Published in final edited form as:

Surgery. 2014 June ; 155(6): 961–973. doi:10.1016/j.surg.2014.02.006.

Chemokines and chemokine receptors: Update on utility and challenges for the clinician

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Chemokines, or chemotactic cytokines, represent a large family of secreted proteins that have a wide range of function in normal physiology. Chemokine functions include direction of immune cell trafficking, angiogenesis, and wound healing, all critically important to patients being considered for surgery or anticancer therapy. In recent years, perturbations in expression of chemokines or their cognate receptors have been associated with several inflammatory disorders and both solid-tumor and hematologic malignancies. As such, chemokines and chemokine receptors have made attractive targets for biomarker identification and drug discovery. To move chemokine laboratory science to the bedside application for biomarker and drug discovery, we will need a more detailed understanding of the mechanisms by which chemokines are expressed and function. Specific areas that require further study include the structure-function relationship of chemokines and their receptors, differential signaling of ligands, concentration gradient-dependent signaling, and the genetic or epigenetic mechanisms that regulate chemokine ligand or receptor gene expression.

Chemokines provide a powerful opportunity for translational investigation. Chemokines are secreted proteins that travel in the circulation, move through the parenchyma and extracellular matrix of tissues, and bind to and activate the extracellular domain of their cognate receptors present on individual cell types. Consequently, chemokines can serve both as potential biomarkers and as promising targets for pharmaceutical intervention. Similar to other cytokines, chemokines altered in expression during specific disease states can serve as useful diagnostic or prognostic biomarkers. For example, chemokines such as CCL2, CCL5, and CCL20 are potential candidate biomarkers in atherosclerosis, diabetes, and inflammatory diseases of the skin and gut. Herein, we outline the numerous inflammatory diseases and cancers associated with aberrant chemokine expression and activity (Table). Our laboratory is currently investigating the role of several chemokines in pancreatic cancer biology, where inflammation is a key component of tumor initiation, growth, and possibly metastasis. We also discuss the existing opportunities for pharmaceutical targeting of aberrant chemokine activity to alleviate disease. In particular, the chemokine CXCL12 is a potential

target for pharmaceutical intervention in several malignancies, including colorectal, breast, and lung cancer. Finally, we explore the emerging pharmaceutical strategies to target more specifically chemokine signaling, leading to a more favorable therapeutic profile.

BIOCHEMISTRY

Relatively small in size (8–14 kDa), chemotactic cytokines are soluble proteins produced and secreted by a variety of cells. The chemokine ligand family currently has 48 characterized members that can be subdivided into 4 groups on the basis of secondary structure involving spacing between conserved cysteines in the N-terminus of the molecule: CC, CXC, CX₃C, and C.^{1–4} Chemokine ligand expression can be inducible or constitutive, whereas some ligands are expressed under conditions of inflammation in certain cell types but homeostatically expressed in others. CXC chemokines can be subclassified by the presence or absence of a glutamic acid-leucine-arginine (ELR) motif preceding the first amino-terminal cysteine residue.⁵

Chemokine receptors are composed of seven transmembrane domain-containing G protein-coupled receptors (GPCRs) and are named on the basis of the secondary structure of their respective ligand binding partners.¹ Approximately 40 kDa in size, chemokine receptors signal through several G protein subunits, leading to a diverse array of effectors.⁶ The conventional signaling pathway attributed to chemokine receptors involves the mobilization of calcium from intracellular stores. Release of calcium is rapid and typically sensitive to blockade of the *G α i* subunit of the heterotrimeric G protein complex. Additional signaling targets have been detailed, and include, but are not limited to, phospholipase C and monomeric GTPases, the mitogen-activated protein kinase and phosphatidylinositol 3-kinase pathways, and several tyrosine kinase pathways.^{7–13}

Although several signaling effectors for chemokines have been identified, their exact mechanism of action and the resulting physiologic functions within specific cell types remains ambiguous. Signaling may be cell-type specific, reflect receptor dimerization, or be altered by unique physiologic contexts, including inflammation, tumor microenvironment, embryogenesis, or angiogenesis. Moving forward, several intracellular and extracellular signaling effectors of chemokines are still being uncovered, in particular with respect to varying diseased states, with the use of unbiased approaches. An example of such a discovery-based approach is the recent mass spectrometric examination of total cellular phosphoproteomic changes after CXCL12 stimulation of T cells.¹⁴

Chemokines bind to the extracellular components of their cognate receptors, which elicits distinct signaling events dependent on each unique chemokine and receptor pairing.^{15,16} Unique pairings of ligands and receptors afford chemokines an assortment of physiologic functions. In some cases, ligands such as CCL2 or CXCL8, are capable of binding to several different receptors.¹⁷ In other situations, such as with CCL20-CCR6, single ligands bind exclusively to a single receptor.

