

■ R E V I E W

Role of MCP-1 in cardiovascular disease: molecular mechanisms and clinical implications

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A B S T R A C T

Many of the major diseases, including cardiovascular disease, are widely recognized as inflammatory diseases. MCP-1 (monocyte chemotactic protein-1) plays a critical role in the development of cardiovascular diseases. MCP-1, by its chemotactic activity, causes diapedesis of monocytes from the lumen to the subendothelial space where they become foam cells, initiating fatty streak formation that leads to atherosclerotic plaque formation. Inflammatory macrophages probably play a role in plaque rupture and the resulting ischaemic episode as well as restenosis after angioplasty. There is strong evidence that MCP-1 plays a major role in myocarditis, ischaemia/reperfusion injury in the heart and in transplant rejection. MCP-1 also plays a role in cardiac repair and manifests protective effects under certain conditions. Such protective effects may be due to the induction of protective ER (endoplasmic reticulum) stress chaperones by MCP-1. Under sustained ER stress caused by chronic exposure to MCP-1, the protection would break down resulting in the development of heart failure. MCP-1 is also involved in ischaemic angiogenesis. The recent advances in our understanding of the molecular mechanisms that might be involved in the roles that MCP-1 plays in cardiovascular disease are reviewed. The gene expression changes induced by the signalling events triggered by MCP-1 binding to its receptor include the induction of a novel zinc-finger protein called MCPIP (MCP-1-induced protein), which plays critical roles in the development of the pathophysiology caused by MCP-1 production. The role of the MCP-1/CCR2 (CC chemokine receptor 2) system in diabetes, which is a major risk factor for cardiovascular diseases, is also reviewed briefly. MCP-1/CCR2- and/or MCPIP-targeted therapeutic approaches to intervene in inflammatory diseases, including cardiovascular diseases, may be feasible.

INTRODUCTION

The development of cardiovascular disease, a major and growing public health problem, involves not only haemodynamic abnormalities, but also a complex interplay

among neurohormonal, inflammatory and biochemical changes acting on cardiac myocytes, the cardiac interstitium or both. This can lead to the development of an innate immune response which is predominantly characterized by the accumulation and activation of

Key words: angiogenesis, atherosclerosis, inflammation, ischaemic cardiomyopathy, monocyte chemotactic protein-1 (MCP-1).

Abbreviations: ACS, acute coronary syndrome; apo, apolipoprotein; CCR2, CC chemokine receptor 2; *cdh*, cadherin; CHD, coronary heart disease; CM-DCFH-DA, chloromethyl 2',7'-dichlorofluorescein diacetate; EAM, experimental autoimmune myocarditis; EC, endothelial cell; ER, endoplasmic reticulum; FasL, Fas ligand; GRP, glucose-regulated protein; HSP, heat-shock protein; HUVEC, human umbilical vein EC; IL, interleukin; JNK, c-Jun N-terminal kinase; LDL, low-density lipoprotein; LDLR, LDL receptor; LV, left ventricular; MCP-1, monocyte chemotactic protein-1; MCPIP, MCP-1-induced protein; MI, myocardial infarction; MMP, matrix metalloproteinase; oxLDL, oxidized LDL; RNS, reactive nitrogen species; ROS, reactive oxygen species; SAPK, stress-activated protein kinase; SMC, smooth muscle cell; TGF- β , transforming growth factor- β ; TNF- α , tumour necrosis factor- α ; VSMC, vascular SMC.

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leucocytes, especially monocytes/macrophages in the heart [1]. Studies have shown that as heart disease progresses the inflammatory cytokine response is activated, causing continuation of deleterious effects on the heart and vasculature, leading to progression of LV (left ventricular) dysfunction and heart failure [2]. Chemokine-induced recruitment of peripheral leucocytes to tissues is a critical step in the development of inflammatory responses. One of the best-studied CC chemokines, MCP-1 (monocyte chemotactic protein-1)/CCL2, binds to its seven transmembrane G-protein-coupled receptor, CCR2 (CC chemokine receptor 2), and the resulting signalling events recruit monocytes to inflammation sites in a variety of chronic inflammatory diseases [3]. There is overwhelming evidence that MCP-1 plays a critical role in the development of cardiovascular disease; however, the molecular mechanisms underlying the role of MCP-1 in the development and progression of the disease remain poorly understood. In the present review, we will summarize recent experimental evidence which suggest that MCP-1/CCR2 signal transduction not only mediates recruitment of monocytes/macrophages during inflammatory processes, but also induces transcriptional gene regulation leading to cell differentiation and death, involved in cardiovascular diseases.

MCP-1: ESTABLISHED PATHOGENIC ROLE IN ATHEROSCLEROSIS

Inflammation is important in the pathogenesis and progression of atherosclerotic disease. Over the past two decades, overwhelming evidence has accumulated that supports the key role MCP-1 plays in the development of atherosclerosis [4–6]. Vascular insults can lead to endothelial dysfunction that causes increased leakage of LDL (low-density lipoprotein) from the vessel lumen into the vessel walls, where they can be converted into oxLDL (oxidized LDL). Vascular insults, such as hyperlipidaemia, also cause the secretion of MCP-1 from ECs (endothelial cells) and SMCs (smooth muscle cells). The secreted MCP-1 is tethered to the proteoglycan on the vessel lumen. Monocytes rolling along the lumen bind CCR2 on the membrane of the leucocyte, which is firmly arrested on the ECs by the actions of CXCR2 (CXC chemokine receptor 2) and its ligands. This MCP-1–CCR2 interaction leads to the activation of the G-protein-coupled receptor, leading to diapedesis of the monocyte between the ECs. The monocytes infiltrating into the subendothelial space differentiate into macrophages, take up oxLDL and become foam cells, the hallmark of the fatty streak in the vessel walls. The fatty streak evolves into a complex lesion by proliferation of SMCs and their migration towards the intima with synthesis of collagen. Continued release of MCP-1 and other cytokines by ECs perpetuates inflammation

and lipid accumulation. In a rabbit model, inhibition of MCP-1 action by administration of a mutant MCP-1 inhibited plaque inflammation, reversed plaque progression and prevented rupture of vulnerable plaques, but did not affect lipid profile. Thus prevention of plaque inflammation alone without lipid-lowering can stabilize the plaque [7].

Genetically modified mice provide strong direct evidence for the role of MCP-1 in atherosclerosis. MCP-1-deficient mice in an LDLR (LDL receptor)-deficient background, when fed a high-cholesterol diet, had 83 % less lipid deposition in the aortic tree than the control LDLR-deficient mice with an intact MCP-1 gene [6]. In an apoB (apolipoprotein B) transgenic atherosclerosis model, similar results were obtained [8]. Decreased macrophage infiltration was associated with MCP-1 deficiency. In support of the role of MCP-1 in atherosclerosis, overexpression of MCP-1 in the arterial wall of hypercholesterolaemic rabbits produced increased macrophage infiltration and accelerated atherosclerosis [9]. CCR2 deficiency in an apoE-deficient background showed decreased macrophage infiltration and protection against plaque formation [10].

