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Matrix Metalloproteinase-9 Modulation by Resident Arterial Cells Is Responsible for Injury-Induced Accelerated Atherosclerotic Plaque Development in Apolipoprotein E–Deficient Mice

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Objective—Although matrix metalloproteinase-9 (MMP-9) has been implicated in atherosclerotic plaque instability, the exact role it plays in the plaque development and progression remains largely unknown. We generated apolipoprotein E (apoE)–deficient (apoE$^{-/-}$) MMP-9–deficient (MMP-9$^{-/-}$) mice to determine the mechanisms and the main cell source of MMP-9 responsible for the plaque composition during accelerated atherosclerotic plaque formation.

Methods and Results—Three weeks after temporary carotid artery ligation revealed that while on a Western-type diet, apoE$^{-/-}$ MMP-9$^{-/-}$ mice had a significant reduction in intimal plaque length and volume compared with apoE$^{-/-}$ MMP-9$^{+/+}$ mice. The reduction in plaque volume correlated with a significantly lower number of intraplaque cells of resident cells and bone marrow–derived cells. To determine the cellular origin of MMP-9 in plaque development, bone marrow transplantation after total-body irradiation was performed with apoE$^{-/-}$ MMP-9$^{+/+}$ and apoE$^{-/-}$ MMP-9$^{-/-}$ mice, which showed that only MMP-9 derived from resident arterial cells is required for plaque development.

Conclusions—MMP-9 is derived from resident arterial cells and is required for early atherosclerotic plaque development and cellular accumulation in apoE$^{-/-}$ mice. (***Arterioscler Thromb Vasc Biol*. 2005;25:1020-1025.**)

Key Words: atherosclerosis ■ MMP-9 ■ bone marrow ■ mouse ■ compartmentalization

Several matrix metalloproteinases (MMPs), namely MMP-1, MMP-2, MMP-3, MMP-7, MMP-9, MMP-11, and MMP-14, are present in human atherosclerotic lesions, leading to the speculation that overexpression of these enzymes is linked to atherogenesis.[1–6] However, experimental models of atherosclerosis demonstrated that mice deficient in apolipoprotein E (apoE) and MMP-3 had larger atherosclerotic plaque, whereas apoE-deficient (apoE$^{-/-}$) mice overexpressing human MMP-1 had a reduction in plaque size.[1,7] Moreover, inactivation of tissue inhibitor of metalloproteinase 1, which can inhibit MMP-1, MMP-3, MMP-9, and MMP-11 activity and is overexpressed in human atherosclerotic lesions, reduced the plaque size in apoE$^{-/-}$ mice.[1] Thus, it is likely that some MMPs are involved in atherogenic processes, whereas others function to inhibit plaque formation.

MMP-9 (gelatinase B) is expressed in late atherosclerotic lesions in humans and has been suggested to mediate plaque instability, a leading cause of acute coronary syndrome and stroke.[2,3] Studies in humans have revealed that polymorphisms in the MMP-9 promoter, which enhance expression, correlate with the development and progression of coronary atherosclerosis.[8,9] Studies with apoE$^{-/-}$ MMP-9$^{-/-}$ mice have demonstrated that MMP-9 is critical to intimal plaque size.[10,11] To determine the mechanisms and the main cell source of MMP-9 responsible for the plaque composition during accelerated atherosclerotic plaque formation, we also cross-bred apoE$^{-/-}$ mice with MMP-9$^{-/-}$ mice. We demonstrate that although MMP-9 expression is derived mostly from bone marrow cells, MMP-9 derived from resident arterial cells dictates the overall plaque composition. Therefore, MMP-9 activity associated with the resident cells (compartmentalization) is required for atherosclerotic plaque development and cellular accumulation in apoE$^{-/-}$ mice.