Regardless of the exclusivity of binding partners in a particular chemokine signaling axis, studies in which the authors examine expression levels of chemokines or receptors must evaluate all members of a particular signaling partnership simultaneously. Because

chemokines direct cell function in a concentration-dependent manner, information regarding chemokine expression must be viewed with this understanding. For example, studies revealing expression of a particular chemokine receptor, while disregarding expression status of the receptor's binding partners, offer little scientific or clinical impact because they may underrepresent a majority of the signaling effects. Thus, any conclusions drawn from such clinical studies examining a single component of a chemokine axis are of limited utility. The delicate balance between available chemokine ligands and cell-surface levels of chemokine receptors is crucial to normal physiologic functions. This veritable "Goldilocks" effect of chemokines---too much or too little chemokine signaling can lead to unintended consequences and exacerbation of disease---can occur as the result of shifts in either ligand or receptor expression. Thus, the complementary nature of chemokine ligands and receptors must be a central consideration for any future studies of chemokine biology and particularly any putative pharmaceutical strategies. Herein, we briefly summarize the known roles of chemokines in human disease pathophysiology and discuss potential clinical implications while also considering joint ligand-receptor relationships.

CHEMOKINE PHYSIOLOGY

Chemokines in immunity

Chemokine-based mediation of cell movement has been characterized in nearly every cell type, although the physiologic function most associated with chemokine stimulation is recruitment of immune cells.¹⁵ Different sets of chemokines are capable of directing the trafficking of unique immune cells, both in inflamed and homeostatic conditions. Constitutively produced chemokines are involved in recruitment of immune cells under nonstressed conditions. These homeostatic chemokines function to repopulate tissues that store immune cells, such as lymph nodes or Peyer's patches.^{16,18} For example, the chemokine CCL21, expressed highly in endothelial cells in lymph node venules, mediates the emigration of T cells from lymph vessels to secondary lymphoid tissues.¹⁶

By contrast, during pathogenic inflammation, such as with infection, trauma, or cancer, production of an alternate set of chemokines may be induced.¹⁹ The production of such inducible chemokines typically is regulated by cytokine production in the local environment of inflammation.²⁰ Inflammatory cytokines such as tumor necrosis factor and interferon- γ induce transcription of a wide variety of chemokines, including CCL2, CCL3, CCL4, CCL5, CCL25, CCL27, CXCL9, and CXCL10.^{21,22} These chemokines then attract activated cells of both the innate and adaptive immune compartments.²³ Importantly, chemokines direct the trafficking of immune cells through both the circulation, in either blood, lymph vessels, and tissue, including environment-specific stroma and extracellular matrices. Unchecked expression of several chemokines, including CCL17, CCL19, CCL20, CCL21, CCL22, CCL25, CCL27, and CCL28, is associated with many different autoimmune and inflammatory disorders, such as colitis and dermatitis (Fig. A).^{22,24}

Chemokines in angiogenesis and embryogenesis

Chemokines are well characterized in their ability to regulate angiogenesis.²⁵⁻²⁷ Although not absolute, the presence or absence of the ELR motif in CXC chemokines correlates with

the ability to be either angiogenic or angiostatic, respectively.²⁸ ELR motif-containing angiogenic chemokines include CXCL1-3 and CXCL5-8, which bind to CXCR1 and CXCR2. Direction of individual events of angiogenesis by these chemokines is partially facilitated by differential binding to each receptor.²⁷ For example, although several ligands can engage both receptors, only ligand binding to CXCR2 leads to endothelial cell migration.²⁹ In addition, the CXCL12-CXCR4 ligand-receptor pair is an independent axis that is angiogenic.^{26,30} Angiostatic chemokines include CXCL4, CXCL9, CXCL10, and CXCL11. CXCL9, CXCL10, and CXCL11 play additional roles outside of angiogenesis, including T-cell chemoattraction via CXCR3 binding.³¹ The balance of available proangiogenic ELR⁺ and angiostatic ELR⁺ chemokines regulates several functions of endothelial cells, including migration, invasion, proliferation, and endothelial tube formation.^{26,27}

