The Role of Chemokines in Fibrotic Wound Healing

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Significance: Main dermal forms of fibroproliferative disorders are hypertrophic scars (HTS) and keloids. They often occur after cutaneous wound healing after skin injury, or keloids even form spontaneously in the absence of any known injury. HTS and keloids are different in clinical performance, morphology, and histology, but they all lead to physical and psychological problems for survivors.

Recent Advances: Although the mechanism of wound healing at cellular and tissue levels has been well described, the molecular pathways involved in wound healing, especially fibrotic healing, is incompletely understood.

Critical Issues: Abnormal scars not only lead to increased health-care costs but also cause significant psychological problems for survivors. A plethora of therapeutic strategies have been used to prevent or attenuate excessive scar formation; however, most therapeutic approaches remain clinically unsatisfactory.

Future Directions: Effective care depends on an improved understanding of the mechanisms that cause abnormal scars in patients. A thorough understanding of the roles of chemokines in cutaneous wound healing and abnormal scar formation will help provide more effective preventive and therapeutic strategies for dermal fibrosis as well as for other proliferative disorders.

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SCOPE AND SIGNIFICANCE

WOUND HEALING UNDERGOES four overlapping phases of hemostasis, inflammation, proliferation, and remodeling in order to repair itself after injury. Embryonic wound healing occurs via regeneration of the same tissue types as original ones, whereas postnatal repair involves scar formation such as hypertrophic scars (HTS) and keloids, which result in critical physical and psychological problems for patients.

TRANSLATIONAL RELEVANCE

A tremendous amount of scientific research has well described the mechanism of wound healing at cellular and tissue levels. However, the

molecular pathways, especially chemokine signaling, involved in wound healing as well as abnormal scar formation are incompletely understood.

CLINICAL RELEVANCE

Although a plethora of therapeutic strategies have been used to prevent or attenuate excessive scar formation, most therapeutic approaches remain clinically unsatisfactory. A thorough understanding of the roles of chemokines in cutaneous wound healing and abnormal scar formation will help provide more effective preventive and therapeutic strategies for dermal fibrosis as well as for other proliferative disorders.

Wound healing and abnormal scar formation

The physical process of wound healing undergoes four overlapping phases of hemostasis, inflammation, proliferation, and remodeling, by which damaged tissue repairs itself after injury. Multiple systems, cells, molecules, and pathways are involved in the process. Briefly, within the first few minutes after injury, platelet extravasation and blood vessel constriction lead to clot formation to stop bleeding before immune cells launch an inflammatory response to debride the wounds by phagocytizing bacteria and cell debris. A cascade of cytokines induces angiogenesis, granulation tissue formation, collagen synthesis, re-epithelialization, and wound contraction in succession to repair and re-surface the wounds. Thereafter, with early wound closure, apoptosis removes the unnecessary cells and collagen is remodeled along lines of tension (Fig. 1). Embryonic wound healing occurs via regeneration of similar tissues in an orderly morphology, whereas postnatal repair involves scar formation by which wound closure is achieved by wound contraction and extracellular matrix (ECM) formation. Pathological healing leads to nonhealing chronic wounds or excessive fibrosis. The latter results in fibroproliferative disorders such as HTS and keloids, which are the dermal form of fibrotic wound healing after the skin injury as illustrated in Fig. 2.

Figure 1. Wound-healing process. Multiple systems, cells, molecules, and pathways are involved in the process. Briefly, platelets and hormones involve blood vessels constriction and clot formation rallied within the first few minutes after injury has a major role to stop bleeding. Then, immune cells launch an inflammatory response to clean the wounds by phagocytizing bacteria and cell debris. Meanwhile, under the actions of cytokines, angiogenesis, collagen production, granulation tissue formation, re-epithelialization, and wound contraction occur. Ultimately, apoptosis removes the excessive cells as collagen is remodeled and realigned along lines of tension in the remodeling wound. To see this illustration in color, the reader is referred to the web version of this article at www.liebertpub.com/wound

