8. 健康危険情報

なし

9. 研究発表

1）国内　口頭発表：　86件（うち、招待講演・シンポジウムなど29件）

原著論文による発表：　1件

それ以外（レビュー等）の発表：　35件

2）国外　口頭発表：　26件（うち、招待講演・シンポジウムなど12件）

原著論文による発表：　45件

それ以外（レビュー等）の発表：　3件

10. 知的財産権の出願・登録状況

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2. 2004,3.18,「薬剤・遺伝子溶出型ステント」江頭健輔, 特願2004-077581
3. 2004.3.30「遺伝子・薬剤放出型ステント」江頭健輔, 特願2004-100040
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【研究成果の刊行に関する一覧表】

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（2）口頭発表（招待講演、シンポジウム等のみ掲載）

＜国内学会＞

1. 江頭健輔：第 8 回日本適応医学会（平成 16 年 6 月 25 日、福島）シンポジウム「再狭窄・動脈硬化の分子機構における血管内皮増殖因子（VEGF）の役割に関する研究の新展開　VEGF は「内皮特異的血管保護因子」あるいは「多機能サイトカイン」か？」

2. 江頭健輔：第 36 回日本動脈硬化学会（日本血管生物医学会）（平成 16 年 7 月 23 日、福岡）動脈硬化病変の分子機構における血管内皮増殖因子（VEGF）の役割- VEGF は「内皮特異的血管保護因子」か- （ジョイントシンポジウム）

3. 江頭健輔、大谷規彰：第 36 回日本動脈硬化学会（平成 16 年 7 月 24 日、福岡）　炎症を標的とした次世代遺伝子溶出型ステントの創製（シンポジウム）

4. 江頭健輔：第 27 回日本高血圧学会（平成 16 年 10 月 7 日、栃木）高血圧性血管傷害における白血球 MCP-1 受容体 CCR2 の重要性- 炎症のニューパラダイム - （シンポジウム）

5. 江頭健輔：第 45 回日本脈管学会（平成 16 年 10 月 29 日、札幌）動脈硬化の最前線- 基礎から臨床- 「炎症制御による次世代遺伝子溶出型ステントの創製（シンポジウム）

6. 江頭健輔：第 24 回日本川崎病研究会（平成 16 年 11 月 12 日、京都）動脈硬化症疾患における炎症の役割　次世代医療としての血管内医療システムの創製（ランチョンセミナー）

7. 大谷規彰、江頭健輔：第 11 回九州血液血管研究会（平成 16 年 11 月 27 日、福岡）再狭窄・動脈硬化の分子機序における血管内皮増殖因子（VEGF）の役割の解明　VEGF は「内皮特異的血管保護因子」か、あるいは「多機能サイトカイン」か？

8. 江頭健輔：第 8 回日本心血管内分泌代謝学会ランチョンセミナー（平成 16 年 11 月 26 日、宮崎）　動脈硬化性疾患における炎症の役割- 次世代医療としての炎症制御による血管内医療システムの創製-

9. 江頭健輔：福岡循環器学術講演会 2004（平成 16 年 12 月 3 日、福岡）ACS 治療のトピックスーPROVE IT の解釈と薬剤溶出型ステント新時代

10. 江頭健輔：第 23 回機能再建材料グループ研究会(平成 17 年 1 月 21 日、茨城)遺伝子薬剤のコーティング技術について

11. 江頭健輔：第 34 回日本心脈管作動物質学会（平成 17 年 2 月 4 日、京都）遺伝子溶出型ステントによる次世代血管内治療システムの創製（シンポジ

ウム）

12. 江頭健輔：第 34 回日本心脈管作動物質学会（平成 17 年 2 月 4 日、京都）心脈管の再生・遺伝子治療（シンポジウム：オーガナイザー）

13. 江頭健輔：第 69 回日本循環器学会総会（平成 17 年 3 月 19 日、神奈川）Local Gene Transfer via Gene-Eluting Stents for Prevention of Restenosis: An Anti-inflammation Strategy for Next-Generation Intravascular Therapy（シンポジウム）

14. 江頭健輔、石橋美奈子：第 69 回日本循環器学会総会（平成 17 年 3 月 20 日、神奈川）Bone marrow-derived monocyte chemoattractant protei-1 receptor CCR2 is critical in angiotensin II-induced vascular remodeling and atherosclerotic process（プレナリーセッション・シンポジウム）

15. 江頭健輔：第 7 回桜ヶ丘循環器研究会（平成 17 年 7 月 9 日、鹿児島）「抗炎症による動脈硬化性病変の治療戦略」－ 再生医工学を駆使した基礎研究成果の臨床応用を目指して－

16. 江頭健輔：第 37 回日本動脈硬化学会総会（平成 17 年 7 月 14-15 日、東京）抗炎症療法による動脈硬化性プラークの進行・不安定化の抑制－ 基礎研究成果の臨床応用を目指して－ （シンポジウム）

