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Role of monocyte chemoattractant protein-1 (MCP-1) in atherosclerosis: Signature of monocytes and macrophages

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Abstract

The monocyte chemoattractant protein-1 (MCP-1/CCL2) is a member of the C-C chemokine family, and a potent chemotactic factor for monocytes. MCP-1 is believed to be identical to JE, a gene whose expression is induced in mouse fibroblasts by platelet-derived growth factor. Two SNPs of CCL2, namely, G-927C and A-2578G, were found to be associated with carotid intima-media thickness, which reflects generalized atherosclerosis and is predictive of future vascular events. Monocyte chemoattractant protein-1 (MCP-1) is the first discovered and most extensively studied CC chemokine, and the amount of studies on its role in the etiologies of atherosclerosis-related diseases have increased exponentially during recent years. This review attempted to provide a perspective of the history, regulatory mechanisms, functions, and therapeutic strategies of this chemokine. The highlights of this review include the roles of MCP-1 in the development of atherosclerosis, cardiovascular diseases, and dyslipidemia. Therapies that specifically or non-specifically inhibit MCP-1 overproduction have been summarized.

Key words: MCP-1, Atherosclerosis, Monocyte, Macrophage, Proinflammatory cytokines

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Topic Review

Chemokines (chemotactic cytokines) are small heparin-binding proteins that constitute a large family of peptides (60–100 amino acids) structurally related to cytokines, whose main function is to regulate cell trafficking. Chemokines were first identified in 1977 with the purification of the secreted platelet factor 4 (PF4/CXCL4) (Wu and others 1977). Since then, studies have identified more than 50 human chemokines and 20 chemokine receptors [1]. Chemokines can be classified into four subfamilies on the basis of the number and location of the cysteine residues at the N-terminus of the molecule and are named CXC, CC, CX3C, and C, in agreement with the systematic nomenclature [2].

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Chemokine and chemokine receptor classification

Chemokines are a family of large peptides (60–100 amino acids (aa)). They are subdivided into four families based on the number and spacing of the conserved cysteine residues in the N-terminal position and are named CXC, CC, CX3C, and C, in agreement with the systematic nomenclature. All chemokines signal through G protein-coupled receptors (GPCR). In general, several chemokines can bind to the same receptor and, conversely, a given chemokine may recognize more than one receptor. However, there are exceptions where unique ligand–receptor pairs exist. To date, more than 50 chemokines and about 20 chemokine receptors have been identified. Their classification and nomenclature can be found in reviews [3].

MCP receptors

Many of the CC chemokine receptors (CCRs) have been cloned based on well-conserved motifs of the earlier identified IL-8 receptors. Many of the genes encoding these CCR proteins of about 360 amino acids are closely linked on chromosome 3p21-22 [1]. All chemokine receptors identified are GPCRs, belonging to the rhodopsin or serpentine receptor family. Generally, these receptors are composed of a short extracellular N-terminus, seven hydrophobic transmembrane domains each connected by three extracellular and three intracellular loops, and a C-terminal intracellular region. Most of the CCRs that bind one or more MCPs are shared by other CC chemokines. As MCPs have a broad cell spectrum, their receptors are expressed on various leukocyte types. In addition, all human MCPs are known to bind to at least two receptors. Also, it should be noted that many receptors such as CCR2 respond to several different ligands. Therefore, understanding implications of these interactions (chemokines-receptors) for them in vivo functions are becoming more challenging. However, it seems that many regulatory mechanisms may come into picture to eliminate redundancy and give each chemokine a unique and specific function [2].

CCL2 mediates its effects through its receptor CCR2, and, unlike CCL2, CCR2 expression is relatively restricted to certain types of cells. There are two alternatively spliced forms of CCR2, namely, CCR2A and CCR2B, which differ only in their C-terminal tails [2]. CCR2A is the major isoform expressed by mononuclear cells and vascular smooth muscle cells [3], whereas monocytes and activated NK cells express predominantly the CCR2B isoform. It is possible that CCR2A and CCR2B may activate different signaling pathway and exert different actions. For example, CCL2 chemotaxis of CCR2A-positive cells occurs without Ca2+ mobilization, but Ca2+ flux is induced in the CCR2B-positive cells [3]. It has been reported that CCL2 is capable of increasing the expression of CCR2A but not CCR2B in synoviocytes obtained from patients with rheumatoid arthritis [4]. It is important to note that CCR2 has dual roles and has both proinflammatory and anti-inflammatory actions. The proinflammatory role of CCR2 is dependent on APCs and T cells, whereas the anti-inflammatory role of CCR2 is dependent on CCR2 expression in regulatory T cells. Further, as many as seven single nucleotide polymorphisms

(SNPs) have been reported for CCR2. However, there is little evidence to suggest that any particular one of them affects clinical disease outcome in patients with acute idiopathic anterior uveitis [5]. CCR2-deficient mice are resistant to the induction of sensory neuropathies [6]. In addition, CCR2-null mice immunized with type II bovine collagen were found to be more susceptible to collagen-induced arthritis than the wild-type mice [7].

