# Chemokines in Wound Healing and as Potential Therapeutic Targets for Reducing Cutaneous Scarring

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Submitted for publication November 8, 2014. Accepted in revised form January 26, 2015.

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**Significance:** Cutaneous scarring is an almost inevitable end point of adult human wound healing. It is associated with significant morbidity, both physical and psychological. Pathological scarring, including hypertrophic and keloid scars, can be particularly debilitating. Manipulation of the chemokine system may lead to effective therapies for problematic lesions.

**Recent Advances:** Rapid advancement in the understanding of chemokines and their receptors has led to exciting developments in the world of therapeutics. Modulation of their function has led to clinically effective treatments for conditions as diverse as human immunodeficiency virus and inflammatory bowel disease. Potential methods of targeting chemokines include monoclonal antibodies, small-molecule antagonists, interference with glycosaminoglycan binding and the use of synthetic truncated chemokines. Early work has shown promising results on scar development and appearance when the chemokine system is manipulated.

**Critical Issues:** Chemokines are implicated in all stages of wound healing leading to the development of a cutaneous scar. An understanding of entirely regenerative wound healing in the developing fetus and how the expression of chemokines and their receptors change during the transition to the adult phenotype is central to addressing pathological scarring in adults.

**Future Directions:** As our understanding of chemokine/receptor interactions and scar formation evolves it has become apparent that effective therapies will need to mirror the complexities in these diverse biological processes. It is likely that sophisticated treatments that sequentially influence multiple ligand/receptor interactions throughout all stages of wound healing will be required to deliver viable treatment options.

## SCOPE AND SIGNIFICANCE

Progressive fibrosis resulting in scarring represents the end point of normal mammalian tissue repair after dermal injury. Although effective in restoring cutaneous barrier function, scar tissue is inferior to healthy skin.[1] Fetal wound healing is entirely regenerative before 24 weeks gestation, without scar tissue formation.[2,3] Behavioral discrepancies have been attributed to differing inflammatory responses and cytokine profiles of fetal and adult wounds. These are controlled by a range of bioactive molecules, including chemokines. This review summarizes current knowledge of chemokine behavior in acute and pathological cutaneous wounds before discussing their application as novel antifibrotic therapeutic agents.



DOI: 10.1089/wound.2014.0568

## TRANSLATIONAL RELEVANCE

Chemokines are bioactive molecules that play key roles throughout wound healing, but particularly within the inflammatory and proliferative phases. First identified by their ability to induce leukocyte migration they have now been shown to have vital roles in leukocyte recruitment, activation and effector function, as well as regulation of angiogenesis and myofibroblast localization.[4–8] Chemokine behavior as agonists or antagonists is variable and dependent on the receptor they bind to.[9] The formation of receptor/ligand dimers and oligomers also influences function.

## CLINICAL RELEVANCE

Chemokines are a large and potentially powerful family of targets for scar reducing therapeutics. Chemokines play a prominent role in normal wound healing, but altered expression is observed in keloid and hypertrophic scars as well as chronic wounds.[10–12] Consequently iatrogenic manipulation of specific chemokine signaling pathways could offer an alternative means to reduce wound fibrosis, chronic wound development, and the incidence of pathological scarring.[13] Complexities of chemokine physiology have delayed development of effective scar-reducing agents.

## DISCUSSION OF FINDINGS AND RELEVANT LITERATURE

### Overview of chemokines

Chemokines are a large family of heparin-binding cytokines known for their small size (8–10 kDa) and four highly conserved cysteine residues.[14] Since interleukin (IL)-8 was first described by Baggiolini[15] knowledge of these complex interacting proteins has increased exponentially.

In 2000 a system of nomenclature was introduced in which each ligand and receptor is identified by its subfamily and an identifying number.[16,17] Recent discoveries and advances, particularly in the area of atypical receptors, has necessitated an update.[18] This method of nomenclature will be utilized throughout this review.

Over 50 chemokines have been identified to date, divided into 4 subgroups based on the arrangement of the first 2 of the 4 cysteine amino acids - CC, CXC, $CX_3C$, and C[19] (Fig. 1).[20] The large CC chemokine family consists of chemokines with the first two cysteine residues adjacent to each other, in comparison to the CXC group, which has a single (variable) amino acid dividing them.[21] The lone member of the $CX_3C$ group ($CX_3CL1$) has three amino acids dividing the first two cysteines. The last group, C, is notable for its members, XCL1 and XCL2, possessing only two of the usual four cysteine residues.[22,23] A detailed discussion of the structure of chemokines is beyond the scope of this review, but is covered comprehensively by Allen *et al.*[24]

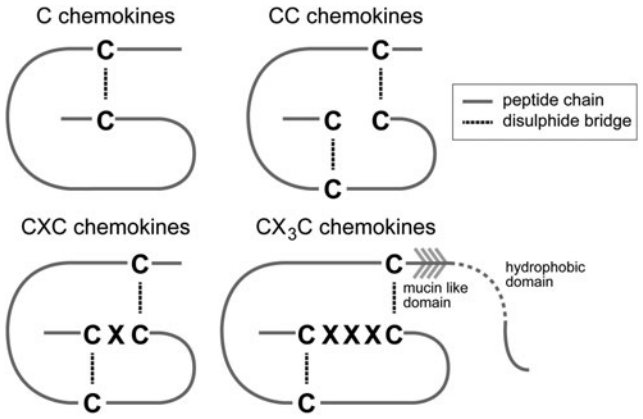


**Figure 1.** The four chemokine subfamilies, including basic structure (Adapted from Martins-Green et al. 2013).[20]

Chemokines may be further classified as either proinflammatory, homeostatic, or a mixture of both. Inflammatory chemokines are produced by activated cells during pathological conditions to recruit leukocytes to inflamed tissues. Homeostatic chemokines are constitutively produced to maintain homeostatic leukocyte trafficking and the architecture of secondary lymphoid organs.[25]

Chemokines exert their chemotactic affects by binding to chemokine receptors, of which over 20 have been discovered to date (Table 1).[18] The classic chemokine receptors are seven transmembrane-spanning proteins coupled to heterotrimeric G proteins that is, G-protein coupled receptors (GPCRs).[25] They are present in the lipid bilayer of target cells with both an extracellular and intracellular component. The extracellular domain consists of the N-terminus and three extracellular loops, while the intracellular region is composed of three loops and the C-terminus.[26] These domains play a role in ligand binding and signal transduction, respectively. Atypical chemokine receptors have the ability to bind chemokines, but do not signal through G proteins.[18] An example is atypical chemokine receptor 1 (ACKR1), previously duffy antigen receptor for chemokines (DARC). These atypical receptors fulfill a number of functions, including scavenging free chemokines in the blood stream.[27]

A two-step model of receptor activation has been proposed. Initially, the chemokine ligand specifically recognizes and binds to the receptor. Next a conformational change in the chemokine allows the