17. 江頭健輔：第 7 回岡山動脈硬化懇話会（平成 17 年 7 月 22 日、岡山）抗炎症療法による再狭窄・動脈硬化の抑制－再生医工学による次世代血管内治療システムの創製を目指して－

18. 江頭健輔：高脂血症フォーラム in 別府（日本医師会生涯教育制度適合学術集会）（平成 17 年 9 月 2 日、別府）抗炎症による動脈硬化性疾患の治療戦略－ スタチンの新たな可能性－

19. 江頭健輔：ヒューマンライフサイエンスフォーラム 2005（平成 17 年 10 月 19 日、大阪）「産学官連携が切り開く遺伝子治療の新展開」遺伝子溶出型ステントがもたらす動脈硬化病変（再狭窄、不安定プラーク）に対する画期的血管機能再生

20. 江頭健輔：第 23 回京浜リピッドクラブ（平成 17 年 10 月 26 日、東京）「抗炎症による再狭窄・動脈硬化の新たな治療対策」－ 再生医工学による新規血管内医療システムの構築を目指して－

21. 江頭健輔：第 28 回日本血栓止血学会学術集会「市民公開講座」（平成 17 年 11 月 6 日、福岡）脳卒中・心筋梗塞を防ぐため Q&A ～参加者募集時に事前公募した一般市民からの質問に沿って～

22. 江頭健輔：第 2 回生活習慣病セミナー（平成 18 年 1 月 27 日、東京）虚血性心疾患治療の病態と治療の最前線－ 病態に則した治療法選択－

23. 江頭健輔：CCB Update for Experts 酸化ストレスと Ca チャネルブロッカー（平成 18 年 2 月 5 日、東京）アゼルニジピンの抗動脈硬化作用：抗炎症・抗酸化作用を中心に

24. 江頭健輔：高血圧フォーラム（平成 18 年 5 月 20 日、滋賀）動脈硬化病変（再狭窄・不安定化）の分子機序と治療に関する最近の知見ー抗炎症をもたらす生体完全吸収性ステントから SRB による血管保護までー（シンポジウム）

25. 江頭健輔：第一回福井早期動脈硬化研究会（平成 18 年 6 月 27 日、福井）虚血性心疾患の病態・治療に関する最新の話題- 生体完全吸収性ナノテク DDS ステントの研究開発を含めて- （シンポジウム）

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4. Egashira K: XVIII World Congress of the International Society for Heart Research (August 9 2004, Australaria) Symposium UAnti-inflammation therapy targeting

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5. Egashira K: Special invited lecture at Shang-Hai medical school and Microport Inc. （October 14,2004、Shanghai,China）難治性心臓病に対する遺伝子溶出ステントとカテーテルの開発

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7. 江頭健輔: 浙江大学医学院 (August 29, 2005) Inflammation and Atherosclerotic Vascular Disease. –special reference to anti-monocyte chemoattractant protein-1 gene therapy-

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＜研究成果の新聞報道など＞

1. 平成18年4月21日掲載、The Science News 科学新聞、「平成18年度　科学技術分野の文部科学大臣表彰」

2. 平成18年6月4日掲載、毎日新聞「冠動脈の“詰まり”レーザー除去」

3. 平成18年11月14日掲載、朝日新聞「ナノ粒子に薬剤封入　心筋梗塞再発防止へ　ホソカワミクロンなど」

4. 平成18年11月14日掲載、化学工業日報「DDSステント　第3世代品を開発　ホソカワミクロンナノパーティクルで」

5. 平成18年11月15日掲載、日刊工業新聞「心筋梗塞治療向け薬物送達システム　ホソカワミクロンが新技術」

6. 平成18年12月15日掲載、産経新聞「九州大と共同で心血管拡張技術　ホソカワミクロン」

7. 平成19年1月16日掲載、毎日新聞「心筋梗塞治療に新技術　ホソカワミクロン　根治治療可能に」

＜別添参照＞

1. ホソカワミクロン「九大医と共同で第三世代型DDSステントの開発に成功
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2. 日経メディカルオンライン
URL: http://medical.nikkeibp.co.jp/leaf/all/gakkai/aha2006/200611/501907.html

