Extracellular Matrix Metalloproteinase Inducer Regulates Matrix Metalloproteinase Activity in Cardiovascular Cells
Implications in Acute Myocardial Infarction

Roland Schmidt, MD; Andreas Bültmann, PhD; Martin Ungerer, MD; Nader Joghetaei, MD; Özgür Bülbül, MS; Sven Thieme, MS; Triantafyllos Chavakis, MD; Bryan P. Toole, MD; Meinrad Gawaz, MD; Albert Schömig, MD; Andreas E. May, MD

Background—Matrix metalloproteinases (MMPs) are thought to promote progression of atherosclerosis and cardiovascular complications such as plaque rupture. It has been suggested that, on tumor cells, the extracellular MMP inducer (EMMPRIN) is involved in MMP synthesis by as yet unknown mechanisms. On cardiovascular cells, regulation of EMMPRIN in vivo or any functional relevance for MMP induction in vitro has not yet been studied. Thus, we studied EMMPRIN expression on monocytes in acute myocardial infarction (MI) and its potential relevance for MMP activation.

Methods and Results—In 20 patients with acute MI, surface expression of EMMPRIN was significantly enhanced on monocytes compared with in 20 patients with chronic stable angina. EMMPRIN upregulation was associated with increased expression of the membrane type 1 MMP (MT1-MMP) on monocytes (flow cytometry) as well as MMP-9 activity (gelatin zymography) in the plasma. At 6 months after successful revascularization, EMMPRIN, MT1-MMP, and MMP-9 had normalized. The secretion of MMP-9 by monocytes was induced by monocyte adhesion to immobilized recombinant EMMPRIN or to EMMPRIN-transfected Chinese hamster ovary cells. Moreover, adherent EMMPRIN-transfected monocytic cells stimulated MMP-2 activity of human vascular smooth muscle cells. Gene silencing of EMMPRIN by small-interfering RNA hindered lipopolysaccharide-induced monocyte secretion of MMP-9, indicating a predominant role of EMMPRIN in MMP-9 induction.

Conclusions—EMMPRIN and MT1-MMP are upregulated on monocytes in acute MI. During cellular interactions, EMMPRIN stimulates MMP-9 in monocytes and MMP-2 in smooth muscle cells, indicating that EMMPRIN may display a key regulatory role for MMP activity in cardiovascular pathologies. (Circulation. 2006;113:834-841.)

Key Words: atherosclerosis • leukocytes • metalloproteinases • myocardial infarction • plaque

Atherosclerosis is an inflammatory progressive disease of the vascular wall. The rupture of the atherosclerotic plaque represents a key process that initiates acute myocardial infarction (MI). The vulnerability of plaques is characterized by the accumulation of inflammatory cells and matrix metalloproteinases (MMPs) that degrade the fibrous cap of the plaque. Within vulnerable plaques, the membrane type 1 MMP (MT1-MMP, MMP-14), MMP-2, and MMP-9 seem to be the predominant MMPs. The major sources for these MMPs are immigrated monocytes/macrophages and vascular smooth muscle cells (SMCs).

Clinical Perspective p 841

MMPs become activated by a complex activation cascade: the proforms of soluble MMPs become activated by soluble proteases such as plasmin or other MMPs or by binding to membrane-anchored MMPs (membrane-type MMPs [MT-MMPs]). For example, the MT1-MMP functions both as a protease and as a receptor and activator of MMP-2. Thereby, MT1-MMP facilitates cell-associated proteolysis with local protease activity; moreover, as recently shown, MT1-MMP facilitates monocye migration and transmigration through activated endothelial cells. MMP-9 is highly expressed and secreted by activated monocytes. In acute MI, MMP-9 has been found to be secreted into the plasma and therefore can serve as a marker of acute MI.

The synthesis of these MMPs appears to be affected by the so-called extracellular MMP inducer (EMMPRIN, CD147). EMMPRIN is a 58-kDa cell surface glycoprotein of the immunoglobulin superfamily. Originally, EMMPRIN ex-
pression has been described on tumor cells, where it can induce the synthesis of MT1-MMP, MMP-9, and MMP-2 in adjacent fibroblasts through homotypic EMMPRIN-EMMPRIN interactions.\textsuperscript{11–13} However, the mechanism of MMP regulation by EMMPRIN is poorly understood. Recently, EMMPRIN expression has been described on macrophages in vitro and in human atheroma.\textsuperscript{14} In vivo, any regulation of EMMPRIN on cardiovascular cells has not been described thus far.

