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COMPREHENSIVE INVITED REVIEW

Chemokines and Their Receptors Are Key Players in the Orchestra That Regulates Wound Healing

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Significance: Normal wound healing progresses through a series of overlapping phases, all of which are coordinated and regulated by a variety of molecules, including chemokines. Because these regulatory molecules play roles during the various stages of healing, alterations in their presence or function can lead to dysregulation of the wound-healing process, potentially leading to the development of chronic, nonhealing wounds.

Recent Advances: A discovery that chemokines participate in a variety of disease conditions has propelled the study of these proteins to a level that potentially could lead to new avenues to treat disease. Their small size, exposed termini, and the fact that their only modifications are two disulfide bonds make them excellent targets for manipulation. In addition, because they bind to G-protein-coupled receptors (GPCRs), they are highly amenable to pharmacological modulation.

Critical Issues: Chemokines are multifunctional, and in many situations, their functions are highly dependent on the microenvironment. Moreover, each specific chemokine can bind to several GPCRs to stimulate the function, and both can function as monomers, homodimers, heterodimers, and even oligomers. Activation of one receptor by any single chemokine can lead to desensitization of other chemokine receptors, or even other GPCRs in the same cell, with implications for how these proteins or their receptors could be used to manipulate function.

Future Directions: Investment in better understanding of the functions of chemokines and their receptors in a local context can reveal new ways for therapeutic intervention. Understanding how different chemokines can activate the same receptor and *vice versa* could identify new possibilities for drug development based on their heterotypic interactions.

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Abbreviations and Acronyms

Ang-2 = angiopoietin-2
AREs = adenine–uridine-rich elements
DARC = Duffy antigen receptor for chemokines
ECM = extracellular matrix
EGF = epidermal growth factor
EST = expressed sequence tag
FGF = fibroblast growth factor
GPCRs = G-protein–coupled receptors
HDAC-1 = histone deacetylase 1
HIF-1α = hypoxic-inducible factor 1-alpha
HTS = hypertrophic scars
IFN-γ = interferon gamma
IL = interleukin
KSRP = KH-type splicing regulatory protein

(*continued*)

SCOPE AND SIGNIFICANCE

Chemokines are a family of small chemotactic cytokines that were discovered in the late 1970s and early 1980s,[1–3] and were originally described as factors that chemoattract and activate cells of the immune system during inflammation. Discovery of new proteins of this family continued at a slow pace through the 1990s, especially those related to homeostasis of the immune system, but it was not until the beginning of the 21st century that we fully realized the wealth of proteins that this family provides, not only as regulators of immune function, but also as having functions that go well beyond. We know today that chemokines play critical roles in many basic biological processes such as angiogenesis and also are critically involved in chronic inflammation, autoimmune diseases, cancer, and viral infections.

In this review, we will first discuss the classification of these proteins and

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Abbreviations and Acronyms (*continued*)
MDNCF = macrophage-derived neutrophil chemoattractant factor
MMPs = matrix metalloproteinases
NFκB = nuclear factor kappa-B
TCR = T-cell receptor
TGF-β = transforming growth factor beta
TNF-α = tumor necrosis factor alpha
VEGF = vascular endothelial growth factor

their receptors, then describe how they are regulated at multiple levels, and address some of the broad functions they perform in a variety of biological processes. In the latter portion of the review, we will focus in more detail on the role of chemokines in normal and abnormal wound healing.

TRANSLATIONAL RELEVANCE

The chemokine network is a good candidate for controlling both a variety of processes involved in inflammation, angiogenesis, and disease. A lack of regulation of this complex network of cytokines can result in chronic inflammation, dysregulation of blood vessel development, and establishment of a chronic environment that leads to impaired healing, generalized fibrotic disease, and cancer. Because these chemokines are small proteins that do not have modifications other than the two disulfide bonds, are stable, and are amenable to large-scale production, it is possible to use these proteins or peptides corresponding to functional regions as adjuvants for wound therapy. Furthermore, the fact that they bind G-protein-coupled receptors (GPCRs) increases the likelihood that their biological pathways can be controllable by small chemical agonists or antagonists.

CLINICAL RELEVANCE

Chemokines are major players in inflammation and angiogenesis. Therefore, changes in their levels or function can lead to chronic inflammation and dysregulated angiogenesis. These alterations can lead to either absent or excessive function, leading to impaired healing, chronic wounds, generalized fibrotic disease in response to injury, excess healing, and development of keloids and cancer. Because chemokines are so closely involved in the regulation of both inflammation and angiogenesis, one could envision that manipulation of this network of cytokines could modulate either of these processes and lead to improvement of these conditions. In the case of wound healing, because chemokines from the CXC and CC families are expressed throughout the wound-healing process in specific temporal and spatial patterns, this network of proteins lends itself to providing regulated control for proper healing.

DISCUSSION OF FINDINGS AND RELEVANT LITERATURE

Classification of chemokines and chemokine receptors

The chemokine superfamily is a group of small (8–10 kDa), positively charged, secreted proteins with a 20%–50% sequence homology, which is reflected in shared structural characteristics. They usually have four cysteines, the first two located near the N-terminus of the molecule, the third in the center of the sequence, and the fourth close to the beginning of the C-terminal helix. These cysteines form disulfide bridges between the first and third cysteine residues and also between the second and fourth cysteine residues, folding the molecule into a globular shape with the N-terminus shaped as a loop, the C-terminus as an α-helix, and the center of the molecule containing three β-pleated sheets. The termini are both exposed to the outside of the molecule and are important in receptor binding (Fig. 1). However, there are a small number of chemokines that contain only two cysteines and others that contain six cysteines.[4–6]

During the early years of their discovery, the chemokines were called by a variety of names, most of them acronyms representing their functions. In 1999, it was decided during a Gordon Conference on these chemotactic proteins that a systematic nomenclature was needed, and

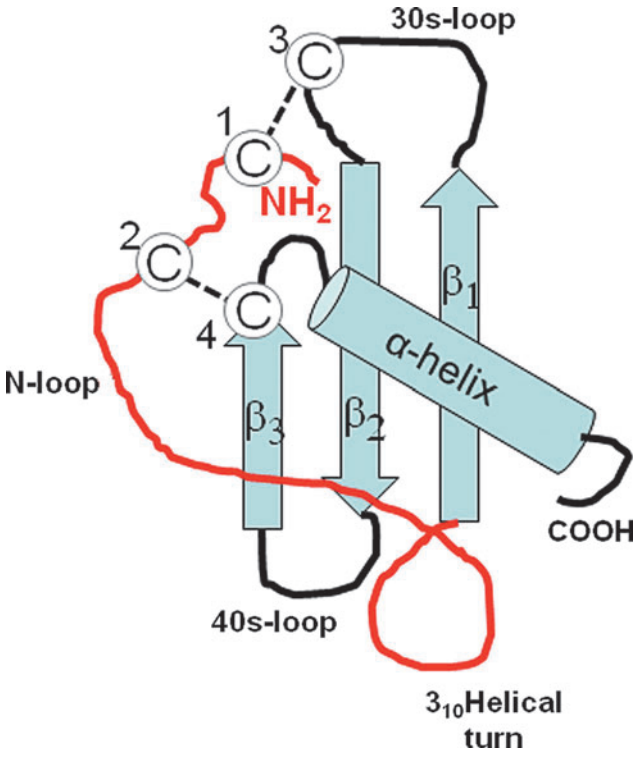

Figure 1. Schematic representation of the structural components of the chemokine molecule. Chemokines are structurally composed of a flexible N-terminus, followed by three antiparallel β-pleated sheets separated by flexible loops and terminate with a long α-helix in the C-terminus. The molecule assumes a globular shape, because cysteine #1, near the beginning of the N-terminus, makes a disulfide bond with cysteine #3 present in the 30s loop, and cysteine #2, also in the N-terminus, establishes a disulfide bond with cysteine #4, which is located close to the C-terminal α-helix.

this was created based on the number of amino acids present between the first two cysteines.[6] Connections between original chemokine names and modern classification are described in Tables 1–3. In this manner, chemokines were divided into four families (Fig. 2). The two largest families are the CXC family (Fig. 2A), in which these two cysteines are separated by any single amino acid, and the CC family (Fig. 2B), in which the first two cysteines are adjacent. The CXC chemokine family is further subdivided into those that contain a glutamic acid (E), a leucine (L), and an arginine (R) immediately before the first cysteine (C). These chemokines are said to be ELR^+, and those that do not contain this sequence are called ELR^-. The presence or absence of this sequence is important for receptor selectivity and/or downstream signaling. The ELR^+ chemokines attract primarily neutrophils and are angiogenic, while the ELR^- chemokines are angiostatic and attract primarily lymphocytes.[7] The remaining two chemokine families are quite small, and include the XC family (Fig. 2C), which has only one of the first two N-terminal cysteines, and the CX3C family (Fig. 2D), in which the first two cysteines are separated by three amino acids. This latter family of chemokines contains only one member, fractalkine, which is tethered to a mucin-like stalk that is linked to a transmembrane domain. The chemokine proper can be released from the stalk by enzymatic digestion and serve as a chemoattractant, or can remain attached as a transmembrane protein and serve as an adhesion molecule.

