delay in epithelialization, and decreased neovascularization. Significant lower expression of CXCR2 is also reported postinjury in diabetic wounds[38] highlighting the importance of specific chemokines required precisely during the early stages of healing for recruiting appropriate inflammatory cells. Neutrophils disappear from the wound site through apoptosis, and it has been shown that apoptotic neutrophils increase their CCR5 receptor expression on the surface, which

binds and sequesters chemokines and also eventually leads to neutrophil apoptosis.

Once the neutrophils disappear, monocytes arrive at the wound site through chemokines, such as CCL2, and differentiate into macrophages that are considered to play a central role in wound repair by producing a battery of growth factors and inflammatory cytokines.[40] Macrophages facilitate the phagocytosis of apoptotic neutrophils and other dead cells at the wound site to clear the debris. At this stage, a large number of monocyte/macrophage-attractant chemokines, namely RANTES (CCL5), macrophage inflammatory protein (MIP-1; CCL4), and monocyte chemoattractant protein (MCP-1 [CCL2] and MCP-3 [CCL7]), are exclusively found to be expressed during the first week after wounding.[10] At this stage, keratinocytes also contribute to the inflammatory network by expressing MCP-1 in the basal keratinocytes at the wound edge. It should also be noted that the MCP-1 not only attracts monocytes but also at later time points attracts mast cells.[41] In early stages of diabetic chronic wounds, it has been noted that there is a damaging delay in essential macrophage response, and it was primarily due to insufficient chemokine expression. A one-time treatment with CCL2 in wounds of diabetic mice significantly stimulated healing in diabetic wounds by restoring the macrophage response.[42] Comparing fibroblasts derived from properly healing and chronic human wounds showed increased expression of MCP-1 in chronic wounds implying that constitutive release of MCP-1 becomes disadvantageous for the healing process.[26] Once macrophage inflammation resolves, lymphocytes appear as the last cell type to arrive at the wound site. Chemokines that are spatially associated with lymphocyte accumulation are MCP-1, IFN-γ–inducible-protein-10 (IP-10; CXCL10), monokine-induced by IFN-γ (Mig-9; CXCL9), and macrophage-derived chemokine (MDC; CCL22). Lymphocyte inflammation is resolved by apoptosis when there is a major shift in the cytokine profile from TNF-α/IL-1 to IFN-γ.

Proliferative phase

This phase is marked by the formation of granulation tissue, a well-vascularized connective tissue containing macrophages and fibroblasts that replace the fibrin clot, a characteristic feature of the proliferative phase of normal wound healing. The presence of granulation tissue in an open wound also allows the process of reepithelialization to begin, as epithelial cells migrate across the new tissue to form a barrier between the wound and the environment.

Angiogenesis and reepithelialization. In normal wounds, endothelial cells promote healing by mediating and regulating the recruitment of inflammatory cells into sites of injury and by the formation of new vessels from the preexisting neovasculature, a process named as angiogenesis. Chemokines play a crucial role in the processes of angiogenesis and reepithelialization.[43] Endothelial cells comprise a broad repertoire of chemokines, which may be expressed after appropriate stimulation. These include CC chemokines, such as MCP-1 and RANTES as well as CXC family members like IL-8, GRO-alpha, IP-10, Mig, and others. IL-8 may furthermore be presented on the surface of endothelial cells to allow interaction with leukocytes to promote recruitment from the intraluminal compartment. MCP-1 contributes to the establishment of a chemokine gradient, which allows subset-specific recruitment of leukocytes to sites of inflammation. Conflicting results exist on endothelial cell expression of CXCR2, as some groups identified its expression in cultured endothelial cells while others could not.[44] However, CXCR-2 (ligand CXCL2 also referred as MIP2-alpha; macrophage inhibitory protein-2) knockout mice showed a significant delay in neovascularization, and in the reepithelialization process, this was postulated that this would be a result of the diminished angiogenic response toward MIP-1, the functional homologues of IL-8 in the murine system. Examining MIP-1 alpha (−/−) and MCP-1 (−/−) mice showed that only MCP-1 (−/−) displayed a significant delay in angiogenesis, reepithelialization, and collagen synthesis but not the MIP-1 alpha (−/−) mice.[45] Interestingly, in MCP-1 (−/−) mice, there was no change in the recruitment of wound macrophages implying that recruitment of monocyte is independent of this chemokine. Low-intensity vibration has shown to improve the delayed wound healing in diabetic mice by increasing the expression of prohealing growth factors and chemokines, namely MCP-1.[46]