**Table 1.** *Chemokine receptors and their associated ligands*

| *Chemokine receptor* | *Associated ligands* |
|---|---|
| CXC subfamily | |
| CXCR1 | CXCL5, CXCL6, CXCL8 |
| CXCR2 | CXCL1, CXCL2, CXCL3, CXCL5, CXCL6, CXCL7, CXCL8 |
| CXCR3 | CXCL4, CXCL4L1, CXCL9, CXCL10, CXCL11 |
| CXCR4 | CXCL12 |
| CXCR5 | CXCL13 |
| CXCR6 | CXCL16 |
| Unknown receptor | CXCL14, CXCL17 |
| CC subfamily | |
| CCR1 | CCL3, CCL4, CCL5, CCL7, CCL8 CCL13, CCL14, CCL15, CCL16, CCL23 |
| CCR2 | CCL2, CCL5, CCL7, CCL8, CCL13, CCL16 |
| CCR3 | CCL4, CCL5, CCL7, CCL11, CCL13, CCL15, CCL24, CCL26, CCL28 |
| CCR4 | CCL17, CCL22 |
| CCR5 | CCL3, CCL4, CCL5, CCL7, CCL14, CCL16 |
| CCR6 | CCL20 |
| CCR7 | CCL19, CCL21 |
| CCR8 | CCL1, CCL18 |
| CCR9 | CCL25 |
| CCR10 | CCL27, CCL28 |
| C subfamily | |
| XCR1 | XCL1, XCL2 |
| $CX_3C$ subfamily | |
| $CX_3CR1$ | $CX_3CL1$ |

N terminus to make the necessary interactions with the receptor resulting in activation.[28] Activation of the receptor causes an exchange of bound guanosine diphosphate (GDP) for guanosine-5′-triphosphate (GTP) in the α-subunit of the G proteins. This results in dissociation of the G proteins and activation of several effector molecules downstream, which stimulates a cascade of signaling events within the cytoplasm of the cell (Fig. 2).[26] What follows is a diverse range of physiological processes, including leukocyte migration and trafficking, leukocyte degranulation, cell differentiation, angiogenesis, and myofibroblast recruitment.[29–31]

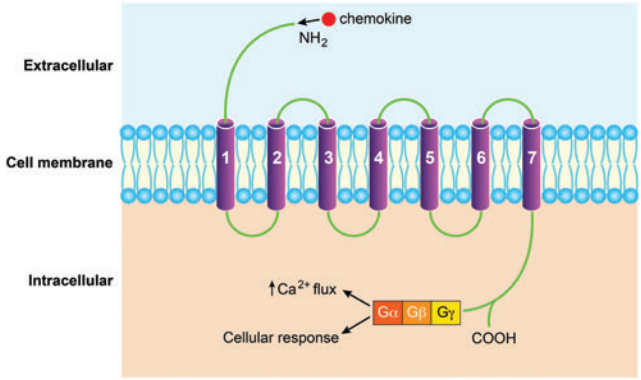


**Figure 2.** Typical chemokine receptor activation. To see this illustration in color, the reader is referred to the web version of this article at www.liebertpub.com/wound

## Chemokines in acute wound healing

Cutaneous wound healing is a dynamic interactive process involving soluble mediators, infiltrating leukocytes, extracellular matrix (ECM), and resident cells—keratinocytes, fibroblasts, endothelial cells, and nerve cells.[32] The traditional model of wound healing involves four overlapping phases, namely hemostasis, inflammation, cellular proliferation, and remodeling.[33] This complex biological process, where multiple parallel and interrelated pathways are activated and synchronized to induce wound repair, requires strict control.[34,35] Chemokines and their effects on leukocyte subsets and resident cells are an integral part of this system.

### *Inflammatory phase*

During the inflammatory phase leukocytes migrate to the area of tissue insult in a characteristic temporal pattern.[32] The predominant cell type transitions from neutrophils to macrophages and finally to lymphocytes.[36,37] Directional cues for leukocyte migration are provided by chemokines, whereas immune cell responses to particular chemokines are determined by their complement of chemokine receptors.[38] In murine models, knockouts of chemokines and their receptors, including CXCR2 and CXCR3, result in delayed or incomplete wound healing. In the case of CXCR4 and its ligand CXCL12, knockout results in perinatal death indicating concurrent roles in development.[38–41]

Leukocyte extravasation from the blood into the tissues is a regulated multistep process involving a series of coordinated interactions between leukocytes and endothelial cells under the guidance of chemokines through soluble gradients and/or immobilized molecules on endothelial luminal surfaces.[42,43]

Neutrophil and monocyte recruitment into inflamed tissues is mainly directed by CXC and CC chemokine subfamilies, respectively.[38] CXC family members, particularly CXCL1, five and eight are released after wounding and are further induced in response to hypoxia, proinflammatory cytokines (IL-1 and tumor necrosis factor-alpha [TNF-α]) and lipopolysaccharides.[44] These neutrophil-attractant chemokines are spatially and temporally expressed, suggesting a sophisticated multistep event in neutrophil recruitment. Proinflammatory cytokines such as TNF-α, suppress CXCR2 expression on neutrophils, whereas CXCL8 simultaneously stimulates the expression of CXCR1 on the cell membrane.[14] This leads to a second chemotactic response enabling neutrophils to migrate to the superficial wound bed. Furthermore, CXCL8–CXCR1interactions induce

granule exocytosis and the respiratory burst, thereby facilitating neutrophil immunological function.[38,45]

Production of monocyte chemoattractant proteins is provoked by similar stimuli to CXC chemokines and involves the secretion of CCL2, CCL7, CCL8, and CCL13.[38] CCL2 is predominant and knockout studies in mice have demonstrated that its absence results in deficient monocyte recruitment.[41] Mouse models have also demonstrated that variable $CX_3CR1$ and CCR2 chemokine receptor expression confers functional heterogeneity upon monocytes.[46] Monocytes with high $CX_3CR1$ expression combined with low levels of CCR1 and CCR2 have been termed resident monocytes, which preferentially locate to normal skin and act as forerunners to resident tissue macrophages. When this pattern of receptor expression is reversed, monocytes migrate to inflamed tissue and trigger an immune response.[47]