Cardiovascular cells, including ECs, VSMCs (vascular SMCs) and cardiac myocytes, can produce MCP-1 in response to a variety of stimuli, and its expression has been identified in advanced murine and human atheroma, which, by triggering and sustaining leucocyte accumulation, may in turn promote chronic inflammation [4]. oxLDL in the arterial wall may up-regulate MCP-1 production in vascular cells and stimulate the local adhesion of monocytes to ECs [11]. Thus MCP-1 may be the mediator linking oxidized lipoproteins and foam cell recruitment into the vessel wall. Studies in transgenic mice overexpressing MCP-1 have provided strong evidence that MCP-1 functions in the recruitment of monocytes to atheroma, resulting in increased foam cell formation and increased atherosclerosis [5]. Knockout of the MCP-1 gene or its receptor CCR2 and anti-MCP-1 gene therapy have been shown to cause a significantly reduced progression of atherosclerosis in murine models with dietary-induced hyperlipidaemia [10,12].

Evidence for the importance of MCP-1 in atherosclerosis in humans comes from many observations. MCP-1 –2518A > G and CCR2 190G > A polymorphisms have been reported to be associated with an increased risk of developing atherosclerosis [13–15]. CCR2 with a replacement of Val⁶⁴ with an isoleucine residue signals less efficiently, and people with this variant have decreased cardiovascular risk [16]. Moreover, MCP-1 has shown some promise as a biomarker for disease monitoring in other inflammatory diseases, such as juvenile rheumatoid arthritis, where plasma levels decrease concordant with improvements in clinical signs and persistent reductions appear to predict longer remission [17]. Indeed, elevated plasma levels of MCP-1

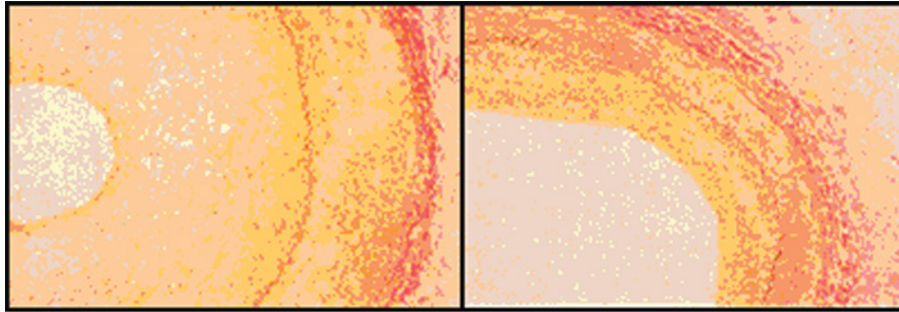


Figure 1 Attenuation of restenosis by antibodies against MCP-1 following angioplasty in a rabbit model

At 1 month after induction of focal atherosclerosis lesions, rabbits underwent balloon angioplasty and the vessels were examined 3 weeks later. A goat anti-(rabbit MCP-1) antibody or equal amounts of pre-immune IgG (30 mg of IgG/kg of body weight), was administered 12 h before angioplasty and again 72 h after angioplasty. Left-hand panel, pre-immune IgG; right-hand panel; anti-(MCP-1) IgG. Restenosis was clearly attenuated by treatment with the anti-(MCP-1) antibody. This Figure was presented at the 66th Scientific Sessions of the American Heart Association, 6–11 November 1993, Atlanta, Georgia, abstract 1-371.

has been found in patients with CHD (coronary heart disease), with the highest levels in those with ACS (acute coronary syndrome) or patients undergoing PTCA (percutaneous coronary transluminal angioplasty) [18,19]. Elevated levels of circulating MCP-1 are positively correlated with most cardiovascular risk factors, measures of coronary atherosclerosis burden and the incidence of CHD [20,21]. In a large population of patients stabilized after ACS, measurement of plasma levels of MCP-1 at baseline, 4 months and 12 months, and correlation with clinical events in the Z phase of the A to Z trail indicated that plasma levels of MCP-1 > 238 pg/ml were independently associated with mortality after adjustment for standard risk predictors and levels of CRP (C-reactive protein) and BNP (B-type natriuretic peptide), suggesting that MCP-1 provides independent prognostic value in both the acute and chronic phases after ACS [22]. The reports that MCP-1 induces ubiquitin and increased activity of ubiquitin–proteasome system in the inflammatory macrophages may destabilize plaques [23] suggest a possible role for MCP-1 in plaque rupture that would trigger myocardial ischaemic episodes.

Balloon angioplasty is used to dilate these plaques in the coronary circulation so as to prevent occlusion of this critical blood supply. However, the benefits of this procedure are hampered by dilatations that end in restenosis which occurs in 30–50% of cases within 6 months of the procedure [24]. The pathogenesis of restenosis after balloon angioplasty involves the migration of medial SMCs across the internal elastic lamina to form a neointima. Proliferation of these cells and their elaboration of an extracellular matrix results in stenosis of the affected area. The presence of an ongoing inflammatory reaction probably maintains SMC migration, proliferation and matrix deposition. In the early 1990s, it was found that balloon injury of the rabbit aorta induced local MCP-1 production [25]. Soon after that we reported that administration of MCP-1 antibodies

retarded restenosis after angioplasty in the rabbit model (Figure 1) [26]. More recent reports have shown that N-terminal-truncated MCP-1 caused significant reduction in neoluminal hyperplasia in mice, rabbits and monkeys [7,24,27]. Anti-CCR2 antibodies also prevented restenosis in primates [28]. Thus anti-MCP-1/CCR2 approaches can be used to prevent restenosis.

MCP-1 AND CARDIAC INJURY

Induction of chemokines is a prominent feature of the inflammatory response associated with ischaemia/reperfusion injury in many tissues. Analysis of patient biopsies and animal models by *in situ* hybridization or immunostaining has identified the expression of MCP-1 mRNA and protein in ischaemic myocardium, which correlates with the accumulation of leucocytes [29]. Experiments from animal models have also shown that myocardial MCP-1 is a major factor responsible for mononuclear cell recruitment into the ischaemic myocardium during the first 5 h of reperfusion [30]. Elevated serum levels of MCP-1 have also been found in patients with CHD and are associated with the risk of MI (myocardial infarction) and dysfunction [20–22]. Genetic evidence also exists linking a CCR2 polymorphism with MI and the end stage of heart failure in humans [14]. In a rat model of experimental MI, administration of a neutralizing antibody to MCP-1 significantly reduced infarct size associated with decreased adhesion molecule expression and macrophage infiltration [31]. In addition, anti-MCP-1 gene therapy significantly reduced ventricular dilation and preserved cardiac function in a mouse model [32]. Mice with disrupted CCR2 also had less post-infarct myocardial remodelling [33].