CXCL4, CXCL10, and CXCL1 were among the first members of the chemokine superfamily to be identified and sequenced.[1,2,8] The first member of the chemokine superfamily known to possess a immune cell-chemoattractant activity was named macrophage-derived neutrophil chemoattractant factor (MDNCF), a chemokine isolated from a medium of lipopolysaccharide-treated monocyte/macrophages that showed the ability to chemoattract neutrophils. Subsequent cloning and sequencing of MDNCF showed that this factor was interleukin (IL)-8 (now referred to as CXCL8) and uncovered its sequence similarity to CXCL4, CXCL10, and CXCL1, and the chicken chemokine chCXCLi2.[9] The identification of these chemokines was quickly followed by the isolation of CCL2, which was purified from a medium conditioned by glioma cells and phytohemagglutinin-treated peripheral blood mononuclear cell and chemoattracted monocytes.[10] Most of the remaining chemokines were identified by searching the mouse and human expressed sequence tag (EST) databases for ESTs similar to the previously identified chemokines.[11] As of yet, at least 47 human chemokines have been identified (Tables 1–3).

Chemokine receptors are seven-transmembrane GPCRs with 77% amino acid identity that associate with heterotrimeric G-proteins. They have a short

Table 1. *CXC chemokine/chemokine receptor superfamily*

Systematic Name	*Human Ligand*	*Mouse Ligand*	*Chemokine Receptor*
CXCL1	GROα/MGSAα	GRO/KC	CXCR2
CXCL2	GROβ/MGSAβ	GRO/KC	CXCR2
CXCL3	GROγ/MGSAγ	GRO/KC	CXCR2
CXCL4	PF4	PF4	CXCR3b
CXCL5	ENA-78	GCP-2/LIX	CXCR2
CXCL6	GCP-2	GCP-2/LIX	CXCR1, CXCR2
CXCL7	NAP-2	N/A	CXCR2
CXCL8	IL-8	N/A	CXCR1, CXCR2
CXCL9	MIG	MIG	CXCR3
CXCL10	IP-10	IP-10	CXCR3
CXCL11	I-TAC	I-TAC	CXCR3
CXCL12	SDF-1α/β	SDF-1α/β	CXCR4
CXCL13	BCA-1	BLC	CXCR5
CXCL14	BRAK	BRAK	N/A
CXCL15	N/A	Lungkine	N/A
CXCL16	N/A	N/A	CXCR6
CXCL17	DMC	DMC	N/A

N/A, not applicable.

Table 2. *CC chemokine/chemokine receptor superfamily*

Systematic Name	*Human Ligand*	*Mouse Ligand*	*Chemokine Receptor*
CCL4	N/A	N/A	N/A
CCL4L1	MIP-1β	MIP-1β	CCR1, CCR5, CCR8
CCL4L3	AT744.2	N/A	CCR1, CCR5
CCL5	RANTES	RANTES	CCR1, CCR3, CCR4, CCR5
CCL6	N/A	C10, MRP-1	CCR1
CCL7	MCP-3	MARC	CCR1, CCR2, CCR3, CCR5
CCL8	MCP-2	MCP-2	CCR1, CCR2, CCR3, CCR5
CCL9/10	N/A	MIP-1γ, MRP-2	CCR1
CCL11	Eotaxin	Eotaxin	CCR2, CCR3, CCR5
CCL12	N/A	MCP-5	CCR2
CCL13	MCP-4	N/A	CCR1, CCR2, CCR3, CCR5
CCL14	HCC-1	N/A	CCR1, CCR5
CCL15	HCC-2	N/A	CCR1, CCR3
CCL16	HCC-4	N/A	CCR1, CCR2, CCR5
CCL17	TARC	TARC	CCR4, CCR8
CCL18	PARC	N/A	CCR3
CCL19	MIP-3β, ELC	MIP-3β	CCR7
CCL20	MIP-3α	MIP-3α	CCR6
CCL21	6Ckine, SLC	6Ckine, SLC	CCR7
CCL22	MDC	ABCD-1	CCR4
CCL23	MPIF-1	CCL6, C10	CCR1
CCL24	Eotaxin-2	N/A	CCR3
CCL25	TECK	MPIF-2	CCR9
CCL26	Eotaxin-3	Eotaxin-3	CCR1, CCR2, CCR3, CCR5
CCL27	CTACK/ILC	CTACK/ILC	CCR10
CCL28	MEC	ALP/CTACK	CCR3, CCR10

acidic N-terminus that faces the extracellular environment and is important in ligand binding, three extracellular loops linked by disulfide bonds, and three intracellular loops, the second of which contains a signature motif of amino acids, the DRYY motif. The C-terminus is rich in Ser and Thr amino acids that, when phosphorylated, contribute to desensitization of the receptor (Fig. 3A). The chemokine G-protein-linked receptors are divided into four families named after the type of the chemokine they bind (Fig. 3B): CCR1–10 (R for receptor) bind CC chemokines; CXCR1–7 bind CXC chemokines; XCR1 binds the XC chemokines, lymphotactins α and β; and CX3CR1 binds fractalkine.[12] The chemokine receptor superfamily also includes a few atypical members, such as the Duffy antigen receptor for chemokines (DARC), which binds several chemokines, but fails to initiate the downstream signaling events.[13] Another such receptor is Decoy-receptor D6 that scavenges

Table 3. *Other chemokine/chemokine receptor superfamilies*

Systemic Name	*Human Ligand*	*Mouse Ligand*	*Chemokine Receptor*
XC chemokine/receptor family			
XCL1	Lymphotactin	Lymphotactin	XCR1
XCL2	SCM-1β	N/A	XCR1
CX3C chemokine/receptor family			
CX3CL1	Fractalkine	Fractalkine	CX3CR1

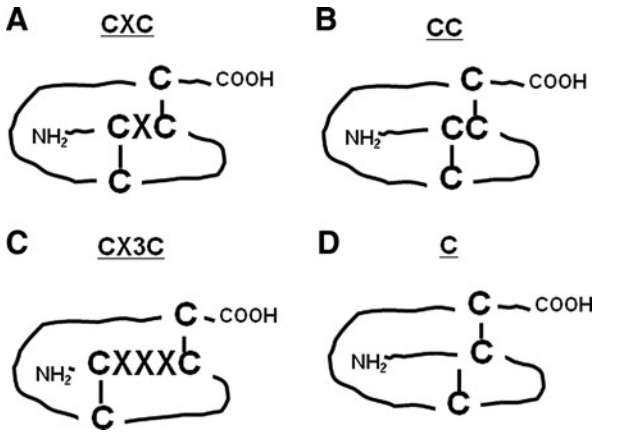

Figure 2. Chemokine superfamily. It is composed of four families; the CXC family **(A)**, in which the first two cysteines are separated by any single amino acid, the CC family **(B)**, in which the first two cysteines are adjacent, the CX3C family **(C)**, in which the first two cysteines are separated by three amino acids, and the C family **(D)**, in which one of the first two cysteines is missing.

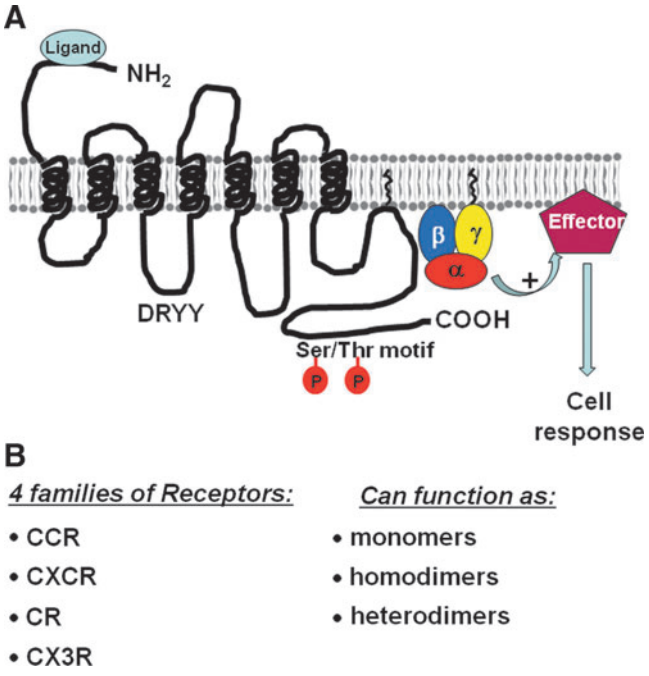

Figure 3. Chemokine receptor structure and families. **(A)** These molecules are seven-transmembrane, GPCRs. They are composed of a short acidic N-terminus facing the outside of the cell, which is important for ligand binding, and three extracellular and three intracellular loops, the second of which contains the DRYY motif that is characteristic of this family. The C-terminus is intracellular and is rich in serine and threonine amino acids which, when phosphorylated, inactivate the receptor. **(B)** They form four families that are named after the ligand families, and they function in monomers, homodimers, and heterodimers. GPCRs, G-protein-coupled receptors.

