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Monocyte chemoattractant protein-1 (MCP-1) in the kidney: does it more than simply attract monocytes?

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MCP-1: a player in the pathogenesis of progression of renal diseases

There is an increasing body of evidence that the CC chemokine monocyte chemoattractant protein-1 (MCP-1) plays a major role in the pathogenesis of progression of renal failure. This is based on observations both in various animal models of renal damage and in different types of human renal disease (for review, see [1]). Locally produced MCP-1 seems to be particularly involved in the initiation and progression of tubulointerstitial damage. The latter has been documented in experiments using transgenic mice with nephrotic serum-induced nephritis: compared with wild-type mice, MCP-1-deficient mice exhibit less tubulointerstitial lesions, but they exhibit no differences in glomerular lesions [2]. There is, however, evidence that MCP-1 also plays a role in the progression of glomerular lesions, since glomerular expression of MCP-1 correlates with the degree of renal damage in inflammatory [3] and non-inflammatory [4] models of glomerular injury. Furthermore, in humans with crescentic glomerulonephritis, MCP-1 is not only expressed in tubular epithelial cells and leukocytes infiltrating the tubulointerstitium, but also in crescents and parietal epithelium [5]. In experimental crescentic glomerulonephritis, administration of antibodies to MCP-1 decreases the extent of proteinuria, reduces glomerulosclerosis and improves renal dysfunction [6].

Of importance, a consistent increase of urinary MCP-1 concentration is found in patients or animals

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with a diseased kidney, and this correlates with the degree of urinary albumin/protein excretion and renal damage [7–10]. In addition to mesangial cells, endothelial cells and infiltrating mononuclear cells [11,12], tubular epithelial cells [12,13] seem to be the major source of MCP-1 in urine. The increased production of MCP-1 by tubular epithelial cells is due to: (i) stimulation by cytokines [14,15]; and (ii) exposure to urinary proteins [16]. Both are mediators of progressive renal damage [17].

Interestingly, therapy with an angiotensin-converting enzyme (ACE) inhibitor or an angiotensin II receptor type 1 (AT_1) antagonist is able to abrogate the renal expression of MCP-1 [18,19]. This may not only be due to reduction of proteinuria. Rat mesangial cells *in vitro* produce increasing amounts of MCP-1 in response to increasing external pressure, suggesting that MCP-1 may be a mediator of the adverse effects of intraglomerular hypertension [20]. The latter is an important factor in the genesis of progressive glomerular damage, which is attenuated by therapy with an ACE inhibitor or an AT_1 receptor antagonist. Taken together, MCP-1 seems to be of pivotal importance in the pathogenesis of progression of renal damage and failure.

To date, the classic perception of MCP-1 was that its main property is the attraction of monocytes/macrophages. These inflammatory cells in turn secrete cytokines and chemokines, fostering inflammation and fibrosis. Increased synthesis of cytokines and chemokines by infiltrating inflammatory cells and resident renal cells has been documented in various forms of glomerulonephritis and tubulointerstitial nephritis. It has been suggested that they are important mediators of progressive renal failure *in vivo* [1]. Recent data [21,22] indicate that the role of MCP-1 goes beyond that of a simple chemoattractant protein.

MCP-1 induces an inflammatory activation of human tubular epithelial cells

Recent data from our group provide evidence that increased concentrations of MCP-1 in the diseased kidney may be an important factor contributing *per se* to increased production of cytokines and adhesion molecules. We demonstrated that MCP-1 specifically activates tubular epithelial cells *in vitro* [21], leading to a time- and dose-dependent increase in secretion of the proinflammatory cytokine interleukin-6 (IL-6) and expression of the intracellular adhesion molecule-1 (ICAM-1) via G_i-protein-, protein kinase C (PKC)- and intracellular Ca^{2+}-dependent mechanisms (Figure 1). MCP-1 activated: (i) the transcription factor nuclear factor-κB (NF-κB), a transcription factor commonly involved in inflammatory and immune responses, and (ii) activating protein-1 (AP-1), a transcription factor involved in inflammatory and growth responses. Both NF-κB and AP-1 were involved in the MCP-1-mediated induction of IL-6. In contrast to IL-6 release, MCP-1-induced ICAM-1 expression was predominantly dependent on NF-κB activation (Figure 2). IL-6 has been suggested to contribute to