In an attempt to examine the role of sustained high levels of MCP-1 resulting from chronic inflammation in the development of heart disease, we generated a

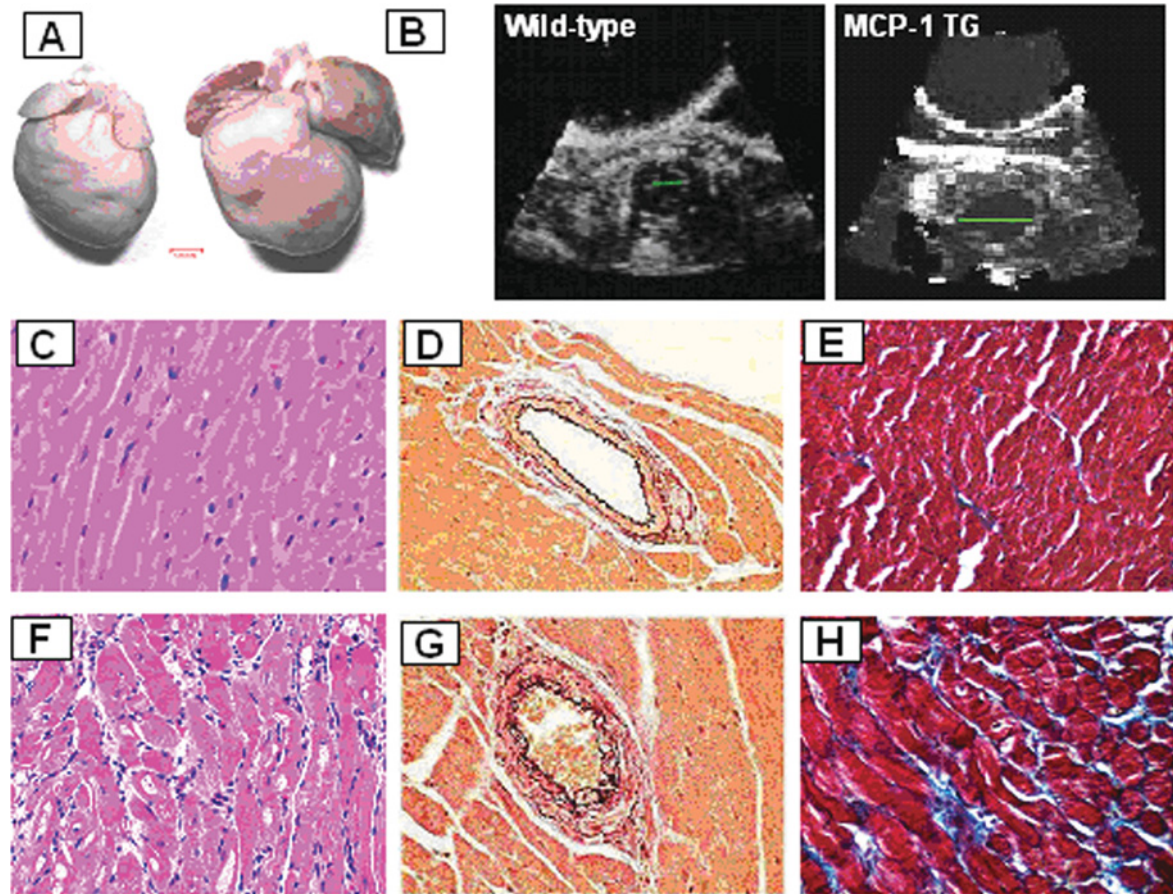


Figure 2 Cardiac-targeted expression of MCP-1 causes ischaemic cardiomyopathy

(A) Gross anatomical inspection revealed marked cardiomegaly in MCP-1-expressing hearts (right) when compared with wild-type control (left). (B) Two-dimensional echocardiogram of wild-type (left) and MCP-1 transgenic (right) mice. An enlargement of the left ventricle in the transgenic mouse heart was observed. The MCP-1 transgenic heart had a lower LV ejection fraction (46 %) than the wild-type control (60 %). Representative photomicrographs in MCP-1 transgenic hearts (C–E) and wild-type controls (F–H) demonstrating infiltrating mononuclear cells within the myocardium (C and F), as assessed by haematoxylin and eosin staining, neointima formation (D and G), as assessed by EVG (elastic Van Gieson) staining, and interstitial fibrosis (E and H), as assessed by Masson's Trichrome staining. Magnification, $\times 40$. Photomicrographs were reprinted from *Am. J. Pathol.* 1998 152: 101–111 with permission from the American Society for Investigative Pathology, and reproduced from [94] with permission, © (2000) Lippincott Williams & Wilkins.

transgenic mouse model with targeted expression of MCP-1 in myocardium [34]. As the animals aged, they developed cardiac hypertrophy and dilatation, and increase in LV mass and ventricular dysfunction (Figures 2A and 2B). Importantly, these effects were shown to be dependent upon gene dosage. When the homozygous transgenic mice with high MCP-1 were compared with the heterozygotes with lower myocardial MCP-1 expression, there was a significantly greater decrease in LV fractional shortening. Most of the heterozygote animals died of heart failure at 12–14 months of age, whereas homozygotes often had loss of ventricular function and died at 6–7 months of age. In the MCP-1-expressing myocardium, we have found infiltrating monocytes/macrophages, thrombotic occlusive arteriolar vasculopathy, cardiomyocyte degeneration and fibrosis (Figures 2C–2H). Further characterization of this model

with ^{31}P NMR analysis of the high-energy metabolic status revealed a decrease in the creatine phosphate/ATP ratio from 2.0 to < 1.0 , indicating that MCP-1-transgenic mice suffer ischaemic injury (V.P. Chacko, R. Weiss and P. E. Kolattukudy, unpublished work). The established markers of ischaemic cardiomyopathy in humans, including macroscopic, microscopic, ultrastructural, biochemical and molecular changes, were also found in the MCP-1 mouse model (Table 1), strongly suggesting that sustained expression of MCP-1 contributes to the development and/or progression of ischaemic heart disease.

MCP-1 AND CARDIAC REPAIR

There is increasing evidence that short-term expression of pro-inflammatory cytokines can be beneficial to

Table 1 Comparison of pathological and molecular alterations in human ischaemic cardiomyopathy and a mouse heart with MCP-1 overexpression

bARK, β -adrenergic receptor kinase; iNOS, inducible NO synthase; RBC, red blood cell; TF, tissue factor; TUNEL, terminal deoxynucleotidyl transferase-mediated dUTP nick-end labelling.

