REVIEW

Chemokines: role in immune cell traffic

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INTRODUCTION

Motility is a hallmark of leukocytes. This property is of crucial importance for all aspects of immunity, and forms the basis for hematopoiesis and immune defense. Breakdown in the control of leukocyte mobilization contributes to chronic inflammatory diseases and, consequently, small molecular weight compounds that selectively interfere with leukocyte recruitment represent a promising approach for the development of novel anti-inflammatory medication. Chemotactic migration of leukocytes largely depends on adhesive interaction with the substratum and recognition of a chemoattractant gradient. Chemokines are secreted proteins of 67 to 127 amino acids and have emerged as key controllers of integrin function and cell locomotion. Numerous distinct chemokines exist, which target all types of leukocytes, including hematopoietic precursors, mature leukocytes of the innate immune system as well as naive, memory and effector lymphocytes. The combinatorial diversity in responsiveness to chemokines ensures the proper tissue distribution of distinct leukocyte subsets under normal and inflammatory/pathological conditions. Besides leukocyte chemoattraction, chemokines also influence migration-unrelated processes, such as angiogenesis, tissue remodeling and tumor metastasis.

CHEMOKINES, THE LARGEST FAMILY OF CYTOKINES

Interleukin-8 (IL-8/CXCL8), the first chemokine, was discovered 15 years ago on the basis of its neutrophil chemoattractant properties. The NH_2-terminal-two of four conserved Cys residues are separated by one amino acid, which typifies IL-8 as a CXC chemokine (Figure 1). The monocyte chemoattractant protein MCP-1/CCL2 with the two NH_2-terminal Cys residues in adjacent position is a CC chemokine and was discovered shortly after IL-8.

The vast majority of the approximately 50 human chemokines fall either into the group of CXC or CC chemokines [1, 2]. In addition, there are two C chemokines, Ltn-α/XCL1 and Ltn-β/XCL2, in which two out of the four conserved Cys residues are missing, and a single CX3C chemokine, called fractalkine/CX3CL1, which has three amino acids separating the two NH_2-terminal Cys residues. Two nomenclature systems are used in the current literature, the traditional abbreviations dating back to the time of chemokine discovery, such as IL-8 and MCP-1, and a systematic nomenclature that combines structural motifs (CXC, CC, XC, CX3C) with L for ligand and the number of the respective gene (http://cytokine.medic.kumamoto-u.ac.jp gives access to recent updates) (Figure 1) [3-5].

Chemokine receptors are designated according to the type of chemokine(s) they bind (CXC, CC, XC, CX3C), followed by R for receptor and a number indicating the order of discovery.

CHEMOKINE RECEPTORS

Chemokine receptors belong to the large family of seven-transmembrane domain receptors, which couple to heterotrimeric GTP-binding proteins (G-proteins) [3, 4, 6, 7]. Experiments with *Bordetella pertussis* toxin indicated that these receptors typically require G-proteins of the Gi-type for signal transduction [3, 4, 6, 8]. Biochemical and functional analysis with IL-8 and related chemokines demonstrated that neutrophils express two types of IL-8 receptors, CXCR1 with selectivity for IL-8 and GCP-2/CXCL6, and CXCR2 with promiscuous binding of IL-8 and numerous other related CXC chemokines [9, 10]. The subsequent cloning of the corresponding receptor cDNAs confirmed these early observations [11, 12]. There are considerable species differences, as demonstrated by the presence of a single IL-8 receptor in rabbits and mice but not in rats or humans, and the lack of an IL-8 homologue in mice but not in rabbits or humans. Sequence information about the first chemokine receptors quickly led to new discoveries, giving rise to a total of 18 currently known human chemokine receptors, including six CXCRs, ten CCRs, one XCR, and one CX3CR.