Macrophages are derived from monocyte differentiation and characterized by reduced CCR2 and increased CCR1 and CCR5 expression in humans.[48,49] This change is likely to reflect stepwise migration into cutaneous tissues with CCL2–CCR2 interaction responsible for the initial recruitment and CCR1/CCR5 involvement to guide accurate tissue localization. Murine skin wound healing models have demonstrated that CCL2, CCL3, and CCL5 also play critical roles in macrophage recruitment.[50–54] Macrophages are essential for normal wound repair providing a source of cytokines, growth factors, and chemokines.[44,50,55] Once recruited, the wound microenvironment strongly influences phenotypic polarization of macrophages enabling functional heterogeneity through differential chemokine, cytokine, and receptor repertoires (Fig. 3).[56] Macrophages of the M1 phenotype (classically activated macrophages) demonstrate powerful microbicidal characteristics, amplify delayed-type hypersensitivity reactions, and promote strong inflammatory responses through TNF-$\alpha$, IL-1B, and IL-6 secretion. M1 polarization is accompanied by production of interferon (IFN)-$\gamma$ responsive chemokines and members of the CC subfamily, including CX3CL1, CXCL9-11, CXCL16, and CCL5, which facilitate type I immune responses.[47,57] In contrast, M2 macrophages (alternatively activated) promote tissue repair, resolution of inflammation, and immunoregulation through high endocytic clearance capacities and reduced proinflammatory cytokine secretion.[47,57] Three M2 subtypes exist (M2a, M2b, and M2c) characterized by different chemokine profiles. M2a and M2b are predominantly associated with immunomodulation and promotion of type II immune responses through CCL17, CCL18, CCL22, CCL24, and CCL1 (specific to M2b) expression. M2c is more important in tissue remodeling involving CXCL13, CCL16, and CCL18.[47] Consequently, manipulation of chemokine signaling to reduce inflammation and promote M2 macrophage polarization is an attractive therapeutic option to reduce fibrosis and scarring (Fig. 3).

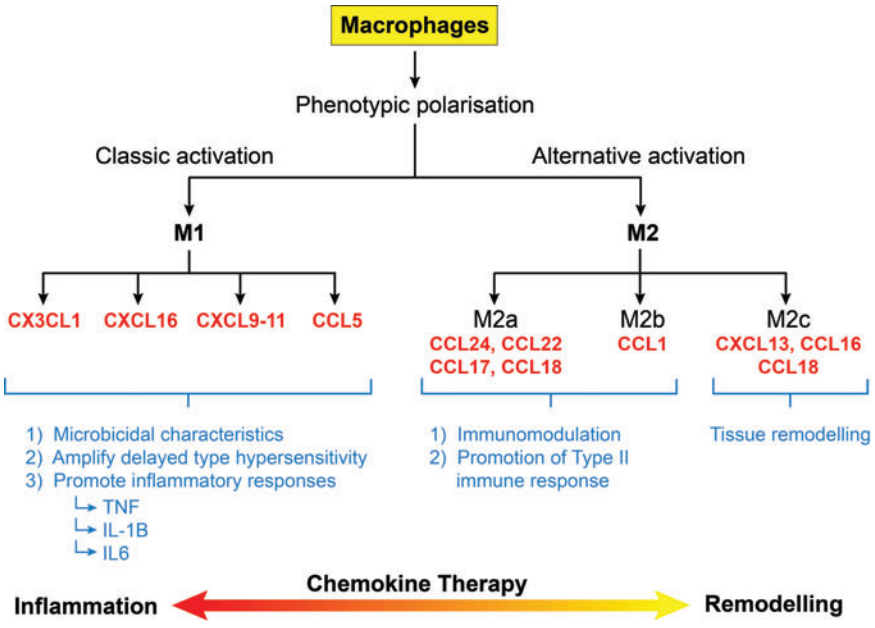


**Figure 3.** Phenotypic polarization of wound macrophages. Manipulation of chemokine signaling to promote M2 macrophage differentiation may be a potential target for reducing fibrosis and scarring. To see this illustration in color, the reader is referred to the web version of this article at www.liebertpub.com/wound

*Proliferative phase*

Cellular proliferation represents the third phase of the classic wound healing model.[58,59] Key physiological processes occurring predominantly during this phase include fibroplasia, angiogenesis, and granulation tissue formation. In the later stages, fibroblasts from wound edges or bone marrow (BM) assume the myofibroblast phenotype, which have contractile properties and contribute to collagen remodeling.[35,60]

Fibroplasia. Fibroplasia is the process resulting in the deposition of ECM proteins, including collagens, proteoglycans, and fibronectin by fibroblasts.[61] It is dependent upon successful angiogenesis, upon which chemokines display key regulatory roles.

Knowledge of chemokine action upon, and secretion by, fibroblasts is poorly studied, but it is apparent that their expression is increased after injury contributing to conditioning of the cellular and cytokine environment within the healing tissue.[62,63] Furthermore, resident fibroblasts are important in initiating inflammation secondary to chemokine-related recruitment of leukocytes through the secretion of CXCL8, CXCL12, CCL2, and CCL11.[63,64] The extent of the inflammatory response is crucial in determining the outcome of fibroplasia, since fibrogenesis is influenced by cytokines, growth factors, and cellular mediators derived from the inflammatory phase. Prolonged ECM deposition results in fibrosis whereas insufficient production leaves wounds at the risk of dehiscence.[65]

Stoeckle and Barker demonstrated in a chorioamniotic membrane model that chicken chemotactic and angiogenic factor (cCAF) is highly expressed by fibroblasts in healing tissue and stimulated granulation tissue formation.[66] It was subsequently found that exogenous cCAF administration stimulated fibroblast differentiation into myofibroblasts and accelerated wound closure *in vivo*.[62] Given cCAF's structural similarity to CXCL8 a similar mechanism may exist in humans. Similarly, CXCR3 receptors expressed by fibroblasts and its ligands CXCL10 and CXCL11, may play a crucial role in wound healing. *In vitro*, CXCL10 has been observed to inhibit epidermal growth factor-related fibroblast motility secondary to the inhibition of calpain proteinases in fibroblasts, thus preventing fibroblast detachment.[67] Furthermore, in mouse models CXCL10 administration limited fibrotic severity in bleomycin-induced pulmonary fibrosis whereas knockout of CXCR3 resulted in impaired wound healing with poor organization of the ECM, reduced collagen content, and altered biomechanical properties.[68]

Chemokines are also important in the control of absolute fibroblast number and consequently the extent of fibroplasia. As stated, CXCL8 may reduce the number of fibroblasts by stimulating the differentiation into myofibroblasts.[62] In contrast, CCL21, a ligand for CCR7, is a potent stimulus for human fibrocyte chemotaxis *in vitro* and promotes migration to sites of injury *in vivo* when injected into cutaneous mouse wounds.[69] Fibrocytes are precursors to fibroblasts and to a lesser extent myofibroblasts.[33] Consequently, influx of these cells to the wound environment may increase the population of resident fibroblasts providing a larger pool of ECM secreting cells.

