Endogenous and Natural Complement Inhibitor Attenuates Myocardial Injury and Arterial Thrombogenesis

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Background—Coagulation disorders and reperfusion of ischemic myocardium are major causes of morbidity and mortality. Lectin pathway initiation complexes are composed of multimolecular carbohydrate recognition subcomponents and 3 lectin pathway–specific serine proteases. We have recently shown that the lectin pathway–specific carbohydrate recognition subcomponent mannose-binding lectin plays an essential role in the pathophysiology of thrombosis and ischemia/reperfusion injury. Thus, we hypothesized that the endogenous mannose-binding lectin (MBL)/ficolin-associated protein-1 (MAP-1) that inhibits complement activation in vitro also could be an in vivo regulator by attenuating myocardial schema/reperfusion injury and thrombogenesis when used at pharmacological doses in wild-type mice.

Methods and Results—In 2 mouse models, MAP-1 preserves cardiac function, decreases infarct size, decreases C3 deposition, inhibits MBL deposition, and prevents thrombogenesis. Furthermore, we demonstrate that MAP-1 displaces MBL/ficolin-associated serine protease (MASP)-1, MASP-2, and MASP-3 from the MBL complex.

Conclusions—Our results suggest that the natural, endogenous inhibitor MAP-1 effectively inhibits lectin pathway activation in vivo. MAP-1 at pharmacological doses represents a novel therapeutic approach for human diseases involving the lectin pathway and its associated MASPs. (Circulation. 2012;126:2227-2235.)

Key Words: blood coagulation ■ complement activation ■ immunity, innate ■ immunology ■ ischemia ■ myocardial infarction

The innate immune response is a “perfect” system that through evolution makes the seminal decision to respond against foreign pathogens.1 Host defense to foreign invaders is mediated by a repertoire of innate immune molecules and receptors able to recognize pathogen-associated molecular patterns, including bacterial surface mannans and glycans, lipopolysaccharide, and bacterial DNA CpG motifs.1,2 Components of innate immunity also can recognize self-tissue after various insults observed in human disease (ie, during autoimmune disease, transplant rejection, and allergy).2–4 Recent evidence from our laboratories shows that in addition to mannose and N-acetylgalactosamine pathogen-associated molecular patterns, mannose-binding lectin (MBL), an initiation molecule in the lectin complement pathway (Figure 1), recognizes endogenous ligands, resulting in induction of inflammatory mediators, tissue injury, vascular remodeling, and thrombogenesis in several models of human disease in vivo.5–10

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Initiation molecules involved in lectin complement pathway activation in humans include MBL and ficolins (ie, ficolin-1,- 2, and -3), whereas in mice MBL-A and -C are the preeminent initiation molecules.11 Each of these initiation molecules is associated with serine proteases called MBL/ficolin-associated serine proteases (ie, MASP-1, -2, and -3), which are involved in the direct activation of C4 and C2 in the lectin complement pathway and conversion of pro–factor D to factor D and cleavage of factor B in the alternative complement pathway.12–15 The direct activation of the alternative pathway has so far been clearly documented only in rodents. Moreover, they have also been shown to be involved in cleavage of prothrombin to thrombin, cleavage of fibrinogen, activation of factor XIII, and cleavage of kininogen (Figure 1).16–21 Inhibition of MBL or MASP-2 protects against myocardial ischemia/reperfusion (MI/R) injury, whereas the natural endogenous inhibitor C1 inhibitor, which inhibits MASPs in addition to other enzymes (ie, C1r/s, factor XIIa, and kallikrein), also preserves myocardial function after MI/R.7,9,10,22,23 Thus, initiation molecules of the lectin complement pathway and their associated MASPs are involved in the activation of multiple biological pathways involved in human disease.
Specific inhibition of the lectin pathway has the functional capacity to regulate and attenuate many different biological pathways and cascade systems in vivo.

Additional truncated protein variants of the MASPs are also associated with MBL and ficolin complexes, including small MBL-associated protein (MAP19) and MBL/ficolin-associated protein-1 (MAP-1; also called MAp44). MAP-1 and small MBL-associated protein are alternative splice variants originating from the MAP1 and MAP2 genes, respectively, and lack the serine protease domains. MAP-1 displaces MASP-2 and inhibits MBL- and ficolin-3–dependent complement activation in vitro, whereas no conclusive evidence has been attributed to small MBL-associated protein. Thus, we hypothesized that MAP-1 is an inhibitor of the lectin pathway in vivo and could be used as a pharmacological inhibitor of MASP-mediated diseases. In the present study, we investigated the use of MAP-1 as an inhibitor of lectin pathway activation in different in vivo models that activate MASP-1 and/or MASP-2 and are MBL dependent.