Downstream second messengers of G protein signaling include phospholipase Cβ (PLCβ) isoforms, Ser/Thr-kinases, phosphatidylinositol 3-kinase-γ (PI3Kγ) and c-Src-related non-receptor tyrosine kinases [6, 7, 13]. Cellular responses to chemokines are typically rapid in onset and transient in duration. The negative control mechanism elicited during chemokine receptor signaling is termed cellular desensitization and is, by itself, also transient, i.e. cells regain responsiveness to a given chemokine after a

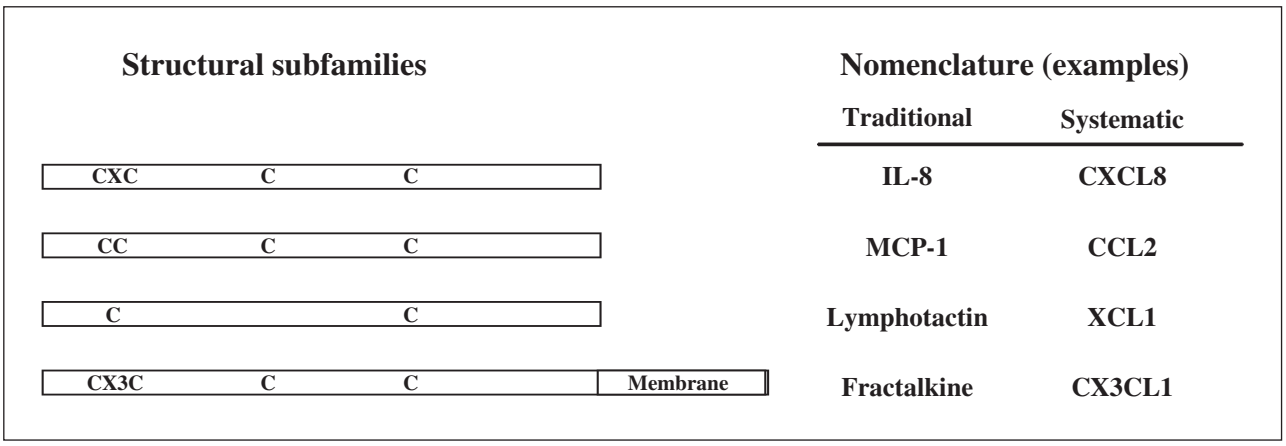

Figure 1

The chemokine family. Based on the arrangement of NH2-terminal Cys residues, the chemokines are divided into the structural subfamilies of CXC, CC, C and CX3C chemokines. For each subfamily, an example of a chemokine is listed by its traditional and systematic name. Currently, the human CXC, CC, C and CX3C subfamilies consist of 14 (CXCL1-14), 28 (CCL1-28), 2 (XCL1-2), and 1 (CX3CL1) members, respectively.

short period of culturing in chemokine-depleted medium. Homologous desensitization is mediated by G protein-coupled receptor kinases (GRKs), which block further signal transduction by phosphorylating Ser/Thr residues in the intracellular C-terminal domains of ligand-occupied chemokine receptors and other G protein-coupled receptors (GPCRs). Uncoupling from G proteins is also observed in a process called heterologous desensitization that involves phosphorylation of ligand-free (nonengaged) chemokine receptors or other GPCRs by alternative protein kinases. GRK-mediated receptor phosphorylation initiates β-arrestin binding, which leads to sequestration or clustering in specialized clathrin-rich membrane microdomains and endocytic uptake of chemokine-receptor complexes. Rapid internalization allows the continuous redistribution of chemokine receptors on leukocytes for maintaining polarized chemokine sensing and directed cell migration along a chemokine gradient.

An additional type of receptor-mediated control is the chemokine "decoy" function of certain chemokine receptors that are unable to induce signal transduction [14]. The Duffy antigen-related chemokine receptor (DARC) on red blood cells and the tissue-expressed D6 receptor, characterized by broad chemokine selectivity, may operate as chemokine scavengers. In addition, a chemokine "decoy" function may also be induced in normal chemokine receptors, as shown in IL-10-treated monocytes and DCs [15]. Perhaps, the switch from functional chemokine receptors to chemokine scavengers may contribute to a change in leukocyte recruitment during immune response progression and wound healing.