Chemokines clearly play a role in neovascularization during embryogenesis, such as vascularization of the gut through the CXCL12-CXCR4 axis, but the functions of chemokines during embryonic development are not limited to the vasculature.³² The CXCL12-CXCR4-CXCR7 axis has been implicated in the development of hematopoietic cells, neurogenesis, cardiogenesis, stem cell homing, receptor-specific primordial tissue organization, and endodermal to mesenchymal cell-cell communication.³³⁻⁴¹ More recently, CX3CL1, CCL14, and CCL4 have been implicated in fetomaternal interactions by directing trophoblast migration along maternal endometria.⁴²

Chemokines in wound healing

Wound healing represents a complex reaction,⁴² typically involving the epithelial barrier of a given organ surface. Chemokines play an integral part in directing wound closure and healing. Processes such as angiogenesis and recruitment of immune cells, outlined previously, require chemokine stimulation and are similarly involved in wound healing and the response to inflammation.⁴³⁻⁴⁵ Epithelial cells also express a wide variety of both chemokine ligands and receptors involved in several steps in the reconstitution of the epithelial barrier. Epithelial cell functions regulated by chemokines during wound healing include cell migration, extracellular matrix deposition, cellular adhesion, and cell proliferation.^{7,46,47} Chemokines, such as CXCL12, CXCL1, and CXCL8, guide these steps of re-epithelialization in tissues such as the gut and skin.^{47,48} As mentioned previously, unchecked expression of inducible chemokines normally involved in immune cell trafficking can lead to chronic inflammatory states, such as is the case with colitis or dermatitis. Similarly, the dysregulated expression of chemokines specifically involved in epithelial wound healing can lead to pathologic states, as occurs with CXCL12 expression in patients with inflammatory bowel disease (Fig. A).⁴⁹ The current theory is that abnormally high levels of CXCL12 disrupts the balance in function of the chemokine, perhaps shifting the ligand from epithelial-centric functions in intestinal epithelial cell migration and/or maturation during wound healing to stimulating recruitment of immune cells to the lamina propria (Fig. A).^{47,49}

Emerging concepts in chemokine biology

As seen in the processes of wound healing, chemokines direct not only the act of cell movement, but many other cellular functions, such as proliferation and cell-cell or cell-matrix adhesion. Chemokines regulate the entire process of cellular trafficking into tissues, including intravasation, gradient-dependent migration, cell-cell adhesion, and extravasation. During cell migration, chemokine receptor binding leads to signaling events that cause changes in the cell machinery that facilitate directed cell movement.⁷ When cells near their destinations and begin interactions with the endothelium, chemokines regulate the transitions that take place to allow the migrating cell to interact with the vascular surface and undergo transendothelial migration.⁵⁰ For example, the shift from selectin-mediated endothelial interaction to integrin-mediated adhesion required during the extravasation process is mediated by chemokines.^{51,52} The mechanisms behind the ability of chemokines to regulate multiple distinct cellular functions, such as migration and adhesion or proliferation and apoptosis, have yet to be fully explored. The observation that chemokines stimulate migration in a biphasic, concentration-dependent manner is an important first step in understanding how chemokines are able to dually regulate cell function.⁵³ CXC chemokines such as CXCL12 have an equilibrium concentration for stimulating migration, beyond which the oligomeric chemokine does not stimulate migration.⁵⁴ It is, therefore, possible that ligand concentration gradients play key regulatory roles in the ability of chemokines to stimulate both migratory and nonmigratory functions.

