Heart failure that develops from myocardial infarction (MI)-induced remodeling of the left ventricle (LV) is a major cause of morbidity and mortality, with ≈70% of heart failure cases originating from MI.1,2 Occlusion of the coronary artery that leads to ischemia of sufficient duration to induce infarction is followed by a progressive physiological wound healing process that can evolve to prolonged pathological remodeling.3 The LV remodeling process incorporates the collective changes in size, shape, and function of the myocardium that follows the injury stimulus.3 LV remodeling is an intricate process that starts with an acute inflammatory response, overlapping with a proliferative phase, which in turn develops into a maturation phase. In the absence of reperfusion, neutrophil influx initiates the inflammatory response and peaks by 24 hours after MI.3 At around day 3 after MI, neutrophil infiltration is followed by the influx of macrophages. Macrophages stimulate fibroblast differentiation to myofibroblasts, triggering synthesis of large amounts of extracellular matrix (ECM) to generate the infarct scar.3 Infiltrating fibrocytes can transform into fibroblasts and

**Abstract:** The first matrix metalloproteinase (MMP) was described in 1962; and since the 1990s, cardiovascular research has focused on understanding how MMPs regulate many aspects of cardiovascular pathology from atherosclerosis formation to myocardial infarction and stroke. Although much information has been gleaned by these past reports, to a large degree MMP cardiovascular biology remains observational, with few studies homing in on cause and effect relationships. Koch’s postulates were first developed in the 19th century as a way to establish microorganism function and were modified in the 20th century to include methods to establish molecular causality. In this review, we outline the concept for establishing a similar approach to determine causality in terms of MMP functions. We use left ventricular remodeling postmyocardial infarction as an example, but this approach will have broad applicability across both the cardiovascular and the MMP fields. (Circ Res. 2014;114:860-871.)

**Key Words:** extracellular matrix ■ fibrosis ■ inflammation ■ matrix metalloproteinases ■ proteomics ■ ventricular remodeling

H
myofibroblasts, contributing to post-MI remodeling. 4–6 Each phase of LV remodeling follows a closely orchestrated cascade to repair the myocardium. As a result, LV remodeling is a complex and intricate process. Comorbidities and age are 2 factors that can interfere with the healing phase. Impaired LV remodeling is frequently caused by pathological inflammation, which impairs deposition and maturation of ECM. 7 The dynamic synthesis of ECM by cardiac fibroblasts and its breakdown by matrix metalloproteinases (MMPs) play essential roles in the successful remodeling of the LV after MI. 8

In addition to providing structural support for cells, the ECM plays a central role in the regulation of cellular functions. 9–11 ECM components include basic structural proteins, such as collagen and elastin, and specialized proteins, such as fibronectin, proteoglycans, and matricellular proteins. All ECM proteins can be degraded by ≥1 MMPs. Therefore, MMP levels and activity after MI directly modulate ECM structure and composition, and consequently cardiac function.

The Cardiac Metalloproteinase Actions (CarMA) Postulates

In 1890, the German bacteriologist Robert Koch proposed 3 postulates to establish a causal relationship between a specific microbe and an infectious disease. 12,13 In this review, we have used postulates of Koch as a framework to explain in parallel how we can depict the action of MMPs in LV remodeling (Table 1). This schema will apply postulates of Koch to the LV remodeling post-MI scenario as a specific example to illustrate the concepts, but this framework has broad implications to other cardiovascular pathologies, as well as MMP functions in other systems. We propose the term cardiac metalloproteinase actions (CarMA) postulates to define this iterative process of proving MMP causality in LV remodeling. We will conclude our review with a discussion on how our postulates can help to drive the MMP field forward.

CarMA Postulate 1: MMP Levels Increase in All Cases of MI in Direct Proportion to Effect

The first Koch postulate requires that the microorganism be found in abundance in all organisms having the disease, but should not be found in healthy organisms. 12,13 Our analogous postulate dictates that MMP protein expression likewise increases in all cases of MI, either in a linear relationship or over a threshold level of expression for cell types, which have low MMP expression in the absence of MI. This postulate is observational because it does not by itself show the direct effect; it merely places the suspect with the victim.

Human and animal models of MI have reported increased levels of several MMPs (Table 2). In particular, MMP-1, MMP-2, MMP-3, MMP-7, MMP-8, MMP-9, MMP-13, and MMP-14 levels increase; and even in the case of MMP-28, where total levels decrease after MI, macrophage-derived MMP-28 increases. 14–19 Most literature reporting MMP expression levels measure these MMPs in the infarcted region or in the plasma. MMP-2, MMP-8, MMP-9, and MMP-13 have been evaluated in both plasma and tissue. 20–22 It is important to note that 16 of the 25 MMPs identified to date have no literature about changes in levels after MI, and absence of results means either that the results were negative or the studies have not been performed.

The increase in an MMP seen after MI could be because of 1 of 2 reasons: (1) there is an influx of cells not present in the normal myocardium that can express the MMP or (2) there is an upregulation of ectopic expression, such that cell types that normally do not express the specific MMP at high levels are now producing it. There is a lot of support for the first reason, with infiltrating neutrophils and macrophages being a predominant source for the upregulation of several MMPs after MI, including MMP-8 and MMP-9. There is less support for the second reason because cardiomyocytes, endothelial cells, and fibroblasts have not been carefully isolated and measurements made for per cell MMP concentrations. Recently, studies have focused on genetic defects leading to overexpression of activated MMPs and have identified MMP-related polymorphisms as a risk factor in the development of MI in humans. The following paragraphs summarize specific changes

| Table 1. A Comparison of Postulates of Koch and the Newly Defined CarMA Postulates |
|--------------------------------|----------------------------------|
| CarMA Postulates               | Currently Known Aspects          |
| 1. The MMP level changes in all cases of MI | MMP-1, MMP-2, MMP-3, MMP-7, MMP-8, MMP-9, MMP-13, and MMP-14 expression or activity change in plasma or LV of patients or animals after MI |
| 2. MMP actions can be mimicked in vitro | Expression and function of some MMPs in isolated cells, such as fibroblasts, neutrophils, and macrophages leading to modulation of cellular functions |
| 3. Modulation of MMPs alters cardiac remodeling | Effect of inhibition or overexpression of MMP activity on LV remodeling |
| 4. MMP proteolytic products regulate cardiac remodeling | Roles of some MMP substrates and their cleavage products in regulating LV remodeling |