On the other hand, CXCR3 is shown to be expressed on vessels at days 7–21 postwounding, and treatment of endothelial cords with CXCR3 ligand IP-10 during the resolving phase of wounds caused dissociation of blood vessels even in the presence of angiogenic growth factors.[47] Along the same lines, mice lacking CXCR3 receptor showed delayed reepithelialization and basement membrane regeneration in excisional wounds.[48] We are still yet to understand the functions of CXCR3 and its ligands in the biology of chronic wounds. Please refer to the article titled "Chemokine Regulation of Angiogenesis During Wound Healing" by Dr. Richard

Bodnar in this issue for a detailed review on the role played by chemokines in angiogenesis during wound healing.

Stromal cell-derived factor-1 (SDF-1/CXCL12), a potent chemokine that express CXCR4 receptor for SDF-1, is considered to play an important role in postinjury leukocyte chemotaxis, migration, and homing of stem cells. A recent study by Su *et al.*[49] showed that SDF-1/CXCR4 signaling pathway facilitates wound healing through augmenting bone marrow-derived stromal cells recruitment to wound tissues and promoted neovascularization. Recent evidence shows that increasing the expression of CXCL12 in wounds of diabetic mice increased stem cell recruitment to the wound and improved wound healing outcome.[50] SDF-1 alpha has also been shown to have a decreased expression in diabetic skin wounds. An interesting study by Castilla *et al.*[51] showed that priming bone marrow-derived stem cells with SDF-1 alpha and injecting subcutaneously into skin wounds of diabetic mice promoted wound healing by improving neovascularization and endothelial progenitor cell recruitment. Similarly, inhibition of SDF-1 alpha in diabetic skin wounds decreased the rate of healing.[52] In contrast, a study by Nishimura *et al.*[53] showed that one-time application of CXCR4 antagonist AMD3100 to the skin wounds of diabetic mice promote wound healing by increasing cytokine production, mobilizing bone marrow EPCs, and by enhancing angiogenesis and vasculogenesis. These results confound each other on the function of CXCR4/SDF-1 axes in wound healing but clearly implies that enhancement of CXCL12 (SDF-1) or CCL2 (MCP-1) expression would be a novel therapeutic strategy to promote healing in chronic wounds.

Remodeling phase

The remodeling phase can last for few months to a year depending on the size of the wound, wherein there is a continuous synthesis and degradation of collagen and other ECM proteins resulting in a mature scar.

Extracellular matrix. The matrix metalloproteinases (MMPs) play an important role in remodeling the ECM. MMPs are involved in tissue repair and remodeling process, such as inflammation, angiogenesis, and re-epithelialization. MMPs, such as MMP-1, −2, and-3, are primarily responsible for degradation and turnover of ECM. In addition, MMPs regulate inflammatory cell function by cleaving MCPs and SDF-1 to reduce cell function. MMP-2 (gelatinase A), MMP-3 (stromelysin-1), and MMP-9 (gelatinase B) cleave MCP to generate CCR-1, 2, and 3 antagonist inhibiting leukocyte function and cleaves SDF-1.[54] TGF-β1 promotes matrix deposition and stabilization by repressing MMPs and stimulating tissue inhibitor of metalloproteinases expression to orchestrate the final remodeling of the ECM.[55] In addition, expression of membrane-type matrix metalloproteinases (MT-MMP) has been found to be important for cell migration in collagen and the remodeling phase of wound healing. MT-MMP activates MMP-2, which has been found to have anti-inflammatory functions and promote collagen reorganization. Nonhealing chronic wounds, in particular diabetic wounds, exhibit impairments in ECM production and increased degradation owing to elevated concentrations of proteolytic MMPs. The decreased tensile strength and the nonability to withstand trauma in chronic wounds are mainly because there is a decreased amount of collagen and in-

