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REVIEW

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Chemokines: a superfamily of chemotactic cytokines

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Abstract Chemokines are a bipartite family of chemotactic proteins that bear the structural hallmark of four cysteine residues, the first two of which are in tandem. The spectrum of action of chemokines encompasses a large number of leukocyte populations, including monocytes, granulocytes, lymphocytes, NK and dendritic cells. Although the spectrum of action of chemokines largely overlaps, clear differences are still present. Chemokines play an important role in the recruitment of leukocytes at the site of inflammation, allergic reaction and tumors. Available information on receptor usage by MCP-1 and related chemokines and signal transduction pathways is reviewed. The better understanding of signaling mechanisms will provide a new basis for the development of therapeutic strategies.

Key words Chemokines · Chemotaxis · Pathology · Signal transduction

Introduction

The extravasation of leukocytes from blood to tissues is best described as a multistep process in which many molecules are involved at any given step. Chemotactic cytokines play a crucial role in this process and activation of chemotactic receptors on leukocytes is mandatory for transmigration [1].

Chemokines are a complex superfamily of chemotactic cytokines mainly involved in immune and inflammatory processes [2, 3]. Structurally, they are characterized by four conserved Cys residues, the first two of which are in tandem. A Cys-X-Cys and Cys-Cys family are distinguished based on the relative position of the Cys tandem, with interleukin (IL-8) and monocyte chemotactic protein-1–8 (MCP-1) as prototypic molecules. Recently a new molecule with only two Cys residues, lymphotactin, has been identified [4].

Chemokines are involved in a variety of disease processes ranging from inflammation to neoplasia and have become an important target for therapeutic intervention and drug design. Due to the complexity of this family, which may include as many as 30 molecules and genes, we will focus this review on the structure and immunobiology of C-C chemokines.

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C-C chemokines

Several independent lines of work led to the identification of MCP-1 and related molecules. In the early 1970s it was noted that supernatants of activated blood mononuclear cells contained attractants active on monocytes and neutrophils [5]. Subsequently a chemotactic factor active on monocytes was identified in culture supernatants of mouse [6] and human [7, 8] tumor lines and was called tumor-derived chemotactic factor (TDCF) [7–9]. TDCF was at the time unique in that it was active on monocytes but not on neutrophils [8] and had a low molecular weight [12 kilodaltons (kDa)] [7, 8]. Moreover, it was implicated in the regulation of macrophage infiltration in murine and human tumors [7, 8, 10]. A molecule with similar cellular specificity and physicochemical properties was independently identified in the culture supernatant of smooth muscle cells (smooth muscle-derived chemotactic factor) [11]. The JE gene had been identified as an immediate-early platelet-derived growth factor (PDGF)-inducible gene in fibroblasts [12, 13]. Thus, in the mid 1980s a gene (JE) was in search of function and a monocyte-specific attractant was awaiting molecular definition. In 1989, MCP-1 was suc-

cessfully purified from supernatants of a human glioma [14], a human monocytic leukemia [15], and a human sarcoma [16–18]: sequencing and molecular cloning revealed its relationship with the long-known JE gene [19–21].

The number of related monocyte chemoattractants, their spectrum of action, cellular source, and role in vivo now extend well beyond those of the initial studies. It is now known that MCP-1, -2, and -3 are produced by different cell types and play a role in a variety of pathophysiological conditions, which include neoplasia and vascular diseases. Most notably, the spectrum of action of these molecules has increased considerably, to include T cells, natural killer (NK) cells, basophils and, for MCP-3, eosinophils and dendritic cells.

Cellular sources

Originally it was thought that several C-C chemokines were selectively expressed by specific cell types, e.g., T cells for RANTES (Regulated on Activation, Normal T cell Expressed and Secreted), hu MIP-1α/LD78, hu MIP-1β/Act-2, and T cell activation gene 3 (TCA3)/I-309. Although human LD78 and Act-2 were identified from lymphocytes, the mouse counterparts (macrophage inflammatory proteins-1α and 1β) were isolated from macrophages stimulated with lipopolysaccharide (LPS). However, mouse JE was first isolated from fibroblasts [12], whereas human MCP-1 was initially derived from tumor cell lines [14, 15, 17, 18]. C-C chemokine can be expressed in a variety of cell types. In addition, certain cell types (e.g., osteosarcoma cells) secrete several C-C chemokines, including MCP-1, -2, -3, and RANTES, as well as a number of CXC chemokines (IL-8, GRO, IP-10, GCP-2) [2, 3, 22, 23].

Monocytes, fibroblasts, and endothelial cells are the predominant normal cellular sources of MCP-1/JE [17, 24–28]. More recent reports indicate that MCP-1 is produced by yet more cell types and by various tumor cell lines (Table 1). Although mouse JE has been identified as a PDGF-induced gene [12], human MCP-1 is predominantly induced in cells by IL-1, tumor necrosis factor-α (TNF-α), or interferon-γ (IFN-γ) [29–34]. Expression of mouse JE and human MCP-1 have been independently studied in virus- or ds RNA-treated cells [12, 35]. MCP-2 was found to be co-expressed with MCP-1 in fibroblasts and mononuclear leukocytes, but lower levels of MCP-2 were observed [25, 36]. Similarly, MCP-3 is co-inducible with MCP-1 in mononuclear leukocytes by phytohemagglutinin and IFN-γ [37]. In mouse mast cells immunoglobulin E plus antigen challenge induces MARC [38], the mouse equivalent of human MCP-3 [39]. It is clear that there is no specific cellular origin of MCP-1, -2, and -3 and that several normal cell types each co-produce these chemokines if appropriately stimulated. The inducibility of chemokines led to the earlier designations of SIS (Small Inducible Secreted) and SIG (Small Inducible Genes) for these molecules.

