Matrix Metalloproteinase-2 Negatively Regulates Cardiac Secreted Phospholipase A2 to Modulate Inflammation and Fever

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Background—Matrix metalloproteinase (MMP)-2 deficiency makes humans and mice susceptible to inflammation. Here, we reveal an MMP-2–mediated mechanism that modulates the inflammatory response via secretory phospholipase A2 (sPLA2), a phospholipid hydrolase that releases fatty acids, including precursors of eicosanoids.

Methods and Results—Mmp2−/− (and, to a lesser extent, Mmp7−/− and Mmp9−/−) mice had between 10- and 1000-fold elevated sPLA2 activity in plasma and heart, increased eicosanoids and inflammatory markers (both in the liver and heart), and exacerbated lipopolysaccharide-induced fever, all of which were blunted by adeno-virus-mediated MMP-2 overexpression and varespladib (pharmacological sPLA2 inhibitor). Moreover, Mmp2 deficiency caused sPLA2-mediated dysregulation of cardiac lipid metabolic gene expression. Compared with liver, kidney, and skeletal muscle, the heart was the single major source of the Ca2+-dependent, ~20-kDa, varespladib-inhibitable sPLA2 that circulates when MMP-2 is deficient. PLA2G5, which is a major cardiac sPLA2 isoform, was proinflammatory when Mmp2 was deficient. Treatment of wild-type (Mmp2+/+) mice with doxycycline (to inhibit MMP-2) recapitulated the Mmp2−/− phenotype of increased cardiac sPLA2 activity, prostaglandin E2 levels, and inflammatory gene expression. Treatment with either indomethacin (to inhibit cyclooxygenase-dependent eicosanoid production) or varespladib (which inhibited eicosanoid production) triggered acute hypertension in Mmp2−/− mice, revealing their reliance on eicosanoids for blood pressure homeostasis.

Conclusions—A heart-centric MMP-2/sPLA2 axis may modulate blood pressure homeostasis, inflammatory and metabolic gene expression, and the severity of fever. This discovery helps researchers to understand the cardiovascular and systemic effects of MMP-2 inhibitors and suggests a disease mechanism for human MMP-2 gene deficiency. (J Am Heart Assoc. 2015;4:e001868 doi: 10.1161/JAHA.115.001868)

Key Words: heart • inflammation • matrix metalloproteinases • PLA2

Matrix metalloproteinases (MMPs) are a family of Zn-dependent endoproteases historically implicated in extracellular matrix remodeling.1 Interestingly, the phenotype of MMP-2 deficiency in humans causes autosomal recessive osteolysis/arthritis syndrome and congenital heart defects.2 Arthritis is a largely inflammatory condition linked to the development of pericarditis, cardiomyopathy, congestive heart failure, and ischemic heart disease.3 MMP-2–deficient mice mimic the human phenotype of arthritis,4 as well as exhibiting lung inflammation associated with impaired immune cell egression from inflamed airways.5 While those observations suggest a protective, anti-inflammatory role of MMP-2, there are also reports indicating that MMP-2 expression can exacerbate myocardial remodeling in mice with pressure overload,6 left ventricular rupture, and late cardiac remodeling after myocardial infarction.7

The mechanism of MMP-2 in these conditions has not been elucidated, but MMP-dependent proteolysis has been recurrently implicated.8 MMP-2–mediated degradation of extracellular matrix proteins, including fibronectin, laminin, and elastin, has been proposed to produce fragments that are chemotactic for cells such as macrophages and play a role in wound healing in the settings of cardiac rupture and myocardial infarction.7 MMP-2 has also been shown to cleave mediators of inflammation like monocyte chemoattractant...
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studies by investigating the liver but unexpectedly found a novel
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Materials and Methods

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Doxycycline hyclate, Escherichia coli EH100 (Ra mutant)
rough strain lipopolysaccharide (LPS), and cholesterol were
obtained from Sigma-Aldrich. EMEM was obtained from ATCC,
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endonuclease analysis. Finally, the linearized recombinant plasmid (by PacI) was transfected into HEK293 cells to generate GFP-expressing adenovirus (AdGFP) or mature human MMP-2–encoding adenovirus (AdMMP-2).

qRT-PCR

Cells were lysed in TRizol. Tissue was extracted by homogenizing 30- to 50-mg pieces of frozen tissue at 4°C in 1 mL of TRizol reagent using the Bullet Blender (Next Advance Inc., NY, USA). RNA was isolated from TRizol according to manufacturer’s instructions, and cDNA was generated from RNA by using random primers and Superscript II reverse transcriptase. Expression instructions, and cDNA was generated from RNA by using random primers and Superscript II reverse transcriptase. Expression analysis of the reported genes was performed with TaqMan qRT-PCR by using the ABI 7900 HT sequence detection system (Applied Biosystems). Gapdh (to confirm interpretation of data relative to Gapdh) were used as internal standards. The RT-PCR data chosen for the figures are relative to Gapdh.

Protein Analysis

Total protein content in tissue homogenate was assessed by using Bio-Rad Protein Assay according to the manufacturer’s instructions or SDS-PAGE followed by Coomassie blue staining.

For determination of PLA2G5 protein, plasma and recombinant PLA2G5 solutions were diluted with a 1:5 volume of SDS-PAGE loading buffer (15% SDS, 8 mol/L urea, 10% 2-mercaptoethanol, 25% glycerol, 0.2 mol/L Tris, pH 6.8), heated at 37°C for 20 minutes, and subjected to 10% Tricine-SDS-PAGE as previously described. After electrophoresis, proteins from the gels were transferred to a nitrocellulose membrane by using the TE22 system (Hoefer). Membranes were visualized with the use of Ponceau S acid staining; scanned to assess protein load; blocked in 5% bovine serum albumin in 20 mmol/L Tris, 150 mmol/L NaCl, pH 7.4, containing 0.1% Tween-20; probed overnight with primary antibodies to PLA2G5; rinsed; probed for 30 minutes with secondary antibodies; and washed in 20 mmol/L Tris, 150 mmol/L NaCl, pH 7.4, containing 0.1% Tween-20 to remove excess of antibody. PLA2G5 immunoreactivity was then detected by using ECL detection reagent.

MMP-2 activity was measured with gelatin zymography, by using SDS-PAGE gels embedded with 2 mg/mL gelatin. After electrophoresis, the gels were washed 3 times in 2.5% Triton X-100 for 20 minutes (to remove excess SDS) and then incubated for 16 hours at 37°C in a buffer of 5 mmol/L CaCl2, 150 mmol/L NaCl, 0.5 mmol/L NaOH, and 25 mmol/L Tris, pH 7.4 (for activity development). Gels were stained with Coomassie brilliant blue to visualize bands with gelatinase activity.

