

Monocyte Chemoattractant Protein-1 Is an Essential Inflammatory Mediator in Angiotensin II-Induced Progression of Established Atherosclerosis in Hypercholesterolemic Mice

Weihua Ni, Shiro Kitamoto, Minako Ishibashi, Makoto Usui, Shujiro Inoue, Ken-ichi Hiasa, Qingwei Zhao, Ken-ichi Nishida, Akira Takeshita, Kensuke Egashira

Objective—Chronic inflammatory processes might be involved in the progression and destabilization of atherosclerotic plaques. Therefore, identification of the mechanism underlying arterial inflammatory function might lead to the development of novel therapeutic strategies. Angiotensin II (AngII) is implicated in atherogenesis by activating the vascular inflammation system, mainly through monocyte chemotaxis. Therefore, we hypothesized that AngII increases plaque size and promotes destabilization of established atheromas by activating the monocyte chemoattractant protein-1 (MCP-1) pathway.

Methods and Results—We report here that 4-week infusion of AngII not only increased plaque size but also induced a destabilization phenotype (ie, increased macrophages and lipids and decreased collagen and smooth muscle cells) of pre-existing atherosclerotic lesions of hypercholesterolemic mice. AngII also enhanced the gene expression of inflammatory cytokines (TNF α , IL-6, etc.) and chemokines (MCP-1, CCR2, etc). Blockade of MCP-1, by transfecting the deletion mutant of the human MCP-1 gene into the skeletal muscles, limited AngII-induced progression and destabilization of established atherosclerotic lesions and suppressed the induction of proinflammatory genes.

Conclusions—These data suggest that MCP-1 functions as a central inflammatory mediator in the AngII-induced progression and changes in plaque composition of established atheroma. (*Arterioscler Thromb Vasc Biol.* 2004; 24:534-539.)

Key Words: atherosclerosis ■ hypercholesterolemia ■ cell adhesion molecules ■ inflammation ■ gene therapy

Atherosclerosis and its complications are the major cause of death in Western countries. Recent evidence suggests that chronic inflammatory processes have an important role in atherosclerotic plaque progression, destabilization, and subsequent rupture/thrombosis, resulting in acute coronary syndrome and stroke.¹ Therefore, identification of the critical inflammatory pathway involved in plaque progression and destabilization of pre-existing established atheromas might aid in the development of novel therapeutic strategies to reduce atherothrombotic complications. Angiotensin II (AngII) is implicated in atherogenesis beyond its hemodynamic effects.² Infusion of AngII into hypercholesterolemic mice dramatically accelerates the development and/or progression of atherosclerotic lesions and the effects of AngII occurred independent of changes in arterial pressure or plasma lipid concentration.^{3,4} The mechanism of AngII-induced enhancement of atherogenesis is probably multifactorial, and includes

hemodynamic effects, endothelial dysfunction and activation,⁵ oxidative stress,⁶ and inflammation.^{7,8} AngII increases monocyte chemotaxis, activates nuclear factor- κ B, and augments production of inflammatory cytokines and chemokines by arterial wall cells and monocytes.⁹⁻¹² AngII is very important in the pathogenesis of atherothrombotic complications, as evidenced by clinical benefits of angiotensin-converting enzyme inhibition¹³ and AngII receptor blockers.¹⁴ There are no reports, however, that address the mechanism of AngII-induced enhancement of atherogenesis and plaque destabilization under in vivo conditions.

Emerging evidence suggests that AngII activates cell inflammatory systems in arterial lesion.^{1,2} Inflammatory changes in arterial lesions are characterized by the recruitment and activation of monocytes/macrophages, which are regulated by monocyte chemoattractant protein-1 (MCP-1).^{15,16} Matrix metalloproteinases (MMP) and tissue factor,

Received December 2, 2003; accepted January 12, 2004.

From the Department of Cardiovascular Medicine (W.N., S.K., M.I., M.U., S.I., K.H., Q.Z., A.T., K.E.), Graduate School of Medical Sciences, Kyushu University, Fukuoka, Japan; and the New Product Research Laboratories (K.N.), Dai-ichi Pharmaceutical Company, Tokyo, Japan.

Consulting Editor for this article was Alan M. Fogelman, MD, Professor of Medicine and Executive Chair, Departments of Medicine and Cardiology, UCLA School of Medicine, Los Angeles, CA.

Correspondence to Dr Kensuke Egashira, Department of Cardiovascular Medicine, Graduate School of Medical Sciences, Kyushu University, 3-1-1, Maidashi, Higashi-ku, Fukuoka 812-8582, Japan. E-mail egashira@cardiol.med.kyushu-u.ac.jp

© 2004 American Heart Association, Inc.

