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Monocyte chemotactic protein-3

Abstract

Monocyte chemotactic protein-3 (MCP-3) belongs to the MCP subgroup of CC chemokines that are structurally closely related but, which differ in receptor usage and hence in biological activities. MCP-3 is one of the most pluripotent chemokines since it activates all types of leukocytes, by binding to at least four different chemokine receptors. The natural protein is heterogeneous due to glycosylation and NH2-terminal processing. Only small amounts of MCP-3 are induced in various cell types by endogeneous (cytokines) or exogeneous (bacteria, viruses) agents. Nevertheless, this omnipotent chemokine, inducible in most body compartments, might play an important role in normal homeostasis

as well as in various pathologies including cancer, auto-immune diseases and chronic inflammation.

Full text

INTRODUCTION

Chemokines constitute a rapidly growing family of low molecular weight proteins, characterized by the conservation of four cysteines, which are important for their tertiary structure [1]. Disulfide bridges are formed between cysteines one and three and between cysteines two and four. Chemokines have been classified into two major subfamilies, namely CXC and CC chemokines, depending on whether their first two NH_2-terminal cysteines are separated by an amino acid (CXC) or are adjacent (CC) [1]. The CXC chemokine subfamily can be further divided into ELR^+ and ELR^-CXC chemokines, based on the presence or absence of the Glu-Leu-Arg (ELR) motif, just in front of the first cysteine. ELR^+CXC chemokines are angiogenic and mainly attract neutrophils, whereas ELR^-CXC chemokines have angiostatic properties and preferentially attract monocytes and lymphocytes. In contrast, CC chemokines are chemotactic for a variety of leukocyte subsets. In addition to the 15 CXC chemokines and 27 CC chemokines already identified, two other chemokines, fractalkine and lymphotactin (alpha and beta), have also been characterized [1]. Lymphotactin lacks cysteines one and three of the typical chemokine structure and is, so far, the only member of the C chemokine subfamily. It exerts chemotactic activity on T lymphocytes and natural killer (NK) cells. Fractalkine, on the other hand, belongs to the CX_3C chemokine subfamily as it exhibits three amino acids between the first two cysteines. It is the only membrane-bound chemokine and chemoattracts T lymphocytes as well as monocytes. In contrast to tumor cells, which often constitutively produce chemokines, normal cells are generally poor chemokine producers, unless they are stimulated with an appropriate endogeneous (cytokine) or exogeneous (plant, viral, or bacterial products) inducer. Instead of remaining in solution, *in vivo*, chemokines are immobilized through low affinity binding to extracellular matrix proteins or to proteoglycans on the endothelium. This provides a mechanism for gradient formation under conditions of high blood flow and, in addition, facilitates the interactions of chemokines with passing leukocytes. Chemokines exert their activities on target cells through high affinity binding to and signaling through G protein-coupled receptors (GPCR) belonging to the serpentine receptor family [2]. So

far, five CXC chemokine receptors (CXCR1-5), ten CC chemokine receptors (CCR1-10), one C chemokine receptor (XCR1) and one CX_3C chemokine receptor (CX_3CR1) have been characterized [2]. Recently, some chemokine receptors were found to function as a coreceptor for entry of HIV-1 (human immunodeficiency virus-1) into macrophages or T-lymphocytes. In the absence of this coreceptor, HIV-1 can bind to its target cell (*via* the primary receptor CD4), but the fusion process cannot take place. Macrophage (M)- tropic (R5) HIV-1 strains, which are able to infect macrophages and primary T cells, but not T cell lines, use CCR5 as a coreceptor, whereas T cell-tropic (X4) HIV-1 strains that can only grow in primary T cells and T cell lines, require CXCR4 for entry [1, 2]. The CC chemokine "monocyte chemotactic protein-3" (MCP-3) binds to CCR5 and is therefore a potential inhibitor of R5 HIV-1 infection. In addition, it is the most pluripotent chemokine, attracting monocytes, lymphocytes, granulocytes, NK cells and dendritic cells [1].