Therefore, we studied whether EMMPRIN expression on monocytes is regulated in vivo under inflammatory conditions such as acute MI and its potential association with MT1-MMP and MMP-9. Moreover, we investigated whether changes in EMMPRIN expression are of functional relevance for MMP activation.

**Methods**

**Patients**

The study group comprised 20 patients presenting with acute MI (Killip class I to IV) within 24 hours after the onset of pain. The diagnosis was based on a history of prolonged ischemic chest pain, ST-segment elevations, and elevation of creatine kinase $>240$ U/mL with a concomitant rise in MB isoenzyme. We included patients who met the first criterion and at least 1 of the other criteria. Blood samples were obtained on admission before revascularization by stent placement. Successful revascularization was defined as Thrombolysis in Myocardial Infarction (TIMI) flow grade III in coronary angiography after stent placement. Twenty patients with chronic stable angina (CSA) and angiographically proven coronary artery disease and 20 healthy volunteers recruited from the hospital staff were studied as control groups. Patients with noncardiac diseases that may have affected results, such as inflammatory disorders, cancer, or infection, were excluded. The baseline clinical, demographic, and angiographic data in the 2 patient groups are shown in Table 1. Approval for blood sampling was obtained from the institutional ethics committee of Technische Universität München. Informed consent was provided according to the Declaration of Helsinki.

**Cells**

Human monocytes were isolated as described.\textsuperscript{15} Briefly, mononuclear cells were isolated by centrifugation of citrate-phosphate-

### TABLE 1. Baseline Characteristics of Study Population

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Acute MI Group (n=20)</th>
<th>CSA Group (n=20)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean age, y (range)</td>
<td>61.8 (38–83)</td>
<td>59 (36–85)</td>
<td>NS</td>
</tr>
<tr>
<td>Sex, M/F</td>
<td>18/2</td>
<td>17/3</td>
<td>NS</td>
</tr>
<tr>
<td>Hypercholesterolemia</td>
<td>11</td>
<td>13</td>
<td>NS</td>
</tr>
<tr>
<td>Arterial hypertension</td>
<td>8</td>
<td>9</td>
<td>NS</td>
</tr>
<tr>
<td>Smoker</td>
<td>9</td>
<td>8</td>
<td>NS</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>3</td>
<td>4</td>
<td>NS</td>
</tr>
<tr>
<td>One-vessel disease</td>
<td>4</td>
<td>4</td>
<td>NS</td>
</tr>
<tr>
<td>Two-vessel disease</td>
<td>5</td>
<td>6</td>
<td>NS</td>
</tr>
<tr>
<td>Three-vessel disease</td>
<td>11</td>
<td>10</td>
<td>NS</td>
</tr>
<tr>
<td>Time from onset of pain to blood sampling, h</td>
<td>$7.7\pm4.7$</td>
<td>...</td>
<td></td>
</tr>
<tr>
<td>Peak creatine kinase, U/L</td>
<td>$1323\pm1104$</td>
<td>...</td>
<td></td>
</tr>
<tr>
<td>Peak CK-MB, U/L</td>
<td>$97.4\pm85.3$</td>
<td>...</td>
<td></td>
</tr>
<tr>
<td>Peak troponin T, U/L</td>
<td>$6.77\pm6.37$</td>
<td>$0.099\pm0.059$</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>CRP on admission, mg/L</td>
<td>$105.8\pm102.7$</td>
<td>$19.81\pm16.1$</td>
<td>&lt;0.01</td>
</tr>
</tbody>
</table>

Target vessel

| LAD                                | 8                     | ...             |       |
| LCx                                | 4                     | ...             |       |
| RCA                                | 6                     | ...             |       |
| Venous bypass graft*               | 2                     | ...             |       |

Medication on admission

| ACE inhibitors                      | 6                     | 14               | <0.025 |
| AT1 receptor blockers               | 0                     | 0                | NS     |
| Nitrates                            | 1                     | 1                |        |
| Statins                             | 4                     | 9                | <0.05  |
| Aspirin                             | 11                    | 14               | NS     |
| $\beta$-Blockers                    | 6                     | 12               | <0.05  |
| Diuretics                           | 4                     | 12               | <0.01  |
| Calcium channel blockers            | 1                     | 2                | NS     |

Values are number of patients unless otherwise indicated. CRP indicates C-reactive protein; LAD, left anterior descending coronary artery; LCx, left circumflex coronary artery; and RCA, right coronary artery.