MCP-1/CCL2

The monocyte chemoattractant protein-1 (MCP-1/CCL2) is a member of the C-C chemokine family, and a potent chemotactic factor for monocytes. MCP-1 is believed to be identical to JE, a gene whose expression is induced in mouse fibroblasts by platelet-derived growth factor [8]. However, the human homolog that has been best characterized as CCL2 was first purified from human cell lines on the basis of its monocyte chemoattractant properties.

CCL2 is the first discovered human CC chemokine. Located on chromosome 17 (chr.17, q11.2), human MCP-1 is composed of 76 amino acids and is 13 kDa in size [9]. MCP belongs to a family composed of at least four members (MCP-1, −2, −3, and −4). The domain structure of human MCPs is shown in Figure 2. The sequence homology between CCL2 and other family members is high and varies between 61% for CCL8 and CCL4, and 71% for CCL7 [10]. The primary protein structures of human CCL2, CCL7, and CCL8 were initially determined using purified natural material, whereas human CCL13 protein sequence was deduced from isolated cDNAs. In addition to these proteins, different molecular mass forms of CCL2 have been purified, but these seem to be caused by O-glycosylation. Glycosylation of CCL2 has been shown to slightly reduce its chemotactic potency. Note that in this review, we will discuss the role of unglycosylated CCL2.

Figure 1.
Amino-acid sequence alignment of human MCPs. Conserved cysteine residues are indicated by alignment and spacing. Consensus sequence residues are in dark grey, whereas conserved and mutated CCL2 residues are shown in black and light grey, respectively.

Chemokine receptor-mediated signal transduction

Relating to second messenger systems modulated by chemokine receptors, one of the first pathways identified was the inhibition of adenylyl cyclase activity to reduce the intracellular cAMP levels, involving Gα i subunit of G proteins [11]. It was also established that the majority of chemokine receptors activate phospholipase C, involving Gqα proteins. The latter leads to the formation of diacylglycerol and inositol 1,4,5-triphosphate with a subsequent increase in protein kinase C activity and transient elevations of cytosolic Ca2+ levels [12]. More distal signaling pathways modulated by chemokines include the activation of the mitogen-activated protein

kinase (PK) cascade, especially the pathway involving extra-cellular signal-regulated kinase 1/2 activation, as well as the phosphorylation of the cytoskeletal-associated kinases [13]. Studies of chemokine receptor activation have also reported the activation of a family of proteins known as Janus kinases and signal transducers and activators of transcriptions [14]. Chemokine receptors also activate the signaling pathway of nuclear factor-κ B.

Mechanisms and pathways for monocyte recruitment

In a study performed by the Van Furth group, it was demonstrated that the half-life of circulating monocytes in humans is about three times longer than in mice, which is estimated to be around 340 million monocytes leaving the circulation daily [15]. Further, in mice, under steady-state conditions, about half of the circulating monocytes are cleared from the blood stream daily [16]. A considerable fraction of circulating monocytes enters the tissues of the body, differentiating into macrophages. In contrast, immature dendritic cells within the tissue are able to leave via afferent lymphatic vessels to the draining lymph nodes, where they mature, present antigens to T cells, and die within a few days of arrival. Thus, a large fraction of monocytes can potentially be cleared as a by-product of immune surveillance.

CCL2 has been demonstrated to recruit monocytes into foci of active inflammation [17]. However, it remains unclear whether monocytes use the same molecular signals to migrate into tissues as part of the constitutive or steady-state efflux from blood. In this regard, evidence has been provided describing the involvement of prostaglandin E2 in the attraction of monocytes to the site of inflammation and their maturity into macrophages [18]. CCL2 secreted in or injected into skin arrives in the draining lymph nodes where it can be presented on the surface of high endothelial venules (HEVs) for recruitment of lymphocytes [19]. Further, they found that CCL2 was the main chemokine responsible for recruiting monocytes. In these studies, the authors showed that only a fraction (~2%) of the circulating monocyte pool is recruited to the lymph nodes [20]. It is not clear whether these cells are representative of the majority of circulating monocytes, or they represent an important subset destined to reach draining lymph nodes. In addition to CCL2, several other chemokines were also shown to be involved in the recruitment of monocytes. In this regard, it has been demonstrated that the stimulation of RANTES leads to recruitment of monocytes/macrophages [21].