Angiogenesis. Reconstruction of wound microvasculature is vital during the proliferative phase of healing to restore nutrient supply to regenerating tissue, promote fibroplasias, and prevent sustained tissue hypoxia.[33] The process by which this occurs is angiogenesis. Regulation depends on a dual, yet opposing balance of angiogenic and angiostatic factors that promote or inhibit neovascularization, respectively.[70] During wound repair this balance is shifted in favor of proangiogenic factors.[71] As wound healing reaches its conclusion there is a marked decline in angiogenesis. This suggests that during wound repair, angiogenesis is tightly controlled and temporally related to the imbalance of expression of angiogenic and angiostatic factors.[70] A comprehensive review of molecular and cellular mechanisms driving angiogenesis has recently been published.[33] It is well established that chemokines, particularly of the CXC subfamily, have a major role in the regulation of angiogenesis. The $NH_2$ terminus of multiple CXC chemokines contains three amino acid residues, Glu-Leu-Arg, immediately before the first cysteine amino acid residue, termed the ELR motif.[72] The CXC chemokines possessing the ELR motif (ELR+) promote angiogenesis, whereas those without the ELR motif (ELR-) inhibit it (Table 2).[72,73] It has been demonstrated that CXCR2 is the primary functional chemokine receptor in mediating

**Table 2.** *ELR+ and ELR chemokines*

| *ELR+ chemokines* | *ELR− chemokines* |
|---|---|
| CXCL1 | CXCL4 |
| CXCL2 | CXCL4L1 |
| CXCL3 | CXCL9 |
| CXCL5 | CXCL10 |
| CXCL6 | CXCL11 |
| CXCL7 | CXCL14 |
| CXCL8 | |

endothelial cell chemotaxis.[74–76] When full-thickness excisional wounds were made on CXCR2$^{-/-}$ mice significant delays in wound healing resulted, including decreased neovascularization.[39] The ELR chemokines exert their angiostatic effects by interaction with CXCR3.[73] This receptor was originally identified on murine endothelial cells[77] and exists in alternative splice forms, CXCR3A, CXCR3B, and CXCR3-alt.[73] It is CXCR3B which mediates the angiostatic activity of most ELR chemokines on human microvascular endothelial cells.[78] Another angiostatic mediator related to the CXC chemokines is ACKR1. This promiscuous receptor found on red blood cells sequesters the ELR+ CXC chemokines and indirectly inhibits angiogenesis.[79,80]

CC chemokines also have a role in angiogenesis. CCL2, acting on CCR2 present on endothelial cells, mediates neovascularization by effecting membrane type-1 matrix metalloproteinase (MT1-MMP).[81] This chemokine also induces expression of vascular endothelial growth factor (VEGF)-A and the transcription factor, monocyte chemotactic protein 1 (MCP-1)-induced protein *in vivo*.[82,83] Ishida *et al.*[84] have demonstrated a significant role for the CCL5/CCR5 interaction during angiogenesis in wound healing. Endothelial progenitor cells (EPCs) have been presumed to be involved in a number of conditions requiring neovascularization, including wound healing, although to date, this has not been validated.[85,86] EPCs are produced in the BM and home to specific injured tissue. *In-vitro,* CCL5 induces migration of EPCs by acting on CCR5 on their cell surface and CCR5-deficient mice exhibit impaired neovascularization during excisional wound healing.[84] CCL11 and CCL16 also have a direct positive effect on angiogenesis.[87]

### *Remodeling phase*

Remodeling begins 2–3 weeks after injury and can last for a year or more. Processes activated after injury slow down and stop as endothelial cells, macrophages, and myofibroblasts undergo apoptosis or exit the wound leaving a relatively acellular collagen-rich mass. This reduction in cellularity is influenced by interactions between CXC chemokines, particularly CXCL10 and CXCL11 with CXCR3, expressed by maturing endothelium and keratinocytes, respectively.[40,88–91] These ligand–receptor interactions reduce fibroblast and endothelial motility while increasing keratinocyte migration.[67,92,93] It is proposed that this signaling axis allows wounds to transition through remodeling resulting in the characteristic appearance of scar tissue with densely packed collagen bundles and reduced elastin.[94,95] Wound contraction secondary to myofibroblast action begins in the proliferative phase, but continues during remodeling.

Re-epithelialization. Re-epithelialization involves keratinocyte migration and proliferation, followed by differentiation to regenerate the epidermis during wound closure. Migration begins within 3–6 h after injury and proceeds throughout wound healing until completed.[96] It is well established that chemokines are crucial to the process of re-epithelialization. In an *in vitro* skin model CXCL1 and CXCL8 induced keratinocyte migration by their interactions with CXCR2.[97] Several other ligand–receptor combinations appear to positively influence keratinocyte migration and proliferation *in vitro*.[98,99] *In vivo* studies in CXCL11$^{-/-}$ and CXCR3$^{-/-}$ mice demonstrated a significant delay in re-epithelialization and deficient dermal maturation in excisional wounds.[40,100]

Chemokine receptors CCR1, CCR10, CXCR1, CXCR2, and CXCR3 are expressed on the surface of mouse keratinocytes and since the same keratinocytes are able to secrete their corresponding monospecific ligands a model of autocrine regulation of re-epithelialization has been postulated.[101] Chemokines may also play a role during non-hematopoietic cell recruitment in wound healing. Bone-marrow derived stem cells (BMDSCs) possess the ability to self-renew and differentiate into multiple cell lines. They reside in adult BM and cutaneous injury leads to increased engraftment of these BMDSCs as epidermal cells.[102] CCL27, and its interaction with CCR10, is the major regulator involved in this migration of keratinocyte precursor cells from the BM to the skin in excisional mice wounds.[103] These BM cells are able to transdifferentiate into keratinocytes at the wound site and intradermal injection of CCL27 accelerated wound healing.[103]

### *$CX_3CL1/CX_3CR1$*

$CX_3CL1$ and its receptor $CX_3CR1$, are the sole members of the $CX_3C$ group. It is discussed separately as it influences numerous stages of wound healing. $CX_3CL1$ is expressed in a soluble chemokine domain form and as a membrane-bound form on the surface of inflamed endothelial cells, epithelial cells, macrophages, and vascular smooth muscle cells.[104] Interaction of the membrane-bound ligand and its receptor mediates cell to cell adhesion of leukocytes.[22,105,106] $CX_3CL1$ is expressed in numerous organs, including the skin whereas the receptor is found on a variety of cells, including monocytes/macrophages.[104] During the inflammatory stage it directly mediates macrophage accumulation and during the proliferative

stage promotes granulation tissue formation, collagen deposition, myofibroblast accumulation, and angiogenesis.[104] Finally, inoculation of wild-type mice with neutralizing anti-$CX_3CR1$ antibodies had dramatic, detrimental, and overarching effects on wound healing.[104]

### Contrasts with fetal wound healing

During fetal wound healing there are several key differences compared with the adult model. These are summarized in Table 3. Due to the underlying differences in cellular chemoattraction and inflammation between adult and fetal wound healing it is highly likely that chemokines are also differentially expressed. When human fetal and adult fibroblasts were cultured *in vitro* it was demonstrated that decreased levels of CXCL8 were expressed by fetal fibroblasts.[107] This would certainly correlate with other literature suggesting a reduced role of inflammation and neutrophils during fetal wound healing and the absence of scar formation. It is clear that many growth factors and cytokines appear to vary in their expression in fetal and adult wounds. While each may be important, their overall significance may be obscured by the complexity of the cytokine milieu, including other unknown or unexamined factors acting in fetal and adult wound healing.[108] It is highly likely that further, as yet undiscovered, chemokines play an important role in the scarless healing of fetal wound healing.