*Venous grafts to RCA.
represents human monocytic cells with a closely related pattern of CD14-positive monocytes) were resuspended with the use of EDTA removed by gentle washing. The remaining cells (85% to 90% fetal calf serum (Clonetics). After 24 hours, nonadherent cells were cultured in supplemented VLE-RPMI-1640. Human coronary artery SMCs and SMC basal medium were from Clonetics. Cells were used cultured in supplemented VLE-RPMI-1640. Human coronary artery SMCs were stably transfected with EMMPRIN as described. EMMPRIN surface expression was routinely confirmed before coculture experiments by real-time polymerase chain reaction (PCR) and Western blot.

Flow Cytometry
Flow cytometry was performed as described with the use of primary mouse anti-human monoclonal antibodies anti-EMMPRIN (clone 1G6.2, from Chemicon) or anti–MT1-MMP (clone 114-1F2, from Oncogene).

Gelatin Zymography
Gelatin zymography was performed as described. Precast SDS gels containing 10% gelatin were from Invitrogen. Equal amounts of plasma from patients with acute MI, patients with CSA, or healthy controls or cell supernatants were loaded on the gels. After electrophoresis, renaturation, and incubation of the gels for 12 hours at 37°C, gelatinolytic activity of MMP-2 and MMP-9 was detected as transparent bands on the Coomassie brilliant blue–stained gels. Gelatin zymographies were quantified (optical density) by ImageJ software.

Real-Time Reverse Transcriptase–PCR
Total RNA was extracted from cells with the use of the RNasey Mini Kit (Qiagen). Contaminating DNA was removed by the Message Clean kit (Gene Hunter). RNA was reverse-transcribed with the use of Omniscript reverse transcriptase (Qiagen) and random hexamers (GIBCO). Real-time PCR was performed by the SybrGreen-PCR core reagents kit according to the manufacturer’s instructions (Applied Biosystems) with the use of the primers shown in Table 2.

Small-Interfering RNA–Mediated Gene Silencing of EMMPRIN
Small-interfering RNA (siRNA) for EMMPRIN exon sequence and nonsilencing control siRNA were obtained from Qiagen (2-For-Silencing). The following EMMPRIN duplex was used: sense, 5’-gcggtgcacactcactcttcgctctcttgac-3’; antisense, 5’-gcgggccgcggggtagctgttctgctcttgact-3’. Nonsilencing control siRNA (Qiagen) was used as negative control. Transfections were performed in 24-well plates with a complex of 1 μg siRNA (20 μmol/L) and 6 μL Effectene Transfection Reagent (Qiagen) 48 hours before the experiments. Successful suppression of EMMPRIN surface expression was routinely confirmed by FACS analysis.

Cloning of EMMPRIN
The EMMPRIN was amplified from a human heart cDNA library (Clontech) by PCR with the use of the forward primer 5’- gcggtgcacactcactcttcgctctcttgac-3’ and the reverse primer 5’- gcggtgcacactcactcttcgctctcttgact-3’ (56°C annealing temperature, 24 cycles). The PCR fragment was cloned in the plasmid pADTrack CMV with KpnI/Xhol to get pADTrack CMV EMMPRIN, the sequence was checked by sequencing, and the expression of the recombinant EMMPRIN was tested by Western blot with the use of peroxidase-conjugated anti-flag M2 antibody (Sigma-Aldrich).