Monocyte chemoattractant protein-1 (MCP-1) inhibits the intestinal-like differentiation of monocytes

Monocytes (MO) migrating into normal, non-inflamed intestinal mucosa undergo a specific differentiation resulting in a non-reactive, tolerogenic intestinal macrophage (IMAC). Recently demonstrated the differentiation of MO into an intestinal-like macrophage (MAC) phenotype *in vitro* in a three-dimensional cell culture model (multi-cellular spheroid or MCS model) [22]. In the mucosa of patients with inflammatory bowel disease (IBD) in addition to normal IMAC, a reactive MAC population as well as increased levels of monocyte chemoattractant protein 1 (MCP-1) is found. After 7 days of co-culture MCS were harvested, and expression of the surface antigens

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CD33 and CD14 as well as the intracellular MAC marker CD68 was determined by flow-cytometry or immunohistochemistry. MCP-1 and MIP-3α expression by HT-29 cells in the MCS was increased by transfection at the time of MCS formation. In contrast to MIP-3α, MCP-1 overexpression induced a massive migration of MO into the three-dimensional aggregates. Differentiation of IMAC was disturbed in MCP-1-transfected MCS compared to experiments with non-transfected control aggregates, or the MIP-3α-transfected MCS, as indicated by high CD14 expression of MO/IMAC cultured inside the MCP-1-transfected MCS [23]. Neutralization of MCP-1 was followed by an almost complete absence of monocyte migration into the MCS. MCP-1 induced migration of MO into three-dimensional spheroids generated from HT-29 cells and inhibited intestinal-like differentiation of blood MO into IMAC. It may be speculated that MCP-1 could play a role in the disturbed IMAC differentiation in IBD mucosa [24].

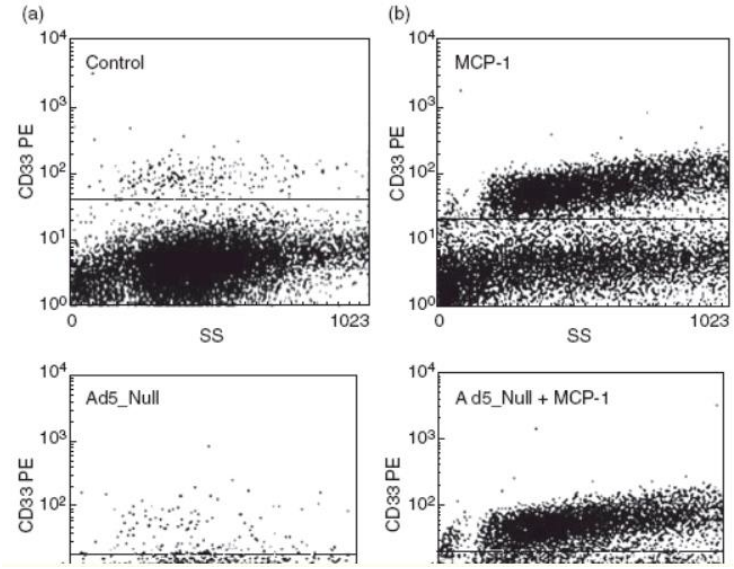

Figure 2.

Macrophages (MAC) inside the aggregates were identified by positive staining for the monocyte (MO)/MAC specific surface marker CD33. (a) $CD33^+$ MO/MAC cultured in non-transfected, Ad5 Null- and macrophage inflammatory protein 3 alpha (MIP-3α)-transfected HT-29 multi-cellular spheroid (MCS) for 7 days. Only a low number of $CD33^+$ cells can be detected. (b) $CD33^+$ MO/MAC in Ad5 MCP-1-, Ad5 Null/Ad5 MCP-1- and Ad5 MIP-3α/Ad5 MCP-1-transfected HT-29 MCS after 7 days of culture. Clearly, many more $CD33^+$ cells can be isolated from the MCS if monocyte chemoattractant protein (MCP)-1 was overexpressed.

MCP-1 inhibits differentiation of IMAC

Differentiation of MO into intestinal-like MAC was determined by the expression of CD14, which is present on blood MO but not detectable on IMAC from normal, non-inflamed mucosa and down-regulated in control HT-29 MCS, as established recently [21]. Immunohistochemical analysis of HT-29-MCS co-cultured with MO/MAC for 7 days showed CD68 positive MAC inside non-transfected control spheroids (Fig. 3a), with no expression of the activation-associated MO/MAC-specific antigen CD14 (Fig. 3b). The vast majority of the cells in the non-transfected

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MCS were epithelial cells, as demonstrated with the epithelial cell marker EP4 (Fig. 3c). Isotype controls supported the specificity of these results (Fig. 3d). In MCP-1-transfected spheroids many more CD68 positive cells could be detected than in the control aggregates (Fig. 3e), most of them expressing CD14 (Fig. 3f). Again, HT-29 cells were stained with EP4 (Fig. 3g). Specificity of the staining was controlled with isotype antibody incubation (Fig. 3h).