Alteration	Human ischaemic cardiomyopathy	MCP-1 mouse model
Clinical	Decrease in fractional shortening Low-frequency heart rate variability Progressive widening of QRS complex	Decrease in fractional shortening Low-frequency heart rate variability Progressive widening of QRS complex
Macroscopic changes	Heart enlargement Coronary atherosclerosis Organized endocardial thrombi Granulation tissue Myocardial fibrosis Collateral circulation	Dilated heart Occlusive coronary lesions Atrial and ventricular thrombi Granulation tissue Myocardial fibrosis High density of medium-size vessels
Microscopic changes	Contraction bands Myocytosis Karyorrhexis Dilated capillaries Pigmented macrophages Mast cell activation	Contraction bands Myocyte vacuolation and disruption TUNEL-positive nuclei Dilated capillaries and sludging RBC phagocytosis in macrophages Mast cell accumulation/degranulation
Ultrastructural changes	Endothelial injury Mitochondrial degeneration Relaxation of myofibrils Apoptosis	Endothelial vacuolation/blebbing Mitochondrial swelling/dense deposits Disorganization of myofibrils Apoptosis
Molecular changes	MCP-1 expression increase TF increase Nitrate/nitrite increase bARK levels increase TNF- α , IL-6 and TGF- β increase Myosin composition change Creatine phosphate/ATP ratio < 1.0 Induction of MMPs Elevated CCR2 expression	MCP-1 expression increase TF increase Increase in ROS/iNOS bARK levels increase TNF- α , IL-6 and TGF- β increase Myosin composition change Creatine phosphate/ATP ratio < 1.0 MMP induction Elevated CCR2 expression

the heart [35]. *In vitro* evidence indicates that MCP-1 protects cultured mouse neonatal cardiac myocytes from hypoxia-induced cell death [36,37]. Investigations from transgenic mouse studies have provided new insights into the role of MCP-1 in cardiac protection. Following ischaemia/reperfusion injury, the hearts from MCP-1 transgenic animals had a decrease in the percentage of infarct size when compared with wild-type control hearts (Figure 3). Immunoblot analyses showed significantly increased levels of TNF- α (tumour necrosis factor- α) and phosphorylated SAPK (stress-activated protein kinase)/JNK (c-Jun N-terminal kinase)1/2 in MCP-1 overexpressing hearts [38], suggesting that cardiac-targeted expression of MCP-1 causes infiltration and activation of leucocytes, resulting in elevated TNF- α secretion and SAPK/JNK1/2 activation, and leading to cardiac protection. Similar findings were also obtained in post-infarct remodelling studies in transgenic mice with cardiomyocyte-targeted expression of MCP-1 (Figure 4)

[39]. MCP-1 transgenic mice have reduced infarct area and scar formation and improved LV dysfunction after MI. Interestingly, the decrease in infarct size and preservation of cardiac function in the MCP-1 transgenic mice was associated with marked myocardial IL-6 (interleukin-6) secretion, STAT-3 (signal transducer and activator of transcription-3) activation and myofibroblast accumulation in the heart [39]. However, a series of studies from MCP-1-deficient mice demonstrated that MCP-1-null mice had less LV remodelling without a change in infarct size [40]. These mice had decreased and delayed infiltration of macrophages, decreased expression of cytokines TNF- α , IL-1 β and IL-10, and decreased myofibroblast accumulation within the healing infarct. This observation was also supported by other approaches that inhibited MCP-1 function, including overexpression of an N-terminal deletion mutant of the MCP-1 gene [32] or genetic deletion of its receptor CCR2 [33], suggesting that MCP-1 causes ventricular remodelling at the same

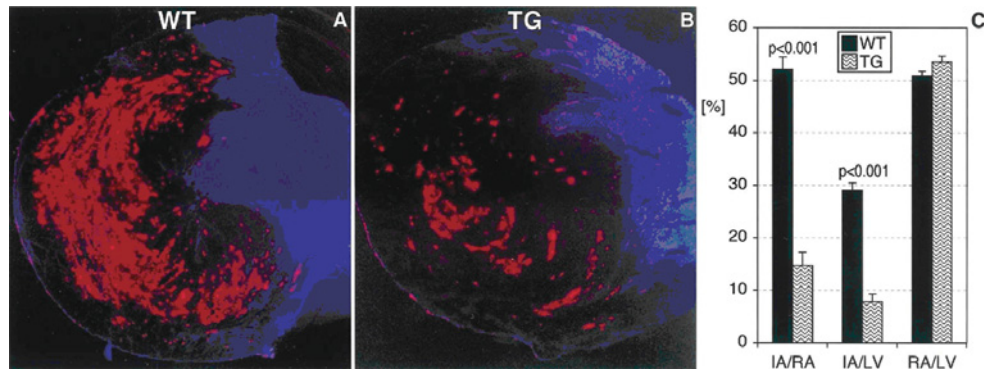


Figure 3 Cardiac-targeted expression of MCP-1 mimics ischaemic preconditioning and limits infarct size

(A and B) Transversal sections of the left ventricle from wild-type (A) and MCP-1 transgenic (B) animals 45 min after LAD (left anterior descending coronary artery) occlusion. Dead myocytes are stained red with propidium iodide. ECs from the non-risk area are stained blue with thioflavin S. The risk area is not stained and appears dark. (C) Quantification of the IA/RA, IA/LV and RA/LV ratios in wild-type and MCP-1 transgenic animals 45 min after ischaemia. IA, infarct area; RA, risk area; LV, left ventricle. Reproduced from [38] with permission, © (2003) European Society of Cardiology.

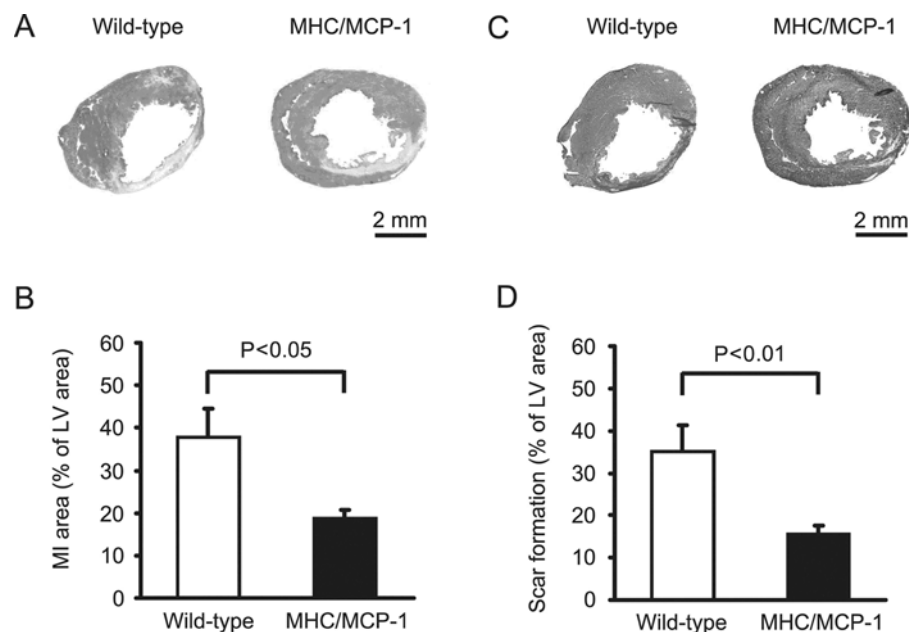


Figure 4 Cardiac-targeted expression of MCP-1 improves post-infarct myocardial remodelling