Arterioscler Thromb Vasc Biol. is available at <http://www.atvbaha.org>

DOI: 10.1161/01.ATV.0000118275.60121.2b

which are produced mainly from lesional macrophages, are believed to greatly contribute to destabilization of human atherosclerotic lesions.¹ Recently, our group and others have also reported that MCP-1 has a vital role in the initiation and progression of atherosclerotic or arteriosclerotic lesions in experimental animals.^{17–24} Essential roles of MCP-1 and its interactions with its receptor (CCR2) in AngII-induced arteriosclerosis are shown in CCR2-deficient mice.²⁵ Therefore, we hypothesized that AngII increases plaque size and promotes destabilization of established atherosclerotic lesions by activating MCP-1. To block the MCP-1/CCR2 signal pathway, an N-terminal deletion mutant of the MCP-1 gene (7ND), which lacks the N-terminal amino acids 2 to 8, was transfected into the skeletal muscle.^{17,18} This mutant MCP-1 binds to its receptor CCR2 and blocks MCP-1-mediated monocyte chemotaxis. In previous studies, we demonstrated that 7ND protein was secreted from the transfected skeletal muscle cells into the circulating blood and subsequently blocked MCP-1-induced chemotaxis in remote organs. Here, we report that AngII infusion into hypercholesterolemic apolipoprotein-E knockout (apoE-KO) mice not only increased plaque size but also promoted atherosclerotic plaque transformation to a more destabilized phenotype, which had more lipid and macrophages and less collagen and fewer smooth muscle cells.¹ In addition to the morphological changes, AngII markedly induced gene expression of several important cytokines and chemokines. Blockade of the MCP-1 pathway limited AngII-induced lesion progression and destabilization and suppressed gene expression of proinflammatory factors.

Methods

Experimental Animals

C57BL/6J apoE-KO and wild-type mice, purchased from Jackson Laboratory (Bar Harbor, Me), were bred and maintained in the Laboratory of Animal Experiments at Kyushu University. The study protocol was reviewed and approved by the Committee on the Ethics of Animal Experiments at Kyushu University Graduate School of Medical Sciences. A part of this study was performed at the Kyushu University Station for Collaborative Research and the Morphology Core at Kyushu University School of Medical Sciences.

Expression Vector

7ND was constructed by recombinant polymerase chain reaction using a wild-type human MCP-1 cDNA as the template and cloned into *Bam*HI (5') and *Not*I (3') sites of the pcDNA3 expression vector (Invitrogen).¹⁸

Treatment

Male apoE-KO mice were fed a normal chow diet (Oriental Yeast) during the experiment. At age 30 weeks, mice were randomly assigned to 1 of 5 groups. The first group (sham group, n=10) received physiological saline via a subcutaneously implanted osmotic minipump (model 2004; Alzet). The second group (AngII+plasmid group, n=10) received AngII dissolved in saline through the minipump for 4 weeks and intramuscular injections of pcDNA3 plasmid DNA at biweekly intervals. The third group (AngII+7ND group, n=10) received AngII dissolved in saline through the minipump for 4 weeks and intramuscular injections of pcDNA3-7ND plasmid DNA (100 μ g) into the femoral muscle at biweekly intervals. The fourth group (ARB group, n=10) received AngII dissolved in saline through the minipump for 4 weeks and AngII receptor blocker (ARB) (olmesartan 7.5 μ g/g) in chow. This

dose of olmesartan has no effect on AngII-induced increase in arterial pressure.²⁶ The fifth group (sham+7ND group, n=10) received physiological saline and intramuscular injections of pcDNA3-7ND plasmid DNA (100 μ g).

In the sham+7ND, AngII+plasmid, and AngII+7ND groups, transgene expression was enhanced by local intramuscular electroporation at the injection site immediately after the injection. Six 100-V, 50-ms electronic pulses were applied to each injection site using an Electroporator CUY21 (BTX).²⁷ The dose of AngII (0.75 mg·kg⁻¹·d⁻¹) provides a serum level of AngII similar to that reported in patients with renovascular hypertension.²⁸ After 4 weeks of saline or AngII infusion, mice were euthanized after collection of blood from the vena cava.

Tissue Preparation

Tissue preparation was performed essentially as previously described.¹⁸ Briefly, after the mice were euthanized, the heart and total aortic segments were rapidly removed after perfusion with phosphate-buffered saline. The total aortic segments (ascending thoracic aorta, arch, descending thoracic aorta, and abdominal aorta) were rapidly frozen in liquid nitrogen for later extraction of RNA. The heart, including the aortic root, was snap-frozen in OCT compound (Tissue-Tek) for histology and immunohistochemistry.