LEUKOCYTE MOBILIZATION AND OTHER CHEMOKINE FUNCTIONS

Chemoattractant activity and the "four Cys-residue" fingerprint arrangement prompted the term chemokines to designate this novel group of chemotactic cytokines [16]. The "classical" chemoattractant agonists lacking the chemokine-typical structural motifs, such as complement component C5a, lipid derivatives leukotrienes and platelet-activating factor, and bacterial N-formyl-methionyl-peptides, do not belong to the chemokine family.

In addition to the structural classification, chemokines are also grouped into functional subsets [6, 17]. Inflammatory chemokines control the recruitment of effector leukocytes in infection, inflammation, tissue injury and tumors. Many of the inflammatory chemokines have broad target cell selectivity and act on cells of the innate as well as the adaptive immune system.

Homeostatic chemokines, by contrast, navigate leukocytes during hematopoiesis in the bone marrow and thymus, during initiation of adaptive immune responses in the spleen and lymph nodes, and in immune surveillance of healthy peripheral tissues. Recent findings indicate that several chemokines cannot be assigned unambiguously to either one of the two functional categories and, therefore, may be referred to as "dual-function" chemokines.

Recruitment of circulating leukocytes to sites of pathogen entry or inflammation involves two separate migration processes, termed extravasation and chemotaxis (Figure 2). Adhesion to the luminal side of blood vessels, transendothelial migration and subsequent chemotaxis of leukocytes are highly complex processes, which are controlled by "outside-in" and "inside-out" signaling events during cellular interactions with chemokines and adhesion ligands.

Triggering of chemokine receptors in leukocytes by endothelia-associated chemokines is a requirement for extravasation and induces a rapid increase in integrin affinity/avidity, which results in firm but transient leukocyte adhesion [18]. Subsequently, the adherent leukocytes move across the endothelial cell layer and the underlying basement membrane and are released into the tissue. Of note, only those types of leukocytes are able to transmigrate at a given vascular site, which are capable of responding to the chemokines present on the local endothelium. In other words, chemokines and their receptors largely determine the selectivity in leukocyte extravasation [3, 4, 6, 17]. For instance, T and B cells with receptors for chemokines present in secondary lymphoid tissues, will not be recruited to peripheral tissues, but instead are destined to recirculate through spleen, lymph nodes and Peyer's patches. By contrast, effector leukocytes bearing receptors for inflammatory chemokines are efficiently recruited to sites of inflammation and disease. This leukocyte traffic