Methods

All procedures were reviewed and conducted according to the standards and principles set forth in the Guide for Care and Use of Laboratory Animals, published by National Institute of Health (publication No. 85–23, revised 1996).

Animals

C57BL/6 (wild-type [WT]) mice (8–12 weeks old; Taconic Farms) were used as background controls for genetically modified MBL-null mice as described previously. The following groups were investigated in the in vivo studies: (1) WT, (2) MBL null, (3) WT+BSA (control protein at 500 μg per mouse IP), (4) WT+MAP-1 (300 or 500 μg per mouse IP), (5) MBL null+recombinant human (rh) MBL (30 μg per mouse IP), and (6) MBL null+rhMBL (30 μg per mouse IP)+MAP-1 (160 μg per mouse IP). Mice were housed 4 per cage and had unlimited access to water and standard mouse chow.

Competition ELISA Assay

Mannan (Sigma-Aldrich M7504) was immobilized on Maxisorp ELISA plates (Nunc, Denmark) at 10 μg/mL overnight at 4°C and served as a ligand for MBL. The plates were washed and blocked in TBS with 0.05% Tween-20 and 2 mmol/L CaCl2 before incubation with 0.5 μg/mL rMBL for 2 hours at 20°C. In 3 different experimental settings, serial dilutions of rMAP-1 and rMASP-1, rMASP-2, or rMASP-3 were mixed in nonadsorbent 96-well plates preceding 2 hours of coincubation at 20°C on the rMBL/mannan ELISA plates.

Next, immunodetection was used to assess the binding of rMAP-1, rMASP-2, or rMASP-3 to rMBL. Binding of rMAP-1 and rMASP-3 was detected with 0.5 μg/mL of a monoclonal antibody (mAb) F3–46 reacting with a shared epitope of MASP-1 and -3 but not cross-reacting with MAP-1. Similar results were obtained with a series of other specific MASP-1/3 mAbs. The mAb-producing hybridomas were generated as described previously. Incubation of the primary mAbs was performed under the standards and principles set forth in the Guide for Care and Use of Laboratory Animals, published by National Institute of Health (publication No. 85–23, revised 1996).

Recombinant Proteins

For these assays, we used in-house–generated human recombinant proteins that were all expressed in CHO-DG44 cells (rMBL, rMASP-1, rMASP-2, rMASP-3, and rMAP-1) as previously described. MAP-1 used in the animal experiments was also produced by the same technique, whereas rhMBL used in the animal experiments was a gift from Enzon, Inc.

Murine MI/R Model

The murine MI/R model was performed with the modifications as described. Briefly, mice were anesthetized with sodium pentobarbital (60 mg/kg) for intubation and then ventilated with positive pressure on an SAR Small Animal Ventilator (model 683; Harvard Apparatus, Holliston, MA) and maintained under anesthesia with isoflurane (1.7 MAC). The chest was opened through a sternotomy, and the chest wall was retracted with a 5–0 black-braided silk suture. An 8–0 black-braided silk suture (USSDG; Norwalk, CT) was passed beneath the left anterior descending coronary artery ~2 mm from the tip of the left atrium. A 1- to 2-mm piece of 0–0 suture (Deknatel; Fall River, MA) was placed on the left anterior descending coronary artery, and the ligation was tightened to occlude the artery. After 45 minutes of ischemia, the ligation was loosened and the 0–0 suture removed. Drainage (20GA I.V. Catheter; BD Insyte) was placed through the skin beneath the sternum. The chest was closed.
closed with a 5–0 black-braded silk suture (Ethicon). The skin was sutured with a 5–0 black-braded silk suture (Ethicon). The drainage catheter was removed from the thoracic cavity after air was removed. The animal was removed from the respirator and allowed to reperfuse for 4 hours. ECG changes (ie, ST segment) were monitored and used to establish ischemia and reperfusion.