HTS are a common and significant negative outcome of skin burn injury to the deep layers of the dermis where prolonged inflammation occurs. Morphologically, HTS are red, raised, uncomfortable scars confined to the boundaries of the original wounds, which can result in functional limitations due to the development of contracture and disfigurement that also lead to cosmetic and psychologic difficulties for burn survivors. The main histological characteristics of HTS include thicker epidermis and dermis, lack of rete ridges in epidermis, hypervascularity, hypercellularity, and excessive ECM formation where abnormal morphology of collagen fibril structure occurs (Fig. 3). Keloids differ from HTS in that they develop after minor injuries or even form spontaneously in the absence of any known injury often in areas of high skin tension. Keloids appear as firm, mildly tender, bulky tumors where the epithelium maybe thinned, leading to focal areas of ulceration. Keloids are benign growth that often extend beyond the boundaries of the original wound. Although keloids can develop in any region where skin injury has occurred, high-tension areas in the skin such as the upper chest and shoulders are especially prone. Histologically, keloid tissue is composed of disorganized whorls of type I and III collagen, which contains pale-staining hypocellular collagen bundles; whereas HTS commonly contain a nodular structure and large numbers of myofibroblasts. HTS are seen in individuals of all ethnic backgrounds; whereas keloids are more common in dark skinned races, suggesting an underlying genetic predisposition.¹

Abnormal scars not only lead to increased health-care costs but also cause significant psychological problems for survivors. A plethora of therapeutic strategies have been used to prevent or attenuate excessive scar formation. However, most therapeutic approaches remain clinically unsatisfactory. Effective care depends on an improved understanding of the mechanism that causes abnormal scars in patients. Although the mechanism of wound healing at cellular and tissue levels has been well described,² the molecular pathways involved in fibrotic healing are incompletely understood. Therefore, in this article, the role of chemokines in cutaneous wound healing and abnormal scar formation will be described.

The role of chemokines in cutaneous wound healing

Chemokines are a family of small proteins with ~8–10 kDa in size. These proteins have historically

Figure 2. Skin fibrotic disorders in a 12-year-old child with keloids after a scald burn and a 28-year-old woman with HTS after a burn injury. HTS, hypertrophic scars. To see this illustration in color, the reader is referred to the web version of this article at www.liebertpub.com/wound

been known under several other names, including small inducible secreted (SIS) family of cytokines,³ small inducible gene (SIG) family of cytokines,⁴ small cytokine (SCY) family of cytokines,⁵ platelet factor-4 (PF-4) superfamily,⁶ and intercrine cytokine family.⁷ Depending on the spacing of their first two cysteine residues, chemokines have been

classified into four main subfamilies: C (XCL), CC (CCL), CXC (CXCL), and CX3C (CX₃CL) chemokines. The C chemokines have only two cysteines: one N-terminal cysteine and one cysteine downstream. The CC chemokine proteins have two adjacent cysteines near their amino terminus. They usually contain four cysteines, but a small number possesses six cysteines. The CXC chemokines have two N-terminal cysteines that are separated by one amino acid, represented by this name with an "X". The fourth group of chemokines have three amino acids between the two cysteines and are termed CX₃C chemokines. Chemokines exert their biological effects by interacting with G protein-linked transmembrane receptors XCR1 for XCL chemokines, CCR for CCL chemokines, CXCR for CXC chemokines, and CX3CR for CX₃C chemokines, which are selectively found on the surfaces of their target cells.

Chemokines, as signaling proteins, are named by their ability to induce chemotaxis of nearby responsive cells. Some of their signaling pathways are considered pro-inflammatory and are formed under certain pathological conditions to recruit immune cells to a site of infection, while others are considered homeostatic to control the migration of cells during normal processes of tissue maintenance or development.^{8,9} Chemokines these days are well known as playing roles in many basic biological processes such as leukocyte trafficking and homing, organ development, angiogenesis, tumorigenesis and metastasis, inflammation, autoimmune response, and viral infection. Recent data demonstrate that recruitment of leukocyte subtypes is tightly regulated by chemokines during wound healing. Moreover, the presence of

Figure 3. Histological characteristics of HTS. The main histological characteristics of HTS include a thicker epidermis and dermis, lack of rete ridges in epidermis, hypervascularity, hypercellularity, atypical ECM, including loss of normal collagen fibril structure, as well as excessive amounts of collagen deposited. ECM, extracellular matrix. To see this illustration in color, the reader is referred to the web version of this article at www.liebertpub.com/wound

chemokine receptors on resident cells (e.g., keratinocytes, endothelial cells) indicates that chemokines also contribute to the regulation of epithelialization, tissue remodeling, and angiogenesis. Thus, chemokines are in an exclusive position to integrate inflammatory events and reparative processes, and are important modulators of human cutaneous wound healing.^{10–12}