(A and C) Heart sections obtained from wild-type and MCP-1 transgenic mice at 14 days after MI stained with haematoxylin and eosin (A) and Masson's Trichrome (C). (B and D) Quantification of the infarct area (B) and scar formation (D). Reproduced from [39] with permission, © (2006) Lippincott Williams & Wilkins.

time that it promotes infarct healing. Thus the above gain-of-function and deterioration studies for MCP-1 suggest that MCP-1 plays an important role in the orchestration and timing of the myocardial stress response, both by providing early cytoprotective signals that are responsible for delimiting tissue injury, but also by providing delayed signals that facilitate tissue repair and/or tissue remodelling once myocardial tissue damage has supervened. The seemingly contradictory effects of

MCP-1 in protection and deterioration can be reconciled by the finding that MCP-1 can induce an ER (endoplasmic reticulum) stress response. Cardiomyocyte-targeted expression of MCP-1 caused highly elevated expression of ER stress chaperones, such as GRP78 (78 kDa glucose-regulated protein), GRP94 (94 kDa GRP), HSP25 (25 kDa heat-shock protein), HSP40 (40 kDa HSP) and HSP70 (70 kDa HSP), in the heart of young animals [41] which also manifested the cardioprotective effects

indicated above [38,39]. Experimental evidence shows that the presence of elevated levels of ER chaperones prior to the ischaemic insult can protect against cell death and thus results in cardioprotective effects. For example, protection by ischaemic preconditioning by inducing the expression of GRP78 and protection from lethal injury by the pre-induction of molecular chaperones in the ER have been demonstrated [42,43]. Expression of GRP78 prior to ischaemic injury has been shown to protect cells in culture, whereas prolonged stress would result in a breakdown of this protection [44]. In animals with cardiac-targeted expression of MCP-1, cardioprotection was found in young animals when the ER stress chaperones were at high levels; however, as the animals aged, the prolonged stress caused by the sustained MCP-1 expression results in the breakdown of protection, leading to the development of heart failure.

MCP-1 IN MYOCARDITIS

Cardiac inflammation, or myocarditis, is a frequent cause of cardiac failure in young adults. A growing body of evidence suggests that autoimmune responses are involved in the pathogenesis of myocarditis and post-infectious cardiomyopathy [45]. EAM (experimental autoimmune myocarditis) is an animal model for human myocarditis and provides an excellent laboratory model to study the mechanisms of inflammation leading to heart failure [46]. Cardiac myosin has been considered as a major carditis-inducing antigen; however, skeletal C protein induces more severe disease than purified skeletal myosin [47]. Immunization with cardiac C protein can cause severe EAM leading to dilated cardiomyopathy [48]. Elevation of MCP-1 was found to be associated with EAM [48,49], and DNA therapy with DNA coding for the MCP-1-binding site on CCR2 rescued EAM animals [48]. Gene therapy with DNA coding for the N-terminal-deleted version of MCP-1 that acts as an MCP-1 antagonist was also shown to reduce the severity of EAM [50]. Myocarditis caused by coxsackievirus [51] and Lyme disease also involves elevated MCP-1 levels, suggesting that MCP-1 plays a control role in myocarditis.

MCP-1 IN ORGAN TRANSPLANT REJECTION

Heart transplantation is a viable option for some patients with end-stage heart failure. However, rejection continues to be a source of significant morbidity and mortality after heart transplantation in spite of the success of immunosuppressive regimens [52]. There are many reports that suggest that MCP-1 is involved in the inflammatory process associated with transplantation which can lead to transplant vasculopathy and tissue destruction resulting

in rejection. Transplant vasculopathy remains a common and frequently fatal but poorly treatable complication of heart transplantation [52]. Recruitment of T-lymphocytes and macrophages is a hallmark of allograft rejection. The expression of many chemokines is up-regulated at some stage during the development of allograft rejection [53]. MCP-1 and CCR2 are among the chemokines and receptors that are overtly and selectively induced in allografts that develop transplant vasculopathy [54]. Early and persistent expression of MCP-1 in cardiac allografts has been implicated in transplant arteriosclerosis and allograft rejection. Over the past 15 years, many studies have reported an elevated expression of MCP-1 and CCR2 associated with organ transplantation in animal models [54–56]. A role for MCP-1/CCR2 in graft vasculopathy and rejection has been implicated by the findings that MCP-1 deficiency, MCP-1 neutralization, gene therapy involving the expression of N-terminal-deleted MCP-1, which acts as an antagonist, and CCR2 deficiency attenuate transplant vasculopathy and prolong graft survival [57–60]. In a pancreatic islet allograft model, elevated expression of MCP-1 and CCR2 was observed [61]. The functional role of MCP-1/CCR2 in rejection was indicated by the finding that the transplanted islet cell survival was dramatically prolonged in CCR2^{-/-} mice when compared with wild-type animals. A considerable number of the CCR2^{-/-} animals had prolonged survival of the islet allografts [61].

Increases in levels of MCP-1 have also been reported in human transplant patients [62–64]. Graft dysfunction in the immediate post-lung transplant period leading to rejection in human patients is associated with elevated levels of MCP-1 [56]. In CCR2^{-/-} mice, evidence was presented to indicate that MCP-1/CCR2 is important in the continuum of acute chronic allograft rejection in lung transplantation [65]. In cases where human myocardial biopsy specimens revealing ischaemic injury were excluded, MCP-1 levels did not show a correlation with the grade of rejection [66].