Infarct Size Measurements
After reperfusion, mice were anesthetized with sodium pentobarbital. The chest cavity was opened, and the left anterior descending coronary artery ligation was reestablished. The heart was flushed retrograde through the thoracic aorta with 500 μL PBS and then perfused with 200 μL 5% Brilliant Blue G (Acros). Hearts were excised and cross-sectioned from base to apex into 1-mm slices with a coronal acrylic matrix (Roboz). Sections were incubated in 1% triphenyltetrazolium chloride (Acros) at 37°C for 15 minutes as described. After triphenyltetrazolium chloride staining, sections were fixed in 10% formalin (Sigma-Aldrich) at 4°C overnight. Each section of the heart was imaged with a Nikon SMZ800 stereoscopic zoom microscope and digital SPOT Insight camera (Diagnostic Instruments, Inc), and areas were calculated with Image J software (National Institutes of Health, Bethesda, MD). Infarct size was determined by calculating total areas of left ventricular free wall, infarcted tissue, nonischemic tissue, and ischemic area at risk. Area at risk was not significantly different between groups (data not shown).

Collection of Blood and Tissue
After reperfusion and echocardiography, the chest cavity was opened, the inferior cava vein was cut, and blood was collected from the thoracic cavity. Hearts were excised and embedded in optimal cutting temperature and frozen in liquid nitrogen–cooled 2-methylbutane.

Ferric Chloride Coagulation Model
The mouse model of localized thrombus formation was used as described. Mice were induced and maintained with isoflurane anesthesia, and blood was collected from the thoracic aorta with 500 μL PBS and then perfused with 200 μL 5% Brilliant Blue G (Acros). Hearts were excised and cross-sectioned from base to apex into 1-mm slices with a coronal acrylic matrix (Roboz). Sections were incubated in 1% triphenyltetrazolium chloride (Acros) at 37°C for 15 minutes as described. After triphenyltetrazolium chloride staining, sections were fixed in 10% formalin (Sigma-Aldrich) at 4°C overnight. Each section of the heart was imaged with a Nikon SMZ800 stereoscopic zoom microscope and digital SPOT Insight camera (Diagnostic Instruments, Inc), and areas were calculated with Image J software (National Institutes of Health, Bethesda, MD). Infarct size was determined by calculating total areas of left ventricular free wall, infarcted tissue, nonischemic tissue, and ischemic area at risk. Area at risk was not significantly different between groups (data not shown).

Histology and Immunohistochemistry
Frozen sections of carotid arteries (5 μm) were fixed with 4% paraformaldehyde for 10 minutes, rinsed with PBS, and then incubated for 1 hour with monoclonal rat anti-mouse MBL-A and MBL-C (Hycult Biotech: 1:100 in PBS/0.05% Triton X-100 supplemented with 1 mMol/L CaCl2). To control for nonspecific staining, isotype control antibody (ie, rat IgG; Vector Laboratories, Burlingame, CA) was used in place of the rat anti-mouse MBL-A and MBL-C mAbs. After a brief rinse, slides were incubated with biotinylated polyclonal rabbit anti-rat IgG (Dako, Carpinteria, CA; 1:600 in PBS for 45 minutes). Tissue sections were incubated with a Vectastain ABC-AP kit (Vector Laboratories), and MBL was detected with a Vector Red alkaline phosphatase substrate kit (Vector Laboratories). All images were captured with a Nikon Eclipse E400 microscope and analyzed with SPOT imaging software.

Myocardial sections (7 μm) were fixed with acetone for 10 minutes, rinsed with PBS, and incubated for 1 hour with polyclonal goat anti-mouse C3 (MP Biomedicals, Solon, OH; 1:500 in PBS/0.05% Triton X-100). After a brief rinse, C3 deposition was detected by the use of donkey anti-goat IRDye800 (Rockland Immunochemicals, Gilbertsville, PA; 1:2000 in PBS for 1 hour). C3 deposition was visualized with an Odyssey infrared imaging system (LI-COR, Lincoln, NE) and analyzed with Image J software.