Chemokines in the hemostasis phase of cutaneous wound healing

Cutaneous wound healing starts with hemostasis immediately after skin injury. Cascading molecular and cellular events are involved in this phase. Besides the neurohormones released responding to the injury, blood elements such as platelets and plasma proteins infiltrate the wounds from locally damaged blood vessels, and they play a crucial role in clot formation and vasoconstriction to stop bleeding. Long before the term chemokine was introduced, PF-4 was mentioned as platelet anti-heparin factor⁵ and later named CXCL4. It has weak chemotactic potency but strongly involves angiostasis,^{13–17} hematopoiesis suppression,¹⁸ inhibition of collagenase activity,¹⁹ and accumulation of deleterious lipoproteins at sites of vascular injury.²⁰ PF-4 appears to function by neutralization of heparin-like molecules on the endothelial surface of blood vessels, thereby inhibiting local antithrombin III activity and promoting coagulation.²¹

Chemokines in the inflammation phase of cutaneous wound healing

The second phase of wound healing is an inflammatory phase that is presented as erythema, swelling, fever, and pain around wound sites, which usually lasts 2–4 days postinjury. This phase features an inflammatory reaction mediated via growth factors, cytokines, and chemokines, which induce the recruitment of different cell types to the wound sites to promote healing.²²

The recruitment of neutrophil granulocytes to sites of tissue injury is one of the earliest events during host defense. Predominantly, CXC chemokines that are stored in blood platelets and become immediately released on activation are likely to dominate neutrophil-dependent host defense at the onset of inflammation in response to acute tissue injury. They are PF-4 and beta-thromboglobulin neutrophil-activating peptide-2 (NAP-2; also named CXCL7).²³ CXCL8, also named interleukin 8 (IL-8), is the first chemokine discovered as a mediator of directional migration of leukocytes to sites of inflammation and injury, as it was previously described as neutrophil-activating factor

(NAF), monocyte-derived neutrophil chemotactic factor (MDNCF), or NAP.^{24–27}

Activation of endothelium is a critical event during the initiation of inflammatory processes and is associated with the induction of cell adhesion molecules and chemotactically active chemokines that promote leukocyte diapedesis from the circulation to sites of evolving inflammation. *In vitro*, both human endothelial cells derived from the skin (HDMECs) and human dermal microvascular endothelial cells (HMEC-1) stimulated with interleukin-1 beta (IL-1 β) and tumor necrosis factor-alpha (TNF- α) expressed high levels of IL-8, growth-regulated oncogene-alpha (GRO- α), and monocyte chemoattractant protein-1 (MCP-1; also named CCL2). C-C motif chemokine 5 (RANTES, also named CCL5) was only weakly induced; however, concomitant treatment with TNF- α and interferon-gamma (IFN- γ) led to upregulation of RANTES. IFN- γ treatment also increased the expression of IFN-inducible protein-10 (IP-10; CXCL10) in these cells. Thus, HDMECs contribute to the dermal cytokine network by selective production of CCL2, CXCL8, CXCL1, CCL5, and CXCL10, which may critically influence the site-specific recruitment of leukocyte subsets.²⁸

Using a skin repair model in adult humans, at day 1 after injury, the CXC chemokines IL-8 and GRO- α are maximally expressed in the superficial wound bed and are spatiotemporally associated with neutrophil infiltration. Macrophage infiltration reaches the highest level at day 2 and is paralleled by MCP-1 mRNA expression in both the basal layer of proliferative epidermis at wound margins and mononuclear cells in wound area. At day 4, perivascular focal lymphocyte accumulation (CD3 expressing cells) correlates with strong focal expression of CXC chemokines Mig (CXCL9) and IP-10, suggesting that a dynamic set of the chemokines contributes to the spatiotemporally different infiltration of leukocyte subsets and, thus, integrates the inflammatory and reparative processes during wound repair.²⁹ By evaluating human burn wound specimens using *in situ* hybridization, both endothelial cells and inflammatory cells contributed to this upregulated MCP-1 signal, which supports the theory that the skin itself is a component of the immune system that is capable of the initiation and maintenance of inflammation at wound sites. Failure to produce MCP-1 or other related mediators by indigenous cutaneous cells might delay the inflammatory response to injury and potentially disrupt other essential phases of wound repair.³⁰ In a human skin wound-healing model, the expression of MCP-1 is

correlated with recruitment of mast cells that are involved in normal human skin wound healing via synthesizing IL-4.³¹

Chemokines exert their biological effects of neutrophil migration via specific receptors. NAF-2 induces neutrophil chemotaxis by differential interaction with IL-8 receptors CXCR1 and CXCR2.³² In a mouse model of excisional skin, both wound-healing chemokine CX3CL1 and its receptor CX3CR1 were highly induced at wound sites. CX3CL1 colocalized with macrophages and endothelial cells, whereas CX3CR1 colocalized mainly with macrophages and fibroblasts. Loss of CX3CR1 function delayed wound closure in both CX3CR1 knockout and wild-type mice infused with anti-CX3CR1-neutralizing Ab. Conversely, the transfer of bone marrow from donor wild-type mice, but not from donor CX3CR1 knockout mice, restored wound healing to normal in CX3CR1 knockout-recipient mice. All these reveal that CX3CL1/CX3CR1 signaling mediates direct recruitment of bone marrow-derived monocytes/macrophages and is involved in wound healing.³³