MCP-1 IN OBESITY, DIABETES AND CARDIOVASCULAR DISEASE

It is well known that obesity-induced diabetes is a major risk factor into the development of cardiovascular disease [67–69]. In recent years, it has become increasingly clear that obesity involves a low-grade systemic inflammatory condition [70]. Visceral-adipose-related inflammation was shown to accelerate atherosclerosis in mice [71]. Both adipocytes and macrophages are sources of cytokine production [72], and pro-inflammatory cytokines promote cellular insulin resistance in fat, muscle and liver [73–75]. High levels of MCP-1 production are associated with obesity in animals and humans [76–78]. Previously, a role for MCP-1 in obesity-induced

insulin resistance was demonstrated [79,80]. Transgenic mice with an adipose-tissue-specific expression of MCP-1 had macrophage infiltration into adipose tissue, increased hepatic triacylglycerol content and insulin resistance. MCP-1-knockout mice fed a high-fat diet had a drastically reduced macrophage accumulation into adipose tissue and hepatic steatosis when compared with high-fat-fed wild-type mice. Furthermore, inhibition of MCP-1 function by the acute expression of a dominant-negative mutant of MCP-1 ameliorated insulin resistance in *db/db* mice and in high-fat-fed wild-type mice [79]. These results strongly suggest that increased expression of MCP-1 in adipose tissue causes macrophage infiltration into adipose tissue, insulin resistance and hepatic steatosis associated with obesity in mice.

That the role of MCP-1 in obesity-induced diabetes is mediated via its binding to the receptor CCR2 was demonstrated by results obtained with CCR2^{-/-} mice [81,82]. CCR2 deficiency resulted in reduced macrophage content, increased adiponectin expression, and ameliorated hepatic steatosis and insulin resistance. Even in mice with established obesity, short-term treatment with a pharmacological antagonist of CCR2 lowered macrophage content of adipose tissue and improved insulin sensitivity [83]. Thus the MCP-1/CCR2 system plays a critical role in obesity-induced diabetes in mice. The role of MCP-1 in macrophage infiltration into adipose tissue has been questioned based on results obtained with MCP-1-deficient mice [83]. However, if another ligand can bind CCR2 and cause the same signalling events, the results obtained with MCP-1-deficient mice do not rule out the important role of the MCP/CCR2 system. In mice, MCP-5 binds to CCR2 and triggers the same signalling process as MCP-1 [84]. Thus results obtained with CCR2-deficient animals would not be the same as those obtained with MCP-1-deficient mice.

The role of MCP-1 in obesity-related diabetes was also observed in humans. The increased level of serum MCP-1 found in humans correlated with markers of the metabolic syndrome, including obesity, insulin resistance, Type 2 diabetes, hypertension and increased serum triacylglycerol concentrations [85]. Expression of CCR2 on monocytes was reported to be elevated in diabetic patients [78]. The molecular mechanisms underlying the role of MCP-1/CCR2 in diabetes are not understood; however, the finding that MCP-1 may lead to ER stress [41] indicates a possible mechanism. In fact, ER stress was reported to link obesity, insulin action and Type 2 diabetes [86]. Chemical chaperones that specifically reduce ER stress restored glucose homeostasis in a mouse model of Type 2 diabetes [87]. The frontline antidiabetic drug pioglitazone, a PPAR- γ (peroxisome-proliferator-activated receptor- γ) agonist, has anti-inflammatory effects. Meta-analysis of data from 19 trials found that pioglitazone reduced death from cardiovascular causes,

heart attack or stroke in diabetic populations [88]. Clearly, these antidiabetic drugs reduce cardiovascular disease through their anti-inflammatory effects.

ROLE OF MCP-1 IN ISCHAEMIC ARTERIOGENESIS AND ANGIOGENESIS

Inflammatory cell infiltration is a feature of the post-ischaemic neovascularization process. Monocytes are recruited to regions of arteriogenesis, where they are thought to secrete growth factors and other bioactive molecules that promote angiogenesis [89]. Early studies suggested that MCP-1 was an angiogenic factor associated with the prominent recruitment of monocytes [90]. Exogenous MCP-1 delivered either intra-arterially or by mini-osmotic pump has been shown to increase monocyte/macrophage recruitment, collateral vessel formation and blood flow to the ischaemic tissue in hindlimb models of ischaemia [91,92]. MCP-1 has been shown to mobilize and transdifferentiate bone marrow monocyte lineage cells into EC-like cells [93]. When monocytes were driven into the heart by cardiomyocyte-targeted expression of MCP-1, the invading monocytes appeared to form erythrocyte-containing vascular-like tunnels [94]. Transfusion of MCP-1-activated monocytic lineage cells accelerates re-endothelialization by transdifferentiation into functional EC-like cells on the injured endothelium [93].

A direct effect of MCP-1 on angiogenesis, which was not related to its monocyte/macrophage recruitment, has also been reported. *In vitro* experiments have shown that MCP-1 promoted the capillary-like structure formation in HUVECs (human umbilical vein ECs) [95–97]. As indicated below, this activity of MCP-1 is mediated via a novel MCP-1-induced zinc-finger protein. MCP-1 also stimulated blood vessel growth in mouse matrigel plug implantation and rabbit corneal models of angiogenesis [95]. MCP-1 also directly influences the angiogenic process through the up-regulation of HIF-1 α (hypoxia-inducible factor-1 α) and VEGF (vascular endothelial growth factor) and activation of the Ets-1 transcription factor [96,98], suggesting that the angiogenic properties of MCP-1 might be mediated through various secondary angiogenic factors. Hence MCP-1 exerts diverse effects on different cell types involved in the post-ischaemic arteriogenesis or angiogenesis.

MECHANISMS OF MCP-1-INDUCED BIOLOGICAL EFFECTS IN THE HEART

Although extensive experimental evidence has suggested that MCP-1 plays multiple functions in cardiopathobiology, the molecular mechanisms underlying these functions are poorly understood. The following section

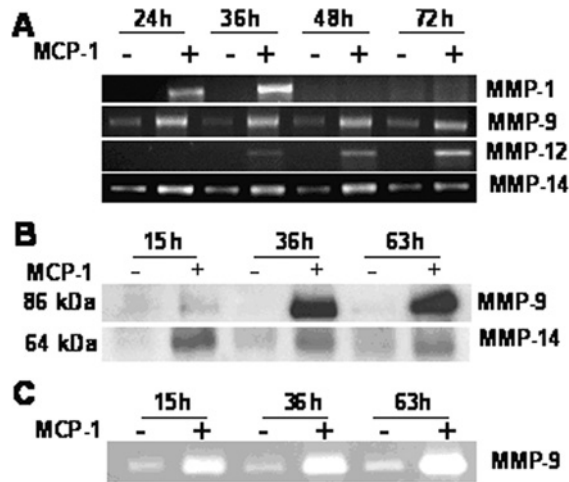


Figure 5 MCP-1 induces MMP expression in human monocytes

(A) RT-PCR analysis of MMP expression in MCP-1 treated (+) and untreated (–) human monocytes. (B) Western blot analysis of MMP-14 in whole-cell lysate of monocytes and MMP-9 in conditioned medium. (C) A zymogram for MMP-9 protease activity in the conditioned medium. Z. Li and P. E. Kolattukudy, unpublished work.

will review the current understanding of the molecular changes induced by MCP-1 in the cardiovascular system.