Specific chemokine axes provide unique opportunities to examine chemokine-driven nonmigratory functions. For example, the functional significance of the near-ubiquitous expression of CXCL12 in normal epithelial cells was previously unknown.⁵⁵ More recent studies suggest that CXCL12 expression may serve as a checkpoint for preventing malignancy; expression of the ligand is a natural stop-gap that is epigenetically silenced in cancer progression and metastasis from primary tissues such as colon, breast, lung, or bone (Fig. *B* and *C*).^{56–59} In particular, re-establishment of CXCL12 expression in colorectal cancer cell lines leads to detachment-based apoptosis or anoikis.^{8,60} As an additional example, CXCL14 has more direct effects in disease prevention.^{11,57,61,62} CXCL14 expressed in the epithelium of a variety of tissues has direct antimicrobial effects during bacterial infection as well as the ability to drive necrosis after forced re-expression in lung cancer.^{57,63} As proteins whose functions occur at the interface between disease and normalcy, CXCL12 and CXCL14 represent unique opportunities for the development of disease-specific biomarkers and pharmaceutical targeting.

CHEMOKINE PATHOPHYSIOLOGY

Chemokines as indicators of disease (biomarkers)

Attention is focusing on the interplay between ligand and receptor expression that influences the ability of individual chemokines to differentially signal. With regard to clinical impact, knowledge of relative concentration levels of particular chemokines may be just as important as determining which chemokines are expressed. Shifts in ligand or receptor expression reflect the basic biology underlying a particular disease. Diseases characterized by local overstimulation of chemokines can lead to pathologies that involve nonspecific

localized cell migration, such as is the case with atopic dermatitis. In contrast, pathologically low local chemokine concentrations, such as is the case of CXCL12 in many cancers, could facilitate the emigration of cells from the local environment, potentially through the physiologically established endocrine gradients. These contrasting mechanisms for disease are discussed in more detail herein (Table).

Chemokines in inflammation

Autoimmune diseases are characterized by a state of unchecked inflammation and increased immune cell infiltration into the tissue parenchyma. Diseases such as Crohn's disease or ulcerative colitis have been shown to have increased expression of the chemokines CCL19, CCL21, CCL25, CCL28, and CXCL8, each of which was correlated with increased mucosal lymphocyte and leukocyte infiltration.^{64–68} Similar examples are found in inflammatory diseases of the skin, with the chemokines CCL17, CCL20, CCL22, CCL27, CXCL9, and CXCL10 thought to regulate the chemoattraction of various dermal infiltrating effector cells.^{69–73} In both psoriasis and atopic dermatitis, excessive recruitment of T cells is driven by chemokines such as CCL17, CCL20, CCL22, and CCL27. In chronic diseases such as type-2 diabetes, multiple sclerosis, and atherosclerosis, increased expression of the chemokines CCL2, CCL5, CXCL9, and CXCL10 correlate with increased infiltration of macrophages and lymphocytes in lesions and plaques.^{74–79} In each of these diseases, permanently increased levels of specific chemokine ligands contribute to ongoing immune cell infiltration.

As summarized by Zlotnik and Yoshie⁴ in the case of CXCL10, pathologically altered levels of chemokines can serve as useful biomarkers. Hepatitis C patients with increased CXCL10 levels in sera demonstrate poor long-term response to standard treatments.⁴ In contrast, CXCL16 levels were found to be decreased in the sera of patients after episodes of either angina pectoris or myocardial infarction compared with healthy adults, although no known mechanism has been proposed for this cardioprotection.⁷⁸

Chemokines in cancer

In contrast, in diseases such as cancer, increased expression of chemokines may lead to immune cell filtration that may correlate with increased survival. After the seminal paper by Müller et al.⁸⁰ that drew attention to the connection between chemokines and cancer, subsequent reports have focused on the role of chemokine receptors in cancer progression while largely ignoring the expression profile of the chemokine ligands that must bind to those receptors to allow for downstream signaling. The paradigm established by the report by Muller et al suggested that analyzing chemokine receptor expression identifies malignant cells capable of metastasis. Since then, CXCR4 expression has been correlated with a variety of epithelial cancers, including skin, breast, ovarian, colorectal, and pancreatic cancers, as well hematologic malignancies such as acute lymphoblastic leukemia and chronic myelogenous leukemia.^{3,81} Clinical correlations focusing solely on CXCR4 receptor expression, however, are mixed, with relatively weak links between increased expression and poor prognosis in breast or colon cancer.^{82–85}