Histology and Immunohistochemistry

Serial cryostat sections (6 μ m) of the aortic root were prepared as described.¹⁸ In brief, atherosclerotic lesions in the aortic root were examined at 5 locations, each separated by 120 μ m, and 5 to 6 serial sections were prepared from each location. Some of these sections were conventionally stained with orcein (for elastic fiber staining) and oil red O (for lipid staining). Interstitial collagens were stained by Picosirius red (Sigma Chemical, St. Louis, Mo) and photographed under polarization microscopy as described previously.²⁹ The remaining sections were used for immunohistochemical analysis. Air-dried cryostat sections were fixed in acetone and stained with the respective antibody: anti-mouse monocyte/macrophage monoclonal antibodies (MOMA-2; Serotec), anti-alpha-SM actin monoclonal antibodies (alkaline phosphatase conjugated; Sigma Chemical), anti-human MCP-1, CCR2, MMP-13 polyclonal antibodies (Santa Cruz Biotechnology, Santa Cruz, CA), anti-mouse MMP-9 polyclonal antibodies (Santa Cruz Biotechnology), and anti-rabbit tissue factor monoclonal antibody (American Diagnostica), as described previously.¹⁸ Respective nonimmune IgGs (Dako) were used as negative controls. After incubation with the appropriate biotinylated affinity-purified secondary antibodies (Nichirei, Tokyo), the sections were incubated with alkaline phosphatase-labeled streptavidin solution (Nichirei) and visualized using a fast red substrate kit (Nichirei). The sections were then counterstained with Mayer hematoxylin.

A single observer blinded to the experiment protocol performed quantitative analysis of atherosclerotic lesions. All images were captured with a Nikon microscope equipped with a video camera and analyzed using Adobe Photoshop 6.0 and National Institute of Health Image Software. Orcein staining was used to delineate the internal elastic lamina for determination of the intimal area. The lipid composition of the lesion was evaluated by calculating the percent of the oil red O positive area versus the total cross-sectional vessel wall area. Similarly, the percent area of macrophage accumulation (MOMA-2-positive area), alpha-SM actin-positive area, and collagen deposition were estimated. In each case, the average value for 4 or 5 locations for each animal was used for analysis.

RNA Extraction and RNase Protection Assay

Total RNA was extracted from the aorta using the acid guanidinium thiocyanate-phenol-chloroform method (Isogen, Nippon Gene). RNase protection assays were performed with 20 μ g of total RNA using a RiboQuant kit with a custom template set according to the manufacturer's protocol (PharMingen; San Diego, Calif). After RNase digestion, protected probes were resolved on denaturing polyacrylamide gels and quantified using a BASS-3000 system (Fuji

Film, Tokyo). The value of each hybridized probe was normalized to that of glyceraldehyde-3-phosphate dehydrogenase (GAPDH) included in each template set as an internal control.

Serum Analysis and Systolic Blood Pressure Determination

Serum total and high-density lipoprotein cholesterol and triacylglycerol concentrations were determined using commercially available kits (Wako). Systolic blood pressure was measured weekly using a tail-cuff system with the mice in a conscious state.

Commercially available ELISA kits (Biosource) were used to measure human MCP-1 and mouse MCP-1 according to manufacturer's instructions. Plasma 7ND concentrations were measured by the use of this human ELISA kit.

Statistical Analysis

Data were expressed as mean \pm SEM. Differences between groups were determined using 2-way analysis of variance and a multiple comparison test; $P < 0.05$ was considered to be statistically significant.

Results

7ND Gene Transfer Limits AngII-Induced Progression and Destabilization of Atherosclerotic Lesions

After a 4-week treatment, cross-sections of the aortic sinus region were examined. The sham group had established lesions characteristic of early fibrous plaques containing necrotic cores and a few foam cells covered by a well-formed fibrous cap (Figure 1A), as previously reported.^{17,30,31} There was a marked increase in the intimal area in the AngII+plasmid group, compared with that observed in the sham and sham+7ND group (Figures 1A and 2). 7ND gene transfer prevented the AngII-induced increase in the intimal area. Treatment with ARB also prevented the AngII-induced enhancement of the intimal area.

We next examined the effects of 7ND gene transfer on AngII-induced changes in plaque composition using immunohistochemical analysis. In particular, lipid deposition, macrophages, smooth muscle cells, and interstitial collagen content were evaluated (Figures 1A and 2). AngII infusion enhanced lipid staining and macrophage staining and attenuated α -SM-positive and collagen-positive areas (Figures 1A and 2). 7ND gene transfer reduced the AngII-induced increases in lipid deposition and macrophage infiltration, and AngII-induced decrease in α -SM actin and collagen (Figures 1A and 2). Treatment with ARB also prevented the AngII-induced changes in lesion composition.

7ND Gene Transfer Attenuates AngII-Induced Upregulation of Cytokine and Chemokine Expression

To explore the mechanisms by which 7ND gene transfer limited progression and destabilization of pre-existing atheromas, we examined gene expression of a number of chemokines, chemokine receptors, and cytokines (Figure 3). RNase protection assay revealed AngII-induced increases in pro-inflammatory genes. 7ND gene transfer significantly attenuated the increased gene expression of inflammatory cytokines and chemokines (Figure 3).