MBL and C3 Deposition Fluorochrome Immunosorbent Assay
MBL and MBL-dependent C3 deposition on mannan-coated 384 microtiter plates was performed as previously described. Human sera (2%) were incubated with vehicle (veronal buffered saline), anti-MBL mAb (3F8; 10 μg/mL), or MAP-1 (5 or 10 μg/mL) for 1 hour at 37°C; placed in mannan-coated wells; and processed for MBL and C3 deposition as described. An additional fluorochrome immunosorbent assay (FLISA) was also performed by replacing the mannan with N-acetylglucosamine-BSA (GlcNAc–BSA) as the MBL ligand coated to the wells as described. Experimental groups for the GlcNAc–BSA FLISA were vehicle (veronal buffered saline), D-mannose (30 mmol/L), or MAP-1 (1, 5, and 10 μg/mL). Results from the GlcNAc–BSA FLISA were processed the same way as the mannan FLISA. Background integrated intensity from the Odyssey readings in both assays consisted of wells coated with veronal buffered saline only and subtracted from all groups. All groups were performed in triplicate; mannan FLISA was repeated 3 times (n = 3) and the GlcNAc–BSA FLISA was repeated 5 times (n = 5).

Statistical Analysis
All statistical analysis was performed with SigmaStat software (SPSS, Chicago, IL). Data are presented as mean ± SEM. Normality and equal variances were checked in each statistical analysis, and 1-way ANOVA, followed by the Student-Newman-Keuls test, was used to establish significance between groups in all figures with the following exceptions. One-way repeated measures ANOVA, followed by the Holm-Sidak test, was used to compare groups in the FeC1 study. The t test was used to compare the 2 WT groups in the analysis of infarct area. C3 deposition on N-acetylglucosamine–BSA plates was analyzed by a Kruskal-Wallis 1-way ANOVA on ranks, followed by the Dunn method to find differences between groups. Values of P < 0.05 were considered statistically significant.

Results
MAP-1/MASP-1, -2, and -3 Compete for MBL Binding
We investigated the direct competition for MBL binding using checkerboard dilutions of rMAP-1 together with rMASP-1, -2, or -3. The MAP-1/MA SP preparations were coincubated on the MBL/mannan surface to mimic the in vivo situation of the formation of an MBL complex with associated proteins on a natural ligand surface. Similar to all 3 MASPs, we observed a clear tendency that MAP-1, in concentrations >80 ng/mL, inhibited MASP binding to MBL (Figure 2). We observed a very strong dose-dependent inhibitory effect of MAP-1 with an almost complete inhibition of the MASP binding in concentrations >7 μg/mL. We also assessed the inhibition range in MAP-1 concentrations >30 ng/mL, at which no inhibition was observed. At concentrations >20 μg/mL, no additional inhibitory effect was evident (Figure 2).
Figure 2. Influence of mannose-binding lectin (MBL)/ficolin-associated protein-1 (MAP-1) on complex formation between MBL and MBL/ficolin-associated serine protease (MASP)-1 (A), MASP-2 (B), and MASP-3 (C). Recombinant (r) MBL was preincubated on immobilized mannan before application of premixed serial dilutions of MAP-1 with MASP-1, -2, or -3. The level of MASP binding to MBL was detected by anti-MASP monoclonal antibodies and measured as OD490–650 nm. Error bars indicate 2 times the SD of duplicate determinations.
fraction in WT mice after MI/R when 500 µg MAP-1 per mouse was used compared with 300 µg MAP-1 per mouse (Figure 3B). The data demonstrate a MAP-1-induced protection from MBL-induced loss of cardiac function after MI/R.

**MAP-1 Reduces Infarct Size**

We also investigated whether MAP-1 protects the myocardium from infarction after MI/R. As we have previously demonstrated, MBL-null mice have very small myocardial infarctions after MI/R (Figure 4A and 4C). The addition of rhMBL (30 µg per mouse) to MBL-null mice increased myocardial infarction size compared with MBL-null mice (Figure 4C). A treatment (160 µg per mouse) of MBL-null mice supplemented with rhMBL significantly protected mice from myocardial infarction compared with MBL-null+rhMBL mice. Similarly, WT mice treated with control protein (ie, BSA) undergoing MI/R displayed larger myocardial infarctions compared with MAP-1–treated WT mice (Figure 4B and 4C). Thus, the natural, endogenous inhibitor, MAP-1, preserves myocardial function, as well as myocardial tissue from the tissue damage associated with MI/R.