Cytokine analysis was outsourced to Eve Technologies (Calgary, Canada) with use of the Mouse Cytokine Array/Chemokine Array 31-Plex (Eve Technologies).

Measurements of Phospholipases and Eicosanoids

Except where indicated otherwise, secretory, cytosolic, and calcium-independent PLA2 activities were measured by using the respective assay kits (Cayman), which use diheptanoyl thio-phosphatidylcholine (PC) or arachidonoyl thio-PC as substrates to distinguish sPLA2 from cPLA2 and iPLA2.

Prostaglandin (PG) E2 in the heart, liver, and hypothalamus was measured by using the Prostaglandin E2 Express ELIA Kit (Cayman). Free 8-isoprostane concentrations in the plasma were measured by using the 8-isoprostane ELIA Kit (Cayman).

To determine the molecular weight of the plasma and cardiac sPLA2 activities, we subjected plasma or heart homogenates to a novel zymographic technique. Hearts were homogenized by using the Bullet Blender in 10 μL/mg sPLA2 assay buffer (100 mmol/L KCl, 10 mmol/L CaCl2, 0.3 mmol/L Triton X-100, 25 mmol/L Tris-HCl, pH 7.4). Bromophenol blue and 20% glycerol were added to the samples for loading into a 10% SDS-PAGE or 10% T (grams of acrylamide and bisacrylamide per 100 mL of solution - in percent), 3% C (ratio of bisacrylamide to acrylamide and bisacrylamide) Tricine-SDS-PAGE gel. After electrophoresis, the gels were reverse-stained by using our previously developed zinc-imidazole reverse stain technique.11 Lanes were then cut into equal-sized sections to cover the full range of molecular weights. The individual sections of gel were incubated for 5 minutes in 200 μL of 100 mmol/L EDTA (to mobilize proteins), washed once with water (to remove excess EDTA), and then washed (2 × 10 minutes) with 2.5% Triton X-100, 10 mmol/L CaCl2, 25 mmol/L Tris, pH 9.0 (to remove excess SDS). Next, 200 μL of sPLA2 assay buffer was added to gel pieces, and the pieces were nebulized by using the Bullet Blender. Samples were centrifuged at 20 000g for 5 minutes, and activity in the eluates (supernatant) was measured by using the sPLA2 assay kit.

Enzyme Inhibition Assays

Indoxam-inhibition concentration-response was constructed for 5 different concentrations by measuring the residual activity with use of the microtiter plate fluorescent assay of sPLA2s with pyrene-labeled phosphatidyl-glycerol as the substrate as described previously.12

Blood Pressure Measurement

Blood pressure was measured by using a computerized tail-cuff system (RTBP 2000; Kent Scientific).

Fever Response to LPS

Body temperature of mice housed at 24±0.5°C was measured rectally after administration of an intraperitoneal injection of E. coli EH100 (Ra mutant) rough strain LPS (Sigma-Aldrich).
To measure the effect of sPLA2 inhibition on the fever response to LPS, we examined mice administered varespladib (10 mg/kg per day, orally for 2 days with the second dose immediately preceding the intraperitoneal injection of LPS). To measure the effect of MMP-2 overexpression on the fever response, we examined mice that were intraperitoneally injected with either AdMMP-2 or AdGFP (≈10⁶ pfu) and then injected 3 days later with LPS (100 μg/kg).

Figure 1. MMP-2 is a negative regulator of phospholipase A₂ activity. A, Enzymatic activities of phospholipase A₂. Left: cPLA₂ and iPLA₂ activity from mouse liver. Right: sPLA₂ activity from mouse plasma. The activity of pools of n=4 mice per genotype was measured in duplicate. *P≤0.05 vs WT. All pairwise multiple-comparison procedures (Holm–Sidak method). B, Left: sPLA₂ activity in plasma samples (pools of n=3 mice per genotype) fractionated by weight using centrifugal filters and measured in duplicate. *P≤0.05 vs WT (3 to 30 kDa). All pairwise multiple-comparison procedures (Holm–Sidak method). Right: Fractionation by molecular weight (nonreducing SDS-PAGE) of sPLA₂ activity in pooled plasma samples (pools of n=3 mice per genotype). After reverse staining (used to visualize plasma protein bands without affecting enzyme activity), protein was eluted from the gel and assessed for sPLA₂ activity (vertical bar diagram). cPLA₂ indicates cytosolic phospholipase A₂; iPLA₂, calcium-independent phospholipase A₂; MMP, matrix metalloproteinase; sPLA₂, secreted phospholipase A₂; WT, wild-type.
Cell Culture Studies

For RNA interference studies, we used a stable cell line of Mmp2 deficiency created from fibroblasts isolated from WT, Mmp2 sold out, Mmp2 knock out, Mmp9 sold out, lungs. These cells were chosen for 4 key traits: (1) preserved ability to proliferate in culture without any detectable phenotypic changes after subsequent passages (up to at least 30 passages), (2) expression of PLA2G5, the major cardiac sPLA2, (3) expression of a proinflammatory phenotype, and (4) amenability to efficient transduction with siRNAs against PLA2G5. The cells used for this study were cultured in EMEM with 10% FBS, 100 U/mL penicillin, and 100 μg/mL streptomycin, at a density of 25 10⁴ cells/cm². Attached fibroblasts were transfected with siRNAs against PLA2G5 or scrambled RNA (control) over a period of 3 days by using Lipofectamine RNAiMAX according to the manufacturer’s instructions.

Statistical Analysis

The main unit of statistical analysis for qRT-PCR and systolic blood pressure measurements was the animal. In qRT-PCR analyses, the RNA isolated for each mouse was measured in triplicate, and the resulting average value was used to compute the mean of said mRNA for each group. Similarly, the mean of the systolic blood pressure for each group was computed from the average of 8 to 10 individual measurements for each mouse in the group. For PLA2s, PGE2, and isoprostanes, the main unit of statistical analysis was the duplicate of pooled samples, as described in the corresponding figure legends. Unless otherwise indicated, the results are reported as mean±SEM values. Results were analyzed by using SigmaPlot 11 software (Systat Software). The specific statistical tests used are indicated in the corresponding figure legends.

