Matrix Metalloproteinase-14 Deficiency in Bone Marrow–Derived Cells Promotes Collagen Accumulation in Mouse Atherosclerotic Plaques

Fabrice Schneider, MD; Galina K. Sukhova, PhD; Masanori Aikawa, MD, PhD; James Canner, MA; Norbert Gerdes, PhD; Sai-Man Timothy Tang, Bsc; Guo-Ping Shi, DSc; Suneel S. Apte, MBBS, DPhil; Peter Libby, MD

Background—Interstitial collagen plays a crucial structural role in arteries. Although in vitro results suggest collagenase activity for membrane-bound matrix metalloproteinase type 1 (MMP-14), in vivo evidence for such a function in atherosclerosis remains scant.

Methods and Results—Because *Mmp14*$^{-/-}$ mice die by 3 weeks of age, this study used lethally irradiated low-density lipoprotein receptor–deficient mice reconstituted with syngeneic bone marrow cells of *Mmp14*$^{-/-}$ or *Mmp14*$^{+/+}$ mice. In both groups, histological analyses of the aortic root revealed similar plaque size and macrophage and smooth muscle cell content after 8 or 16 weeks of atherogenic diet. By 16 weeks, however, the plaques of low-density lipoprotein receptor–deficient mice engrafted with *Mmp14*$^{-/-}$ bone marrow (n=12) contained significantly more interstitial collagen than those receiving *Mmp14*$^{+/+}$ bone marrow (n=14; $P<0.05$). In vitro, bone marrow–derived macrophages from *Mmp14*$^{-/-}$ mice had significantly less interstitial collagenase activity than those from *Mmp14*$^{+/+}$ mice both basally ($P<0.01$) and on tumor necrosis factor-α stimulation ($P<0.05$). Western blot analysis and gelatin zymography of aortic extracts revealed that MMP-14 deficiency yielded decreased activation of pro–MMP-13 but not of pro–MMP-2 or pro–MMP-8.

Conclusion—MMP-14 from bone marrow–derived cells can influence the collagen content of mouse atheroma, a critical component of plaque stability. **(*Circulation*. 2008;117:931-939.)**

Key Words: atherosclerosis ■ collagen ■ metalloproteinases ■ pathology ■ plaque

Rupture of atherosclerotic plaques in coronary arteries causes most fatal acute myocardial infarctions.[1,2] Such plaques characteristically have a thin fibrous cap, a large lipid-rich core, and abundant macrophages. Because interstitial collagen confers tensile strength on the fibrous cap, collagenolysis in the fibrous cap likely participates critically in plaque disruption.[3,4] Matrix metalloproteinases (MMPs) can degrade all components of arterial extracellular matrix, and considerable evidence supports their involvement in plaque remodeling. Three members of the MMP family denoted interstitial collagenases (MMP-1, MMP-8, and MMP-13) can cleave triple-helical fibrillar collagen at the neutral pH of the extracellular milieu.[5,6] In addition to these secreted, soluble enzymes, the membrane-anchored or membrane type 1 MMP, MMP-14, also can exhibit collagenase activity.[7–9] Human and animal studies have localized these collagenases in atherosclerotic plaques.[10–14] Mice genetically altered to express collagenase-resistant collagen (*Col*$^{R/R}$) have increased collagen content in atheromata in vivo.[15] Mice lacking MMP-13 accumulate more collagen with a more organized supramolecular structure in plaque than those wild type for this key interstitial collagenase.[16] MMP-14 localizes in human plaque[17] and on peripheral blood monocytes during myocardial infarction.[18] Experimental studies showed enhanced expression of MMP-14 during arterial remodeling after balloon injury[19] and in the myocardium after ischemia/reperfusion.[20] However, the contribution of MMP-14 to collagen metabolism during atherosclerosis in mice remains unexplored. Mice genetically deficient in MMP-14 (*Mmp14*$^{-/-}$) typically die 3 weeks after birth of unknown causes. Before death, these mice show significant growth impairment and wasting.[21,22] These findings hamper the analysis of atherogenesis in compound mutant mice for *Mmp14*$^{-/-}$ and low-density lipoprotein receptor–deficient

Received April 29, 2007; accepted November 27, 2007.

From the Donald W. Reynolds Cardiovascular Clinical Research Center and Fondation Leducq Transatlantic Network on Atherothrombosis (F.S., G.K.S., M.A., J.C., N.G., S.-M.T.T., G.-P.S., P.L.), Brigham and Women's Hospital, Harvard Medical School, Boston, Mass; Service de Chirurgie Vasculaire (F.S.), Hôpital Henri Mondor, Créteil, France; Department of Biomedical Engineering (S.S.A.), Lerner Research Institute Cleveland Clinic, Cleveland, Ohio; and Center for Molecular Medicine (N.G.), Karolinska Institute, Stockholm, Sweden.

Guest Editor for this article was Donald D. Heistad, MD.

Correspondence to Peter Libby, MD, Brigham and Women's Hospital, 77 Ave Louis Pasteur, NRB 741, Boston, MA 02115. E-mail plibby@rics.bwh.harvard.edu

Circulation **is available at http://circ.ahajournals.org** **DOI: 10.1161/CIRCULATIONAHA.107.707448**

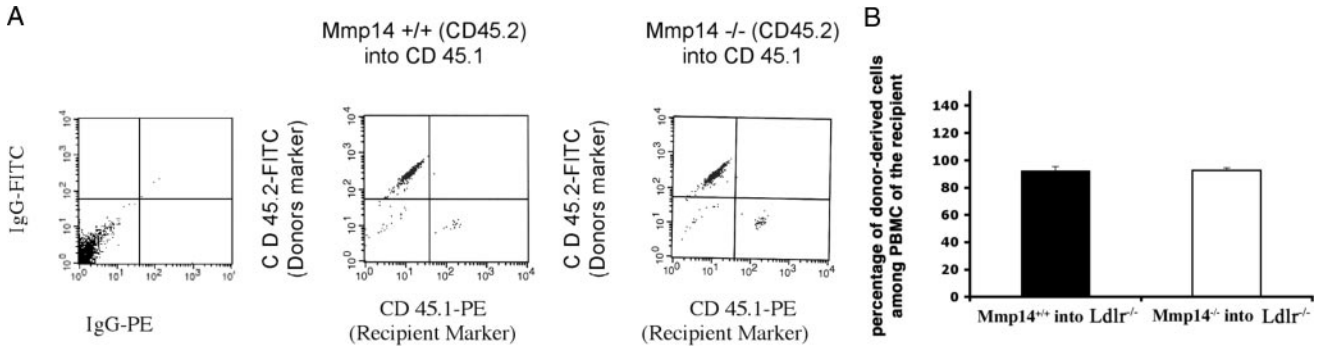

Figure 1. Successful reconstitution of bone marrow–derived cells after γ-irradiation in CD45.1-positive recipient mice. Bone marrow–derived cells from 2- to 3-week-old *Mmp14*$^{+/+}$ or *Mmp14*$^{-/-}$ (CD45.2-positive/CD45.1-negative) mice were transplanted into CD45.1-positive/CD45.2-negative mice. Blood was harvested after 5 weeks. A, Representative plots of peripheral blood cells stained with anti–CD45.1-PE and anti–CD45.2-FITC, analyzed by FACS. B, Quantitative analysis of percentage of donor-derived cells among recipient peripheral blood monocytic cells (n=3 for both groups).

mice (*Mmp14*$^{-/-}$/*Ldlr*$^{-/-}$). The present study circumvented this constraint by using bone marrow from *Mmp14*$^{-/-}$ mice to examine the role of MMP-14 expressed by bone marrow–derived cells in atheroma formation. Macrophages, which arise from bone marrow, appear to express the bulk of MMP-14 in atheroma.[23] This approach permitted us to test the hypothesis that MMP-14 derived from bone marrow participates in collagen catabolism in plaques using lethally irradiated *Ldlr*$^{-/-}$ mice reconstituted with bone marrow from *Mmp14*$^{-/-}$ mice.