Methods

Preparations for Carotid Atherosclerotic Plaque Development

All procedures were approved by the animal studies committee at Washington University. F0 mice used were apoE$^{-/-}$ mice (background C57BL/6) and MMP-9$^{-/-}$ mice[12] (129 SvEv). We then generated F1 mice that were heterozygous for MMP-9 and heterozygous for apoE (apoE$^{+/-}$ MMP-9$^{+/-}$) with a mixed background (50% C57BL/6 and 50% 129 SvEv). F2 breeding pairs for mice deficient

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in the MMP-9 gene and the apoE gene (apoE$^{-/-}$ MMP-9$^{-/-}$; dKO) and littermate mice deficient in apoE gene only (apoE$^{-/-}$ MMP-9$^{+/+}$; sKO) were based on these F1 parental lines (1:16 ratio). Southern blots of tail DNA for germline transmission of the MMP-9 allele was performed as described previously.[12] RT-PCR of tail DNA was performed for the apoE allele as described previously[13] and confirmed by serum cholesterol levels on regular chow. F3 mice were then generated from F2 breeding pairs of dKO and sKO mice and genotyped again by RT-PCR of tail DNA for the MMP-9 and apoE alleles before any use. The following sense and antisense primers were used: MMP-9: 5′-GCATACTTGTACCGCTATGG -3′; 5′-TAACCGGAGGTCCAAACTGG-3′ and neomycin: 5′-GAAGGG-ACTGGCTGCTATTG-3′; 5′-AATATCACGGGTAGCCAACG-3′. The dKO mice were healthy and indistinguishable from the sKO mice in appearance. Experiments were performed in male mice of 8 to 10 weeks of age without knowledge of genotype.

One week before the surgery (temporary ligation placed on the middle of the left common carotid artery),[13] animals were started on a high-fat diet (42% of total calories from fat; 0.15% cholesterol; Harlan-Teklad; Madison, Wis). The ligature was removed after 2 days, restoring the carotid blood flow. At 0 days (sham surgery without ligation), 2 days (restoration of flow), 4 days, 7 days, and 3 weeks, animals were euthanized, and carotid arteries were either harvested for zymography (snap-frozen) or were perfusion-fixed via the left ventricle at 100 mm Hg pressure as described previously.[13] Before perfusion fixation, blood was drawn from the left ventricle for analysis of levels with use of an Affinity Cholesterol Reagent Procedure 402 (Sigma-Aldrich). Standards were obtained from a cholesterol standard solution (200 mg/dL; Wako Chemicals).

Gelatin Substrate Zymography

Frozen tissue samples were pulverized under liquid nitrogen (LN_2) and extracted in ice-cold 50 mmol/L Tris-HCl buffer, pH 7.5, containing 1.0 mol/L NaCl, 2.0 mol/L urea, 0.1% (wt/vol) Brij-35, 0.1% EDTA, and protease inhibitor cocktail P8340 (Sigma-Aldrich). After centrifugation at 10 000*g* for 1 hour at 4°C, the supernatant was concentrated by centrifugation using a 5000-MW cutoff membrane. Samples (10 μg) were resolved by SDS-PAGE under nonreducing conditions, using 12% polyacrylamide gels containing 1 mg/mL gelatin. Gels were washed with Triton X-100 to remove the SDS, then incubated overnight (37°C) in 50 mmol/L Tris-HCl, pH 8.5, 5 mmol/L $CaCl_2$, and 0.5 mmol/L $ZnCl_2$. Zones of lysis were visualized after staining the gels with 0.5% Coomassie blue R-250. The quantitative evaluation of the gelatinolytic was performed by scanning the gel using a Bio-Rad GS 700 imaging densitometer (Bio-Rad). Dilutions of culture medium conditioned by HT 1080 cells were used as an internal standard.

