Introduction

Marco Baggiolini

Università della Svizzera Italiana, Via Lambertenghi 10A, 6904 Lugano, Switzerland

The beginning

In the first week of December 1987, two papers (one from the old, the other from the new world) presented the partial sequence of a novel protein, which was isolated from the culture supernatants of stimulated human monocytes and acted on neutrophil leukocytes. It was originally called NAF (neutrophil activating factor) [1], or MDNCF (monocyte-derived neutrophil chemotactic factor) [2]. At about the same time, two other laboratories reported the isolation of what turned out to be the same protein [3, 4]. The name was changed to NAP-1 (neutrophil-activating peptide number one) in the wise expectation to find analogues, but the new chemo-attractant became widely known by the fashionable and rather inappropriate name of interleukin-8 (IL-8).

After establishing the sequence, we rushed to a full analysis of the biological properties of IL-8 and found that its pattern of activity was qualitatively identical to that of known chemo-attractants for leukocytes, like the complement fragment C5a and N-formylmethionyl peptides [5]. The only difference was that IL-8 was selective for neutrophils, whereas the other attractants were non-specific. The effects of IL-8 were prevented by pretreatment of the cells with *Bordetella pertussis* toxin, a clear indication that they were mediated by a G-protein coupled receptor [5]. The initial observations, which were summarised in a JCI "Perspective" [6], attracted much interest. We needed large quantities of pure IL-8, which was produced biologically [7] and by chemical synthesis [8], and we concentrated on the study of IL-8 structure–activity relationships and, together with many others laboratories, on the search for IL-8-related chemokines.

In a decade of mining, human chemokines surfaced as a mega-family of 50 or so ligands and 20 receptors, all involved in leukocyte traffic [9]. The chemokines rapidly became a hot issue in immunology, pathology and medicine. Their biological relevance is perhaps best emphasised by the multiple interactions of viruses with the chemokine system, which evolved the expression of chemokines, receptors, antago-

Chemokine Biology – Basic Research and Clinical Application, Volume I
edited by Bernhard Moser, Gordon L. Letts and Kuldeep Neote

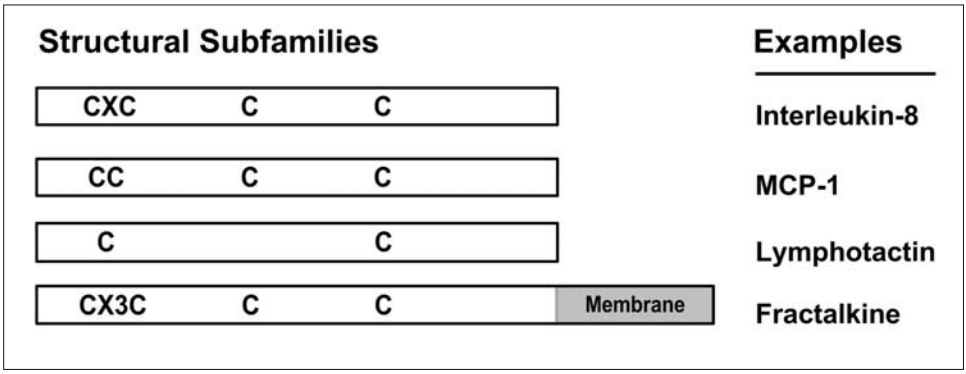

Figure 1
Chemokine subfamilies. The boxes represent the amino acid sequences, C indicates the position of cysteines that form intra-molecular disulphide bonds, and X stands for other amino acids. For each subfamily one representative example is named.

nists and even chemokine-binding proteins to gain control of leukocyte traffic. Viruses also learned to use chemokine receptors to infect cells [10].

The field moved in unexpected directions eventually showing that chemokines are involved in lymphocyte homing and in the house-keeping traffic that maintains the immune system effective. Roles for chemokines have also been suggested in haematopoiesis, morphogenesis, metastasis formation and angiogenesis. It has been shown that chemokine antagonists have anti-inflammatory and HIV-suppressing activity, and the development of low molecular weight antagonists has given rise to a major industrial effort toward therapy. The issue of targeting chemokines for therapeutic purposes is amply treated in Volume II of the present work.