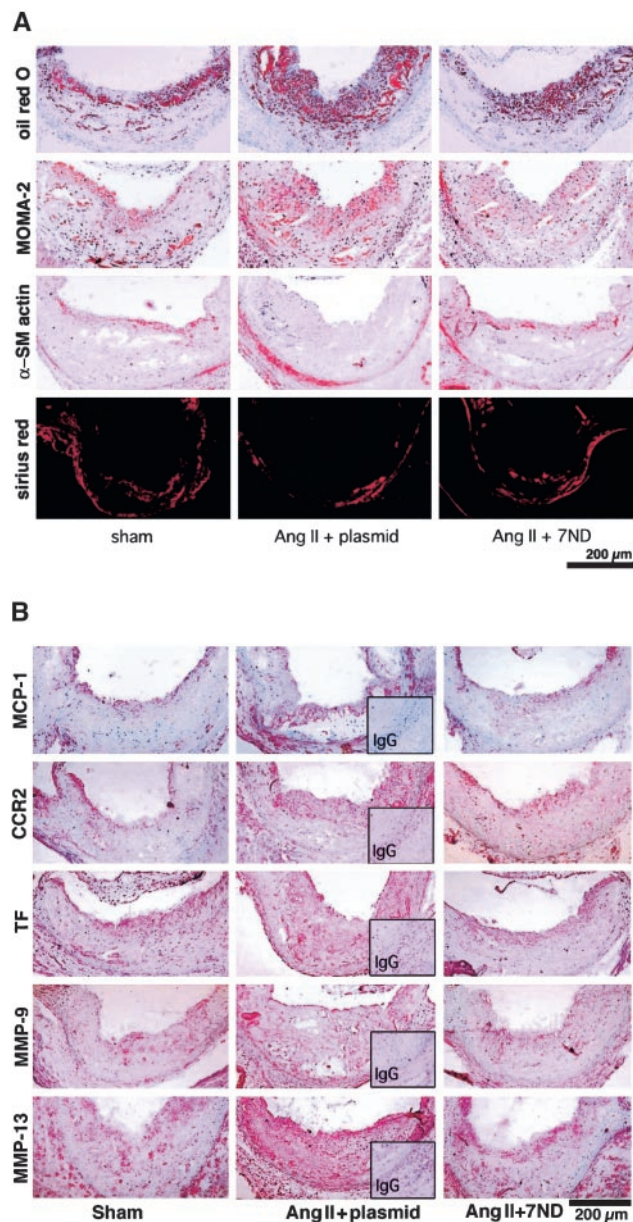


Figure 1. Histopathologic and immunohistochemical pictures of atherosclerotic lesions in the aortic root. A, 7ND gene transfer suppressed AngII-induced destabilization of the atheroma. From the top to the bottom panel, photomicrographs of atherosclerotic lesions stained with oil red O, immunostained with anti-murine macrophage antibody (MOMA-2) or anti-human α -SM actin antibody. Interstitial collagen was visualized using polarization microscopy after staining with Picrosirius red. Bar=200 μ m. B, 7ND gene transfer suppressed the AngII-induced increase in immunoreactive MCP-1, CCR2, MMP-9, MMP-13, and tissue factor. Bar=200 μ m.

Because 7ND gene transfer reduced MCP-1 gene expression, immunohistochemical staining for MCP-1 and its receptor (CCR2) was performed (Figure 1B). As expected, AngII infusion increased immunoreactive MCP-1 and CCR2 in the intimal lesions. Incomplete colocalization of MCP-1 and CCR2 suggests that CCR2 expression might increase not only in lesional monocytes but also in activated cells such as vascular smooth muscle cells. 7ND gene transfer reduced the

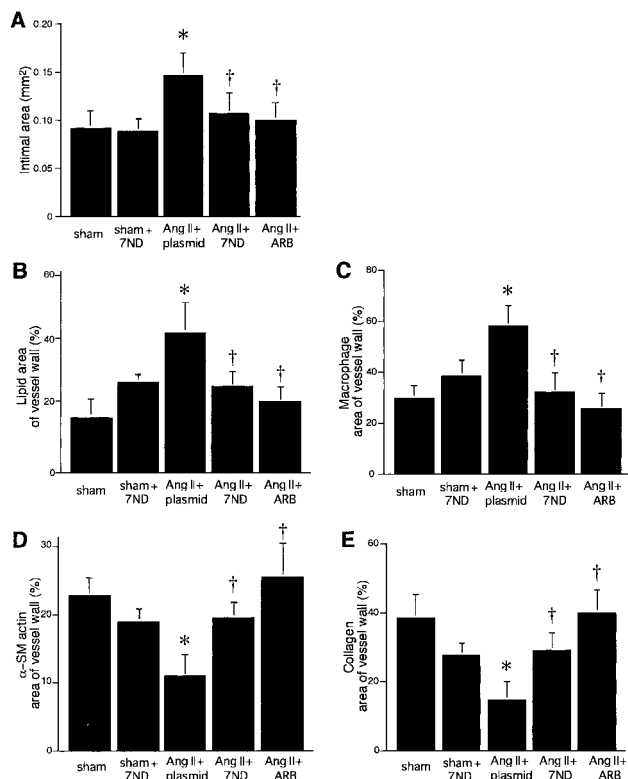


Figure 2. Quantitative comparison of atherosclerotic lesion size and lesion composition in sham-operation, sham+7ND, AngII+plasmid, AngII+7ND, and ARB groups. Data are reported as mean±SEM, n=8 to 10. *P<0.05 versus the sham group. †P<0.05 versus AngII+plasmid group.