CarMA indicates cardiac metalloproteinase actions; LV, left ventricle; MI, myocardial infarction; and MMP, matrix metalloproteinase.
in individual MMPs in the post-MI setting, by clinical observations first followed by evidence in animal models.

Clinically, MMP-1 has mainly been studied in plasma. MMP-1 levels were higher in the plasma of patients with acute coronary syndrome.23 MMP-1 serum levels predicted the extent of coronary atherosclerosis and were significantly higher in male patients at 6 months when compared with those at 4 days after MI.24,25 Interestingly, MMP-1 promoter polymorphisms MMP-1607 1G/2G, MMP-519 A/G, and MMP-340 T/C have been associated with risk of early MI although the authors did not examine what effect these polymorphisms had on MMP-1 levels.26

MMP-1 is mainly expressed in leukocytes, fibroblasts, and endothelial cells in the post-MI LV. In rats, MMP-1 activity in the infarct LV begins at day 2, peaks at day 7, and declines through day 14 when activity levels return to baseline.27,28 MMP-1 activity parallels the proliferative phase of tissue repair that occurs during myocardial healing. Fibroblasts and endothelial cells are the main cell types present during the proliferation phase, synthesizing new ECM proteins that will spatially replace dead myocytes and form de novo connective tissue to generate a vascularized infarct scar. The new blood vessels formed support the heavy cellular load and develop collateral circulation to the ischemic site. The formation of new vessels requires endothelial cell proliferation and degradation of multiple ECM proteins.29 Studies on MMP-1 in the post-MI LV have been hampered by the fact that human MMP-1 is divergent from mouse MMP-1, in that the mouse has 2 MMP-1 isoforms: MMP-1a and MMP-1b.30 In mice, MMP-1a shares 59% homology and MMP-1b shares 57% homology with human MMP-1.

MMP-2 is constitutively expressed under normal conditions and is synthesized by cardiomyocytes, endothelial cells, vascular smooth muscle cells, and fibroblasts.31–33 Post-MI, MMP-2 levels increase both in human plasma and in infarcted LV.34 Myocytes and myofibroblasts are sources of MMP-2 after MI.35 Plasma MMP-2 levels were shown to correlate strongly with MI size and LV dysfunction in a ST-segment–elevation MI population.36 The MMP-2 1575 gene polymorphism, which increases MMP-2 levels in plasma, correlates with MI occurrence in a male Mexican population.37

Peterson et al.16 reported MMP-2 mRNA and protein levels are elevated within 24 hours after MI and peak around day 14 after MI in rats. In mice, MMP-2 activity rapidly increases within 4 days after MI, peaks at day 7, and remains elevated until day 14.38 Because MMP-2 has high constitutive activity, it has been thought of as the MMP housekeeping gene to oversee normal tissue turnover.35

In a study of 271 patients aged <45 years, 2 MMP-3–related polymorphisms—Leu125Val PECAM1 and A1/A2 FVII—were identified as MI-related and showed strong influence in plaque formation.39 A clinical study in adolescents with ventricular arrhythmia identified plasma MMP-3 as a biomarker of arrhythmia in patients with hypertrophic cardiomyopathy.40 Animal models have shown MMP-3 levels to increase at day 2 after MI in the myocytes of infarcted region and to remain elevated through day 14 after MI.35,41