Table 2. *Select chemokines function during wound healing*

		Functions	
Ligand	*Receptors*	*Normal (Acute) Skin Wound Healing*	*Chronic Skin Wound Healing*
CXCL2/MIP2-alpha[39]	CXCR2	Chemoattractants for polymorphonuclear leukocytes and hematopoietic stem cells	Not studied
CXCL8/IL-8[14]	CXCR1/CXCR2	Chemoattractants for neutrophils	Excessive production leads to abnormal initiation and resolution of inflammation.[37] Significantly decreased expression is also noted in rabbit diabetic ear wound model.[38]
CXCL9/IP-9[48]	CXCR3	Resolution of inflammatory phase	Not studied
CXCL10/IP-10[47]	CXCR3	Resolution of inflammatory phase	Not studied
CXCL12/SDF1[49]	CXCR4	Migration and homing of stem cells	Decreased expression in diabetic skin wounds.[50]
CCL2/MCP-1[10]	CCR2	Chemoattractants for monocytes/macrophages	Increased expression promotes wound healing in diabetic wounds by restoring macrophage response.[42]
CCL7/MCP-3[10]	CCR1/CCR2	Chemoattractants for monocytes/macrophages	Not studied
CCL4/MIP-1[10]	CCR5	Chemoattractants for monocytes/macrophages	Not studied
CCL5/RANTES[10]	CCR5/CCR3/CCR1	Chemoattractants for monocytes/macrophages	Not studied

MIP-1, macrophage inflammatory protein-1; RANTES, regulated on activation, normal T-cell expressed and secreted; SDF-1, stromal cell-derived factor-1.

Table 3. *Synopsis of current preclinical and clinical studies showing promise in healing chronic wounds through changes in the expression of chemokines*

Study Title	*Study Design*	*Chemokines Implicated*	*Outcome*
Weinheimer-Haus *et al.*[46]	Application of low-intensity vibration (diabetic mouse skin wound)	Increased expression levels of MCP-1	Enhanced healing in diabetic wounds
Stenstresser *et al.*[64]	Skin electroporation of a plasmid encoding hCAP-18/LL-37 host defense peptide (diabetic mouse skin wound)	Increased expression levels of SDF-1 alpha and CXCR4 receptor	Promoted healing in diabetic wounds
Castilla *et al.*[51]	*Ex vivo* priming of BMDSCs with SDF-1 alpha (diabetic mouse skin wound)	Increased expression levels of SDF-1 alpha in wounds	Promoted healing in diabetic wounds
Lima *et al.*[65]	Topical application of insulin (preclinical model; diabetic mouse skin wound and clinical trial; diabetic ulcer patients)	Increased expression levels of SDF-1 alpha	Improved healing in diabetic wounds
Schürmann *et al.*[66]	Oral administration of linagliptin (diabetic mouse skin wound)	Decreased expression levels of MIP-2	Beneficial in healing of diabetic wounds
Nishimura *et al.*[53]	Application of CXCR4 antagonist AMD3100 (diabetic mouse skin wound)	Increased expression levels of SDF-1 alpha	Promoted healing in diabetic wounds
Bermudez *et al.*[52]	Inhibition of SDF-1 alpha levels in a diabetic mouse skin wound	Inhibition of SDF-1 alpha levels	Decreased the rate of healing in diabetic wounds
Nguyen *et al.*[67]	Topical silencing of p53 in diabetic mouse skin wound	Increased expression levels of SDF-1 alpha	Improved healing in diabetic wounds
Liu *et al.*[68]	Topical injection of SDF-1 alpha engineered bone marrow-derived fibroblasts in diabetic mouse skin wound	Increased expression levels of SDF-1 alpha	Improved healing in diabetic wounds
Restivo *et al.*[50]	Topical application of CXCL12 expression plasmid in diabetic mouse skin wound	Increased expression levels of CXCL12	Improved healing in diabetic wounds
de Leon *et al.*[69]	Treating chronic wounds with platelet-rich plasma (PRP) gel (clinical trial)	Increase in the levels of chemokines reported	PRP can restart the healing process in chronic wounds
Badillo *et al.*[70]	Treatment with lentiviral construct containing SDF-1 alpha gene (diabetic mouse skin wound)	Overproduction of SDF-1 alpha levels	Improved healing in diabetic wounds

creased MMP production. Chronic wound fluid has shown to impair cell proliferation and angiogenesis and also showed increased levels of MMPs.[56] Chemokines are responsible for both production and degradation of ECM. Many of the chemokines that were discussed above have either a direct chemokine-induced MMP production by resident cells (fibroblasts, keratinocytes, and endothelial) within the wound or through sustained inflammatory cell infiltrate. Increasing or decreasing the expression of appropriate chemokines would benefit the wounds to lay down an appropriate matrix for a favorable healing outcome. More extensive research is required to understand the specific functions of chemokines in ECM production in both acute and chronic wounds. Table 2 summarizes the select chemokines function in acute and chronic wound healing. Table 3 summarizes the current preclinical and clinical studies showing the changes in the expression levels of certain chemokines through various treatment modalities.