**MAP-1 Prevents Complement Activation After MI/R**

Complement activation after MI/R results in C3 deposition via an MBL complex– and MASP-2–dependent mechanism. WT mice treated with MAP-1 displayed significantly less C3 deposition after MI/R compared with WT mice treated with control protein (ie, BSA; Figure I in the online only Data Supplement). Thus, as previously demonstrated in vitro, MAP-1 also prevents MBL- and MASP-2–dependent complement activation in vivo after MI/R.

**MAP-1 Prevents Occlusive Arterial Thrombogenesis**

Previous studies have demonstrated that FeCl₃-induced occlusive thrombogenesis is mediated by the MBL complex and MASP-1 in vivo. WT+BSA mice developed occlusive thrombogenesis and succession of carotid artery blood flow 15 minutes after application of 3.5% FeCl₃. WT mice treated with MAP-1 (500 µg per mouse) demonstrated no decrease in carotid artery blood flow after FeCl₃ application. These results demonstrate that MAP-1 significantly prevents coagulation in vivo (Figure 5).

**MAP-1 Preserves Myocardial Function**

Hearts from MBL-null mice are protected from MI/R–induced loss of cardiac function compared with WT mice. In contrast, MBL-null mice developed a WT phenotype and significantly decreased myocardial ejection fraction after MI/R (Figure 3A) when given rhMBL, similar to previously observed results. MAP-1 (160 µg per mouse) significantly prevented MI/R-induced loss of cardiac function in rhMBL (30 µg per mouse)–supplemented MBL-null mice (Figure 3A, left). Ejection fractions after MI/R in WT mice treated with MAP-1 were significantly higher compared with WT mice treated with saline or a control protein, BSA (Figure 3A, right). We observed a nonsignificant difference in the ejection fraction in WT mice after MI/R when 500 µg MAP-1 per mouse was used compared with 300 µg MAP-1 per mouse (Figure 3B). The data demonstrate a MAP-1-induced protection from MBL-induced loss of cardiac function after MI/R.
MAP-1 Attenuates MBL Deposition on GlcNac-BSA

We have previously demonstrated that MAP-1 does not prevent MBL deposition on mannan-coated plates, so the inhibition of MBL deposition on carotid arteries was an unexpected observation (Figure 6). MBL and C3 deposition on mannan-coated plates was summarized in Figure 7A. MBL and C3 deposition on mannan was significantly inhibited by mAb 3F8. In contrast, MBL deposition on mannan was not inhibited by MAP-1. Consistent with our findings in Figure 2, MAP-1 significantly attenuated C3 deposition on mannan-coated plates in a dose-related manner. We also investigated whether MAP-1 could inhibit MBL deposition on a structurally different ligand, GlcNac-BSA. As shown in Figure 7B, MAP-1 (10 μg/mL) significantly attenuated MBL deposition and the resulting C3 deposition on GlcNac-BSA. These data demonstrate that in addition to inhibiting MASP incorporation into the MBL complex, MAP-1 inhibits MBL deposition on some MBL ligands and inhibits the resulting complement activation and C3 deposition.

Discussion

MAP-1, also known as Map44, is a recently discovered protein that inhibits lectin complement pathway activation and is highly expressed in striated muscle, including the

Figure 4. Assessment of myocardial infarction after myocardial ischemia/reperfusion in mannanose-binding lectin (MBL)-null and wild-type (WT) mice. Brilliant Blue G dye (blue) denotes the nonischemic area; red and white demonstrate the area at risk (AAR; ischemic tissue). White (unstained) tissue denotes infarcted tissue; red, viable tissue. A, Representative myocardial sections (apex to base) from individual hearts after staining for infarcted tissue in MBL-null mice treated with recombinant human (rh) MBL, rhMBL+MBL/ficolin-associated protein-1 (MAP-1), or saline. B, Representative myocardial sections (apex to base) from individual hearts after staining for infarcted tissue in WT mice treated with MAP-1 (500 μg per mouse IP) or control protein (BSA; 500 μg per mouse IP). C, Percentage of infarcted left ventricle (LV). The percentage of the infarcted area was calculated from LV weight, AAR, area of infarct, and noninfarcted area. The AAR was not significantly different within the groups. All data are mean±SEM of n=3 per group. **P<0.001 vs MBL null +rhMBL or MBL null; *P<0.01 vs WT+rhMBL.
myocardium. Although the functional aspects of this protein are largely unknown, the present study demonstrates that at pharmacological doses, MAP-1 functions as a novel, endogenous, natural inhibitor of the lectin pathway via several mechanisms. First, in vitro studies demonstrate that MAP-1 competitively inhibits all 3 MASPs from interacting with the MBL complex. Second, in vivo MAP-1 also inhibits MBL deposition in the FeCl₃ model of occlusive thrombogenesis. Third, MAP-1 effectively inhibits MBL complex–mediated pathophysiologic outcomes in vivo after MI/R and inhibits thrombogenesis. Thus, MAP-1 is a novel, endogenous, natural inhibitor of the lectin complement pathway in vivo.