inflammatory CC chemokines.[14] This function can be critical, because these receptors can remove excess chemokines, thereby limiting the effects of these powerful modulators of the immune system. In addition, viral chemokine receptor-like proteins that signal in the absence of ligand binding, as well as those chemokine receptors that serve as coreceptors for viral entry (*e.g.*, HIV), have also been identified.[12] Finally, chemokines can also exert their effects by binding to heparan moieties on the surface of the extracellular matrix (ECM) molecules and transmembrane proteins, and in this manner enhance their effects on immune cells and/or generate haptotactic gradients that influence chemotaxis.[15] Seven-transmembrane receptors are highly amenable to inhibition by small pharmacological agents, making the receptors excellent drug targets and enabling modulation of chemokine functions.[16–18]

Although some chemokine receptors bind one chemokine exclusively, many chemokines bind more than one receptor, and many of the receptors bind more than one chemokine within the same family, creating a redundancy within the chemokine superfamily. When the ligand binds to the receptor, the heterotrimeric G protein, which is composed of Gα, β, and γ, is activated. Binding of the chemokine to its GPCR enables Gα, which in the nonactivated state is bound to GDP, to release this guanine nucleotide and bind GTP, thereby promoting the dissociation of Gα from G$\beta\gamma$ and the activation of both signaling molecules. This, in turn, triggers a series of downstream signaling pathways involving the Rho family of small GTPases, leading to a response that generally results in directional cell movement or chemotaxis.[19,20] Regulation of the chemokine GPCR function is tightly linked to a variety of adaptor proteins that facilitate their internalization.[21] The function of these receptors is also regulated by desensitization between themselves and with other receptor types that involves phosphorylation of the Ser and Thr residues in the C-terminus of the receptor, which is located inside the cell.[22–24]

Regulation of chemokine and chemokine receptor expression

Despite their importance in a variety of normal biological processes and in disease, the complexity involved in chemokine regulation is only recently being appreciated. Chemokines that function in inflammation are very tightly regulated in a dose- and time-dependent manner, strongly suggesting that their actions are affected by the microenvironmental conditions.[15] Expression of these inflammatory or inducible chemokines is stimulated by a variety of signals, including bacterial products, the inflammatory cytokines tumor necrosis factor alpha (TNF-α), IL-1, interferon gamma (IFN-γ), and IL-6, in response to stress, and by thrombin, a component of the coagulation cascade.[25–29] The levels of chemokine expression can be regulated by repression, transcription activation, post-transcriptional activation, translation, and post-translation mechanisms, and by a variety of environmental factors.

In general, repression occurs in the cells that are not stimulated. For example, the *CXCL8* gene is kept in check by the nuclear factor kappa-B (NFκB)–repressor factor binding to its own element in the promoter of this chemokine to inhibit activation by the NFκB transcription factor, by a similar mechanism involving Octomer-1 repressor (Oct-1), and by deacetylation of histones by histone deacetylase 1 (HDAC-1). During transcription activation, in addition to NFκB, the AP-1, STAT1, and Elk1 transcription factors are involved in stimulation of transcription of chemokines induced by a variety of stimuli.[27,29,30–32] Further, in response to hypoxia-induced stress, chemokine expression is increased through NFκB and AP-1, in conjunction with the transcription factor hypoxic-inducible factor 1-alpha (HIF-1α).[33–35]

Regulation of chemokine expression at the post-transcriptional level involves alterations in mRNA stability. For example, the CXCL1 and CXCL8 mRNA levels increase upon IL-1β stimulation, and the levels remain high up to 8 h in the continued presence of IL-1β, even when coincubated with actinomycin D, an inhibitor of transcription.[36] In the case of CXCL1, both the intrinsic mRNA instability and the increased stability conferred by IL-1β require the 5′-UTR of the mRNA, as well as the adenine–uridine-rich elements (AREs) within the 3′-UTR.[37,38] The 3′-UTR of IL-8/CXCL8 also contains AREs important for mRNA stability,[39,40] apparently due to their interactions with the mRNA-stabilizing proteins AUF1 and HuR.[40] The CXCL8 mRNA stability is also regulated by destabilizing proteins, including KH-type splicing regulatory protein (KSRP) and tristetraprolin, which interact with the AREs under basal conditions, leading to rapid deadenylation and degradation.[41] In the presence of IL-1β, the CXCL8 mRNA is likely stabilized by both a decreased interaction with KSRP[41] and an increased interaction with HuR.[42] Interestingly, HuR is overexpressed in many human tumors, where it stabilizes CXCL8 mRNA. It is also possible that the enhanced mRNA stability may participate in pathogenesis of wound healing by increasing

the production and secretion of this angiogenic chemokines.

Alternative RNA splicing is another component of chemokine post-transcriptional regulation that affects the chemokine activity and tissue distribution.[43] In the CXC family, CXCL12 is alternatively spliced to yield six splice variants that result in the production of six CXCL12 isoforms, CXCL12α, β, γ, δ, ε, and φ, which differ in their C-termini due to differences in the splicing of exon 4.[44,45] CXCL12α and β are expressed strongly in the skin, liver, pancreas, kidney, spleen, lung, and bone marrow, whereas CXCL12γ is primarily expressed in the heart, and CXCL12δ, ε, and φ in the pancreas. The differential tissue expression of these isoforms suggests that they possess nonoverlapping functions, although further studies are needed in this area.[45] RNAs of some CC chemokines also undergo alternative splicing. CCL4 and CCL4L are two very closely related chemokines that have different isoforms due to alternative splicing. Both possess the splice variants lacking exon 2, leading to frameshifts whose products are thought to be nonfunctional.[46] *CCL4L2*, an allele of CCL4L that appears more frequently in the HIV-infected population, has a base substitution in the splice acceptor site of the second intron, generating multiple splice variants with reduced expression that may be nonfunctional.[46] Alternative splicing of CCL20 results in two isoforms that have similar effects on T-cell chemotaxis *in vitro*; any differences in the expression or function of these isoforms *in vivo* remain unclear.[47] Likewise, both isoforms of CCL23 promote chemotaxis and calcium mobilization in neutrophils, monocytes, and lymphocytes, although the longer isoform exerts a more potent effect on monocyte chemotaxis.[48] The CCL27 splice isoforms, in contrast, appear to have some nonoverlapping functions. One is a normal chemokine with a signal peptide that generates a secreted protein, but in the other, the signal peptide has been substituted by a sequence that directs this isoform to the nucleus where it turns on transcription.[49,50]

Post-translational regulation of chemokines is also a key factor in regulating their function. This regulation can involve cleavage by proteases, changes in storage, release, and receptor presentation, adhesion to extracellular molecules, and binding to decoy receptors. Activation by cleavage involves removal of amino acids, primarily from the N-terminus. For example, the CXCL8 sequences encode for 99 amino acids, but the protein is secreted after removal of a 22-amino-acid signal peptide to yield a protein of 77 amino acids. After secretion, this protein can be processed by removal of one to eight amino acids at the N-terminus, generating several isoforms. CXCL8 (1–77) and (6–77) are the most common forms.[9] Endothelial cells and fibroblasts predominantly produce the 77aa isoform, whereas human monocytes, neutrophils, and lymphocytes predominantly produce the 72aa CXCL8 isoform. A variety of enzymes have been shown to generate these cleaved isoforms, including thrombin, plasmin, and matrix metalloproteinases (MMPs) 1, 9, 13, and 14.[51,52] These isoforms of CXCL8 are highly active in chemoattracting and activating neutrophils; indeed, this chemokine is the strongest chemoattractant and activator of human neutrophils.[53,54] *In vitro*, CXCL8 is known to stimulate a transient increase in Ca^{2+} upon interaction with its G_i-dependent receptors on neutrophils, followed by release of MMP-9-containing granules, neutrophil shape changes, and chemotaxis. Because MMP-9 cleaves CXCL8 into the more potent forms of the chemokine, this process leads to a feedback loop that amplifies the biological effects of this CXCL8 on neutrophils. It is also noteworthy to point out that a comparison of the intracellular Ca^{2+} release and receptor-binding capability indicates that the N-terminal-truncated, more active, isoforms of CXCL8 signal primarily through CXCR1.[55] Other chemokines with important roles in wound healing that undergo N-terminal processing are CXCL9, CXCL10, and CXCL11. These chemokines not only undergo processing at the N-terminus, but they are also cleaved at the C-terminus, thereby promoting differential activities. In contrast, CCL2, which is critical for monocyte chemotaxis, loses the activity when processed at the N-terminus, whereas processing at the C-terminus does not affect the activity.[52] The post-translational modification by glycosylation is rare in chemokines. The best-known modification of this type occurs in CCL2, which contains an *O*-glycosylation site in the C-terminus. However, this glycosylation does not appear to have a major impact on the chemotactic activities of this protein.[56] A modification that affects the function of CXCL8, CXCL10, and CXCL11 is citrullination or deimination of these chemokines. Although citrullination does not appear to alter binding of the respective receptors, it does inhibit the chemotactic activities of these chemokines for their respective leukocytes, and thus reduces the ability of these leukocytes to respond during acute or chronic inflammation.[57,58] Binding to decoy receptors is another very important post-translational regulatory process of chemokine function. The decoy receptors most likely to play a

significant role in wound healing are the DARC receptor and the Decoy receptor 6 (D6). These receptors lack the DRY motif and do not use the classic signal transduction pathways activated by the other GPCRs. It is thought that these receptors bind chemokines, internalize them, and thereby inhibit their function, but it is also possible that by binding the chemokines, they keep them out of circulation. This is particularly relevant with chemokines bound to DARC, as this receptor is present in both red blood cells and the endothelial cells lining the blood vessels.[59–62] Another important post-translational modification in chemokine function is related to their ability to bind to proteoglycans on the surface of cells, particularly endothelial cells. In this manner, gradients can be formed that serve as directional cues for chemotaxis of leukocytes during inflammatory and homeostatic responses. Further, binding of chemokines to proteoglycans can enable proper or improved presentation of the chemokine to its receptor, thereby enhancing function.[63] For example, oligomerization of CCL5 by binding to proteoglycans is required for signaling through CCR1, leading to T-cell arrest, but it is not needed for the chemotaxis and migration of these cells induced by CCL5 through its other receptor, CCR5.[64] Finally, it also is known that inhibition of secretion is another way to regulate chemokine function. For example, surface proteins of *Lactobacillus casei* were found to be anti-inflammatory by decreasing the intracellular CXCL10 protein stability, resulting in inhibition of protein secretion and function.[65]