### Table 2. MMP Expression After MI

<table>
<thead>
<tr>
<th>MMP No.</th>
<th>Other Names</th>
<th>Level After MI</th>
<th>Animal Model</th>
<th>Measured in Humans</th>
<th>Method</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>MMP-1</td>
<td>Collagenase 1</td>
<td>LVI: ↑, Plasma: ↑</td>
<td>Rat, mice</td>
<td>Yes</td>
<td>Immunoblot, ELISA</td>
<td>28,132,133</td>
</tr>
<tr>
<td>MMP-2</td>
<td>Gelatinase A</td>
<td>LVI: ↑, Plasma: ↑</td>
<td>Rat, mice</td>
<td>Yes</td>
<td>Immunoblot, ELISA</td>
<td>28,132,133</td>
</tr>
<tr>
<td>MMP-3</td>
<td>Stromelysin 1</td>
<td>LVI: ↑</td>
<td>Rabbit</td>
<td>No</td>
<td>Zymography</td>
<td>41</td>
</tr>
<tr>
<td>MMP-7</td>
<td>Matrilysin</td>
<td>LVI: ↑, Mice: ↑</td>
<td>No</td>
<td>Immunoblot</td>
<td>14</td>
<td></td>
</tr>
<tr>
<td>MMP-8</td>
<td>Neutrophil collagenase; collagenase 2</td>
<td>LVI: ↑</td>
<td>Rat, sheep</td>
<td>No</td>
<td>Immunoblot</td>
<td>45,46</td>
</tr>
<tr>
<td>MMP-9</td>
<td>Gelatinase B</td>
<td>LVI: ↑, Plasma: ↑</td>
<td>Rat, mice, dog, rabbit</td>
<td>Yes</td>
<td>Immunoblot, ELISA</td>
<td>28,132,133</td>
</tr>
<tr>
<td>MMP-10</td>
<td>Stromelysin 2</td>
<td>Plasma: =</td>
<td>...</td>
<td>Yes</td>
<td>ELISA</td>
<td>138</td>
</tr>
<tr>
<td>MMP-11</td>
<td>Stromelysin 3</td>
<td>Unknown</td>
<td>...</td>
<td>...</td>
<td>...</td>
<td></td>
</tr>
<tr>
<td>MMP-12</td>
<td>Macrophage elastase</td>
<td>Unknown</td>
<td>...</td>
<td>...</td>
<td>...</td>
<td></td>
</tr>
<tr>
<td>MMP-13</td>
<td>Collagenase 3</td>
<td>LVI: ↑</td>
<td>Sheep</td>
<td>No</td>
<td>Immunoblot</td>
<td>46</td>
</tr>
<tr>
<td>MMP-14</td>
<td>MT-1-MMP</td>
<td>LVI: ↑</td>
<td>Rat, sheep</td>
<td>No</td>
<td>Immunoblot</td>
<td>16, 46</td>
</tr>
<tr>
<td>MMP-15</td>
<td>MT2-MMP</td>
<td>Unknown</td>
<td>...</td>
<td>No</td>
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<tr>
<td>MMP-16</td>
<td>MT3-MMP</td>
<td>Unknown</td>
<td>...</td>
<td>No</td>
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<td></td>
</tr>
<tr>
<td>MMP-23</td>
<td>CA-MMP</td>
<td>Unknown</td>
<td>...</td>
<td>No</td>
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<tr>
<td>MMP-26</td>
<td>Matrilysin-2</td>
<td>Unknown</td>
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<td></td>
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<tr>
<td>MMP-27</td>
<td>Unknown</td>
<td>Unknown</td>
<td>...</td>
<td>No</td>
<td>...</td>
<td></td>
</tr>
<tr>
<td>Myocyte-derived MMP-28</td>
<td>Epilysin</td>
<td>LVI: ↓</td>
<td>Mice</td>
<td>No</td>
<td>Immunoblot</td>
<td>19</td>
</tr>
<tr>
<td>Macrophage-derived MMP-28</td>
<td></td>
<td>LVI: ↑</td>
<td>Mice</td>
<td>No</td>
<td>Immunoblot</td>
<td></td>
</tr>
</tbody>
</table>

CA-MMP indicates cysteine array matrix metalloproteinase; LVI, left ventricle infarct region; MI, myocardial infarction; and MT, membrane-type.
MMP-7 is also a biomarker for cardiac disease. Elevated serum MMP-7 levels were observed in 144 patients with LV hypertrophy, and MMP-7 was identified as a marker of LV structural remodeling. Tissue analysis in animal models has shown elevated MMP-7 levels after MI in the remote and infarcted myocardium. Interestingly, this MMP is expressed both in cardiomyocytes, which explains the increased levels in the remote tissue, and in macrophages.

MMP-8 is a major player during the inflammatory response. Studies in humans showed that increases in MMP-8 activity in the infarct area after MI lead to increased susceptibility to cardiac rupture. MMP-8 human plasma levels are significantly increased 1 day after MI.

Early after injury, the major cellular source of MMP-8 is the neutrophil. As such, MMP-8 expression levels in rats increase 6-fold after 6 hours and peak at 12 hours after MI. In sheep, MMP-8 has been shown to be expressed by macrophages during the later stages of remodeling.

In a clinical study of acute ST-segment-elevation MI, plasma MMP-9 levels peaked on days 1 and 4 after MI. MMP-9 activity was positively correlated with LV volume. Blankenberg et al demonstrated that MMP-9 links to the development of LV dysfunction and late survival. This establishes that MMP-9 increases in direct proportion to the effect.

Of all the MMPs evaluated to date, MMP-9 has been the MMP most frequently tracked with the development of LV dysfunction. Rodent models have shown MMP-9 expression to increase after MI, peaking at days 1 to 7 after MI with neutrophils and macrophages being the main source of MMP-9 after MI.

Patients with pressure-overload hypertrophy and a significantly reduced LV ejection fraction showed increased mRNA levels of MMP-1, MMP-13, and MMP-14. Other human studies have correlated increased levels of MMP-13 with cardiomyopathies.

Similar to what is observed in humans, animal models have shown increased MMP-13 activity after MI. MMP-13 is expressed in cardiac fibroblasts. In a rat model, MMP-13 showed a biphasic profile, initially increasing 1 to 2 days after MI followed by a second peak at 2 weeks. In an ovine model, MMP-13 levels were persistently increased ≤1 month after MI.

The membrane-type MMP, MMP-14, slowly increases after MI, peaking at 16 weeks in rats. In pigs, both expression and activity of MMP-14 increase after MI. MMP-14 is expressed in fibroblasts and in myocardies. In a sheep model of MI, MMP-14 increased in the border and infarcted regions when compared with that in the control region and the levels significantly correlated with the extension of LV remodeling.

MMP-28 in normal mouse heart is mainly expressed in the infarcted myocardium, and as such, total MMP-28 levels decrease the MMP-28 derived from macrophages in-creases from day 3 after MI, when this cell type infiltrates into the infarct region.

The results obtained to date highlight the fact that some MMPs increase (MMP-1, MMP-2, MMP-3, MMP-7, MMP-8, MMP-9, MMP-13, MMP-14, and macrophage-derived MMP-28), whereas others decrease (myocyte MMP-28). Of all that have been measured, all MMPs show changes from baseline values, which establishes the first postulate for these individual MMPs.