Our data extend previous studies demonstrating an important role of the MBL complex in myocardial infarction. MAP-1 in the present study significantly preserved myocardial function, inhibited C3 deposition, and decreased myocardial infarct size in WT mice after MI/R. Along these lines, MASp-2 inhibition or deletion also decreases myocardial infarct size and is responsible for the formation of a C3 convertase necessary for C3 deposition after MBL complex interactions with its ligand. MAP-1 dose-dependently inhibits the association of MASP-2 with the MBL complex in the present study and significantly decreased C3 deposition in vivo after MI/R. Thus, MAP-1, when given at pharmacological doses, functionally inhibits the formation of a functional MBL complex and the resulting complement activation after MI/R in vivo.

Although many studies have investigated the role and function of MASP-2 in the activation of the lectin complement pathway, the functions of MASP-1 and MASP-3 have been demonstrated only recently. MASP-1 and -3 activate the alternative complement pathway. Because MAP-1 dose-dependently inhibits assembly of MASP-1 and -3 into the MBL complex, MAP-1 also likely inhibits this amplification loop, which plays a major role in tissue damage and inflammation after MI/R. The multiple inhibitory mechanisms of action of MAP-1 within the complement system make it a potentially very effective and efficient inhibitor before, during, and after initiation of complement activation. MASP-1 and MASP-2 play significant roles in coagulation. MASP-2 and MASP-1 contribute to the generation of thrombin from prothrombin. MASP-1 dose-dependently cleaves/activates fibrinogen and factor XIII albeit at a slower rate than thrombin in vitro. MAP-1 may also stabilize clot formation by activating thrombin-activatable fibrinolysis inhibitor and thus inhibiting fibrinolysis. We have previously demonstrated that MAP-1 is responsible for FeCl₃-induced occlusive thrombogenesis in vivo. In the present study, MAP-1 dose-dependently inhibited the incorporation of both MASP-1 and MASP-2 into the MBL complex. Furthermore, MAP-1 inhibited occlusive thrombogenesis of the carotid artery after FeCl₃ application in WT mice. Thus, MAP-1 is a functional, native, endogenous inhibitor of coagulation in vivo.

Interestingly, we also observed that MAP-1 inhibited MBL deposition on the vascular endothelium after FeCl₃ application. This observation was unexpected because MAP-1 does not prevent MBL binding to mannann (Figure 7A) and is consistent with our previous findings. In contrast to mAb 3F8, which inhibits both MBL and C3 deposition on mannann, MAP-1 did not inhibit MBL binding to mannann-coated plates but dose-dependently attenuated C3 deposition (Figure 7A). The attenuation of C3 deposition on mannann-coated plates is consistent with the inhibition of MASP incorporation into the MBL complex, as shown in Figure 2, but does not explain the inhibition of MBL binding in the FeCl₃ carotid artery study (Figure 6). The binding avidity of MBL to mannann is significantly higher compared with GlcNAc-BsA. We hypothesize that MAP-1 may inhibit MBL binding on MBL ligands that display lower-avidity binding. Indeed, we observed that MAP-1 significantly attenuated MBL and C3 deposition on GlcNAc-BsA–coated plates. The GlcNAc-
BSA complex is a MBL ligand that displays lower avidity compared with mannan, probably because of the irregular spacing of the GlcNAc attached to BSA. These data suggest that MAP-1, in addition to inhibition of MASPs incorporation into MBL complexes, attenuates MBL binding onto some ligands, which could be particularly relevant for endogenous ligands.