Chemokine functions

Chemokines are frequently divided into two functionally different categories, homeostatic and proinflammatory, based upon their expression profiles and functionality. Homeostatic chemokines are expressed constitutively and function in leukocyte development and trafficking within the lymph nodes, whereas the proinflammatory chemokines exhibit inducible expression and function in leukocyte recruitment and other inflammation-associated events.[66] For example, within the CXC chemokine family, CXCL12 is considered to be a homeostatic chemokine, as both CXCL12α and its receptor CXCR4 are constitutively expressed by a variety of cells. The homeostatic function of CXCL12α involves its role in trafficking and homing of stem and progenitor cells, including embryonic cells during development, hematopoietic stem cells within the bone marrow, and circulating progenitor cells to the areas of injury.[67] However, this chemokine has also been associated with cancer progression and tumor metastasis.[68,69] In contrast to the more homeostatic effects of CXCL12α, the majority of the remaining CXC chemokines are considered to be proinflammatory. For example, CXCL8 is not constitutively expressed, but exhibits inducible expression in multiple cell types, including neutrophils, epithelial cells, endothelial cells, fibroblasts, and specific cancer cells, largely in response to proinflammatory stimuli such as TNF-α, IL-1, bacterial products, and thrombin.[28,29,70] Although CXCL8 was initially purified and characterized based upon a proinflammatory function that of neutrophil chemotaxis,[70] the expression of both this chemokine and its receptors in non-neutrophil cell types suggests additional functions. Indeed, the effects of CXCL8 on endothelial cells have been studied extensively, and include the stimulation of angiogenesis, as well as vascular permeability, which is important in both inflammation and angiogenesis.[9,71–77] Additional known functions of CXC chemokines include inhibition of angiogenesis (CXCL4, 9, 10, and 11), stimulation of myofibroblast differentiation (CXCL8), stimulation of B-lymphocyte chemotaxis (CXCL12 and13), regulation of thymocyte migration within the thymus (CXCL12), and increased migration of specific T-lymphocyte subsets (CXCL8, CXCL10, and CXCL13).[5,12,78,79]

Like the CXC chemokines, CC chemokines can have either homeostatic or proinflammatory functions, or both. The first of the CC chemokines to be identified, CCL2, was purified and characterized based on its ability to chemoattract monocytes, and is thus considered a proinflammatory chemokine.[10,80] Proinflammatory CC chemokines are now known to be induced in multiple cell types, including monocytes, endothelial cells, fibroblasts, epithelial cells, and smooth muscle cells, in response to inflammatory stimuli, and are also released from platelets in response to thrombin. Their receptors exhibit similarly broad expression. These chemokines stimulate a variety of cellular responses, including chemotaxis of monocyte/macrophages, T-lymphocytes, eosinophils, basophils, and smooth muscle cells, endothelial cell, basophil, and mast cell activation, smooth muscle cell proliferation, and angiogenesis.[81–84] Some CC chemokines, such as CCL17, 18, 19, and 21, are constitutively expressed in many tissues and organs, suggesting homeostatic functions either exclusively or in addition to proinflammatory functions.[66] Because these chemokines are expressed constitutively and also induce leukocyte chemotaxis, they are thought to participate in tissue-specific physiological leukocyte homing and/or trafficking.[85] For example, the CCR7 ligands CCL19 and CCL21 facilitate the movement of

naïve T-lymphocytes from blood vessels into the lymph nodes, and their subsequent colocalization with antigen-presenting cells.[86]

The XC family, XCL1 and XCL2, can function as proinflammatory chemokines.[87] XCL1 stimulates chemotaxis of T-lymphocytes and natural killer cells (NK) cells *in vitro* and *in vivo*.[88] and also promotes chemotaxis of B-cells and neutrophils *in vitro*. However, XC chemokines may also have homeostatic functions; for example, XCL1 is upregulated in $CD4^+$ T cells after T-cell receptor (TCR) ligation in the absence of CD28-mediated costimulation, and appears to both inhibit activation/proliferation and promote apoptosis in $CD4^+$ cells lacking this costimulatory signal.[89,90]

The CX3C chemokine CX3CL1 may also have homeostatic and proinflammatory functions. This chemokine is constitutively expressed by a variety of cell types, including endothelial cells, smooth muscle cells, neurons, and intestinal epithelial cells, but it also stimulates chemotaxis of monocytes, NK cells, and $CD8^+$ T-lymphocytes,[91,92] and promotes NK-cell activation and cytotoxic function.[93] However, the induction of monocyte chemotaxis by CX3CL1 may also have homeostatic roles. For example, CX3CR1 is important in the constitutive monocyte crawling along the vasculature, a type of immune surveillance that facilitates their rapid extravasation in response to infection[94]; this may result from the interaction of monocyte CX3CR1 with constitutively expressed endothelial CX3CL1.

Chemokine function is also modulated by the presentation of their receptors on the surface of the cells. Although these GPCRs normally are in a monomeric form, they can also form dimers and higher-order oligomers, and, in this fashion, modulate chemokine function.[5,95] For example, both CXCR1 and CXCR2 bind CXCL8 and can form both homodimers and heterodimers with each other.[96,97] However, CXCR2 internalizes much faster than CXCR1 upon ligand binding.[98] This suggests that upon ligand binding, signaling through CXCR1 may remain active for a longer period of time than does signaling through CXCR2, which may compensate, at least in part, for the fact that CXCR1 is, in general, less abundant on the cell surface than CXCR2. CCR2, the receptor that binds CCL2, a chemokine critical in chemoattracting monocytes, is known to interfere with the function of CCR5 and CXCR4, the two chemokine receptors that serve as coreceptors for HIV entry into macrophages and lymphocytes, respectively. An antibody to CCR2 induces oligomerization of this receptor with CCR5 and CXCR4 and prevents HIV infection.[99] Another example is the DARC receptor that exists on the surface of cells in an oligomeric form and functions as an antagonist to CCR5 signaling through hetero-oligomerization.[100] When DARC hetero-oligomerizes with CCR5, CCR5-induced chemotaxis and calcium signaling are impaired, suggesting that DARC, although normally not a signaling receptor, can modulate chemokine receptor-dependent functions in addition to serving as a sink for chemokines.

Chemokine expression and function during wound healing in humans

Phases of normal cutaneous healing

Cutaneous wound healing occurs via sequential, overlapping phases, starting with hemostasis, followed by inflammation, re-epithelialization, granulation tissue formation, and remodeling (Fig. 4). Blood coagulation and clot formation occur rapidly after wounding to prevent blood loss, leading to hemostasis. During inflammation, leukocytes leave adjacent blood vessels and migrate to the site of injury, where they facilitate the removal of both microorganisms and cellular debris and secrete a plethora of cytokines that are critical for proper healing. The inflammatory phase is then resolved via decreased extravasation as well as increased removal of inflammatory cells by apoptosis. As inflammation is resolving, the proliferative phase is initiated, which involves both re-epithelialization, the process whereby epithelial cells proliferate and migrate to cover the wound, and formation of the healing/granulation tissue, a tissue characterized by proliferation of fibroblasts, angiogenesis, ECM deposition, and wound contraction. During the remodeling phase, the granulation tissue is converted to a mature scar; this occurs via cell apoptosis, regression of the neovasculature, removal of the transitional matrix, and deposition of new matrix molecules.