MONOCYTE CHEMOTACTIC PROTEIN-3

Discovery of MCP-3

In 1992, human monocyte chemotactic protein-3 (MCP-3) was identified as a protein with monocyte chemotactic activity, copurified with the CC chemokines MCP-1 and MCP-2 from stimulated human osteosarcoma cells [3]. Because of its high sequence similarity with MCP-1 (71%) and MCP-2 (58%), the protein was named MCP-3. The cDNA and gene encoding human MCP-3 were cloned on the basis of the amino acid sequence determined for the purified MCP-3 protein [4-6]. The cDNA for the putative murine homologue of human MCP-3 was isolated by different groups and was named MARC (mast cell activation-related chemokine), FIC (fibroblast-inducible cytokine) or murine MCP-3 [7]. Human and murine MCP-3 show 59% homology at the protein level. In 1994, a cDNA for a fourth "monocyte chemotactic protein", which shared 59% identical amino acids with human MCP-3, was isolated and designated MCP-4 [8]. Recently, MCP-1, MCP-2, MCP-3 and MCP-4 have been renamed CCL2, CCL8, CCL7 and CCL13, respectively [9].

MCP-3 gene

The gene encoding human MCP-3 is located on chromosome 17q11.2-12, clustered with the other CC chemokine genes [6]. It codes for a precursor

protein of 99 amino acids and consists of three exons and two introns. The first exon contains the 5'untranslated region and a sequence coding for the signal peptide and the first two amino acids of the mature protein (Figure 1). The second exon encodes amino acids 3 to 42 of the mature protein whereas the third exon consists of the coding sequence for the COOH-terminal region of the protein and the 3'untranslated region. A polyadenylation signal and several destabilizing AT-rich sequences have been described in the 3'untranslated region of the human MCP-3 gene [8]. The TATA-box present in the MCP-3 promoter region controls the rate of basal transcriptional activity and is characteristic of rapidly transcribed genes. In addition, other regulatory elements, including a CAAT-box, an AP-1-like sequence and an Ets-like motif have also been described for the MCP-3 gene [8].

MCP-3 protein

Human MCP-3 is produced as a precursor protein containing 99 amino acids, including an aminoterminal hydrophobic signal sequence [8]. After cleavage of the signal peptide, a mature protein of 76 amino acids is secreted. The NH_2-terminal amino acid of MCP-3 is a pyroglutamic acid, a cyclic derivative of glutamine, which is not susceptible to Edman degradation. Therefore, different proteolytic fragments of natural MCP-3 were sequenced in order to determine its amino acid sequence [3]. The primary structures of human MCP-1, MCP-2, MCP-3 and MCP-4 are compared in Figure 2.

Expression of human MCP-3 in COS cells resulted in the isolation of four different recombinant MCP-3 glycotypes (11, 13, 17 and 18 kDa), due to *N*- and *O*-glycosylation. This is in agreement with the fact that MCP-3 contains one *N*-glycosylation site ($Asn_6Thr_7Ser_8$) and several potential *O*-glycosylation sites. Unglycosylated MCP-3 has a theoretical relative molecular mass (M_r) of 8956.4 and a high theoretical iso-electric point (pI = 9.74) because of the large number of lysine and arginine residues.

Owing to its NH_2-terminal pyroglutamic acid, MCP-3 cannot be cleaved by the serine protease CD26/dipeptidyl peptidase IV (DPP IV), which removes the NH_2-terminal dipeptide from many chemokines [10]. However, human MCP-3(1-76) is processed by gelatinase A into MCP-3(5-76), leading to loss of activity [11]. After cleavage by this metalloproteinase, MCP-3 still binds to its receptors, but no longer induces calcium fluxes or chemotaxis. Thus, cleaved MCP-3 acts as a

natural chemokine antagonist that reduces inflammation. Alternatively, addition of extra residues at the NH_2-terminus of MCP-3 also resulted in inactivation [12].