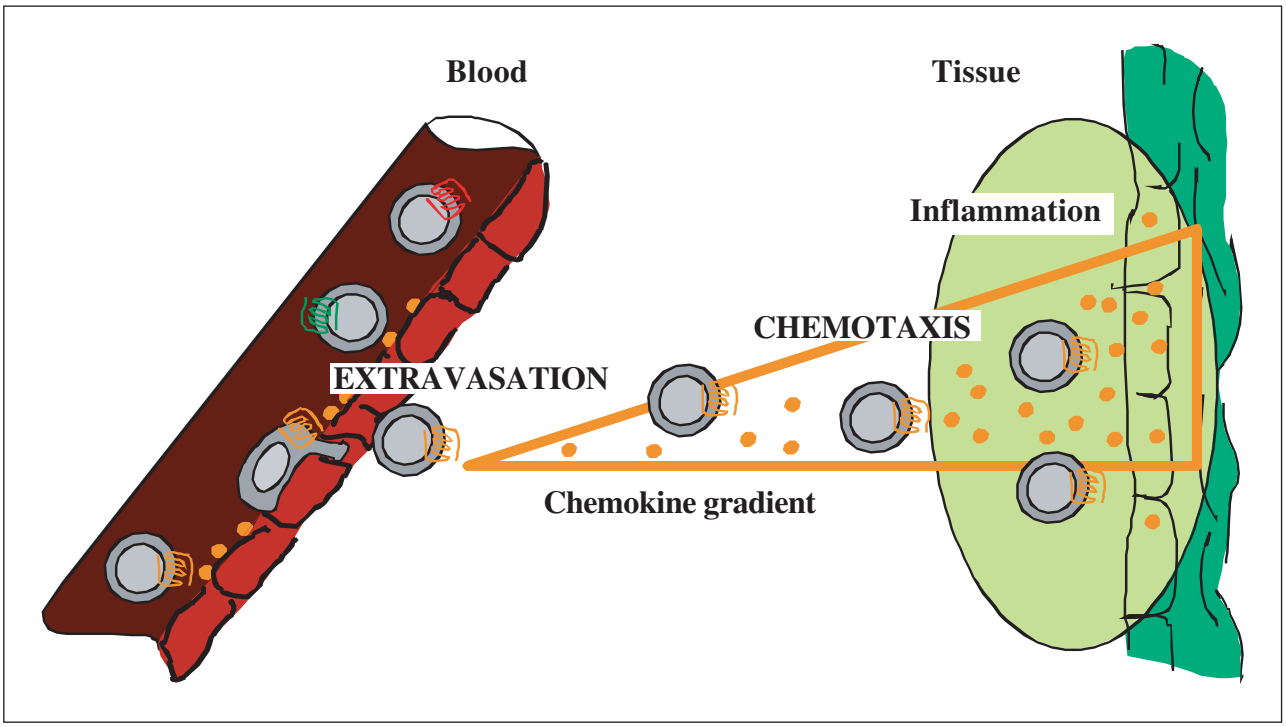

Figure 2

Recruitment and localization of circulating leukocytes. The recruitment process involves two distinct and sequential processes, termed extravasation and chemotaxis. During extravasation, blood leukocytes interact with adhesion molecules on the luminal side of blood vessels. To resist shear forces, integrin function is upregulated *via* chemokine receptor signaling, which leads to firm attachment of leukocytes to the blood vessel wall and subsequent transendothelial migration. Once in the perivascular area, leukocytes orient themselves according to chemokine gradients and migrate in the direction of increasing chemokine concentration.

control function forms the basis for the development of chemokine-based anti-inflammatory therapy (see below).

Chemokines are also thought to contribute to hematopoiesis by controlling the localization of leukocyte precursors within distinct microenvironments in the bone marrow and thymus. Of note, SDF-1/CXCL12 and its receptor CXCR4 were shown to be critically involved in myelo- and B lymphopoiesis whereas mostly dual-function chemokines and their receptors (including CXCR3, CCR4, CCR8, CCR9 as well as CXCR4) control thymocyte development [19-21].

Finally, recent reports have demonstrated the involvement of chemokines in tumor metastasis, a finding that seems to be related to the chemokine-typical control of leukocyte traffic [22, 23]. Possibly, chemokine-mediated tissue cell relocation or retention may also contribute to organogenesis, angiogenesis and tissue remodeling (see below).

Migration-unrelated chemokine activities include the modulation of leukocyte effector functions (cytotoxicity, mediator release, cytokine production), tissue cell growth, differentiation, and survival (organogenesis, angiogenesis), and human immunodeficiency virus (HIV) infection [2, 6, 24-27]. HIV-1 requires the co-receptors CCR5 and CXCR4 for entry into CD4+ target cells and the chemokines, which bind to these co-receptors (RANTES/CCL5, MIP-1α/CCL3 and MIP-1β/CCL4 for CCR5, and SDF-1 for CXCR4), interfere with HIV infection. In infected individuals, the level of these HIV suppressor chemokines correlates indirectly with disease progression, suggesting that inhibitors for CCR5 and CXCR4 may be used in the treatment of this disease. Migration-unrelated functions are poorly understood and are major topics in current chemokine research.