Editorial p 863
Clinical Perspective p 939

Methods

Animal Preparation

All experiments conformed to a protocol approved by the Standing Committee on Animals of Harvard Medical School. *Mmp14*$^{+/-}$ mice[24] backcrossed 7 generations into congenic C57BL/6 mice were crossbred to generate *Mmp14*$^{-/-}$ mice and *Mmp14*$^{+/+}$ littermates. *Ldlr*$^{-/-}$ C57BL/6 mice 6 to 10 weeks of age (Jackson Laboratories, Bar Harbor, Me) were lethally irradiated (2 times at 700 rad 3 hours apart) and received bone marrow (5×10^6 cells per mouse IV) derived from *Mmp14*$^{-/-}$ (n=29) and *Mmp14*$^{+/+}$ (n=31) donor mice 2 to 3 weeks of age. After bone marrow reconstitution (5 weeks), mice consumed a high-cholesterol diet (Research Diets, New Brunswick, NJ; 1.25% cholesterol, 0% cholate) for 16 weeks (n=26) or 8 weeks (n=34). Mouse plasma was collected for cholesterol measurements at day 0, after bone marrow reconstitution, and at the time of death.[12] For the in vitro study, we used *Mmp13*$^{-/-}$ mice with congenic C57BL/6 background.[16] In separate experiments, we verified the effect of MMP-14 deficiency on reconstitution of bone marrow–derived cells by transplanting bone marrow–derived cells from *Mmp14*$^{+/+}$ or *Mmp14*$^{-/-}$ mice (both CD45.2) into *Ldlr*$^{-/-}$ mice (CD45.1; n=3 each group).[25] The reconstitution of peripheral blood monocytes was >92% in both groups (Figure 1).

Fluorescence-Activated Cell Sorter Analysis

We performed fluorescence-activated cell sorter analysis (FACS) as previously described.[25] Briefly, 20 μL diluted mouse blood (1:1 in FACS buffer [2% BSA, 0.1% sodium azide in PBS]) and 1 μL Fc block (eBioscience, San Diego, Calif) were incubated at room temperature (15 minutes) before fluorescently labeled antibodies were added (15 minutes). After incubation with FACS lysis buffer, the cells were washed twice with FACS buffer and analyzed by FACS. CD45.2-FITC and CD45.1-PE antibodies and corresponding isotype controls were purchased from eBioscience.

Tissue Preparation and Histological Assays

The aortic roots were prepared as described previously.[26,27] Briefly, mice were perfused at physiological pressure with normal saline via the left ventricle, and the hearts and aortas were removed en bloc. The aortic root was embedded in optical cutting temperature compound (Sakura, Torrance, Calif). To evaluate intimal lesion size, frozen sections of aortic root were incubated with oil red O (0.5% in glycerol). Immunohistochemical studies used rat anti-mouse monoclonal antibody to Mac3, a macrophage marker (1:1000, BD PharMingen, San Diego, Calif), and smooth muscle cell (SMC) α-actin staining with primary antibody FITC-conjugated α-actin mouse monoclonal (1:500, Sigma, St Louis, Mo), followed by anti-FITC biotin–conjugated secondary antibody (1:400, Sigma) and a rabbit anti–MMP-14 polyclonal antibody (1:500, Chemicon, Temecula, Calif). We analyzed fibrillar collagen content using picrosirius red staining of sections, which were viewed under polarized light. Quantitative analyses used Image-Pro Plus Software (Media Cybernetics, Bethesda, Md). Two blinded observers recorded the percentage of the total area with positive color for each section.

Reverse-Transcription Polymerase Chain Reaction

Total RNA was extracted from whole mouse aortas (pooled, n=3 per group) and reverse transcribed. Real-time reverse-transcription polymerase chain reaction (RT-PCR) used SYBR Green PCR Master Mix and MyiQ Single Color Detection System (BioRad, Hercules, Calif). Oligonucleotide primers used to recognize mouse mRNAs included the following: MMP-2, 5′-GCA-CCC-TTG-AAG-AAG-TAG-CTA-TG-3′ and 5′-GCA-GGA-GAC-AAG-TTC-TGG-AGA-TA-3′; MMP-8, 5′-CAA-CCT-ATT-TCT-CGT-GGC-TG-3′ and 5′-TGC-AGG-TCA-TAG-CCA-CTT-AG-3′; MMP-9, 5′-AAC-ACA-CAG-GGT-TTG-CCT-TC-3′ and 5′-CGT-CGT-GAT-CCC-CAC-TTA-CT-3′; MMP-12, 5′-TTT-CTT-CCA-TAT-GGC-CAA-GC-3′ and 5′-GGT-CAA-AGA-CAG-CTG-CAT-CA-3′; MMP-13, 5′-TCC-CTT-GAT-GCC-ATT-ACC-AGT-C-3′ and 5′-AAA-AAG-AGC-TCA-GCC-TCA-ACC-TG-3′; MMP-14, 5′-AGG-GTT-CCT-GGC-TCA-TGC-3′ and 5′-ACA-GCG-GCC-GCA-CTC-ACA-3′; cathepsin K, 5′-CCA-GTG-GGA-GCT-ATG-GAA-GA-3′ and 5′-AAG-TGG-TTC-ATG-GCC-AGT-TC-3′; α1 procollagen I, 5′-TCT-TTC-TCC-TCT-CTG-ACC-G-3′ and 5′-AAG-GTG-CTG-ATG-GTT-CTC-C-3′; and GAPDH, 5′-TGG-GTG-TGA-ACC-ATG-AGA-AG-3′ and 5′-GCT-AAG-CAG-TTG-GTG-GTG-C-3′.

Western Blotting and Gelatin Zymography

Whole aortas from *Mmp14*$^{-/-}$→*Ldlr*$^{-/-}$ and *Mmp14*$^{+/+}$→*Ldlr*$^{-/-}$ (n=3 each group) were harvested and snap-frozen in liquid nitrogen. After pulverization, samples were homogenized into radioimmunoprecipitation assay buffer (Boston Bioproducts, Boston, Mass) with EDTA-free protease inhibitor cocktail (Roche, Indianapolis, Ind) for 30 minutes at 4°C and centrifuged at 3000*g* for 30 minutes. For Western blotting, total protein (20 μg per well) was separated by

standard SDS-PAGE and blotted to polyvinylidene difluoride membranes (Bio-Rad, Hercules, Calif) using a semidry blotting apparatus. Blots were blocked in 5% (wt/vol) defatted dry milk in PBS/0.1% Tween 20 (Sigma-Aldrich, St Louis, MO) overnight and incubated with the respective primary antibody (2 hours). The secondary peroxidase-conjugated antibody (1:10 000, Jackson Immunoresearch, West Grove, Pa) was added for another hour. Finally, immunoreactive proteins were visualized with the Western Lightning Chemiluminescence Reagent Plus (PerkinElmer Life Sciences, Boston, Mass). We used rabbit polyclonal antibody against MMP-14 (1:1000, Chemicon), goat polyclonal antibodies against MMP-13 (1:2000, Chemicon) and MMP-8 (1:100, R&D Systems, Minneapolis, Minn), and a rabbit polyclonal antibody against α-tubulin (1:500, Santa Cruz Biotechnology, Santa Cruz, Calif) as a loading control. For gelatin zymography, we used a previously described protocol.[28] Briefly, equal amounts of total protein were separated under nonreducing conditions by SDS-PAGE containing gelatin (1 mg/mL, BioRad). After washing with renaturation buffer (BioRad; 30 minutes), we incubated the gel in development buffer (24 hours) and then stained it with Coomassie brilliant blue 0.5% and destained in 25% methanol/10% acetic acid.

Macrophage Culture and In Vitro Collagenase Assay

Bone marrow–derived macrophages were harvested from the femurs and tibias of *Mmp13*$^{-/-}$, *Mmp14*$^{-/-}$, and wild-type mice. After incubation in red cell lysis buffer (ammonium chloride 0.155 mol/L in PBS), we centrifuged (20 minutes) the samples on Ficoll medium (LSM, ICN Biomedicals, Aurora, Ohio) and collected the monocytic cells. Macrophages were selected using medium with macrophage-colony stimulating factor (25 ng/mL, Cell Sciences, Canton, Mass) for 6 days. Determination of the collagenolytic capacity used cells maintained in serum-free medium overnight, unstimulated or stimulated with tumor necrosis factor-α (TNF-α; 10 ng/mL) and then incubated with fluorescein-labeled nondenatured collagen type-I (0.3 mg/mL, Calbiochem, La Jolla, Calif) for 48 hours in the presence or absence of 1,10-phenanthroline (0.1 mmol/L, Sigma), a broad metalloenzyme inhibitor. Digested collagen fragments were measured at 485-nm excitation and 530-nm emission in a fluorescent plate reader.