The tertiary structure of human MCP-3 was determined by ^{1}H-NMR (nuclear magnetic resonance) spectroscopy [13]. These analyses indicated that, in contrast to most chemokines, MCP-3 exists as a monomer at high concentrations (up to 2-20 mg/ml). The study also demonstrated that the monomeric structure of MCP-3 is similar to that of other CC chemokines since it also contains three beta-strands and a COOH-terminal alpha-helix (Figure 3). However, it differs from the other CC chemokines in its NH_2-terminal region. Indeed, while this region is disordered and flexible in MCP-3, it is structured in other CC chemokines such as MCP-1, RANTES and MIP-1beta.

Cellular sources of MCP-3

Human MCP-3 protein was originally copurified with MCP-1 and MCP-2 from the supernatant of osteosarcoma cells, induced with a cytokine mixture [3]. The amount of MCP-3 recovered was comparable to that of MCP-2 but was about 10-fold lower than the amount of MCP-1. Stimulation of the osteosarcoma cells with interleukin (IL)-1beta, interferon (IFN)-beta, phorbol 12-myristate 13-acetate (PMA) or virus resulted in the expression of MCP-3 mRNA [8]. In addition, several other tumor cell lines have been shown to express MCP-3 mRNA (Table 1). In promonocytic U937 cells, MCP-3 mRNA was found to be transiently induced by PMA and super-induced by addition of cycloheximide [5]. Furthermore, MCP-3 mRNA was also expressed in the myelomonocytic leukemia THP-1 cell line after stimulation with PMA or lipopolysaccharide (LPS) [8].

Following induction with Mycobacterial lipoarabinomannan (LAM), LPS, IL-1beta, TNF-alpha or IFN-gamma, monocytes were shown to express low levels of MCP-3 transcripts [14, 15]. Conversely, the anti-inflammatory cytokines IL-4, IL-10 or IL-13 suppressed this MCP-3 expression in monocytes [14]. Secretion of MCP-3 *protein* by mononuclear leukocytes was observed after stimulation with IL-1beta, IFN-alpha, IFN-beta, measles virus, poly I-C or Con A [16]. Surprisingly, LPS did not induce production of MCP-3 protein in these cells.

Furthermore, a synergistic induction of MCP-3 protein was observed in fibroblasts, stimulated with a combination of IL-1beta and IFN-gamma [16]. Stimulation of human umbilical vein endothelial cells (HUVEC) with IL-1beta, IFN-

gamma, TNF-alpha, or LPS resulted in expression of the MCP-3 gene [14]. In airway smooth muscle cells, IL-1beta, IFN-gamma and TNF-alpha were found to induce MCP-3 mRNA. Dexamethasone significantly inhibited this cytokine-induced accumulation of MCP-3 mRNA, in contrast to IL-4, IL-10 and IL-13 which had no inhibitory effect [17]. Finally, platelets constitutively expressed low levels of MCP-3 mRNA [18].

In vitro *activities of MCP-3*

Human MCP-3 was first described as a protein with potent chemotactic activity for monocytes, but not for neutrophils. However, it has now become clear that MCP-3 has a very broad range of target cells, including most leukocytic cell types (Table 2) [8, 19-28].

With a minimal effective concentration of 1 and 0.1 ng/ml, MCP-3 is a very potent chemoattractant for monocytes and T lymphocytes, respectively. Freshly isolated $CD4^+$ and $CD8^+$ lymphocytes as well as several T cell clones have been shown to migrate towards MCP-3 [8]. In monocytes and T cell clones, an increase in $[Ca^{2+}]_i$ has been reported following addition of MCP-3. Moreover, MCP-3 has been shown to induce the release of several enzymes in monocytes and T lymphocytes, enabling these cells to digest extracellular matrix components and to migrate into tissues [8, 19-21].

Freshly isolated NK cells do not respond to MCP-3. However, after cell culture in the presence of IL-2, these cells are sensitized and are able to migrate towards MCP-3 [22-23]. MCP-3 was also shown to induce $[Ca^{2+}]_i$ increases in cloned NK cells [8]. In dendritic cells, directional migration and an increase in $[Ca^{2+}]_i$ was observed in response to MCP-3.