SUMMARY

The potential of the chemokine system as drug targets in chronic wounds is achievable but requires more in-depth understanding of the chemokine interactions and its receptors in both acute and chronic wound settings. To date, only a few of the $\sim$50 human chemokines' functions have been explored. This may be a possible reason for the present stalemate and inability to achieve a successful outcome for healing chronic wounds. More importantly, chemokines can be considered as adjuvants to stimulate a nonhealing wound environment provided that the timely and spatially different expression pattern of chemokines is captured as detected in a physiological wound milieu.

ACKNOWLEDGMENTS AND FUNDING SOURCES

The author expresses her thanks to Dr. Richard Bodnar, Department of Pathology, VA Pittsburgh Health Care System, Pittsburgh, for his time and help in critically reviewing the article. She also expresses thanks to the Department of Plastic Surgery for providing the resources to complete this review article.

AUTHOR DISCLOSURE AND GHOST WRITING

The author has no disclosed commercial associations that could lead to a conflict of interest. The author is solely responsible for writing the article and did not use ghost writers.

ABOUT THE AUTHOR

Latha Satish, MSc, PhD, joined the Department of Pathology as a postdoctoral research fellow at the University of Pittsburgh after completion of the PhD program at the University of Madras, Chennai, India. She then joined the Allegheny Research Institute, Pittsburgh, as Research Associate and then was promoted as Research Assistant Professor in the same institute. Dr. Satish joined the Department of Plastic Surgery as Research Assistant Professor at the University of Pittsburgh in 2011. Her long-term research interest has been to uncover the genes and signaling pathways responsible for skin fibrosis and scarless wound healing along with studying chronic wounds and biofilms. Her interest is also to uncover the biological mechanisms leading to Dupuytren's contracture (palmar fascia fibrosis).

TAKE HOME MESSAGES

- The best approach to modulate inflammatory responses in a nonhealing wound can be achieved through targeting chemokines.
- Chemokines have the ability to convert chronic inflammation to acute inflammation.
- Various chemokines are expressed throughout the process of wound healing, and one-time application of a candidate chemokine can stimulate the nonhealing environment by producing factors necessary for healing.
- A much better understanding of chemokine function in the chronic wound biology is required to identify the candidate drug.
- Chemokines that currently show promising evidence for future clinical trials in treating chronic wounds are CXCL12 and CCL2.