Statistical Analysis

Data are presented as mean±SD. Differences between groups were determined with the Mann-Whitney *U* test. Values of $P<0.05$ were considered significant.

The authors had full access to and take full responsibility for the integrity of the data. All authors have read and agree to the manuscript as written.

Results

Characteristics of Mice

Body weight and plasma cholesterol did not differ between groups (*Mmp14*$^{+/+}$→*Ldlr*$^{-/-}$ and *Mmp14*$^{-/-}$→*Ldlr*$^{-/-}$) consuming an atherogenic diet for 8 or 16 weeks. Total serum cholesterol level increased with the duration of the atherogenic diet and did not differ between groups (Table).

Table. Characteristics of Mice Used in the Present Analysis

	Mmp14$^{+/+}$→*Ldlr*$^{-/-}$	*Mmp14*$^{-/-}$→*Ldlr*$^{-/-}$	*P*
n	31	29	
Body weight, g			
Day 0	22.77±2.29	23.19±1.63	NS
+4 wk	22.42±1.71	23.36±2.95	NS
+12 wk	25.08±1.81	25.85±1.53	NS
+20 wk	24.8±2.4	25.6±2.6	NS
Total cholesterol, mg/dL			
Day 0	177.12±53.85	191.18±87.07	NS
+4 wk	218.84±61.37	204.07±103.53	NS
+12 wk	535.16±148.19	611.62±194.57	NS
+20 wk	1176±526.39	1127±661.97	NS

Mice consumed a high-fat diet for 8 weeks (n=17 for each group) or 16 weeks (n=14 for *Mmp14*$^{+/+}$→*Ldlr*$^{-/-}$; n=12 for *Mmp14*$^{-/-}$→*Ldlr*$^{-/-}$). Data are presented as mean±SD. NS indicates not significant.

Compound Mutant *Ldlr*$^{-/-}$ Mice With *Mmp14*$^{-/-}$ Bone Marrow–Derived Cells Expressed Less MMP-14 Than Those Receiving *Mmp14*$^{+/+}$ Bone Marrow–Derived Cells

Our experimental approach supposes that bone marrow–derived macrophages furnish most of the MMP-14 in the plaque. In the mice constructed for this study, only the bone marrow–derived cells from *Mmp14*$^{-/-}$ mice lack MMP-14. Many arterial SMCs and endothelial cells do not originate from bone marrow[29] but could produce MMP-14, especially in inflammatory environments such as atheromata.[30,31] Macrophages localized in the atherosclerotic intima of *Ldlr*$^{-/-}$ mice reconstituted from *Mmp14*$^{-/-}$ donors did not contain MMP-14, whereas in intimal lesions of *Ldlr*$^{-/-}$ mice receiving *Mmp14*$^{+/+}$ bone marrow, macrophages showed strong immunostaining for MMP-14 (Figure 2A). Quantitative analysis of aortic extracts demonstrated that the aortic wall of *Ldlr*$^{-/-}$ mice receiving *Mmp14*$^{-/-}$ bone marrow contained significantly less MMP-14 mRNA (Figure 2B) and protein (Figure 2C) compared with mice reconstituted with *Mmp14*$^{+/+}$ bone marrow.

MMP-14 Deficiency in Bone Marrow–Derived Cells Does Not Influence Atherosclerotic Lesion Size or Cellular Composition

Aortic root lesion size was similar in *Ldlr*$^{-/-}$ mice receiving *Mmp14*$^{+/+}$ bone marrow and *Ldlr*$^{-/-}$ mice receiving *Mmp14*$^{-/-}$ bone marrow (Figure 3A). Quantitative image analysis revealed similar intimal areas in *Ldlr*$^{-/-}$ mice reconstituted with *Mmp14*$^{+/+}$ or *Mmp14*$^{-/-}$ after 8 weeks (0.13±0.08 and 0.12±0.11 mm^2, respectively) or 16 weeks (0.33±0.10 and 0.25±0.12 mm^2, respectively) on atherogenic diet (Figure 3B). These lesions contained similar macrophage and SMC content at 16 weeks (Figure 3C). Quantitative image analysis confirmed a similar percentage of positive area for SMCs (α-actin$^+$) and macrophages (Mac-3$^+$) in plaques of mice receiving *Mmp14*$^{+/+}$ and *Mmp14*$^{-/-}$ bone marrow cells at 8 and 16 weeks (2.16±2.21% versus 2.46±2.03% and 5.38±1.89% versus 4.89±2.14% at 8 and 16 weeks, respectively, for SMCs; 5.26±2.2% versus 4.08±2.13% and 7.12±3.39% versus 6.08±2.13% at 8 and 16 weeks, respectively, for macrophages) (Figure 3D). Our results agree with our previous study that found similar plaque burden and cell content in *Mmp13*$^{-/-}$/*ApoE*$^{-/-}$ and *Mmp13*$^{+/+}$/*ApoE*$^{-/-}$ mice.[16]

MMP-14 Deficiency in Bone Marrow–Derived Cells Increases Collagen Content in the Atherosclerotic Intima

Aortic atheromata of *Ldlr*$^{-/-}$ mice receiving *Mmp14*$^{-/-}$ bone marrow cells displayed greater accumulation of fibrillar