Chemokine basics

Chemokines consist of approximately 70–130 amino acids including four conserved cysteines [11, 12]. As secretory proteins, they are synthesised with a leader sequence of 20–25 amino acids, which is cleaved off before release. Two main subfamilies, CXC and CC chemokines, are distinguished according to the position of the first two cysteines, which are separated by one amino acid (CXC) or adjacent (CC) (Fig. 1) [11, 12]. Two disulphide bonds, linking Cys1 to Cys3 and Cys2 to Cys4, confer to the chemokines their characteristic three-dimensional structure with a rigid core. The amino-terminal domain is short (3–10 amino acids) and structurally disordered, while the carboxyl-terminal helix consists of 20–60 amino acids. All

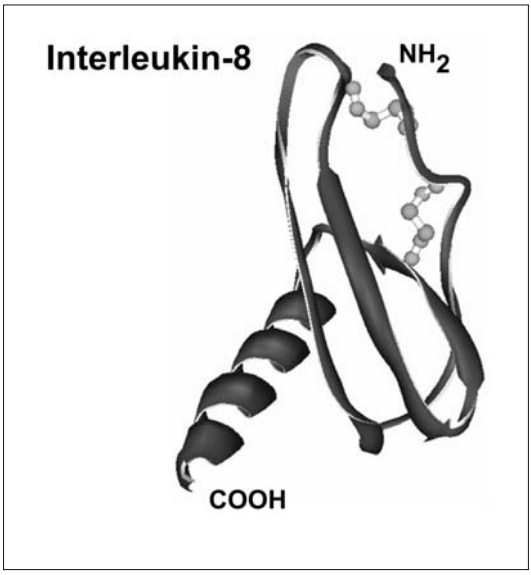

Figure 2
Three-dimensional structure of IL-8. In solution, all chemokines fold in this manner. The following, functionally relevant domains are visible: The receptor recognition (docking) region located within the exposed loop after the second cysteine, the receptor triggering region corresponding to the short amino-terminal sequence (NH_2), the prominent core consisting of three anti-parallel β-strands connected by loops, and a carboxyl-terminal α-helix (COOH). The characteristic disulphide bonds keep chemokines in their biologically active conformation.

chemokines are folded in this manner (Fig. 2) [13]. Few variants of the chemokine structure paradigm have been described. Lymphotactin has two, instead of four, conserved cysteines [14, 15], while fractalkine and CXCL16 are membrane-bound and have three and two amino acids, respectively, between the first two cysteines [14, 16–18]. The biological significance of these variants is largely unknown, but the adhesive properties of membrane-anchored chemokines may be relevant for leukocyte extravasation [19, 20].

Two chemokine nomenclature systems are used: the traditional abbreviations, such as IL-8 and MCP-1, which date back to the time of chemokine discovery, and a systematic nomenclature based on the structural motifs CXC, CC, XC, CX3C or CX2C, followed by 'L' (for ligand) and the number of the respective gene, e.g., CXCL8 for IL-8, CCL2 for MCP-1. The most common original names, together with the systematic designations, are presented in Table 1, and a complete listing with the most recent updates can be found at http://cytokine.medic.kumamoto-u.ac.jp. Chemokine receptors are designated according to the type of chemokine(s)

