MCP-2/CCR8 AXIS IS ACTIVATED IN EXPERIMENTAL RENAL AND VASCULAR INFLAMMATION.

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INTRODUCTION

Chronic inflammation is a main feature of renal diseases and cardiovascular related pathologies. The family of monocyte chemotactic proteins (MCPs) are the most relevant cytokines involved in inflammatory and immune regulation. Most of the studies have been focus on MCP-1, and its receptor (CCR-2), as potential therapeutic target for inflammatory diseases. However, information about other members is scarce. Moreover, there are differences between the members of this family. Murine MCP-2 (also named CCL8) has been involved in dermal inflammation and cancer. In experimental atherosclerosis MCP-2 is induced in advanced lesions, but there is no data in renal diseases. Recent studies have described murine MCP-2 as a new CCR8 ligand, whether human MCP-2 is a CCR2 ligand (Table 1).

Human MCPs	
CHEMOKINE	RECEPTOR
MCP-1/CCL2	CCR1, CCR2, CCR3
MCP-2/CCL8	CCR1, CCR2, CCR3, CCR5
MCP-3/CCL7	CCR1, CCR2, CCR3
MCP-4/CCL13	CCR3

Murine MCPs	
CHEMOKINE	RECEPTOR
MCP-1/CCL2	CCR2
MCP-2/CCL8	CCR8
MCP-3/CCL7	CCR2
MCP-5/CCL12	CCR2

TABLE 1. Human and murine family of MCPs, with their receptors

Our aim was to evaluate whether MCP-2, and its potential receptor CCR-8, could be involved in renal and vascular inflammation.

METHODS

- In vivo studies were done in C57BL/6 mice. The model of systemic infusion of Angiotensin II (AngII; 100 mg/kg/min subcutaneously, by osmotic minipumps), peritoneal dialysis model for 30 days and the model of unilateral ureteral obstruction (UUO) were done. Th17 response was blocked by a neutralizing antibody against its effector cytokine IL-17A.
-In vitro studies were done in murine endothelial cells (Mile Sven-1 line) and vascular smooth muscle cells (VSMCs).
- To evaluate in vivo MCP-2 responses, systemic administration of recombinant murine MCP-2 (550 ng/g mouse; i.p) into mice was done. In this model, some animals were treated with Parthenolide, an inhibitor of the nuclear factor-κB (NF-κB) pathway (3,5 µg/g bw per day, i.p.), starting 1 day before MCP-2 administration, and studied 48 hours later (Image 1).

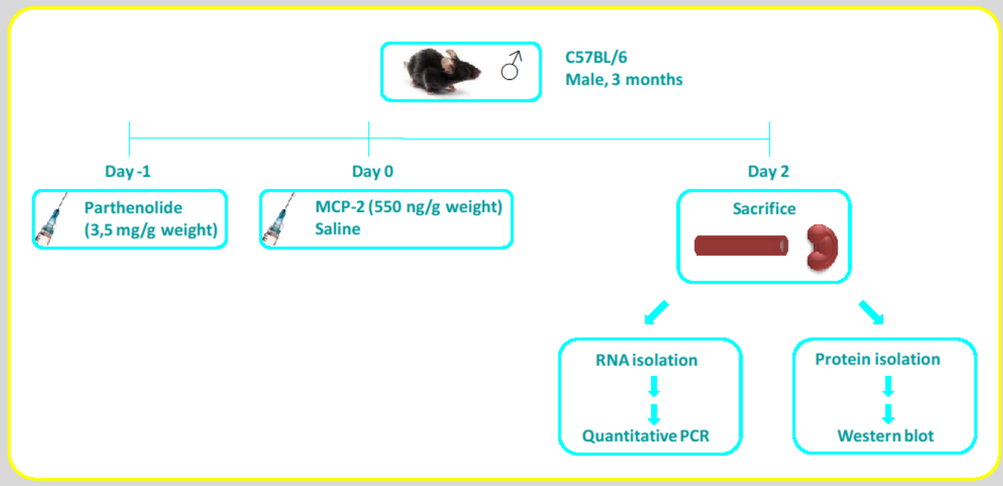

IMAGE 1. Systemic administration model of MCP-2 into mice.

RESULTS

1. MCP-2 AND CCR8 LEVELS IN DIFFERENT EXPERIMENTAL MODELS OF INFLAMMATION.

The gene expression of CCL8 in three experimental models of inflammation was increased. These rising levels were decreased by a neutralising antibody against IL-17A (Figure 1).