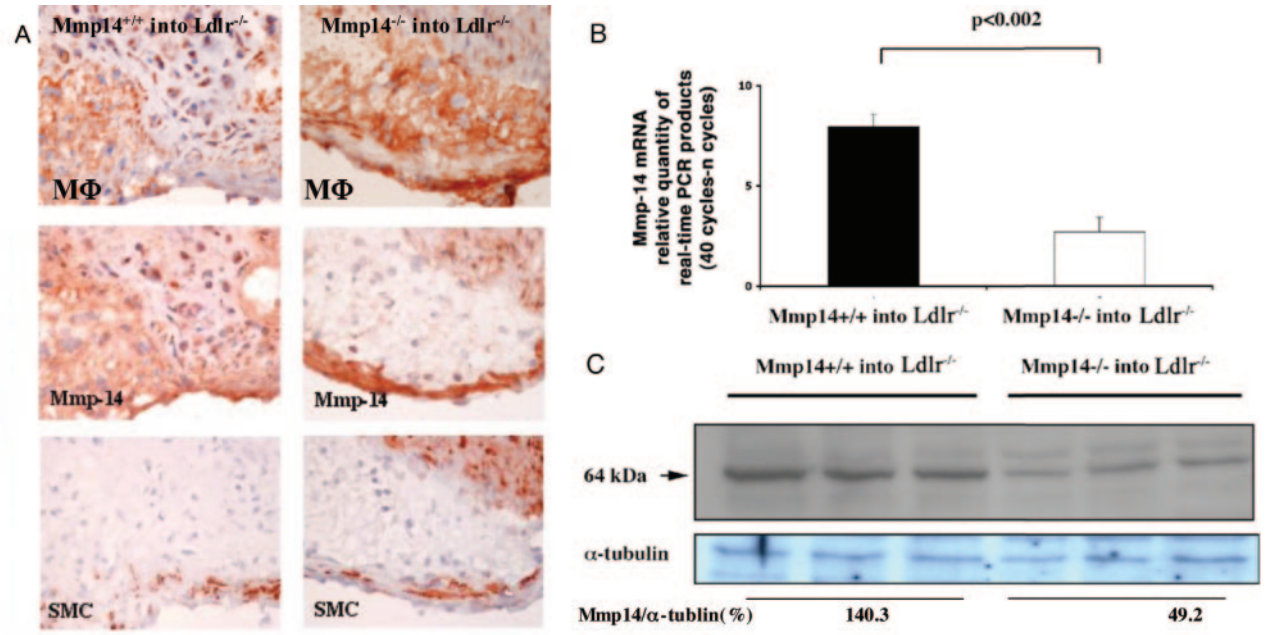

Figure 2. MMP-14 expression in the aortas of *Ldlr*$^{-/-}$ mice receiving *Mmp14*$^{+/+}$ or *Mmp14*$^{-/-}$ bone marrow cells. A, Immunoreactive MMP-14 colocalizes mainly with macrophages (MΦ) in the intima of *Ldlr*$^{-/-}$ mice transplanted with *Mmp14*$^{+/+}$ bone marrow and fed an atherogenic diet for 16 weeks (left). Most macrophages in the aortic intima of *Ldlr*$^{-/-}$ mice receiving *Mmp14*$^{-/-}$ bone marrow lack detectable MMP-14 for the duration of atherogenic diet consumption (right). SMCs stained positively for MMP-14 in both groups. These micrographs are representative of 3 mice analyzed from each group. High power=×40. B, Aortas (n=3, pooled) from *Ldlr*$^{-/-}$ mice receiving *Mmp14*$^{-/-}$ bone marrow contained significantly less MMP-14 mRNA, measured by real-time RT-PCR, compared with *Ldlr*$^{-/-}$ mice receiving *Mmp14*$^{+/+}$ bone marrow. Bars represent mean±SD. C, Transplantation of *Mmp14*$^{-/-}$ bone marrow cells into *Ldlr*$^{-/-}$ mice reduced the level of MMP-14 protein (64 kDa) detected by Western blot analysis. The same amount of total protein was loaded as shown by immunoreactive band to α-tubulin. Data represent results obtained from 3 mice from each group. Quantitative analysis revealed that transfer of *Mmp14*$^{-/-}$ bone marrow reduced the ratio of plaque MMP-14 and α-tubulin (mean percent) in plaques.

collagen, as shown by picrosirius red staining under polarized light (Figure 4A). Quantitative analysis showed that MMP-14 deficiency in bone marrow–derived cells increases collagen content expressed as the percentage of intimal area (Figure 4B). As observed in infarcted hearts of MMP-9–deficient mice,[32] targeted deletion of a single MMP may cause "compensatory" changes in expression of other MMPs or other enzymes involved in collagen turnover. After 16 weeks of atherogenic diet, RT-PCR analysis of mRNA expression in aortic extracts detected similar levels of all tested MMPs (MMP-2, -8, -9, -12, and -13) and a cysteine proteinase, cathepsin K, also implicated in arterial wall remodeling.[33] Nor did MMP-14 deficiency affect interstitial collagen gene expression because aortas of both experimental groups had similar procollagen Iα-mRNA levels (Figure 5).

MMP-14 Deficiency Decreases Collagenase Activity of Bone Marrow–Derived Macrophages

Because macrophages furnish most MMPs in atheromata, further experiments compared the collagenase activity of bone marrow–derived macrophages from *Mmp13*$^{-/-}$, *Mmp14*$^{-/-}$, and wild-type mice using a fluorescein-labeled collagen type I as substrate to monitor collagen degradation (Figure 6). Bone marrow–derived macrophages from *Mmp14*$^{-/-}$ mice had significantly lower collagenolytic activity than those from wild-type or *Mmp13*$^{-/-}$ mice. Although treatment with the proinflammatory cytokine TNF-α increased collagenolysis by macrophages, collagen-degrading activity remained higher in macrophages from wild-type mice compared with *Mmp14*$^{-/-}$ and *Mmp13*$^{-/-}$ mice. After addition of 1,10-phenanthroline, a nonselective metalloenzyme inhibitor, degradation of collagen in the 3 different conditions decreased almost to the level obtained without stimulation, suggesting that MMPs account for most of the TNF-α–induced collagenolytic activity.

MMP-14 Deficiency Alters Activation of MMP-13 but Not of MMP-2 or MMP-8

We previously demonstrated a key role of MMP-13 in the regulation of plaque collagen content.[16] Western blot analysis revealed that the level of latent MMP-13 (≈57 kDa) increased in aortic extracts of mice receiving *Mmp14*$^{-/-}$ bone marrow cells, whereas levels of active MMP-13 (≈45 kDa) decreased considerably. The level of a truncated form of MMP-13 (≈20 kDa) was similar in both groups (Figure 7A). Gelatin zymography demonstrated similar levels of latent and active MMP-2 in both groups (pro-Mmp2, ≈72 kDa; active Mmp2, ≈60 kDa) (Figure 7B), indicating that MMP-14 influences MMP-13 activation either directly or indirectly during atherogenesis. In the atherosclerotic aorta, however, MMP-14 does not appear critical to MMP-2 activation under these conditions. Western blot analysis revealed similar levels of active MMP-8 between the groups (Figure 7C).

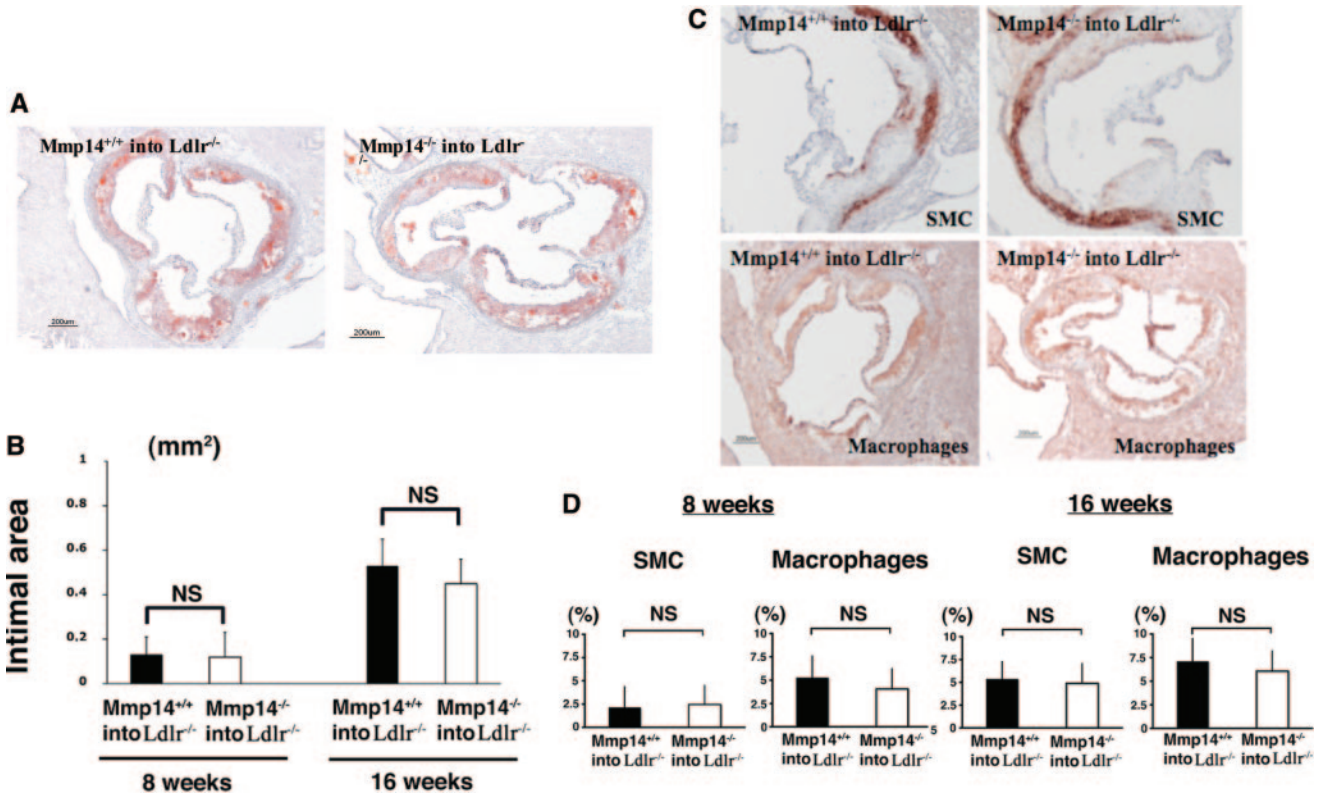

Figure 3. The size and macrophage and SMC accumulation in the intima of the aortic root in *Ldlr*$^{-/-}$ mice receiving *Mmp14*$^{+/+}$ or *Mmp14*$^{-/-}$ bone marrow. A, Oil Red O staining shows similar plaque size in the intima in both groups after 16 weeks of atherogenic diet. Scale bar=200 μmol/L. B, Quantitative analysis of the intimal area showed no difference between *Ldlr*$^{-/-}$ mice receiving *Mmp14*$^{+/+}$ or *Mmp14*$^{-/-}$ bone marrow and fed an atherogenic diet for 8 weeks or 16 weeks. Bars represent mean±SD. C, Immunohistochemical analysis of macrophages (Mac-3) or SMCs (α-actin) shows similar accumulation of positive cells in the intima of control and experimental group mice after 8 and 16 weeks of atherogenic diet. D, Quantitative analysis of the Mac-3 and α-actin immunopositive areas in the intima of *Ldlr*$^{-/-}$ mice receiving *Mmp14*$^{+/+}$ or *Mmp14*$^{-/-}$ bone marrow. Results express the percentage of positive area for macrophage and SMC staining according to the total area of intima. Bars represent mean±SD. Scale bar=200 μmol/L.