Table 1 - Human chemokines divided into CXC, CC, C, and CX3C subfamilies

Systematic [1]	Classical [2]	
CXC Chemokines		
CXCL1-3	GRO α, β, γ	Growth-related proteins α, β, γ
CXCL5	ENA-78	Epithelial cell-derived neutrophil-activating peptide 78
CXCL6	GCP-2	Granulocyte chemotactic protein 2
CXCL7	NAP-2	Neutrophil-activating peptide 2
CXCL8	IL-8	Interleukin 8
CXCL9	Mig	Monocyte/macrophage-activating, IFN-γ-inducible protein
CXCL10	IP10	IFN-γ-inducible 10 kDa protein
CXCL11	I-TAC	IFN-γ-inducible T cell alpha chemoattractant
CXCL12	SDF-1	Stromal cell-derived factor 1
CXCL13	BCA-1	B cell-attracting chemokine 1
CXCL14	BRAK	Breast and kidney-expressed chemokine
CC Chemokines		
CCL1	I-309	Intercrine-β glycoprotein 309
CCL2	MCP-1	Monocyte chemoattractant protein 1
CCL3,-4	MIP-1α, β	Macrophage inflammatory proteins 1α,1β
CCL5	RANTES	Regulated on activation, normal T cell expressed and secreted
CCL7	MCP-2	
CCL8	MCP-3	
CCL11	Eotaxin	Eosinophil chemoattractant protein
(CC chemokines continued)		
CCL13	MCP-4	
CCL14-16	HCC-1, 2, 4	Hemofiltrate CC chemokines 1, 2, 4
CCL17	TARC	Thymus and activation-regulated chemokine
CCL18	DC-CK1	Dendritic cell-derived CC chemokine 1
CCL19	ELC	EBI1(CCR7)-ligand chemokine
CCL20	LARC	Liver and activation-regulated chemokine
CCL21	SLC	Secondary lymphoid tissue chemokine
CCL22	MDC	Macrophage-derived chemokine
CCL23	MPIF-1	Myeloid progenitor inhibitory factor-1
CCL24	Eotaxin 2	
CCL25	TECK	Thymus-expressed chemokine
CCL26	Eotaxin 3	
CCL27	CTACK	Cutaneous T cell-attracting chemokine
CCL28	MEC	Mammary enriched chemokine
C Chemokines		
XCL1	Lymphotactin	(SCM-1α/single cysteine motif 1α)
XCL2	SCM-1β	Single cysteine motif 1β
CX3C Chemokine		
CX3CL1	fractalkine	

[1] *Systematic nomenclature is further defined at http://cytokine.medic.kumamoto-u.ac.jp.*
[2] *One representative out of several classical designations is listed for each chemokine.*

they bind (CXC, CC, XC, CX3C), followed by 'R' (for receptor) and a number reflecting the order of discovery.

Chemokines act via seven-trans-membrane domain receptors coupled to GTP-binding proteins. Most receptors recognise more than one chemokine and several chemokines bind to more than one receptor [21]. Structure–activity relationship studies have shown that CXC and CC chemokines have two sites of interaction with their receptors, one in the amino-terminal domain and the other within the exposed loop following the second cysteine. Both sites are kept in close proximity by the disulphide bonds. The loop region, which is conformationally rigid, appears to interact first and to function as a receptor-docking domain. This interaction restricts the mobility of the chemokine and presumably facilitates the binding of the amino-terminal domain that triggers a response (Fig. 3). All chemokines signal via receptors that are coupled to GTP-binding proteins of the Gi type and are sensitive to *B. pertussis* toxin. The signalling cascade induced by chemokines is typical for this class of seven-trans-membrane domain receptors [22].

Within the tissues, chemokines bind to glycosaminoglycans on the surface of cells and in the extracellular matrix by ionic interaction with basic residues in the core region and/or the carboxyl-terminal helix (Fig. 3) [23, 24]. Bound chemokines retain their full chemotactic activity and remain confined to the site where they are produced and released [25, 26]. This property explains the long-lasting, locally focused response to chemokines.

Receptor expression and chemokine driven leukocyte traffic regulation

In terms of function it is useful to differentiate between inflammatory and homeostatic chemokines. Inflammatory chemokines assure the recruitment of defence cells to sites of infection, tissue injury, inflammation and other disturbances of homeostasis. They are produced by a wide variety of tissue cells and by immigrating leukocytes at sites of pathological changes, act on receptors with broad selectivity, such as CXCR1, CXCR2, CXCR3, CCR1, CCR2, CCR3 and CCR5, and attract granulocytes, monocytes and lymphocytes. Homeostatic chemokines control the traffic of lymphocytes and their precursors during haematopoiesis in the bone marrow, the lymphoid and certain non-lymphoid tissues. They are expressed constitutively at homing sites within healthy tissues and act on receptors of high selectivity, which recognise a single, or at the most two, chemokines.

Initially chemokines were perceived as mediators of effector cell responses and the study of receptor expression was largely confined to phagocytes. Blood phagocytes express different sets of chemokine receptors. CXCR1 and CXCR2, the receptors or CXCL8/IL-8, are characteristic for neutrophils. Monocytes express CCR1, CCR2 and CCR5, eosinophils CCR1 and CCR3, while basophils express CCR1, CCR2 and CCR3. These patterns of receptors are characteristic for the different