In the present study, we demonstrate multiple functional aspects of MAP-1 in vivo, which are graphically represented in Figure 1. In addition to attenuating myocardial injury and complement activation after MI/R, MAP-1 functionally inhibits occlusive thrombogenesis in vivo. Thus, MAP-1 has a variety of inhibitory properties associated with the MBL complex, particularly MASP-1, MASP-2, and MASP-3. These 3 serine proteases are also associated with the ficolins, which leads to complement activation and likely other biological activities. We cannot exclude additional inhibitory functions of MAP-1 that may be associated with MASPs activity. The present data clearly demonstrate that the native, natural, endogenous inhibitor MAP-1 has multiple functional outcomes associated with coagulation and complement activation in vivo. These pharmacological actions may have a significant impact on the treatment of cardiovascular diseases associated with complement and coagulation abnormalities.

Acknowledgments
We acknowledge the expert technical assistance of Margaret Morrissey during the course of these studies. We thank Dr Lea Munthe-Fog for help with graphical design.

BSA complex is a MBL ligand that displays lower avidity compared with mannan, probably because of the irregular spacing of the GlcNAc attached to BSA. These data suggest that MAP-1, in addition to inhibition of MASPs incorporation into MBL complexes, attenuates MBL binding onto some ligands, which could be particularly relevant for endogenous ligands.

In the present study, we demonstrate multiple functional aspects of MAP-1 in vivo, which are graphically represented in Figure 1. In addition to attenuating myocardial injury and complement activation after MI/R, MAP-1 functionally inhibits occlusive thrombogenesis in vivo. Thus, MAP-1 has a variety of inhibitory properties associated with the MBL complex, particularly MASP-1, MASP-2, and MASP-3. These 3 serine proteases are also associated with the ficolins, which leads to complement activation and likely other biological activities. We cannot exclude additional inhibitory functions of MAP-1 that may be associated with MASPs activity. The present data clearly demonstrate that the native, natural, endogenous inhibitor MAP-1 has multiple functional outcomes associated with coagulation and complement activation in vivo. These pharmacological actions may have a significant impact on the treatment of cardiovascular diseases associated with complement and coagulation abnormalities.

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Figure 7. Mannose-binding lectin (MBL) and MBL-dependent C3 deposition on mannan- (A) or GlcNAc-BSA– (B) coated plates. A, On mannan-coated plates (A), monoclonal antibody 3F8 (10 μg/mL) significantly inhibited MBL (top) and C3 deposition (bottom). MBL/ficolin-associated protein-1 (MAP-1) did not inhibit MBL deposition on mannan-coated plates but significantly attenuated C3 deposition in a dose-related manner. *P<0.001 vs all groups within the same panel. Other statistical comparisons are given in the figure. n=3 in triplicate. II indicates integrated intensity. B, On GlcNAc-BSA–coated plates, D-mannose (d-Man; 30 mmol/L) significantly attenuated MBL and C3 deposition. MAP-1 (10 μg/mL) significantly attenuated C3 deposition on GlcNAc-BSA–coated plates. D-mannose was used as a control because it will not inhibit potential ficolin-induced C3 deposition on GlcNAc-BSA. *P<0.001 vs control; n=5 in triplicate.

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Disclosures
Dr Skjoedt and Garred are listed as inventors on a patent application on the use of MAP-1 as an antiinflammatory agent. The other authors report no conflicts.

References
Reperfusion of ischemic tissues induces tissue injury that is mediated by complement activation. We have identified a novel, endogenous, natural complement inhibitor that displaces the 3 serine proteases (ie, mannose-binding lectin/ficolin-associated serine protease-1, -2, and -3) from the mannose-binding lectin complex in a dose-dependent manner. Furthermore, at pharmacologic concentrations, mannose-binding lectin-associated protein-1 prevents arterial thrombogenesis, as well as myocardial injury after ischemia and reperfusion in vivo. The mannose-binding lectin complex has been associated with several clinical diseases, and mannose-binding lectin-associated protein-1 may represent a novel molecular mechanism to modulate its activity in vivo.
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Supplemental Figure 1. Myocardial C3 deposition following MI/R. Panel A. A representative myocardial section following immunohistochemical staining for murine C3. C3 deposition was visualized by Odyssey imaging.
Supplemental Figure 1 Panel B. Summary of C3 deposition in WT mice treated with MAP-1 or control protein (BSA). Image J analysis was used to quantitative pixel counts. Sections stained for C3 analyzed using Image J software. Date are mean +/- SEM from 6-9 mice/group. *P<0.05 compared to BSA