Immunohistochemistry and Histomorphometry

The entire length of the carotid arteries was processed and embedded in paraffin. Serial sections 5-μm thick were obtained every 100 μm throughout the vessel and were stained with Verhoeff-van Gieson elastic stain.[14] For immunohistochemistry, sections were stained for smooth muscle cells (SMCs; SM α-actin; 1:500 dilution; Sigma-Aldrich) and for macrophages (Mac-3; 1:1000 dilution; PharMingen) as described previously.[15] Volumetric measurements for intimal atherosclerotic plaque and intraplaque Mac-3, foam cell, SM α-actin staining were performed on digitized images using Olympus Microsuite software (Soft Imaging Systems).

Sirius Red Staining for Collagen

Sections were stained with 2% Sirius red (Fast red F3B) in saturated aqueous picric acid for 60 minutes at room temperature and destained in 2 changes of 30% (v/v) acetic acid over 10 minutes. Sections were dehydrated, cleared in xylene, and mounted. Quantification of collagen content was performed using picrosirius red staining and digital image microscopy with polarized light. Volumetric measurements for collagen content were performed on digitized images using Olympus Microsuite software.

Histological Quantitation

Histological slides were viewed using the Olympus BX60-F3 microscope (Olympus Optical Co. Ltd.). An Olympus U-POT polarizing filter and a U-ANT analyzer were used to view collagen slides with circularly polarized light. Images were captured using the Olympus CV-12 camera with an Olympus U-CMAD-2 adapter and an Olympus U-TV 0.5x photo tube. Images were transferred to a Dell personal computer (model 6312KL-04W-B66) and viewed on a Dell Ultra Sharp monitor (model 2000FP). Images were captured using Olympus Microsuite Basic software version 3.1. Mac-3 and SM α-actin areas were measured using a mouse-driven tracing method similar to that used to analyze intimal plaque area (Olympus Microsuite Basic). Because of the more disperse nature of collagen and SM α-actin stains, these areas were quantified using Optimas software version 6.5.172 (Media Cybernetics). A region of interest was selected to include only the intimal plaque area. SM α-actin staining was quantified by setting hue thresholds to include the maximum amount of staining in every slide. Collagen staining was quantified similarly. Data were collected to analyze percent positive staining area per region of interest.

Irradiation and Bone Marrow Transplantation

ApoE$^{-/-}$ MMP-9$^{-/-}$ and apoE$^{-/-}$ MMP-9$^{+/+}$ mice underwent lethal gamma irradiation (≈9.5 Gy) from a cesium source, followed 6 hours later by transplantation with femoral bone marrow cells obtained from 8- to 10-week-old apoE$^{-/-}$ MMP-9$^{-/-}$ and apoE$^{-/-}$ MMP-9$^{+/+}$ donors (5×10^6 cells per recipient; 0.3 to 0.5 mL by intravenous injection). Two groups were studied: apoE$^{-/-}$ MMP-9$^{-/-}$ donor marrow transplanted to apoE$^{-/-}$ MMP-9$^{+/+}$ recipients, and apoE$^{-/-}$ MMP-9$^{+/+}$ donor marrow transplanted to apoE$^{-/-}$ MMP-9$^{-/-}$ recipients. Mice were housed in a specific pathogen–free barrier environment. All nontransplanted mice died 7 to 14 days after irradiation. Animals achieving successful engraftment, a return to a normal complete blood count after the total body irradiation and bone marrow transplantation (BMT), underwent temporary carotid ligation 6 weeks after transplantation, and carotids were harvested 1 and 3 weeks later.

Statistical Analysis

Results are shown as mean±SEM. Excel 2000 statistical package was used for the quantitative analyses of intimal plaque volume, intraplaque foam cell, Mac-3, SM α-actin, and collagen-stained volume (Student's *t* test).