Discussion

This study demonstrates that MMP-14 influences the collagen level in mouse atherosclerotic plaque. Moreover, MMP-14–mediated interstitial collagenase activity in plaque depends substantially on bone marrow–derived cells. Indeed, bone marrow–derived inflammatory cells provide most of the MMP-14 within the arterial wall under atherogenic conditions.[30,34] Vascular SMCs also express considerable MMP-14 during mouse development.[35,36] However, the role of *Mmp14* in these cells will have to await the development of conditionally inactivated *Mmp14* mice, which are presently unavailable. In the meantime, successful transplantation of bone marrow—a critical step in this study—enabled analysis of the role of bone marrow–derived cells in experimental atherogenesis. An earlier study showed that MMP-9 participates importantly in the recruitment and maturation of bone marrow stem cells.[37] The bone marrow reconstitution experiments reported here indicate that trafficking of bone marrow cells to the atherosclerotic plaque does not require MMP-14 expression.

Deficiency of MMP-14 in macrophages does not affect the size or cellular content of plaques. Earlier work in *Mmp14*$^{-/-}$ mice indicated that MMP-14 acts as a collagenase during osteogenesis, soft tissue remodeling,[21,38] and degradation of the basement membrane to initiate neoangiogenesis.[22] Furthermore, MMP-14 has collagenolytic activity in vitro.[39,40] The present study establishes in vivo that MMP-14 influences collagen content of atherosclerotic plaques. A previous study compared the atherosclerotic lesion in the aorta between *Ldlr*$^{-/-}$ mice and littermate *Ldlr*$^{-/-}$ mice receiving total irradiation and bone marrow reconstitution.[41] The collagen layer was significantly thicker in untreated *Ldlr*$^{-/-}$ mice than in *Ldlr*$^{-/-}$ mice receiving total irradiation and bone marrow reconstitution, suggesting that bone marrow reconstitution may have influenced SMC involvement in plaque fibrosis.

Interpretation of the present data requires careful consideration of several potential confounders. Indeed, unexpected decreases in myocardial collagen after myocardial infarction in *Mmp9*$^{-/-}$ mice likely resulted from a compensatory increase in MMP-13 in infarcted tissue.[32] Therefore, we measured the RNA level of the other interstitial collagenases MMP-8 and MMP-13 and the potentially collagenolytic cysteine proteinase cathepsin K. MMP-14 deficiency affected neither the level of the other enzymes tested nor the level of procollagen-I mRNA. Thus, collagen accumulation in the atherosclerotic plaques of mice receiving *Mmp14*$^{-/-}$ bone marrow–derived cells did not appear to result from compensatory changes in other collagenases or in interstitial collagen gene expression. In vitro study of bone marrow–derived macrophages documented decreased collagen breakdown by

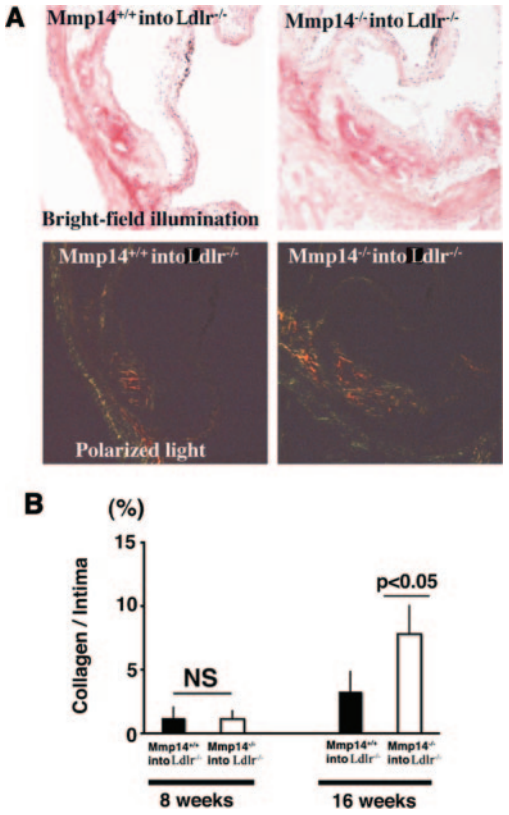

Figure 4. Collagen accumulation in the aortic intima of *Ldlr*$^{-/-}$ mice receiving *Mmp14*$^{+/+}$ or *Mmp14*$^{-/-}$ bone marrow cells. A, Picrosirius red staining viewed under bright light (top). Picrosirius red staining viewed under polarized light in the same sections shows fibrillar collagen (bottom). After 16 weeks of atherogenic diet, the intima of *Ldlr*$^{-/-}$ mice receiving *Mmp14*$^{-/-}$ bone marrow cells (right) contained more interstitial collagen than mice receiving *Mmp14*$^{+/+}$ bone marrow cells (left). B, Quantitative analysis of picrosirius red birefringence in the intima shows similar amounts of collagen in *Ldlr*$^{-/-}$ mice receiving *Mmp14*$^{+/+}$ or *Mmp14*$^{-/-}$ bone marrow at 8 weeks of atherogenic diet. At 16 weeks, the aortic intima of *Ldlr*$^{-/-}$ mice receiving *Mmp14*$^{-/-}$ bone marrow cells contained more collagen than mice receiving *Mmp14*$^{+/+}$ bone marrow cells. Bars represent mean±SD.

Mmp14$^{-/-}$ cells compared with wild-type and *Mmp13*$^{-/-}$ cells under unstimulated conditions or after TNF-α stimulation (Figure 6).

As previously shown, membrane type 1 MMP (MMP-14) participates in the activation of the latent forms of MMP-2 (progelatinase A) and MMP-13.[42–44] To test whether MMP-14 acts in the plaque directly as a collagenase or also acts by processing pro–MMP-13 and/or MMP-2, we examined the activation of MMP-13 and MMP-2 in the atherosclerotic aortas of mice reconstituted with MMP-14–deficient or wild-type bone marrow. Interestingly, deficiency of MMP-14 decreased the levels of activated MMP-13 but did not affect MMP-2 activation in atherosclerotic lesions, suggesting a dual role of MMP-14 in collagenolysis and plaque stabilization. Notably, MMP-14 deficiency did not affect MMP mRNA levels, including MMP-13, as demonstrated by real-time RT-PCR. Although MMP-14 deletion decreased accumulation of a cleaved form of MMP-13 in mouse atheromata, lack of MMP-14 did not affect MMP-8 activation. This result indicates that the situation in atheromata in vivo may differ

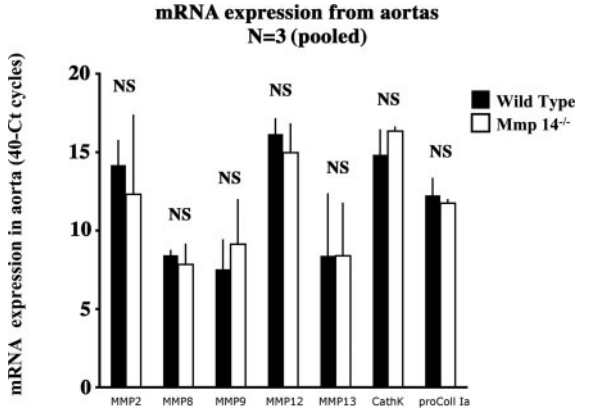

Figure 5. Real-time RT-PCR for mRNAs that encode molecules related to matrix remodeling and/or MMP-14 function. Aortas from *Ldlr*$^{-/-}$ mice receiving *Mmp14*$^{+/+}$ and *Mmp14*$^{-/-}$ bone marrow cells (n=3, pooled) and fed an atherogenic diet for 16 weeks contained similar mRNA levels of MMP-2/gelatinase-A, MMP-8/collagenase-2, MMP-9/gelatinase-B, MMP-12, MMP-13/collagenase-3, cathepsin K (CathK), and α1 chain of type I procollagen (pro Coll). Bars indicate mean±SD; NS, not significant.