REFERENCES

1. Goova MT, Li J, Kislinger T, et al. Blockade of receptor for advanced glycation end-products restores effective wound healing in diabetic mice. Am J Pathol 2001;159:513–525.
2. Blakytny R, Jude E. The molecular biology of chronic wounds and delayed healing in diabetes. Diabet Med 2006;23:594–608.
3. Ehrenreich M, Ruszczak Z. Update on tissue-engineered biological dressings. Tissue Eng 2006;12:2407–2424.
4. Smiell JM, Wieman TJ, Steed DL, et al. Efficacy and safety of becaplermin (recombinant human platelet-derived growth factor-BB) in patients with non-healing, lower extremity diabetic ulcers: a combined analysis of four randomized studies. Wound Repair Regen 1999;7:335–346.
5. Menke NB, Ward KR, Witten TM, Bonchev DG, Diegelmann RF. Impaired wound healing. Clin Dermatol 2007;25:19–25.
6. Pease JE, Williams TJ. The attraction of chemokines as a target for specific anti-inflammatory therapy. Br J Pharmacol 2007;147:S212.
7. Charo IF, Ransohoff RM. The many roles of chemokines and chemokine receptors in inflammation. N Engl J Med 2006;354:610–621.
8. Rosenkilde MM, Schwartz TW. The chemokine system—a major regulator of angiogenesis in health and disease. APMIS 2004;112:481–495.
9. Gerard C, Rollins BJ. Chemokines and disease. Nat Immunol 2001;2:108–115.
10. Engelhardt E, Toksoy A, Goebeler M, et al. Chemokines IL-8, GROalpha, MCP-1, IP-10, and Mig are sequentially and differentially expressed during phase-specific infiltration of leukocyte subsets in human wound healing. Am J Pathol 1998; 153:1849–1860.
11. Wetzler C, Kampfer H, Stallmeyer B, et al. Large and sustained induction of chemokines during impaired wound healing in the genetically diabetic mouse: prolonged persistence of neutrophils and macrophages during the late phase of repair. J Invest Dermatol 2000;115:245–253.
12. Borish LC, Steinke JW. Cytokines and chemokines. J Allergy Clin Immunol 2003;111 (2 suppl):S460.
13. Baggiolini ML. Chemokines in pathology and medicine. J Int Med 2001;250:91–104.
14. Martins-Green M, Petreaca M, Wang L. Chemokines and their receptors are key players in the orchestra that regulates wound healing. Adv Wound Care (New Rochelle) 2013;2:327–347.
15. Roy I, Evans DB, Dwinell MB. Chemokines and chemokine receptors: update on utility and challenges for the clinician. Surgery 2014;155:961–973.
16. Reddy MA, Natarajan R. Epigenetic mechanisms in diabetic vascular complications. Cardiovasc Res 2011;90:421–429.
17. Margolis DJ, Bilker W, Santanna J, Baumgarten M. Venous leg ulcer: incidence and prevalence in the elderly. J Am Acad Dermatol 2002;46:381–386.
18. Agren MS, Eaglstein WH, Ferguson MW, et al. Causes and effects of the chronic inflammation in venous leg ulcers. Acta Derm Venerol Supppl (Stockh) 2000;210:3–17.
19. Smith PD. Update on chronic-venous-insufficiency-induced inflammatory processes. Angiology 2001; 52:S35–42.
20. Robson MC. Wound infection. A failure of wound healing caused by an imbalance of bacteria. Surg Clin North Am 1997;77:637–650.
21. Dow G, Browne A, Sibbald RG. Infection in chronic wounds: controversies in diagnosis and treatment. Ostomy Wound Manage 1999;45:23–27.
22. Vande Berg JS, Rudolph R, Hollan C, Haywood-Reid PL. Fibroblast senescence in pressure ulers. Wound Repair Regen 1998;6:38–49.
23. Vande Berg JS, Rose MA, Haywood-Reid PL, et al. Cultured pressure ulcer fibroblasts show replicative senescence with elevated production of plasmin, plasminogen activator inhibitor-1, and transforming growth factor-beta1. Wound Repair Regen 2005;13:76–83.
24. Stanley A, Osler T. Senescence and the healing rates of venous ulcers. J Vasc Surg 2001;33:1206–1211.
25. Wall IB, Moseley R, Baird DM, et al. Fibroblast dysfunction is a key factor in the non-healing of chronic venous leg ulcers. J Invest Dermatol 2008; 128:2526–2540.
26. Schwarz F, Jennewein M, Bubel M, et al. Soft tissue fibroblasts from well healing and chronic human wounds show different rates of myofibroblasts *in vitro*. Mol Biol Rep 2013;40:1721–1733.
27. Raffetto JD, Mendez MV, Marien BJ, et al. Changes in cellular motility and cytoskeletal actin in fibroblasts from patients with chronic venous insufficiency and in neonatal fibroblasts in the presence of chronic wound fluid. J Vasc Surg 2001;33:1233–1241.
28. Pastar I, Stojadinovic O, Yin NC, et al. Epithelialization in wound healing: a comprehensive review. Adv Wound Care (New Rochelle) 2014;3:445–464.
29. Schultz GS, Wysocki A. Interactions between extracellular matrix and growth factors in wound healing. Wound Repair Regen 2009;17:153–162.
30. Fivenson DP, Faria DT, Nickoloff BJ, et al. Chemokine and inflammatory cytokine changes during

chronic wound healing. Wound Repair Regen 1997; 5:310–322.