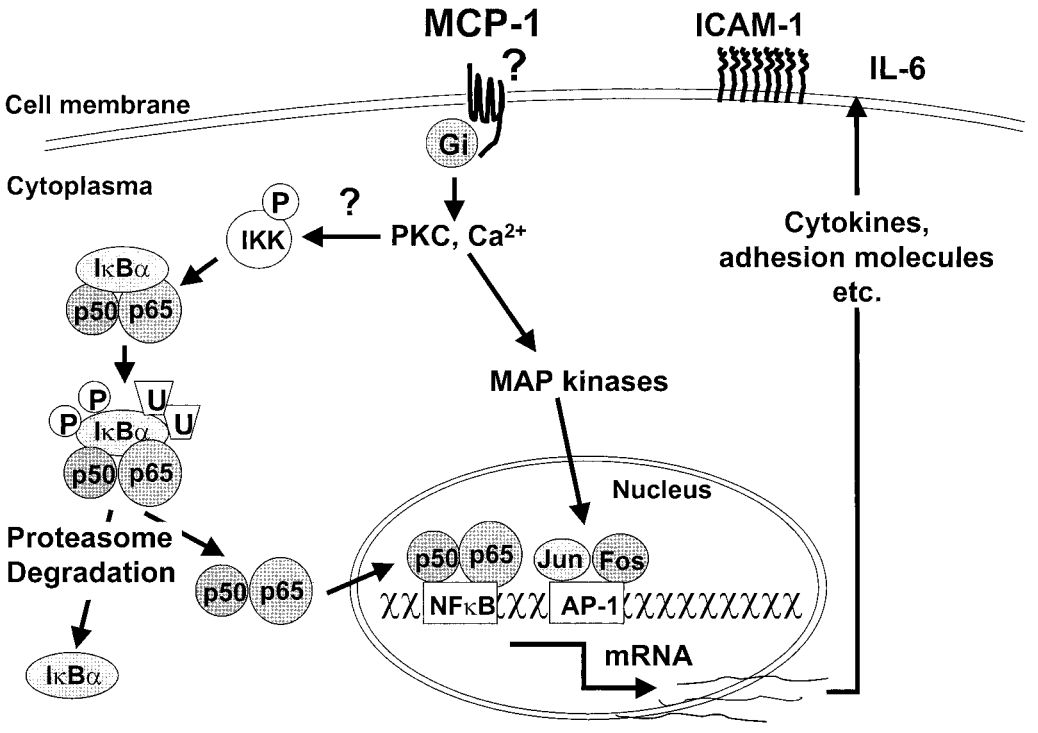

Fig. 1. Scheme of the intracellular signalling pathways of MCP-1 in a human tubular epithelial cell.

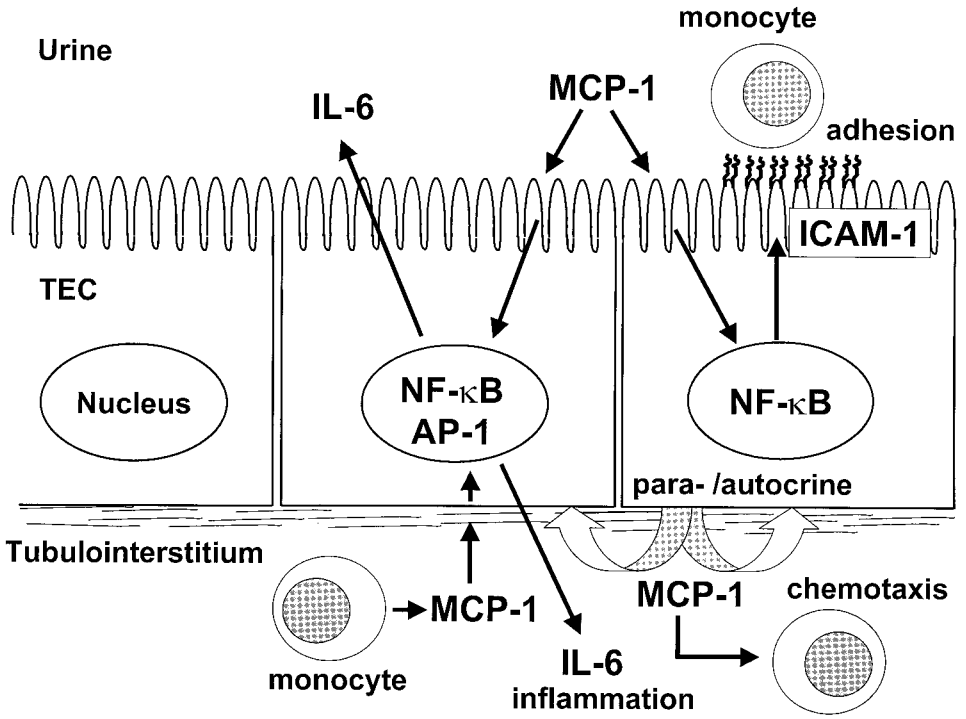

Fig. 2. Scheme of the direct and indirect effects of MCP-1 on human tubular epithelial cells (TECs).

progression of renal diseases [23,24] because its renal expression correlates with the degree of mesangial hyperproliferation, tubular atrophy, and the intensity of interstitial infiltrates [25,26]. De novo expression of the adhesion molecule ICAM-1 by tubular epithelial cells and increased expression by interstitial and glomerular cells has been observed in different forms of glomerulonephritis, tubulointerstitial inflammation and renal allograft rejection [27]. Thus, the observed effects of MCP-1 on tubular epithelial cells *in vitro* may be critical steps during progression *in vivo*.