Results

MMP-2 Is a Negative Regulator of PLA2 Activity and Eicosanoid Synthesis

The PLA2 family of intracellular (cytosolic [cPLA2] and calcium-independent [iPLA2]) and extracellular (secretory [sPLA2]) enzymes catalyze the hydrolysis of phospholipids to liberate fatty acids esterified at the sn-2 position. Mmp2−/− mice

Figure 2. A, Effect of different nutritional regimens on plasma sPLA2 activity. The activity in pools of n=4 (for chow-fed and 2.5-day 0.15% cholesterol–fed mice) or n=3 (for fasted-refed mice and 4-week 2% cholesterol–fed mice) was measured in duplicate. *P≤0.05 vs WT, t test. B, Effect of diet on hepatic MMP-2 expression. Gelatin zymography indicating hepatic MMP-2 activity levels of WT mice that were fed, fasted, fasted-refed, or cholesterol fed. Pools of n=5 for fasted mice, n=4 for fed, fasted-refed, and cholesterol-fed mice. MMP indicates matrix metalloproteinase; sPLA2, secreted phospholipase A2; WT, wild-type.
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Figure 3. MMP-2 is a negative regulator of eicosanoid synthesis. A, Hepatic PGE2 concentration in mice. Pools of n=4 for each genotype were measured in duplicate. *P<0.05 vs WT (control). Multiple comparisons vs control group (Holm–Sidak method). B, Plasma free 8-isoprostane concentration in mice. Pools of n=4 for each genotype were measured in duplicate. *P<0.05 vs WT, t test. C, WT mice were administered the sPLA2 inhibitor varespladib (10 mg/kg per day) for 2 days. Left: Plasma sPLA2 activity. Right: Hepatic PGE2 concentration. Pools of n=3 for each treatment group were measured in duplicate. *P<0.05 vs WT–Varespladib (control). †P<0.05 vs Mmp2−/−− Varespladib (control). All pairwise multiple-comparison procedures (Holm–Sidak method). MMP indicates matrix metalloproteinase; PGE2, prostaglandin E2; sPLA2, secreted phospholipase A2; WT, wild-type.

showed a ≈25% elevation of hepatic cPLA2 and iPLA2 activity and dramatically elevated plasma sPLA2 activity (Figure 1A).

The plasma sPLA2 activity of Mmp2−/− mice was found at a molecular weight range of 3 to 30 kDa through centrifugal filtration (Figure 1B, left).

Development of a novel combination of techniques, involving nonreducing SDS-PAGE followed by reverse-stain and sPLA2 activity assay, revealed that Mmp2−/− plasma sPLA2 has a molecular weight of ≈20 kDa (Figure 1B, right). Plasma sPLA2 activity was less elevated in Mmp7−/− and Mmp9−/− mice, indicating a predominant role of MMP-2 (Figure 1A). Notably, plasma sPLA2 activity was also elevated in Mmp2−/− mice subjected to diverse nutritional regimens including fasting-refeeding and excess cholesterol supplementation (Figure 2A). In these nutritional regimens, MMP-2 levels remained unchanged in WT mice (Figure 2B). These data consistently indicate that MMP-2 is a negative regulator of sPLA2 activity.

The sn-2 position of phospholipids is enriched in polyunsaturated fatty acids (eg, arachidonic acid), which is the precursor of all eicosanoids, including PGE2 (which is synthesized downstream of cyclooxygenases) and 8-isoprostanes (which are formed independent of cyclooxygenases).14 In Mmp2−/− mice, hepatic PGE2 (Figure 3A) and plasma 8-isoprostanes (Figure 3B) were both increased. However, PGE2 levels in Mmp7−/− and Mmp9−/− mice were not as elevated (Figure 3A). Treatment of Mmp2−/− mice with the sPLA2 inhibitor varespladib normalized the levels of plasma sPLA2 and hepatic PGE2 (Figure 3C). These data showed that MMP-2 deficiency causes excess sPLA2 activity, which, in turn, elevates PGE2.

MMP-2 Is a Negative Regulator of Fever

At baseline, Mmp2−/− mice exhibited signs of a proinflammatory state as indicated by the increased mRNA expression of the genes encoding tumor necrosis factor (TNF)-α (Tnf), interleukin (IL)-1β (Il1b), monocyte chemoattractant protein-1 (MCP-1) (Ccl2), regulated on activation, normal T cell (C-C motif) ligand 6 (CCL6) (Ccl6) in the heart and liver and downregulated expression of liver X receptor-α (Nr1h3) (Figure 6). A pattern supporting a proinflammatory state in Mmp2 deficiency was also suggested by the protein levels of IL-1β, RANTES, IP-10, G-CSF, MCP-1, MIG, LIX, and IL-13 (Figure 7).

Compared with Mmp2−/− mice, in Mmp7−/− or Mmp9−/− mice, the hepatic expression of Tnf, Il1b, and Ccl2 was not
elevated and that of *Nr1h3* was not decreased (Figure S1 and data not shown).

In response to bacterial LPS, *PGE*₂ synthesized by the PLA₂/cyclooxygenase/PGE synthase pathway promotes fever and inflammation.¹⁵ We administered LPS (30 μg/kg) to WT and *Mmp2⁻⁻⁻* mice and searched for signs of inflammation, endoplasmic reticulum stress, or lipid metabolic dysregulation by using qRT-PCR and a cytokine array. Five hours after LPS administration, hepatic and cardiac mRNA expression of the genes encoding TNF-α (*Tnf*), IL-1β (*Il1b*), MCP-1 (*Ccl2*), RANTES (*Ccl5*), and CCL6 (*Ccl6*) was exacerbated in *Mmp2⁻⁻⁻* hearts (Figure 6). At the protein level, *Mmp2⁻⁻⁻* hearts also had pronounced expression of IL-1β, RANTES, Eotaxin, VEGF, IP-10, MIG, and MIP-2. The levels of G-CSF, MCP-1, TNF-α, LIX, IFN-γ, and IL-13 were less induced and IL-17 was unchanged versus WT mice (Figure 7). These data revealed that the inflammatory response to LPS depends strongly on whether MMP-2 is expressed.

In response to LPS (30 μg/kg), *Mmp2⁻⁻⁻* mice housed at constant ambient temperature (24±0.5°C) had exacerbated fever with highly elevated sPLA₂ and PGE₂ (Figures 8A through 8C and S2). Similarly, doxycycline administration to WT mice delayed the resolution of fever (Figure S3A) and increased mRNA expression of the genes encoding TNF-α (*Tnf*), IL-1β (*Il1b*), and MCP-1 (*Ccl2*) (Figure S3B).

The difference in febrile response between WT and *Mmp2⁻⁻⁻* mice observed at an LPS dose of 30 μg/kg was not seen at a dose of 100 μg/kg (Figure 8D). Strikingly, pretreatment with varespladib (10 mg/kg per day) completely blocked the development of LPS (100 μg/kg)-induced fever in *Mmp2⁻⁻⁻* but not WT mice (Figure 8D).

Transduction with AdMMP-2, which resulted in hepatic overexpression of human MMP-2, ameliorated LPS-induced fever and reduced PGE₂ levels in the brain (Figure S4).