【研究成果の刊行物・別刷】

次のページ以降に番号のところに○をつけた論文の別刷りを添付します。

Molecular Mechanisms Mediating Inflammation in Vascular Disease

Special Reference to Monocyte Chemoattractant Protein-1

Kensuke Egashira

Abstract—There are several clinical challenges for the treatment of intractable cardiovascular diseases, including restenosis, atherosclerotic complications resulting from plaque rupture, severe tissue ischemia, and heart failure. Emerging evidence suggests that an inflammatory process is involved in the pathogenesis of such intractable diseases. In particular, inflammatory responses to arterial injury, which cause continuous recruitment and activation of monocytes mainly through activation of the monocyte chemoattractant protein-1 (MCP-1) pathway, have a central role in restenosis and atherogenesis. We recently devised a new strategy for anti–MCP-1 therapy by transfecting an N-terminal deletion mutant of the MCP-1 gene into skeletal muscles. This mutant MCP-1 lacks the N-terminal amino acids 2 to 8, called 7ND, and works as a dominant-negative inhibitor of MCP-1. We demonstrated that 7ND gene transfer suppresses monocyte infiltration/activation after arterial injury and markedly inhibits experimental restenosis in animals after balloon injury or stent placement. Furthermore, 7ND gene transfer not only attenuated the development of early atherosclerotic lesions but also limited progression of preexisting atherosclerotic lesions and changed the lesion composition into a more stable phenotype in hypercholesterolemic mice. Vascular inflammation mediated by MCP-1 might create a positive feedback loop to enhance restenotic and atherosclerotic changes through activating lesional monocytes. Therefore, vascular inflammation mediated by MCP-1 has a central role in the development of experimental restenosis, atherosclerosis, and plaque destabilization, leading to acute coronary syndrome. This strategy for gene therapy might be useful against human restenosis, thereby opening a new therapeutic window for antirestenosis and antiatherosclerosis paradigms. (*Hypertension.* **2003;41[part 2]:834-841.**)

Key Words: monocyte ■ arteriosclerosis ■ restenosis ■ gene therapy ■ inflammation

Inflammatory changes in the arterial wall have a central role in the development of restenosis and atherosclerosis.[1,2] A considerable body of evidence supports the notion that various mediators such as adhesion molecules, cytokines, and chemokines are involved in the initiation and progression of atherosclerotic lesions.[1,2] Monocyte chemoattractant protein-1 (MCP-1) is the most important chemokine that regulates migration and infiltration of monocytes/macrophages. MCP-1 belongs to a CC chemokine subfamily of chemokines. MCP-1 is the specific chemotactic factor for monocytes/macrophages and has an important role in the pathogenesis of chronic inflammatory disorders.[3,4] The effects of MCP-1 are mediated mainly through CC chemokine receptor 2 (CCR2).[3,4] MCP-1 causes chronic vascular inflammation and induces thrombosis, proliferation and migration of vascular smooth muscle cells, angiogenesis, and oxidative stress (Figure 1). Previous studies indicate that (1) MCP-1 production from endothelial cells, smooth muscle cells, and lesional leukocytes increases in the presence of endothelial dysfunction and atherosclerotic risk factors (Figure 1); (2) MCP-1 expression is increased in atherosclerotic lesions[5,6] and injured arteries[7,8]; and (3) eliminating MCP-1 function decreases neointimal hyperplasia after injury and atheroma formation in mice.[9–12] We demonstrated that MCP-1 has an important role in coronary arteriosclerosis in a rat model of NO synthesis inhibition.[13–17]

Emerging evidence suggests that MCP-1–mediated inflammatory disorders are involved in restenosis and atherosclerosis, as well as in other treatment-intractable cardiovascular diseases, such as posttransplantation arteriosclerosis, vascular remodeling owing to hypertension, myocarditis/cardiomyopathy, and cardiac dysfunction and remodeling after myocardial infarction.[18–23] Therefore, therapeutic strategies targeting MCP-1 might become useful and practical treatments for cardiovascular diseases that are intractable with conventional therapies. In this regard, we devised a new strategy for anti–MCP-1 gene therapy by transfecting mutant MCP-1 gene.[17,24] This strategy might be useful for clarifying the role of MCP-1 under pathophysiologic conditions in vivo. In this review, we describe the role of MCP-1 in cardiovascular

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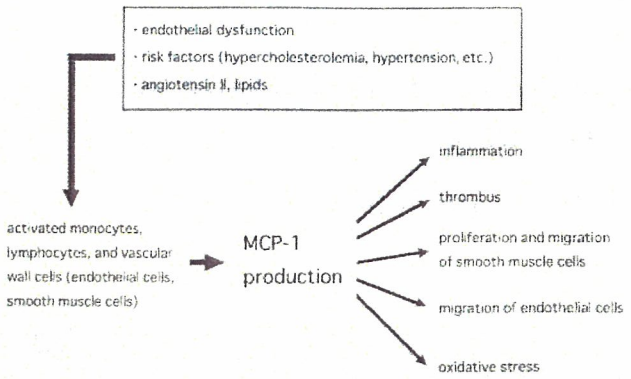


Figure 1. The role of the MCP-1 pathway in the pathogenesis of atherosclerosis, plaque destabilization, thrombosis, and restenosis.

diseases and introduce recent work that addresses the usefulness of anti–MCP-1 gene therapy. The study protocol was reviewed and approved by the Committee on Ethics on Animal Experiments, Kyushu University Faculty of Medicine, and the experiments were conducted according to the Guidelines of American Physiological Society. A part of this study was performed at the Kyushu University Station for Collaborative Research.

