Chemokines – linking receptors to response

MALCOLM L. WATSON *Department of Pharmacy & Pharmacology, University of Bath, Bath, UK*

The accumulation and activation of inflammatory cells has long been recognized as a key event in host defence and tissue injury. Prior to 1987, leucocyte chemotaxis was attributed largely to the local generation of complement fragments, lipids such as leukotriene B_4, or bacterial-derived peptides. A number of chemotactic activities in biological fluids or cell culture supernatants had also been reported, but at that time these activities were often mistakenly assumed to be caused by known cytokines such as interleukin (IL)-1. Two papers[1,2] published in 1987 described the identification of a neutrophil selective chemoattractant protein derived from monocytes. The protein became most commonly known as IL-8 (and has now been reborn as CXCL8, *vide infra*) and its identification was soon followed by the identification of a number of structurally and functionally related proteins with selective chemoattractant activity for other leucocyte types, including monocytes, lymphocytes and eosinophils. These findings provided, for the first time, a plausible mechanism for the ability of one leucocyte subtype to dominate certain types of inflammatory reactions. The subsequent naming of these proteins as chemokines reflected the activity of most members of the family as *chemo*tactic cyto*kines.*[3] Over 40 members of the chemokine family have been identified in the human and there is now little doubt regarding their collective importance as central players in the inflammatory response.

Anyone new to the chemokine literature will be struck by the plethora of chemokine names based on cellular source or target. Nomenclature has proved problematic from the first convergence of different laboratories working on the same or different members of the family.[4] Historically, chemokines have been named according to target (neutrophil-activating factor; melanoma growth stimulating activity; monocyte chemotactic protein; eotaxin), source (platelet factor 4; monocyte-derived chemokine; lungkine), stimulus for production (Mig – monokine induced by interferon-γ; Epstein–Barr virus-induced receptor ligand chemokine), structural features (6Ckine – chemokine with 6 cysteines; SCM – single C motif) and any combination of these (monocyte-derived neutrophil chemotactic factor; ENA-78 – epithelial cell-derived neutrophil attractant with 78 amino acids; IP-10, interferon-γ-inducible protein of 10 kDa). While useful within one research area, none of these systems is sustainable when the true diversity of their sources and target cell promiscuities are recognized. A systematic nomenclature for chemokines and their receptors[5,6] has now gained wide acceptance and is outlined in the review by Ajeubor & Swain in this issue of *Immunology*. This nomenclature system is an extension of the previous common convention of referring to chemokine subfamilies according to the spacing of conserved cysteine motifs, with the postfix L (for ligand) or R (for receptors) and a number to identify individual members. Hence, CXCL and CXCR are used for the ligands and receptors when the first two cysteines in the chemokine are separated by one amino acid, CCL and CCR when adjacent, and CX3CL and CX3CR when separated by three amino acids. A fourth subfamily of chemokines, with a single cysteine in the N-terminal region, are the XCL chemokines and their receptor is denoted XCR to avoid confusion with complement receptors. It is worth noting that the receptor nomenclature has received a much more rapid adoption than that for the ligands. A key benefit of the new nomenclature is in helping ensure that workers in different research areas know exactly what molecule is under discussion. However, unlike the imminent adoption of the Euro as the single currency across much of Europe, there will probably be dual, triple or even quadruple names in use for some time to come. This situation may decrease readability of articles, but does serve to remind the reader of the history of individual chemokines. Hence, the bland 'CCL2' will probably retain the explanation 'monocyte chemotactic protein-1' for some years, and 'the eotaxin receptor' conveys much more than 'the CCL11 receptor CCR3'.

In addition to those summarized in Ajeubor & Swain's review (this issue), few new human chemokines or receptors have been reported in the last year (Table 1). CXCL16[7,8] has a transmembrane domain reminiscent of CX3CL1 (fractalkine) and is expressed on the membrane of antigen-presenting cells, but also may be shed under appropriate

Received 12 November 2001; revised 19 November 2001; accepted 20 November 2001.