Results

MMP-9 Levels During Atherogenesis in ApoE$^{-/-}$ Mice

We used apoE$^{-/-}$ MMP-9$^{+/+}$ mice to assess the levels of MMP-2 and MMP-9 activity after temporary ligation while on a high-fat diet. By gelatin substrate zymography, MMP-9 was not detected in nonligated carotids, but its levels increased soon after temporary ligation, peaking at 4 days and then tapering a bit thereafter (Figure 1A). In contrast, MMP-2 was detected in nonligated carotids, but similar to MMP-9, MMP-2 levels increased after injury, peaking at 7 days. As expected, MMP-9 was not detected in apoE$^{-/-}$ MMP-9$^{-/-}$ (dKO) mice after temporary ligation, whereas MMP-9 was clearly detected in apoE$^{-/-}$ MMP-9$^{+/+}$ (sKO) mice (Figure 1B).

MMP-9 Deficiency Reduces Plaque Volume in ApoE$^{-/-}$ Mice

To determine whether the induction of MMP-9 levels affected intimal atherosclerotic plaque formation, we performed temporary ligation on apoE$^{-/-}$ MMP-9$^{-/-}$ mice and age-matched apoE$^{-/-}$ MMP-9$^{+/+}$ mice. Twenty-one days after

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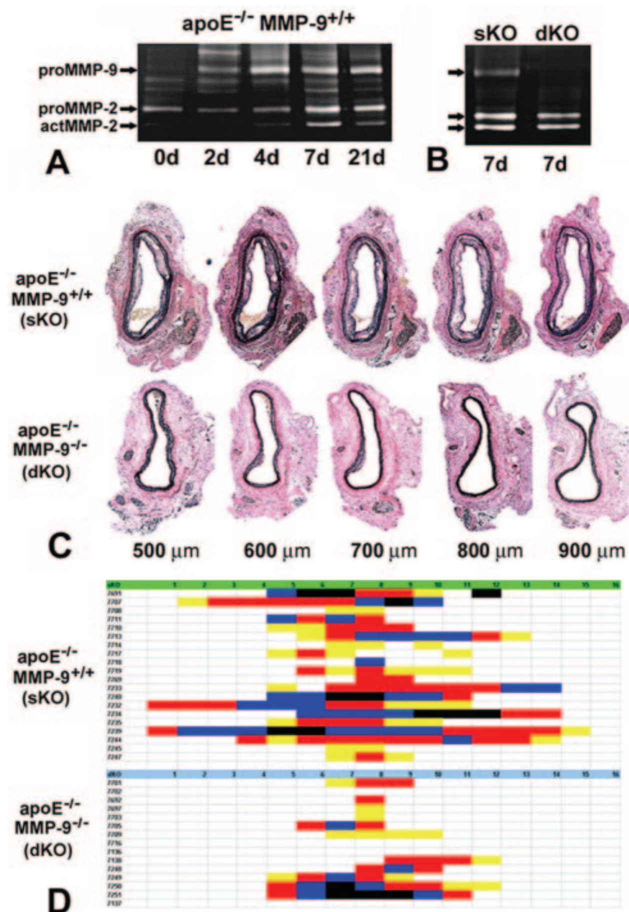

Figure 1. A, Gelatin substrate zymography of the apoE$^{-/-}$ MMP-9$^{+/+}$ carotids after temporary ligation was performed. MMP-9 expression was absent in nonligated, sham mice (0 days [0d]), but its levels increased and peaked at 7d after temporary ligation. Pro–MMP-2 (proMMP-2) was seen before and after temporary ligation, but the levels of activated MMP-2 (actMMP-2) increased after ligation (n=5 per time point). B, Seven days after the temporary ligation, MMP-9 activity was detected in the apoE$^{-/-}$ MMP-9$^{+/+}$ (sKO) mice, and it was not detected in the apoE$^{-/-}$ MMP-9$^{-/-}$ (dKO) mice. C, Cross-sections of apoE$^{-/-}$ MMP-9$^{+/+}$ (n=20) and apoE$^{-/-}$ MMP-9$^{-/-}$ (n=15) common carotid arteries at 3 weeks after temporary ligation (magnification ×200). The representative sections are 100 μm apart and 500 to 900 μm proximal to the carotid bifurcation. The temporary ligation is usually at 700 μm proximal to the bifurcation. D, Lengths of plaque in apoE$^{-/-}$ MMP-9$^{+/+}$ (n=20) and apoE$^{-/-}$ MMP-9$^{-/-}$ (n=15) common carotid arteries at 3 weeks after temporary ligation are represented by color boxes. Each box represents 100 μm in length. The black box denotes a cross-sectional plaque area of >0.03 mm^2; blue box, an area between 0.02 and 0.03 mm^2; red box, an area between 0.01 and 0.02 mm^2; yellow box, an area between 0.005 and 0.01 mm^2; white box, an area of <0.005 mm^2.

