CC Chemokine Receptor 2 Is Required for Macrophage Infiltration and Vascular Hypertrophy in Angiotensin II–Induced Hypertension


Abstract—Recent studies have identified the presence of macrophages in the arterial wall of hypertensive animals and suggested that as is the case in atherosclerosis, macrophage products may be important mediators of the adaptive response of the arterial wall. In support of this, we have previously shown that the expression of monocyte chemoattractant protein-1 is upregulated in the arteries of hypertensive animals. We hypothesized that macrophage recruitment is a critical step in the pathogenesis of hypertension. To obtain insights into this potential mechanism, we made use of mice deficient in the CC chemokine receptor 2 (CCR2), the receptor for monocyte chemoattractant protein-1. Hypertension was induced with the subcutaneous administration of angiotensin II \((0.75 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{d}^{-1})\) for 7 days. Using in situ hybridization with a probe for c-fms to identify macrophages, we found that hypertension-induced macrophage infiltration of the arterial wall was virtually eliminated in CCR2-deficient mice. In addition, vascular hypertrophy was reduced by \(\approx 65\%\) compared with wild-type animals. These data demonstrate that CCR2 is essential for the recruitment of macrophages into the arterial wall in the setting of hypertension. Furthermore, the decreased hypertrophic response suggests that vascular hypertrophy occurs in part as a consequence of macrophage infiltration. In angiotensin II–induced hypertension, CCR2-mediated responses are critical to the process of macrophage recruitment and vascular hypertrophy and may represent one mechanism by which at least some forms of hypertension may lead to the development of atherosclerosis. \((\text{Hypertension}. \ 2000;36:360-363.)\)

Key Words: proteins ■ angiotensin II ■ hypertrophy ■ macrophages

It is becoming increasingly apparent that hypertension, like atherosclerosis, is associated with the presence of an inflammatory response in the arterial wall.\(^1,2\) The initial phase of this inflammatory response is characterized by the accumulation of macrophages in the arterial wall.\(^3-5\) The importance of this macrophage infiltration in the development of hypertensive vasculopathy remains unknown. Furthermore, the precise factors that control macrophage recruitment into the vascular wall have not been determined.

We recently demonstrated that the expression of monocyte chemoattractant protein-1 (MCP-1) is upregulated at both the message and protein level in aortic tissues of hypertensive animals.\(^4\) This response was seen in animals made hypertensive with the infusion of either angiotensin II or norepinephrine. MCP-1 is a potent macrophage chemoattractant that has been previously implicated in the development of atherosclerosis.\(^6-9\) We hypothesized that in at least some forms of hypertension, MCP-1 upregulation and subsequent macrophage infiltration may represent a key step in the development of vascular hypertrophy. Implicit in this hypothesis is the assumption that vascular hypertrophy is dependent to a significant degree on the production of growth factors by resident macrophages.

To gain further insights into the potential contributions of monocytic inflammatory responses to hypertensive vascular changes in angiotensin II–induced hypertension, we made use of mice deficient in the CC chemokine receptor 2 (CCR2).\(^10\) The only known agonist for this receptor is MCP-1. This model provides the opportunity to determine the functional importance of angiotensin II–induced macrophage recruitment into the arterial wall. We hypothesized that when angiotensin II–mediated hypertension is induced in CCR2-deficient mice, there would be a marked reduction in macrophage accumulation in the arterial wall as well as a decrease in vascular hypertrophy.

Methods

Animals

Mice deficient in CCR2 were generated as described previously.\(^10\) The animals were the progeny of breeder pairs that had been back

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Systolic Blood Pressure Measurements

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<th>Control</th>
<th>Angiotensin II</th>
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<tr>
<td>Wild type</td>
<td>107±3</td>
<td>129±8*</td>
</tr>
<tr>
<td>CCR2 deficient</td>
<td>114±3</td>
<td>151±14*</td>
</tr>
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Mean systolic blood pressures were obtained with the tail-cuff technique. Data are presented as mean±SEM. *P<0.001 compared with animals not treated with angiotensin II (n=6 per group).

crossed into the C57BL/6 line for 8 generations to ensure genetic homogeneity. All animals used in the present study were male progeny derived from homozygous breeding pairs. Animals were used at 4 to 8 weeks of age. C57BL/6 wild-type animals were used as control animals and were age and sex matched for all experiments. Six animals were included in each experimental group.

Angiotensin II Model of Hypertension

Hypertension was induced through the use of continuous angiotensin II infusion delivered via an osmotic minipump (Alzet) as previously described. Briefly, mice were anesthetized with ketamine (80 mg/kg IP) and xylazine (10 mg/kg IP). With sterile technique, osmotic minipumps that contained sufficient angiotensin II to deliver a dose of 0.75 mg·kg⁻¹·d⁻¹ were placed in the subcutaneous space over the abdomen. Blood pressures were measured with a computerized, noninvasive tail-cuff system (BP 2000; Visitech Systems).

Microscopic Analysis

Animals were killed with CO₂ inhalation. The thorax was opened, a 21-gauge needle was placed into the left ventricle, and the inferior vena cava was severed. The animals were then perfused with normal saline at 100 mm Hg until the perfusate was clear. The entire animal was removed en bloc and embedded in paraffin. Individual sections were made of the proximal third of the descending thoracic aorta.

For morphometric analysis of vascular hypertrophy, sections were stained with hematoxylin and eosin. Three serial sections from the proximal descending thoracic aorta of each animal were photographed and stored in a digital format. Arterial wall thickness was then measured in a blinded fashion with NIH Image software.