Therefore, in the absence of MMP-2, LPS-induced fever is signaled through a varespladib-inhibitable (ie, sPLA₂-dependent) pathway, which would otherwise be inhibited by MMP-2. The inhibitory effect of MMP-2 in fever is obliterated when the LPS dose is sufficiently high. These data identify MMP-2 as a novel negative regulator of LPS-induced fever.

**The Heart Is a Major Source of sPLA₂ Activity**

We compared sPLA₂ activity in plasma, liver, heart, kidney, and skeletal muscle. Strikingly, the sPLA₂ activity in *Mmp2⁻⁻⁻* hearts was 10²⁻ to 10³-fold greater versus WT hearts and versus *Mmp2⁻⁻⁻* plasma, depending on the substrate
Figure 5. A, Analysis by qRT-PCR of hepatic levels of human and mouse MMP-2 level. B, Left: Analysis by gelatin zymography of MMP-2 levels in plasma, heart, and liver. Right: Analysis by qRT-PCR of human MMP2 expression in heart and liver. Mice were intraperitoneally injected with AdMMP-2 or AdGFP (≈10⁸ pfu). The data corresponds to Mmp2⁻/⁻ mice sacrificed 5 days after adenoviral injection. n=4 mice for each genotype. AdGFP indicates green fluorescent protein–expressing adenovirus; AdMMP, MMP-2–encoding adenovirus; MMP, matrix metalloproteinase; ND, not detected; qRT-PCR, quantitative real-time polymerase chain reaction; rhMMP-2, recombinant human MMP-2.

(Figure 9), sPLA₂ activity of liver, kidney, and skeletal muscle were not different between WT and Mmp2⁻/⁻ mice (Figure 9). The sPLA₂ elevated in Mmp2⁻/⁻ mice hydrolyzed both the sPLA₂–specific substrate diheptanoyl thio-PC and arachidonoyl thio-PC (Figure 9). The apparent Michaelis-Menten constant (Kₘₐₚp) of plasma sPLA₂ for diheptanoyl thio-PC was equal in Mmp2⁻/⁻ and WT mice (303 and 273 μmol/L, respectively). Plasma values were similar to those of Mmp2⁻/⁻ and WT hearts (259 and 308 μmol/L, respectively). These data indicated that the plasma and cardiac sPLA₂ enzymes were the same. The cardiac sPLA₂ depended on calcium for its activity (Figure 10A) and was active over a broad pH range with maximal activity at pH 7.5 (Figure 10B) and a molecular weight of ≈20 kDa (Figure 10C). In haploinsufficient (Mmp2⁻/⁻) mice, the levels of cardiac sPLA₂ activity were as approximately half those of Mmp2⁻/⁻ mice (Figure 11A).

PLA2G5, Which Is a Major Cardiac sPLA₂ Isoform, Contributes to Inflammation in MMP-2 Deficiency

Secreted PLA₂s comprise a family of at least 11 lipid hydrolases in humans and mice: PLA2G1B, PLA2G2(A, C, D, E, F), PLA2G3, PLA2G5, PLA2G10, and PLA2G12(A, B). Some of these enzymes might not be good candidate targets of MMP-2 in our studies. (1) The C57BL/6 mouse strain is a natural knock-out for PLA2G2A; thus, this sPLA₂ cannot account for the observed effects. (2) Neither PLA2G2F, PLA2G3, nor PLA2G12¹²,¹⁶ could be excluded because they...
are not effectively inhibited by varespladib (which blunted sPLA2 activity and PGE2 levels in Mmp2−/− mice [Figure 3C]). (3) PLA2G2E could be excluded because it promotes obesity,17 while MMP-2 deficiency protects against obesity.18 (4) PLA2G2D could also be excluded because it ameliorates inflammation,19 whereas MMP-2 deficiency results in a proinflammatory state (Figures 6 and 7). (5) PLA2G12A is unlikely because this has very low PLA2 activity.

qRT-PCR analysis of the entire sPLA2 gene family expressed in C57BL/6 mice indicated that their expression was equal in WT and Mmp2−/− hearts (Figure 11B).

Inhibition studies with the pan-sPLA2 inhibitor indoxam suggested that cardiac sPLA2 is most likely a mixture of indoxam-resistant and indoxam-sensitive sPLA2s with a net IC50 for indoxam of 2 μmol/L (Figure 11C). Possible indoxam-resistant candidates are PLA2G1B, PLA2G2D, PLA2G2F, and PLA2G10, all of which have an IC50 for indoxam of ≥1 μmol/L.12 Possible indoxam-sensitive components are PLA2G2E (IC50≈0.035 μmol/L) and PLA2G5 (IC50≈0.170 μmol/L).12 Therefore, cardiac sPLA2 is either a mixture of sPLA2s or a completely novel (ie, a nonclassic) member of the sPLA2 family.

The mRNA for Pla2g5 is highly expressed in cardiac and vascular atherosclerotic tissue.20 In the absence of PLA2G2A (as in our mouse model), PLA2G5 might be a major contributor to the composite sPLA2 activity that circulates in plasma20 and could thus contribute to inflammation in Mmp2 deficiency, at least as a minor component. Indeed, showing that PLA2G5 could contribute to exacerbated inflammation characteristic of Mmp2 deficiency, PLA2G5 expression knock-down with siRNA (Figure 12A) caused significant downregulation of inflammatory markers in Mmp2−/− but not in WT cell cultures (Figure 12B).

Studies summarized in Figure 13 show that recombinant human MMP-2 did not cleave or inactivate recombinant human PLA2G5 or PLA2G10. Addition of recombinant human MMP-2 to plasma ex vivo also did not inhibit plasma sPLA2 activity. To examine the possible involvement of plasma factors, like activators or inhibitors of sPLA2, in the elevation of sPLA2 in Mmp2 deficiency, we further spiked plasma of WT or Mmp2−/− mice with recombinant human PLA2G5 for 4 to 16 hours. We were unable to detect any loss of the plasma PLA2G5 immunoreactivity or enzymatic activity (Figure 13A through 13C). Western immunoblotting and time-resolved fluorescence immunonassay revealed similar yet very small amounts of PLA2G5 in plasma and cardiac homogenates from WT and Mmp2−/− mice; these data were in line with the
notion that MMP-2 deficiency does not regulate sPLA₂ expression (Figure 13C and 13D). Overall, the data obtained by targeting PLA2G5 showed that MMP-2 does not cleave or inactivate sPLA₂ in plasma, suggesting that MMP-2 might regulate sPLA₂ activity in tissue. Addition of recombinant human MMP-2 to Mmp2⁻/⁻ heart homogenates had no effect on the activity or the molecular weight of cardiac sPLA₂ (Figure 13E). Therefore, we hypothesized that MMP-2 inhibits the release of active sPLA₂ from the heart and tested this through the ex vivo analysis of myocardial releasates (Figure 14A).