AngII-induced increase in immunostaining for MCP-1 and CCR2.

Immunohistochemical staining for MMP and tissue factor were also performed (Figure 1B). AngII infusion increased immunoreactive MMP-9, MMP-13, and tissue factor. 7ND

gene transfer reduced the AngII-induced increase in MMP and tissue factor.

Beneficial Effects of 7ND Gene Transfection Had No Relation With Serum Lipid Levels or Systolic Blood Pressure

There were no statistically significant differences in serum total cholesterol levels among the groups (sham group, 480±21; AngII+plasmid group, 503±18; AngII +7ND, 488±19; ARB group, 478±22 mg/dL). 7ND gene transfer did not affect AngII-induced increases in systolic blood pressure (sham group, 102±3; AngII+plasmid group, 153±8; AngII +7ND, 157±6; ARB group, 110±8 mm Hg at 4 weeks of treatment).

7ND Gene Transfer Increases Plasma Concentrations of 7ND

Concentrations of 7ND and MCP-1 in plasma and transfected muscle were measured. In wild-type mice transfected with 7ND without AngII infusion, 7ND was detected in transfected muscle and plasma 3, 7, and 14 days after transfection (Table). Plasma MCP-1 concentrations did not change during the course of experiments.

Discussion

We demonstrated that blockade of MCP-1×7ND gene transfer limited AngII-induced progression of pre-existing advanced atherosclerotic lesions in hypercholesterolemic mice. Because 7ND gene transfer did not affect serum lipid or blood pressure levels, AngII-induced enhancement of atherosclerosis must be a direct effect of AngII on cells in the atherosclerotic vascular wall. These data suggest that the essential role of MCP-1 in AngII increases plaque size of the pre-existing atheroma.

A clinically significant finding of the present study is that AngII increased markers of plaque instability. Qualitative changes in plaque are more likely than a decrease in plaque size or the degree of stenosis to contribute greatly to a reduction in cardiovascular events caused by atherothrombotic complications. Lesional macrophages might be a major source of cytokines, MMP, and tissue factor. Increased degradation of interstitial collagen by MMP and decreased cytokine synthesis have been demonstrated in human atherosclerotic lesions prone to rupture.¹ Resultant decreases in interstitial collagen might weaken the plaque's biomechanical strength, which in turn increases the likelihood of plaque rupture.¹ Tissue factor regulates plaque's thrombogenicity.³² MCP-1 increases tissue factor expression and activity in human vascular smooth muscle cells.³³ We recently reported that 7ND gene transfer inhibited progression and destabilization of advanced atheroma by reducing inflammation in apoE-KO mice that were not received AngII.²⁴ We demonstrate the preservation of interstitial collagen in the atherosclerotic plaque associated with decreased expression of MMP and tissue factor by 7ND gene transfer. Therefore, MCP-1-induced inflammation appears to be an essential step not only in AngII-induced progression but also in AngII-induced changes in composition of established atheroma.

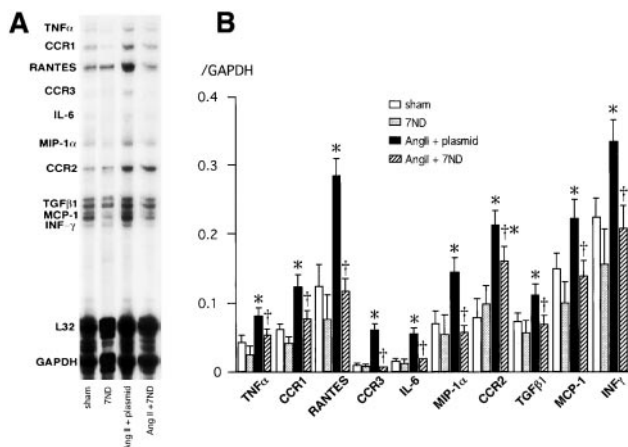


Figure 3. Effects of 7ND gene transfer on the AngII-induced gene expression of several chemokines (RANTES and MCP-1), chemokine receptors (CCR1, CCR2, and CCR3), and cytokine (TNF-α, IL-6, IL-1β, TGF-β1) in the aorta. A, Representative autoradiograph of RNase protection assay. B, Summary of densitometric analysis. Data are expressed as the ratio of each mRNA to the corresponding GAPDH mRNA. *P<0.05 versus the sham group. †P<0.05 versus AngII+plasmid group; n=6 to 7.