### Chemokines in chronic wounds

A chronic wound is defined as a break in skin continuity of greater than 42 days duration or of frequent recurrence.[109,110] These debilitating wounds have failed to progress through the normal stages of healing and, therefore, enter a state of pathological inflammation.[111] This leads to delayed or incomplete healing with poor anatomical and functional outcomes as well as detrimental effects on patient quality of life.[112,113] Multiple etiologies underlie chronic wound development, but over 90% are secondary to diabetes, venous insufficiency or pressure.[114] Increasingly prevalent risk factors, including obesity and diabetes, in combination with an aging population, suggests chronic wounds will impose a progressively larger economic burden upon healthcare providers in the future.[114]

The normal function of the inflammatory phase is to prepare the wound bed for healing by removing debris, necrotic tissue, and bacterial contaminates, as well as recruiting fibroblasts.[111] It is an essential process, but must be tightly controlled. Neutrophils play a vital role and during normal wound healing their population declines following successful transition into the proliferation stage. In chronic wounds, activated neutrophils persist throughout the healing process,[115] leading to an excess of activated neutrophils, which cause an accumulation of MMPs.[116] This, combined with downregulation of tissue inhibitor of metalloproteinase (TIMP) expression, creates an environment with a relative excess of MMP activity leading to the degradation of crucial wound components and further neutrophil recruitment generating a positive feedback loop and chronic inflammation.[111,117]

The use of MMP-deficient animals has demonstrated that MMPs can affect cytokines and chemokines as well as ECM proteins, establishing another method by which inflammatory processes can be influenced.[118] More specifically MMPs have been shown to inactivate specific chemokines, generate antagonistic derivatives, and produce truncated chemokine variations that are more potent.[118] Examples of chemokine inactivation by MMPs include CXCL12, which has been shown to be inactivated by MMP-1–3, MMP-9, MMP-13, and MMP-14.[119] MMP-9 also inactivates CXCL chemokines, including CXCL1 and CXCL4.[120] Finally,

**Table 3.** *Comparison of adult and fetal wound healing*

| | *Adult wound healing* | | *Fetal wound healing* | |
|---|---|---|---|---|
| Collagen content | Type I + + + | Type III + | Type I + | Type III + + + |
| Hyaluronic acid | + | | + + + | |
| Extracellular matrix modulators | MMP + | TIMP + + + | MMP + + + | TIMP + |
| Adhesion proteins | ↓ upregulation | | ↑ upregulation | |
| Platelets | ↑ PDGF, TGF-$\beta$1 and TGF-$\beta$2 | | ↓ degranulation and aggregation | |
| Inflammatory cells | + + + | | + | |
| Interleukins | ↑ proinflammatory cytokines | | ↑ anti-inflammatory cytokines | |
| Transforming Growth Factor-$\beta$ | TGF-$\beta$1 & $\beta$2 + + + | | TGF-$\beta$1 & $\beta$2 + TGF-$\beta$3 + + + | |
| Gene expression | ↑ genes involved in cell growth & proliferation | | ↑ ↑ ↑ genes involved in cell growth & proliferation | |
| Progenitor cells | + | | + + + | |
| CXCL8 | + + + | | + | |

MMP, matrix metalloproteinases; TIMP, tissue inhibitor of metalloproteinase; PDGF, platelet-derived growth factor; TGF, transforming group factor; ↑, increase; ↓, decrease.

CXCL9 and CXCL10 are degraded by MMP-8 and MMP-9,[121] which in the case of CXCL9, decreases chemotactic ability. A number of inactivated chemokines act as functional antagonists through binding to their original receptors, thereby blocking noncleaved chemokines or affecting chemotactic gradients. For example, MMP-2 has been shown to process CCL7 into an antagonistic form.[122] Finally, MMPs have been shown to increase the biological activity of certain chemokines. MMP-9 has been demonstrated to influence CXCL8, resulting in significantly increased chemotactic activity.[120] A truncated CXCL8 variant with increased activity has also been generated by MMP-8, MMP-13, and MMP-14.[123,124] In summary, the variable actions of MMPs on chemokines affects the progression of inflammatory responses and influences the type of cells, which are recruited and activated.[118]

Much of our understanding of acute wound healing comes from *in vivo* animal models. Chronic wounds on the other hand are challenging to simulate as they do not occur naturally in the animal world.[117] Despite this, a number of models have been developed although their findings are sometimes contradictory and cannot be directly translated to humans.

A murine model involving animals deficient in TNFSF14 (LIGHT) has been developed.[125] LIGHT mediates VEGF, a growth factor that induces macrophage apoptosis *in vitro*.[126] Since macrophage apoptosis is important in the resolution of inflammation during wound healing, the authors propose that LIGHT-deficient wounds mimic chronic healing/nonhealing wounds in humans.[125] This work also implicates chemokines in chronic wound pathogenesis. Excess proinflammatory chemokine production (CXCL8, CCL2, and CXCL10) persisted from the early stages following injury, resulting in excess leukocyte infiltrates. Excessive neutrophil infiltration is associated with unregulated MMP production and high levels of reactive oxygen species leading to increased inflammation,[127,128] whereas an increased duration of macrophage presence may lead to further protease-mediated damage of the healing tissue.[129] Crucially, premature and excessive CXCL10, resulted in early chemoattraction of T-lymphocytes,[125] which are usually associated with the remodeling phase of wound healing.[13] This suggests a disorganized healing process. In contrast Pradhan *et al.*, demonstrated significantly increased baseline expression of CXCL8 and its receptors CXCR1 and CXCR2 in diabetic rabbits compared with nondiabetic animals. However, after wounding there was almost unchanged expression of these chemokines.[130] Consequently, the acute inflammatory response was significantly blunted with adverse effects on wound healing rates. Correction of this deficiency may represent a novel therapeutic approach. Indeed, the application of talactoferrin to wounded diabetic and nondiabetic mice modulated the early inflammatory response with evidence of increased CXCL8 expression associated with improved wound closure.[131] Furthermore, improved healing has been demonstrated in diabetic ulcers with the application of a talactoferrin gel in humans.[132]