Role of MCP-1 in Cardiovascular Disease

In animal and human atherosclerotic lesions, ≈80% of leukocytes are monocytes/macrophages, and 10% to 20% of them are memory T-lymphocytes.[25] Atheroma-forming cells (endothelial cells, smooth muscle cells, and macrophages) express MCP-1 and CCR2, and activity in this pathway is increased in atherosclerotic lesions.[26] Oxidative stress, oxidized inflammatory lipids, and redox-sensitive transcription factors (NF-κB, AP-1, etc) reportedly contribute to increased expression of MCP-1. Furthermore, activation of the MCP-1/CCR2 pathway induces adhesion molecules,[27] proinflammatory cytokines,[27,28] chemokines, and matrix metalloproteinases[29] and thus accelerates atherosclerosis in hypercholesterolemic animals.[30,31] More importantly, MCP-1 induces tissue factor and inflammatory cytokines such as interleukin-6 in human arterial smooth muscle cells.[32] Abrogation of the MCP-1/CCR2 pathway inhibits the early development of atherosclerotic lesions in mice.[9,10] These findings suggest that MCP-1 contributes not only to vascular inflammation but also to the development of atherosclerosis, plaque

Plasma Concentrations of MCP-1 and 7ND after 7ND Transfection in Mice

		No. of Days After 7ND Transfection			
	Baseline	3	7	14	28
MCP-1, pg/mL	76±5	85±7	88±6	77±5	80±6
7ND, pg/mL	<20.0*	226±21	220±20	140±12	<20.0*

Values are mean±SE, n=6 to 8. Plasma concentrations of 7ND released by the transfected skeletal muscle were measured by the use of human MCP-1 ELISA kit (Biosource). Plasma MCP-1 concentrations were measured with murine MCP-1 ELISA kit (Biosource). Wild-type mice (C57BL/6J) were transfected with intramuscular injections of pcDNA3–7ND plasmid DNA (100 μg) into the femoral muscle. Transgene expression was enhanced by intramuscular electroporation at the injection site immediately after injection.[46]

*Below detectable limits.