### CarMA Postulate 2: MMP Action Can Be Mimicked In Vitro

The second Koch postulate requires that the microorganism be isolated from a diseased organism and grown in pure culture. In the case of MI, our postulate dictates that isolated cardiac cells stimulated with factors that induce MMPs display biological functions in vitro that are similar to what is observed during cardiac remodeling in vivo. The effect of MMP stimulation on isolated cardiac-related cells is summarized in Table 3. Although the true translation of this postulate would be that treating cells with MMPs or MMP inhibitors would show the same effects as seen in vivo, studies in this arena have focused on stimuli that increase or decrease MMP levels and subsequently show an effect on cell function.

Cardiac fibroblasts represent >50% of the cells in the normal mammalian heart, and fibroblast numbers dramatically increase after MI. Cardiac fibroblasts express MMP-1, MMP-2, MMP-3, MMP-7, MMP-8, MMP-9, and MMP-14 under normal conditions. MMP-2 followed by MMP-14 and MMP-1 were the most abundant MMPs in human cardiac fibroblasts. MMP-3, MMP-7, MMP-8, and MMP-9 showed low expression. MMP expression in fibroblasts isolated from post-MI LV remains to be evaluated.

Cardiac fibroblasts undergo a transition from a fibroblast to a myofibroblast phenotype during LV remodeling, and stimuli such as inflammatory cytokines that stimulate this phenotype transition also regulate the expression of several MMPs. Transforming growth factor-β (TGF-β) stimulates MMP expression and activity. In fibroblasts, MMP-2, MMP-9, and MMP-14 can process TGF-β to its active form by processing latent TGF-β–binding protein. Treatment with tumor necrosis factor (TNF-α), a proinflammatory factor, that plays a significant role in vivo in the genesis of postischemic inflammation, led to a pronounced increase in the expression of MMP-3, MMP-7, MMP-8, and MMP-9, whereas c2-fold increase was observed in MMP-1, MMP-2, and MMP-14.

In vitro, cardiac fibroblast survival is associated with increased expression and activity of MMP-2. Cardiac fibroblast cultures cultured in a type I collagen lattice upregulated activated MMP-2 and MMP-14. Using a multitude of MMP null skin fibroblasts, the Weiss laboratory showed that secreted collagenases and gelatinases (MMP-2, MMP-8, MMP-9, and MMP-13) provide potent matrix-resorptive activity, whereas only MMP-14 was necessary for focal collagenolytic activity required for cell migration. Insulin-like growth factor-1, a factor released from its binding protein in the ECM by MMP-1, MMP-2, MMP-8, and MMP-9, modulates fibroblast function. Treatment with insulin-like growth factor-1 increased fibroblast adhesion to several ECM proteins and induced the expression of collagen and integrins.

In summary, MMP expression and activity in cardiac fibroblasts can be induced in vitro resulting in modulation of cellular functions, such as adhesion, migration, cytokine production, and ECM secretion.

In addition to the cardiac fibroblast, the neutrophil and the macrophage are key inflammatory cell types that regulate LV remodeling after MI. Isolated canine neutrophils incubated with postischemic cardiac lymph showed increased MMP-9 levels. Neutrophil-derived MMP-8–activated lipopolysaccharide...
induced CXC chemokines to regulate the initial inflammatory response and to promote tissue responsiveness. Neutrophil activation in vitro leads to translocation of active MMP-8 from specific granules to the plasma membrane. Studies performed in neutrophils isolated from healthy donors showed that adenosine, a naturally produced nucleoside, inhibited release of MMP-9, whereas TNF-α and interleukin-8 stimulated MMP-9 expression. Human neutrophils showed 10-fold increased expression of MMP-9 on stimulation with proinflammatory mediators—TNF-α, lipopolysaccharide, or platelet-activating factor.IMMUNOPHILLOPLASMINOGEN REGULATES MACROPHAGE MIGRATION by factors that induce nuclear factor-κB or activation in inflammation by the activation of MMP-9.

80 Tor protein-1.79 Plasminogen regulates macrophage migration stimulated by factors that induce nuclear factor-κB or activation in inflammation by the activation of MMP-9.

80 Tor protein-1.79 Plasminogen regulates macrophage migration stimulated by factors that induce nuclear factor-κB or activation in inflammation by the activation of MMP-9.

Table 3. Effect of MMPs on Isolated Cells

<table>
<thead>
<tr>
<th>Cells</th>
<th>Inducer/Inhibitors</th>
<th>Effect on MMPs</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cardiac fibroblasts</td>
<td>Proinflammatory cytokines</td>
<td>↑ MMP-1, MMP-3, and MMP-9</td>
<td>56</td>
</tr>
<tr>
<td></td>
<td>TGF-β1</td>
<td>↑ MMP-2 and MMP-14</td>
<td>61</td>
</tr>
<tr>
<td></td>
<td>Type I collagen lattice</td>
<td>↑ MMP-2, MMP-14, and TIMP-2</td>
<td>66</td>
</tr>
<tr>
<td></td>
<td>IGF-1</td>
<td>↑ MMP-1, MMP-2, MMP-8, and MMP-9</td>
<td>68</td>
</tr>
<tr>
<td>Human cardiac fibroblasts</td>
<td>TNF-α</td>
<td>↑ MMP-1, MMP-8, MMP-3, MMP-7, and MMP-9</td>
<td>57,64</td>
</tr>
<tr>
<td>Neutrophils</td>
<td>Postischemic cardiac lymph</td>
<td>↑ MMP-9</td>
<td>70</td>
</tr>
<tr>
<td></td>
<td>PFA/LPS/PMA</td>
<td>MMP-8 translocation</td>
<td>72</td>
</tr>
<tr>
<td></td>
<td>TNF-α and IL-8</td>
<td>↑ MMP-9</td>
<td>73–75</td>
</tr>
<tr>
<td></td>
<td>Adenosine</td>
<td>↓ MMP-9</td>
<td>73–75</td>
</tr>
<tr>
<td>Human neutrophils</td>
<td>TNF-α/LPS/PAF</td>
<td>↑ MMP-9</td>
<td>57,64</td>
</tr>
<tr>
<td>Macrophages</td>
<td>PMA</td>
<td>↑ MMP-1 and MMP-3</td>
<td>78</td>
</tr>
<tr>
<td></td>
<td>IFN-γ</td>
<td>↓ MMP-1 and MMP-3</td>
<td>78</td>
</tr>
<tr>
<td>Human macrophages</td>
<td>IFN-γ/LPS</td>
<td>↑ MMP-1, MMP-3, MMP-7, MMP-10, MMP-12, MMP-14, and MMP-25, ↓ TIMP-3</td>
<td>77</td>
</tr>
<tr>
<td></td>
<td>IL-4</td>
<td>↑ MMP-11, MMP-12, MMP-25, and TIMP-3</td>
<td>77</td>
</tr>
<tr>
<td></td>
<td>NF-κB</td>
<td>↑ MMP-1 and MMP-3</td>
<td>79</td>
</tr>
</tbody>
</table>