A consistent finding in pressure ulcers, the diabetic foot and venous stasis ulcers is an accumulation of senescent fibroblasts.[133–135] These fibroblasts demonstrate decreased proliferative rates and a dysfunctional chemokine secretory profile involving excess CCL2 release and reduced CXCL8 production compared with wounds healing normally.[136,137] Furthermore, high levels of functional CCL2, CCL5, CCL18, CCL20, CCL27, CXCL1, and CXCL12 have been reported in chronic wound debridement tissue. This extract was able to stimulate migration of healthy dermal fibroblasts and bioactive molecule secretion from cellular skin substitutes suggesting that senescent cells also show aberrant responses to chemokines.[101] Interestingly, there was little variation in chemokine concentration between donors in this study, despite differing wound etiologies, size, and duration.[101]

Finally, it is not just inflammatory processes that are deranged in chronic wounds. Altered angiogenesis is well recognized as a contributor to delayed healing in diabetic and venous ulcers.[138,139] CXCL12, which exclusively binds to CXCR4, plays a crucial role in EPC migration[140] and dysfunction in its signaling pathways has been implicated in aged and diabetic wound healing in preclinical models.[141] A relative lack of CXCL12, as found in diabetic wounds, lead to decreased cellular migration and angiogenesis as well as increased inflammation.[142] During diabetic wound healing, administration of CXCL12 directly to the wound reversed EPC recruitment impairment in mice.[143] This beneficial effect has been replicated by lentiviral-mediated gene transfer of CXCL12 in diabetic mice wounds resulting in improved early healing.[144] This hypothesis has been further developed using a novel cell-based therapy where *ex-vivo* BMDSCs primed with CXCL12 were injected subcutaneously into full-thickness diabetic mice wounds.[145] These CXCL12-primed BMDSCs significantly promoted wound healing, neovascularization, and EPC recruitment.[145] These and other cited literature, suggest a therapeutic role for CXCL12 and other chemokines in chronic wound management.[146] This is summarized in Fig. 4.

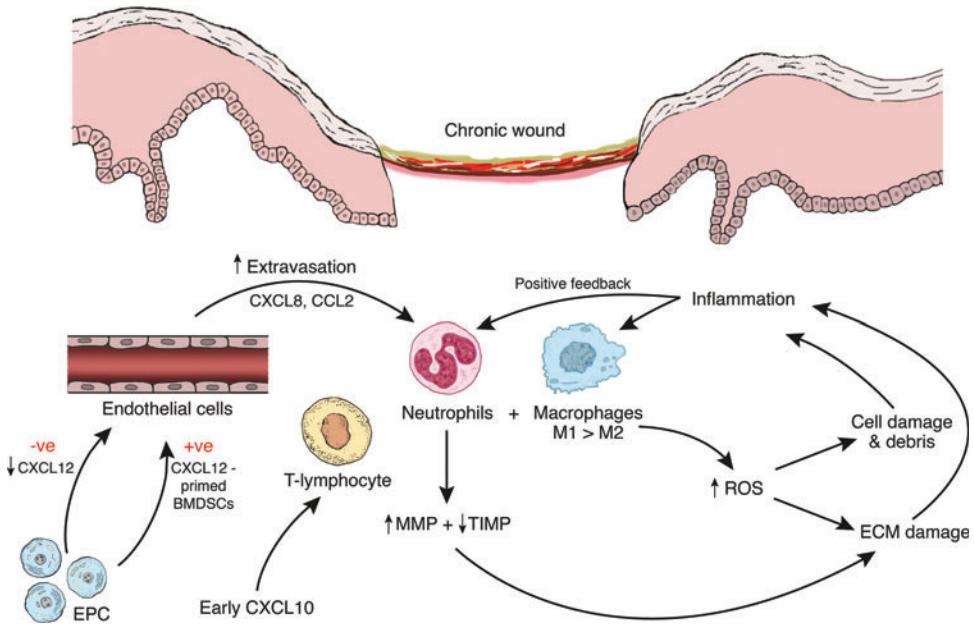


**Figure 4.** Chemokines in chronic wounds. Unbalanced MMP/TIMP expression leads to increased tissue degradation and inflammation. This results in further activated neutrophils/macrophages. A positive feedback loop is created leading to a chronic inflammatory milieu. Influencing chemokine expression may lead to therapeutic options. To see this illustration in color, the reader is referred to the web version of this article at www.liebertpub.com/wound

## Chemokines and pathological scarring

Hypertrophic scars are red, raised, uncomfortable scars that are confined to the boundaries of the original wound.[11] Keloid scars are benign collagenous tumors that form in the reticular layer of the dermis during a prolonged wound healing process in persons with a genetic predisposition.[147,148] They spread beyond the margins of the original wound and are associated with increased collagen deposition and fibroproliferation.[149–151] Both are associated with significant morbidity.

Keloid scars are characterized by overproliferation of fibroblasts, leukocyte infiltration, and prolonged excessive collagen synthesis.[152,153] Due to their fundamental function of chemoattraction, it has been hypothesized that chemokines are involved in this process.[154] It has been shown that CXCL1 and its receptor CXCR2 are more abundant in keloid fibroblasts and, therefore, postulated that the inflammatory component of scarring is crucial to the development of keloid scars.[154] Fibroblasts from keloid scars have been shown to have increased levels of CXCL8 compared with normal human fibroblasts.[10] This highlights a possible role for CXCL8 in leukocyte recruitment and activation in keloid scars.

It is hypothesized that hypertrophic scarring results from the overproduction of ECM during fibroplasia, secondary to abnormalities in epidermal–dermal communication within which CXCR3 plays a critical role.[95] To investigate the effect of a lack of CXCR3 receptors, wound healing and scarring was compared between $CXCR3^{-/-}$ and wild-type mice with full-thickness excisional wounds.[95] In the absence of CXCR3, wounds remained immature with an inflammatory milieu and went on to develop a hypertrophic scar phenotype, similar to those observed in humans.[95] Taking this idea to its logical conclusion, the authors postulated that transplantation of normal fibroblasts to a $CXCR3^{-/-}$ wound may lead to the correction of fibroblast-generated matrix dysfunction, resulting in improved healing. Not only did these normal fibroblasts survive within the wound milieu, they had a positive effect on healing, including matrix remodeling, improved collagen alignment, and increased tensile strength.[155] This raises the possibility of using transplanted fibroblasts as cellular therapies to stimulate more functional and regenerative healing responses.[155]

Hypertrophic scars are hypercellular due to increased numbers of fibroblasts and recruitment of peripheral nonhematopoietic cells. Once again, the recruitment of cells hints at the possible role of chemokines during hypertrophic scar development. It has been previously demonstrated that CXCL12 can be beneficial in chronic wounds, where there is a lack of recruitment of effector cells. Therefore, it is logical to assume that it can be disadvantageous in situations where excessive cellular recruitment is a problem. Within biopsies of hypertrophic scars from human burn patients,

increased CXCL12/CXCR4 signaling was demonstrated compared with normal skin.[11] This increased signaling was downregulated following administration of IFN α 2B and coincided with remodeling of hypertrophic scars.[11] Therefore, it is argued that CXCL12/CXCR4 interactions are involved in promoting recruitment of cells that contribute to the development of these abnormal scars. However, somewhat contradictory work into the role of CXCL12 in wound healing and scarring has been published.[156] Following the evidence that CXCL12 was beneficial in chronic, hard-to-heal wounds, they studied the effect of continuously delivered CXCL12 mounted on an alginate dressing on wound healing and scar appearance in full-thickness incisional porcine wounds. They were able to show that treated pig wounds healed faster and with less fibrosis than control wounds.[156] Interestingly, histological assessment did not reveal increased vascular density in CXCL12-treated wounds[156] suggesting a mechanism other than EPC homing for the beneficial effects.