The physiological or pathological inducers for MCPs can be classified into several groups. Cytokines such as IL-1, TNF-α, and IFN-γ are potent stimulators of several C-C chemokines, including MCP-1, MCP-2, and MCP-3.

Table 1 Cellular sources of monocyte chemotactic proteins (MCPs) (*IL-1β* interleukin-1β, *TNF-α* tumor necrosis factor-α, *IFN-γ* interferon-γ, *PDGF* platelet-derived growth factor, *GM-CSF* granulocyte-macrophage colony-stimulating factor, *LPS* lipopolysaccharide, *ConA* concanavalin A, *PHA* phytohemagglutinin, *LDL* low-density lipoprotein, *PMA* phorbol myristate acetate

Producer cell types	Chemokine production			Inducer
	MCP-1	MCP-2	MCP-3	
Epithelial cells	+			IL-1β, TNF-a, IFN-γ
Keratinocytes	+			TNF-α, IFN-γ
Melanocytes	+			IL-1α, TNF-α
Fibroblasts	+	+	+	IL-1α, IL-1β, TNF-α, IFN-γ, PDGF, IL-4, IL-10, virus, dsRNA
Monocytes/macrophages	+	+	+	IL-1β, TNF-α, IFN-γ, IFNβ, GM-CSF, LPS, ConA, PHA, virus, dsRNA
Granulocytes		(+)		LPS
Endothelial cells	+	(+)		IL-1β, TNF-α, IL-4, LDL, thrombin, LPS, PMA, shear stress
Smooth muscle cells	+			IL-1β, TNF, LPS, LDL
Mesothelial cells	+			IL-1β, TNF-α, IFN-γ
Mesangial cells	+			IL-1β, TNF-α, IFN-γ, IgG, thrombin, LPS
Chondrocytes	+			IL-1β, TNF-α, PDGF, TGF-β, LPS, retinoic acid
Osteoblasts	+			IL-1β, TNF-α
Lipocytes	+			IL-1β, TNF-α, IFN-γ
Astrocytes	+			TNF-α
Tumor cells				
– Carcinoma (Hep-2)	+	(+)		IL-1β, IFN-γ, PMA
– Sarcoma (MG-63)	+	+		IL-1β, IFN-γ, virus
– Melanoma (Bowes)	+	+	+	
– Myelomonocytic leukemia (THP-1)	+	+	+	IFN-γ, LPS, PMA
– Glioma (U-105MG)	+			

Furthermore, other cytokines such as IL-4, IL-10, granulocyte-macrophage colony-stimulating factor (GM-CSF) PDGF, and transforming growth factor-β (TGF-β) induce expression of MCP-1 in certain cell types [40–43]. Synergy between cytokines (e.g., IL-1β and IFN-γ) for MCPs induction has been observed. Several types of infections (viral, bacterial), products derived from bacteria (e.g., LPS), viruses (e.g., ds RNA), and plants (e.g., mitogen), and various other immunomodulators also directly or indirectly induce MCP-1 and MCP-2 (Table 1). Production of MCP-1 and MCP-3 can also be downregulated by inhibitory cytokines (e.g., IL-13) or by glucocorticoids such as dexamethasone [37, 43]. Although often co-produced, the expression of MCP-1, -2, and -3 can be differently regulated, both qualitatively and quantitatively, depending on the inducer and the cellular source. For example, in connective tissue cells IL-1β was the best inducer of MCP-1, but IFN-γ was a better inducer of MCP-2. MCP-2 is produced at a lower absolute concentration than MCP-1 by these cells [25].

Protein structure

Human MCP-1 is a glycoprotein of 76 residues with four cysteines forming two intramolecular disulfide bridges [19, 20]. Several glycosylated forms of MCP-1 have been reported, ranging from 9 kDa to 17 kDa on sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). The addition of *O*-linked sugar and sialic acid residues contributes to the different molecular weight forms of MCP-1 [14, 44–46]. MCP-1 is a basic protein (pI = 10.6) with affinity for heparin.

Natural MCP-2 and -3 proteins were first co-purified from conditioned medium of osteosarcoma cells and identified by amino acid sequence analysis [47]. MCP-2 and MCP-3 contain 76 amino acids, including the four cysteines characteristic of the chemokine family. Both peptides display high sequence similarity to MCP-1 (62% and 71% identity, respectively). MCP-2 and MCP-3 are slightly more basic than MCP-1, with theoretical pIs of 10.8 and 10.9, respectively. Based upon the theoretical relative molecular mass (8,893 daltons) and on the apparent molecular weight of 7.5 kDa on SDS-PAGE [47, 48], no *O*-glycosylation is expected for MCP-2. Natural human MCP-3 occurred as an 11-kDa protein on SDS-PAGE [47]. Although the cDNA-derived protein sequence contains one amino terminal *N*-glycosylation site [37, 39], natural 11-kDa MCP-3 did not appear to be *N*-glycosylated [49]. Moreover, folded synthetic MCP-3 also appeared as an 11-kDa protein on SDS-PAGE, although the theoretical relative molecular mass is only 8,935 daltons [48]. In addition to the unglycosylated MCP-3 form, Minty et al. [37] detected multiple forms (11, 13, 17, and 18 kDa) after expression in COS cells. Here, both *N*- and *O*-glycosylation were involved. Electrospray mass spectrometry of the unglycosylated protein confirmed the amino terminal pyroglutamate and the existence of two disulfide bridges.