Plasma Concentrations of MCP-1 and 7ND After 7ND Transfection in Wild-Type Mice

	Baseline	Days After 7ND Transfection			
		3	7	14	28
Plasma MCP-1, pg/mL	88±5	92±6	94±6	90±6	86±5
Plasma 7ND, pg/mL	<20.0 (below detectable limit)	124±14	120±11	66±9	<20.0 (below detectable limit)
Muscular tissue 7ND, pg/mg protein	<20.0 (below detectable limit)	362±28	260±22	105±20	<20.0 (below detectable limit)

Values are mean±SE, n=6 to 8.

AngII causes endothelial dysfunction by decreasing nitric oxide synthase activity, mainly through oxidative stress, which in turn results in inflammation in the arterial wall.^{1,2} We previously demonstrated an increase in tissue Ang-II activity after blockade of nitric oxide synthesis.³⁴ In the present study, 7ND gene transfer suppressed AngII-induced increase in MCP-1, CCR2, and other cytokine expression. Because recent data¹ suggest that lesional inflammatory cells release enzymes such as angiotensin-converting enzyme that generate AngII,^{2,35,36} local AngII levels increase as macrophages become activated by oxidized LDL.³⁵ Our data strongly support the notion that an increased concentration and/or activity of AngII creates a positive-feedback mechanism for further increases in AngII generation, atherogenesis, and atherothrombotic events.

In conclusion, the present data demonstrate the essential role of MCP-1-mediated inflammation in AngII-induced progression of established atheroma in hypercholesterolemic mice. Because the activity and formation of AngII is enhanced at the inflamed human atherosclerotic sites that are prone to rupture, it is reasonable to propose that increased local action of AngII contributes greatly to the process of plaque rupture and subsequent cardiovascular ischemic complications by acting as an inflammatory mediator. Thus, AngII might augment inflammatory functions of atherosclerotic lesions in the presence of risk factors such as hypercholesterolemia. The clinical benefit of angiotensin-converting enzyme inhibitors or AngII receptor blockers might be caused by anti-inflammatory activity.

Acknowledgments

This study was supported by grants-in-aid for Scientific Research (14657172, 14207036) from the Ministry of Education, Science, and Culture, Tokyo, Japan, by Health Science Research Grants (Comprehensive Research on Aging and Health, and Research on Translational Research) from the Ministry of Health Labor and Welfare, Tokyo, Japan, and by the Program for Promotion of Fundamental Studies in Health Sciences of the Organization for Pharmaceutical Safety and Research, Tokyo, Japan.