**MMP-2 Is a Negative Regulator of the Release of Active sPLA₂ From the Myocardium**

Freshly isolated specimens of Mmp2⁻/⁻ myocardium released dramatically more sPLA₂ activity ex vivo than did similar specimens isolated from WT mice (Figure 14B). This release of sPLA₂ activity from Mmp2⁻/⁻ hearts, as well as the expression of inflammatory markers, was reduced by adenovirus-mediated expression of human MMP-2 (Figure 14C). The secreted enzyme was equal in molecular weight (Figure 14C) to the sPLA₂ isolated from cardiac
**Figure 8.** MMP-2 is a negative regulator of fever. A, Body temperature was measured rectally in WT and Mmp2−/− mice before and after intraperitoneal injection of LPS (30 μg/kg) at the indicated times. \( n=3 \) mice per genotype. \(*P<0.05\) vs Time=0 minute. One-way repeated-measures ANOVA. \( \bar{P}≤0.05\) vs WT. All pairwise multiple-comparison procedures (Holm–Sidak method). B, Plasma sPLA2 activity in untreated mice or LPS (30 μg/kg)-treated mice 5 hours after LPS administration. Pools of \( n=3 \) mice were measured in duplicate. \(*P<0.05\) vs WT–LPS, \( t \) test. \( \bar{P}≤0.05\) vs Mmp2−/−–LPS, \( \bar{t} \) test. C, Hypothalamic PGE2 levels in untreated mice or LPS (30 μg/kg) treated mice 5 hours after LPS administration. Pools of \( n=3 \) mice were measured in duplicate. \(*P<0.05\) vs WT–LPS, \( t \) test. \( \bar{P}≤0.05\) vs Mmp2−/−–LPS, \( \bar{t} \) test. D, Body temperature was measured rectally in WT and Mmp2−/− mice before and after intraperitoneal injection of LPS (100 μg/kg) at the indicated times. \( n=3 \) mice per genotype. Selective blockade of LPS-induced fever in Mmp2−/− (but not WT) mice by the pan-sPLA2 inhibitor varespladib. Mice received varespladib (10 mg/kg per day) orally for 2 days, with the second dose immediately preceding intraperitoneal injection of LPS. \(*P<0.05\) vs Time=0 minute. One way repeated measures ANOVA. \( \bar{P}≤0.05\) vs Mmp2−/−–Varespladib. All pairwise multiple-comparison procedures (Student-Newman-Keuls method). ANOVA indicates analysis of variance; LPS, lipopolysaccharide; MMP, matrix metalloproteinase; PGE2, prostaglandin E2; sPLA2, secreted phospholipase A2; WT, wild-type.

homogenates (Figure 1B), suggesting that the active sPLA2 in the heart does not require proteolytic maturation for secretion to occur. MMP-2 haploinsufficiency caused a reduction in ex vivo myocardial sPLA2 release (Figure 14D). In the presence of Brefeldin A, an inhibitor of the classic secretory pathway, the myocardial release of sPLA2 was significantly blunted (Figure 14D).

The **MMP-2 Inhibitor Doxycycline Replicates Aspects of the Phenotype of Mmp2 Deficiency**

The experiment described in Figure 4A revealed that doxycycline administration can induce an MMP-2 deficiency phenotype in WT mice. To further characterize whether doxycycline induces phenotypic transformation in the heart, we administered doxycycline (50 mg/kg per day for 2 weeks) to WT mice and correlated their plasma and cardiac sPLA2 levels to cardiac inflammatory marker expression (Figure 15A). We used Mmp2−/− mice to define whether doxycycline induction of sPLA2 was due to MMP-2 inhibition (Figure 15B). We made 3 important observations. (1) Doxycycline upregulated plasma and cardiac sPLA2 activity only in Mmp2−/− mice, not in Mmp2+/− mice. (2) The elevation of plasma sPLA2 activity by doxycycline preceded that of cardiac sPLA2. (3) Induction of proinflammatory genes in the heart was detectable only after cardiac
sPLA₂ activity was significantly elevated (as assessed by qRT-PCR analysis of Ccl2, Tnf, Il1b, Ccl5, Ccl6, and Cxcl10) (Figure 15B).

Therefore, when MMP-2 is deficient, the heart is a major source of highly active sPLA₂. Plasma sPLA₂ activity may result from a systemic contribution, including the heart. Development of cardiac inflammation may require prior induction of cardiac sPLA₂ activity.

**Emerging Functions of the MMP-2/sPLA₂ Axis in Cardiovascular Regulation**

**Direct evidence of the proinflammatory potential of cardiac sPLA₂**

Treatment of mice with varespladib decreased the otherwise elevated levels of PGE₂ (Figure 16A) and Tnf, Il1b, Ccl2, Ccl5, Ccl6, and Ccl24 mRNA in the heart of Mmp2⁻/⁻ mice (Figure 16B).

To confirm that cardiac sPLA₂ is indeed sufficient to cause inflammation, we purified the active enzyme from Mmp2⁻/⁻ hearts by using a preparative zymographic technique and injected it into WT mice (Figure 17A). The purified enzyme, which had the same KM app as the heart homogenate and plasma sPLA₂s, had the opposite effect to sPLA₂ inhibition with varespladib: it increased plasma sPLA₂ activity and caused cardiac inflammation in recipient WT mice (Figure 17B and 17C).

**Role of the MMP-2/sPLA₂ axis in cardiac metabolic homeostasis**

Metabolic and inflammatory processes intercept at the level of several transcription factors such as liver X receptor and sterol-regulatory binding proteins (encoded by Nr1h3 and Srebf, respectively).²¹,²² To determine whether the MMP-2/sPLA₂ axis impacted cardiac lipid metabolic as well as proinflammatory gene expression, we conducted qRT-PCR analysis of these transcription factors in mice administered
the sPLA2 inhibitor varespladib. Analysis of lipid metabolic genes by qRT-PCR indicated cardiac metabolic dysregulation in Mmp2 knockout mice. This dysregulation was normalized by varespladib, showing that the MMP-2/sPLA2 axis influences cardiac lipid metabolism as well as inflammation (Figure 18A).