The murine equivalent of human MCP-1 has been identified as the competence gene JE, regulated by PDGF [12, 50]. Surprisingly the JE gene codes for an amino terminal blocked protein with an additional carboxy terminal tail (49 amino acids) compared with human MCP-1. The corresponding biologically active protein has been isolated from virally infected fibroblasts [35]. The murine homologue of MCP-3 has been isolated from macrophages using a cDNA probe for human MCP-3 [51]. The sequence was found to be identical to that of MARC, derived from mast cells challenged with immunoglobulin E and antigen [38] and FIC isolated from fibroblasts stimulated with serum [52]. Mouse JE and MARC show 55% and 59% amino acid sequence similarity with human MCP-1 and MCP-3, respectively [39].

Receptors

Receptor binding studies and cross-desensitization, evaluated as an increase in intracellular calcium concentration or measurement of biological responses such as chemotaxis or histamine release, have been useful for characterizing chemokine receptors and their promiscuous usage by different agonists. More recently, the identification and cloning of multiple membrane receptors has helped to elucidate, but not to completely clarify, the complex pattern of chemokine cell activation. Two distinct receptors (type A and B) have been reported that bind with high affinity to IL-8 [53, 54]. The two proteins have a molecular mass of about 40 kDa and share 77% sequence identity. IL-8 receptor B also binds with high affinity the other members of the C-X-C chemokine family (NAP-2 and GRO). Four different receptors for C-C chemokines have been identified. The first, the CC CKR1, initially known as the MIP-1α/RANTES receptor [55, 56], also binds MCP-3 with high affinity [57]. This protein maps on chromosome 3p21 [55] and similar to the other known chemotactic receptors is a member of the GTP-binding protein-coupled receptor superfamily. The receptor is related to the IL-8 and the C5a and FMLP receptors with a degree of amino acid identity of approximately 30% and 23%, respectively. Two cDNA encoding two MCP-1 receptors (CC CKR2A and B) with alternatively spliced carboxyl tails were cloned in Mono Mac 6 cells, a monocytic cell line. The two receptors are approximately 50% identical to the MIP-1α/RANTES receptor and are expressed in THP-1 cells and monocytes [58]. Recently, it was reported that CC CKR2B is a promiscuous receptor for MCP-1 and MCP-3 [59]. CC CKR3 is a eosinophil-selective chemokine receptor that binds MIP-1α, MIP-1β, and RANTES [60]. This receptor shows 63%, 51%, and 31% sequence identity to CC CKR1, CC CKR2B, and IL-8 (A and B) receptors, respectively. More recently, a receptor promiscuous for MIP-1α, RANTES and MCP-1 was cloned in a human basophilic cell line [61]. This protein shows 40%–50% homology with the other chemokine receptors and is expressed in monocytes and in T and B lymphocytes.

The MIP-1α/RANTES receptor showed a high level of amino acid identity, 33% (56% in the amino terminal extracellular region), with a protein encoded by US28, an open reading frame of human cytomegalovirus [55, 56]. Expression of US28 cDNA in 293 or K562 cells showed that this receptor binds and signals after the interaction with MIP-1α, RANTES, and MCP-1, but not with IL-8, a C-X-C chemokine [56, 62]. Similarly, herpes samiri virus encodes a promiscuous calcium-mobilizing receptor for IL-8, GROα, and NAP-2 [63]. These observations, together with the finding that other viruses are able to express genes which encode for cytokines or cytokine receptors, indicate that mammalian gene piracy is a general evolutionary mechanism acquired by viruses to elude immunological and inflammatory host responses [64, 65].

A promiscuous chemokine receptor with an estimated molecular weight of approximately 35 kDa was identified on the surface of red blood cells [66, 67]. Radiolabelled IL-8, GROα, NAP-2 (C-X-C) and RANTES, MCP-1, but not MIP-1α (C-C), bind reversibly to 1,000–9,000 sites/cell with a K_d of 5 nM. In contrast to leukocyte populations, both C-C and C-X-C chemokines displace each other in a heterologous manner. Overall, the sequence has 27% identity with that of IL-8RB and 23% identity with the MIP-1α/RANTES receptor. The red blood cell chemokine receptor was identified as the Duffy blood group antigen, the erythrocyte receptor for *Plasmodium vivax* [68]. The physiological role and importance of chemokine binding to the Duffy antigen remains undefined. However, recent data showing the expression of this receptor by cells other than red blood cells suggest that this protein may play a role in controlling leukocyte extravasation into tissues.