Adenovirus Generation
For recombination, electrocompetent Escherichia coli BJ5183 (Stratagene) was cotransformed with 1 μg of Pmel linearized plasmid pADTrack CMV EMMPRIN and 0.1 μg pAdeasy1 at 2500 V, 200 Ω, and 25 μFD (E coli pulser; Bio-Rad). The positive plasmid pAdeasy1 EMMPRIN was retransformed in E coli DH5α and amplified. For transfection (effectene transfection reagent; Qiagen) of 293 cells, pAdeasy1 EMMPRIN was digested with PacI. After 7 days, cells were harvested, the pellet was resuspended in PBS, and the cells were lysed. After centrifugation, the lysate was stored at −80°C. For plaque selection of recombinant virus, infected 293 cells were overlaid with growth medium containing 0.5% agarose (1:1 mix of modified Eagle’s medium 2% [GIBCO Life Technologies] supplemented with 20% serum, 2× penicillin/streptomycin, 2× 1-glutamine, and 1% agarose in water 1%). Five to 14 days after infection, the cell layer was monitored for formation of plaques that were picked, resuspended in 0.5 mL PBS, and stored at −80°C. The plaques were used for further amplification rounds on 293 cells.

Generation of Chimeric EMMPRIN-Fc Fusion Protein
CHO codon–optimized Fc was synthesized (Medigenomix) and cloned KpnI/EcoRV in the plasmid pcDNA5-FRT (Invitrogen; KpnI/Xhol banded) to get pcDNA-FRT Fc opt. For cloning of the extracellular domain of human EMMPRIN, a PCR was performed with the primers 5’-gcggtgcacactcactcttcgctctcttgac-3’ and 5’-gcggtgcacactcactcttcgctctcttgact-3’ with the use of pADTrack CMV EMMPRIN as template DNA at 56°C annealing temperature and 24 cycles. The PCR fragment was cloned in the plasmid pcDNA-FRT Fc with KpnI/NotI to get pcDNA-FRT EMMPRIN-Fc opt and checked by sequencing.

Cloning of Human Fc Fragments
For cloning of a control fragment, the leader peptide of CD40 was amplified from a human heart cDNA library (Clontech, Palo Alto, Calif) by PCR with the use of the primer 5’-gcggtgcacactcactcttcgctctcttgac-3’ and 5’-gcggtgcacactcactcttcgctctcttgact-3’. The PCR fragment was cloned in the plasmid pcDNA-FRT Fc with KpnI/NotI and checked by sequencing.

Western Blot
Cells were lysed in RIPA buffer (20 mmol/L Tris-HCl, pH 8.0, 150 mmol/L NaCl, 1% NP-40, 0.5% deoxycholate, 0.1% SDS, 1 mmol/L EDTA, 10 μg/mL leupeptin, 2 μg/mL aprotinin, 1 mmol/L phenylmethylsulfonyl fluoride). Protein was quantitated with the use of Bio-Rad protein assay reagents. Cell lysates were subjected to precasted 10% SDS-PAGE gels under reducing conditions. Prestained molecular markers (Invitrogen) were used to estimate the molecular weight of samples. Proteins were transferred to Hybond-ECL membrane (Amersham-Pharmacia) in running buffer with 20% methanol. After nonspecific sites were blocked, blots were incubated with mouse monoclonal antibodies anti-human EMMPRIN (HIM6, Becton Dickinson, 10 μg/mL), anti-human MT1-MMP (clone 114-1F2, Oncogene; 10 μg/mL), anti-human MMP-9 (polyclonal, Chemicon, 10 μg/mL), and 5% nonfat milk in TBST. Blots were incubated with peroxidase-conjugated secondary antibodies (1:10,000) and visualized by ECL (Amersham).
EMMPRIN-induced MMP-2 activity of SMCs. After 12 hours, supernatants were harvested and forwarded to gelatin zymography. To determine EMMPRIN-induced MMP-2 activity of SMCs.

**Cell-to-Cell Adhesion**

EMMPRIN-transfected CHO cells or nontransfected CHO cells were cultured in 6-well plates until confluence, fixed with 4% paraformaldehyde, and washed 3 times with PBS. Isolated human monocytes (1 × 10⁶ per well) were allowed to adhere to the fixed CHO cells. After 12 hours, supernatants were harvested for gelatin zymography to determine EMMPRIN-induced MMP-9 activity of monocytes. In other experiments, MonoMac6 cells were infected with increasing amounts of adenovirus EMMPRIN vector (multiplicity of infection [MOI] 0, 10, 50). Successful overexpression of EMMPRIN was confirmed by Western blot and real-time reverse transcriptase–PCR. EMMPRIN-overexpressing monocytic cells were fixed with 4% paraformaldehyde, washed, and coincubated with cultivated human vascular SMCs. After 12 hours, cell supernatants were harvested for gelatin zymography to determine EMMPRIN-induced MMP-2 activity of SMCs.