Abbreviations: CCL, CC chemokine ligand; CCR, CC chemokine receptor; CXCL, CXC chemokine ligand; CXCR, CXC chemokine receptor; IL, interleukin.

Correspondence: Dr Malcolm L. Watson, Department of Pharmacy and Pharmacology, University of Bath, Claverton Down, Bath BA2 7AY, UK. E-mail: M.L.Watson@bath.ac.uk

Table 1. Recently characterized human chemokines and receptors*

Novel chemokine/receptor	Expression	Receptor or ligand	Activity
CXCL16	Antigen-presenting cells: membrane bound or released	CXCR6 (STRL33/Bonzo/TYMSTR)	Activated T-cell chemotaxis
CCL28 (MEC)	Mucosal epithelium	CCR10 CCR3	Memory T-cell and eosinophil chemotaxis
CCR11	Heart, small intestine	CCL19 (ELC) CCL21 (SLC) CCL25 (TECK)	Binding only

*See http://csp.medic.kumamoto-u.ac.jp/for a full listing of chemokines, receptors and their interactions.
ELC, Epstein–Barr virus-induced receptor ligand chemokine; MEC, mucosae-associated chemokine; SLC, secondary lymphoid tissue chemokine; STRL33, seven transmembrane domain receptors from lymphocytes clone 33; TECK, thymus-expressed chemokine; TYMSTR, T-lymphocyte-expressed seven-transmembrane domain receptor.

conditions. CXCL16 induces chemotaxis of activated T cells via the human immunodeficiency virus (HIV) co-receptor STRL33/Bonzo/TYMSTR, which is therefore now designated CXCR6. The specificity of this ligand–receptor pair is particularly unusual, in that CXCL16 has no agonist activity at other chemokine receptors and no other endogenous ligands are known to bind CXCR6.[8] A novel CC chemokine, mucosae-associated epithelial chemokine (MEC, designated CCL28), was independently identified by two groups.[9,10] CCL28 is produced by epithelial cells and induces chemotaxis of memory T lymphocytes and eosinophils via CCR10 and CCR3. Finally, another CC receptor has been identified,[11,12] tentatively designated CCR11. This protein binds CCL19, CCL21 and CCL25, although the functional responses elicited by other ligands described in one of the original studies were the result of initially unsuspected expression of CCR2.[13] A comprehensive and up-to-date listing of chemokines, receptors and their interactions is available on the Web (http://csp.medic.kumamoto-u.ac.jp/).[14]

The name chemokine has proved useful in highlighting what is doubtless the most recurrent theme in the activity of these proteins – as chemotaxins – and many studies restrict their interest to their activity on the most mobile of mammalian cells, the leucocytes. However, several founder members of the chemokine family were identified on the basis of activities on other cells or their cellular source, prior to the recognition of their leucocyte chemoattractant activity. Hence, CXCL1, CXCL2 and CXCL3 were characterized as melanoma growth stimulating activity and products of *gro* growth-related oncogenes,[15] and CXCL4 was characterized as a platelet α-granule product and has limited chemotactic activity.[16] It is probable that activities besides induction of leucocyte chemotaxis are, for many chemokines and their receptors, at least as important in disease pathophysiology; this topic has been discussed in the context of T-cell biology by Ward and associates.[17] CXCR4, which is the receptor for CXCL12 (stromal cell derived factor-1), has received particular attention because of its role in HIV-1 infection as well as in embryonic development.[18] Signalling by CXCR4 is a central theme in the review by Curnock *et al*. in this issue of *Immunology*.

Besides leucocytes, chemokine receptors have been identified on a number of structural cells, including those of epithelial, endothelial, fibroblast, smooth muscle and neuronal origin. While much of the early data were confined to identification of mRNA for chemokine receptors using reverse transcription–polymerase chain reaction (RT–PCR) techniques or binding of chemokine ligands, several studies (some of which are summarized in Table 2) have now provided evidence of expression of receptors and signalling responses to chemokine receptor ligation. Less well characterized are the physiological or pathological consequences of chemokine receptor activation in these cells. While direct chemotactic activity on tumour cells and endothelial cells is relevant to metastasis and angiogenesis, feedback regulation of chemokine production or growth regulation appear to be important functions in other cell types.