Recent studies have begun to analyze chemokine ligand expression in the context of cancer. As described previously, the epithelial expression of CXCL12, the cognate ligand for CXCR4, is suppressed in several cancers. Importantly, CXCL12 expression is correlated with improved clinical outcomes in osteosarcoma and breast cancer.^{58,86–88} Studies using mammary, melanoma, pancreatic, and colorectal cancer cell lines demonstrated that endogenously or exogenously reintroducing CXCL12 prevents tumor cells from homing to sites of common metastasis such as the liver or lung (Fig. *B* and *C*).^{9,56,59,89} Thus, in the case of colorectal cancer, decreased autocrine expression of CXCL12 in malignant cells may allow the cells to follow paracrine (localized) and endocrine (distant) gradients and circumvent a physiologic fail-safe mechanism for cells that become non-adherent (Fig. *B* and *C*).

Other examples in which increased chemokine expression is associated with improved clinical outcomes in cancer patients include CXCL13, CXCL14, and CXCL16.^{57,87,90} Although the mechanisms for the apparent benefits provided by these chemokines are largely unknown, some evidence has been uncovered for CXCL16; increased levels of CXCL16 secreted by tumor cells and detected in either tumor tissue or patient sera lead to increased infiltration of effector T cells in breast, colorectal, and renal cancers.^{90–92} The invading immune cells allow for an antitumor effect, leading to significant tumor regression. In breast cancer in particular, CXCL16 expression also was increased after ionizing radiation, suggesting that the induced chemokine expression facilitates infiltration of cytotoxic T cells and a successful anti-tumor cell immune reaction.⁹²

Increased expression of receptors such as CCR6 and CCR7 correlates with liver and lymph node metastasis in several solid-tumor malignancies, such as colorectal cancer and melanoma, respectively.^{24,93} The role that their cognate ligands, CCL20 and the pair of CCL19 and CCL21, respectively, may play in tumor cell biology, however, is unknown. Given the importance of gradient concentration in the ability of cells to migrate in a directed fashion, a reasonable hypothesis would be that the cognate ligands for CCR6 and CCR7 are repressed in cancers that have metastasized to liver or lymph nodes (Fig. *B* and *C*). Depending on the original expression of the ligands in precancerous cells, further study of these ligands may provide a novel opportunity for discovering biomarkers linked with disease progression.

Future studies may be best designed to analyze multiple chemokine pathways simultaneously.⁹⁰ Using arrays to analyze expression in either human cell lines or tissue may be a better way to elucidate the specific chemokine genes that are most altered in expression relative to others.

CHEMOKINES AS PHARMACEUTIC TARGETS

Eliciting cell movement can lead to both positive and negative physiologic outcomes. Efforts to target chemokines will encounter the aforementioned “Goldilocks” effect that occurs in chemokine biology. Thus, approaches to targeting chemokine signaling designed to modulate disease should tread carefully between the balance to either enhance or inhibit aberrant chemotaxis.

Targeting chemokine receptors

The conventional model for targeting chemokine signaling stems from chemokine receptors being classified as members of the super family of GPCRs. Although numerous pharmaceutical agonists and antagonists exist for other families of GPCRs, most notably the β -adrenergic receptor family,⁹⁴ there has been little progress in the movement of inhibitors of chemokine receptors into the clinic in the last 15 years.

The most recognized example for pharmaceutically targeting of chemokine receptor is the small molecule inhibitor AMD3100 (plerixafor). AMD3100 was proposed originally for treatment of patients with HIV, given the ability of the virus to use CXCR4 as a coreceptor for internalization.⁹⁵ In clinical trials, however, treatment with AMD3100 had poor efficacy and resulted in dose-limiting cardiac side effects.⁹⁶ Subsequent studies established the ability of AMD3100 to mobilize hematopoietic stem cells because of the drug's ability to block localized CXCL12 signaling in bone marrow, thus allowing cells to enter the circulation.⁹⁷ This finding allowed AMD3100 to be proposed for use as part of short-term regimens for autologous stem cell transplantation applied to patients with leukemia or multiple myeloma.⁹⁸ In the case of AMD3100, the serendipitous consequences of systemically blocking chemokine receptor signaling were of benefit, albeit in a short-term setting, and was unrelated to the originally targeted disease. AMD3100 is one of many examples of attempts to block chemokine signaling through receptor inhibition.⁹⁹ The only currently approved clinical drug that targets a chemokine receptor with the goal of blocking a specific disease process involves the CCR5 inhibitor drugs. The CCR5 small molecular inhibitor UK-427857 (maraviroc) has been approved for use as an HIV coreceptor blocker in approved patients whose disease has proven resistant to conventional therapies.¹⁰⁰ Beyond the use of small molecule inhibitors, several neutralizing antibodies against chemokine receptors have been tested in early-phase clinical trials,⁹⁹ including an antibody designed for blocking CCR2 activity in a variety of inflammatory diseases.¹⁰¹