Signal transduction in human monocytes

The molecular mechanisms responsible for monocyte activation by chemokines have been recently elucidated [22, 69]. Here, only the most recent findings will be discussed. Elevation of intracellular calcium concentration has been reported to be one of the earliest events after receptor engagement by most of the C-C chemokines [52, 55, 56, 58, 70–81]. This response is rapid, transient, and sensitive to *Bordetella pertussis* toxin (PTox) [52, 73, 75, 78, 82], suggesting that chemokine receptors are associated with PTox-sensitive GTP-binding proteins. In support of this observation it was reported that monocyte chemotaxis in response to MCP-1, MCP-3, RANTES, and MIP-1α is inhibited in a concentration-dependent manner by PTox [70, 77, 78], while under the same experimental conditions cholera toxin (CTox) was ineffective [70, 78]. However, the chemotactic response of monocytes to MCP-2 [70] and of IL-2-activated NK cells to MCP-1, RANTES, and MIP-1α [83] were recently reported to be sensitive to CTox, suggesting that chemokine receptors can be associated with both PTox- and CTox-sensitive GTP-binding proteins. Inhibition of forskolin-induced cyclic AMP generation by MCP-1 was also recently reported to be a precocious effect of MCP-1B receptor activation [82]. This is consistent with the activation of a $G_{\alpha i}$GTP-binding protein. The role of cyclic AMP metabolism in chemokine-induced biological responses awaits further investigation.

In initial studies it was found that in human monocytes activated with MCP-1 the influx of calcium across the plasma membrane rather than the release of calcium from intracellular stores appeared to be the main mechanism responsible for intracellular calcium elevation [70, 77]. In parallel, MCP-1 stimulation of human monocytes did not result in a detectable metabolism of phosphatidyl inositol biphosphate [77]. More recently, single cell analysis of human monocytes selected by avid adherence has shown that MCP-1 also mobilizes calcium from intracellular stores [84]. In addition, MCP-1B receptor expressed in 293 cells induces the discharge of intracellular calcium stores, although this was not associated with inositol trisphosphate production [82].

Calcium influx was required for arachidonate accumulation by MCP-1 in human monocytes [85]. Arachidonic acid release by C-C chemokines (MCP-1, MCP-3, RANTES, and MIP-1α) was rapid (<15 s), reached the plateau at 2–3 min, and was inhibited by PTox [70, 85, 86]. Platelet activating factor, a product of membrane phospholipid metabolism, increased in a synergistic fashion both arachidonic acid release and chemotactic response by MCP-1, MCP-3, RANTES, and MIP-1α [85, 86]. Recently, it was observed that 5-oxo-ETE, a product of 5-hydroxy-eicosanoid dehydrogenase, could also strongly increase both monocyte migration and arachidonic acid release by MCP-1 and MCP-3, but not by FMLP (S. Sozzani, unpublished work). These results, together with the finding that phospholipase A_2 inhibitors block both monocyte polarization and chemotaxis [85], support arachidonic acid as a second messenger for monocyte migration to chemokines.

Elevation of intracellular calcium might also be required to sustain receptor-induced protein kinase activation. Staurosporine, C-I, and H-7, inhibitors of serine/threonine kinases, and genistein and erbstatin, inhibitors of tyrosine kinases, were found to inhibit monocyte migration in response to MCP-1, RANTES, MIP-1α, MCP-2, and MCP-3 [70, 78]. These results indicate a possible role for protein kinases in the induction of monocyte migration. The exact role and nature of the protein kinases involved await further investigation.

In vitro effects

Chemotaxis, the eponymous function of chemokines, is the most extensively studied activity. Functions such as expression of cytokines, enzymes, and adhesion molecules have been studied in a less extensive and systematic fashion. Table 2 summarizes concisely the spectrum of action of C-C chemokines emerging from studies of human molecules. The activity of human IL-8, the prototypic C-X-C

chemokine, is also shown for comparison. Chemokines of animal origin have been studies less extensively [87–89]: the discordant results obtained for the effect of MIP-1 on neutrophils in mouse versus man suggest that significant differences in the spectrum of action of chemokines may exist among species. Human C-C chemokines have been studied in a number of in vitro and in vivo rodent experiments [7, 16, 51, 90, 91]. Studies conducted mainly with MCP-1, but to some extent also with MCP-3 and RANTES, suggest that, while the mouse molecules are fully active in humans, human C-C chemokines are considerably less active in the mouse [87, 88]. These results caution against underestimating the potential of these molecules in heterologous systems.

Monocytes

All members of the C-C chemokine family which have been adequately tested share the capacity to induce leukocyte migration with distinct, but overlapping, spectra of action (Table 2). Human and murine MCP-1, MCP-2, MCP-3, RANTES, and I-309 elicit directional migration of mononuclear phagocytes and are inactive on neutrophylic granulocytes [2, 3, 92]. MCP-1 affects several functions of mononuclear phagocytes related to recruitment or to effector activity (Table 3). Natural and recombinant MCP-1 augments expression of the integrins CD11b and c in human monocytes [93, 94]. Interaction and localized digestion of extracellular matrix components is essential for phagocyte extravasation and progression in tissues. MCP-1 induces production of gelatinase and urokinase-type plasminogen activator (uPA). Concomitantly, MCP-1 augments expression of the cell surface receptor for uPA [95]. Induction of gelatinase was also observed with MCP-2 and -3 [39, 47]. Thus, C-C chemokines arm monocytes with the molecular tools which allow localized and polarized digestion of extracellular matrix components during recruitment. In tumor tissues, the release of lytic enzymes by MCP-1 stimulated tumor-associated macrophages (TAM) may provide a ready-made pathway for invasion of tumor cells [96] and thus contribute to augmented metastasis associated with inflammation [97]. MCP-1 induces a respiratory burst in human monocytes, although it is a weak stimulus compared with other agonists [16, 80]. MCP-1 induces low levels of IL-1 but not TNF [93, 98]. Natural MCP-1 was also reported to induce IL-6 [93]. However, in another study recombinant MCP-1 had little effect on IL-6 release (M. Sironi et al., unpublished data). Human MCP-1 induced monocyte cytostasis for a tumor line [16] or synergized with bacterial products (but not with