from results of an in vitro study on human tear fluid MMP-8 during wound healing after acute eye injury[45] that suggested MMP-14–dependent activation of MMP-8. Because our previous analysis indicated a role for *Mmp13* activity in the atherosclerotic plaque, the present findings suggest that MMP-13 activation may mediate, at least in part, the effect of MMP-14 on collagen metabolism during atherogenesis. Unlike humans, mice lack MMP-1; hence, MMP-13 appears to

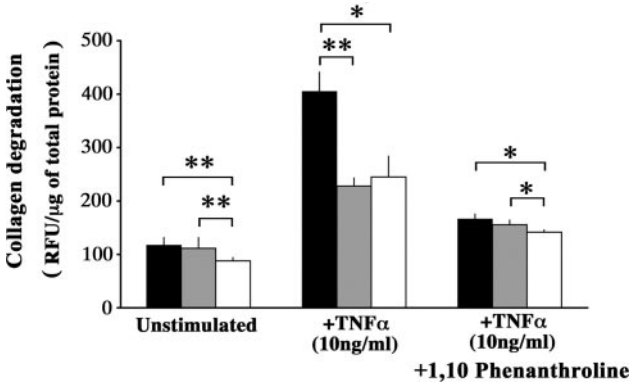

Figure 6. Collagen degradation by bone marrow–derived macrophages in vitro. Interstitial collagenolytic activity was determined after 48 hours of incubation of bone marrow–derived macrophages from wild-type (black bar), *Mmp13*$^{-/-}$ (gray bar), and *Mmp14*$^{-/-}$ mice (white bar) by release of soluble fluorescent material from FITC-labeled native collagen type I. Under unstimulated conditions, bone marrow–derived macrophages from *Mmp14*$^{-/-}$ mice showed less collagen breakdown compared with bone marrow–derived macrophages from *Mmp13*$^{-/-}$ and wild-type mice. After stimulation with TNF-α, collagenolysis by bone marrow–derived macrophages from wild-type mice exceeded that of cells from *Mmp13*$^{-/-}$ or *Mmp14*$^{-/-}$ mice. Addition of a metalloenzyme inhibitor (1,10-phenanthroline) decreased the collagenase activity of both groups. However, the collagenase activity of bone marrow–derived macrophages from *Mmp14*$^{-/-}$ mice remained lower than enzymatic activity of cells from wild-type and *Mmp13*$^{-/-}$ mice. Data are presented as mean±SD (n=3). Comparison of the respective study groups used the Mann-Whitney test; *$P<0.05$, **$P<0.01$. RFU indicates relative fluorescent units.

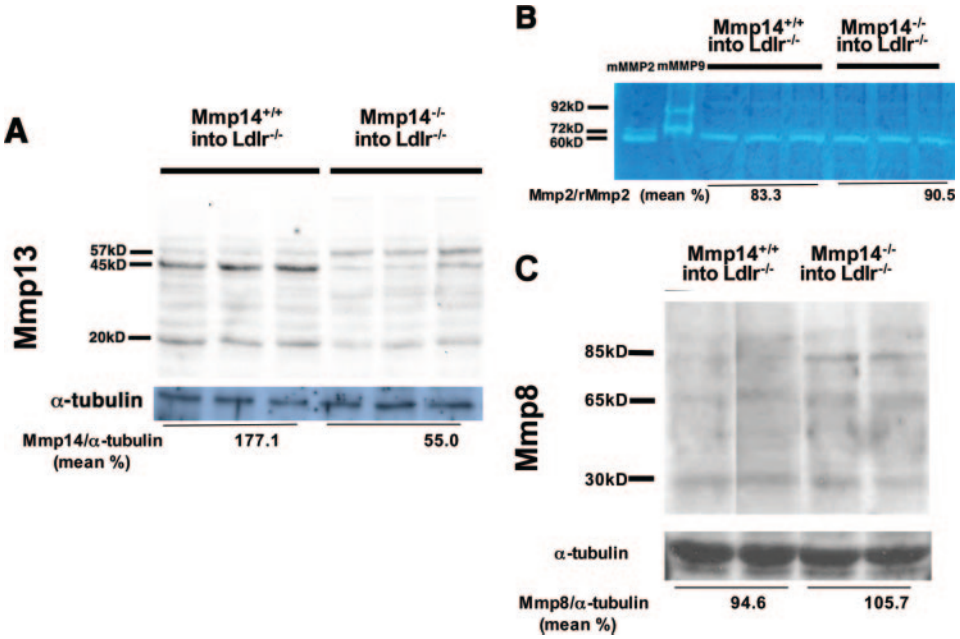

Figure 7. MMP-2, MMP-13, and MMP-8 activation in aortic protein extracts from *Ldlr*$^{-/-}$ mice receiving *Mmp14*$^{+/+}$ and *Mmp14*$^{-/-}$ bone marrow cells. A, Western blot analysis detected less of the presumed active form (45 kDa) and higher levels of the proform (57 kDa) of MMP-13 in mice receiving *Mmp14*$^{-/-}$ bone marrow cells compared with mice receiving *Mmp14*$^{+/+}$ bone marrow cells. The level of a truncated form of 20 kDa was similar between both groups. Equal protein loading was affirmed by assessing α-tubulin. Quantitative analysis revealed that transfer of *Mmp14*$^{-/-}$ bone marrow reduced the ratio of active MMP-13 and α-tubulin (mean percent) in plaques. B, Gelatin zymography detected similar levels of the active form of MMP-2 in mice receiving *Mmp14*$^{+/+}$ or *Mmp14*$^{-/-}$ bone marrow cells. The bands were identified by comparison with bands from digested recombinant mouse MMP-2 and MMP-9. Quantitative analysis indicated that the ratios of active MMP-2 and recombinant MMP-2 were similar in the 2 groups (mean percent). C, Western blot analysis detected no difference in level of the presumed active form (65 kDa) and higher levels of the proform (85 kDa) of MMP-8 in mice receiving *Mmp14*$^{-/-}$ bone marrow cells compared with mice receiving *Mmp14*$^{+/+}$ bone marrow cells. The level of a truncated form of 30 kDa was similar between groups. Equal protein loading was affirmed by assessing α-tubulin. Quantitative analysis showed that the ratios of active MMP-8 and α-tubulin were similar in the 2 groups (mean percent).

subserve in mice the functions of MMP-1 in humans. These considerations illustrate that the pathophysiological principles demonstrated here may not apply directly to humans. Although several tissue and cell types require MMP-14 for activation of pro–MMP-2, our present data suggest that alternate mechanisms operate in atheromata.[21,24] In addition, a recent study described how MMP-14 had developmental effects that did not depend on its role in pro–MMP-2 activation during lung and submandibular gland maturation.[46] Taken together, these data suggest that effects of macrophage-derived MMP-14 other than pro–MMP-2 activation dominate in atherosclerosis. Because furin activates MMP-14 intracellularly in the trans-Golgi, MMP-14 can exert its proteolytic activity in the pericellular space as soon as it anchors in the cell membrane.[47] A recent study showed that the furin-like proconvertase PC5 also can activate MMP-14 in vascular SMCs.[48]

Human coronary artery plaques that have caused fatal thrombosis typically have a thin fibrous cap, reduced levels of intact interstitial collagen, and abundant levels of MMP-13[12] and MMP-14[17] and display biochemical signatures of collagenolysis in situ. The present study found that MMP-14 deficiency in bone marrow–derived cells (primarily macrophages) does not influence atherosclerotic lesion size or cellular composition but does, with time, substantially increase lesional content of fibrillar collagen. These results agree with our previous findings that collagenase resistance or MMP-13 deficiency promoted collagen accumulation, a key molecular determinant of plaque stability, but not atheroma burden.[15,16]

The present study used cholesterol-fed *Ldlr*$^{-/-}$ mice, an established mouse model of hypercholesterolemia, to induce accumulation of plaque macrophages, a major source of MMPs. Macrophage infiltration in hypercholesterolemia promotes MMP production in arteries.[1–4] Indeed, we reported early on that oxidatively modified low-density lipoprotein could promote MMP-14 expression in vascular cells in culture.[30] Our own work and studies of others have demonstrated that lipid lowering reduces MMP expression and yields collagen accumulation in atheromata.[3]

Taken together with studies using *Mmp13*$^{-/-}$ and *Col*$^{R/R}$ mice,[15,16] the present work further supports the involvement of collagenases from the MMP family in arterial collagen remodeling and illustrates a novel aspect of collagen metabolism in atherosclerosis. These results shed new mechanistic light on the molecular and cellular mechanisms that promote collagen degradation and thus may influence the biomechanical properties of plaques. The role of bone marrow–derived cells, principally macrophages, in regulating collagen accumulation demonstrated here underscores the role of inflammation in clinically critical aspects of plaque biology.