To identify macrophages in an accurate and specific manner, in situ hybridization with c-fms, which encodes the monocyte colony-stimulating factor receptor, was used to identify vascular macrophages in a specific and sensitive manner that would allow for the quantification and localization of the site of infiltration. Essentially no macrophages were seen in the aortic wall of wild-type animals under control conditions. In contrast, when wild-type animals were made hypertensive with angiotensin II infusions, macrophages were readily identified throughout the arterial wall (Figures 1A and 1B). In the wild-type animals treated with angiotensin II, the majority of c-fms-positive cells were seen in the adventitia (arrow, B). In CCR2-deficient mice treated with angiotensin II, there was a marked decrease in the number of macrophages (C).

Results

Angiotensin II treatment resulted in a uniform increase in systolic blood pressure of treated animals; the mean blood pressures are shown in the Table. Previous studies from our laboratory have demonstrated that 7 days of angiotensin II infusion results in a maximal blood pressure response. Although there was a trend for the CCR2-deficient animals to exhibit a slightly greater hypertensive response, there was no statistically significant difference in the peak blood pressure response between the wild-type and the CCR2-deficient animals (20±8% versus 33±13% increase, P=0.38).

In situ hybridization for c-fms, which encodes the monocyte colony-stimulating factor receptor, was used to identify vascular macrophages in a specific and sensitive manner that...
ric analysis are shown in Figure 3. There is marked vascular hypertrophy in the wild-type animals. In contrast, the CCR2-deficient mice exhibited a highly significant reduction in hypertrophy. Mean data are summarized in Figure 4.

**Discussion**

The study data demonstrate that in an angiotensin II model of experimental hypertension, the CCR2 receptor is necessary for the accumulation of macrophages and the subsequent development of vascular hypertrophy. We have previously shown that MCP-1 expression is upregulated in the aortic tissues of hypertensive animals. Currently, the only identified ligand for the CCR2 receptor is MCP-1. Thus, the present study demonstrates the potential functional importance of MCP-1 as a chemoattractant for macrophages in vivo. In animals that lack CCR2, the recruitment of macrophages into the arterial wall of hypertensive animals was almost completely eliminated. Furthermore, we demonstrated that in these animals, vascular hypertrophy was markedly reduced. Taken together, these findings demonstrate the presence of CCR2 is an absolute requirement for macrophage infiltration and subsequent vascular hypertrophy in an angiotensin II model of hypertension. They also raise the possibility that MCP-1 is the critical chemoattractant for macrophages in angiotensin II-induced hypertension.

The mechanisms responsible for the upregulation of MCP-1 expression in experimental hypertension are likely complex and involve several different signaling pathways. Angiotensin II has been directly implicated in the expression of MCP-1 in vascular cells. In addition, mechanical strain directly induces the expression of MCP-1 in cultured vascular cells. Thus, it is possible that both mechanical and humoral factors may be important in the regulation of MCP-1 in vivo. Studies performed by Olleserhaw et al have shown that in an aortic coarctation model of hypertension, vascular hypertrophy occurs above the level of the coarctation (where wall strain is increased) but not below the level of the coarctation despite the fact the 2 regions are in the same humoral milieu. Similarly, we have shown that MCP-1 expression is upregulated in both an angiotensin II model and a norepinephrine model of hypertension. It appears that even though both angiotensin II and wall strain may result in the upregulation of MCP-1 expression in vivo, the mechanoregulation of MCP-1 may be of greater importance under these experimental conditions.

The finding that macrophage recruitment represents a necessary requirement for the development of vascular hypertrophy provides strong support for the hypothesis that the vascular pathology of angiotensin II–induced hypertension, like atherosclerosis, occurs in part as the result of the development of an inflammatory state in the vascular wall. This hypothesis is also supported by the earlier observations that inflammatory cells are present in the arterial wall of hypertensive animals as well as more recent observations that reactive oxygen species play a critical role in the hypertensive response. Perhaps more important, the commonality of disease mechanisms of hypertension and atherosclerosis suggests a potential mechanism by which hypertension can accelerate the development of atherosclerosis. This is further supported by the recent observations that in

**Figure 2.** Quantification of macrophage infiltration showing mean number of macrophages per aortic cross section as identified with c-fms hybridization. Values are mean ± SEM of trilicate sections. A total of 6 animals for each group were used for the data analysis, except for the wild-type control animals, for which specimens from only 4 animals were available for analysis. *P < 0.001.

**Figure 3.** Hematoxylin and eosin-stained sections of the proximal descending thoracic aorta obtained from wild-type animals and CCR2-deficient mice that were either made hypertensive with angiotensin II infusions (Ang II) or untreated (control).

**Figure 4.** Mean arterial wall thickness (intima plus media) for both wild-type and CCR2-deficient animals. Values are mean ± SEM of triplicate measurements obtained from 6 animals per group. *P < 0.001.
2 different murine models of atherosclerosis, MCP-1 is essential to the development of atherosclerotic lesions.25,26

It is important to point out that the overall process of macrophage infiltration of the arterial wall is only part of a much larger process. Monocyte infiltration of the arterial wall is a complex process and involves several sequential steps, including chemotaxis, adhesion, infiltration, sustenance, and possibly proliferation. In addition, there are likely additional mediators of each step of this process (eg, adhesion molecules). Therefore, although a CCR2 agonist may be necessary for macrophage recruitment, it is likely that it is only 1 component of a far more complex process.

In summary, we used a CCR2-deficient animal model to demonstrate the critical contribution of macrophage recruitment to vascular hypertrophy in angiotensin II–induced hypertension. These results also raise the possibility that CCR2-mediated chemotaxis may be 1 mechanism whereby hypertension contributes to the development of atherosclerosis.

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References

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