Several mechanisms regulate chemokine activity in inflammation, including downregulation of chemokine expressions by endogenous IL-10 in cutaneous inflammatory response of murine wound healing,³⁴ receptor unresponsiveness, and downregulation by high concentrations of ligands.⁹ Furthermore, pro-inflammatory cytokines such as TNF- α not only induce IL-8 but also may conversely suppress CXCR2 expression on neutrophils.³⁵ Metalloproteinases downregulate inflammation in wound healing via gelatinase A cleavage of MCP-3 (also named CCL7) *in vitro*.³⁶

Chemokines in the proliferation phase of cutaneous wound healing

Proliferation phase overlaps with the end of inflammatory phase after injury. In this phase, fibroblasts proliferate in the wounds and excrete glycoprotein and collagen to form provisional ECM. Concurrently, endothelial cells migrate to wound areas and result in angiogenesis. The new blood vessels, local cells, and the provisional ECM form granulation tissue. The formation of granulation tissue into the wounds enables basal keratinocytes from the wound edges and dermal appendages to proliferate and migrate across provisional matrix. At first, contraction occurs without myofibroblast involvement; however, fibroblasts, stimulated by growth factors, differentiate into myofibroblasts, which resemble smooth muscle cells and are primarily responsible for contraction.³⁷

IFN- γ -inducible protein-9 (IP-9), also known as CXCL11, IFN-inducible T-cell alpha chemoattract-

ant (I-TAC), beta-R1, and H-174, and its receptor CXCR3 are expressed by basal keratinocytes during re-epithelialization in mice and humans. It is produced after mechanical wounding of a keratinocyte monolayer as a wound response factor. It limits epidermal growth factor-induced fibroblast motility, promotes motility in undifferentiated keratinocytes, and enhances growth factor-induced motility in undifferentiated keratinocytes. CXCL11 simultaneously promotes re-epithelialization as a mediator of epidermal-dermal communication during wound repair.³⁸ CXCL11 was found to be a key ligand in the CXCR3 signaling system for wound repair, promoting re-epithelialization and modulating the maturation of the superficial dermis.³⁹ Delayed re-epithelialization and basement membrane regeneration were also reported in mice lacking CXCR3.⁴⁰ IL-8 and GRO- α in humans and MIP-2 (CXCL2) and KC (CXCL1) in the murine system are neutrophil attractants. They also elicit responses in keratinocytes as ligands interacting with keratinocyte CXCR2 receptors, and, thus, play a role in re-epithelialization during wound repair.¹²

Chemokines are of importance in two major roles of endothelial cells during the complex events of wound healing.¹² Endothelium mediates and regulates the recruitment of leukocytes from the intraluminal compartment to tissues, by expressing CC chemokines such as MCP-1 and RANTES as well as CXC family members such as IL-8, GRO- α , IP-10, and Mig after appropriate stimulation.^{28,30,41–43} Endothelial cells form new vessels during wound repair mediated by CXC pro-angiogenic chemokines, including CXCL1-3, 5–8 and their receptors, CXCR1 and CXCR2.⁴⁴ Engelhardt *et al.*²⁹ reported that initial wound neoangiogenesis is associated with high levels of IL-8 and GRO- α expression. Since all CXC chemokines, which contain a Glu-Leu-Arg (ELR) motif, bind to the CXCR2, they appear to be responsible for mediation of the angiogenic activity.⁴⁵ Beyond direct angiogenic effects, chemokines indirectly promote the recruitment of macrophages by macrophage inflammatory protein-1 alpha (MIP-1 α , also named CCL3) and MCP-1 to wound sites, which, in turn, act as a source of angiogenic cytokines.^{46,47}

Although stromal cell-derived factor-1 (SDF-1, also named CXCL12) lacks ELR motif as other CXC subfamily members, it specifically binds to CXCR4 receptors on endothelial cells, induces endothelial cell chemotaxis, and is important for vascularization in the gastrointestinal tract.^{48–51} Recent data also support the involvement of SDF-1 in the homing of bone marrow-derived stem cells to wound sites during skeletal, myocardial, vascular,

lung, and skin wound repair as well as some fibrotic disorders via its receptor CXCR4.^{52–56}