IFN-γ indicates interferon-γ; IGF-1, insulin-like growth factor-1; IL, interleukin; LPS, lipopolysaccharide; NF-κB, nuclear factor κB; PAF, platelet-activating factor; PMA, phorbol myristate acetate; TGF-β, transforming growth factor-β; TIMP, tissue inhibitor of metalloproteinase; and TNF-α tumor necrosis factor-α.

On the basis of these reports, we found that several MMPs are elevated in isolated MI-relevant cells when stimulated with factors known to affect cardiac remodeling, which establishes the second postulate.

CarMA Postulate 3: Modulation of MMPs Alters the Course of Cardiac Remodeling

The third Koch postulate requires that the cultured microorganism causes disease when introduced into a healthy organism. In the case of MI, our postulate decrees that interventions blocking or enhancing MMP functions will significantly affect LV remodeling.

Reviews by Jourdan-Lesaux et al3 and Phatharajaree et al48 discuss these studies extensively, and we have summarized those findings in Table 4. Several studies performed using broad-spectrum MMP inhibitor have shown beneficial effects on cardiac remodeling after MI.36 The administration of the broad-spectrum MMP inhibitor, CP-471 474, in mice immediately after MI attenuated LV dilation at 4 days after MI.81 This inhibitor inhibits MMP-1, MMP-2, MMP-3, MMP-9, and MMP-13.81 Another study in pigs showed that administration of an MMP inhibitor (PD166793) 5 days after MI led to reduction in MI size and expansion rate by 2 weeks after MI.82 PD 166793 binds to the active domain of MMP-2, MMP-3, and MMP-13 with high affinity and to MMP-1, MMP-7, MMP-9, and MMP-14 with low affinity.83 When treatment was continued for 2 months, the effect persisted to the late phase of MI healing, as evidenced by reduced LV chamber dilation.82

Yarbrough et al84 demonstrated that the MMP inhibitor PGE530742, which blocks MMP-2, MMP-3, MMP-9, and MMP-13, but not MMP-1 or MMP-7, reduces progression of LV end-diastolic volume after MI. PGE530742 is a phosphoamide-based inhibitor that binds with high affinity by forming a tetrahedral complex leading to MMP inhibition.85 The relatively specific MMP-2 inhibitor, 2R-2-[5-[4-[ethyl-methylamino]phenyl]thiophene-2-sulfonamido]-3-methylbutyric acid, showed beneficial changes to remodeling. 2R-2-[5-[4-[Ethyl-methylamino]phenyl]thiophene-2-sulfonamido]-3 methylbutyric acid inhibits MMP-2, MMP-9, and MMP-14 but not MMP-1, MMP-3, or MMP-7. Inhibition of MMP-2 activity increased survival rate and reduced cardiac rupture and macrophage infiltration after MI.86 Tetracyclines,
such as doxycycline, can also regulate coronary artery disease by inhibiting MMP-2, MMP-8, MMP-9, and MMP-13 through their ability to chelate zinc.\textsuperscript{87-89} Cardiac remodeling can be regulated by indirect modulation of MMP expression and activity. The cannabinoid receptor agonist rimonabant decreased MMP-9 activity and TGF-β1 expression in rats, leading to reduced collagen content, attenuation of ECM destruction, and fibrosis 6 weeks after MI.\textsuperscript{90} Salvinolic acid A serves as a competitive inhibitor of MMP-9 and prevented LV remodeling after MI, in part, by preventing fibroblast proliferation and myofibroblast transdifferentiation.\textsuperscript{91} The mechanism by which MMP-9 modulates cardiac fibroblast proliferation and phenotype after MI is not yet known.