temporary ligation, we saw a significant reduction in the intimal plaque volume in apoE$^{-/-}$ MMP-9$^{-/-}$ mice (0.004±0.001 mm^3) compared with apoE$^{-/-}$ MMP-9$^{+/+}$ mice (0.011±0.002 mm^3; $P<0.02$; Figure 1C). The length of the plaque was also reduced in the apoE$^{-/-}$ MMP-9$^{-/-}$ mice (796±86 μm versus 393±76 μm; $P<0.01$; Figure 1D). There were no significant differences in the arterial remodeling as determined by internal and external elastic lamina measurements of the 2 groups (data not shown). Moreover, there were no differences in the total cholesterol levels (apoE$^{-/-}$ MMP-9$^{+/+}$ 1089±134 mg/dL; apoE$^{-/-}$ MMP-9$^{-/-}$ 1249±200 mg/dL) and in the triglyceride levels (apoE$^{-/-}$ MMP-9$^{-/-}$ 105±38 mg/dL; apoE$^{-/-}$ MMP-9$^{+/+}$ 103±42 mg/dL).

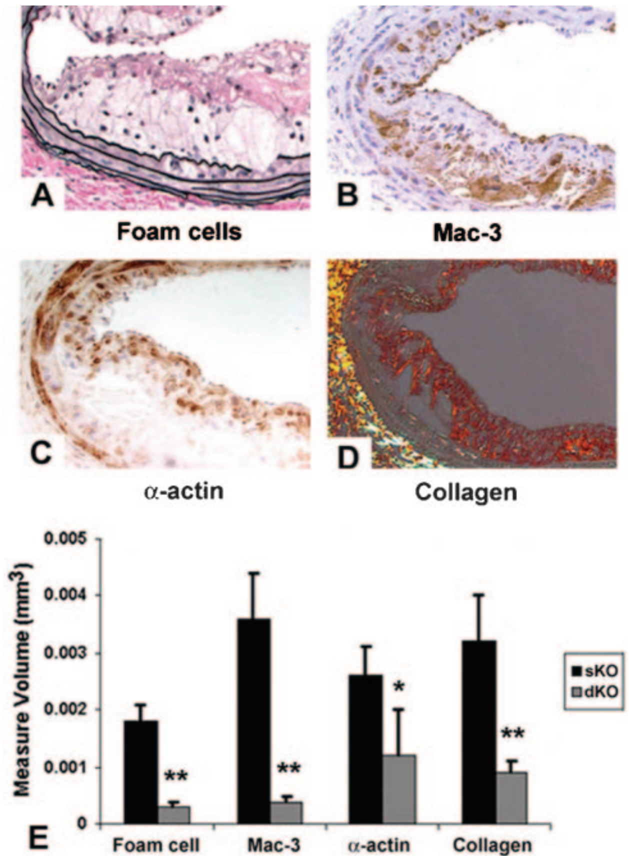

Figure 2. A, Representative section of apoE$^{-/-}$ MMP-9$^{+/+}$ common carotids at 3 weeks after temporary ligation stained Verhoff van Gieson. B, Adjacent section of apoE$^{-/-}$ MMP-9$^{+/+}$ common carotids at 3 weeks after temporary ligation stained with anti–Mac-3 antibody (stained brown). C, SM-specific α-actin to identify SMCs (stained brown). D, Sirius red staining to identify collagen under dark field (stained orange). All magnifications are ×400. E, A 3D intraplaque measurement showed that apoE$^{-/-}$ MMP-9$^{-/-}$ mice (n=20) had significantly less foam cell, macrophage, SMC, and collagen accumulation in the plaque compared with apoE$^{-/-}$ MMP-9$^{+/+}$ mice at 3 weeks after temporary ligation (mean±SEM; *$P<0.05$; **$P<0.02$).

MMP-9 Deficiency Attenuates Intraplaque Cell Accumulation