**The MMP-2/sPLA2/cyclooxygenase axis regulates blood pressure homeostasis**

Cyclooxygenase-derived prostanoids are major regulators of systemic blood pressure in both normal physiology and pathophysiology, with some prostaglandins being vasoconstrictive (eg, thromboxane A2), whereas others are vasodilatory (eg, PGE2 and PGI2). Treatment with indomethacin (to inhibit cyclooxygenase-dependent production of eicosanoids) or varespladib at a regimen that inhibited PGE2 (Figure 16A) triggered acute hypertension selectively in Mmp2−/−, but not in WT, mice (Figure 18B).

**Discussion**

We discovered MMP-2 to be a major negative regulator of systemic sPLA2, which, in Mmp2 deficiency, leads to massive levels of sPLA2 activity in the myocardium and

**Figure 10.** Biochemical characteristics of the sPLA2 elevated in Mmp2 deficiency. A, Cardiac sPLA2 requires Ca++ for activity. The sPLA2 activity in Mmp2−/− heart homogenate was measured in the presence of EGTA (to chelate calcium) or an excess of calcium. Pools of n=3 were measured in duplicate. Similar results were obtained in 3 separate experiments. B, Cardiac sPLA2 has a broad pH optimum in the basic pH range. The sPLA2 activity of Mmp2−/− mouse heart homogenate (pool of n=3 mice) was measured at the indicated pH values in duplicate. Similar results were obtained in 3 separate experiments. C, Molecular weight of cardiac sPLA2 activity. Strategy for analytical isolation of cardiac sPLA2. Hearts were excised and homogenized, cardiac proteins (from 1.5 mg tissue) were resolved by molecular weight (non-reducing SDS-PAGE). After reverse staining (for protein band visualization without loss of activity), protein was eluted from the gel and assessed for sPLA2 activity (vertical bar diagram). MMP indicates matrix metalloproteinase; ND, not detected; sPLA2, secreted phospholipase A2; WT, wild-type.
plasma. As a result, Mmp2-deficient mice exhibit dysregulation of inflammatory and lipid metabolic genes in the heart, exacerbated fever, and a compensatory reliance on eicosanoids for blood pressure homeostasis. We found the heart to be a major source of circulating sPLA2, which can be readily unmasked when MMP-2 deficiency is

**Figure 11.** Expression characteristics of the sPLA2 elevated in Mmp2 deficiency. A, Dependence on MMP-2 expression. Cardiac sPLA2 activity (bottom) and plasma MMP-2 levels (top) in mice lacking neither copy (Mmp2+/+, n=4), 1 copy (Mmp2+/−, n=3) or 2 copies (Mmp2−/−, n=4) of Mmp2. Pooled samples were measured in duplicate. *P<0.05 vs WT, t test. B, Lack of regulation by transcription. qRT-PCR analysis of the entire family of known sPLA2s expressed in C57BL mice. PLA2G2A is not presented as the gene for this enzyme is disrupted in C57BL strain. n=4 mice per genotype. C, Enzyme inhibition assay with indoxam suggests that cardiac sPLA2 may be a mixture of various sPLA2 enzymes. MMP indicates matrix metalloproteinase; ND, not detected; qRT-PCR, quantitative real-time polymerase chain reaction; sPLA2, secreted phospholipase A2; WT, wild-type.

**Figure 12.** Evidence of proinflammatory actions of PLA2G5 in Mmp2-deficient cells. A, qRT-PCR analysis of Pla2g5 expression in mouse fibroblasts treated with siRNA sequences to inhibit the expression of PLA2G5. Sequence 2 was selected for further analyses. Pools of n=4 were measured in triplicate. B, qRT-PCR analysis of inflammatory marker genes, fibrosis marker genes, and MMPs in fibroblasts treated with siRNA to inhibit the expression of PLA2G5. Pools of n=4 were measured in triplicate. *P<0.05 vs WT+control siRNA. †P<0.05 vs Mmp2−/−+control siRNA. All pairwise multiple comparisons vs control group (Holm–Sidak method). MMP indicates matrix metalloproteinase; ND, not detected; qRT-PCR, quantitative real-time polymerase chain reaction; WT, wild-type.
caused by either pharmacological inhibition or gene knock-out.

To validate the conclusions of this research, we combined integrative physiology studies with biochemical analysis including the development of an approach for high-resolution 1-step isolation of bioactive sPLA$_2$ from complex biological sources such as heart homogenates and plasma. To demonstrate the unique relevance of MMP-2, we conducted parallel studies of $Mmp2^{-/-}$, $Mmp7^{-/-}$, $Mmp9^{-/-}$, and haploinsufficient ($Mmp2^{+/+}$) mice. To exclude that the observed regulation of systemic sPLA$_2$ was a mere compensatory result of chronic $Mmp$ gene deficiency, we further examined mice treated with the FDA-approved broad-spectrum MMP inhibitor doxycycline. We show that this latter model replicates many traits of $Mmp2$ gene deficiency, yet doxycycline failed to elevate plasma and cardiac sPLA$_2$ in $Mmp2^{-/-}$ mice, revealing a highly specific and dominant role of MMP-2 in the regulation of systemic sPLA$_2$. To firmly establish the validity of these findings, we further performed gain-of-function studies with WT and $Mmp2^{-/-}$ mice (and cell cultures) involving either transduction of human MMP-2 with an adenoviral vector. These series of complementary and

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**Figure 13.** Effect of MMP-2 on sPLA$_2$ activity. A, Recombinant human PLA2G5 or PLA2G10 (4 μmol/L) were incubated for 4 hours with or without MMP-2 (400 nmol/L). Plasma of WT or $Mmp2^{-/-}$ mice was incubated for 16 hours with or without MMP-2 (100 nmol/L). Results shown are representative of 3 separate experiments. B, Western blot with PLA2G5 antibody showing lack of cleavage of recombinant human PLA2G5 by MMP-2. C, Determination of PLA2G5 content in mouse plasma by Western blot with PLA2G5-specific antibodies. The analysis suggests similar yet negligible PLA2G5 immunoreactivity in plasma of WT and $Mmp2^{-/-}$ mice. Note that PLA2G5 immunoreactivity was not affected by incubation with MMP-2. D, Determination of PLA2G5 in the heart of WT and $Mmp2^{-/-}$ mice by time resolved fluorescence immunoassay (TRFIA). The analysis suggests similar yet negligible PLA2G5 immunoreactivity in heart homogenates of either WT or $Mmp2^{-/-}$ mice; this was in line with the qRT-PCR data for Pla2g5 (shown in Figure 11B). E, MMP-2 does not cleave cardiac sPLA$_2$. Lack of effect of incubation with recombinant human MMP-2 on the sPLA$_2$ from $Mmp2^{-/-}$ heart homogenates (pool of n=3 hearts). Heart homogenate was incubated with or without MMP-2 (400 nmol/L) for 4 hours. Left: Total sPLA$_2$ activity. Right: Electrophoretic migration of sPLA$_2$ activity on Tricine-SDS-PAGE (scatter plot). MMP indicates matrix metalloproteinase; qRT-PCR, quantitative real-time polymerase chain reaction; sPLA$_2$, secreted phospholipase A$_2$; WT, wild-type.
redundant studies unambiguously demonstrated the existence of a novel heart-centric MMP-2/sPLA2 signaling axis that modulates cardiac inflammation and metabolism, systemic blood pressure homeostasis, and fever.