With a minimal effective concentration of 30 ng/ml, MCP-3 was found to be an efficient chemoattractant for eosinophils and basophils [8, 24-27]. MCP-3 also induced an increase in $[Ca^{2+}]_i$ in these cells. In addition, both unprimed and IL-3-treated basophils showed an increased histamine release in reponse to MCP-3. Finally, although CC chemokines were previously thought to be ineffective on neutrophils, MCP-3 was shown to chemoattract neutrophils from 10 ng/ml onwards, but its efficacy (*i.e.* maximal chemotactic response) was inferior to that of the CXC chemokine IL-8 [28].

Receptor usage of MCP-3

To exert its pleiotropic activities on the different target cells, MCP-3 binds and signals through various receptors. The CC chemokine receptors CCR1, CCR2, CCR3 and CCR5 have all been shown to bind MCP-3 [2, 29].

CCR1 was the first CC chemokine receptor isolated and was initially identified as the MIP-1alpha/RANTES receptor. Later, it was shown that other chemokines, including MCP-3, MCP-2, "hemofiltrate CC chemokine-1" (HCC-1), HCC-2 and "myeloid progenitor inhibitory factor-1" (MPIF-1) bind CCR1 as well. Various leukocytic cell types, such as monocytes, T lymphocytes, eosinophils, basophils and dendritic cells express CCR1 [2].

The CC chemokine receptors CCR2A and CCR2B are derived from a single gene *via* alternative splicing, and vary only in their COOH-terminal cytoplasmic region [2]. CCR2B appears to be the predominant form. CCR2A and CCR2B were both identified as MCP-1 receptors but later, MCP-2, MCP-3 and MCP-4 were also found to be functional CCR2 ligands. CCR2 is expressed on monocytes, T lymphocytes, basophils and dendritic cells. CCR2B has HIV-1 coreceptor activity *in vitro*, but the activity and strain specificity are low compared to CCR5 and CXCR4, the major coreceptors for R5 and X4 HIV-1 strains, respectively.

Although originally cloned as the eotaxin receptor, CCR3 has now been shown to be a functional receptor for several other CC chemokines, including eotaxin-2, eotaxin-3, RANTES, MCP-2, MCP-3, MCP-4 and HCC-2 [2]. In addition, several HIV-1 strains can use CCR3 for entry of microglial cells *in vitro*. However, *in vivo* usage by HIV-1 has not been established. CCR3 is expressed on eosinophils, basophils, mast cells, T lymphocytes, dendritic cells and microglial cells.

Human CCR5 was identified as a receptor for MIP-1alpha, MIP-1beta and RANTES, and was subsequently found to be the major coreceptor for entry of M-tropic (R5) HIV-1 strains in permissive cells [2]. At present, it is known that other CC chemokines, including MCP-2, MCP-3, MCP-4, eotaxin and MCP-1 also bind to CCR5 (MCP-2 > MCP-3 > MCP-4 > > > eotaxin > MCP-1). MCP-3 appears to be an antagonist for CCR5, since no significant signaling through CCR5 or downregulation of CCR5 was observed in CCR5 transfectants in response to MCP-3 [29].

Role of MCP-3 in human diseases

Since MCP-3 can bind to several potential HIV-1 coreceptors (CCR5, CCR2B and CCR3), its capacity to inhibit HIV-1 infection was analyzed. At a concentration of 1 mug/ml, MCP-3 can compete with gp120 for binding to CCR5. However, at this concentration, it was found to be a weak inhibitor of R5 HIV-1 infection in T cells [29]. This observation was probably a consequence of the inability of MCP-3 to downregulate CCR5. At higher concentrations, MCP-3 (1-5 mug/ml) was even shown to enhance the replication of both R5 and X4 viruses [30]. However, for some R5 and X4 HIV-1 strains, low concentrations of MCP-3 (< 100 ng/ml) were able to inhibit HIV-1 entry into permissive cells [31]. The basis for this protective effect of MCP-3 is currently unclear. A possible mechanism might be that these HIV-1 strains interact with another MCP-3 receptor (CCR2B or CCR3) as well, and use such coreceptors to enter the target cells. On the other hand, MCP-3 may affect HIV-1 infection *via* signaling through the CCR2 receptor and subsequent desensitization of the CCR5 and/or CXCR4 pathway. Indeed, heterologous desensitization of CCR5 and CXCR4 signaling by the CCR2 receptor has recently been reported. In addition, MCP-3 has been shown to enhance the HIV-1-specific cytotoxicity of cytotoxic T lymphocytes (CTLs) *via* CCR3 [31].