References

- Libby P. Current concepts of the pathogenesis of the acute coronary syndromes. *Circulation*. 2001;104:365–372.
- Dzau VJ. Theodore Cooper Lecture: Tissue angiotensin and pathobiology of vascular disease: a unifying hypothesis. *Hypertension*. 2001;37:1047–1052.
- Daugherty A, Manning MW, Cassis LA. Angiotensin II promotes atherosclerotic lesions and aneurysms in apolipoprotein E-deficient mice. *J Clin Invest*. 2000;105:1605–1612.
- Weiss D, Kools JJ, Taylor WR. Angiotensin II-induced hypertension accelerates the development of atherosclerosis in apoE-deficient mice. *Circulation*. 2001;103:448–454.
- Laursen JB, Rajagopalan S, Galis Z, Tarpey M, Freeman BA, Harrison DG. Role of superoxide in angiotensin II-induced but not catecholamine-induced hypertension. *Circulation*. 1997;95:588–593.
- Rajagopalan S, Kurz S, Munzel T, Tarpey M, Freeman BA, Griending KK, Harrison DG. Angiotensin II-mediated hypertension in the rat increases vascular superoxide production via membrane NADH/NADPH oxidase activation. Contribution to alterations of vasomotor tone. *J Clin Invest*. 1996;97:1916–1923.
- Kim JA, Berliner JA, Nadler JL. Angiotensin II increases monocyte binding to endothelial cells. *Biochem Biophys Res Commun*. 1996;226:862–868.
- Chen XL, Tummala PE, Olbrych MT, Alexander RW, Medford RM. Angiotensin II induces monocyte chemoattractant protein-1 gene expression in rat vascular smooth muscle cells. *Circ Res*. 1998;83:952–959.
- Ruiz-Ortega M, Bustos C, Hernandez-Presa MA, Lorenzo O, Plaza JJ, Egido J. Angiotensin II participates in mononuclear cell recruitment in experimental immune complex nephritis through nuclear factor-kappa B activation and monocyte chemoattractant protein-1 synthesis. *J Immunol*. 1998;161:430–439.
- Hahn AW, Jonas U, Buhler FR, Resink TJ. Activation of human peripheral monocytes by angiotensin II. *FEBS Lett*. 1994;347:178–180.
- Hernandez-Presa M, Bustos C, Ortego M, Tunon J, Renedo G, Ruiz-Ortega M, Egido J. Angiotensin-converting enzyme inhibition prevents arterial nuclear factor-kappa B activation, monocyte chemoattractant protein-1 expression, and macrophage infiltration in a rabbit model of early accelerated atherosclerosis. *Circulation*. 1997;95:1532–1541.
- Kim S, Izumi Y, Yano M, Hamaguchi A, Miura K, Yamanaka S, Miyazaki H, Iwao H. Angiotensin blockade inhibits activation of mitogen-activated protein kinases in rat balloon-injured artery. *Circulation*. 1998;97:1731–1737.
- Yusuf S, Sleight P, Pogue J, Bosch J, Davies R, Dagenais G. Effects of an angiotensin-converting-enzyme inhibitor, ramipril, on cardiovascular events in high-risk patients. The Heart Outcomes Prevention Evaluation Study Investigators. *N Engl J Med*. 2000;342:145–153.
- Dahlof B, Devereux RB, Kjeldsen SE, Julius S, Beevers G, Faire U, Fyhrquist F, Ibsen H, Kristiansson K, Lederballe-Pedersen O, Lindholm LH, Nieminen MS, Omvik P, Oparil S, Wedel H. Cardiovascular morbidity and mortality in the Losartan Intervention For Endpoint reduction in hypertension study (LIFE): a randomised trial against atenolol. *Lancet*. 2002;359:995–1003.
- Gerard C, Rollins BJ. Chemokines and disease. *Nat Immunol*. 2001;2:108–115.
- Mukaida N, Harada A, Matsushima K. Interleukin-8 (IL-8) and monocyte chemoattractant and activating factor (MCAF/MCP-1), chemokines essentially involved in inflammatory and immune reactions. *Cytokine Growth Factor Rev*. 1998;9:9–23.
- Egashira K, Koyanagi M, Kitamoto S, Ni W, Kataoka C, Morishita R, Kaneda Y, Akiyama C, Nishida KI, Sueishi K, Takeshita A. Anti-monocyte chemoattractant protein-1 gene therapy inhibits vascular remodeling in rats: blockade of MCP-1 activity after intramuscular transfer of a mutant gene inhibits vascular remodeling induced by chronic blockade of NO synthesis. *FASEB J*. 2000;14:1974–1978.
- Ni W, Egashira K, Kitamoto S, Kataoka C, Koyanagi M, Inoue S, Imaizumi K, Akiyama C, Nishida Ki K, Takeshita A. New Anti-Monocyte Chemoattractant Protein-1 Gene Therapy Attenuates Atherosclerosis in Apolipoprotein E-Knockout Mice. *Circulation*. 2001;103:2096–2101.
- Boring L, Gosling J, Cleary M, Charo IF. Decreased lesion formation in CCR2^{-/-} mice reveals a role for chemokines in the initiation of atherosclerosis. *Nature*. 1998;394:894–897.