Chemokines in the remodeling phase of cutaneous wound healing

Remodeling overlaps with the proliferation phase in which type III collagen is replaced by type I collagen.⁵⁷ Disorganized collagen fibers are rearranged, cross-linked, and aligned along tension lines.⁵⁸ Hypercellularity in remodeling wounds is reduced by apoptosis.⁵⁹

Important chemokines during the remodeling phase are CXCL11 produced by basal keratinocytes, and CXCL10 produced by neovascular endothelium, which interact with the chemokine receptor CXCR3. Stimulation of CXCR3 signaling converts fibroblasts from a migratory to a contractile state after an increase of mature dermal collagen fibers, increases keratinocyte migration by activation of m-calpain, and inhibits endothelial cell migration and proliferation.²² In an *in vivo* study, full-thickness excisional wounds in the CXCR3 (–/–) knockout mice remained hypercellular and presented immature matrix components. They also presented poor remodeling and re-organization of collagen, which resulted in a healed dermis lacking tensile strength.⁶⁰

In the phase of tissue remodeling, as wound closure becomes complete, lymphocytes constitute the most abundant leukocyte subset in human wounds. MCP-1, IP-10, Mig, and macrophage-derived chemokine (MDC, also named CCL22) are found to be spatially associated with lymphocyte accumulation.^{12,29}

The role of chemokines in abnormal scar formation

Chemokines derived from infiltrating cells in the dermis may further enhance cellular infiltrates and proinflammatory or fibrogenic cytokine release, leading to fibroblast activation. Many studies have emphasized the pathogenic role of MCP-1 (CCL2)/CCR2 axis in the induction of fibrosis, scleroderma, liver cirrhosis, atherosclerosis, pulmonary fibrosis, renal fibrosis, and colon fibrosis in human and animal models.^{61–65} Utilizing a murine dermal fibrosis model induced by a subcutaneous injection of bleomycin into the dorsal skin of MCP-1 knockout mice, diminished induction of skin fibrosis was found in mice, suggesting that MCP-1 as a key determinant in the development of skin fibrosis was induced by bleomycin *in vivo*.⁶⁶ Mechanical force significantly modulates both T-cell-dependent inflammation and scar formation in another murine HTS model, in which as Th2 che-

mokine, MCP-1 signaling was involved.⁶⁷ Focal adhesion kinase (FAK) was found activated after cutaneous injury, and mechanical loading potentiated this process. Fibroblast-specific FAK knockout mice had substantially less inflammation and fibrosis than control mice in a model of HTS formation, and FAK was found acting through extracellular-related kinase to mechanically trigger the secretion of MCP-1. Similarly, MCP-1 knockout mice formed minimal scars, indicating that inflammatory chemokine pathways are a major mechanism by which FAK mechanotransduction induces fibrosis.⁶⁸

After burn injury, paired normal and HTS tissue from patients was collected and dermal fibroblasts were isolated and cultured. The mRNA and protein levels of Toll-like receptor 4 (TLR4) and its adaptor protein myeloid differentiation factor 88 (MyD88) were significantly increased in HTS fibroblasts compared with normal fibroblasts. MCP-1 and IL-8 were also significantly increased in HTS fibroblasts compared with normal skin (NS) fibroblasts, and were upregulated by lipopolysaccharide (LPS) *in vitro*. However, when transfected with MyD88 small interfering RNA (siRNA), and then stimulated with LPS, HTS fibroblasts expressed significantly decreased mRNA and protein of these molecules compared with the cells only stimulated by LPS, demonstrating that the TLR4 pathway contributes to hypertrophic scarring through inflammatory signaling.⁶⁹

Microvessel number as well as IL-8, MCP-1, and MIP-1 α mRNA levels were significantly higher in pathological scars than those in the NS and surgical scars, and these chemokines play important roles in promoting neovascularization of pathological scars.⁷⁰ Yamamoto *et al.* showed that stem cell factor (SCF) upregulates MCP-1 expression and synthesis in mast cells, which acts on fibroblasts to enhance type I collagen mRNA expression, indicating an important interaction of fibroblasts and mast cells, via SCF and MCP-1 in the induction of fibrosis.⁷¹ In another study, blockade of mast cell activation reduces cutaneous scar formation and levels of inflammatory cytokines, including CXCL1. Compared with the control, the treatment causes a significant decrease in scar width along with accelerated collagen re-organization.⁷²