Novel targeting of chemokines ligands

Alternate strategies that target chemokine signaling include generation of inhibitors that antagonize ligand rather than receptor activity. For example, a neutralizing antibody for CXCL10 has shown promise in early-phase trials for the treatment of ulcerative colitis and rheumatoid arthritis.¹⁰² With the field in its infancy, several strategies for ligand activity manipulation through small molecules have been formulated.^{103,104} One such manipulation of strategy stems from the characterization of the sulfotyrosine binding pocket that is conserved in all chemokines.¹⁰⁵ Generation of inhibitors to the sulfotyrosine binding pocket lends potential versatility to targeting either individual chemokines or entire subgroups of ligands.

More recent strategies for targeting dysregulated signaling of chemokines in disease include the development of biologic or gene-based chemokine therapies. The rationale behind these forms of therapy is partly to restore physiologic gradient concentrations of chemokines rather than systemically negate signaling through protein inhibition. Introducing chemokines systemically could circumvent side effects that would occur from systemic blockade of chemokine receptor signaling, as occurred with AMD3100. In cases in which disease leads

to increased trafficking of cells in circulation, such as colitis, dermatitis, or cancer, recombinant chemokine would confuse the established abnormal gradients and arrest further cell movement.

Gene therapies offer one mechanism for locally reintroducing chemokines to treat human disease. Using vaccine or viral methods, the authors of several studies in mouse models have demonstrated the ability of gene-delivered chemokines to recruit immune cells to ameliorate disease, by the use of *CCL21* for melanoma,¹⁰⁶ *CXCL10* to improve HPC vaccine immune reaction,¹⁰⁷ and *CXCL12* or *CXCR4* to treat heart failure.^{108,109} A plasmid encoding *CXCL12* has shown clinical efficacy in treating patients with heart failure in a phase 1 clinical trial.¹¹⁰

Chemokines as biologic therapies wherein synthesized recombinant chemokines are administered directly to patients is an emerging pharmaceutical field. Preclinical studies in colorectal cancer and melanoma in which authors used several recombinant variants of CXCL12 have demonstrated the ability of biologic-based therapies to prevent tumor metastasis.^{9,89} The chemokines used in this study included monomeric and dimeric variants of CXCL12. These variants, representing the low and high ends of the chemokine concentration gradients that are believed to control biphasic migration, were able to regulate cancer cell migration in mouse models. The monomeric variant of CXCL12 is cardioprotective in rat models of cardiac ischemia.¹¹¹ Our model, underscoring the importance of concentration gradients, is supported by recent studies demonstrating that, in mice, dimeric CCL2 prevents CCR2-mediated recruitment of leukocytes,¹¹² and, in primate models, modified variants of CCL5 prevent sexual transmission of HIV.^{113,114} Given their ability to differentially manipulate chemokine-directed cell migration, future development of variants such as these will lead to more efficient pharmaceutical targeting of chemokine signaling.

In summary, at the clinical level, pharmaceutical targeting of chemokine signaling has yet to mirror the diversity of the β -adrenergic or neurotransmitter fields.⁹⁴ The conventional approach of antagonizing chemokine receptors has had limited success, underscoring the need for more novel approaches to generating chemokine pharmaceuticals. Chemokine ligand-based therapies, using biologics or small molecules, offer potential for treating diseases while avoiding many of the shortcomings of receptor inhibition.