Early administration of carvedilol in pigs with acute MI showed reduced monocyte chemotactic protein-1 and MMPs.\textsuperscript{92} Carvedilol is a β blocker and vasodilator that has a unique property of guanine nucleotide modulatable binding.\textsuperscript{93} On the basis of the mechanism of action of carvedilol, we found that its effect on MMPs after MI is probably indirect, but the exact molecular mechanisms that underlie these effects have not yet been established.\textsuperscript{94} Inhibition of endothelin receptor type A by an antagonist prevented LV dilation after MI in rats through inhibition of MMP activation.\textsuperscript{95} At 7 days after MI, Wistar rats showed reduced collagen accumulation after treatment with an endothelin receptor type A antagonist through an unknown mechanism.\textsuperscript{96} TGF-β stimulates MMP-2 and MMP-9 and inhibits MMP-1 and MMP-3 synthesis in vitro, and in a rat model of ischemia-reperfusion TGF-β pretreatment reduced LV dysfunction by blocking MMP-1–mediated cardiomyocyte necrosis.\textsuperscript{97}

MMP inhibition does have some negative effects, and clinical trials have highlighted the difficulties in separating out the divergent functions. MMP inhibition leads to delayed healing in vascular and dermal wounds because MMP activity regulates the migration of inflammatory cells and smooth muscle cells into the wound.\textsuperscript{98} MMP inhibition on collagen deposition has detrimental effects because it prevents the accumulation of collagen necessary for scar formation.\textsuperscript{99} MMP inhibition may affect expression or activity of other signaling factors, including TNF-α, TGF-β, and interleukin-1β, which can induce an imbalance in the ECM turnover process.\textsuperscript{100} The timing of intervention to modulate MMP expression or activity plays a prominent role because some MMP activities are essential for beneficial cardiac remodeling after MI.\textsuperscript{101}

Several MMP null or transgenic mice have been generated to evaluate the effects of gene deletion and introduction in the post-MI setting.\textsuperscript{86,102} Particularly, these studies are beneficial in understanding whether global deletion of a specific MMP can rescue a disease phenotype, and whether reintroducing the MMP at a determined time-point can alter disease development. MMP-2 null mice have attenuated LV rupture and reduced late remodeling after MI.\textsuperscript{86} MMP-7 null mice show improved survival after MI through antiarrhythmic effects mediated through connexin-43 preservation.\textsuperscript{103} MMP-9 null mice have attenuated LV enlargement and reduced collagen accumulation after MI.\textsuperscript{104} MMP-28 deletion exacerbated LV dilation, LV dysfunction and rupture through a defective inflammatory response and suppressed M2 macrophage activation.\textsuperscript{105} More studies using genetic approaches are warranted to move the field toward clinical translation.

Transgenic MMP-1 or MMP-2 mice both develop a cardiac failure phenotype over time, even in the absence of superimposed injury.\textsuperscript{106-108} Alternatively, transgenic overexpression of MMP-9 in macrophages attenuated the inflammatory response and improved LV function after MI.\textsuperscript{7} This report revealed that MMP responses in the MI setting are likely to be

Table 4. Effect of MMP Modulation on Cardiac Remodeling

<table>
<thead>
<tr>
<th>Intervention</th>
<th>Model</th>
<th>Time After MI</th>
<th>Effect Observed</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Broad-spectrum MMP Inhibitor (CP-471 474)</td>
<td>Mice</td>
<td>4 d</td>
<td>↓ LV dilation</td>
<td>81</td>
</tr>
<tr>
<td>Broad-spectrum MMP Inhibitor (PD166793)</td>
<td>Pig</td>
<td>5 d</td>
<td>↓ MI size and expansion</td>
<td>82</td>
</tr>
<tr>
<td>MMP inhibitor (PGE-530742)</td>
<td>Pig</td>
<td>10 d</td>
<td>↓ LV end diastolic volume</td>
<td>53</td>
</tr>
<tr>
<td>Broad-spectrum MMP Inhibitor (CP-471 474)</td>
<td>Rabbit</td>
<td>4 wk</td>
<td>↓ LV dilation, ↑ neovascularization</td>
<td>139</td>
</tr>
<tr>
<td>TISAM</td>
<td>Mice</td>
<td>3 d</td>
<td>↑ Survival, ↓ rupture</td>
<td>86</td>
</tr>
<tr>
<td>MMP-2 null</td>
<td>Mice</td>
<td>28 d</td>
<td>↓ LV rupture</td>
<td>100</td>
</tr>
<tr>
<td>MMP-1 Tg</td>
<td>Mice</td>
<td>No additional injury</td>
<td>↑ Systolic dysfunction, severe LV remodeling</td>
<td>102</td>
</tr>
<tr>
<td>MMP-2 Tg</td>
<td>Mice</td>
<td>No additional injury</td>
<td>↑ Systolic dysfunction, severe LV remodeling</td>
<td>103,104</td>
</tr>
<tr>
<td>MMP-7 null</td>
<td>Mice</td>
<td>7 d</td>
<td>↑ Survival, preserved myocardial conduction patterns</td>
<td>111</td>
</tr>
<tr>
<td>MMP-9 null</td>
<td>Mice</td>
<td>≤15 d</td>
<td>↓ LV enlargement, collagen accumulation</td>
<td>101</td>
</tr>
<tr>
<td>Macrophage-specific MMP-9 Tg</td>
<td>Mice</td>
<td>5 d</td>
<td>↑ LV function, ↓ inflammation</td>
<td>2,105</td>
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<tr>
<td>MMP-28 null</td>
<td>Mice</td>
<td>≤28 d</td>
<td>↑ LV dysfunction and rupture</td>
<td>15</td>
</tr>
<tr>
<td>MT1-MMP Tg</td>
<td>Mice</td>
<td>≤14 d</td>
<td>↑ LV remodeling, fibrosis, ↑ survival</td>
<td>46</td>
</tr>
<tr>
<td>TIMP-1 null</td>
<td>Mice</td>
<td>≤14 d</td>
<td>↑ LV remodeling</td>
<td>48</td>
</tr>
<tr>
<td>TIMP-2 null</td>
<td>Mice</td>
<td>1 wk</td>
<td>↑ Infarct expansion, LV dysfunction and inflammation</td>
<td>107</td>
</tr>
<tr>
<td>TIMP-3 null</td>
<td>Mice</td>
<td>5 and 30 d</td>
<td>↑ LV rupture</td>
<td>140</td>
</tr>
<tr>
<td>TIMP-4 null</td>
<td>Mice</td>
<td>3–7 d</td>
<td>↑ LV rupture</td>
<td>109</td>
</tr>
</tbody>
</table>