Expression/function during normal wound healing

Hemostasis. Immediately after injury, bleeding occurs, and platelets and plasma fibronectin are released along with prothrombin (Fig. 5). In the tissue, prothrombin is activated to form thrombin, which then cleaves fibrinogen to generate fibrin that with the platelets and the plasma fibronectin forms the clot. Of the injury-response chemokines, CXCL4 participates in the coagulation process and prevents the premature development of blood vessels. In addition, *CXCL4L1*, a nonallelic gene variant of CXCL4 inhibits angiogenesis during wound healing that is more potent than CXCL4 itself and

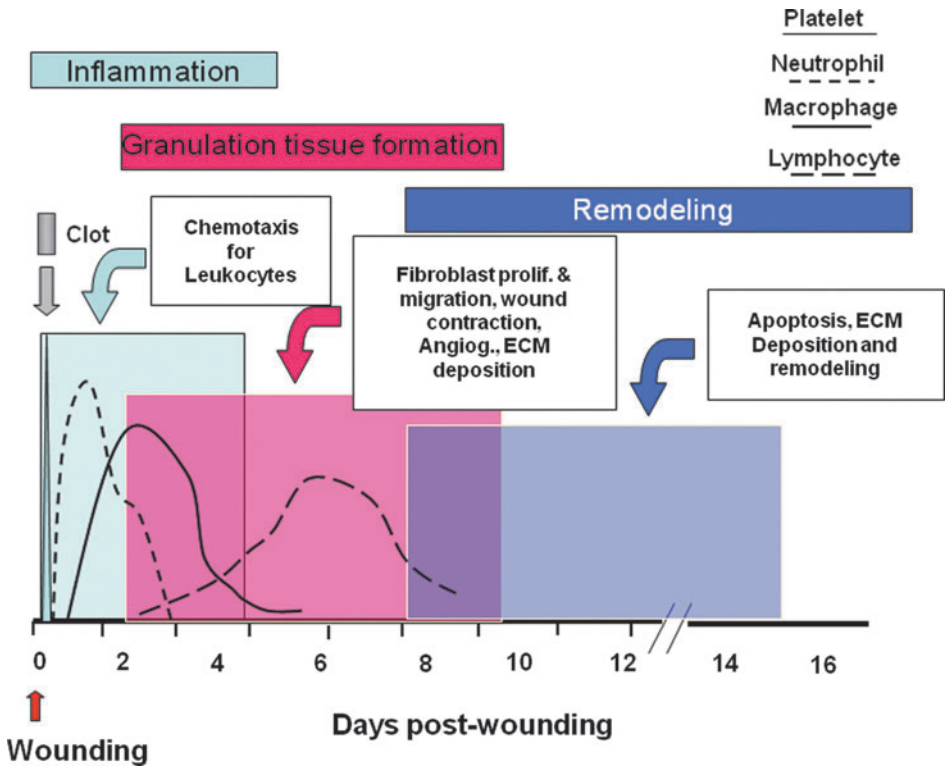

Figure 4. Phases of normal cutaneous wounds. Shortly after wounding and clot formation, the inflammatory phase of healing begins with neutrophils coming in first, followed by macrophages. This phase is followed by re-epithelialization and granulation tissue formation in which the keratinocytes migrate to cover the wound, and the wound tissue begins its repair by cell proliferation, ECM production, and blood vessel development. Finally, during the remodeling phase, much of the extracellular elements are removed by apoptosis, and the ECM is remodeled to produce the scar. ECM, extracellular matrix.

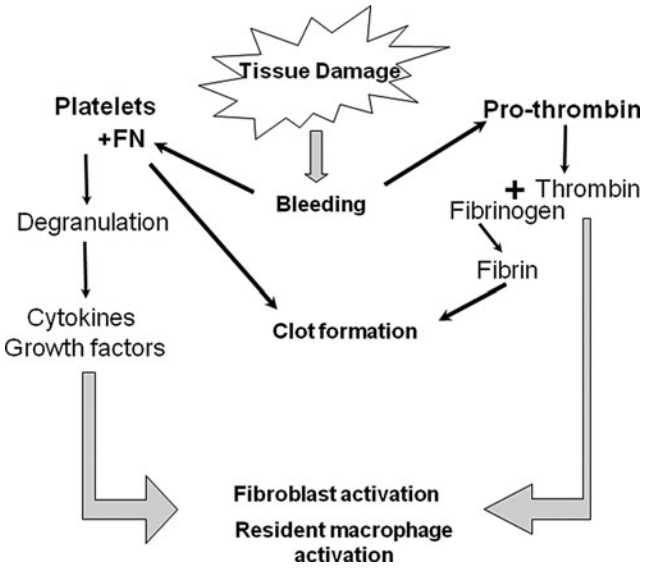

Figure 5. Immediate response to wounding. After tissue damage, bleeding occurs, and prothrombin is activated to thrombin, which cleaves fibrinogen in the tissue to make fibrin. Fibrin, in turn, with the fibronectin and activated platelets, forms the clot. The degranulation of the platelets releases cytokines and growth factors that, in conjunction with thrombin, activate fibroblasts and resident macrophages present in the tissue.

is very effective in inhibiting tumor growth of melanomas and lung carcinomas.[101] When the platelets are released, they degranulate and discharge a plethora of cytokines that, along with thrombin, activate resident macrophages, keratinocytes, and fibroblasts (Fig. 6). These activated wound cells produce a variety of inflammatory mediators, such as prostaglandins, leukotrienes, interleukins, growth factors, and cytokines that induce expression and activation of adhesion molecules in the endothelial cells of nearby blood vessels, and also increases the endothelial permeability.[102] The endothelial cell adhesion molecules E- and P-selectins mediate weak adhesion of leukocytes to endothelial cells, causing the leukocytes to roll along the endothelium. The endothelial intercellular adhesion molecule and vascular cell adhesion molecule then bind integrins expressed by neutrophils, the first leukocytes to arrive at the wound site, to mediate their firm adhesion, facilitating their migration through the endothelium.[103] Chemokines produced at the sites of injured tissue then promote the directional migration of these leukocytes to the wounded area (Fig. 7). In humans, two such proinflammatory chemokines are CXCL8 and CCL2.[28,29,104]

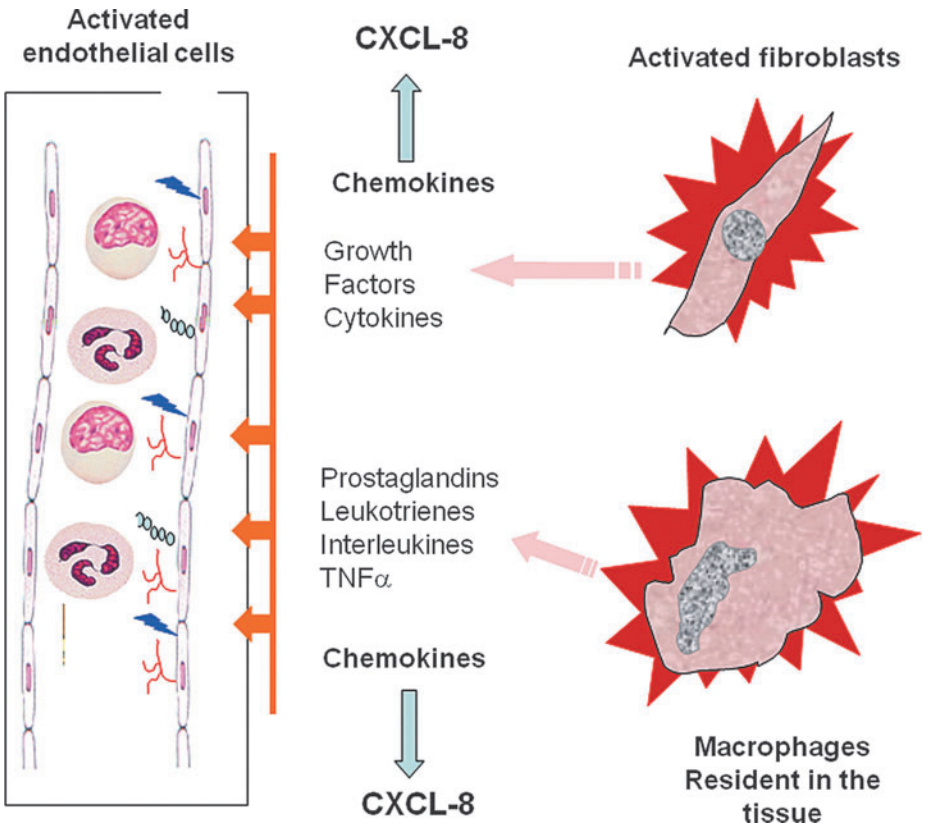

Figure 6. Chemoattraction of leukocytes into the sites of injury. Activated fibroblasts and macrophages produce more growth factors and cytokines along with prostaglandins, leukotrienes, interleukins, and TNF-α. These mediators stimulate endothelial cells to produce and/or activate cell surface adhesion molecules that capture the leukocytes. Simultaneously, chemokines produced locally chemoattract these leukocytes, generating the inflammatory phase of healing. TNF-α, tumor necrosis factor-α.