In conclusion, the last decade has witnessed several key advancements in understanding the mechanisms behind chemokine activity in normal physiology as well as disease. However, critical gaps in knowledge still exist and require further study. The downstream signaling effectors of chemokines are still being defined, particularly when considering differing cell types, differing disease states, and nonmigratory functions. Similarly, the mechanisms behind the ability of cells to respond differently to varying concentrations of the same chemokine are not well understood. Further study of concentration-based signaling and resulting functionality, as well as structure and physiologic function of chemokine ligands, is necessary to fully understand the potential of alternative strategies for generation of chemokine specific pharmaceuticals. The concept of chemokine ligands existing in monomer-dimer equilibria, with each side of the equilibrium differentially influencing cell

function, is needed in in vivo settings to understand the relationship between varying biochemical quaternary states of specific chemokine ligands and the transition from health to disease. A greater shift toward the understanding of chemokine ligand pharmacology in vivo is required as opposed to the current paradigm of focusing attention on receptor-based activity alone. From a clinical perspective, expression analysis must include not only all components of a given axis at once but also multiple axes. Evaluating the expression of a single gene or a single axis may preclude the ability to detect the most effective biomarkers in a particular disease and ignore potential functional redundancies. At present, chemokines are poised to make substantive impacts as both biomarkers and the basis for novel pharmaceutical strategies in treating human disease.

Acknowledgments

We thank Dr Sam Hwang, Dr Brian Volkman, and Anthony Getschman for their critical evaluation and suggestions for the manuscript. We also thank Mary Bryson of Bryson Biomedical for her assistance with the figure illustrations. Dr Dwinell is co-founder with Dr Brian Volkman and Dr Francis Peterson of Protein Foundry, LLC (www.proteinfoundry.com), a producer of molecular grade chemokines for use in biomedical research. The authors have no other conflicts of interest to declare.

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Fig.

Schematic overview of chemokine function in intestinal epithelium. Pathologically established gradients of chemokine proteins lead to aberrant migration of cells in multiple ways. Herein, we describe the differing roles of chemokines by using the intestinal epithelium as a model tissue site of dysregulated chemokine production. (A) Acute or chronic inflammation resulting from epithelial barrier disruption, pathogen infection, or other stimuli, leads to increased chemokine secretion. As a result, new local chemokine gradients attract immune cells from the circulatory system into to the site of inflammation. (B) A contrasting gradient is seen during human cancer malignancy, specifically in the CXCL12-CXCR4 axis. Dysplastic lesions contain heterogeneous groups of cells, including some that lose expression of homeostatically produced chemokines, such as CXCL12. Within malignant tumors, those cells that lack CXCL12 ligand expression and retain CXCR4 receptor expression are able to migrate toward existing local gradients of chemokine expression. Primary sources of CXCL12 gradients include neighboring fibroblasts and endothelial cells. Thus, cancer cells lacking ligand expression are capable of following a new gradient into circulation. (C) Those cancer cells that lack CXCL12 ligand expression and survive in circulation can then migrate to distant organs with constitutively high CXCL12 expression, such as the liver, lung, or bone. Within the vessels of these distant sites, cancer cells then follow a new chemokine gradient established by both the endothelial and parenchymal cells, engendering metastases.

Table

Changes in chemokine ligand and receptor expression in human disease

Chemokine ligand	Chemokine receptor(s)	Human disease/ condition associated	Receptor change	Ligand change	Clinical finding
CCL1	CCR8				
CCL2	CCR2, CCR4	Atherosclerosis, type 2 diabetes	–	↑CCL2	↑ chemokine levels in plaques, adipose tissue, and circulation leading to macrophage recruitment ^{76,79}
CCL3	CCR2, CCR4, CCR5				
CCL4	CCR5, CCR8?				
CCL5	CCR1, CCR3, CCR4, CCR5	Atherosclerosis, multiple sclerosis	↑CCR1, ↑CCR5	↑CCL5	Inflammatory chemokine production leading to lymphocytic and monocytic infiltration. Infiltrating monocytes have ↑ CCR1 and ↑ CCR5 ^{74,77,78}
CCL6	CCR1, CCR2, CCR3				
CCL7	CCR1, CCR2, CCR3				
CCL8	CCR1, CCR2, CCR3				
CCL11	CCR3				
CCL12	CCR2				
CCL13	CCR2, CCR3				
CCL14	CCR1				
CCL15	CCR1, CCR3				
CCL16	CCR1				
CCL17	CCR4	Psoriasis, atopic dermatitis	–	↑CCL17	Keratinocytic/endothelial chemokine production leading to lymphocytic infiltration ^{71,73}
CCL18					
CCL19	CCR7	Colitis	–	↑CCL19	Mucosal CCL19 production leading to CCR7-dependent lymphocyte cell infiltration ⁶⁴
CCL20	CCR6	Psoriasis	–	↑CCL20	Epithelial CCL20 production leading to T-cell infiltration ⁷²
		Colorectal cancer	↑CCR6	?	↑ tumor expression of receptor correlated with liver metastasis ⁹³
		Colitis	–	↑CCL20	Mucosal CCL20 production leading to CCR6-dependent dendritic cell infiltration ²²
CCL21	CCR7	Colitis	–	↑CCL21	Mucosal CCL21 production leading to CCR7-dependent lymphocyte cell infiltration ⁶⁴
		Melanoma, gastric cancer, nonsmall cell lung cancer, esophageal cancer, chronic lymphocytic leukemia	↑CCR7	?	Tumor cell expression of CCR7 leads to lymph node metastasis ¹¹⁵