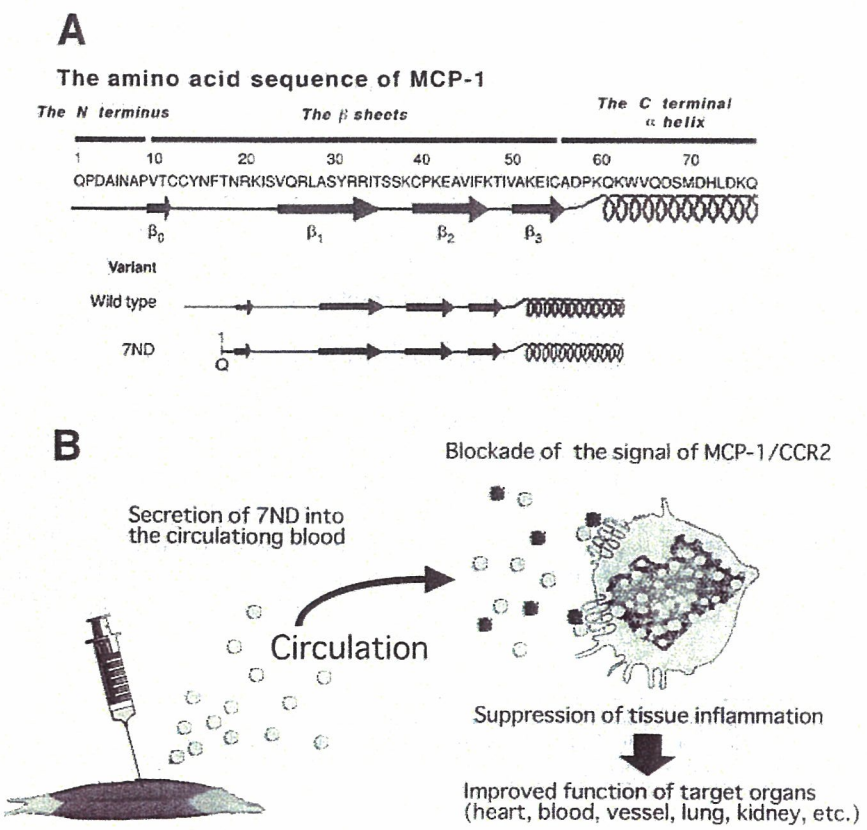
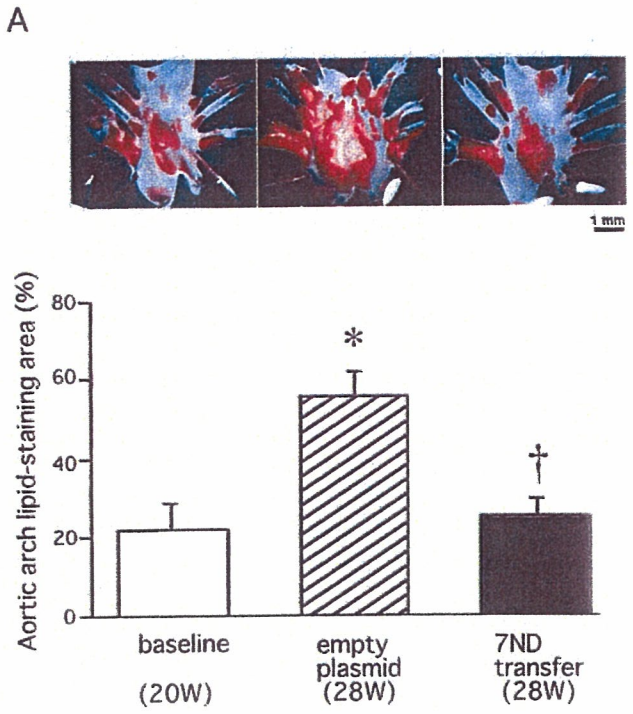
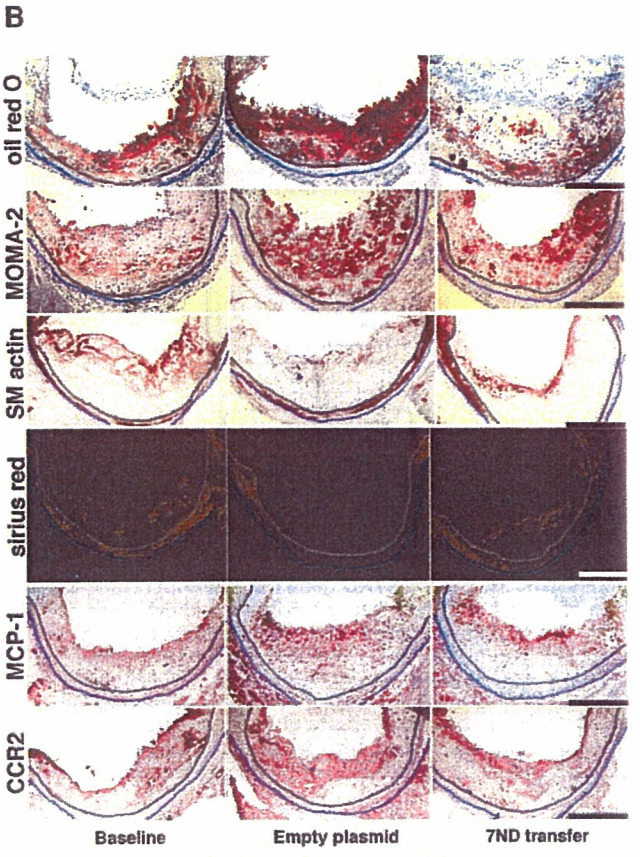


Figure 2. Structure of MCP-1 and 7ND (A), and schema for strategy of anti–MCP-1 gene therapy by 7ND gene transfer (B). A, 7ND is an N-terminal deletion mutant of human MCP-1 that lacks the N-terminal amino acids 2 to 8 and acts as a dominant-negative inhibitor for MCP-1. Intramuscular transfection of 7ND gene therefore suppresses monocyte chemotaxis in remote organs by blocking the MCP-1/CCR2 signal pathway. B, To achieve effective blockade of the MCP-1/CCR2 signal pathway, we transfected the expression plasmid vector encoding 7ND gene into skeletal muscle. We reported that 7ND protein is secreted from the transfected skeletal muscle cells into the circulating blood, blocks the MCP-1/CCR2 signal pathway in remote target organs or tissues, and suppresses monocyte recruitment into the target organs or tissues