I/R indicates ischemia/reperfusion; LV, left ventricle; MI, myocardial infarction; MT1-MMP, membrane type-1-matrix metalloproteinase; Tg, transgenic; TIMP, tissue inhibitor of metalloproteinase; and TISAM, ((2R)-2-[5-[4-[ethyl-methylamino]phenyl]thiophene-2-sulfonylamino]-3-methylbutyric acid.)
both beneficial and detrimental, depending on the time when the MMP is expressed and what substrates are nearby and available for processing. Cardiac-restricted overexpression of MMP-14 caused adverse remodeling, increased fibrosis, and reduced survival.2,105

In conjunction with this postulate, mechanisms that increase MMP activity should promote cardiac remodeling. The extracellular MMP inducer, increases in the LV of patients with acute MI and may play a critical role in LV remodeling after MI.106 Tissue inhibitor of metalloproteinase (TIMP)-1 deletion, as a mechanism to increase MMP activity, aggravated LV remodeling after MI, presumably through stimulating ECM turnover.104 TIMP-2 null mice show greater infarct expansion, exacerbated LV dysfunction, and increased inflammatory response after MI.107 TIMP-3 and TIMP-4 deficiency leads to increased cardiac rupture.108,109 MicroRNA (miR)-21 was recently identified to regulate MMP-2 and increase its expression in cardiac fibroblasts after MI in an ischemia-reperfusion mice model via regulation of phosphatase and tensin homolog signaling pathway in infarct zone.110 Whether and how other miRNAs regulate MMPs has not been extensively explored.

On the basis of these reports, we found that the modulation of MMPs regulates LV remodeling, which establishes the third postulate.

CarMA Postulate 4: MMP Proteolytic Products Regulate Cardiac Remodeling

The fourth Koch postulate requires that the microorganism be reisolated from the inoculated, diseased experimental host and identified as being identical to the original specific causative agent.12,13 In the case of MI, our postulate indicates that MMP substrate cleavage products should regulate LV remodeling, and that MMP effects can be rescued by altering substrate levels. For example, adding back substrate would rescue the MMP null phenotype, and removing substrate would rescue the MMP transgenic phenotype.111 This postulate finalizes the complete mechanistic approach by explaining how MMPs function but is complicated by the fact that many MMPs increase after MI and each MMP has multiple substrates, some of which remain to be identified.

The analogy to reisolation of a microorganism from a diseased individual would be to identify the MMPs associated with a particular cardiovascular disease. However, identification is not enough. The temporal profile of the MMP during the course of disease, as well as cellular source and substrates, are vital information for a full understanding of the disease and for development of diagnostic and prevention tools. The identification of MMPs and MMP-cleavage products as biomarkers in human patients may provide increased diagnostic capabilities for the early detection of disease. This is still a largely unexplored area in both the clinical and the basic science fields of MMP research. However, the studies performed to date provide important information about MMP upregulation in patients.

MMP-3, MMP-9, and TIMP-4 concentrations in plasma samples of patients with atrial fibrillation showed predictive capabilities of these factors for the early recurrence of atrial fibrillation.112 Stress-induced cardiomyopathy patients showed lower MMP-1 and MMP-8 levels but higher TIMP-4 levels with elevated LV end-diastolic pressure when compared with controls.113 In post-MI patients, a specific plasma profile was observed, with decreased MMP-8 and MMP-9 levels and increased TIMP-4 levels.114 The future identification of early changes in levels of MMPs in patients may help to reveal details about the functional mechanisms affected before the onset of cardiac disease.

After MI, ECM undergoes proteolysis directed by MMPs that leads to the generation of ECM peptide fragments called matricryptins or matrikines.115 Matricryptins are substrate fragments produced from the cleavage of such ECM proteins as collagens (I, IV, XVII, and XV), connective tissue glycoproteins (fibronectin, thrombospondin-1, laminin, and secreted protein acidic and rich in cysteine), and elastin.116 Matricryptins serve as bioactive signaling molecules to regulate the post-MI inflammatory and scar formation responses.117

Most studies to date evaluating matricryptins roles have evaluated effects on angiogenesis because a large number of angiogenic factors are released from ECM by MMPs. Endostatin, a fragment of collagen XVIII produced through proteolytic cleavage by elastase, suppresses adverse LV remodeling and heart failure in a rat MI model.117 Other angiogenic factors produced include angiotatin and tumstatin.

Fibronectin plays an important role in wound healing process after MI and is an MMP substrate (MMP-7 and MMP-9, in particular).111,118,119 Fibronectin fragments influence remodeling by regulating monocyte migration into the infarcted myocardium, which improves survival of injured cardiac myocytes.120 Fibronectin fragments trigger the feedback mechanism to induce the fibronectin expression.119 A study in fibronectin extracellular domain A null mice showed that the absence of the extracellular domain A improves survival and cardiac performance by regulating ECM turnover and inflammatory response after MI.121

MMP substrates are not limited to ECM proteins. Interleukin-1β is cleaved by MMP-9 to generate its active form.122 The proinflammatory cytokine interleukin-1β regulates LV remodeling after MI.123 Interleukin-1β has also been shown to induce MMP-1, MMP-3, MMP-8, MMP-9, MMP-13, and MMP-14 in a number of cells and tissues, indicating a positive feedback mechanism.15,123-125 Interleukin-1β is associated with interstitial fibrosis in the chronic phase of a rat MI model.123-125 Treatment with anti–interleukin-1β decreased expression of collagen type III in both infarct and noninfarcted remote areas.123-125 In the acute phase of MI, anti–interleukin-1β treatment caused increased incidences of LV rupture and dilation.123-125 Interleukin-1β also coordinates with TNF-α to regulate ECM remodeling and fibrosis.125