31. King A, Balaji S, Le LD, Crombleholme TM, Keswant SG. Regenerative wound healing: the role of IL-10. Adv Wound Care (New Rochelle) 2014;3:315–323.
32. Lundberg JE, Roth TP, Dunn RM, Doyle JW. Comparison of IL-10 levels in chronic venous insufficiency ulcers and autologous donor tissue. Arch Dermatol Res 1998;290:669–673.
33. Park SA, Raghunathan VK, Shah NM, et al. PDGF-BB does not accelerate healing in diabetic mice with splinted wounds. PLoS One 2014;9:e104447.
34. Barrientos S, Brem H, Stojadinovic O, Tomic-Canic M. Clinical application of growth factors and cytokines in wound healing. Wound Repair Regen 2014;22:569–578.
35. Jones SA, Wolf M, Qin S, MacKay CR, Baggiolini M. Different functions for the IL-8R of human neutrophil leukocytes: NADPH oxidase and phospholipase D are activated through IL-8R1 but not IL-8R2. Proc Natl Acad Sci U S A 1996;93:6682–6686.
36. Hasegawa M, Higashi K, Matsushita T, et al. Dermokine inhibits ELR(+) CXC chemokine expression and delays early skin wound healing. J Dermatol Sci 2013;70:34–41.
37. Petreaca ML, Do D, Dhall S, et al. Deletion of a tumor necrosis superfamily gene in mice leads to impaired healing that mimics chronic wounds in humans. Wound Repair Regen 2012;20:353–366.
38. Pradhan L, Cai X, Wu S, et al. Gene expression of pro-inflammatory cytokines and neuropeptides in diabetic wound healing. J Surg Res.2011;167:336–342.
39. Devalaraja RM, Nanney LB, Du J, et al. Delayed wound healing in CXCR2 knockout mice. J Invest Dermatol 2000;115:234–244.
40. Sunderkötter C, Steinbrink K, Goebeler M, et al. Macrophages and angiogenesis. J Leukoc Biol 1994; 55:410–422.
41. Trautmann A, Toksoy A, Engelhardt E, et al. Mast cell involvement in normal human skin wound healing: expression of monocyte chemoattractant protein-1 is correlated with recruitment of mast cells which synthesize interleukin-4 *in vivo*. J Pathol 2000; 190:100–106.
42. Wood S, Jayaraman V, Huelsmann EJ, et al. Pro-inflammatory chemokine CCL2 (MCP-1) promotes healing in diabetic wounds by restoring the macrophage response. PLoS One 2014;9:e91574.
43. Gillitzer R, Goebeler M. Chemokines in cutaneous wound healing. J Leukoc Biol 2001;69:513–521.
44. Murdoch C, Finn A. Chemokine receptors and their role in inflammation and infectious diseases. Blood 2000;95:3032–3043.
45. Low QE, Drugea IA, Duffner LA, et al. Wound healing in MIP-1alpha(−/−) and MCP-1(−/−) mice. Am J Pathol 2001;159:457–463.
46. Weinheimer-Haus EM, Judex S, Ennis WJ, Koh TJ. Low-intensity vibration improves angiogenesis and wound healing in diabetic mice. PLoS One 2014;9:e91355.
47. Bodnar RJ, Yates CC, Rodgers ME, Du X, Wells A. IP-10 induces dissociation of newly formed blood vessels. J Cell Sci 2009;122:2064–2077.
48. Yates CC, Whaley D, Hooda S, Hebda PA, Bodnar RJ, Wells A. Delayed reepithelialization and basement membrane regeneration after wounding in mice lacking CXCR3. Wound Repair Regen 2009; 17:34–41.
49. Su X, Zhu F, Zhang M, et al. Stromal cell-derived factor-1 enhances wound healing through recruiting bone marrow-derived stem cells to the wound area and promoting neovascularization. Cells Tissues Organs 2013;197:103–114.
50. Restivo TE, Mace KA, Harken AH, Young DM. Application of the chemokine CXCL12 expression plasmid restores wound healing to near normal in a diabetic mouse model. J Trauma 2010;69:392–398.
51. Castilla DM, Jun Liu Z, Tian R, Li Y, Livingstone AS, Velazquez OC. A novel autologous cell based therapy to promote diabetic wound healing. Ann Surg 2012;256:560–572.
52. Bermudez DM, Xu J, Herdrich BJ, Radu A, Mitchell ME, Liechty KW. Inhibition of stromal cell-derived factor-1α further impairs diabetic wound healing. J Vasc Surg 2011;53:774–784.
53. Nishimura Y, Ii M, Qin G, et al. CXCR4 antagonist AMD3100 accelerates impaired wound healing in diabetic mice. J Invest Dermatol 2012;132:711–720.
54. McQuibban GA, Gong JH, Wong JP, Wallace JL, Clark-Lewis I, Overall CM. Matrix metalloproteinase processing of monocyte chemoattractant proteins generates CC chemokine receptor antagonists with anti-inflammatory properties *in vivo*. Blood 2002;100:1160–1167.