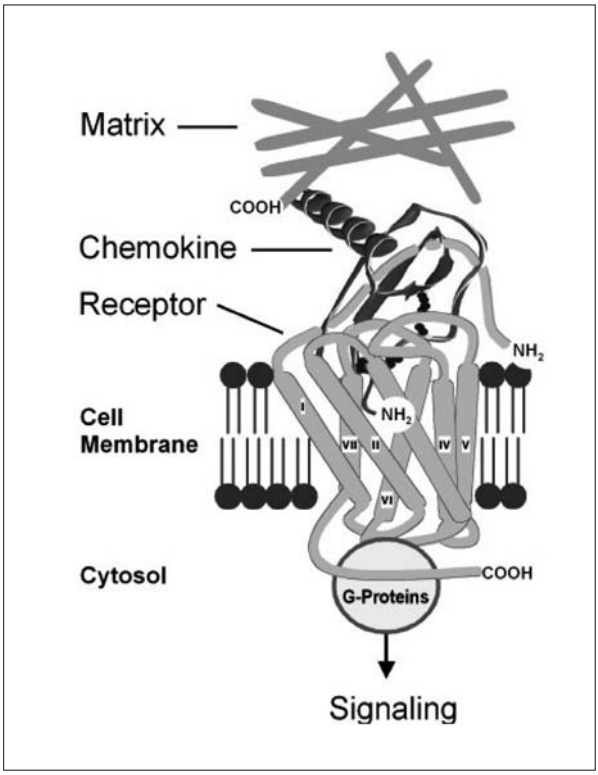

Figure 3
Interaction of chemokines with seven-trans-membrane domain receptors. The scheme shows the chemokine interacting with the receptor through its amino-terminal region and with extracellular glycosaminoglycans through heparin-binding regions, which are mostly localized in the carboxyl-terminal region (COOH). The chemokine-triggered receptor initiates the signaling cascade by activating a G-protein.

types of phagocytes and are sufficiently different to explain the selective recruitment of a single type of phagocyte, for instance, eosinophils in allergic inflammation or monocytes in chronic infectious lesions [27].

The results of studies on the responses of blood lymphocytes to chemokines were highly controversial until it was realised, that in these cells the expression of chemokine receptors changes considerably in dependence of differentiation and functional specialisation. It was first observed that culturing blood T cells in the presence of IL-2 progressively increases the expression of several receptors for inflammatory chemokines, such as CCR1, CCR2, CCR5 and CXCR3, and the chemotactic response to the respective ligands, e.g., CCL2/MCP-1, CCL3/MIP-1α,

CCL5/RANTES and CXCL10/IP10 [28]. The effect of IL-2 is reversible: Receptor numbers and responsiveness rapidly decline when the cytokine is withdrawn and are fully restored when it is supplied again. These observations indicated that chemokine receptor expression could be used to define different stages of T cell differentiation and the acquisition of particular functional properties.

Following up on these ideas, it was subsequently shown that Th1 and Th2 cells, as obtained by culturing in the presence of IL-2 and interferon-γ or IL-2 and IL-4, respectively, have different patterns of chemokine receptors: CCR5 and CXCR3 being characteristic for Th1 and CCR3 and CCR4 for Th2 cells [29, 30]. It was then shown that chemokine receptor detection by immunochemistry may be used for the identification of subtypes of T cells in tissues. Biopsies of rheumatoid synovium, which is rich in Th1 lymphocytes, stain strongly for CCR5, while a marked staining for CCR3 is detected at sites of allergic inflammation, where Th2 lymphocytes are recruited together with eosinophils [31].

CCR1, CCR2, CCR5 and CXCR3, the receptors that are up-regulated in T cells after treatment with IL-2, respond to inflammatory chemokines, which are induced at sites of infection and inflammation to recruit defence cells. When the T cells are stimulated with antibodies against CD3 and CD28, mimicking activation via the T cell receptor, they down-regulate the first set of receptors and up-regulate CCR7. A similar mechanism guides the traffic of dendritic cells. Inflammatory chemokines attract immature dendritic cells, expressing CCR1, CCR2 and CCR5, into inflamed tissues. The cells then mature, acquiring the capacity to capture and process antigens, and to present antigenic epitopes, and are thus ready to move on. CCR1, CCR2 and CCR5 are down-regulated and replaced by CCR7 and the mature dendritic cells migrate into the draining lymph nodes in response to CCL19/ELC and CCL21/SLC via CCR7 [32].