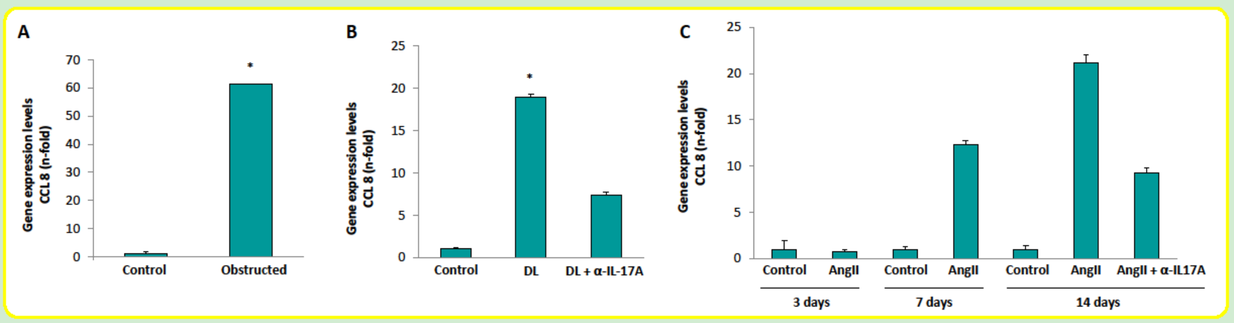

FIGURE 1. MCP-2 increases in different experimental models of inflammation. (A) kidneys of UUO model, (B) peritoneum of PD model and (C) aorta of AngII-systemic infusion model. *p <0.05 *vs. Control.*

In peritoneum of PD model, CCR8 expression was decreased as a response to the damage with dialysis liquids and treatment with anti-IL-17A. In aorta of AngII-systemic administration model CCR8 receptor was increased in AngII-treated mice compared to control (Figure 2).

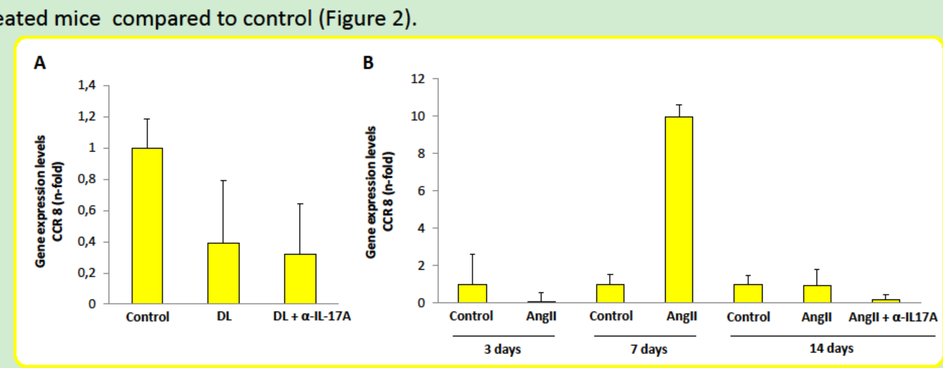

FIGURE 2. CCR8 levels in different experimental models of inflammation. (A) peritoneum of PD model and (B) aorta of AngII-systemic infusion model. *p <0.05 *vs. Control.*

2. MCP-2 INCREASED INFLAMMATORY AND FIBROTIC FACTORS AND CCR8 EXPRESSION IN ENDOTHELIAL AND VASCULAR SMOOTH MUSCLE CELLS.

In cultured endothelial cells MCP-2 increased the gene expression of several proinflammatory genes in a time- and dose-dependent fashion. In these experiments, MCP-2 produced the highest inflammatory effect at 100 ng/ml for 3-6 hours (Figure 3). In time-dependent experiments, CCL8 increased profibrotic genes (figure 4).

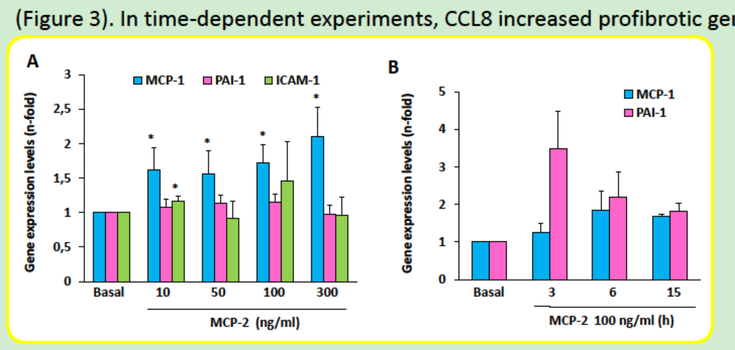

FIGURE 3. MCP-2 produces the highest proinflammatory action at 100 ng/ml and between 3-6 hours. Endothelial cells were treated with rising doses of MCP-2 for 6 hours (A) and 3, 6, 15 hours at 100 ng/ml (B). n = 2–3 experiments; *p <0.05 *vs. Basal.*