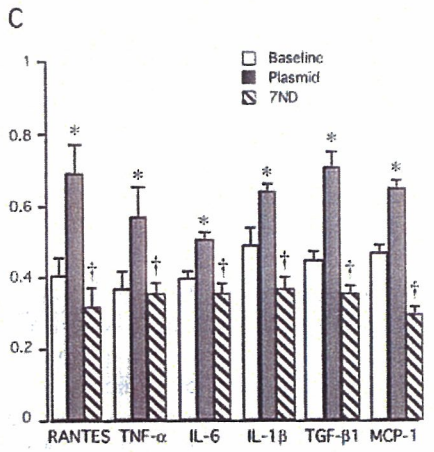


Figure 3. A, Transfection with the 7ND gene inhibits progression of established atherosclerotic lesions in the aortic arch of apoE-KO mice. Photomicrographs of the intraluminal surface of the aortic arch stained with oil red O. Quantitative comparison of atherosclerotic lesion size and lesions in baseline, empty plasmid, and 7ND-transfected ApoE-KO mice are also shown. Data are reported as mean±SEM, n=9 to 10. *$P<0.05$ vs the baseline; †$P<0.05$ vs empty plasmid group. Modified from Inoue et al[46]. B, 7ND transfection changes the composition of the atheroma such that it is more stable. From the top to bottom, photomicrographs of atherosclerotic lesions stained with oil red O or immunostained with anti-murine macrophage antibody (MOMA-2), anti-human α-SM actin antibody, anti–MCP-1 antibody, and anti-CCR2 antibody. Interstitial collagen was visualized by using polarization microscopy after staining with picro sirius red. Internal and external elastic layers are highlighted with blue and black lines, respectively. Bar, 200 μm. Modified from Inoue et al.[46] C, Effect of 7ND transfection on chemokine (RANTES and MCP-1) and cytokine TNF-α IL-6, IL-β TGF-β gene expression in the abdominal aorta. Data are expressed as the ratio of each mRNA to the corresponding GAPDH mRNA. *$P<0.05$ vs the baseline and 7ND-transfected group. Modified from Inoue et al.[46]

destabilization, and thrombosis (Figure 1), which results in acute coronary syndrome.

Inflammation also contributes to the development of restenotic changes after balloon injury or stenting. Inflammatory and proliferative cells in the injured artery are shown to express MCP-1 after injury. Interestingly, a rapid and prolonged production of MCP-1 is reported in patients who present with restenosis after balloon angioplasty.[24,33] Cipollone et al[24] demonstrated that patients with restenosis have a prolonged increase in plasma MCP-1, whereas nonrestenotic patients have only a transient increase in plasma MCP-1. Thus, human arteries with underlying hypercholesterolemia and/or atherosclerosis are likely to represent prolonged production of MCP-1 after arterial injury. Therefore, elucidating the underlying mechanism of prolonged production of MCP-1 after vascular injury would open the way to identify molecular mechanisms of restenosis. Furukawa et al[7] demonstrated that repeated injections of polyclonal antibodies against rat MCP-1 reduced neointimal formation in a rat model of carotid artery balloon injury. We[11] and others[12] demonstrated that mice lacking CCR2 displayed diminished neointimal hyperplasia formation after femoral arterial injury. There might be important differences between injury associated with balloon dilatation and that associated with stent implantation. In addition to mechanical injury, a foreign body response to stent prosthesis induces intense inflammation in the arterial wall, with ensuing production of cytokines and growth factors that subsequently induce proliferation and migration of vascular smooth muscle cells.[34–37] As a result, neointimal hyperplasia is more than 2-fold greater after stent implantation than after balloon angioplasty.[36,38] Inhibition of

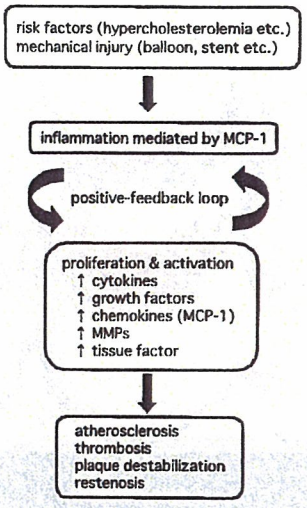


Figure 4. Schematic diagram of our hypothesis regarding the role of the MCP-1/CCR2 pathway in the development/progression of atherosclerosis and thrombosis, plaque destabilization, and restenosis. Because 7ND gene transfer suppressed expression of MCP-1 and the other chemokines and cytokines, it is likely that MCP-1–mediated inflammation creates a positive feedback loop (a vicious cycle) to enhance vascular inflammation and atherogenesis possibly through activating lesional monocytes. The beneficial effects of 7ND gene transfer on restenosis and established atherosclerotic lesions might be causedy mainly by suppression of monocyte recruitment and activation.

cellular proliferation with the immunosuppressant sirolimus might be an effective strategy to suppress in-stent restenosis.[39–42] Experimental data suggest that the beneficial effects of sirolimus-eluting stents are mediated at least in part by antiinflammatory effects.[39] Inhibition of the MCP-1 or CCR2 pathways attenuate in-stent neointimal hyperplasia in nonhuman primates.[43] These data suggest that MCP-1 and CCR2 have a pivotal role in the pathogenesis of restenosis after balloon injury or stent-induced injury.