**Cell Adhesion to Recombinant EMMPRIN-Fc Fusion Protein**

SMCs or isolated monocytes were allowed to adhere to immobilized acellular EMMPRIN-Fc fusion protein (5 μg/mL) and to Fc fragments of human IgG (2 μg/mL) as a negative control with and without additional specific activity–blocking anti–MT1-MMP monoclonal antibody (5 μg/mL) for 24 hours. Afterward, cell supernatants were subjected to Western blot analysis and gelatin zymography.

**Statistical Analysis**

Results with normally distributed continuous variables were reported as mean ± SD and were analyzed by unpaired t test or ANOVA followed by the Scheffé test, as appropriate. Surface expressions of MT1-MMP and EMMPRIN were compared by simple linear regression analysis. In general, P < 0.05 was regarded as significant.

**Results**

**EMMPRIN, MT1-MMP, and MMP-9 in Acute MI**

Monocyte expressions of EMMPRIN and MT1-MMP were studied in patients presenting with acute MI (n = 20) compared with patients with CSA and healthy control persons (n = 20 each). Flow cytometric analysis revealed significantly enhanced surface expression of EMMPRIN in acute MI (Figure 1A), which was associated with enhanced surface expression of MT1-MMP (Figure 1B). Figure 1C demonstrates that EMMPRIN expression in acute MI correlates positively with MT1-MMP expression. Enhanced monocytic surface expression of EMMPRIN was associated with increased MMP-9 activity in the plasma of patients with acute MI (Figure 1D; P < 0.01). No differences of EMMPRIN and MT1-MMP surface expression were found between patients with CSA and healthy controls. At 6 months after successful revascularization, cell surface expressions of both EMMPRIN and MT1-MMP were found to be normalized (Figure 2A; P > 0.05) in accordance with a normalization of the MMP-9 activity (Figure 2B). These data show for the first time that EMMPRIN is regulated on cardiovascular cells in vivo under pathophysiological conditions such as acute MI, which correlates positively with enhanced expression of MT1-MMP and MMP-9.

**EMMPRIN Induces MMP-9 Secretion From Monocytes**

On the basis of these findings, we studied the potential functional consequences of EMMPRIN regulation for MMP activity in vitro. We hypothesized that enhanced cellular expression of EMMPRIN (as found in acute MI) may regulate MMP expression and activity of monocytes. To test this, CHO cells were stably transfected with human EMMPRIN. Successful transfection was confirmed on the mRNA and protein level by real-time PCR (Figure 3A) and by Western blotting (Figure 3B). The cells were fixed with paraformaldehyde (4%) before coincubation with isolated human monocytes. After 12 hours of coincubation, cell culture supernatants were harvested and forwarded to gelatin zymography.
zymography. Figure 3C shows enhanced MMP-9 activity of monocytes that are adherent to EMMPRIN-expressing CHO cells in comparison to nontransfected cells. These data indicate that cell surface–associated EMMPRIN induces MMP-9 secretion in adjacent monocytes during cellular interactions.

To further study the functional relevance of EMMPRIN in an isolated system, EMMPRIN was cloned, and recombinant chimeric Fc-EMMPRIN fusion protein was manufactured. Consistent with the adhesion experiments to cell-associated EMMPRIN, adhesion of human monocytes to the immobilized recombinant Fc-EMMPRIN strongly induced protein secretion and activity of MMP-9 (Figure 3D, 3E) compared with adhesion to immobilized Fc fragments or poly-L-lysine (not shown). Together, these results emphasize the ability of EMMPRIN to stimulate monocytic MMP-9 activity.