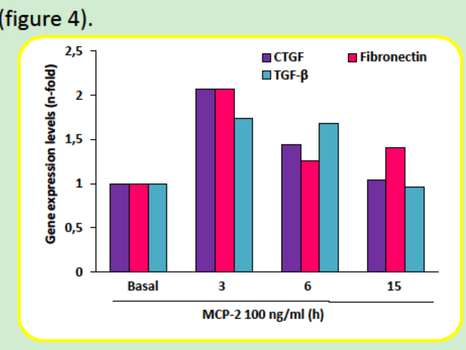

FIGURE 4. MCP-2 increases the gene expression of profibrotic factors. This figure shows a representative experiment.

In this cells, CCR8 expression is lower, but these levels increase exponentially when MCP-2 is added (Figure 5).

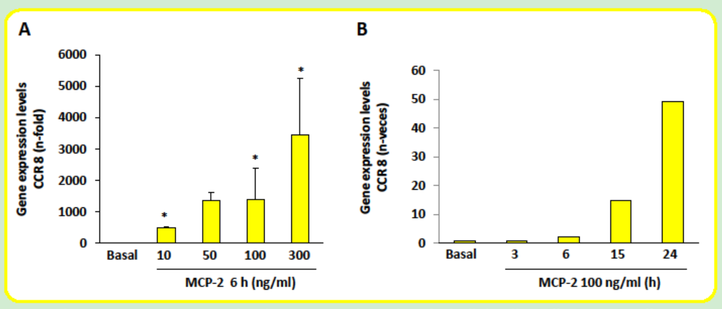

FIGURE 5. MCP-2 increases CCR8 expression in mouse cultured endothelial cells. Mile Sven 1 cells were treated with rising concentrations of CCL8 (A) and for 3, 6, 15 and 24 hours (B). n = 2–3 experiments; *p < 0.05 *vs. Basal.*

In addition, vascular smooth muscle cells were estimulated with MCP-2. In time-dependent experiments, MCP-2 at 100 ng/ml increased the phosphorylation of P65 (NF-κB subunit) protein expression levels (Figure 6).

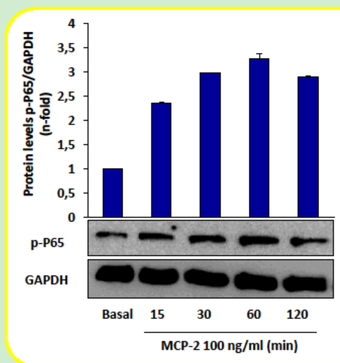

FIGURE 6. MCP-2 increases p-P65 protein expression in time-dependent pattern in vascular smooth muscle cells. VSMC were treated for 15, 30, 60 and 120 minutes. n = 2 experiments.

Moreover, these cells were estimulated with MCP-2 at 100 ng/ml for 24 hours. MCP-2 increased CCR8, PCNA and p-P65 protein expression levels (Figure 7).

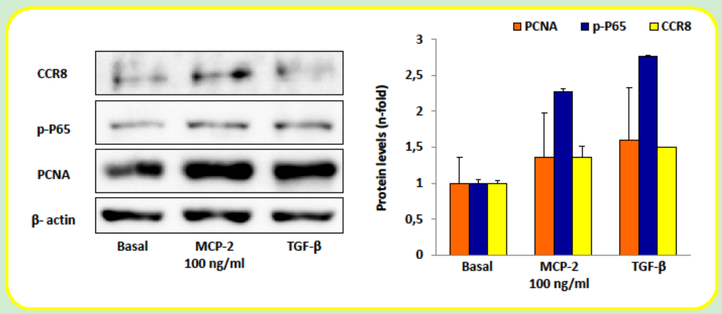

FIGURE 7. MCP-2 at 24 hours regulates proliferation, inflammation and CCR8 expression in VSMC. Protein expression levels of PCNA, p-P65 and CCR8. n = 2 experiments.

3. SYSTEMIC ADMINISTRATION OF MCP-2 INTO MICE REGULATED PROINFLAMMATORY FACTORS AND CCR8 EXPRESSION.

In MCP-2-injected mice, renal and aortic gene expression of injury markers and proinflammatory factors were significantly increased after 48 hours. As observed *in vitro*, CCR8 gene levels were overexpressed by MCP-2. NF-κB blockade by Parthenolide downregulated renal and aortic gene expression to control levels (Figures 8 and 9).