We previously reported upregulated SDF-1/CXCR4 signaling during wound healing in burn patients. In paired HTS and site-matched NS sections from recovering burn patients, SDF-1 was expressed in the epidermis, dermis, hair follicles, blood vessels, and sweat glands of NS; however, significantly upregulation occurred in HTS tissue

compared with NS from the same patients. Burn injury increased CD14⁺CXCR4⁺ cells, particularly CD14⁺hiCXCR4⁺ cells in patient peripheral blood. The SDF-1 α serum level strongly correlated with the severity of burn injury and patient age (Fig. 4). *In vitro*, dermal fibroblasts constitutively expressed SDF-1 α and deep dermal fibroblasts expressed more SDF-1 α than superficial fibroblasts. LPS increased SDF-1 α gene expression in fibroblasts. In addition, recombinant SDF-1 α and LPS-stimulated fibroblast-conditioned medium upregulated peripheral blood nuclear cell mobility.⁷³ Local dermal fibroblasts are activated by inflammatory signals after injury and produce SDF-1, which attracts CXCR4 expressing monocytes homing to the wound site from the systemic circulation. Thus, fibroblasts appear to be not only targets but also producers of chemokines.^{74,75} We also found that the increased SDF-1 expression in postburn HTS tissue was downregulated by subcutaneous IFN- α 2b, and resulted in a significant improvement in scarring in seven of nine patients.^{76–78} Burn injury increased CD14⁺CXCR4⁺ cells, particularly CD14⁺hiCXCR4⁺ cells in peripheral blood, which were downregulated by IFN- α 2b in a time-dependent manner (Fig. 5). In addition, fibrocytes were identified by dual fluorescent staining for procollagen 1 and leukocyte-specific protein-1 (LSP-1) in the samples of NS and HTS tissue from patients before and after IFN- α 2b treatment. A significant reduction in numbers of fibrocytes in the HTS tissue from patients was found after treatment with systemic IFN- α 2b (Fig. 6). These specific hematopoietic-derived circulating cells are found in burn patients and appear to be the source of fibrocytes found in HTS tissue of human and animal model, where they play an important role in scar formation.^{79–83} So far, we understand that deep burn injury activates dermal fibroblasts through TLRs by inflammatory signals derived from infectious pathogens such as LPS and damage associated molecular pathogens (DAMPs) such as denatured ECM. Activated fibroblasts, in turn, release chemokines such as SDF-1, which leads to the recruitment of blood-borne mononuclear cells into wounds, which are the progenitor cells of macrophages and fibrocytes. These cells contribute to hypertrophic scarring directly or indirectly by differentiation of fibrocytes to fibroblasts and myofibroblasts, or regulation of fibroblasts to fibrosis by many growth factors, including transforming growth factor beta (TGF- β) (Fig. 7).⁸⁴

Blocking SDF-1/CXCR4 pathway by CTCE-9908, a CXCR4 antagonist, minimized HTS formation *in vivo* in a human HTS-like nude mouse model, in which split-thickness human skin is transplanted into full-

thickness dorsal excisional wounds and it develops fibrotic scars that resemble human HTS.^{82,85} In this study, wounds and scar formation were monitored using digital photography at multi-time points after split-thickness human skin tissue were transplanted into full-thickness dorsal excisional wounds in athymic mice, which were treated by CTCE-9908 or vehicle. At 1 week after grafting, human skin grafts were flat, moist, and well vascularized and they gradually resembled the surrounding mouse skin over time. At 8 weeks, the grafts in the control mice started to contract and eventually hypertrophic, firm, contracted, and raised scar developed; whereas grafts on the CTCE-9908-injected mice remained flat with a limited contraction. CTCE-9908 significantly attenuated scar formation (Fig. 8), reduced the accumulation of macrophages and myofibroblasts, enhanced the remodeling of collagen fibers, and downregulated the gene and protein expression of fibrotic growth factors in the human skin grafts. These findings reveal a potential therapeutic value of CXCR4 antagonist in the prevention and treatment of dermal form fibrosis and possibly other fibroproliferative disorders.* The role of SDF-1/CXCR4 signaling in scar formation during an acute phase of remodeling after myocardial infarction in rats treated with the selective CXCR4 receptor antagonist AMD3100 found that AMD3100 treatment reduced infarct size, improved systolic function, and selectively inhibited the increase in scar protein synthesis.⁸⁶