We have not yet discovered the mechanism by which MMP-2 inhibits sPLA2 activation and systemic release. Although we have data pointing to inflammation being secondary to high sPLA2 in Mmp2-deficient mice, further studies are warranted to firmly establish whether preexisting inflammation in Mmp2-deficient mice can be causative of high sPLA2 levels (Figure 19).

Phospholipase A2 was discovered in the 19th century as a major active component in snake venom. The subsequent identification of sPLA2 in mammalian systems began with pancreatic PLA2G1B and then PLA2G2A from human synovial fluid. Members of the family of secreted PLA2 enzymes depend on calcium for activity and have 6 to 8 disulfide bridges and a histidine-aspartate dyad that catalyzes the hydrolysis of water, which enables a nucleophilic attack that releases fatty acids from the sn-2 position of membrane glycerophospholipids.16 Secretory PLA2 isoenzymes have a low molecular mass of $\approx 16$ kDa.

Despite catalyzing the same biochemical reaction, it is increasingly evident that sPLA2s play nonredundant, complex roles in inflammation and metabolism that depend on the target tissue and their relative affinity for specific cell membrane phospholipids; the latter being a function of the head group and the specific fatty acid in the sn-2 position.
PLA2G2A, PLA2G5, and PLA2G10, which are considered to be the major contributors to plasma PLA2 activity in humans, have different induction profiles; for example, PLA2G5 is induced by a Western diet, whereas PLA2G2A is induced by proinflammatory stimuli (eg, bacterial LPS). While PLA2G3 and PLA2G5 have the potential to promote atherogenesis, PLA2G10 may limit atherogenesis.

Like PLA2G5, PLA2G2E is diet inducible. In obesity, PLA2G2E and PLA2G5 are both upregulated in adipose tissue, where PLA2G2E alters lipoprotein phospholipids and facilitates lipid accumulation in adipose tissue and liver and PLA2G5 counteracts adipose tissue inflammation and hyperlipidemia.

Similarly, in the context of immune complex–induced arthritis, where PLA2G2A is a mediator, PLA2G5 may protect against inflammation by promoting immune complex clearance.

Our findings position the heart at the center of a paradigm where cardiac sPLA2 acts in a hormonal fashion to affect inflammatory and metabolic gene expression, fever, and blood pressure homeostasis under negative regulation by MMP-2, and possibly other MMPs (as demonstrated by the lesser effects of MMP-7 and MMP-9). PLA2G5 is the major sPLA2 expressed in the heart. Our siRNA studies conducted with

Figure 15. The MMP-2 inhibitor doxycycline replicates aspects of the phenotype of Mmp2 deficiency. A, Analysis of WT mice administered doxycycline (50 mg/kg per day) for 0 to 15 days (2 weeks). (n=4 mice per time point). The sPLA2 activity of pools was measured in duplicate. *P≤0.05 vs day 0. One-way repeated-measures ANOVA. All pairwise multiple-comparison procedures (Student-Newman-Keuls method). qRT-PCR analysis of indicated inflammatory markers was conducted for individual mice. *P≤0.05 vs day 0. All pairwise multiple comparisons (Holm–Sidak method). B, Analysis of Mmp2−/− mice administered doxycycline (50 mg/kg per day) for 0 to 15 days (2 weeks). (n=3 per time point). The sPLA2 activity of pools was measured in duplicate. qRT-PCR analysis of indicated inflammatory markers was conducted for individual mice. *P≤0.05 vs day 0. All pairwise multiple comparisons (Holm–Sidak method). ANOVA indicates analysis of variance; MMP, matrix metalloproteinase; qRT-PCR, quantitative real-time polymerase chain reaction; sPLA2, secreted phospholipase A2; WT, wild-type. DOI: 10.1161/JAHA.115.001868
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PLA2GS-expressing Mmp2+/+ and Mmp2−/− cell cultures indicated that this sPLA2 contributes to the quantitative trait of MMP-2 deficiency in a significant way. However, we cannot exclude that MMP regulation is a general mechanism controlling the release of multiple sPLA2 isoforms (not just PLA2GS) from tissue. This notion is supported by our enzyme inhibition studies with indoxam. Further, when MMP-2 is inhibited with doxycycline, plasma sPLA2 increases earlier than cardiac sPLA2 activity, which ultimately precedes cardiac inflammation. Overall, the data point to MMP-2 being a major negative regulator for systemic release of active sPLA2 from tissue.

All human and mouse sPLA2s have an N-terminal signal peptide and are, therefore, predicted to be secreted through the conventional endoplasmic reticulum–Golgi pathway. To date, little is known about how sPLA2 secretion is regulated. Nothing is known about how cardiac sPLA2 activity affects the heart or the impact of the cardiac released sPLA2 on systemic homeostasis. We have found that the release of sPLA2 from myocardium occurs rapidly (reaching significance within 2 hours) and does not require stimulation. Myocardial sPLA2 release was unmistakably elevated in Mmp2−/− mice but inhibited in WT and in Mmp2−/− mice forced to express human MMP-2. Confirming that MMP-2 negatively regulates sPLA2 secretion in vivo, we found that plasma sPLA2 is upregulated in Mmp2−/− mice as well. Moreover, the K_M app of the cardiac sPLA2 was equal to that of the plasma sPLA2, indicating that the heart is a major source of systemic released sPLA2. Using a pharmacological inhibitor of MMP-2 and adenovirus-mediated overexpression of human MMP-2, we further showed that MMP-2–mediated proteolysis is necessary for suppressing the release of active sPLA2 from tissue into the circulation and MMP-2 does not act via presecretion or postsecretion degradation of sPLA2 or by regulating sPLA2 transcription. Our data support the notion that sPLA2 acts in a paracrine or endocrine fashion to alter the expression of inflammatory and lipogenic genes in target tissues, including the heart. Like sPLA2, MMP-2 is secreted into the circulation and may thereby affect sPLA2 and the inflammatory and metabolic states in other tissues.