Inflammation. Like thrombin, CXCL8 also stimulates endothelial permeability through both modulation of adherin-junction endothelial cell adhesion and subsequent cell contraction, thereby facilitating leukocyte exit from the circulation.[76,105] Indeed, CXCL8 is an important mediator of pulmonary edema, a form of pathological permeability, after lung injury.[106,107] After extravasation, the inflammation-related chemokines induced shortly after injury (*e.g.*, CXCL1, 4, 5, 6, 7, and 8) chemoattract the endothelium-associated leukocytes to the site of injury. To achieve this, the chemokines form relatively stable gradients through interactions with proteoglycans, thereby promoting directional cell migration.[108–110] In humans, CXCL8 (and to a lesser extent CXCL1), is a key and potent chemoattractant for neutrophils through both the CXCR1 and CXCR2 receptors, and is a very effective stimulator of neutrophil activation through CXCR1, its more specific receptor.[111] Newly arrived neutrophils also secrete CXCL8, leading to a further neutrophil influx.[108] Once present, neutrophils employ various strategies to kill bacteria and decontaminate the wound, including the secretion of proteases and antimicrobial peptides, as well as generation of reactive oxygen intermediates via the respiratory burst.[112] Neutrophil apoptosis occurs spontaneously in the absence of inflammatory mediators; this spontaneous apoptosis appears to be mediated by cathepsin D release from neutrophil granules, which then facilitates the cleavage and activation of caspase 8, resulting ultimately in caspase 3 activation, DNA fragmentation, and apoptosis.[113] It is noteworthy that proinflammatory factors, including chemokines such as CXCL8, delay neutrophil apoptosis; this may result from a corresponding delay in cathepsin D release from neutrophil granules.[113] Other studies have shown that apoptotic neutrophils have increased the surface levels of the CCR5 receptor, which binds and sequesters chemokines and potentially also leads to neutrophil apoptosis.[114]

Neutrophil influx is followed by monocyte chemotaxis to the wound via CC chemokines, such as CCL2, which can be released by neutrophils at

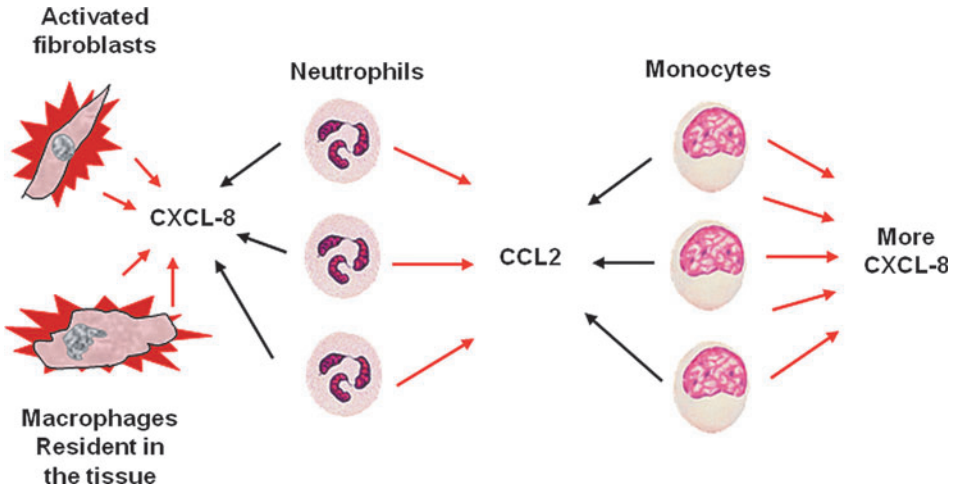

Figure 7. Early inflammatory phase of healing. Neutrophils are the first leukocytes to arrive at the sites of injury, where they eliminate infection and produce chemokines such as CCL2, which attracts monocytes from the blood into the tissue. These monocytes differentiate into macrophages that, in turn, secrete a plethora of cytokines and growth factors that are important in the development of the granulations tissue and, eventually, in attracting lymphocytes. Shortly after that, the final phase of healing, remodeling, ensues.

early times during the healing process, by the monocytes themselves, and by keratinocytes at later stages of healing.[108,115,116] In the wound tissue, monocytes differentiate into macrophages, which remove apoptotic neutrophils and other dead cells, function as antigen-presenting cells, and secrete cytokines and growth factors that promote later stages of inflammation and wound repair. Phagocytosis of the apoptotic neutrophils by macrophages then leads to removal of these chemokines from the area of inflammation, preventing further leukocyte influx. This phagocytic process also stimulates macrophage release of soluble FasL, which accelerates neutrophil apoptosis and may promote apoptosis of other leukocytes, potentially that of the macrophages themselves.[117] Recently, we have shown that vascular endothelial growth factor (VEGF), a growth factor that is produced during this stage of healing to stimulate angiogenesis, also stimulates the macrophages to express LIGHT, a member of the TNF-α family of cytokines, which binds to Lymphotoxin-β receptor and induces macrophage death.[118]

As the macrophage inflammation resolves, CCL3, 4, and 5 are then produced in the granulation tissue, which then chemoattract lymphocytes, the last type of leukocytes to arrive at the wound site. The lymphocytes then exert a specific response against microbes and other foreign material in the wound, the B lymphocytes via antibody production in response to antigen binding to the B-cell receptor, and the T lymphocytes through production of cytokines and stimulation of cytolytic activity in response to the interactions between the TCR and major histocompatibility complex-bound antigen on antigen-presenting cells. T-cell activation and proliferation in response to TCR ligation are further dependent upon costimulatory signals from either the antigen-presenting cell, such as B7 proteins interacting with T-cell CD28, or from an adjacent T-cell. Lymphocyte-induced inflammation is then resolved by apoptosis when IFN-γ and TNF-α are produced at the wound site.

Re-epithelialization. Re-epithelialization is an important process during wound healing, and it occurs during inflammation and granulation tissue formation. Epidermal growth factor (EGF) and transforming growth factor (TGF-β) released by platelets stimulate the keratinocytes at the wound edge to proliferate and migrate to cover the wounded area. Additional stimulatory factors, including CXCL8, fibroblast growth factor (FGF), and keratinocyte growth factor, produced by neutrophils, macrophages, endothelial cells, and fibroblasts, maintain the proliferative and promigratory signals.[108] During the re-epithelialization process, the keratinocytes migrate beneath the provisional ECM, composed primarily of fibrin and fibronectin, with vitronectin, tenascin, and collagen type III present in lesser amounts. Keratinocyte interactions with the matrix molecules are mediated by their corresponding integrin receptors, and are required for re-epithelialization, which also depends on the secretion of new ECM proteins. For example, keratinocyte migration requires their *de*

novo secretion of laminin-5 (also called 332) at the leading edge, providing a substrate for the migration and proliferation of the keratinocytes.[119,120] In addition, re-epithelialization and wound healing require the activity of various proteases, including the serine protease plasmin. Re-epithelialization is significantly delayed in plasminogen-deficient mice,[121] likely due to the inability of keratinocytes to degrade and thus migrate between the fibrin matrix and the underlying dermal tissue, as mice deficient in both plasminogen and fibrinogen exhibit more normal healing.[122] MMPs are also important for normal re-epithelialization; wound treatment with a broad-spectrum metalloproteinase inhibitor significantly delayed re-epithelialization, and similar healing delays were observed in mice expressing a collagenase-resistant mutant collagen I. More recently, a series of studies have shown that the chemokine CXCL11 is produced by basal keratinocytes and promotes migration of undifferentiated keratinocytes through calpain activation, and thus plays an important role in the development of the epithelium after injury.[123,124] This chemokine functions through CXCR3; mice that are null for this receptor exhibit delayed re-epithelialization and basement membrane deposition after wounding.[125,126]

Granulation tissue formation. During granulation tissue formation, new blood vessels develop from pre-existing vessels through a multistep process known as angiogenesis. In this process, blood vessels adjacent to the wound site interact with a variety of angiogenic factors secreted by fibroblasts and macrophages (*e.g.*, CXCL8, VEGF, and basic FGF), keratinocytes (*e.g.*, CXCL8 and VEGF), and endothelial cells themselves (*e.g.*, CXCL8 and VEGF). These factors may be stimulated, in part, by decreased wound oxygen tension through the activation of HIF-1α.[127] Both CXCL8 and VEGF promote vascular permeability, a process known to occur early in angiogenesis, by inducing de-adhesion and contraction of endothelial cells lining these blood vessels.[76] CXCL8 stimulates angiogenesis by increasing the endothelial cell permeability[118] both *in vitro* and *in vivo* and by stimulating endothelial cell migration and tube formation *in vitro*.[77] Further, this chemokine also chemoattracts fibroblasts to the forming granulation tissue and induces their secretion of cellular fibronectin, tenascin, and collagen I.[128] Cellular fibronectin, in particular, is an important substrate for the migration of fibroblasts into the wound site.[9,128] Fibroblast proliferation, migration, and ECM deposition are further enhanced by TGF-β and platelet-derived growth factor, derived from tissue macrophages and/or activated platelets. TGF-β, in conjunction with CXCL8, then promotes the differentiation of a subset of fibroblasts in the granulation tissue into myofibroblasts, cells that secrete a variety of ECM components and express α-smooth muscle actin, enabling cell contraction that facilitates wound closure.[129,130] Myofibroblast differentiation also requires the interaction with cellular fibronectin containing the ED-A domain, as inhibition of either this form of fibronectin or the corresponding integrin receptors prevents TGF-β1–mediated myofibroblast differentiation.[131,132]

Remodeling. Remodeling of the wound tissue occurs over a prolonged time period and involves ECM turnover coupled with a significant decrease in cellularity, the latter of which results from the apoptosis of residual inflammatory cells and myofibroblasts as well as regression of the neovasculature.[129] Fibroblasts and myofibroblasts within the granulation tissue produce matrix-degrading enzymes that mediate the removal of the provisional matrix.[129] These cells also synthesize the matrix components characteristic of mature connective tissue, including fibronectin and collagens I and III.[133] Myofibroblasts undergo apoptosis during this time, which is thought to result from a release of mechanical tension after their contraction of the nascent matrix occurring during wound contraction and closure.[129,134] For example, *in vitro* release of mechanical tension in collagen gels containing myofibroblasts induced their apoptosis.[135] *In vivo*, splinting wounds in an open state prevent myofibroblast apoptosis; removal of the splints and release of the granulation tissue lead to increased apoptosis.[136] It has also been shown that chemokines that signal through the CXCR3 receptor are important in the processes involved in dermal and epidermal maturation during wound healing. For example, CXCL10 inhibits EGF-dependent fibroblast migration by inhibiting the detachment of the trailing edge of the cells through protein kinase A (PKA) and calpain activation.[137] Further, inhibition of CXCL11 during wound healing resulted in poor dermis–epidermis maturation with reduced basement membrane components and persistence of the provisional matrix in the dermis.[138]