Chemokine ligand	Chemokine receptor(s)	Human disease/ condition associated	Receptor change	Ligand change	Clinical finding
CCL22	CCR4	Psoriasis	–	↑CCL22	Keratinocytic/endothelial chemokine production leading to lymphocytic infiltration ⁷¹
CCL23	CCR1				
CCL24	CCR3				
CCL25	CCR9	Colitis	–	↑CCL25	Mucosal chemokine production leading to lymphocyte cell infiltration ⁶⁵
CCL26	CCR3				
CCL27	CCR10	Dermatitis	–	↑CCL27	Keratinocytic CCL27 production leading to T-cell infiltration ⁷⁰
CCL28	CCR3, CCR10	Colitis	–	↑CCL28	Mucosal chemokine production leading to lymphocyte cell infiltration ⁶⁶
CXCL1	CXCR1, CXCR2				
CXCL2	CXCR2				
CXCL3 CXCL4	CXCR2				
CXCL5	CXCR2				
CXCL6	CXCR1, CXCR2				
CXCL7	CXCR1, CXCR2				
CXCL8	CXCR1, CXCR2	Colitis	–	↑CXCL8	Mucosal chemokine production leading to leukocyte cell infiltration ¹¹⁶
CXCL9	CXCR3	Psoriasis, multiple sclerosis	–	↑CXCL9	Inflammatory chemokine production leading to lymphocytic infiltration ^{71,74}
CXCL10	CXCR3	Psoriasis, multiple sclerosis	–	↑CXCL10	Inflammatory chemokine production leading to lymphocytic infiltration ^{71,74}
		Hepatitis C	–	↑CXCL10	Increased chemokine levels in sera correlates with poor response to standard treatment ⁴
CXCL11	CXCR3, CXCR7				
CXCL12	CXCR4, CXCR7	Colorectal cancer, breast cancer, osteosarcoma, lung cancer, pancreatic cancer	↑CXCR4	↓CXCL12	Parallel ↑ in receptor expression and ↓ in ligand expression in tumor cells lead to ↑ distant metastasis ^{56–59,80,88}
		Melanoma, ovarian cancer, acute lymphoblastic leukemia, chronic myelogenous leukemia	↑CXCR4	?	↑ expression of receptor in tumor cells ^{3,4}
CXCL13	CXCR5	Breast cancer	↑CXCR5	↑CXCL13	In HER2-positive patients, ↑ tumoral ligand and receptor expression correlates with ↑ survival ⁸⁷
CXCL14		Lung cancer	–	↓CXCL14	Chemokine is silenced in tumor cells, correlated with ↓ tumor necrosis ⁵⁷
CXCL15					

Chemokine ligand	Chemokine receptor(s)	Human disease/ condition associated	Receptor change	Ligand change	Clinical finding
CXCL16	CXCR6	Colorectal cancer, renal cancer, breast cancer	–	↓CXCL16	↑ tumoral ligand secretion (detected in tissue or serum) correlates with ↑ survival or ↑ radiation response ⁹⁰⁻⁹²
CXCL17		Myocardial infarction/angina pectoris	–	↑CXCL16	Decreased chemokine levels in sera after episodes ⁷⁸
XCL1	XCR1				
XCL2	XCR1				
CX3CL1	CX3CR1				