LEUKOCYTE RELOCATION DURING IMMUNE RESPONSES

The immune system is highly complex, both in terms of effector cell variety and the multitude of leukocyte differentiation and maturation stages. Leukocyte development from precursors in the bone marrow and thymus is characterized by a flexibility in leukocyte migration properties, which enables coordinated relocation of individual precursors during their sequential steps of maturation. The need for precursor relocation is reflected by changes in the chemokine receptor profile and the selection of chemokines present in distinct microenvironments [19-21]. Importantly, chemokines with constitutive expression at distinct sites in primary lymphoid tissues and their receptors on precursors largely determine the temporal and spatial program of differentiation by controlling precursor-stromal cell interactions. Mature leukocytes in peripheral blood possess the proper repertoire of chemokine receptors and adhesion molecules for immediate involvement in immune defense.

Both inflammatory and homeostatic chemokines are involved in leukocyte traffic control during initiation of immune responses [2, 6, 17, 27]. Cells of the innate immune system (granulocytes, NK cells, monocytes/macrophages, immature DCs) are equipped with numerous receptors for inflammatory chemokines, which enable their "first line of defense" function at the site of pathogen entry. Of interest is the activation-induced switch in migratory behavior in DCs (Figure 3) [2-6, 27]. As sentinels in peripheral tissues, notably in the skin, lung and gastrointestinal tract, immature DCs quickly localize via receptors for inflammatory chemokines at the site of patho-