Role of MCP-1-mediated infiltrating mononuclear cells

Monocyte/macrophage infiltration is critical to the initiation and progression of ischaemic heart disease. MCP-1 binding to CCR2 initiates a series of signalling events that not only causes chemotactic migration of monocytes, but also triggers changes in gene expression in the monocytes leading to the production of biologically active molecules that affect neighbouring cells in the heart. *In vitro*, MCP-1 activates the respiratory burst of monocytes and induces the expression of the pro-inflammatory cytokines IL-6 and IL-1 β [99], which in turn may regulate the expression of TGF- β (transforming growth factor- β). Treatment of human monocytes with MCP-1 induces the expression of MMPs (matrix metalloproteinases), which have been shown to play important roles in cardiac remodelling [100]. Several MMPs, including MMP-1, MMP-9, MMP-12 and MMP-14, were up-regulated by treatment of human monocytes with MCP-1 (Figure 5A) (Z. Li and P. E. Kolattukudy, unpublished work). Western blot analysis confirmed the induction of MMP-9 and MMP-14 from MCP-1-treated monocytes (Figure 5B). Similar changes are observed in MCP-1 transgenic mice and patients with ischaemic cardiomyopathy, which have an active inflammatory reaction associated with MCP-1 production and leucocyte infiltration (Table 1). Early studies in a mouse MI model showed apoptosis of the infiltrating inflam-

matory cells during the acute stage and myofibroblasts during the subacute stage [101]. In the MCP-1 transgenic mouse model, infiltrating monocytes undergo apoptosis, releasing FasL (Fas ligand) that causes apoptosis of ECs and VSMCs leading to vasculopathy and thrombosis and results in ischaemia [102]. Inhibition of infiltrating mononuclear cell apoptosis by monocyte-targeted expression of anti-apoptotic Bcl-2 prevented apoptosis of vascular cells and attenuated the development of ischaemic heart failure [103]. Prevention of vascular cell apoptosis by cardiac-targeted expression of soluble Fas to decoy FasL released by the apoptotic monocytes also rescued MCP-1 transgenic mice from developing cardiomyopathy and heart failure [102]. These observations suggest that MCP-1-mediated infiltration of monocytic cells and the biologically active molecules released from dying monocytes are associated with deleterious effects on LV function and the progression of heart failure.

Effects of MCP-1 on the biology of the major cell types in the heart

In addition to its critical role in mononuclear cell recruitment, MCP-1 also exerts important actions on the major cell types in the heart. MCP-1 treatment induces the expression of MMPs in cultured murine cardiac myocytes and VSMCs. More importantly, conditioned medium from MCP-1-treated monocytes caused a further induction of pro-inflammatory cytokines and MMPs in cultured cardiac myocytes and aortic SMCs (Z. Li and P. E. Kolattukudy, unpublished work), suggesting that the MCP-1-induced release of biologically active molecules from monocytes can affect gene expression in the surrounding cells in the myocardium. Gene array data from MCP-1 transgenic mice hearts revealed that MCP-1 expression in cardiomyocytes potently induces a set of genes that encode a class of proteins called ER-stress-response proteins [41], which are known to protect against a subsequent challenge from further stress that is by itself lethal [42–44]. Interestingly, a recent study has shown that MCP-1-expressing hearts had a decreased expression of the NADPH oxidase family proteins Nox1, gp91^{phox} and Nox3 compared with the hearts of wild-type mice following ischaemia/reperfusion injury [104], suggesting that overexpression of MCP-1 protects against further accumulation of oxidative stress proteins induced by ischaemia/reperfusion. This observation was supported by our recent finding that administration of cerium oxide nanoparticles, a potent antioxidant, via the tail vein resulted in decreased oxidative stress and ER stress responses in the heart, resulting in the preservation of cardiac dysfunction in MCP-1 transgenic mice [105].

Evidence from animal models and *in vitro* experiments suggests that MCP-1 can directly and indirectly promote cardiac fibrosis in ischaemic cardiomyopathy [106–108]. Both neutralizing MCP-1 antibodies and CCR2

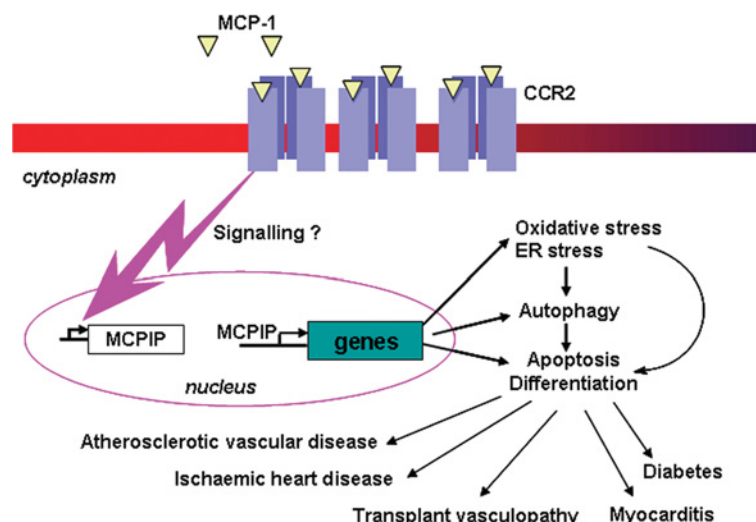


Figure 6 MCP-1-induced signalling events in cardiovascular disease

deficiency can reduce fibrosis [109,110]. The profibrotic functions of MCP-1 are thought to reflect its role in mononuclear cell recruitment and activation. MCP-1 can directly stimulate macrophages to secrete TGF- β 1, which can subsequently promote the production of extracellular matrix [111]. MCP-1 has also been shown to stimulate collagen expression via the endogenous up-regulation of TGF- β [112] and enhance the expression of MMPs in fibroblasts [113]. Pathological stimuli in the heart can activate cardiac fibroblasts which differentiate into myofibroblasts, participating as key cells in tissue healing. Activated myofibroblasts produce extracellular matrix proteins, and myofibroblast apoptosis has been considered as a mechanism responsible for the evolution of granulation tissue into a scar [114]. Transgenic mice with the cardiac overexpression of MCP-1 had increased myocardial IL-6 secretion and accumulation of cardiac myofibroblasts, whereas MCP-1-null mice had reduced myofibroblast density, suggesting that MCP-1 may modulate fibroblast phenotype and activity [39,40]. This was confirmed by studies showing that treatment with MCP-1 directly promoted the differentiation of cardiac fibroblasts into myofibroblasts [39]. MCP-1 has also been suggested to be involved in the recruitment of bone-marrow-derived fibroblast precursors ('fibrocytes'), capable of differentiating into fibroblasts involved in the fibrotic process [106]. Therefore MCP-1 exerts diverse effects on different cell types involved in cardiac fibrotic remodelling.