Given the plethora of chemokines and the promiscuity of receptors, it is unlikely that interactions restricted to single ligand–receptor pairs ever occur outside very closely controlled *in vitro* systems. Another layer of complexity and subtlety to chemokine signalling is added by the potential for homo- or heterodimerization of chemokine receptors. Martínez and co-workers[33,34] have demonstrated that stimulation of cells bearing appropriate receptors with more than one chemokine can lead to receptor heterodimerization, forming for example a CCR2 and CCR5 heterodimer. This particular heterodimer results in an amplified signal compared with activation via either homodimer formed following single ligand stimulation. Furthermore, homo- and heterodimers can activate distinct pathways and consequently elicit dissimilar cellular responses. Hence, the cellular response to a single chemokine is unlikely to reflect accurately that seen in an inflammatory situation where multiple chemokines and receptors are present. An interesting corollary of this is found in individuals expressing the CCR2 mutation, CCR2V64I. Heterodimerization of this receptor with CCR5 or CXCR4 may account for the protective effect of this mutation against HIV strains that do not utilize (non-mutated) CCR2 for cellular entry.[33]

Table 2. Functional chemokine receptors on human tissue cells*

Cell type	Chemokine receptor	Chemokine ligands	Evidence	References
Astrocyte	CXCR4	CXCL12 (SDF-1)	Calcium response	19,20,21
	CCR5	CCL4 (MIP-1β)	glutamate release	
		CCL5 (RANTES)	survival	
Neuron	CXCR2	CXCL1 (MGSA)	Calcium response	19,22,23
	CXCR4	CXCR12	Apoptosis	
	CCR5	CCL4		
Smooth muscle	CCR1	CCL3 (MIP-1α)	Ligand binding	24
	CCR2?	CCL2 (MCP-1)	Calcium response	
Endothelial	CXCR2	CXCL1	Chemotaxis	25
			Angiogenesis	
Skin fibroblast	CCR2?	CCL2	Collagenase and IL-1 production	26
Synovial fibroblast	CXCR4	CXCL12	ERK signalling	27
	CCR2	CCL2	Enhanced IL-6 and	
	CCR5	CCL5	CXCL8 release	
Gut epithelial	CXCR4	CXCL12	Calcium response	28
Airway epithelial	CCR3	CCL11 (eotaxin)	Calcium response	29
Melanoma	CXCR2	CXCL1	Receptor phosphorylation,	30,31
	CXCR4	CXCL12	growth stimulation	
Breast cancer cells	CCR7	CCL21 (6Ckine)	Ligand-induced actin	32
	CCR10	CCL27 (ESkine)	polymerization, chemotaxis	

*Receptor mRNA expression and immunoreactivity is also reported in these and other studies.

6Ckine, chemokine with 6 cysteines; ERK, extracellular signal-regulated kinase; ESkine, embryonic stem cell chemokine; IL, interleukin; MCP, monocyte chemotactic protein; MGSA, melanoma growth stimulatory activity; MIP, macrophage inflammatory protein; RANTES, regulated on activation, normal T-cell expressed and secreted; SDF, stromal cell-derived factor.

The reviews published in this issue of *Immunology* assess current developments in the understanding of chemokine biology and their roles in disease. Key pathways in the intracellular signalling response to chemokine receptors are reviewed by Curnock *et al.* Their article highlights the importance of phosphoinositide-3-kinase isoforms and their downstream targets in the cellular response to chemokine receptor ligation. Two further reviews explore some of the many disease areas where modulation of the chemokine response may be exploited therapeutically. Lloyd describes the role of chemokines in lung inflammation; and the article by Ajuebour & Swain describes the actions of chemokines and chemokine receptors in intestinal and hepatic inflammation. As reported in these articles, chemokine receptor antagonists are now entering clinical trials, and results from such studies will help determine in what diseases chemokines play significant roles and, most importantly, whether they present a useful therapeutic target.