Effector and central memory T cells (TEM and TCM, respectively) can be distinguished according to their chemokine receptor outfit, which reflects their different role in a secondary immune response [33]. TEM cells have effector function. They produce IL-4 and interferon-γ, and may store perforin, and, owing to the absence of CCR7, can be recruited rapidly into inflamed tissues for immediate defence in response to inflammatory chemokines. By contrast, the CCR7-positive central memory T cells (TCM) have no immediate effector function. They represent a clonally expanded memory cell pool, are attracted to lymph nodes after a secondary antigen challenge, and can stimulate dendritic cells to produce IL-12, provide help to antigen-specific B cells, and generate a new wave of effector T cells [33].

Control of lymphocyte traffic in disease-unrelated processes

Homeostatic chemokines control the relocation and recirculation of lymphocytes in the context of maturation, differentiation and activation, and ensure their correct

positioning within discrete areas of primary and secondary lymphoid organs [34, 35].

The recognition that chemokines direct the homeostatic traffic of lymphocytes goes back to the work by Lipp and colleagues [36] who found that the deletion of the gene of the putative chemokine receptor BLR1 (which was renamed CXCR5 after identification of its ligand chemokine, CXCL13/BCA-1 [37, 38]) impaired the formation of Peyer's patches and inguinal lymph modes because of the inability of CXCR5-deleted B cells to home into follicular areas. Subsequent work elucidated the role of another receptor for homeostatic chemokines, CCR7, which binds CCL19/ELC and CCL21/SLC [39]. Follicle formation in lymphoid tissues depends on immigration and settling of B and T cells. Both types of lymphocytes bear CCR7, they are recruited in response to CCL21/SLC expressed in high-endothelial venules and migrate to the parafollicular area in response to CCL19/ELC and CCL21/SLC. The B cells, which also bear CXCR5, are attracted into the follicles, where CXCL13/BCA-1 is expressed.

It was subsequently found that T cells acquire CXCR5 on activation, in particular on contact with antigen-presenting dendritic cells. Such cells can thus enter the follicles in response to CXCL13/BCA-1 and fulfil a helper function to B cells by enhancing antibody production. Some re-enter circulation as a small pool of memory cells [40, 41]. CXCR5-bearing T cells represent a novel type of effectors. They differ from Th1 and Th2 cells as they markedly enhance antibody production when co-cultured with B cells and do not express cytokines that are characteristic of Th1 or Th2 cells [42]. Owing to their follicular homing properties and function, these cells are called follicular B helper T cells (TFH). The possible involvement of TFH cells in immune pathology, including autoimmune diseases with B cell involvement is presently under study.

Peripheral immune surveillance T cells

The skin, the gut and the lung are the main sites of pathogen entry into the body owing to their huge contact area to the outside. Immune defence in these tissues is assured by dedicated lymphoid structures (like the mucosa-associated lymphoid tissue of the lung and the gastrointestinal tract) and by a large population of resident T cells, which are distributed throughout the tissue. The mechanism of the tissue-specific entry of immune surveillance T cells is studied by searching for chemokines that are constitutively expressed by the endothelia of blood micro-vessels, the main site of leukocyte extravasation, and by determining the pattern of chemokine receptor expression of the resident T cells. In the skin, most T cells cluster around post-capillary venules of the superficial dermal plexus. *In situ* studies have shown that these cells express CCR8, and that CCL1/I-309, its only ligand, is produced constitutively in blood micro-vessels (as well as in Langerhans cells and melanocytes of

healthy epidermis) but not in keratinocytes or fibroblasts [43]. No other chemokine and receptor combination appears to satisfy the requirements for constitutive expression, local distribution and selectivity. It is thus assumed that the homeostatic traffic of skin-homing T cells is based, at least in part, on the recruitment of circulating CCR8 expressing T cells in response to cutaneous CCL1/I-309 [44]. One would expect that similar mechanisms regulate the selective homing of T cells into the gut and the lung. It has been shown that effector T cells home into the small intestine in response to CCL25/TECK acting via its receptor, CCR9 [45, 46], but the role of CCR9 and its ligand chemokine in the homeostatic traffic of gut-selective T cells is still a matter of debate. The studies of the skin indicate that peripheral immune surveillance T cells (TPS), in contrast to TCM and TEM cells, fulfil a "first line of defence" function, like other sentinel cells, and it is thus reasonable to assume that TPS cells are present in other frontier tissues [44].