To determine whether MMP-9 influences the intraplaque content, we assessed the accumulation of foam cells and macrophages, markers of an early fatty streak. There were significantly fewer intraplaque foam cells and macrophages in apoE$^{-/-}$ MMP-9$^{-/-}$ mice (n=15) compared with apoE$^{-/-}$ MMP-9$^{+/+}$ mice (n=20; Figure 2A, 2B, and 2E). This reduction was not attributable to any change in numbers or differential of circulating leukocytes between the 2 groups of apoE$^{-/-}$ mice (data not shown). Furthermore, to address the effect of MMP-9 on SMC and collagen content in the atherosclerotic plaque, we quantified the plaque staining for SM-specific α-actin and the sirius red staining for collagen under dark field (Figure 2C through 2E). We show that

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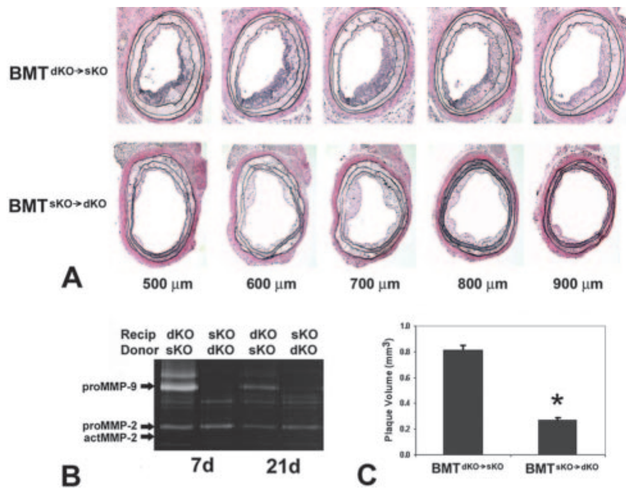

Figure 3. Reciprocal BMT with apo$E^{-/-}$ MMP-$9^{+/+}$ (sKO) and apo$E^{-/-}$ MMP-$9^{-/-}$ (dKO) mice was performed. After 6 weeks, temporary carotid ligation was performed. Three weeks later, carotids were harvested from sKO recipients (Recip; n=8) reconstituted from dKO bone marrow donor ($BMT^{dKO\rightarrow sKO}$) and dKO recipients (n=8) reconstituted from sKO bone marrow donor ($BMT^{sKO\rightarrow dKO}$). A, The representative sections are 100 μm apart and 500 to 900 μm proximal to the carotid bifurcation. The temporary ligation is usually at 700 μm proximal to the bifurcation. B, Gelatin substrate zymogram was performed with these carotid tissues harvested at 7 days (7d) and 21d after injury. Pro–MMP-9 (proMMP-9) expression was predominantly associated with the sKO bone marrow donor. C, However, significantly greater plaque burden was seen with the sKO recipient (dKO donor) compared with the dKO recipient (sKO donor; *$P<0.05$).

apo$E^{-/-}$ MMP-$9^{-/-}$ mice had significantly less SMC and collagen accumulation in the plaque compared with the control apo$E^{-/-}$ MMP-$9^{+/+}$ mice (*$P<0.05$).