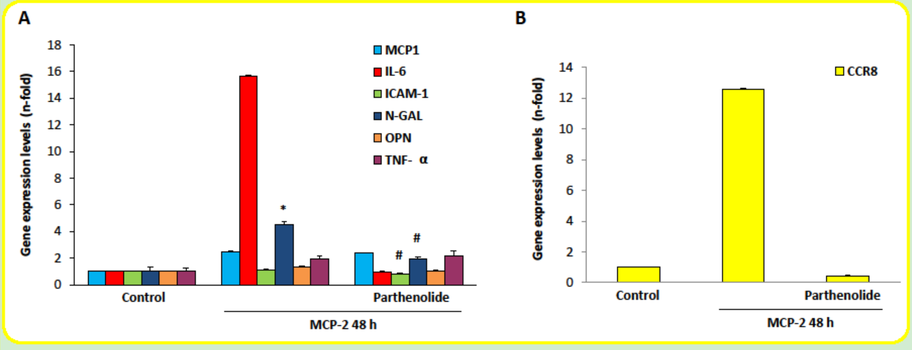

FIGURE 8. MCP-2 increases the gene expression of proinflammatory factors and CCR8 receptor in mice kidneys. mRNA levels of MCP-1, IL-6, ICAM-1, N-GAL, Osteopontin (OPN), TNF-α (A) and CCR8 receptor (B). *p < 0.05 vs control mice and #p < 0.05 vs MCP-2-injected mice.

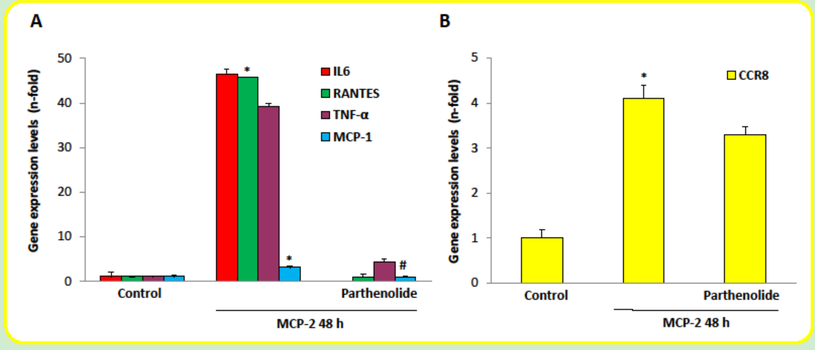

FIGURE 9. MCP-2 increases the gene expression of proinflammatory factors and CCR8 receptor in mice aorta. mRNA levels of IL-6, RANTES, TNF-α, MCP-1, (A) and CCR8 receptor (B). *p < 0.05 vs control mice and #p < 0.05 vs MCP-2-injected mice.

In kidneys of MCP-2 treated-mice, p-P65 protein levels are increased after 48 hours. However, CCR8 protein levels were similar as control (Figure 10).

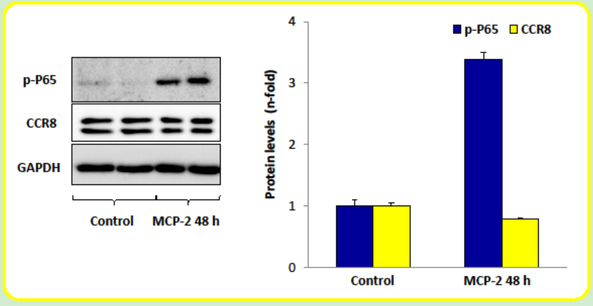

FIGURE 10. MCP-2 increased *in vivo* p-P65 protein levels but CCR8 protein levels were the same as control in mice kidneys.

CONCLUSIONS

Our in vitro and in vivo studies show that:

- MCP-2 tissue levels were increased associated with inflammation. This increase was not correlated with a rising level of CCR8 expression in all tissues.
- MCP-2 increased the expression of proinflammatory, profibrotic genes and CCR8 receptor in endothelial and vascular smooth muscle cells.
- MCP-2 in vivo, by intraperitoneal administration in mice, regulated proinflammatory factors and CCR8 expression, which were diminished by Parthenolide.

In experimental models of renal and vascular damage MCP-2 /CCR8 axis is activated linked to inflammation, suggesting a potential role of MCP-2 in these pathologies.

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