Table 2 The spectrum of action of chemokines (*Neu* neutrophils, *Eo* eosinophils, *Ba* basophils, *Mo* monocytes, *Ly* lymphocytes, *NK* natural killer cells, *DC* dendritic cells, *ND* not determined)[a]

Family	Molecule	Neu	Eo	Ba	Mo	T Ly	NK	DC	B Ly
Cys-X-Cys[c]	IL-8	2+	±	+-	–	+	±	–	–
	IP-10	–	ND	ND	2+	2+	ND	–	ND
Cys-Cys	MCP-1	–	–	2+	3+	3+	2+	–	–
	MCP-2	–	+	+	3+	3+	2+	–	ND
	MCP-3	2+	2+	2+	3+	3+	2+	3+	ND
	RANTES	–	2+	2+	+	2+	2+	3+	+
	MIP-1α	±[b]	+	+	+	+	2+	3+	+
	MIP-1β	–	–	–	+	2+	–	ND	–

[a] The activity considered is migration
[b] Disputed
[c] The spectrum of action of IL-8 and IP-10 (C-X-C) is shown for comparison

Table 3 Effects of MCPs on leukocyte functions other than chemotaxis (*NO* nitric oxide, *uPA* urokinase-type plasminogen activator, *uPA-R* uPA receptor)

Chemokine	Cells	Function	References
MCP-1	Monocytes	Oxidative burst	[16, 80]
		Lysosomal enzyme release	[16, 162]
		Inhibition of NO synthase	[100]
		Gelatinase, uPA, uPA-R	[39, 47, 95]
		Adhesion molecules (CD11b and c)	[93, 94]
		IL-1 and IL-6	[93, 98]
		Tumor cytostasis and/or lysis	[16, 98, 99]
	Basophils	Histamine release	[103, 163]
MCP-2	Monocytes	Gelatinase	[47]
		Lysosomal enzyme release	[162]
	Basophils	Histamine release	[107]
MCP-3	Monocytes	Gelatinase	[47]
		Lysosomal enzyme release	[162]
	Basophils	Histamine release	[47, 107]

IFNγ) in stimulating mouse macrophage cytotoxicity [99] or human monocytes [98]. In an interesting and intriguing recent study, human MCP-1 inhibited the induction of nitric oxide (NO) synthase in the macrophage cell line J774 [100]. If confirmed, this suggests that MCP-1 could account for both the recruitment and concomitant partial functional deactivation of TAM.

Early descriptions of the chemokine subsequently identified as MCP-1 showed its selective activity on mononuclear phagocytes versus polymorphonuclear leukocytes as a distinctive and, at the time, unique property [6, 7]. However, more recent studies have shown that MCPs are active on multiple leukocyte populations.

Basophils and eosinophils

Unlike RANTES and MIP-1α, MCP-1 is not active on human eosinophils [74]. However, MCP-1 appears to have chemotactic properties for basophils [101], being active from 3 nM onwards with an optimal concentration of 10–30 nM. RANTES was active at the same concentration but resulted in a higher number of migrating cells, whereas MIP-1α caused basophil migration comparable to MCP-1 at 3 times lower concentrations [102]. MCP-1 (10 nM to 1 μM) was also able to induce histamine release from human basophils. This could be partially inhibited by preincubation with IL-8 or RANTES [103, 104]. When basophils were pretreated with IL-3, IL-5, or GM-CSF, the histamine releasing effect of MCP-1 was doubled, and basophils were also activated to release leukotriene C_4 (LTC_4) [75, 105]. MCP-1 was a stronger basophil agonist than IL-8, RANTES, MIP-1α, MIP-1β, complement fragment 3a (C3a), and anti-IgE receptor antibodies, but was somewhat weaker than C5a. Trigging of basophils with 30 nM MCP-1 induced a significant increase in intracellular calcium.

MCP-2 induced in vitro chemotaxis of eosinophils from 30 nM onwards, but was less potent than RANTES [106]. Like MCP-1, MCP-2 was chemotactic for basophils (from 10 nM onwards). MCP-2 (100 nM) was also able to induce enhanced (calcium-dependent) histamine secretion from human basophils. No effect could be detected on mouse peritoneal mast cells [107]. Synthetic [48] as well as recombinant [37] MCP-3 were potent (minimal effective concentration of 3 nM) chemotactic proteins for eosinophil and basophil granulocytes. MCP-3 also induced an increase in intracellular calcium in these cells [76, 107]. MCP-3 caused an enhanced histamine release from both unprimed and IL-3-treated basophils. Moreover, MCP-3 induced the release of LTC_4 from IL-3-treated basophil granulocytes [76, 107].