MCP-1 does not only exhibit direct effects on tubular epithelial cells, but also on another cell type that plays a role in progressive renal damage, i.e. vascular smooth muscle cells [22]. Stimulation of vascular smooth muscle cells with MCP-1 induced proliferation and resulted in a concentration- and time-dependent release of IL-6. Similar to tubular epithelial cells, this effect was mediated via G_i-protein, PKC and NF-κB. MCP-1 also induced extracellular signal-regulated kinase (ERK), which, along with IL-6 release, was G_i-protein-dependent. MCP-1-induced, proliferation of vascular smooth muscle cells, which was ERK-dependent. MCP-1 stimulated the binding activity of NF-κB and of AP-1. NF-κB was involved in IL-6 release by MCP-1, whereas proliferation was dependent on AP-1, demonstrating that, as in tubular epithelial cells, MCP-1 induces differential activation of NF-κB and AP-1 in vascular smooth muscle cells. Thus, these latter data do not only propose a new mechanism for the proatherogenic effect of MCP-1 in the progression of cardiovascular disease, but also implicate MCP-1 as a factor leading to vascular damage in the diseased kidney.

Which receptor(s) is responsible for the mediation of effects of MCP-1 on human tubular epithelial cells?

Chemokines exert their effects through binding to G-protein-coupled receptors on the surface of leukocytes targeted for activation and migration. These receptors, once activated, trigger a set of cellular reactions that result in inositol triphosphate formation, intracellular calcium release, and PKC activation [28]. The classic MCP-1 receptors belong to the family of heptahelical, pertussis-sensitive, G-protein-coupled receptors [29]. The MCP-1 receptor on tubular epithelial cells appears to be coupled to G_i-protein activation [21]. These findings are in accordance with our observations in vascular smooth muscle cells [22], as well as data of others [29,30].

As in leukocytes, MCP-1-induced up-regulation of IL-6 synthesis and ICAM-1 expression by human tubular epithelial cells are dependent on PKC and intracellular Ca^{2+} (Ca_i^{2+}) [21]. Similarly, Schecter *et al.* [30] demonstrated that the induction of tissue factor by MCP-1 in human vascular smooth muscle cells required Ca_i^{2+} mobilization and that it was PKC-dependent. Therefore, although currently unproven, it is likely that MCP-1-signalling is mediated by the classical PKC subgroups α, β or γ.

To date, in renal tissue of humans and experimental animals, expression of CCR1-5 mRNA transcripts were detected only in infiltrating mononuclear cells. Two MCP-1 receptors, generated by alternative splicing and designated as CCR2A and -B, have been cloned in human monocytes [31]. On the basis of our polymerase chain reaction and flow cytometry

analysis studies, the MCP-1 receptor on human tubular epithelial cells is distinct from these two receptors [21]. Furthermore, the MCP-1 receptor on human tubular epithelial cells is also distinct from the CCR1, CCR3, CCR4 and CCR5 receptors. Antibodies against CCR1–5 failed to inhibit the specific effects of MCP-1 on tubular epithelial cells. However, binding studies for MCP-1 revealed that cultured human tubular epithelial cells express a MCP-1 binding protein on the cell surface. The possibility that the effects of MCP-1 on tubular epithelial cells are mediated via an endosome-lysosomal pathway was ruled out [21]. Taken together, the above results implicate that it is likely that the MCP-1 receptor on human tubular epithelial cells is different from previously cloned CC chemokine receptors. The nature of the MCP-1 receptor(s) on human tubular epithelial cells, which seems to be G_i-protein coupled, remains to be determined (Figure 1).

Conclusion

MCP-1 can activate tubular epithelial cells directly *in vitro*. This action of MCP-1 on a resident renal cell had so far remained unknown, but had been described in cultured vascular smooth muscle cells [22,30]. The MCP-1-mediated activation of tubular epithelial cells is consistent with the notion that MCP-1 contributes to tubulointerstitial inflammation, which is a hallmark of progressive renal disease [17]. Importantly, tubulointerstitial rather than glomerular damage correlates best with the loss of renal function and the risk of progression to end-stage renal failure. Actually, there is a strict correlation between tubular atrophy, interstitial fibrosis, the extent of interstitial infiltrates and renal dysfunction [32]. The direct effects of MCP-1 on vascular smooth muscle cells [22] may not only be of importance in the progression of cardiovascular damage in general, but also in the progression of vascular lesions in the kidney, which could contribute further to progression of renal failure.

Previous studies focused on the role of MCP-1 in renal inflammation and its induction of inflammatory signals [1]. Our recent data suggest that MCP-1 is more than just a chemoattractant. Rather, MCP-1 can directly elicit an inflammatory response by inducing cytokine and adhesion molecule expression in the kidney. This is an important new mechanism in the pathogenesis of tubulointerstitial inflammation.

Note added in proof

Krüger *et al*. [33] recently reported that recipients of a renal allograft who are carriers of the MCP-1-2518 (G/G) polymorphism, i.e. a genotype characterized by increased production of MCP-1 by mononuclear cells, have a significantly reduced mean graft survival compared with the heterozygous (A/G) or wild-type (A/A) allele carriers. In contrast, carriers of the 64I mutation of the CCR2 receptor, a genotype which presumably alters CCR2 expression or function, had no increase of mean renal allograft survival. These *in vivo* data support our *in vitro* findings [21], i.e. specific inflammatory activation of human tubular epithelial cells by MCP-1 despite the lack of CCR2 receptors on these cells.

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