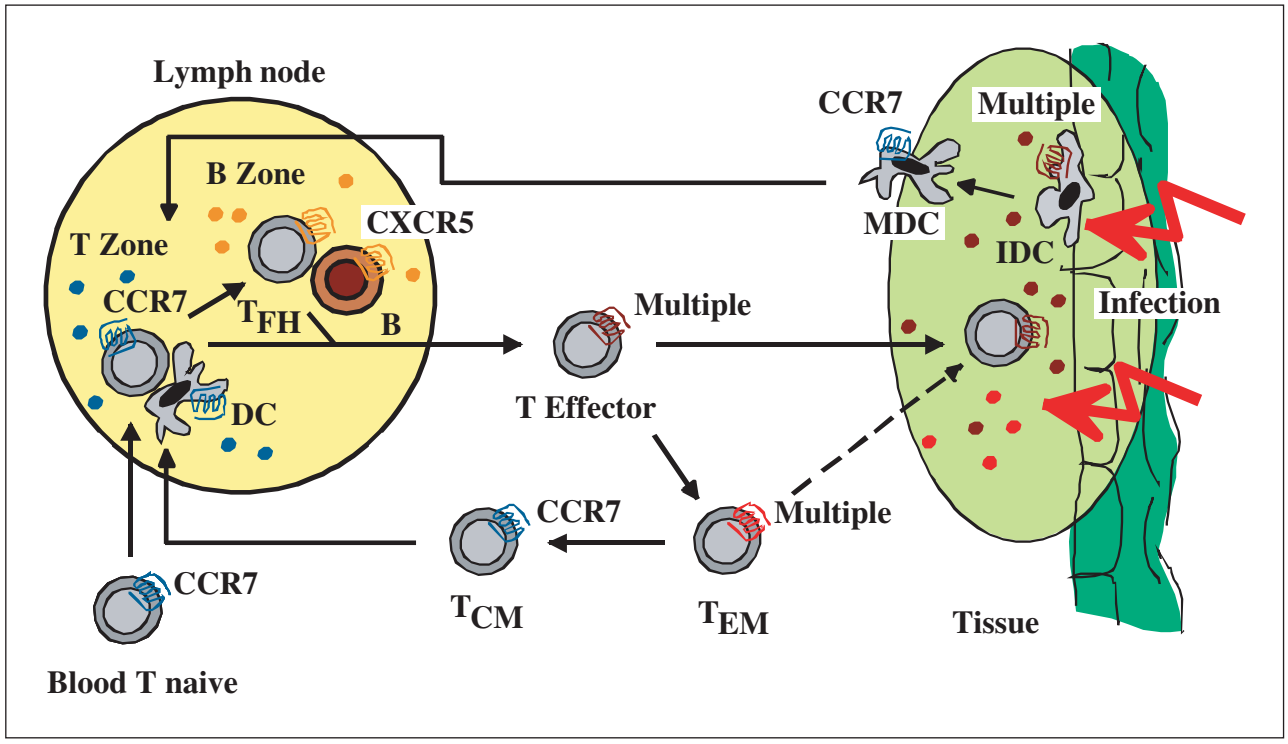

Figure 3

Initiation of adaptive immune responses. Dendritic cells (DCs) fulfill important functions as sentinel cells in peripheral tissues and as initiator of T cell responses in local lymph nodes. Stimulation of T cells within the T zone results in the transitory CXCR5- expressing T_{FH} cells, which are the precursors of effector/memory T cells. By contrast to naive and T_{FH} cells, effector T cells and certain memory T cells (T_{EM} cells) express numerous receptors for inflammatory chemokines for their efficient relocation to inflammatory sites. During the primary immune response, some of the effector T cells become T_{EM} cells and, eventually, long-lived T_{CM} cells, which express CCR7 for recirculation through secondary lymphoid tissues (splenic T Zone, lymph nodes, Peyer's patches).
Multiple, multiple chemokine receptor expression; IDC, immature DCs; MDC, mature DCs; T_{FH} cells, follicular B helper T cells; T_{EM} cells, effector memory T cells; T_{CM} cells, central memory T cells.

gen entry or tissue damage [28, 29]. Antigen uptake and processing as well as maturation into potent T cell stimulators is rapidly induced by the local inflammatory conditions. Importantly, this DC maturation results in the secretion of high levels of inflammatory chemokines, which leads to cellular desensitization towards these chemokines, and expression of CCR7 (see below), which enables their exit from the site of infection and their co-localization with T cells in reactive lymph nodes.

Naive T cells continuously recirculate through secondary lymphoid tissues (spleen, lymph nodes, Peyer's patches) and are largely excluded from peripheral tissues (skin, lungs and gastrointestinal tract) (Figure 3) [30, 31]. Their lymph node-homing phenotype is largely defined by the expression of CCR7, which recognizes ELC/CCL19 and SLC/CCL21, as well as by the expression of a set of adhesion molecules (CD62L, LFA-1 or α4β7), which interact with the corresponding vascular ligands present at these sites [28, 32, 33]. During contact with newly recruited antigen-presenting (mature) DCs, CD4+ T helper (Th) cells rapidly acquire follicular homing properties by expression of CXCR5, the selective receptor for the follicular chemokine BCA-1/CXCL13 (Figure 3). These newly primed T cells, referred to as follicular B helper T (T_{FH}) cells, are recruited to the B cell compartment by means of BCA-1, co-localize with B cells and support antibody responses [34-36].

Two types of antigen-experienced T cell subsets are produced during adaptive immune responses, namely large numbers of short-lived effector T cells, which express the full complement of receptors for inflammatory chemokines for direct participation in ongoing immune responses, and long-lived memory T cells for rapid involvement in recall (vaccination) responses (Figure 3) [37, 38]. Peripheral blood memory T cells are further divided into two major subsets characterized by different migratory and functional properties. Central memory T (T_{CM}) cells express CCR7 (and CD62L or α4β7) for continuous recirculation through spleen, lymph nodes and Peyer's patches, whereas effector memory T (T_{EM}) cells lack these lymph node-homing receptors but instead express a variety of inflammatory receptors for immediate recruitment to inflammatory sites [39, 40]. Recent evidence in mice suggests the following order in T cell differentiation during primary immune responses: naive (antigen-inexperienced) T cells - > effector T cells - > TEM cells - > TCM cells [41].