T lymphocytes

MCP-1, -2, and -3 were reported to induce directional migration of freshly isolated $CD4^+$ and $CD8^+$ T lymphocytes and T cell clones in vitro [108, 109]. In T clones, MCPs induced calcium fluxes that were sensitive to the action of PTox [108]. MCP-1 was also purified as the main attractant for T lymphocytes from cultures of mitogen-activated peripheral mononuclear cells [110]. Natural purified MCP-1 induced transendothelial migration of T lymphocytes with a memory phenotype ($CD45RO^+$) in a 4-h assay [110] and induced T lymphocyte accumulation in vivo when inoculated in mice with severe combined immunodeficiency [109]. These findings suggest MCPs are important determinants of T lymphocyte distribution in pathophysiological conditions.

NK cells

NK cells were tested by our group for their ability to migrate in response to MCP-1, MCP-2, and MCP-3 [111]. Purified NK cells (>80% $CD16^+$ and $CD56^+$ and <2% $CD3^+$ and $CD14^+$) were tested in a new double filter assay. Freshly isolated NK cells showed only a minor response to MCP-1, however, if NK cells were cultured in vitro in the presence of IL-2 (7–10 days), they acquired a strong response to MCP-1. Migration to MCP-1 showed a typical bell-shaped concentration curve with maximal migration observed at 50 ng/ml MCP-1. At peak concentration, a consistent fraction (approximately 30%) of the input cell population responded to the agonist. These results were confirmed and extended by Maghazachi et al. [83] who showed that IL-2-activated NK cells and NK 3.3 cells migrate in response to MCP-1, MIP-1α, and RANTES. MCP-1 and RANTES also induced chemokinesis of NK cells [83]. IL-2-cultured NK cells showed specific binding sites for labelled MCP-1, and cell migration was inhibited in a concentration-dependent manner by both CTox and PTox. Collectively, these results show that NK cells express specific receptors for MCP-1. By reverse transcriptase polymerase chain reaction, we recently found that IL-2-cultured NK cells express MCP-1 receptor transcripts [112].

Dendritic cells

Recently we have observed that in vitro cultured dendritic cells ($CD1a^+$, MHC class II $L243^+$, $CD14^-$, $CD3^-$, and $CD20^-$) migrate in response to MCP-3, RANTES, and MIP-1α but not to MCP-1 and MCP-2. Peak active concentrations and the percentage of input cells migrating in response to chemokines were comparable to those observed with monocytes. Active cytokines were also able to induce a significant increase in cytosolic calcium concentration [113]. Dendritic cells exert a sentinel function by picking up antigens in nonlymphoid organs and triggering naive T cell-mediated immune responses. To accomplish this, dendritic cells need to localize in tissues and subsequently to migrate to lymphoid organs. It is very likely that chemokines will play an important role in directing dendritic cell traffic. The effect of chemokines on other biological functions peculiar to these cells, such as macropinocytosis or antigen presentation, are at present unknown.

In vivo effects and significance

Most available information on the in vivo production and role of C-C chemokines relates to MCP-1. There is evidence that MCP-1 may play a role in neoplastic diseases, inflammatory reactions, and atherosclerosis (Table 4). However, with a few notable exceptions, available information is indirect and correlative in nature.

Neoplasia

Analysis of mechanisms of recruitment of macrophages in tumors was one pathway that led to the identification of MCP-1 [7, 10, 14, 18, 114]. Several lines of evidence suggest that MCP-1 can represent an important determinant of the levels of TAM [10, 114]. In early studies with murine tumors or human tumors in nude mice a correlation was found between MCP-1 activity and percentage of TAM, a finding confirmed in subsequent experiments with the MCP-1 probe [115]. Subcutaneous inoculation of tumor-derived human MCP-1, MCP-2, and MCP-3 led to macrophage infiltration [16, 47]. Finally, and conclusively, transfer of the mouse or human MCP-1 gene was associated with augmented levels of macrophage infiltration [90, 91]. High expression of MCP-1 was associated with abrogation of tumorigenicity of CHO cells [90] but not of malignant mouse tumors [91]. At low tumor inocula, MCP-1 gene transfer was associated with higher tumorigenicity and lung colonizing ability, despite a lower growth of resulting lesions [91, 116]. These findings were interpreted in the light of the dual influence that TAM can exert on tumor growth [10, 114].

Leukocyte infiltration is associated with administration of cytokines such as interferons, IL-2, or IL-4, by conventional routes or following gene transfer. Interferon and IL-2 induce endogenous chemokines in a renal cancer model [117]. Thus secondary induction of chemokines may play a pivotal role in leukocyte recruitment in tumors treated with cytokines other than chemokines.

Various human tumor lines express MCP-1 in vitro spontaneously or after exposure to inflammatory signals, and some do in vivo. The latter include gliomas, histiocytomas, sarcomas, and melanoma [14, 16, 118, 119]. Expression of MCP-1 was recently found in Kaposi's sarcoma (KS) in vivo and in KS-derived spindle cell cultures [120]. For these studies, we used a novel anti-MCP-1 monoclonal antibody (mAb) (5D3) and assays based on it [121]. Since KS is characterized by a conspicuous macrophage infiltrate and is believed to represent a cytokine-propelled disease, production of MCP-1 may be particularly significant in this disease.