In addition, as reactive targets, the proper expression and function of chemokine receptors are equally important. An *in vivo* study presented the lack of CXCR3, which resulted in hypertrophic and hypercellular scarring characterized by on-going wound regeneration, cellular proliferation, and scars.⁸⁷ Wenzel *et al.* found an enhanced expression of CCR4 on lesional CD8⁺ T lymphocytes in skin lesions from a subset of chronic discoid lupus erythematosus (CLE) patients with scarring. Significantly increased numbers of circulating CD8⁺ T cells expressing CCR4 and increased levels of thymus- and activation-regulated chemokine (TARC, also named CCL17) were detected in patients with disseminated discoid CLE. Thus, CCR4 and TARC/CCL17 play a role in the pathophysiology of CLE, particularly the cytotoxic CD8⁺ T cells expressing CCR4 appear to be involved in scarring subtypes of CLE.⁸⁸

*The Therapeutic Potential of a C-X-C Chemokine Receptor type 4 (CXCR-4) Antagonist on Hypertrophic Scarring *in vivo*. Unpublished manuscript, Wound Healing Research Group, Division of Critical Care Medicine, Department of Surgery, University of Alberta, British Canadian BioSciences Corp, Canada.

Figure 4. SDF-1/CXCR4 signal in human NS and HTS tissue. **(A)** Immunohistochemistry staining for SDF-1 in skin tissue from burn patients. Paired biopsy specimens of HTS tissue and site-matched NS were obtained from burn patients. **(B)** Flow cytometry analysis for CD14 and CXCR4 double-stained cells in the PBMCs from burn patients and controls. **(C)** Enzyme-linked immunosorbent assay for SDF-1 α level in the serum from burn patients. NS, normal skin; PBMCs, peripheral blood mononuclear cells; SDF, stromal cell-derived factor. To see this illustration in color, the reader is referred to the web version of this article at www.liebertpub.com/wound

Figure 5. IFN- α 2b treatment downregulates SDF-1/CXCR4 signal in burn patients. **(A)** Immunohistochemistry staining for SDF-1 in HTS tissue from burn patients before and after IFN- α 2b treatment. **(B)** Under a light microscope, a 500 $\times$ 1500 pixel field was defined through the whole layers of sections, and the signal was quantified by MetaMorph analysis. The data are displayed as a percentage of target staining in tissue. **(C)** Representative flow cytometry dot plots of CD14 $^{+}$ CXCR4 $^{+}$ cells in PBMCs from a burn patient before and after IFN- α 2b treatment. **(D)** Regression analysis showing the changes individually of CD14 $^{+}$ hiCXCR4 $^{+}$ cell populations of total PBMCs from burn patients treated with or without IFN- α 2b treatment. Data represent mean $\pm$ SE ($n=3$). To see this illustration in color, the reader is referred to the web version of this article at www.liebertpub.com/wound

Figure 6. Immunofluorescent detection of fibrocytes in HTS tissue from burn patients treated with IFN- α 2b. **(A)** Immunofluorescent staining and confocal microscopy analysis of fibrocytes in HTS biopsy. NS and HTS samples from patients before and after IFN- α 2b treatment were stained for LSP-1 and for procollagen-1 and examined by confocal microscopy. **(B)** Quantification of fibrocytes in HTS sections before and after IFN- α 2b treatment using MetaMorph Image Software as described in the “Materials and Methods” section. Data represent mean $\pm$ SD ($n=9$). $^{**}p<0.01$. LSP, leukocyte-specific protein. To see this illustration in color, the reader is referred to the web version of this article at www.liebertpub.com/wound

Figure 7. Mechanism of dermal fibrosis. Deep burn injury activates dermal fibroblasts through TLRs by inflammatory signals derived from infectious pathogens such as LPS and DAMPs, including endogenous molecules such as denatured ECM. Activated fibroblasts, in turn, release chemokines such as SDF-1, which leads to the recruitment of blood-borne cells into wounds. These cells may be the progenitor cells of macrophages and fibrocytes, and they contribute to hypertrophic scarring by directly differentiating to fibroblasts and myofibroblasts, or indirectly regulating fibroblasts to fibrosis by many growth factors, including TGF- β . DAMPs, damage-associated molecule pathogens; LPS, lipopolysaccharide; TGF, transforming growth factor; TLR, toll-like receptor. To see this illustration in color, the reader is referred to the web version of this article at www.liebertpub.com/wound

Figure 8. Morphology of wounds and scars from human HTS-like nude mouse model. Wounds and scar formation were monitored using digital photography at multi-time points after split-thickness human skin grafts were transplanted into full-thickness dorsal excisional wounds in athymic mice, which were treated by CXCR4 antagonist (CTCE-9908) or vehicle control. Throughout the experimental period, human skin grafts remained viable. At 1 week after grafting, the dressings were removed. The grafts were flat, moist, and well vascularized from 2 weeks. Over time, the graft color gradually resembled the surrounding mouse skin. At 8 weeks, the grafts in the control mice started to contract and eventually hypertrophic, firm, contracted, and raised scars developed. Grafts on the CTCE-9908-injected mice remained flat with a limited contraction. To see this illustration in color, the reader is referred to the web version of this article at www.liebertpub.com/wound