Cellular Source of MMP-9 Activity

We performed reciprocal BMT with apo$E^{-/-}$ MMP-$9^{-/-}$ (dKO) and apo$E^{-/-}$ MMP-$9^{+/+}$ (sKO) mice. There were no significant differences in the leukocyte count or in the rest of the complete blood counts between the repopulated mice (data not shown). We determined that MMP-9 expression in the carotid tissue is dependent predominantly on the bone marrow–derived cells (Figure 3B). Pro-MMP expression was prominent at 7 and 21 days after temporary ligation in dKO mouse recipients reconstituted with sKO mouse donors ($BMT^{sKO\rightarrow dKO}$), whereas sKO mouse recipients reconstituted with the bone marrow from dKO mouse donors ($BMT^{dKO\rightarrow sKO}$) had minimal pro–MMP-9 expression. Therefore, the source of MMP-9 appears to be the bone marrow–derived cells, possibly inflammatory cells, such as macrophages. However, when we measured the plaque size in these mice 3 weeks after the ligature removal, sKO mice reconstituted with dKO bone marrow ($BMT^{dKO\rightarrow sKO}$) had significantly greater plaque volume than those sKO mice reconstituted with dKO bone marrow ($BMT^{sKO\rightarrow dKO}$). Therefore, increased plaque burden was associated with the MMP-$9^{+/+}$ recipient rather than the MMP-$9^{+/+}$ bone marrow donor, demonstrating that plaque composition in this animal model is intimately linked to MMP-9 generated by resident arterial cells (eg, SMCs, endothelial cells, etc) and not the bone marrow–derived cells.

Discussion

The atherosclerotic plaques seen in high-fat diet–fed apo$E^{-/-}$ mice resulting from a temporary ligation are reminiscent of early fatty streaks and also more advanced plaques with abundant foam cells and a fibrous cap (Figure 1).[13] This approach with a focal mechanical injury at the site of the temporary ligation generates a significant atherosclerotic plaque in 3 weeks compared with the 6 to 12 months needed for de novo plaque development on high-fat diet alone. Using this model of focally accelerated atherosclerotic plaque formation, we demonstrate that a lack of MMP-9 led to a reduction in atherosclerotic plaque volume and, more significantly, to a reduction in intraplaque cell accumulation of resident cells and bone marrow–derived cells in the apo$E^{-/-}$ mice.

Although MMP-9 has been suggested to regulate leukocytosis[16] and stem cell mobilization,[17] MMP-$9^{-/-}$ mice show no defect in neutrophil or macrophage influx into elastase-injured aorta or into skin or lung in models of acute injury and inflammation or tumor progression.[18–22] However, our data indicate that MMP-9, at least in the vascular disease model used here, is required for broad cellular accumulation in the plaque. Interestingly, MMP-9 is expressed by inflammatory cells and SMCs in human atherosclerotic plaques and in neointimal lesions in various animals models, including apo$E^{-/-}$ mice.[23] Therefore, we asked the question, "Is the function of macrophage-derived MMP-9 distinct from that of the SMC-derived MMP-9?"

Our studies confirm that MMP-9 is not expressed to a significant degree in the normal mouse carotid artery, but that it increases early after temporary ligation and even more so between 2 and 7 days. This appears to coincide with leukocyte infiltration of the injured carotid artery, supporting the view that inflammatory cells may be responsible for the increased production of MMP-9 at later intervals. More convincing evidence that inflammatory cells are responsible for MMP-9 expression arises from our studies in mice subjected to irradiation and reciprocal BMT, although bone marrow–derived SMCs and fibroblast progenitor cells can just as well be the source.