20. Gu L, Okada Y, Clinton SK, Gerard C, Sukhova GK, Libby P, Rollins BJ. Absence of monocyte chemoattractant protein-1 reduces atherosclerosis in low-density lipoprotein receptor-deficient mice. *Mol Cell*. 1998;2:275–281.
21. Mori E, Komori K, Yamaoka T, Tani M, Kataoka C, Takeshita A, Usui M, Egashira K, Sugimachi K. Essential role of monocyte chemoattractant protein-1 in development of restenotic changes (neointimal hyperplasia and constrictive remodeling) after balloon angioplasty in hypercholesterolemic rabbits. *Circulation*. 2002;105:2905–2910.
22. Egashira K, Zhao Q, Kataoka C, Ohtani K, Usui M, Charo IF, Nishida K, Inoue S, Katoh M, Ichiki T, Takeshita A. Importance of monocyte chemoattractant protein-1 pathway in neointimal hyperplasia after periarterial injury in mice and monkeys. *Circ Res*. 2002;90:1167–1172.
23. Usui M, Egashira K, Ohtani K, Kataoka C, Ishibashi M, Hiasa K, Katoh M, Zhao Q, Kitamoto S, Takeshita A. Anti-monocyte chemoattractant protein-1 gene therapy inhibits restenotic changes (neointimal hyperplasia) after balloon injury in rats and monkeys. *FASEB J*. 2002;16:1838–1840.
24. Inoue S, Egashira K, Ni W, Kitamoto S, Usui M, Otani K, Ishibashi M, Hiasa K, Nishida K, Takeshita A. Anti-monocyte chemoattractant protein-1 gene therapy limits progression and destabilization of established atherosclerosis in apolipoprotein E-knockout mice. *Circulation*. 2002;106:2700–2706.
25. Bush E, Maeda N, Kuziel WA, Dawson TC, Wilcox JN, DeLeon H, Taylor WR. CC chemokine receptor 2 is required for macrophage infiltration and vascular hypertrophy in angiotensin II-induced hypertension. *Hypertension*. 2000;36:360–363.
26. Usui M, Egashira K, Tomita H, Koyanagi M, Katoh M, Shimokawa H, Takeya M, Yoshimura T, Matsushima K, Takeshita A. Important role of local angiotensin II activity mediated via type 1 receptor in the pathogenesis of cardiovascular inflammatory changes induced by chronic blockade of nitric oxide synthesis in rats. *Circulation*. 2000;101:305–310.
27. Zhao Q, Egashira K, Inoue S, Usui M, Kitamoto S, Ni W, Ishibashi M, Hiasa K, Ichiki T, Shibuya M, Takeshita A. Vascular endothelial growth factor is necessary in the development of arteriosclerosis by recruiting/activating monocytes in a rat model of long-term inhibition of nitric oxide synthesis. *Circulation*. 2002;105:1110–1115.
28. Capers QT, Alexander RW, Lou P, De Leon H, Wilcox JN, Ishizaka N, Howard AB, Taylor WR. Monocyte chemoattractant protein-1 expression in aortic tissues of hypertensive rats. *Hypertension*. 1997;30:1397–1402.
29. Junqueira LC, Bignolas G, Brentani RR. Picrosirius staining plus polarization microscopy, a specific method for collagen detection in tissue sections. *Histochem J*. 1979;11:447–455.
30. Paigen B, Morrow A, Holmes PA, Mitchell D, Williams RA. Quantitative assessment of atherosclerotic lesions in mice. *Atherosclerosis*. 1987;68:231–240.
31. Nakashima Y, Plump AS, Raines EW, Breslow JL, Ross R. ApoE-deficient mice develop lesions of all phases of atherosclerosis throughout the arterial tree. *Arterioscler Thromb*. 1994;14:133–140.
32. Aikawa M, Voglic SJ, Sugiyama S, Rabkin E, Taubman MB, Fallon JT, Libby P. Dietary lipid lowering reduces tissue factor expression in rabbit atheroma. *Circulation*. 1999;100:1215–1222.
33. Schecter AD, Rollins BJ, Zhang YJ, Charo IF, Fallon JT, Rossikhina M, Giesen PL, Nemerson Y, Taubman MB. Tissue factor is induced by monocyte chemoattractant protein-1 in human aortic smooth muscle and THP-1 cells. *J Biol Chem*. 1997;272:28568–28573.
34. Takemoto M, Egashira K, Usui M, Numaguchi K, Tomita H, Tsutsui H, Shimokawa H, Sueishi K, Takeshita A. Important role of tissue angiotensin-converting enzyme activity in the pathogenesis of coronary vascular and myocardial structural changes induced by long-term blockade of nitric oxide synthesis in rats. *J Clin Invest*. 1997;99:278–287.
35. Diet F, Pratt RE, Berry GJ, Momose N, Gibbons GH, Dzau VJ. Increased accumulation of tissue ACE in human atherosclerotic coronary artery disease. *Circulation*. 1996;94:2756–2767.
36. Kitazono T, Padgett RC, Armstrong ML, Tompkins PK, Heistad DD. Evidence that angiotensin II is present in human monocytes. *Circulation*. 1995;91:1129–1134.

Arteriosclerosis, Thrombosis, and Vascular Biology



JOURNAL OF THE AMERICAN HEART ASSOCIATION

Monocyte Chemoattractant Protein-1 Is an Essential Inflammatory Mediator in Angiotensin II-Induced Progression of Established Atherosclerosis in Hypercholesterolemic Mice

Weihua Ni, Shiro Kitamoto, Minako Ishibashi, Makoto Usui, Shujiro Inoue, Ken-ichi Hiasa, Qingwei Zhao, Ken-ichi Nishida, Akira Takeshita and Kensuke Egashira

Arterioscler Thromb Vasc Biol. 2004;24:534-539; originally published online January 22, 2004;
doi: 10.1161/01.ATV.0000118275.60121.2b

Arteriosclerosis, Thrombosis, and Vascular Biology is published by the American Heart Association, 7272
Greenville Avenue, Dallas, TX 75231

Copyright © 2004 American Heart Association, Inc. All rights reserved.

Print ISSN: 1079-5642. Online ISSN: 1524-4636

The online version of this article, along with updated information and services, is located on the
World Wide Web at:

<http://atvb.ahajournals.org/content/24/3/534>

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in *Arteriosclerosis, Thrombosis, and Vascular Biology* can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the [Permissions and Rights Question and Answer](#) document.

Reprints: Information about reprints can be found online at:
<http://www.lww.com/reprints>

Subscriptions: Information about subscribing to *Arteriosclerosis, Thrombosis, and Vascular Biology* is online at:
<http://atvb.ahajournals.org/subscriptions/>