Anti–MCP-1 Gene Therapy by Intramuscular Transfection of Mutant MCP-1 Gene

Because MCP-1–mediated inflammation appears to have a central role in the pathogenesis of cardiovascular inflammation and its disease process, we sought a new therapeutic strategy to target the MCP-1/CCR2 pathway. An N-terminal deletion mutant of MCP-1, called 7ND, which lacks the N-terminal amino acids 2 to 8, forms inactive heterodimers with wild-type MCP-1 and exerts its inhibitory activity as a dominant-negative inhibitor under in vitro conditions (Figure 2A).[44] We therefore evaluated the use of gene therapy to block MCP-1 activity in vivo by using intramuscular transfection of this mutant MCP-1 gene. The use of skeletal muscle as a biofactory to produce a secreted protein has been reported previously. From a clinical point of view, this strategy (the delivery of plasmid DNA by intramuscular injection) is simple and shown to be nontoxic. No gene delivery systems of clinical use with acceptable safety for local gene delivery to coronary artery lesions are available at the present time. We demonstrated that (1) intramuscular transfection of plasmids encoding the human 7ND gene into skeletal muscle resulted in secretion of 7ND protein into the circulating blood, and (2) the 7ND protein binds to the MCP-1 receptor on monocytes or target cells and, thus, (3) achieved an effective and sufficient blockade of MCP-1 activity in remote organs (Figure 2B).[17] The therapeutic effects of this strategy may depend on the protein secreted into circulation by the transgene. To confirm the efficacy of transgene, we measured plasma MCP-1 and 7ND concentrations in mice after intramuscular transfection of 7ND gene (Table). Plasma MCP-1 concentrations did not change during the course of experiments, whereas 7ND was detected in plasma 3, 7, and 14 days after transfection.

This strategy also suppressed monocyte recruitment into the coronary vessels and the development of coronary arteriosclerosis in a rat model of chronic inhibition of NO synthesis.[17] Furthermore, there were no apparent side effects during the period of the study. On the basis of these pioneering studies, this strategy might be a useful and feasible form of gene therapy against inflammation and related diseases mediated by MCP-1 in humans. This strategy might also be useful for clarifying the role of MCP-1 under pathophysiologic conditions in vivo, especially in organs into which direct gene transfer is difficult.

Effect of 7ND Gene Transfer on Atherosclerosis and Plaque Destabilization

Although mice lacking MCP-1 or CCR2 display reduced initial atheroma formation,[9,10] whether postnatal blockade of MCP-1 could be a unique site-specific therapy against atherosclerosis was unknown. Apolipoprotein E–knockout (ApoE-KO) mice develop hypercholesterolemia and atherosclerotic lesions similar to those observed in humans and are widely used for studying the pathogenesis of atherosclerosis. Therefore, we tested the effectiveness of 7ND gene transfer on the development of atherosclerosis in this model.[45] ApoE-KO mice (7 or 8 weeks of age) were fed a Western-type diet and randomized into 2 groups. The mice were injected with PBS or 7ND gene (5 μg 7ND vector plasmid encapsulated in hemagglutinating virus of Japan-liposome) into the femoral muscles at weeks 0 and 3 after the start of a Western-type diet. After 6 weeks on the diet, the control group mice had typical fatty atherosclerotic lesions in the aortic root. Macrophage infiltration and MCP-1 immunostaining were observed in atherosclerotic lesions. The 7ND gene transfer effectively blocked MCP-1 activity and inhibited the formation of atherosclerotic lesions, but had no effect on serum lipid concentrations. Furthermore, this strategy increased the lesional extracellular matrix content, suggesting that blockade of MCP-1 reduced markers of plaque destabilization. These data suggest that MCP-1 can be a novel therapeutic target for atherosclerosis.

Investigation of molecular mechanisms underlying later complications of atherosclerosis is clinically very important, because atherosclerotic complications such as acute myocardial infarctions and stroke develop during the later stages of atherosclerosis. Lesion composition rather than size or degree of the stenosis of the lesion is believed to determine the likelihood of plaque rupture and subsequent thrombotic complications such as acute coronary syndrome.[1] Therefore,