The role of chemokines in the growth and metastasis of tumors is very complex and includes tumor promoting as well as tumor inhibiting effects. MCP-3 is known to attract a broad range of leukocyte subsets which potentially recognize and destroy tumor cells. Therefore, the anti-tumor activity of MCP-3 was assessed by transfecting mastocytoma cells with the MCP-3 gene and growing these cells in syngeneic hosts. Interestingly, tumor cells underwent rejection, associated with an increased infiltration of tumor-associated macrophages, T cells, eosinophils, and neutrophils into the tumor tissues [32, 33]. In addition, dendritic cells accumulated in perivascular areas.

Multiple sclerosis (MS) is an autoimmune disease of the central nervous system, characterized by perivascular inflammatory cell infiltrates and plaques of demyelination. Several chemokines, including MCP-3, are elevated in acute and chronic MS lesions [34]. Moreover, an increased expression of CCR2, CCR3 and CCR5 was observed in the central nervous system of MS patients, which suggests a role for these chemokine receptors and their ligands in the pathogenesis of MS.

MCP-3 is implicated in several lung diseases as well, such as non-atopic and atopic asthma, characterized by chronic inflammation and local tissue eosinophilia [35]. The presence of elevated amounts of MCP-3 and other eosinophil attracting chemokines in both atopic and non-atopic asthma might contribute to the infiltration of eosinophils in the lung. In addition, increased levels of MCP-3 have been reported in other inflammatory diseases, including ulcerative colitis and bilial cirrhosis, suggesting an immunoregulatory role in these diseases [36, 37]. In Table 3, diverse human pathologies in which MCP-3 protein was detected, are summarized.

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Figures

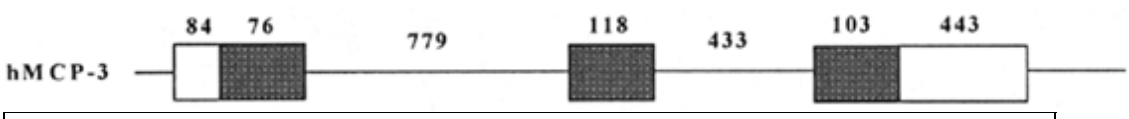


Figure 1

Gene structure of human MCP-3. The exon sequences (boxes) are divided into untranslated sequences (light boxes) and translated sequences (shaded boxes). The intron sequences are indicated as a horizontal line. Lengths of segments are in bp.

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          1    5    10   15   20   25   30   35   40   45   50   55
hMCP-1    QPDAINAPVTCCYNFTNRKISVQRLASYRRITSSKCPKEAVIFKTIVAKEICADPKQKW
hMCP-2    QPDSVSIPITCCFNVINRKIPIQRLESYTRITNIQCPKEAVIFKTKRGKEVCADPKERW
hMCP-3    QPVGINTSTTCCYRFINKKIPKQRLESYRRTTSSHCPREAVIFKTKLDKEICADPTQKW
hMCP-4    QPDALNVPSTCCFTFSSKKISLQRLKSY VITTSRCPQKAVIFRTKLGKEICADPKEKW

          60   65   70   75
hMCP-1    VQDSMDHLDKQTQTPKT
hMCP-2    VRDSMKHLDQIFQNLKP
hMCP-3    VQDFMKHLDKKTQTPKL
hMCP-4    VQNYMKHLGRKAHTLKT
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Figure 2

Primary structures of human MCP-1, MCP-2, MCP-3 and MCP-4. The amino acid sequences of human MCP-1, MCP-2, MCP-3 and MCP-4 are aligned (one letter code). The conserved cysteine residues are underlined.