Acknowledgments

We thank Eugenia Shvartz for technical assistance and Joan Perry for editorial assistance. Dr Andreas Zirlik assisted with the *Ldlr*$^{-/-}$ (CD45.1-positive) mouse strain husbandry.

Sources of Funding

This work was supported by a grant from the National Heart, Lung, and Blood Institute (HL-080472), a Transatlantic Network of Excellence in Atherothrombosis grant from the Fondation Leducq (Dr Libby), and a Harold M. English grant from Harvard Medical International (Dr Schneider). Dr Gerdes received a PhD scholarship from the Schering Foundation.

Disclosures

None.

References

1. Falk E, Shah PK, Fuster V. Coronary plaque disruption. *Circulation*. 1995;92:657–671.
2. Libby P, Theroux P. Pathophysiology of coronary artery disease. *Circulation*. 2005;111:3481–3488.
3. Aikawa M, Libby P. The vulnerable atherosclerotic plaque: pathogenesis and therapeutic approach. *Cardiovasc Pathol*. 2004;13:125–138.
4. Libby P. Molecular bases of the acute coronary syndromes. *Circulation*. 1995;91:2844–2850.
5. Visse R, Nagase H. Matrix metalloproteinases and tissue inhibitors of metalloproteinases: structure, function, and biochemistry. *Circ Res*. 2003; 92:827–839.
6. Nagase H, Woessner JF Jr. Matrix metalloproteinases. *J Biol Chem*. 1999;274:21491–21494.
7. Knäuper V, Will H, Lopez-Otin C, Smith B, Atkinson SJ, Stanton H, Hembry RM, Murphy G. cellular mechanisms for human procollagenase-3 (MMP13) activation. *J Biol Chem*. 1996;271:17124–17131.
8. Sato H, Takino T, Okada Y, Shinagawa A, Yamamoto E, Seiki M. A matrix metalloproteinase expressed on the surface of invasive tumor cells. *Nature*. 1994;370:61–65.
9. Strongin AY, Marmer BL, Grant GA, Goldberg GI. Plasma membrane-dependent activation of the 72-kDa type IV collagenase is prevented by complex formation with TIMP2. *J Biol Chem*. 1993;268:14033–14039.
10. Galis ZS, Sukhova GK, Lark MW, Libby P. Increased expression of matrix metalloproteinases and matrix degrading activity in vulnerable regions of human atherosclerotic plaques. *J Clin Invest*. 1994;94: 2493–2503.
11. Nikkari ST, O'Brien KD, Ferguson M, Hatsukami T, Welgus HG, Alpers CE, Clowes AW. Interstitial collagenase (MMP-1) expression in human carotid atherosclerosis. *Circulation*. 1995;92:1393–1398.
12. Aikawa M, Rabkin E, Voglic SJ, Shing H, Nagai R, Schoen FJ, Libby P. Lipid lowering promotes accumulation of mature smooth muscle cells expressing smooth muscle myosin heavy chain isoforms in rabbit atheroma. *Circ Res*. 1998;83:1015–1026.
13. Sukhova GK, Schonbeck U, Rabkin E, Schoen FJ, Poole AR, Billinghurst RC, Libby P. Evidence for increased collagenolysis by interstitial collagenases-1 and -3 in vulnerable human atheromatous plaques. *Circulation*. 1999;99:2503–2509.
14. Herman MP, Sukhova GK, Libby P, Gerdes N, Tang N, Horton DB, Kilbride M, Breitbart RE, Chun M, Schonbeck U. Expression of neutrophil collagenase (matrix metalloproteinase-8) in human atheroma: a novel collagenolytic pathway suggested by transcriptional profiling. *Circulation*. 2001;104:1899–1904.
15. Fukumoto Y, Deguchi J, Libby P, Rabkin-Aikawa E, Sakata Y, Chin MT, Hill CC, Lawler PR, Varo N, Schoen FJ, Krane SM, Aikawa M. Genetically determined resistance to collagenase action augments interstitial collagen accumulation in atherosclerotic plaques. *Circulation*. 2004;110: 1953–1959.
16. Deguchi J, Aikawa E, Libby P, Vachon JR, Inada M, Krane SM, Whittaker P, Aikawa M. Matrix metalloproteinase-13/collagenase-3 deletion promotes collagen accumulation and organization in mouse atherosclerotic plaques. *Circulation*. 2005;112:2708–2715.
17. Rajavashisth TB, Xu XP, Jovinge S, Meisel S, Xu XO, Chai NN, Fishbein MC, Kaul S, Cercek B, Sharifi B, Shah PK. Membrane type 1 matrix metalloproteinase expression in human atherosclerotic plaques: evidence for activation by proinflammatory mediators. *Circulation*. 1999;99: 3103–3109.
18. Schmidt R, Bultmann A, Ungerer M, Joghetaei N, Bulbul O, Thieme S, Chavakis T, Toole BP, Gawaz M, Schomig A, May AE. Extracellular matrix metalloproteinase inducer regulates matrix metalloproteinase activity in cardiovascular cells: implications in acute myocardial infarction. *Circulation*. 2006;113:834–841.
19. Jenkins GM, Crow MT, Bilato C, Gluzband Y, Ryu WS, Li Z, Stetler-Stevenson W, Nater C, Froehlich JP, Lakatta EG, Cheng L. Increased expression of membrane-type matrix metalloproteinase and preferential localization of matrix metalloproteinase-2 to the neointima of balloon-injured rat carotid arteries. *Circulation*. 1998;97:82–90.
20. Deschamps AM, Yarbrough WM, Squires CE, Allen RA, McClister DM, Dowdy KB, McLean JE, Mingoia JT, Sample JA, Mukherjee R, Spinale FG. Trafficking of the membrane type-1 matrix metalloproteinase in ischemia and reperfusion: relation to interstitial membrane type-1 matrix metalloproteinase activity. *Circulation*. 2005;111:1166–1174.
21. Holmbeck K, Bianco P, Caterina J, Yamada S, Kromer M, Kuznetsov SA, Mankani M, Robey PG, Poole AR, Pidoux I, Ward JM, Birkedal-Hansen H. MT1-MMP-deficient mice develop dwarfism, osteopenia, arthritis, and connective tissue disease due to inadequate collagen turnover. *Cell*. 1999;99:81–92.
22. Hiraoka N, Allen E, Apel IJ, Gyetko MR, Weiss SJ. Matrix metalloproteinases regulate neovascularization by acting as pericellular fibrinolysins. *Cell*. 1998;95:365–377.
23. Ray BK, Shakya A, Turk JR, Apte SS, Ray A. Induction of the MMP-14 gene in macrophages of the atherosclerotic plaque: role of SAF-1 in the induction process. *Circ Res*. 2004;95:1082–1090.
24. Zhou Z, Apte SS, Soininen R, Cao R, Baaklini GY, Rauser RW, Wang J, Cao Y, Tryggvason K. Impaired endochondral ossification and angiogenesis in mice deficient in membrane-type matrix metalloproteinase I. *Proc Natl Acad Sci U S A*. 2000;97:4052–4057.
25. Bavendiek U, Zirlik A, LaClair S, MacFarlane L, Libby P, Schonbeck U. Atherogenesis in mice does not require CD40 ligand from bone marrow-derived cells. *Arterioscler Thromb Vasc Biol*. 2005;25:1244–1249.
26. Sukhova GK, Zhang Y, Pan JH, Wada Y, Yamamoto T, Naito M, Kodama T, Tsimikas S, Witztum JL, Lu ML, Sakara Y, Chin MT, Libby P, Shi GP. Deficiency of cathepsin S reduces atherosclerosis in LDL receptor-deficient mice. *J Clin Invest*. 2003;111:897–906.
27. Sukhova GK, Wang B, Libby P, Pan JH, Zhang Y, Grubb A, Fang K, Chapman HA, Shi GP. Cystatin C deficiency increases elastic lamina degradation and aortic dilatation in apolipoprotein E-null mice. *Circ Res*. 2005;96:368–375.
28. Shimizu K, Shichiri M, Libby P, Lee RT, Mitchell RN. Th2-predominant inflammation and blockade of IFN-gamma signaling induce aneurysms in allografted aortas. *J Clin Invest*. 2004;114:300–308.
29. Hoognagle MH, Thomas JA, Wamhoff BR, Owens GK. Origin of neo intimal smooth muscle: we've come full circle. *Arterioscler Thromb Vasc Biol*. 2006;26:2579–2581.
30. Rajavashisth TB, Liao JK, Galis ZS, Tripathi S, Laufs U, Tripathi J, Chai NN, Xu XP, Jovinge S, Shah PK, Libby P. Inflammatory cytokines and oxidized low density lipoproteins increase endothelial cell expression of membrane type 1-matrix metalloproteinase. *J Biol Chem*. 1999;274: 11924–11929.
31. Shofuda K, Yasumitsu H, Nishihashi A, Miki K, Miyazaki K. Expression of three membrane-type matrix metalloproteinases (MT-MMPs) in rat vascular smooth muscle cells and characterization of MT3-MMPs with and without transmembrane domain. *J Biol Chem*. 1997;272:9749–9754.
32. Ducharme A, Frantz S, Aikawa M, Rabkin E, Lindsey M, Rohde LE, Schoen FJ, Kelly RA, Werb Z, Libby P, Lee RT. Targeted deletion of matrix metalloproteinase-9 attenuates left ventricular enlargement and collagen accumulation after experimental myocardial infarction. *J Clin Invest*. 2000;106:55–62.
33. Cheng XW, Kuzuya M, Sasaki T, Arakawa K, Kanda S, Sumi D, Koike T, Maeda K, Tamaya-Mori N, Shi GP, Saito N, Iguchi A. Increased expression of elastolytic cysteine proteases, cathepsins S and K, in the neointima of balloon-injured rat carotid arteries. *Am J Pathol*. 2004;164: 243–251.
34. Uzui H, Harpf A, Liu M, Doherty TM, Shukla A, Chai NN, Tripathi PV, Jovinge S, Wilkin DJ, Asotra K, Shah PK, Rajavashisth TB. Increased expression of membrane type 3-matrix metalloproteinase in human atherosclerotic plaque: role of activated macrophages and inflammatory cytokines. *Circulation*. 2002;106:3024–3030.
35. Apte SS, Fukai N, Beier DR, Olsen BR. The matrix metalloproteinase-14 (MMP14) gene is structurally distinct from other MMP genes and is co-expressed with the TIMP-2 gene during mouse embryogenesis. *J Biol Chem*. 1997;272:25511–25517.
36. Lehti K, Allen E, Birkedal-Hansen H, Holmbeck K, Miyake Y, Chun TH, Weiss SJ. An MT1-MMP-PDGF receptor-beta axis regulate mural cell investment of the microvasculature. *Genes Dev*. 2005;19:979–991.
37. Heissig B, Hattori K, Dias S, Friedrich M, Ferris B, Hackett NR, Crystal RG, Besmer P, Lyden D, Moore MA, Werb Z, Rafii S. Recruitment of