**SMC Secretion of MMP-2 on Adhesion of EMMPRIN-Transfected Monocytes**

Within the vulnerable atherosclerotic plaque, degradation of the protective fibrous cap is thought to also be affected by MMP activity of vascular SMCs, which produce substantial amounts of MMP-2. We therefore studied the impact of EMMPRIN-expressing monocytes on SMC secretion of MMP-2 during cellular interactions. Human monocytic MonoMac6 cells were transfected with increasing concentrations of EMMPRIN adenovirus (MOI 0, 10, 50), yielding increasing concentrations of EMMPRIN surface expression, as confirmed by Western blotting (Figure 4A). Nontransfected or transfected monocyte cells were fixed with paraformaldehyde (4%), washed, and coincubated with monolayers of human vascular SMCs. After 12 hours, cell culture supernatants were harvested and analyzed by gelatin zymography. In fact, we found an increased MT1-MMP surface expression of EMMPRIN-stimulated SMCs (data not shown) and enhanced MMP-2 protein secretion and activity of SMCs after cellular adhesion to EMMPRIN-transfected monocytic cells, which was in the range of maximum SMC stimulation achieved with lipopolysaccharide (Figure 4B). Moreover, specific activity–blocking anti–MT1-MMP monoclonal antibody revealed a crucial role for MT1-MMP in EMMPRIN-mediated MMP-2 activation (Figure 4C) within SMCs. These data indicate that enhanced expression of EMMPRIN on monocytes (as has been observed in acute MI) is operative and can induce MMP activity in adjacent cells of the vascular wall, eg, SMCs.
EMMPRIN Is Required for Induction of MT1-MMP and MMP-9 in Monocytes

These results encouraged us to further study the relevance of EMMPRIN within the complex activation cascade of MMPs. EMMPRIN gene silencing was performed with the use of siRNA as described in Methods. The lipopolysaccharide-induced increase of the mean fluorescence for EMMPRIN expression was only 52.5% in EMMPRIN siRNA–pretreated cells in comparison with the increase in control siRNA–treated monocytes. In accordance with our ex vivo data, we found that EMMPRIN gene silencing hindered an adequate induction of MT1-MMP surface expression in response to 12-hour stimulation with lipopolysaccharide (Figure 5A).

MT1-MMP upregulation was inhibited by 42% (±28%). Consistently, analysis of cell culture supernatants revealed that lipopolysaccharide-induced secretion and activity of MMP-9 were primarily abrogated by EMMPRIN gene silencing (Figure 5B, 5C). The application of a control siRNA had no inhibitory effect. These data emphasize a predominant role of EMMPRIN for MMP activation in monocytes, suggesting that the presence of EMMPRIN is needed for MMP activation.

Discussion

MMPs make an essential contribution to the pathophysiology of atherosclerosis.2,3 Specifically, the presence of MT1-MMP, MMP-2, and MMP-9 within vascular walls has been linked to an unstable plaque phenotype, which is prone to rupture.9,20–22 Monocytes/macrophages and SMCs appear to represent the major sources of MMP activity within the artery wall.
plaque. The present study highlights the role of EMMPRIN in cardiovascular diseases. Specifically, our study shows for the first time that EMMPRIN is quantitatively upregulated on monocytes under pathophysiological conditions in vivo: EMMPRIN surface expression is enhanced in acute MI and normalizes after successful therapy. In vivo, EMMPRIN upregulation is accompanied by MT1-MMP upregulation and by enhanced plasma activity of MMP-9. The fact that EMMPRIN expression correlated with MT1-MMP expression suggests common activation pathways. In vitro, we have shown for the first time a functional relevance for EMMPRIN for both monocyte and SMC function: activation of EMMPRIN-transfected monocyctic cells to vascular SMCs stimulated SMC secretion of MMP-2. Similarly, monocyte adhesion to EMMPRIN-transfected CHO cells or to immobilized recombinant EMMPRIN induced monocyte secretion and activity of MMP-9. Moreover, suppression of EMMPRIN by gene silencing abrogated the lipopolysaccharide-mediated induction of MT1-MMP and MMP-9 in monocytes, suggesting that the presence of EMMPRIN is required for MMP stimulation in monocytes. These data indicate that cellular upregulation of EMMPRIN in vivo (eg, in acute MI) may be operative and induce a cascade of MMP activation.