REFERENCES

1 Yoshimura T, Matsushima K, Tanaka S, Robinson EA, Appella E, Oppenheim JJ, Leonard EJ. Purification of a human monocyte-derived neutrophil chemotactic factor that has peptide sequence similarity to other host defense cytokines. Proc Natl Acad Sci USA 1987; **84:**9233–7.

2 Walz A, Peveri P, Aschauer H, Baggiolini M. Purification and amino-acid sequencing of NAF, a novel neutrophil-activating factor produced by monocytes. Biochem Biophys Res Commun 1987; **149:**755–61.

3 Lindley IJD, Westwick J, Kunkel SL. Nomenclature announcement – the chemokines. Immunol Today 1993; **14:**24.

4 Westwick J, Li SW, Camp RD. Novel neutrophil-stimulating peptides. Immunol Today 1989; **10:**146–7.

5 Zlotnik A, Yoshie O. Chemokines: a new classification system and their role in immunity. Immunity 2000; **12:**121–7.

6 Murphy PM, Baggiolini M, Charo IF *et al.* International Union of Pharmacology. XXII. Nomenclature for chemokine receptors. Pharmacol Rev 2000; **52:**145–76.

7 Matloubian M, David A, Engel S, Ryan JE, Cyster JG. A transmembrane CXC chemokine is a ligand for HIV-co-receptor Bonzo. Nat Immunol 2000; **1:**298–302.

8 Wilbanks A, Zondlo SC, Murphy K *et al.* Expression cloning of the STRL33/BONZO/TYMSTR ligand reveals elements of CC, CXC, and CX3C chemokines. J Immunol 2001; **166:**5145–54.

9 Wang W, Soto H, Oldham ER *et al.* Identification of a novel chemokine (CCL28), which binds CCR10 (GPR2). J Biol Chem 2000; **275:**22313–23.

10 Pan J, Kunkel EJ, Gosslar U *et al.* A novel chemokine ligand for CCR10 and CCR3 expressed by epithelial cells in mucosal tissues. J Immunol 2000; **165:**2943–9.

11 Gosling J, Dairaghi DJ, Wang Y, Hanley M, Talbot D, Miao Z, Schall TJ. Cutting edge. Identification of a novel chemokine receptor that binds dendritic cell- and T cell-active chemokines including ELC, SLC, and TECK. J Immunol 2000; **164:**2851–6.

12 Schweickart VL, Epp A, Raport CJ, Gray PW. CCR11 is a functional receptor for the monocyte chemoattractant protein family of chemokines. J Biol Chem 2000; **275:**9550–6.