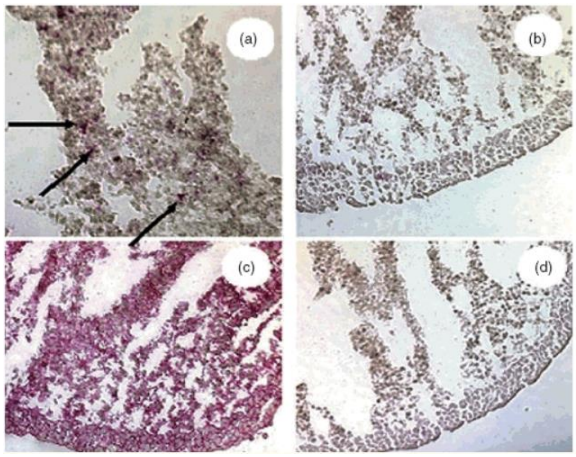

Figure 3.

Immunohistochemical staining of multi-cellular spheroid (MCS) co-cultured with monocytes (MO) for 7 days. Aggregates were stained for the intracellular MO/macrophage (MAC) specific marker CD68 and the differentiation and activation associated surface antigen CD14. (a–d) Non-transfected control MCS; (e–h) Ad-MCP-1-transfected MCS. (a) In non-transfected control MCS $CD68^+$ MAC could be identified inside the aggregates after 7 days of co-culture; (b) no expression of CD14 could be detected in these cells; (c) staining of EP4 revealed the epithelial character of the vast majority of cells; (d) isotype control staining; (e) transfection of HT-29 cells with Ad MCP-1 resulted in a higher rate of monocyte migration into the aggregates; (f) a much higher number compared to non-transfected MCS expressed CD14; (g) EP4 staining; (h) isotype control (original magnification ×400).

Elevated levels of MCP-1 have been found in atopic reactions such as allergic rhinitis or allergic asthma and in atherosclerotic plaques, where MAC and lymphocytes are the main inflammatory cells [13]. During IBD the proinflammatory chemokine MCP-1 is up-regulated on RNA [25] and protein level [26] in intestinal mucosa; however, it is not always accompanied by increases in chemokine serum levels [27]. MCP-1 induces strong migration of MO into the inflamed tissue and is one of the factors responsible for the maintenance of the inflammation [20].

An increase in MIP-3α protein production was observed in HT-29 and Caco-2 cells after stimulation with TNF and IL-1β. MIP-3α protein levels were also elevated in primary intestinal epithelial cells from patients with IBD. The increased production of MIP-3α in epithelial cells may also play an important role in lymphocyte activation and recruitment to the colonic epithelium in IBD [28]. In a recent study we have shown a differentiation-dependent induction of Mip-3α expression in intestinal macrophages and a co-localization of Mip-3α/$CD68^+$ (macrophages) and $CD45R0^+$ cells (memory T cells) in the lamina propria [30]. Mip-

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3α overexpression was associated with an increased recruitment of memory T cells in the spheroid model, whereas neutralization of Mip-3α abolished this effect [29]. These data indicate clearly that under identical conditions MIP-3α is chemotactic for T cells but not for monocytes/macrophages.

MCP-1 might play a very important role for the recruitment of MO into the intestinal mucosa, as overexpression was followed by a very dramatic increase in the recruitment of MO into the MCS and neutralization of MCP-1 almost completely prevented invasion of MCS by MO. In addition, the differentiation of MO into anergic IMAC taking place in the healthy gut is disturbed by the presence of the proinflammatory chemokine MCP-1 but not by MIP-3α [30].

The presence of MCP-1 in the inflamed tissue could directly inhibit the intestinal-like differentiation of MO. MCP-1 is involved in the inflammatory process of atherosclerosis through the induction of scavenger receptor expression via the ERK pathway and differentiation of MO into foam MAC [31].

On the other hand, the presence of increased numbers of MO inside the MCP-1-transfected aggregates could influence the differentiation process, hallmarking an indirect MCP-1 effect. Inhibition of MO/MAC differentiation is not due to transfection of HT-29 cells with adenoviral constructs, as transfection with an empty control virus or the Ad5 MIP-3α virus did not disturb the differentiation process.

Competing interests

The authors declare that they have no competing interests.

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