### Chemokines as therapeutic targets

The excessive infiltration of leukocytes is a hallmark of many autoimmune and chronic inflammatory diseases. Several approaches have been postulated to prevent cellular recruitment to inflamed tissues, including the modulation of chemokines and their receptors.[9] However, despite great interest, effective treatments that target the chemokine family have remained elusive.

Potential methods of influencing the chemokine system. Potential strategies for influencing the chemokine system are highlighted in Fig. 5. In recent years the pharmaceutical industry has made many developments in the use of therapeutic monoclonal antibodies (mAbs) to inhibit specific aspects of immune cell function, although their use to intervene in the chemokine system is a relatively new strategy.[25] These therapeutic antibodies can act either directly or indirectly on their targets. They can bind to and block their target protein, that is, direct targeted therapy, or they can influence critical biological processes that is, antibody-dependent cell-mediated cytotoxicity or complement-dependent toxicity.[157] Certain characteristics of chemokine receptors, including their limited availability as purified proteins and low immunogenicity, have hampered the development of novel therapeutic agents.[157] However, following increased interest in this area, neutralizing mAbs have been used to inhibit leukocyte recruitment in a number of disease processes in animal models and have been incorporated into human trials. A mAb to CXCL8 (ABX-IL8, Abgenix) has been shown to inhibit neutrophil and monocyte infiltration into the lungs of patients suffering from COPD, reducing the severity of dyspnea, but having no influence on lung function or health status.[158] The same antibody has been tested in the context of cutaneous disease that is, psoriasis, see below.

Unlike adhesion molecules and cytokine receptors, GPCRs can be blocked by small-molecule antagonists, and much work has been focused on this area of the chemokine system. Certain chemokine receptors, including CXCR3 and CCR5, have the ability to bind several ligands at nonoverlapping binding sites.[159,160] Due to a discrepancy in size, small molecules are unable to compete with the larger chemokine ligands at an orthosteric site. However, they can successfully antagonize chemokine activity by interaction at an allosteric

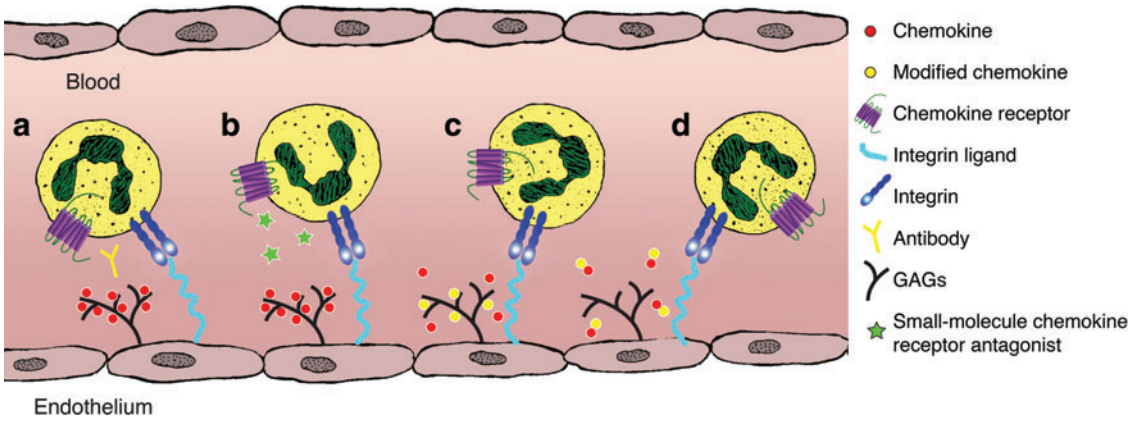


**Figure 5.** Diagrammatic representation of therapeutic strategies to influence the chemokine system **(a)** mAbs act either directly or indirectly to prevent ligand/receptor binding **(b)** small-molecule antagonists bind to an allosteric site, preventing chemokines binding to the main orthosteric site **(c)** modified, nonfunctioning chemokines prevent native chemokines from binding to GAGs **(d)** Truncated chemokines can bind to chemokine receptors acting as antagonists. To see this illustration in color, the reader is referred to the web version of this article at www.liebertpub.com/wound

binding site causing modulation of the main chemokine orthosteric site.[161] This induced change also overcomes the problem of ligand promiscuity as these inhibitors prevent all ligands from binding to a particular receptor.[161] Following discovery of the chemical profile of chemokines, the identification of small molecules to antagonize their receptors has gathered momentum.[162]

Chemokines binding to glycosaminoglycans (GAGs) is vital for normal chemokine function.[163] They help to establish a chemotactic gradient, protect chemokines from proteolytic cleavage, and have a role in oligomerization.[164–167] It has been postulated that interfering with GAG-chemokine interactions could be a potential therapeutic target, however, inherent complexities of this system has prevented significant progress.[25]

While not affecting their ability to bind to their receptor(s), the truncation of the N terminus eliminates the signaling potential of many chemokines.[25] It has been demonstrated that re-engineered chemokines can be biologically active as chemokine agonists,[168] while this approach has also produced potent antagonists also.[169]

Obstacles to developing chemokine-based therapies. Most of the chemokine receptor targets identified for the treatment of human disease processes have been validated in animal models.[170] The majority of compounds developed targeting these receptors have shown good efficacy in animal and *in vitro* models, but have ultimately failed to translate into clinically effective therapeutics. There are a number of reasons to explain this frustration. There are clear similarities between the chemokine systems of man and mice and work in murine models has contributed significantly to our understanding of chemokine biology. However, due to speciation and differing evolutionary pressures, the gene organization of human and mice chemokine clusters is very different.[171] Clearly, not all observations made in murine models can be extrapolated to application in man and hence, many identified targets have failed to progress to fruition.