stem and progenitor cells from the bone marrow niche requires MMP-9 mediated release of kit-ligand. *Cell*. 2002;109:625–637.
38. Atkinson SJ, Patterson ML, Butler MJ, Murphy G. Membrane type 1 matrix metalloproteinase and gelatinase A synergistically degrade type 1 collagen in a cell model. *FEBS Lett*. 2001;491:222–226.
39. Jiang A, Pei D. Distinct roles of catalytic and pexin-like domains in membrane-type matrix metalloproteinase (MMP)-mediated pro-MMP-2 activation and collagenolysis. *J Biol Chem*. 2003;278:38765–38771.
40. Ohuchi E, Imai K, Fujii Y, Sato H, Seiki M, Okada Y. Membrane type 1 matrix metalloproteinase digests interstitial collagens and other extracellular matrix macromolecules. *J Biol Chem*. 1997;272:2446–2451.
41. Schiller NK, Kubo N, Boisvert WA, Curtiss LK. Effect of gamma-irradiation and bone marrow transplantation on atherosclerosis in LDL receptor-deficient mice. *Arterioscler Thromb Vasc Biol*. 2001;21:1674–1680.
42. D'Ortho M, Stanton H, Butler M, Atkinson SJ, Murphy G, Hembry RM. MT1-MMP on the cell surface causes focal degradation of gelatin films. *FEBS Lett*. 1998;421:159–164.
43. Fosang AJ, Last K, Fuji Y, Seiki M, Okada Y. Membrane-type 1 MMP (MMP-14) cleaves at three sites in the aggrecan interglobular domain. *FEBS Lett*. 1998;430:186–190.
44. Pei D, Weiss SJ. Transmembrane-deletion mutants of the membrane-type matrix metalloproteinase-1 process progelatinase A and express intrinsic matrix-degrading activity. *J Biol Chem*. 1996;271:9135–9140.
45. Holopainen JM, Moilanen JA, Sorsa T, Kivelä-Rajamäki M, Tervahartiala T, Vesaluoma MH, Tervo TMT. Activation of matrix metalloproteinase-8 by membrane type 1-MMP and their expression in human tears after photorefractive keratectomy. *Invest Ophthalmol Vis Sci*. 2003;44:2550–2556.
46. Oblander SA, Zhou Z, Galvez BG, Starcher B, Shannon JM, Durbeej M, Arroyo AG, Tryggvason K, Apte SS. Distinctive functions of membrane type 1 matrix-metalloprotease (MT1-MMP or MMP-14) in lung and submandibular gland development are independent of its role in pro-MMP-2 activation. *Dev Biol*. 2005;277:255–269.
47. Yana I, Weiss SJ. Regulation of membrane type-1 matrix metalloproteinase activation by proprotein convertases. *Mol Biol Cell*. 2000;11:2387–2401.
48. Stawowy P, Meyborg H, Stibenz D, Borges Pereira Stawowy N, Roser M, Thanabalasingam U, Veinot JP, Chretien M, Seidah NG, Fleck E, Graf K. Furin-like proprotein convertases are central regulators of the membrane type matrix metalloproteinase-pro-matrix metalloproteinase-2 proteolytic cascade in atherosclerosis. *Circulation*. 2005;111:2820–2827.

CLINICAL PERSPECTIVE

Many data support the concept that the collagen content of the fibrous cap of an atherosclerotic plaque governs its propensity to rupture and causes thrombotic complications. The mechanisms that regulate the plaque collagen content thus have considerable clinical interest. Members of the matrix metalloproteinase (MMP) family possess interstitial collagenase activity capable of breaking down this critical component of the plaque structure. Previous work has highlighted a role for MMP-13, a secreted interstitial collagenase, in collagen breakdown in mouse atherosclerotic plaques. The present study tested the role of a membrane-bound MMP (MMP-14) present in plaques in regulating the interstitial collagen content of atheromatous lesions. Animals that lack MMP-14 do not survive to adulthood, so these experiments used animals with bone marrow cells lacking MMP-14 to test the hypothesis that this enzyme contributes to collagen catabolism in mouse atheromata. After 16 weeks of an atherogenic diet, low-density lipoprotein receptor–deficient mice engrafted with MMP-14–deficient bone marrow contain significantly more interstitial collagen compared with those receiving wild-type bone marrow. The effect of MMP-14 on collagen metabolism may be indirect through activation of the latent zymogen form of MMP-13 as shown by biochemical experiments. Thus, MMP-14 from bone marrow–derived cells can influence the collagen content of atheromata, a critical component of plaque stability.