Figure 3

Tertiary structure of human MCP-3. The tertiary structure of MCP-3 was determined by NMR spectroscopy (Swiss-Prot database [13]).

Table 1
Cellular sources and regulators of human MCP-3

Cell type		Inducer (+)/Inhibitor (–)	Level	Ref
Monocytes	+	LAM; IL-1β; TNF-α; IFN-γ; LPS	mRNA	[8]
	–	IL-4; IL-10; IL-13	mRNA	[14]
Mononuclear cells	+	IL-1β, IFN-α, IFN-β, measles virus, poly I-C, Con A	protein	[16]
Fibroblasts	+	IL-1β + IFN-γ	protein	[16]
Endothelial cells (HUVEC)	+	IL-1β; TNF-α; IFN-γ; LPS	mRNA	[14]
Airway smooth muscle cells	+	IL-1β, IFN-γ, TNF-α	mRNA	[17]
	–	dexamethasone	mRNA	
Platelets		constitutively	mRNA	[18]
Myelomonocytic leukemia cells (THP-1)	+	LPS; PMA	mRNA	[8]
Osteosarcoma cells (MG-63)	+	IL-1β	protein	[3]
	+	IL-1β; IFN-β; virus; PMA	mRNA	[8]
Promonocytic leukemia cells (U937)	+	PMA	mRNA	[5]

Table 2
***In vitro* activities of human MCP-3**

Cell type	Activity	MEC[a] (ng/ml)	Ref.
Monocytes	Chemotaxis	1-3	[8]
	$[Ca^{2+}]_i$ increase	< 50	[8]
	Degranulation:		
	– *N*-acetyl-β-D-glucosaminidase[b]	10	[19]
	– Arachidonic acid	50	[20]
	– Gelatinase B	ND[c]	[3]
T lymphocytes	Chemotaxis	0.1-1	[8, 21]
	$[Ca^{2+}]_i$ increase	ND	[8, 21]
T lymphocyte clones	Chemotaxis	1	[8]
	$[Ca^{2+}]_i$ increase	5	[8]
	Degranulation:		
	– Granzyme A[b]	1,000	[23]
	– *N*-acetyl-β-D-glucosaminidase[b]	1,000	[23]
Neutrophils	Chemotaxis	10	[8, 28]
	$[Ca^{2+}]_i$ increase	ND	[8, 28]
Eosinophils	Chemotaxis	30-100	[8, 24]
	$[Ca^{2+}]_i$ increase	10	[8, 24]
	Respiratory burst	< 50	[25]
Basophils	Chemotaxis	30	[26, 27]
	$[Ca^{2+}]_i$ increase	< 500	[8, 27]
	Degranulation: Histamine	1,000	[26]
Basophils (IL-3-primed)	Degranulation:		
	– Histamine	100	[26, 27]
	– Leukotriene C_4	100	[26, 27]
NK cells (IL-2-stimulated)	Chemotaxis	30	[8, 22, 23]
	$[Ca^{2+}]_i$ increase	ND	[8, 22, 23]
NK cell clones	Chemotaxis	10	[8]
	$[Ca^{2+}]_i$ increase	1-3	[8]
	Degranulation:		
	– Granzyme A[b]	100	[23]
	– *N*-acetyl-β-D-glucosaminidase[b]	100	[23]
Dendritic cells	Chemotaxis	1-10	[8]
	$[Ca^{2+}]_i$ increase	< 100	[8]

[a] MEC: minimal effective concentration; [b] cytochalasin B-treated cells; [c] ND: not determined.

Table 3
Presence of MCP-3 in human pathology

Body system	Disease	Ref.
Central nervous system	Multiple sclerosis	[34]
Respiratory system	Asthma	[35]
	Chronic sinusitis	[38]
Gastro-intestinal system	Ulcerative colitis	[36]
	Bilial cirrhosis	[37]
Visual system	Vernal keratoconjunctivitis	[39]

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human recombinant interleukin-1 beta, myelopoiesis, myelodepression, cancer chemotherapy.

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