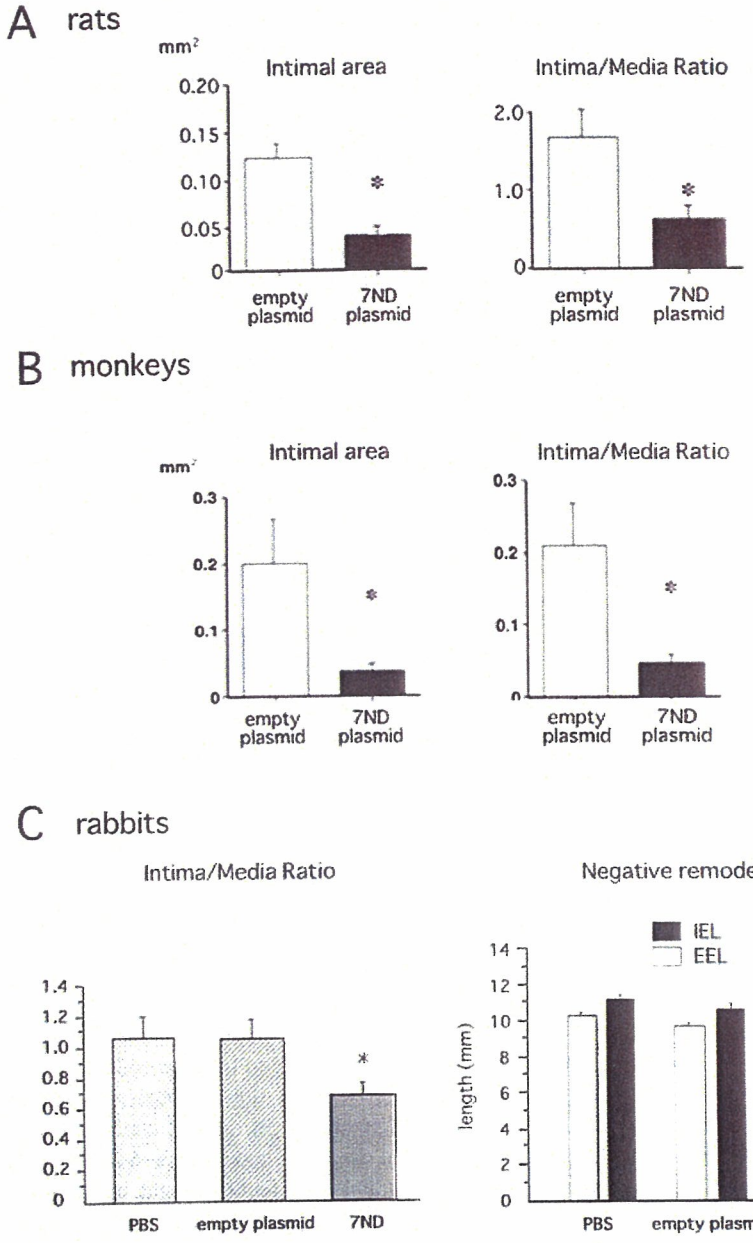


Figure 5. A, Effect of the intramuscular transfer of the 7ND gene on intimal area and intima/media ratio 28 days after balloon injury in rats transfected with empty plasmid or 7ND plasmid (n=8 each). **P*<0.01 vs the empty plasmid treatment. Modified from Usui.[48] B, Effect of the intramuscular transfer of the 7ND gene on intimal area and intima/media ratio 28 days after balloon injury in monkeys (n=6). **P*<0.01 vs the empty plasmid treatment. Modified from Usui.[48] C, Effect of intramuscular transfer of 7ND gene on neointimal formation (intima/media ratio) and negative remodeling on day 28 after balloon injury in rabbits. IEL indicates internal elastic lamina; EEL, external elastic lamina. **P*<0.05, ***P*<0.01 vs PBS or empty plasmid. Modified from Mori.[49]

we tested the hypothesis that blockade of MCP-1 limits progression and destabilization of established lesions in ApoE-KO mice.[46] ApoE-KO mice were fed a normal chow diet during the experiment. At 20 weeks of age, the baseline group of mice was killed to determine the extent of baseline established lesions. Other mice were randomly assigned into 2 groups. The 7ND-transfected group received intramuscular injections of naked pcDNA3 to 7ND plasmid DNA (100 μg) into the femoral muscle at biweekly intervals for up to 8 weeks. Plasma MCP-1 concentrations did not change during the course of experiments, whereas 7ND was detected in plasma up to 2 weeks after transfection. Blockade of MCP-1 by 7ND gene transfer limited progression of preexisting atherosclerotic lesions independent of serum cholesterol levels (Figure 3A). In addition, blockade of MCP-1 changed the lesion composition into a more stable phenotype, ie, containing fewer macrophages and lymphocytes, less lipid, and more smooth muscle cells and collagen. This finding warrants clinical attention because interstitial collagen in the shoulder region is considered to be a critical determinant of fibrous cap integrity.[1] This strategy decreased expression of CD40, the CD40 ligand, tissue factor, and matrix metalloproteinases-9 and -13 in the atherosclerotic plaque (Figure 3B), and normalized the increased chemokine (RANTES and MCP-1) and cytokine (TNFα, IL-6, IL-1β, and TGFβ-1) gene expression (Figure 3C). Suppression of the expression of MCP-1 and the other chemokines and cytokines by 7ND gene transfer implies that MCP-1–mediated inflammation creates a positive feedback loop to enhance vascular inflammation and atherogenesis, possibly through activating lesional monocytes (Figure 4). The beneficial effects of 7ND gene transfer on established atherosclerotic lesions might be owing mainly to the suppression of monocyte recruitment and activation. These data suggest that anti–MCP-1 therapy not only limits