Volume I focuses on the functions of chemokines in immunobiology, as the title indicates, with particular attention to the control of T cell traffic in inflammation and homeostasis. In view of major recent progress, the properties of newly-defined T cell subsets with *bona fide* effector and/or memory functions, namely TCM, TEM and TPS cells will be discussed in relation to Th1 and Th2 cells. A special chapter is dedicated to NK cells and γδ T cells, which share certain features with effector T cells. Adaptive immunity, including immune homeostasis and antimicrobial defence, fully depends on antigen-presentation and co-stimulation by dendritic cells and, therefore, an update on the control of dendritic cell traffic by chemokines is presented. Chemokine-induced cellular responses are mediated by selective receptors. The complex molecular networks involving soluble and membrane-bound mediators that are activated on chemokine receptor triggering are considered in a separate chapter. Since considerable progress has been made recently in the study of the homeostatic functions of chemokines, the local, constitutive production of chemokines in the tissues, in particular by the endothelial cells of micro-vessels, and its role in leukocyte transendothelial migration has been given special consideration. A chapter considers the modification of chemokines and chemokine activities by proteases, as well as the phenomenon of inhibition or potentiation of chemokine-induced responses by other chemokines or chemokine derivatives. These interactions will eventually deepen our understanding of leukocyte recruiting in inflammation, when several chemokines are produced concomitantly. The last part of the volume is dedicated to chemokine-mediated responses that involve tissue cells and microbes. New insides are presented on the cross-talk between G-protein-coupled receptors on neurons and leukocytes, the influence of virus-encoded chemokines on the immune system of the host, the function of chemokine receptors in tissue cells, and the involvement of chemokines and related peptides in antimicrobial defence. The state-of-the-art view on chemokine immunobiology should provide the context for discussing pathology and therapy-related aspects of chemokine research, which are the main focus of Volume II.

References

1 Walz A, Peveri P, Aschauer H, Baggiolini M (1987) Purification and amino acid sequencing of NAF, a novel neutrophil-activating factor produced by monocytes. *Biochem Biophys Res Commun* 149: 755–761

2 Yoshimura T, Matsushima K, Tanaka S, Robinson EA, Appella E, Oppenheim JJ, Leonard EJ (1987) Purification of a human monocyte-derived neutrophil chemotactic factor that has peptide sequence similarity to other host defense cytokines. *Proc Natl Acad Sci USA* 84: 9233–9237

3 Schröder J-M, Mrowietz U, Morita E, Christophers E (1987) Purification and partial biochemical characterization of a human monocyte-derived, neutrophil-activating peptide that lacks interleukin 1 activity. *J Immunol* 139: 3474–3483

4 Van Damme J, Van Beeumen J, Opdenakker G, Billiau A (1988) A novel, NH2-terminal sequence-characterized human monokine possessing neutrophil chemotactic, skin-reactive, and granulocytosis-promoting activity. *J Exp Med* 167: 1364–1376

5 Thelen M, Peveri P, Kernen P, von Tscharner V, Walz A, Baggiolini M (1988) Mechanism of neutrophil activation by NAF, a novel monocyte-derived peptide agonist. *FASEB J* 2: 2702–2706

6 Baggiolini M, Walz A, Kunkel SL (1989) Neutrophil-activating peptide-1/interleukin 8, a novel cytokine that activates neutrophils. *J Clin Invest* 84: 1045–1049

7 Lindley I, Aschauer H, Seifert JM, Lam C, Brunowsky W, Kownatzki E, Thelen M, Peveri P, Dewald B, von Tscharner V et al (1988) Synthesis and expression in *Escherichia coli* of the gene encoding monocyte-derived neutrophil-activating factor: Biological equivalence between natural and recombinant neutrophil-activating factor. *Proc Natl Acad Sci USA* 85: 9199–9203

8 Clark-Lewis I, Moser B, Walz A, Baggiolini M, Scott GJ, Aebersold R (1991) Chemical synthesis, purification, and characterization of two inflammatory proteins, neutrophil activating peptide 1 (interleukin-8) and neutrophil activating peptide 2. *Biochemistry* 30: 3128–3135

9 Baggiolini M (2001) Chemokines in pathology and medicine. J Intern Med 250: 91–104

10 Murphy PM (2001) Viral exploitation and subversion of the immune system through chemokine mimicry. *Nat Immunol* 2: 116–122