Freshly isolated ovarian carcinoma cells, primary cultures, and some established cell lines were shown in early studies to release tumor-derived chemotactic factor (TDCF) activity [7–9]. These observations were recently revised [122]. Immunohistochemistry and in situ hybridization demonstrated that ovarian carcinoma cells and, in some tumors, also stromal elements express MCP-1. High levels of MCP-1 were measured in the ascites (but not in blood) of patients with ovarian cancer but not in the peritoneal fluid of patients with nonmalignant conditions. Production of MCP-1 and recruitment of TAM is likely to play an important role in progression of this disease because macrophage-derived cytokines promote the growth of ovarian carcinoma and its secondary implantation in peritoneal organs [123].

Table 4 Evidence for an in vivo role of MCPs

	Species	Molecule	Disease	Evidence	Selected references
Neoplasia	Mouse	MCP-1	Various histologies	Correlations; *gene transfer*[a]	[90, 91]
	Man	MCP-1	Histocytoma	In situ expression	[119]
			Glioma	In situ expression	[14, 164]
			Sarcomas	In situ expression	[165]
			Melanoma	In situ expression	[118]
			Kaposi's sarcoma	In situ expression	[120]
			Ovarian carcinoma	In situ expression	[9, 122]
Atherosclerosis	Primates	MCP-1	Hypercholesterolemia	In situ expression	[127]
	Rabbit	MCP-1	Plaques	In situ expression	[129]
	Man	MCP-1	Plaques	In situ expression	[128–130]
Inflammatory/ immune reactions	Mouse	MCP-1	Encephalomyelitis	In situ expression	[132]
	Rat	MCP-1	Pulmonary alveolitis	In situ expression; *antibody blocking*[a]	[135]
			Pulmonary granuloma	In situ expression; *antibody blocking*[a]	[136]
			Allograft	In situ expression	[131]
				Kidney ischemia	In situ expression [137]
	Man	MCP-1	Rheumatoid arthritis	In situ expression	[43, 145]
			Idiopathic pulmonary fibrosis	In situ expression	[133, 134, 140]
			Chronic active hepatitis	In situ expression	[141, 142]
			Schistosoma egg granulomas	In situ expression; *antibody blocking*	[149]

[a] Direct evidence of in vivo functions of MCPs is italicized

The available information suggests that MCP-1 is an important determinant of macrophage infiltration in murine and, at least some, human tumors. Human tumor lines of epithelial origin (breast, colon, ovary [7, 9], release small molecular weight chemoattractant(s). Only for ovarian carcinoma was the TDCF recently identified as MCP-1. Whether MCP-1 or a related chemokine explains these observations and is involved in macrophage recruitment in common epithelial cancers remains to be defined. Tumor-derived MCP-1 could downregulate important anti-tumor pathways (e.g., NO [100]), induce production of growth stimulatory cytokines (e.g., IL-1 or IL-6 in ovarian cancer [93, 98]), and stimulate the production of proteolytic enzymes which could promote a process of counter-current invasion [39, 47, 95, 96]. Thus, MCP-1 and related molecules produced by certain tumors may play a role in the immunobiology of neoplastic tissues which extends beyond the mere recruitment of mononuclear phagocytes.

Examination of macrophage function and inflammation in neoplastic disorders, as well as in other inflammatory conditions, reveals a paradoxical situation in which recruitment at the tumor site co-exists with a systemic defect in the ability to mount local inflammatory reactions [10, 124]. We speculated that chemokines produced continuously in tumors may also contribute to the systemic impairment of macrophage function observed in advanced neoplasia [10]. In support of this hypothesis, chemoattractants were recently found to cause rapid release of the IL-1 decoy receptor and of the p75 TNF receptor [125, 126], which could buffer the action of these inflammatory mediators.

Atherosclerosis

Recruitment of monocytes is the first recognizable event in the natural history of atherosclerosis. Vessel wall elements (endothelial cells, smooth muscle cells) produce abundant amounts of MCP-1 in response to inflammatory cytokines and modified lipids. MCP-1 has been detected in arterial walls in animal models of atherosclerosis [127–129]. Moreover, MCP-1 has been detected in human atheromatous plaques [128–130]. Interestingly, in plaques MCP-1 expression is most prominent in subendothelial macrophage and endothelial cells. Their relative expression is dependent on the progression level of the atherosclerotic lesion [128].

Inflammatory and immune reactions

Expression of MCP-1 was detected in a variety of animal models of inflammatory and immune reactions, including cardiac allografts [131], allergic encephalomyelitis [132], bleomycin-induced pulmonary fibrosis [133, 134], pulmonary granuloma and immune complex alveolitis [135, 136], renal ischemia [137], and bacteremia [138]. In a rodent model of glomerulonephritis and in kidney biopsies from patients with inflammatory glomerulopathies, MCP-1 expression was upregulated and was associated with a prominent monocyte infiltrate [139]. More recently, increased levels of MCP-1 were observed in the urine of patients with lupus nephritis. MCP-1 was detected only in the active form of the disease and was decreased by glucocorticoid administration (A. Mantovani, G. Remuzzi, unpublished work). In human diseases, in situ hybridization and polyclonal antisera have revealed MCP-1 mRNA and/or protein in idiopathic pulmonary fibrosis [140], chronic active hepatitis [141, 142], skin delayed-type hypersensitivity reactions [143], and rheumatoid arthritis [43, 144–146]. In the latter disease, it is of interest that MCP-1 expression could be induced in synovial fibroblasts while synovial macrophages constitutively express the chemokine [144]. Blood levels of MCP-1 in humans have been studied using sandwich ELISAs based on polyclonal antisera and/or mAb, with discrepant results as to its presence in normal serum [147, 148]. Free anti-MCP-1 IgG is present in normal human donors and decreases following intravenous inoculation of endotoxin, with a concomitant rapid increase in MCP-1 levels [147]. These findings raise the interesting possibility that anti-chemokine autoantibodies represent a regulatory pathway for these mediators.