The marked reduction in macrophages and foam cells we observed could be attributable to the lack of MMP-9 in inflammatory or SMC progenitor cells in the initial phase, which may use the proteinases to propagate proinflammatory signals. Indeed, MMPs are increasingly being recognized as regulators of inflammation rather than as effectors of matrix destruction. For instance, the release of MMP-2 and MMP-7 by resident cells controls leukocyte influx into the lung by generating gradients of specific chemokines, and many MMPs can directly modify several chemokines, leading to altered activity.[24–27] Furthermore, Chana et al[28] reported that mesangial matrix contains several potential monocyte-binding domains that may be regulated by MMPs, and thus, contribute to monocyte entrapment and modulate cell activation. MMP-9 could regulate similar proinflammatory mechanisms in the artery wall altering the vascular matrix composition.

Ironically, when we the measured the plaque volume in these BMT animals, increased plaque burden was associated

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with the MMP-$9^{+/+}$ recipient mice rather than the MMP-$9^{+/+}$ bone marrow donor, demonstrating that plaque composition in this animal model is intimately linked to MMP-9 generated by the resident cells (eg, SMCs, endothelial cells, etc) and not the bone marrow–derived cells. Therefore, despite the greater MMP-9 expression associated with the bone marrow cells as demonstrated by zymography (Figure 3B), MMP-9 released and held by the resident cells may be more important for regulating the specificity of proteolysis than the affinity of the enzyme–substrate interaction and, in turn, regulating the plaque composition. After all, cells do not release protease indiscriminately, especially enzymes like MMPs with such a defined substrate specificity. Rather, they require precise interactions to accurately degrade, cleave, or process specific substrate in the pericellular space (compartmentalization).[29] Indeed, an emerging concept is that MMPs are anchored to the cell membrane or membrane proteins, such as integrins, thereby targeting their catalytic activity to specific substrates within the pericellular space. In recent years, specific MMP–integrin interactions have been reported: MMP-9 to CD44,[30] MMP-7 to cell surface proteoglycans,[31] and MMP-1 to integrin $\alpha_{2\beta1}$,[32] among others.

The exact mechanisms that lead to increased extracellular matrix that make up the atherosclerotic plaque burden remain largely unknown. The contribution of early migration and proliferation of medial SMCs, which are a major source of matrix proteins and possibly bone marrow–derived SMC progenitor cells, are often suggested to be key to plaque formation.[33,34] Recently, 2 groups reported that neointima formation and SMC accumulation and migration are reduced in MMP-$9^{-/-}$ mice.[35,36] We also observed reduced SMC accumulation in the atherosclerotic plaque in apo$E^{-/-}$ MMP-$9^{-/-}$ mice. We further observed reduced intraplaque collagen accumulation in apo$E^{-/-}$ MMP-$9^{-/-}$ mice. However, there is no MMP-9 protein substrate identified to account for this phenotype because interstitial collagen is not an MMP-9 substrate. Perhaps decreased collagen accumulation in apo$E^{-/-}$ MMP-$9^{-/-}$ mice is a byproduct of reduced intraplaque SMC accumulation.

Our findings and those of others[10,11] support the conclusion that MMP-9 is a critical player in the development and progression of atherosclerotic plaques. Our results support the recent findings suggesting that genetic variations in humans that effect MMP-9 expression influence the development and progression of atherosclerosis[8] and that elevated levels of MMP-9 in serum are seen in patients with severe coronary stenosis.[9,37] We further showed that the resident cells and not the bone marrow–derived cells were indeed the source of MMP-9 affecting the plaque composition in this model of injury-induced accelerated atherogenesis. As stated previously, we used a mixed background (50% C57BL/6 and 50% 129 SvEv) for this rapid atherogenesis. Hence, the caveat is that our findings may be unique to our animal model or the mixed animal background. However, we believe that strategies aimed at blocking MMP-9 activity should not be limited to the inflammatory cells but also to the SMCs and other resident cells, which may be of potential benefit in early development of atherosclerotic plaque, and in late complications of plaque instability alike, in those highly susceptible individuals.

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