Many of the potential targets of therapeutic intervention in the chemokine system are based on the classical paradigm of a single ligand interacting with a single receptor leading to a consistent and predictable cellular response. Although they may function as monomers, it is now well established that many chemokine receptors also act as dimers or higher order oligomers.[172] In fact, all tested chemokine receptors form oligomers in a ligand-independent manner,[173–177] and heterodimers can form even between CC and CXC subclasses.[172] Another relevant phenomenon is that of crosstalk between different receptors. This refers to the ability of a particular receptor to influence the signaling and function of another receptor.[172] The principles of oligomerization and crosstalk challenges the logic of inhibiting one ligand/receptor to bring about a desired therapeutic effect.

Success stories in chemokine-targeted therapy. Undoubtedly, the most developed field of chemokine therapeutics is in the treatment of the human immunodeficiency virus (HIV). Two chemokine receptors have been implicated in viral entry into host cells, namely CXCR4 and CCR5,[24] affecting T-cells and macrophages, respectively.[178] A naturally occurring mutation of CCR5, CCR5-Δ32, is uncommon in HIV-1-infected patients and individuals homozygous for the mutation are rarely affected by HIV-1.[179] This observation led to the development of numerous small-molecule antagonists of CCR5. The first to be approved by the European Medicines Agency has been Maraviroc, developed by Pfizer, which is used in treatment-experienced patients infected with CCR5-tropic viruses only.[157] Further antagonists continue to be developed.[180] When HIV translates into the more advanced AIDS, CXCR4 becomes the more relevant receptor.[181] Although potent inhibitors of CXCR4 exist, their clinical utility is limited by severe side effects. Indeed, since CXCR4 or CXCL12 knockout mice are not viable, significant doubt remains over whether this pathway can be safely manipulated in the long term.[157]

The use of chemokine therapeutics in inflammatory disease processes is far less developed. The receptor CCR9 is expressed on a subset of circulating lymphocytes and is recognized as being responsible for mediating homing of these cells to the intestinal mucosa.[182] Its sole ligand CCL25 is highly expressed within the intestine.[183] CCX282-B (Vercirnon) is a small-molecule antagonist to CCR9, which inhibits CCR9- and CCL25-dependent chemotaxis. In a recent randomized control trial it was demonstrated to be safe, efficacious, and well tolerated by patients suffering from Crohn's disease through the oral route although it failed to reach its primary endpoints.[184] This work has demonstrated that therapies targeting the chemokine system in inflammatory disease processes is a realistic goal.

Existing nonchemokine therapies for cutaneous scarring. Traditional approaches toward scar reduction are well established. They involve pressure/silicon dressings, onion extracts, vitamin E-based remedies, and corticosteroids. However, the clinical efficacy and evidence base for these strategies is

limited.[185] As detailed above, there are significant differences in the healing profiles of adult and fetal wounds. Recent therapies have attempted to reproduce fetal healing that is, regeneration, in adults. In particular, modulating the ratios of the subsets of transforming group factor beta (TGF-$\beta$) in the wound microenvironment has received much attention. Recombinant human TGF-$\beta$3, Avotermin, has been demonstrated to be safe and numerous double-blind, placebo-controlled prospective trials has demonstrated statistically significant improvement in scar appearance at a range of doses and dose frequencies.[186]

**TAKE-HOME MESSAGES**

- Chemokines and their receptors have been implicated in all aspects of wound healing and scarring, both normal and pathological. Despite our ever-expanding knowledge in this large family of chemotactic cytokines, their exact nature and influences remain elusive.
- Current novel therapeutics for the treatment of cutaneous scarring attempt to revert adult wound healing to the regenerative phenotype of the fetus. There is currently a lack of knowledge with regard to the role of chemokines in fetal healing. Advances in this area may lead to clinically effective treatments in the future.
- Future therapies will need to address the issues of chemokine/receptor oligomerization and cross talk. It is likely that they will need to influence multiple ligand/receptor interactions simultaneously or sequentially.

The key to any modulation of scar formation is the coordination of numerous cellular and molecular processes. Direct cytoplasmic coupling between cells mediated by gap junctions provides a direct line for the spread of biological information within tissues.[187] This is now recognized as an important aspect of cutaneous injury response, and connexins appear to have an influence on scar formation.[188] Several connexins appear to be expressed in the skin, including Cx43, found in the epidermis and dermis and the potential for targeting this connexin has been extensively researched.[189–191] Numerous antisense-based therapies targeting Cx43 are in development.[188]

## SUMMARY

It is clearly apparent that chemokines are intrinsically involved in the process of cutaneous wound healing and scar formation. The way in which they mediate chemoattraction and cellular responses to injury are vital to a satisfactory outcome following wounding. It has been shown that they can have positive and negative impacts on this complex physiological process, and novel therapies that influence either the expression or suppression of these cytokines will likely play a significant role in the treatment of problem scars in the future. However, to date numerous works in the literature report contradicting observations of the functions of chemokines. This suggests that the exact biological pathways by which chemokines assert their influence are yet to be fully realized. This must be addressed before clinically effective, readily available therapies can be a realistic goal.

Research into other applications of chemokines in health may lead to benefit in the setting of wound healing. Work into bioengineering has demonstrated potential gains producing synthetic chemokines that are more effective than their native counterparts.[168]

Something which has become clear from work in other areas of wound healing therapy for example, growth factors, is that targeting a single element of a complex process is unlikely to yield clinically significant results outside of experimental conditions. It is likely that to effectively interfere with the aberrant physiology leading to problematic scars, multiple chemokines and/or receptors will need to be targeted either simultaneously or sequentially.[18] Despite these obstacles, chemokines will almost certainly play a significant role in wound healing therapies of the future.

## ACKNOWLEDGMENTS AND FUNDING SOURCES

No funding was required for this review article.

## AUTHOR DISCLOSURE AND GHOSTWRITING

No competing financial interests exist. The content of this article was expressly written by the authors listed. No ghostwriters were used to write this article.

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### Abbreviations and Acronyms

ACKR1 = atypical chemokine receptor 1
AIDS = acquired immunodeficiency syndrome
BM = bone marrow
BMDSCs = bone marrow-derived stem cells
cCAF = chicken chemotactic and angiogenic factor
Cx43 = connexin 43
DARC = duffy antigen receptor for chemokines
ECM = extracellular matrix
EPCs = endothelial progenitor cells
GAGs = glycosaminoglycans
GDP = guanosine diphosphate
GPCRs = G-protein-coupled receptors
GTP = guanosine-5′-triphosphate
HIV = human immunodeficiency virus
IFN-$\gamma$ = interferon gamma
IL = interleukin
mAbs = monoclonal antibodies
MCP-1 = monocyte chemotactic protein 1
MMPs = matrix metalloproteinases
MT1-MMP = membrane type 1-matrix metalloproteinase
PDGF = platelet-derived growth factor
TGF-$\beta$ = transforming group factor-beta
TIMP = tissue inhibitor of metalloproteinase
TNFSF14 = tumor necrosis factor ligand superfamily member 14
TNF-$\alpha$ = tumor necrosis factor-alpha
VEGF = vascular endothelial growth factor