55. Overall CM, Wrana JL, Sodek J. Transcriptional and post-transcriptional regulation of 72-kDa gelatinase/type IV collagenase by transforming growth factor-beta 1 in human fibroblasts. Comparisons with collagenase and tissue inhibitor of matrix metalloproteinase gene expression. J Biol Chem 1991;266:14064–14071.
56. Trengove NJ, Stacey MC, MacAuley S, et al. Analysis of the acute and chronic wound environments: the role of proteases and their inhibitors. Wound Repair Regen 1999;7:442–452.
57. Dogan S, Demirer S, Kepenekci I, et al. Epidermal growth factor-containing wound closure enhances wound healing in non-diabetic and diabetic rats. Int Wound J 2009;6:107–115.
58. Ishida Y, Kondo T, Takayasu T, Iwakura Y, Mukaida N. The essential involvement of cross-talk between IFN-gamma and TGF-beta in the skin wound-healing process. J Immunol 2004;172:1848–1855.
59. Barone EJ, Yager DR, Pozez AL, et al. Interleukin-1alpha and collagenase activity are elevated in chronic wounds. Plast Reconstr Surg 1998;102: 1023–1027.
60. Liu T, Yang F, Li Z, Yi C, Bai X. A prospective pilot study to evaluate wound outcomes and levels of serum C-reactive protein and interleukin-6 in the wound fluid of patients with trauma-related chronic wounds. Ostomy Wound Manage 2014;60:30–37.
61. Lim YC, Bhatt MP, Kwon MH, et al. Proinsulin C-peptide prevents impaired wound helaing by activating angiogenesis in diabetes. J Invest Dermatol 2014 [Epub ahead of print]; DOI: 10.1038/jid.2014.285.
62. Stojadinovic O, Brem H, Vouthounis C, et al. Molecular pathogenesis of chronic wounds: the role of beta-catenin and c-myc in the inhibition of epithelialization and wound healing. Am J Pathol 2005;167:59–69.
63. Motzkau M, Tautenhahn J, Lehnert H, Lobmann R. Expression of matrix-metalloproteases in the fluid of chronic diabetic foot wounds treated with a protease absorbent dressing. Exp Clin Endocrinol Diabetes 2011;119:286–290.
64. Steinstraesser L, Lam MC, Jacobsen F, et al. Skin electroporation of a plasmid encoding hCAP-18/LL-37 host defense peptide promotes wound healing. Mol Ther 2014;22:734–742.
65. Lima MH, Caricilli AM, de Abreu LL, et al. Topical insulin accelerates wound healing in diabetes by enhancing the AKT and ERK pathways: a double-blind placebo-controlled clinical trial. PLoS One 2012;7:e36974.
66. Schürmann C, Linke A, Engelmann-Pilger K, et al. The dipeptidyl peptidase-4 inhibitor linagliptin attenuates inflammation and accelerates epithelialization in wounds of diabetic ob/obmice. J Pharmacol Exp Ther 2012;342:71–80.
67. Nguyen PD, Tutela JP, Thanik VD, et al. Improved diabetic wound healing through topical silencing of p53 is associated with augmented vasculogenic mediators. Wound Repair Regen 2010;18:553–559.
68. Liu ZJ, Tian R, An W, et al. Identification of E-selectin as a novel target for the regulation of postnatal neovascularization: implications for diabetic wound healing. Ann Surg 2010;252:625–634.
69. de Leon JM, Driver VR, Fylling CP, et al. The clinical relevance of treating chronic wounds with an enhanced near-physiological concentration of platelet-rich plasma gel. Adv Skin Wound Care 2011;24:357–368.
70. Badillo AT, Chung S, Zhang L, Zoltick P, Liechty KW. Lentiviral gene transfer of SDF-1alpha to wounds improves diabetic wound healing. J Surg Res 2007;143:35–42.

Abbreviations and Acronyms

bFGF = basic fibroblast growth factor
ECM = extracellular matrix
EGF = epidermal growth factor
GM-CSF = granulocyte–macrophage colony-stimulating factor
IFN = interferon
IL = interleukin
MCP-1 = monocyte chemoattractant protein-1
MIP-1 = macrophage inflammatory protein-1
MMPs = matrix metalloproteinases
MT-MMP = membrane-type matrix metalloproteinases
PDGF = platelet-derived growth factor
RANTES = regulated on activation, normal T-cell expressed and secreted
SDF-1 = stromal cell-derived factor-1
TNF = tumor necrosis factor
VEGF = vascular endothelial growth factor