11 Baggiolini M, Dewald B, Moser B (1994) Interleukin-8 and related chemotactic cytokines – CXC and CC chemokines. *Adv Immunol* 55: 97–179

12 Baggiolini M, Dewald B, Moser B (1997) Human chemokines: An update. *Annu Rev Immunol* 15: 675–705

13 Clark-Lewis I, Kim K-S, Rajarathnam K, Gong J-H, Dewald B, Moser B, Baggiolini M, Sykes BD (1995) Structure-activity relationships of chemokines. *J Leukocyte Biol* 57: 703–711

14 Kelner GS, Kennedy J, Bacon KB, Kleyensteuber S, Largaespada DA, Jenkins NA,

Copeland NG, Bazan JF, Moore KW, Schall TJ et al (1994) Lymphotactin: A cytokine that represents a new class of chemokine. *Science* 266: 1395–1399

15 Kennedy J, Kelner GS, Kleyensteuber S, Schall TJ, Weiss MC, Yssel H, Schneider PV, Cocks BG, Bacon KB, Zlotnik A (1995) Molecular cloning and functional characterization of human lymphotactin. *J Immunol* 155: 203–209

16 Bazan JF, Bacon KB, Hardiman G, Wang W, Soo K, Rossi D, Greaves DR, Zlotnik A, Schall TJ (1997) A new class of membrane-bound chemokine with a CX3C motif. *Nature* 385: 640–644

17 Pan Y, Lloyd C, Zhou H, Dolich S, Deeds J, Gonzalo JA, Vath J, Gosselin M, Ma JY, Dussault B et al (1997) Neurotactin, a membrane-anchored chemokine upregulated in brain inflammation. *Nature* 387: 611–617

18 Matloubian M, David A, Engel S, Ryan JE, Cyster JG (2000) A transmembrane CXC chemokine is a ligand for HIV-coreceptor Bonzo. *Nat Immunol* 1: 298–304

19 Imai T, Hieshima K, Haskell C, Baba M, Nagira M, Nishimura M, Kakizaki M, Takagi S, Nomiyama H, Schall TJ et al (1997) Identification and molecular characterization of fractalkine receptor CX3CR1, which mediates both leukocyte migration and adhesion. *Cell* 91: 521–530

20 Fong AM, Robinson LA, Steeber DA, Tedder TF, Yoshie O, Imai T, Patel DD (1998) Fractalkine and CX3CR1 mediate a novel mechanism of leukocyte capture, firm adhesion, and activation under physiologic flow. *J Exp Med* 188: 1413–1419

21 Murphy PM, Baggiolini M, Charo IF, Hebert CA, Horuk R, Matsushima K, Miller LH, Oppenheim JJ, Power CA (2000) International union of pharmacology. XXII. Nomenclature for chemokine receptors. *Pharmacol Rev* 52: 145–176

22 Thelen M (2001) Dancing to the tune of chemokines. *Nat Immunol* 2: 129–134

23 Chakravarty L, Rogers L, Quach T, Breckenridge S, Kolattukudy PE (1998) Lysine 58 and histidine 66 at the C-terminal α-helix of monocyte chemoattractant protein-1 are essential for glycosaminoglycan binding. *J Biol Chem* 273: 29641–29647

24 Amara A, Lorthioir O, Valenzuela A, Magerus A, Thelen M, Montes M, Virelizier JL, Delepierre M, Baleux F, Lortat-Jacob H et al (1999) Stromal cell-derived factor-1a associates with heparan sulfates through the first b-strand of the chemokine. *J Biol Chem* 274: 23916–23925

25 Webb LMC, Ehrengruber MU, Clark-Lewis I, Baggiolini M, Rot A (1993) Binding to heparan sulfate or heparin enhances neutrophil responses to interleukin 8. *Proc Natl Acad Sci USA* 90: 7158–7162

26 Middleton J, Neil S, Wintle J, Clark-Lewis I, Moore H, Lam C, Auer M, Hub E, Rot A (1997) Transcytosis and surface presentation of IL-8 ky venular endothelial cells. *Cell* 91: 385–395

27 Baggiolini M, Dahinden CA (1994) CC chemokines in allergic inflammation. *Immunol Today* 15: 127–133

28 Loetscher P, Seitz M, Baggiolini M, Moser B (1996) Interleukin-2 regulates CC chemokine receptor expression and chemotactic responsiveness in T lymphocytes. *J Exp Med* 184: 569–577