An MCP-1-induced transcription factor mediates stress responses in the heart

Many studies have suggested that MCP-1 may also mediate biological effects other than leucocyte chemotaxis [95–99,111,113]. Using a gene array approach,

we have discovered that signalling initiated by MCP-1 binding to CCR2 in human peripheral blood monocytes triggered the induction of a novel zinc-finger protein called MCPIP (MCP-1-induced protein) [115]. This protein is the first member of a novel family of zinc-finger proteins [116], and was found to be a transcription factor that can induce cell death [115]. MCPIP is notable for its expression in the infiltrating inflammatory cells and the major cell types in the myocardium and has been shown to have pro-apoptotic activity [115,117]. Increased MCPIP expression was found to be associated with apoptotic cardiomyocytes in the myocardium of MCP-1 transgenic mice and failing human hearts [115]. Recently, we have explored the molecular mechanisms by which MCPIP causes cardiomyocyte death (C. W. Younce and P. E. Kolattukudy, unpublished work). Adenovirus-mediated expression of MCPIP in H9c2 cells caused cell death. Experimental evidence indicates that MCPIP causes the production of ROS (reactive oxygen species) and RNS (reactive nitrogen species) via the induction of NADPH oxidase and iNOS (inducible NO synthase). This oxidative stress causes ER stress that leads to autophagy and cell death. ER stress induction was determined by the expression of the ER stress chaperones GRP78, GRP94, HSP25 and HSP70. Induction of autophagy was reported by the induction of beclin1 and LC3 cleavage required for autophagolysosome formation. Inhibition of ROS and RNS production inhibited ER stress, as well as autophagy and cell death. Selective inhibitors of ER stress inhibited autophagy and cell death, and inhibitors of autophagy prevented MCPIP-induced autophagolysosome formation and cell death. The MCPIP-induced signalling events that lead to the development of cardiovascular disease are described in Figure 6. There are indications that the MCPIP-induced processes that lead to cell death in H9c2 cells might also be

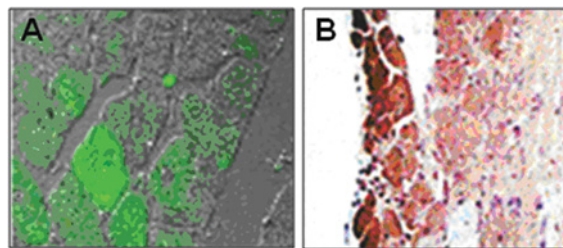


Figure 7 Free radical detection by CM-DCF-DA (A) and RNS detection by anti-nitrotyrosine antibody staining (B) in the myocardium of transgenic mice with cardiomyocyte-targeted expression of MCP-1

Superposition of Nomarsky and fluorescence images is shown in (A). N. Moldovan, J. Bower, P. Goldschmidt and P. E. Kolattukudy, unpublished work.

involved in the cardiovascular cell death associated with chronic inflammation in animals. In the MCP-1 mouse model of ischaemic heart disease, ROS and RNS formation in the heart tissue has been detected by CM-DCFH-DA (chloromethyl 2',7'-dichlorofluorescein diacetate) staining and nitrotyrosine staining of the heart tissue (Figure 7) (N. Moldovan, J. Bower, P. Goldschmidt and P. E. Kolattukudy, unpublished work). Elevated levels of ER chaperones have been found in such hearts [41]. Administration of antioxidant cerium oxide nanoparticles attenuated ER stress and the development of heart disease strongly suggesting that such processes are involved in the development of inflammation-induced heart disease [105]. To directly test for the critical role of MCP-1 in the development of heart disease, we have generated transgenic mice with cardiac-targeted expression of MCP-1 in the myocardium under the control of MHC promoter. Such animals developed progressive ventricular dilatation and dysfunction accompanied by cardiac myocyte death (S. Graham, J. Niu and P. E. Kolattukudy, unpublished work).

MCP-1 can also cause differentiation that is also mediated via MCP-1. MCP-1-induced tube formation in HUVECs was accompanied by the induction of MCP-1 and was inhibited by the knockdown of MCP-1 expression with specific siRNA (small interfering RNA) [97]. Forced expression of MCP-1 induced tube formation by HUVECs. In fact, MCP-1 expression stimulated the proliferation and migration of HUVECs involved in tube formation. Chromatin immunoprecipitation analysis indicated that *cdh12* (cadherin 12) and *cdh19* genes were targets of MCP-1. MCP-1 expression induced both *cdh12* and *cdh19* in HUVECs, and knockdown of *cdh12* and *cdh19* inhibited tube formation induced by MCP-1 expression, indicating that MCP-1-induced angiogenesis is probably mediated, at least in part, via the induction of *cdh12* and *cdh19* [97]. Whether other differentiation processes induced by MCP-1, such as

transdifferentiation of monocytes into ECs, are also mediated via MCP-1 remain to be established.

MCP-1 AS A POSSIBLE TARGET FOR THERAPEUTIC INTERVENTIONS

In animal models, interference with MCP-1 or its receptor that showed beneficial effects against the development of cardiovascular disease did not manifest any changes in the lipid profile. Thus drugs that target MCP-1/CCR2-mediated processes should give benefits in addition to those obtained by lipid-lowering drugs. However, statins, the most commonly used anticholesterol drugs, also suppress the expression of MCP-1 and CCR2 in cell cultures and patients [118]. MCP-1/CCR2-directed therapy is likely to be successful against restenosis after angioplasty. Direct anti-MCP-1 therapy was found to be effective in animal models. When an N-terminal-truncated MCP-1 was expressed by administering an expression plasmid into skeletal muscle, the truncated form was expressed in such a way that it could be measured in plasma. In two animal models, this approach showed substantial reductions in lesion formation in coronary arteries and aortas [7]. Antibodies against MCP-1 or CCR2 are other possible therapeutic agents [3,12]. Whether such agents can be used effectively in humans remains questionable.

In recent years, small molecules have been discovered that interfere with MCP-1–CCR2 interactions [3,119, 120]. If such small molecules turn out to be safe and effective anti-MCP-1/CCR2 therapy could become practical in the near future.

SUMMARY AND FUTURE DIRECTIONS

MCP-1 binding to its receptor CCR2 initiates a series of signalling pathways that not only causes chemotactic migration of the target cells, but also regulates gene expression that orchestrates homeostatic responses within the heart, leading to beneficial effects or deleterious effects. Various stimuli in the heart cause the elaboration of MCP-1, leading to recruitment of monocytes/macrophages and the induction of MCP-1, which cause an ER stress response in the heart. The short-term beneficial effects of MCP-1 signalling may be lost if myocardial expression of these molecules either becomes sustained and/or excessive, in which case the salutary effects of these proteins may be contravened by triggering death signalling, leading to deleterious effects. Future studies are needed to evaluate whether MCP-1 can be an appropriate target for therapeutic intervention in such diseases.

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