In addition to the substrates mentioned above, there are a wide variety of substrates that have been identified for MMP-9, including thrombospondins, laminins, tenascins, cytokines, and growth factors.51 However, the effect of MMP-9 proteolysis of these substrates on LV remodeling has not been reported. On the basis of these reports, we found that several cleavage products of MMPs regulate LV remodeling, which establishes the fourth postulate.
Challenging Dogma: Moving Beyond Matrix Proteolysis

In the 21st century, Fredericks and Relman suggested a revised version of postulates of Koch based on the modern nucleic acid–based microbial detection methods. The revised postulates provided more sensitivity and specificity in identifying the cause of the microbial disease. Similarly in the MMP field, further understanding is required to predict successfully how modifications could be beneficial or adverse to the post-MI response. The first postulate has been fairly well defined, and we have a generally detailed understanding of the temporal and spatial patterns in the post-MI setting for about a third of the MMPs. The second, third, and fourth postulates still require large voids to be filled before we have a clear understanding of the mechanisms of MMP actions after MI (Figure).

There are several areas in which additional in vivo and in vitro studies are needed, to provide a complete understanding of MMP mechanisms. For one, not all MMPs have been measured after MI and their cell sources identified, which will tell us which cells produce which MMPs under which settings. Importantly, the interactions between a specific MMP and the particular cardiac cell involved remain to be examined. Previous studies have shown that although fibroblasts are the major source of MMPs, MMPs can also regulate fibroblast functions. Membrane type-1-MMP can cleave fibronectin and trigger fibrosis. MMP-2 and MMP-9 cause collagen synthesis by regulating TGF-β. Overexpression of TIMPs 1 to 4 also cause increased collagen synthesis and fibroblast differentiation. These studies provide evidence of complex MMP mechanisms. For one, not all MMPs have been measured after MI and their cell sources measured are as an output measurement. Cell-based targeting studies are also needed that isolate cells directly from the post-MI LV to examine MMP levels under ex vivo conditions. These studies will explain how MMPs influence each other in different cell types and whether this interaction changes by cell type or time. This approach will also provide us information about which MMPs should be promoted or inhibited and at what times this intervention should occur. To develop therapies to protect from adverse remodeling precisely, more quantitative and mechanistic approaches are required to understand the dynamic interaction between MMPs and the different cell types.

MMP inhibition is 1 approach to delineate the role of a specific MMP in different pathological conditions. Catalytic MMP activity is regulated at 4 levels: gene expression, compartmentalization, zymogen activation, and enzyme inactivation. Pathologically, MMPs are primarily regulated at the transcriptional level, with increased expression in response to hypoxia, cytokines, and growth factors. Inhibition of a specific MMP can be achieved with the use of null mouse models, blocking peptides, and siRNAs. These methods will directly affect mRNA stability, protein translation, pro-MMP zymogen activation, trafficking, secretion, and inhibitor binding.

Although these approaches are promising, the failure of clinical trials to translate basic science findings has been frustrating. A more detailed understanding of MMP functions remains to be acquired. Apart from studying the effect of deletion and overexpression of MMPs in a post-MI heart, research should focus on understanding the effects of delivery of MMPs or MMP-specific inhibitors to the post-MI heart. A study in Fischer rats showed that injection of collagen to the infarcted heart improved LV stroke volume and ejection fraction. Such localized treatment studies are required to delineate the processes of LV remodeling in the post-MI setting. Which MMPs target what ECM components at which days after MI is still an unresolved question.

In conclusion, LV remodeling after MI is a complex process. A more complete understanding of MMP substrates is required, to know which substrates are cleaved in the post-MI setting, by which MMPs and at what time(s), and for what purpose. Although there is a lot of information on some MMP substrates, such as those for MMP-2 and MMP-9, we do not know which substrates are the most relevant. For other MMPs, such as MMP-28, few substrates have been identified to date. In addition, identifying MMP substrates is not enough. Knowledge on the biological functions of the MMP-generated substrate peptides is crucial. The identification of the MMP-cleavage sites is required to understand the effect of MMP cleavage on the substrate and its potential for downstream signaling. Currently, only a few substrates and their cleavage sites are known. The MEROPS database integrates information of MMPs and their substrates, including known cleavage sites (http://merops.sanger.ac.uk). One concept to be highlighted in this review is that MMPs process a wide range of substrates and thus affect myocardial biology in ways not only directly related to collagen structure. A more complete understanding of the MMP substrate axis will likely identify specific substrates whose inhibition or overexpression could provide therapeutic means to prevent adverse LV remodeling. MMP substrates may also serve as useful diagnostic indicators to assess treatment response.

In conclusion, LV remodeling after MI is a complex process regulated by a multitude of factors, including MMPs. Targeted future directions. CarMA indicates cardiac metalloproteinase actions; ECM, extracellular matrix; LV, left ventricle; MI, myocardial infarction; and MMP, matrix metalloproteinase.
studies are clearly warranted to identify the mechanisms of MMP actions at both in vitro and in vivo levels, which will help to improve outcomes for the post-MI patient. By suggesting a paradigm to establish cause and effect relationships between remodeling events and specific MMP actions, our postulates can help to further our understanding of both the MMP field generally and the MI field specifically.

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None.

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Translating Koch's Postulates to Identify Matrix Metalloproteinase Roles in Postmyocardial Infarction Remodeling: Cardiac Metalloproteinase Actions (CarMA) Postulates
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