Figure 9. The roles of chemokines in wound healing and scar formation. To see this illustration in color, the reader is referred to the web version of this article at www.liebertpub.com/wound

Table 1. Chemokine expression by cells during cutaneous wound healing

Cell Sources	Chemokines
Activated platelets	PF-4/CXCL4, NAP-2/CXCL7
Macrophages	CX3CL1
Th2 cells	MCP-1/CCL2
Mast cells	MCP-1/CCL2
Neutrophils	IL-8/CXCL8
Endothelial cells	IL-8/CXCL8, GRO- α /CXCL1, MCP-1/CCL2, RANTES/CCL5, IP-10/CXCL10, Mig/CXCL9, CX3CL1
Keratinocytes	IP-9/CXCL11, IP-10/CXCL10,
Fibroblasts	SDF-1/CXCL12,
Keloid fibroblasts	MCP-1/CCL2

In keloids, IL-1 β stimulated a statistically significant increase in MCP-1 production by both normal and keloid-derived fibroblasts, which is suggested to lead to keloid formation.⁸⁹ An increase in MCP-1 and CCR2 signal was found in the keloid tissues. Coculture of keloid CD14⁺ cells and normal fibroblasts enhanced fibroblast proliferation and a parallel increase in extracellular MCP-1. Further, MCP-1 enhanced fibroblast proliferation via Akt (protein kinase B) activation. Blockade of either MCP-1 or Akt signaling suppressed fibroblast proliferation induced by CD14⁺ cells from patients, indicating that enhanced MCP-1 release by keloid CD14⁺ cells augments fibroblast proliferation, which might initiate keloid development.⁹⁰ MGSA/GRO- α (CXCL1) was present in myofibroblasts and lymphocytes in keloid tissues, which positively correlated with the degree of inflammatory infiltrate in the lesions. Keloids also exhibited intensive immunoreactivity for the CXCR2 receptor in endothelial cells and inflammatory infiltrates with occasional staining of myofibroblasts. Nirodi *et al.* postulate that the inflammatory component is important in the development of keloid lesions and chemotactic cytokines participate in this process.⁹¹

SUMMARY

Collectively, chemokines are necessary for wound healing contributing to coagulation, leuko-

cyte infiltration, re-epithelialization, neoangiogenesis, stem cell recruitment, collagen re-organization, and lymphocyte-mediated immunoresponse. However, hyperactivity of chemokines may lead to abnormal wound healing such as fibrotic scars with the appearance of prolonged and hyperactively inflammatory response, hypervascularity, hypercellularity, collagen accumulation and disorganization, fibrocyte recruitment, and CD8 T-cell-mediated immunoresponse (Fig. 9). Chemokines appear to be released not only by recruited blood-borne cells such as platelets, macrophages, lymphocytes, and neutrophils into the wounds but also by resident wound cells such as fibroblasts, keratinocytes, and endothelial cells (Table 1).

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Abbreviations and Acronyms

Akt = protein kinase B
CLE = chronic discoid lupus erythematosus
DAMPs = damage-associated molecular
pathogens
ECM = extracellular matrix
FAK = focal adhesion kinase
GRO = growth-regulated oncogene
HDMECs = human endothelial cells derived
from the skin
HMECs = human dermal microvascular
endothelial cells
HTS = hypertrophic scars
IFN = interferon
IL = interleukin

IP = IFN-inducible protein
I-TAC = interferon-inducible T-cell
alpha chemoattractant
LPS = lipopolysaccharide
LSP = leukocyte-specific protein
MCP = monocyte chemoattractant protein
MDC = macrophage-derived chemokine
MDNCF = monocyte-derived neutrophil
chemotactic factor
MIP-1 α = macrophage inflammatory protein-1
alpha
MyD88 = myeloid differentiation factor 88
NAF = neutrophil-activating factor
NAP = neutrophil-activating peptide
NS = normal skin

PBMCs = peripheral blood mononuclear cells
PF = platelet factor
RANTES = regulated on activation normal T cell
expressed and secreted
SCF = stem cell factor
SCY = small cytokine
SDF = stromal cell-derived factor
SIG = small inducible gene
siRNA = small interfering RNA
SIS = small inducible secreted
TARC = thymus- and activation-regulated
chemokine
TGF = transforming growth factor
TLR = toll-like receptor
TNF = tumor necrosis factor