Helper T cells of the Th1 and Th2 phenotype express a characteristic set of chemokine receptors, which mainly bind inflammatory chemokines, and some of these appear to contribute to immune response polarization (see below) [2, 6, 17, 42]. CXCR3, CXCR6 and CCR5 are frequent on Th1 cells. Of note, CXCR6 binds the membrane-anchored chemokine CXCL16 and, thus, may primarily control Th1 cell adhesion and transendothelial migration. By contrast, Th2 cells frequently express CCR3, CCR4 and CCR8. CCR3 is highly selective for Th2 cells, whereas CCR4 and CCR8 are also found on Th1 cells, skin-homing T cells and peripheral blood regulatory ($CD4^+$ $CD25^+$) T cells. Collectively, the "polarized" expression of the chemokine recep-

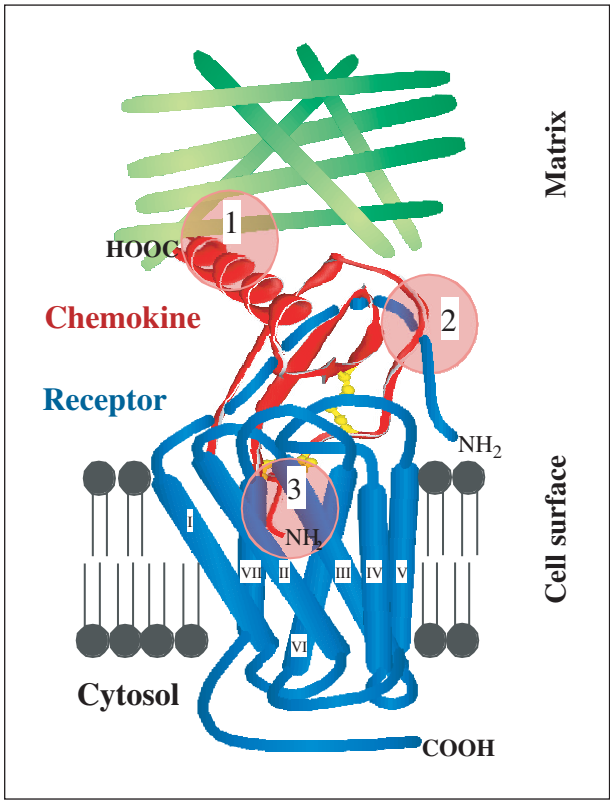

Figure 4
Structural segregation of chemokine anchorage and receptor triggering. (1) Matrix fixation site in the COOH-terminal α-helix or core structure, (2) N-loop region defined by second and third Cys residues, and (3) NH_2-terminus, which interacts with the chemokine-binding pocket present in the transmembrane region of the chemokine receptor.

tors is not strict and a simple, non-overlapping assignment of inflammatory chemokine receptors to distinct effector/memory T cell populations cannot be made. This may explain in part the marginal "phenotypes" seen in mice with single deficiencies in genes for inflammatory chemokines or chemokine receptors and further suggests that multiple chemokine receptors need to be targeted for effective anti-inflammatory intervention (see below) [43, 44].

ANTAGONISTS AND CHEMOKINE-BASED THERAPIES

The characteristics for chemokines are two functionally conserved regions that are essential for induction of leukocyte migration, one mediating receptor binding and the other enabling cell surface or extracellular matrix fixation (Figure 4) [43-45]. The core structure and COOH-terminal α-helix in chemokines are equipped with glycosaminoglycan-binding domains for their immobilization on extracellular matrices and cell surfaces.