Also occurring during this remodeling phase is regression of the neovasculature, a process associated with endothelial cell apoptosis.[139] The angiostatic chemokines, CXCL9, 10, and 11, are important in this process[140]; they are stimulated by IFN-γ and TNF-α, which are found in the wound

tissue at this stage of healing. The effects of these three chemokines on dissociation of newly formed blood vessels are mediated by CXCR3-B, a variant of the CXCR3 receptor that is expressed in a cell-cycle-dependent manner, which, upon ligand binding, inhibits endothelial cell proliferation. CXCL10 interaction with CXCR3 stimulates μ-calpain activation, resulting in cleavage of the cytoplasmic domain of integrin β_3 and also stimulates caspase 3, an enzyme critical in apoptotic events.[140] Stimulation of PKA and μ-calpain by this pair of chemokine/chemokine receptors also leads to the dissociation of the microvessels. In addition, myofibroblast-derived proteases present during the remodeling phase may facilitate the degradation of the prosurvival matrix molecules and/or growth factors, thereby decreasing endothelial cell survival by the removal of prosurvival stimuli and thus leading to vessel regression. Matrix degradation may also generate matrix fragments that induce apoptosis directly, as has been suggested for endostatin, a fragment of type XVIII collagen,[141] and for tumstatin, a fragment of type IV collagen.[142] In contrast to the cell survival effects of intact matrix components, these matrix fragments are thought to function as integrin antagonists that promote apoptosis.[143,144] Another potential cause of vessel regression is the synthesis of regression-inducing factors, including angiopoietin-2 (Ang-2). Ang-2 is produced by endothelial cells in an autocrine manner, and its expression is associated with endothelial cell apoptosis and vascular regression *in vivo*,[145,146] perhaps due to decreased vessel association with pericytes.[147,148]

These studies show that the roles of chemokines in injury repair are not confined to their well-characterized roles in leukocyte chemotaxis and angiogenesis. It is becoming clear that chemokines also play an integral part in regulating the organization of the healing wounds, as well as its re-epithelialization (Fig. 8).

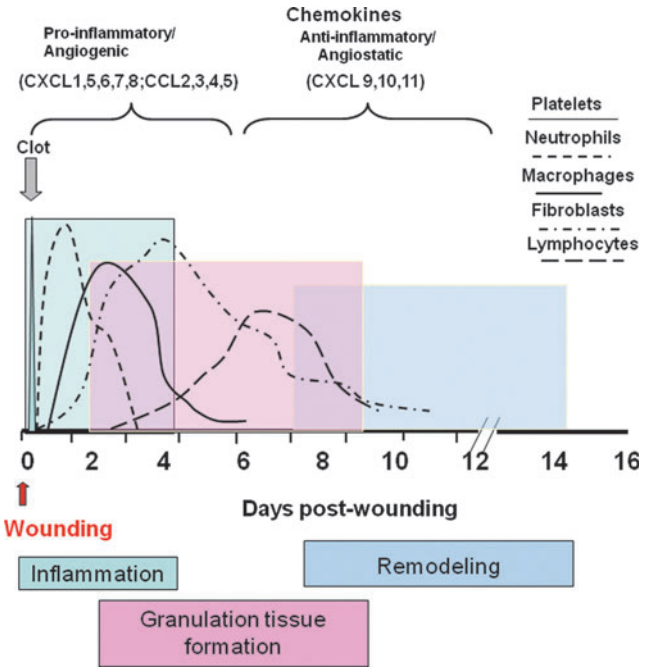

Figure 8. Chemokines and their roles in the various phases of wound healing. During clot formation, a series of events that lead to the inflammatory phase of wound healing occurs. Platelet granules release a number of molecular mediators, among them the chemokine CXCL4, which is anti-angiogenic and ensures that blood vessels do not develop prematurely. CXCL1 and 8 are also produced shortly after wounding and chemoattract neutrophils, which rid the wound of bacteria and foreign materials. Neutrophils, in turn, produce CCL2, a potent chemoattractant of monocyte/macrophages. These leukocytes clean up the debris and dead neutrophils, and, along with other cells in the wound (*e.g.*, fibroblasts), produce a plethora of growth factors and cytokines, among which are the chemokines CXCL1, 5, 6, 7, and 8, and CCL2, 3, 4, and 5. CCL2 will continue to attract more macrophages until fibrinogen in the wound disappears, at which time macrophage arrival stops. At this time in the progression of the healing process, CXCL8, along with vascular endothelial growth factor, stimulates angiogenesis. CCL3, 4, and 5 are instrumental in bringing in lymphocytes that are the last major type of leukocytes present in the wound tissue. CXCL9, 10, and 11 are produced somewhat later during healing and are known to be anti-inflammatory and antiangiogenic or angiostatic. These chemokines are present in the right place at the right time to ensure that angiogenesis is terminated and inflammation is resolved.

Expression/function of chemokines during impaired wound healing

As described above, normal wound healing requires the sequential stimulation and resolution of multiple phases, which, in turn, depend on the appropriate presence and function of different cell types under the influence of numerous regulatory molecules, many of which are chemokines. Alterations in any aspect of this process, including the types of molecules and cells present, their duration, localization, and/or function, can result in pathological healing states, such as in the various types of chronic wounds and in excessive healing such as in fibrosis, keloids, scleroderma, and psoriasis.

After the inflammatory cells have cleared the wound of microorganisms and cellular debris, the emigration of inflammatory cells from the blood vessels must be halted, and wound inflammatory cells must be removed, leading to the resolution of inflammation. The removal of neutrophils and macrophages is particularly important: neutrophils because of their production of tissue-damaging proteases and reactive oxygen intermediates,[149] and macrophages because of their secretion of inflammatory cytokines and proteases as well as stimulation of T-cell activation. Therefore, failure to remove these cells can lead to excessive tissue

damage and deterrence of the healing process that can then lead to impaired healing.

Nonhealing and chronic wounds. A number of chemokines have been shown to be involved in impaired wound healing. CXCL11 is important in dermal–epidermal interactions and in maturation of the healing tissue. When this chemokine was inhibited using an antisense technology, it was found that the dermis was immature and still contained provisional ECM molecules. In addition, re-epithelialization was delayed with the basement membrane being deficient in Laminin V and Collagen IV.[138] It has also been shown that CXCL12 is important in the development of the granulation tissue microvessels, and that the transactivation of this chemokine is dependent on HIF-1α activation.[150,151] Moreover, a decrease in CXCL12 has also been associated with poor healing after ionizing radiation-induced injury.[152] More recently, we have shown that deletion of the tumor necrosis factor superfamily member 14 (*TNFSF14*/*LIGHT*) gene leads to impaired wounds in mice.[153] These wounds show excessive production of three chemokines CXCL8, CCL2, and CXCL10, very early after wounding; this may be key to the abnormal initiation and resolution of inflammation. Indeed, the high levels of CXCL10 early in the healing process may be a critical characteristic of impaired healing, because the normal function of this chemokine is to attract lymphocytes to the wound tissue in the later stages of healing to finalize the process. The presence of lymphocytes at the same time as neutrophils, which produce a variety of enzymes designed to prevent infection, and macrophages, which produce a series of cytokines that are important in the development of the granulation tissue, most likely will cause confusion in the process that may lead to impaired healing. This is reflected in the fact that these wounds show defective basement membranes in which collagen IV and α smooth muscle actin are significantly decreased. The latter may explain the weak dermal–epidermal interaction and the leaky blood vessels we observe. Further, the granulation tissue is rich in Col III when it should have primarily Col I, and the latter, when present, does not form fibrils.

Regarding the role of chemokines in chronic wound development, there are a variety of reports indicating that some chemokines are elevated and others are decreased, and some of the reports are conflicting. However, a study performed in 1997[154] systematically examined the levels of chemokines in the wound fluid of venous ulcers that were undergoing treatment. These investigators found high levels of the angiostatic chemokines, CXCL4 and 10, between weeks 0 and 3, but thereafter the angiogenic chemokines, CXCL7 and 8, were highest in the wound fluid. These changes favored healing. These investigators also found that the levels of the CC chemokines CCL2, CCL4, and CCL5 decreased with healing. Although these findings are only correlative, they suggest that chemokines may play a critical role in the pathogenesis of chronic venous ulcers through their angiogenic properties and their ability to promote and resolve inflammation.