13 Schweickart VL, Epp A, Raport CJ, Gray PW. CCR11 is a functional receptor for the monocyte chemoattractant protein family of chemokines. J Biol Chem 2001; **276:**856.
14 Nomiyama H, Tanase S, Yoshie O, Sakaki Y. Cytokine signalling pathway database. http://csp.medic.kumamoto-u.ac.jp/
15 Richmond A, Balentien E, Thomas HG *et al.* Molecular characterisation and chromosomal mapping of melanoma growth stimulatory activity, a growth-factor structurally related to beta-thromboglobulin. EMBO J 1988; **7:**2025–33.
16 Walz A, Dewald B, von Tscharner V, Baggiolini M. Effects of the neutrophil-activating peptide NAP-2, platelet basic protein, connective tissue-activating peptide III and platelet factor 4 on human neutrophils. J Exp Med 1989; **170:**1745–50.
17 Ward SG, Bacon K, Westwick J. Chemokines and T lymphocytes: more than an attraction. Immunity 1998; **9:**1–11.
18 Horuk R. Physiology – chemokines beyond inflammation. Nature 1998; **393:**524–5.
19 Klein RS, Williams KC, Alvarez-Hernandez X, Westmoreland S, Force T, Lackner AA, Luster AD. Chemokine receptor expression and signaling in macaque and human fetal neurons and astrocytes. Implications for the neuropathogenesis of AIDS. J Immunol 1999; **163:**1636–46.
20 Bakhiet M, Tjernlund A, Mousa A, Gad A, Stromblad S, Kuziel WA, Se A, Andersson J. RANTES promotes growth and survival of human first-trimester forebrain astrocytes. Nat Cell Biol 2001; **3:**150–7.
21 Bezzi P, Domercq M, Brambilla L *et al.* CXCR4-activated astrocyte glutamate release via TNF-α: amplification by microglia triggers neurotoxicity. Nat Neurosci 2001; **4:**676–8.
22 Hesselgesser J, Halks-Miller MI, Vecchio V, Peiper SC, Hoxie J, Kolson DL, Taub D, Horuk R. CD4-independent association between HIV-1 gp120 and CXCR4: functional chemokine receptors are expressed in human neurons. Curr Biol 1997; **7:**112–21.
23 Hesselgesser J, Taub D, Baskar P, Greenberg M, Hoxie J, Kolson DL, Horuk R. Neuronal apoptosis induced by HIV-1 gp120 and the chemokine SDF-1 is mediated by the chemokine receptor CXCR4. Curr Biol 1998; **8:**595–8.
24 Hayes IM, Jordan NJ, Towers S *et al.* Human vascular smooth muscle cells express receptors for CC chemokines. Arterioscler Thromb Vasc Biol 1998; **18:**397–403.
25 Addison CL, Daniel TO, Burdick MD *et al.* The CXC chemokine receptor 2, CXCR2, is the putative receptor for ELR+CXC chemokine-induced angiogenic activity. J Immunol 2000; **165:**5269–77.
26 Yamamoto T, Eckes B, Mauch C, Hartmann K, Krieg T. Monocyte chemoattractant protein-1 enhances gene expression and synthesis of matrix metalloproteinase-1 in human fibroblasts by an autocrine IL-1α loop. J Immunol 2000; **164:**6174–9.
27 Nanki T, Nagasaka K, Hayashida K, Saita Y, Miyasaka N. Chemokines regulate IL-6 and IL-8 production by fibroblast-like synoviocytes from patients with rheumatoid arthritis. J Immunol 2001; **167:**5381–5.
28 Jordan NJ, Kolios G, Abbot SE, Sinai MA, Thompson DA, Petraki K, Westwick J. Expression of functional CXCR4 chemokine receptors on human colonic epithelial cells. J Clin Invest 1999; **104:**1061–9.
29 Stellato C, Brummet ME, Plitt JR, Shahabuddin S, Baroody FM, Liu MC, Ponath PD, Beck LA. Cutting edge: expression of the C-C chemokine receptor CCR3 in human airway epithelial cells. J Immunol 2001; **166:**1457–61.
30 Mueller SG, Schraw WP, Richmond A. Melanoma growth-stimulatory activity enhances the phosphorylation of the class-II interleukin-8 receptor in nonhematopoietic cells. J Biol Chem 1994; **269:**1973–80.
31 Norgauer J, Metzner B, Schraufstatter I. Expression and growth-promoting function of the IL-8 receptor beta in human melanoma cells. J Immunol 1996; **156:**1132–7.
32 Muller A, Homey B, Soto H *et al.* Involvement of chemokine receptors in breast cancer metastasis. Nature 2001; **410:**50–6.
33 Mellado M, Rodriguez-Frade JM, Vila-Coro AJ, Martin de Ana A, Martinez A. Chemokine control of HIV infection. Nature 1999; **400:**273–4.
34 Mellado M, Rodriguez-Frade JM, Vila-Coro AJ, Fernandez S, Martin de Ana A, Jones DR, Toran JL, Martinez A. Chemokine receptor homo- or heterodimerization activates distinct signaling pathways. EMBO J 2001; **20:**2497–507.