Glycosaminoglycans are negatively charged carbohydrate moieties on glycoproteins and are portrayed as essential "chemokine-presenting and -clustering" sites that enable chemokine gradient formation within tissues. Receptor binding and triggering are mediated by distinct epitopes in the NH_2-terminal region of chemokines, which are in a position opposite to the glycosaminoglycan-binding sites [2, 45]. Structure-activity studies in chemokines revealed the importance of the NH_2-terminal domain preceding the first Cys residue and the "N-loop" defined by the second and third Cys residues, for receptor interactions, and led to a two-step chemokine-binding model (Figure 4) [46]. In a first "docking" step, an epitope in the N-loop of the chemokine interacts with the chemokine receptor, which is thought to lead to conformational changes in the receptor (and possibly also in the chemokine). The subsequent "triggering" step involves the binding of the NH_2-terminal region of the chemokine to the (now accessible) receptor-binding pocket. The two binding sites in chemokines are small (consisting of few amino acids) and are kept in close proximity by the two highly conserved disulphide bonds [46].

Modifications in the NH_2-terminal regions yielded numerous chemokine variants with antagonistic properties, demonstrating potential therapeutic application [2, 43, 45]. Obviously, the therapeutic potential of peptides is limited. Instead, the global effort of the pharmaceutical industry is focused on non-peptide inhibitors of low molecular weight, which target chemokine receptors involved in the control of leukocyte recruitment to inflammatory sites or those functioning as HIV-1 co-receptors [44]. So far, current drug development programs are concentrated on single chemokine receptors, and ongoing clinical trials will tell us more about their strength as novel anti-inflammatory medication. However, given the functional redundancy in the chemokine receptors controlling inflammatory infiltrates, compounds with multiple receptor-blocking activities need to be considered as more efficient anti-inflammatory agents.

Also, recent studies have revealed natural chemokines with antagonistic activity, further underscoring the importance of chemokines as controllers of leukocyte navigation. On the one hand, multiple NH_2-terminally truncated forms of chemokines were identified in natural sources, which are thought to be the product of inflammation- or tissue cell-derived proteases [1, 47-51]. Many of these natural chemokine truncation variants have antagonistic activity and, therefore, chemokine-selective proteases are proposed to be important factors in the control of leukocyte traffic. On the other hand, several intact and active chemokines were demonstrated to act also as antagonists. For instance, Mig/CXCL9, IP-10/CXCL10 and ITAC/CXCL11, the ligands for CXCR3, are antagonists for CCR3, the receptor for eotaxin/CCL11 and several other CC chemokines [52]. Since CXCR3 and CCR3 are differentially expressed on Th1 and Th2 cells, these findings suggest that the CXCR3 ligands contribute to Th1-type immune response polarization by blocking the migration of $CCR3^+$ (Th2) cells. Other natural chemokines with agonistic and antagonistic activities are eotaxin/CCL11 and eotaxin-3/CCL26, which attract eosinophils, basophils and Th2 cells *via* CCR3, while blocking $CCR2^+$ cells [53], and MCP-3/CCL7, a potent agonist for CCR1, CCR2 and CCR3 that blocks $CCR5^+$ cells [54]. The combination of stimulatory and inhibitory properties represents yet another level of control that influences cellular traffic during immune responses.

PERSPECTIVES

The inventory of the molecular components of the chemokine system is largely known, and many aspects of

immunity that depend on chemokine-driven leukocyte traffic, relocation and co-localization can now be studied. Hot topics in future chemokine research may include T and B cell development and homeostasis. Also, there is no doubt about the importance of chemokines in the initiation of T cell responses, but there is still an obvious lack of information about their contribution to immune response resolution after successful pathogen elimination. Future research will focus on macrophages with anti-inflammatory capacity, IL-10-producing Th cells and regulatory T cells. Another unresolved issue relates to the leukocyte traffic control that enables efficient immune surveillance in peripheral tissues. Finally, the presence of chemokines in a great variety of immune pathologies is well documented. In a next step, we will have to dissect individual inflammatory conditions into discrete schemes of effector cell recruitment in order to define the single or combinatorial chemokine components, which contribute to chronic diseases.

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