Although MCP-1 has been found in a variety of inflammatory conditions, there are only a few studies providing direct evidence of its in vivo importance. In a rat model of immune complex-induced alveolitis, anti-MCP-1 antibody reduced the severity of the disease [135]. In another study an anti-MCP-1 antiserum partially inhibited lung granuloma formation in rats [136]. Anti-MCP-1 antiserum also reduced the inflammatory reaction in *Schistosoma* egg granulomas [149].

Therapeutic strategies

Given the involvement of chemokines in a wide range of inflammatory diseases, it is not surprising that considerable efforts are being made to exploit these molecules therapeutically. The main strategies under evaluation are summarized in Table 5 and are briefly discussed here.

Inhibition of synthesis

Classic immunosuppressive and anti-inflammatory drugs are potent inhibitors of the production of certain chemokines, such as IL-8 and MCP-1. Active molecules include glucocorticoid hormones, FK 506, and cyclosporine A [137, 150–154]. The identification of 5′ regulatory sequences has allowed definition to some extent of the molecular targets. Given the promiscuity of transcription factors such as NFkB it is at present unclear whether this approach will eventually lead to the development of selective anti-chemokine agents.

Antibodies

Antibodies to C-C chemokines are invaluable in defining their role in pathophysiology. mAbs directed against IL-8

Table 5 Therapeutic strategies aimed at chemokines (*mAb* monoclonal antibody)

Strategy	Molecules	Chemokine	Pathology	Selected references
Inhibition of synthesis	Glucocorticoids, FK506	IL-8, MCP-1	Various	[137, 150–154]
Blocking antibodies	mAb	IL-8 MCP-1	Ischemia/reperfusion,	[155, 156] [135, 136, 149]
Antagonists: peptides	Analogue	MCP-1 IL-8	ND	[157, 158] [166]
Antagonists: simple chemicals	?	IL-8	ND	(T. Wells, personal communication)
Hematopoietic precursor inhibition or recruitment	MIP-1α/LD78	MIP-1α/LD78	Myelosuppression; blood precursor transplantation	[159] [161]
Gene therapy	MCP-1	MCP-1	Neoplasia	[90, 91]

have been investigated systematically for their potential to modify pathology in animal models. Anti-IL-8 mAb were found to be beneficial in a range of pathological conditions, including ischemia reperfusion injury, inflammatory kidney diseases, septic shock, and delayed-type hypersensitivity reactions [155, 156]. The latter observation is intriguing and surprising in view of the marginal role generally attributed to neutrophils in this type of reaction.

Antagonists

Considerable efforts have been made to develop chemokine antagonists. This has been partially prompted by the nature of the receptors which belong to a class of classical pharmacological targets. Chemokines with altered sequence can act as antagonists, e.g., N terminally altered MCP-1 [157, 158]. Recently, based on the three-dimensional structure of a peptide agonist, a first simple chemical with low but significant capacity to compete for receptor binding of IL-8 was described (T. Wells, personal communication). The identification of eotaxin (and its receptor) as a specific eosinophil attractant will probably generate further impetus to develop chemokine antagonists.

Hematopoiesis

Various chemokines affect hematopoietic precursors, but this is a prominent property of MIP-1α [159, 160]. MIP-1α inhibits the proliferation of normal early hematopoietic precursors. It has therefore been suggested that it may be useful to protect normal stem cells from damage of cytotoxic chemotherapy.

MIP-1α, IL-8, and probably other chemokines cause the recruitment from the bone marrow into the blood of hematopoietic precursors [161]. They may therefore represent an alternative to G-CSF to obtain precursors from the blood for transplantation.

Gene therapy

Transfer of chemokine (MCP-1, IP-10) genes into tumors caused growth retardation or regression [90, 91]. The effect was highly dependent upon the tumor system (unpublished work). The recent identification of chemokines active on dendritic cells may provide tools to direct dendritic cell traffic in immunization strategies.

Concluding remarks

C-C chemokines are chemotactic proteins with overlapping spectra of action which include monocytes, T cells, NK cells, and basophils as common targets. Activity on dendritic cells is at present restricted to MCP-3, RANTES, and MIP-1α. These spectra of action suggest that these molecules may play an important role not only in inflammatory and neoplastic conditions but also in the generation and expression of immune and allergic reactions. As such they now represent a prime target for the development of novel therapeutic strategies. While chemical antagonists remain the holy grail for the future, it is likely that the first anti-chemokine strategy to undergo antergo clinical evaluation is likely to be antibodies. Redundancy and promiscuity of receptor usage represent formidable stumbling blocks for the development of effective anti-chemokine strategies.

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