Given this state of affairs, it is clear that to understand how the various chronic wounds develop, specific animal models for those wounds are needed. In our recently developed mouse model of impaired wounds (see above), we found that the impaired wounds become chronic when they are infected with biofilm-forming bacteria. Simultaneously, we found that the chemokine eotaxin was the primary chemokine elevated in the chronic wounds relative to the nonhealing wounds.[153] This chemokine is important in combating multicellular organisms, which in the case of chronic wounds could be envisioned that the biofilm is a multicellular organism.

Burns. It has been known for some time that young and aged burned mice show an increase in CCL2 at 1 day postburn.[155] It has also been shown that CXCL12 is expressed in the hair follicles and endothelial cells of the blood vessels in the area of the burn and in the eosinophils and mononuclear cells found in the wound bed.[156] Blocking CXCL12 resulted in improved healing with diminished inflammation and improved re-epithelialization. Moreover, CXCL1 and CXCR2 are stimulated during the healing of human burn wounds, especially during the processes of inflammation, epithelialization, and angiogenesis.[157,158] It has also been shown that CX3CR1 in burn wound healing is associated with dermal angiogenesis, and that when this chemokine receptor is missing, there is a decrease in the number of myeloid cells coming to the wound site and also decreased angiogenesis.[159]

Atherosclerosis. Atherosclerotic plaques are another example of nonhealing wounds in arteries. Inflammation is a critical component of this impaired healing, and chemokines are squarely in the center of this process. Many chemokines have been associated with the development of atherosclerotic lesions, including initiation.[160–162] Injury to the endothelial cells leads to the production of chemokines such as CXCL8 and CCL2 that are chemotactic for neutrophils and macrophages, respectively. These two types of leukocytes play critical roles in the initial

response to the endothelial cells. When neutrophils are activated and degranulate, they release peptides called α-defensins; these peptides have been found in the intima layer of atherosclerotic arteries, and when in circulation, they bind to low density lipoprotein (LDL) and make it more adherent to the endothelial cells.[163] Accumulation of LDL is a major feature of atherosclerotic plaque development. CCL2 and its receptor CCR2, however, have been the most studied chemokine/receptor pair in atherogenesis.[164] Knockout mice for these proteins in background mice genetically susceptible to developing atherosclerotic plaques develop much smaller lesions.[165] It has also been shown that statins inhibit CCL2 expression.[27] Finally, it has been shown that by inhibition of neutrophil and macrophage recruitment after angioplasty or stent placement through inhibition of CCR2, there was an inhibition of restenosis.[166] More recently, other chemokines have been implicated in plaque initiation and progression. CCL5 on endothelial cells interacts with CCR1, 3, and 5 on neutrophils, monocytes, and T-lymphocytes, inducing their firm adhesion and migration into the arterial wall. CX3CL1 interaction with its receptor, CX3CR1, has been found not only to be important in adhesion and migration of $Ly6C^{hi}$ monocytes into the arterial wall but also to promote the survival of macrophages.[160,161]

TAKE-HOME MESSAGES

- Chemokines are expressed in a temporal and spatial manner during wound healing and play many different functions during healing. Some are proinflammatory, some anti-inflammatory, some angiogenic, some angiostatic, and some play a role in remodeling. Because these processes occur sequentially during wound healing, this family of small cytokines could be considered one of the major regulators of healing. In addition, their activation of G-protein receptors is highly significant for the clinic, because these receptors are very amenable to the development of small molecules that serve as agonists or antagonists of function.
- Because chemokines are small proteins without modifications, their agonist/antagonist peptides can be made by recombinant means and directed to fold properly and be easily available for treatment.
- Although not much has been done in the clinic regarding the use of chemokines and/or their receptors as a basis for treatment, these molecules have great potential. Chronic inflammation and alteration in angiogenesis can potentially be reduced or eliminated by interfering with proinflammatory or angiogenic chemokines and/or their receptors by using (1) small molecules that interfere with receptor function; (2) modified antagonists of chemokines or their N-termini; (3) neutralizing monoclonal antibodies directed to either the chemokine or its receptor.
- More studies on the role of chemokines and their receptors need to be performed to use them effectively in the clinic.

Excess healing. In some excess healing conditions, the myofibroblasts fail to undergo apoptosis, leading to their prolonged presence in the wound and production of excessive ECM, resulting in fibrosis and abnormal scar formation. Indeed, keloids and hypertrophic scars (HTS) represent such a situation. They exhibit reduced fibroblast/myofibroblast apoptosis, suggesting that defective apoptosis in these cells may contribute to the excessive scarring seen in these conditions.[167,168] Keloids are scars that continue to grow due in part to proliferation of both fibroblasts and myofibroblasts and excess collagen production. Unlike normal scar, cells within the keloid show expression of both CXCL1 and CXCL2 and their receptors CXCR1 and 2.[169,170] Fibrosis due to injury or inflammation results in collagen accumulation and scarring that interferes with the ability of the skin to heal properly; it also interferes with the function of organs, such as the lung or kidney to function. Overexpression of chemokines is associated with many types of fibrosis. Elevated expression of CXCL8 and CCL3 in lavage fluid of lungs is characteristic of patients whose idiopathic pulmonary fibrosis is progressing and CXCL10 and CXCL9 are also upregulated in bleomycin-induced lung fibrosis.[171,172] CXCL1, 5, and 8 are overexpressed in fibrotic pancreatitis.[173] Neutralization of chemokine activity can greatly reduce the fibrotic accumulation in these diseases. For example, inhibition of CCL3 and CCL2 in bleomycin-induced lung fibrosis significantly reduces inflammatory cell accumulation and fibrosis.[174,175] Likewise, it has recently been shown that wounds made in mice deficient in CXCR3 have abnormal healing that leads to scarring. This defect was reversed when wild-type fibroblasts were added to the wound. These wounds contained the ECM that resembled that of the wild-type mice and healed normally.[176] It has also been shown that CXCL12 is elevated in HTS, and that interactions with its receptor, CXCR4, result in signaling pathways that are involved in the development of HTS by promoting migration of the $CD14^+$ and $CXCR4^+$ cells from the blood circulation into the wound tissue. These cells differentiate into fibrocytes and myofibroblasts, leading to development of HTS.[177]

Scleroderma is another condition in which excess fibrosis develops. It is an autoimmune disease in which fibroblasts produce excessive ECM, especially collagens, resulting in fibrosis of the skin and

other organs. Depending on which organs are affected, scleroderma can be fatal. Several chemokines have elevated expression associated with scleroderma. In culture, the basal production of CXCL8 was significantly increased in scleroderma fibroblasts compared to controls, and these fibroblasts produce CXCL1 that, in turn, chemoattracts macrophages. Serum levels of CXCL8, CCL2, 3, and 4 were significantly elevated in patients with scleroderma compared with normal individuals,[178–180] and the levels of CXCL8, CCL3, and 3 were higher in lung lavage fluids from scleroderma patients, especially those with lung complications.[181–182] In skin samples from affected patients, the expression of CCL2 and 5 is high with CCL2 being expressed in the epidermis, inflammatory mononuclear cells, and vascular endothelial cells, and CCL5 in the epidermis.[183,184] Although no expression of CCL2 was found in healthy individuals, this chemokine was expressed in uninvolved as well as involved skin areas from 10 of 11 scleroderma patients.[185,186] Interestingly, exogenously administered CCL2 stimulated expression of its mRNA in scleroderma fibroblasts, but not in normal fibroblasts.[185] Because most of these chemokines are not expressed in normal tissue, their presence may cause some of the pathology of this disease. In fact, a genetic predisposition may be partially explained by the significant association between scleroderma and two polymorphisms occurring close to each other in the *CXCR2* gene.[186]

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.

ABOUT THE AUTHORS

Manuela Martins-Green came to the United States on a Fulbright Fellowship, received a PhD from the University of California–Davis, was a National Research Service Awardee at the Lawrence Berkeley National Laboratory, and was an adjunct assistant professor at Rockefeller University before joining the University of California–Riverside (UCR) faculty in 1993. She is currently Professor of Cell Biology at UCR. Dr. Martins-Green is a Fellow of the American Association for the Advancement of Science (AAAS), has been President of the UCR faculty Senate, won both the Distinguished Service Award and the Innovative Teaching Award at UCR, and is currently on the Board of Directors of the Wound Healing Society (WHS) and of the University of California Institute of Global Health. She has been chair of several committees of WHS and is currently the newsletter editor. Dr. Martins-Green continues to pursue the role of chemokines in wound healing and angiogenesis, a field she pioneered in the late 1980s. Her research has been funded by NCI, NIGMS, NAIAD, AHA, and TRDRP, and has been highlighted four times since 2001 in the Press Book of the American Society for Cell Biology. **Melissa Petreaca** received a PhD from the Martins-Green laboratory in 2008. As a graduate student, she investigated the role of the chemokine CXCL8/IL-8 in endothelial permeability and the importance of vascular endothelial growth factor (VEGF) in inflammation resolution during wound healing, for which she received the WHS Young Investigator Award (2007). She then received the Wound Healing Foundation 3M Award to investigate the importance of TNFSF14/LIGHT in inflammation and wound healing. Dr. Petreaca is currently a Visiting Assistant Professor of Biology at Pomona College. **Allen Wang** is a graduate student in the Martins-Green laboratory studying the role of the chemokine CXCL12/SDF1α and its receptor CXCR4 as targets of the metastatic pathway inhibited by pomegranate juice and individual components of the juice.

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