29 Bonecchi R, Bianchi G, Bordignon PP, D'Ambrosio D, Lang R, Borsatti A, Sozzani S, Allavena P, Gray PA, Mantovani A et al (1998) Differential expression of chemokine receptors and chemotactic responsiveness of type 1 T helper cells (Th1s) and Th2s. *J Exp Med* 187: 129–134
30 Sallusto F, Lenig D, Mackay CR, Lanzavecchia A (1998) Flexible programs of chemokine receptor expression on human polarized T helper 1 and 2 lymphocytes. *J Exp Med* 187: 875–883
31 Loetscher P, Moser B, Baggiolini M (2000) Chemokines and their receptors in lymphocyte traffic and HIV infection. *Adv Immunol* 74: 127–180
32 Sallusto F, Mackay CR, Lanzavecchia A (2000) The role of chemokine receptors in primary, effector, and memory immune responses. *Annu Rev Immunol* 18: 593–620
33 Sallusto F, Geginat J, Lanzavecchia A (2004) Central memory and effector memory T cell subsets: function, generation, and maintenance. *Annu Rev Immunol* 22: 745–763
34 Moser B, Loetscher P (2001) Lymphocyte traffic control by chemokines. *Nat Immunol* 2: 123–128
35 Moser B, Wolf M, Walz A, Loetscher P (2004) Chemokines: multiple levels of leukocyte migration control. *Trends Immunol* 25: 75–84
36 Förster R, Mattis AE, Kremmer E, Wolf E, Brem G, Lipp M (1996) A putative chemokine receptor, BLR1, directs B cell migration to defined lymphoid organs and specific anatomic compartments of the spleen. *Cell* 87: 1037–1047
37 Legler DF, Loetscher M, Roos RS, Clark-Lewis I, Baggiolini M, Moser B (1998) B cell-attracting chemokine 1, a human CXC chemokine expressed in lymphoid tissues, selectively attracts B lymphocytes via BLR1/CXCR5. *J Exp Med* 187: 655–660
38 Gunn MD, Ngo VN, Ansel KM, Ekland EH, Cyster JG, Williams LT (1998) A B-cell-homing chemokine made in lymphoid follicles activates Burkitt's lymphoma receptor-1. *Nature* 391: 799–803
39 Förster R, Schubel A, Breitfeld D, Kremmer E, Renner-Müller I, Wolf E, Lipp M (1999) CCR7 coordinates the primary immune response by establishing functional microenvironments in secondary lymphoid organs. *Cell* 99: 23–33
40 Schaerli P, Willimann K, Lang AB, Lipp M, Loetscher P, Moser B (2000) CXC chemokine receptor 5 expression defines follicular homing T cells with B cell helper function. *J Exp Med* 192: 1553–1562
41 Breitfeld D, Ohl L, Kremmer E, Ellwart J, Sallusto F, Lipp M, Forster R (2000) Follicular B helper T cells express CXC chemokine receptor 5, localize to B cell follicles, and support immunoglobulin production. *J Exp Med* 192: 1545–1552
42 Moser B, Schaerli P, Loetscher P (2002) CXCR5(+) T cells: follicular homing takes center stage in T-helper-cell responses. *Trends Immunol* 23: 250–254
43 Schaerli P, Ebert L, Willimann K, Blaser A, Roos RS, Loetscher P, Moser B (2004) A skin-selective homing mechanism for human immune surveillance T cells. *J Exp Med* 199: 1265–1275
44 Ebert LM, Schaerli P, Moser B (2005) Chemokine-mediated control of T cell traffic in lymphoid and peripheral tissues. *Mol Immunol* 42: 799–809

45 Svensson M, Marsal J, Ericsson A, Carramolino L, Broden T, Marquez G, Agace WW (2002) CCL25 mediates the localization of recently activated CD8alphabeta(+) lymphocytes to the small-intestinal mucosa. *J Clin Invest* 110: 1113–1121

46 Johansson-Lindbom B, Svensson M, Wurbel MA, Malissen B, Marquez G, Agace W (2003) Selective generation of gut tropic T cells in gut-associated lymphoid tissue (GALT): requirement for GALT dendritic cells and adjuvant. *J Exp Med* 198: 963–969