This research suggests the intriguing possibility that the heart is involved in promotion of fever by way of being a major source of sPLA2. To date, sPLA2s have not been solidly implicated in fever. In fact, in our experiments, varespladib did not alleviate LPS-induced fever in WT mice. However, we found that in Mmp2−/− mice, varespladib prevented fever, showing that sPLA2 does indeed contribute to fever if MMP-2 is lacking. A PubMed search of (Varespladib OR LY315920 OR LY333013 OR A-001 OR A-002 OR S-3013 OR S-5920) AND fever did not yield any results as of January 20, 2015. The current data strongly support the notion that sPLA2 is a regulator of fever when MMP-2 is lacking and reveal a novel role of MMP-2 in this process.

Human MMP-2 gene deficiency causes osteolysis/arthritis syndrome and cardiac defects, with severe symptoms presenting from early childhood. Individuals with Winchester syndrome and cardiac defects, with severe symptoms presenting from early childhood, lack a functional MMP-2. How lack of MMP-2 causes these syndromes is unknown. Conceivably, excessive sPLA2 activity in MMP-2–deficient individuals promotes inflammation that could exacerbate bone degeneration and fever, predispose to cardiac dysfunction, and cause hypertension in response to nonsteroidal anti-inflammatory drug treatment.

Figure 16. Emerging functions of the MMP-2/sPLA2 axis in cardiac inflammatory gene expression. A, Cardiac PGE2 concentrations in mice treated with or without varespladib (10 mg/kg per day for 2 days). Pools of n=3 mice per genotype were measured in duplicate. B, qRT-PCR of inflammatory marker genes in mice treated with or without varespladib (10 mg/kg per day for 2 days). n=3 mice per genotype. *P<0.05 vs WT; †P≤0.05 vs Mmp2−/−; ‡Varespladib (control). All pairwise multiple comparisons vs control group (Holm–Sidak method). MMP indicates matrix metalloproteinase; PGE2, prostaglandin E2; qRT-PCR, quantitative real-time polymerase chain reaction; sPLA2, secreted phospholipase A2; WT, wild-type.
inflammatory drugs, as shown here for indomethacin. The powerful effects of varespladib in Mmp2\(^{-/-}\) mice suggest that this drug could be an effective antipyretic and anti-inflammatory treatment in human MMP-2 deficiency.

Preclinical studies indicated that pan-sPLA\(_2\) inhibition with varespladib could reduce atherosclerotic lesions in rodents.\(^{28}\) Varespladib effectively decreases total cholesterol, atherogenesis, and aneurysm formation in apolipoprotein E mouse models.\(^{3}\) Early trials in humans yielded promising results for varespladib as a treatment in coronary heart disease. The PLASMA (Phospholipase Levels and Serological Markers of Atherosclerosis) and PLASMA II trials indicated that varespladib was effective at reducing the plasma concentrations of PLA2G2A, LDL cholesterol, and non-HDL cholesterol.\(^{29,30}\) Those studies provided the rationale and impetus for the VISTA-16 (Vascular Inflammation Suppression to Treat Acute
Coronary Syndrome in 16 weeks) trial, which tested the potential cardiovascular benefits of varespladib given for 16 weeks to acute coronary syndrome patients. In the VISTA-16 trial, varespladib decreased the levels of plasma LDL cholesterol and C-reactive protein but caused an increase in myocardial infarction events, resulting in termination of the trial.31 These adverse clinical results reflect the different roles of sPLA2 isoforms, such that pan-sPLA2 inhibition does not exclusively produce effects beneficial in the treatment of coronary heart disease.25 However, it remains to be determined whether administration of sPLA2 inhibitors (specifically, varespladib) is beneficial in specific sets of patients for which no treatments are currently available. Such is the case of individuals with MMP-2 deficiency.

In cardiac disease pathogenesis, MMP-2 activity has been suggested to be protective in some contexts and deleterious in others. Some deleterious effects of MMP-2 have been explained by excessive cleavage of extracellular matrix.6,7,32 A protective role of MMP-2 expression is the facilitation of inflammatory cell egression from inflamed tissue as has been shown for cardiac interstitial tissue in a model of cytokine-induced cardiomyopathy32 and lung parenchyma in allergen-induced lung inflammation.5 MMP-2 may also be beneficial by degrading chemokines, such as monocyte-chemoattractant protein (MCP)-3, to dampen tissue inflammation8; this mechanism may be relevant in viral cardiomyopathy.33

The possibility of a bidirectional interaction between proinflammatory mediators and sPLA2 isoenzymes and the emerging role of metalloproteinases, such as MMP-2, in the control of this interaction is a fascinating new avenue for research. However, our findings are consistent with inflammation being secondary to high sPLA2 activity in MMP-2 deficiency. Complementary experiments support this notion: (1) Mmp2−/− mice exhibited signs of liver inflammation (Figure 6) but hepatic sPLA2 activity was normal (Figure 9). (2) Inhibition of MMP-2 by doxycycline induced a cardiac...
proinflammatory phenotype in WT mice reminiscent of Mmp2 deficiency. However, this cardiac proinflammatory phenotype was observed only in mice exhibiting sufficiently elevated cardiac sPLA2 (Figure 15). (3) Administration of cardiac sPLA2 microisolated from Mmp2+/− hearts triggered cardiac inflammation in recipient WT mice (Figure 17). (4) LPS, which is a proinflammatory stimulus, did not acutely increase sPLA2 activity in WT or Mmp2−/− mice, at least within the time frame (ie, 5 hours) studied (Figures 6 through 8). Further, addition of a proinflammatory cytokine regulated by MMP-2 cleavage (MCP-3) to Mmp2+/+ and Mmp2−/− fibroblast cultures for up to 48 hours did not elevate sPLA2 activity (unpublished observations) (Evan Berry, BSc, and Carlos Fernandez-Patron, PhD, unpublished data, 2014).

Therefore, the data point to MMP-2 acting as a negative regulator of the activation of a mixture of intracellular sPLA2s, which are proinflammatory, at least under the set of conditions studied. Once secreted from tissue, these sPLA2s may act in paracrine fashion. The interactions between MMP-2 and sPLA2 likely form an autoregulatory loop. Indeed, previous research suggests that at least some sPLA2s (eg, PLAG1B) may stimulate pro-MMP-2 to MMP-2 activation through the PI3K/AKT pathway.

Negative regulation of the systemic release of sPLA2, a family of enzymes with complex protective and deleterious actions on inflammation and metabolic pathways, may further help to explain the complexity of MMP-2 (and MMP inhibitors) actions in the cardiovascular system and their dependence on the pathophysiologic context.

In short, a heart-centric MMP-2/sPLA2 axis may modulate blood pressure homeostasis, inflammatory and metabolic pathways, and may further help to explain the complexity of MMP-2 (and MMP inhibitors) actions in the cardiovascular system and their dependence on the pathophysiologic context.

Disclosures

None.

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