The role of EMMPRIN for MMP activation was described originally on tumor cells.10 EMMPRIN-expressing tumor cells appear to induce MMP-2, MMP-9, and MT1-MMP in adjacent fibroblasts by as yet unknown pathways.12 Additionally, the extent of cell surface expression of EMMPRIN on tumor cells is a marker of poor outcome in various tumors, eg, ovarian carcinoma.23,24 The potential role of EMMPRIN for cardiovascular pathologies has not yet been characterized. Previously, it was shown in vitro that EMMPRIN is upregulated on monocytes/macrophages by atherogenic stimuli such as modified LDL and during monocyte differentiation.25,26 In human atheroma, EMMPRIN has been histomorphologically identified and appeared to be colocalized with macrophages and MMP-9 activity, suggesting functional relevance.14 Our data indicate that the upregulation of EMMPRIN in acute MI may have multiple consequences. Because we could clearly demonstrate in vitro that intercellular interactions involving EMMPRIN induce MMPs in both monocytes (MMP-9) and SMCs (MMP-2), it is tempting to assume that EMMPRIN expression is functionally involved in the accompanying MT1-MMP expression and MMP-9 secretion in acute MI. However, the mechanism of how EMMPRIN may affect MMP activation in acute MI remains unclear. The fact that the hindering of EMMPRIN abrogates the induction of protein secretion and activity of MMP-9 by lipopolysaccharide suggests that EMMPRIN may not only directly induce MMPs as an extracellular inductor but may also represent a key element that is required for MMP induction.

Lately, MT1-MMP has been described as facilitating monocyte migration through activated endothelial cells in vitro.8 Our data suggest that EMMPRIN is crucially involved in MT1-MMP regulation and that MT1-MMP is involved in EMMPRIN-mediated MMP-2 activation within adjacent SMCs. Thus, EMMPRIN and MT1-MMP may allow monocyte migration into the subendothelial space. Within the vessel wall, cellular interactions with adjacent monocytes/macrophages or SMCs may stimulate a cascade of MMP activation involving MT1-MMP, MMP-2, and MMP-9 that may promote plaque progression and destabilization. Together, EMMPRIN may induce a vicious circle leading to extracellular matrix degradation within the atherosclerotic plaque.

Limitations
On admission, patients with CSA (compared with patients with acute MI) had preferably received specific therapy such as ACE inhibitors and statins. Obviously, we cannot fully exclude any influence of the medication on EMMPRIN and MMPs. However, we did not observe any significant differences in EMMPRIN expression or MMP activation in acute MI patients who were on ACE inhibitors or statins in comparison to acute MI patients without previous medication. In addition, we have not recognized any differences between the CSA group and healthy control persons (n = 20), who did not take any medications (data not shown). Therefore, we are convinced that our findings are at least primarily caused by MI rather than medication.

Degradation of the extracellular matrix and MMP facilitated migration of mononuclear cells are fundamental for the development of atherosclerosis, for plaque rupture, for restenosis after percutaneous coronary intervention, and for the inflammatory response to myocardial damage after ischemia and reperfusion.6 This study shows for the first time that EMMPRIN and MT1-MMP on monocytes may represent novel biomarkers of acute MI. EMMPRIN is essentially involved in MMP activation in monocytes and in vascular SMCs. Future studies must show whether EMMPRIN and MT1-MMP display targets for the prevention of unwanted MMP activity during cardiovascular processes.

Acknowledgments
This work was supported by a grant to Dr May from the Wilhelm-Sander Stiftung (2004.801.1). We thank M. Hölderle for expert technical assistance and Dr G. Lehmann for statistical calculations. The authors had full access to the data and take full responsibility for its integrity. All authors have read and agree to the manuscript as written.

Disclosures
None.

References
Approximately 70% of all fatal myocardial infarctions (MIs) are caused by rupture of atherosclerotic plaques. As a consequence, understanding the mechanisms of plaque rupture is crucial to the detection and prevention of this phenomenon. Plaque vulnerability is characterized by the accumulation of inflammatory cells and matrix metalloproteinases (MMPs). Within vulnerable plaques, the membrane type 1 MMP (MT1-MMP, MMP-14), MMP-2, and MMP-9 seem to be the predominant MMPs. In this report, Schmidt and coworkers provide a concept of how the extracellular MMP inducer (EMMPRN/CD147) may act both as a marker of MI and as an active factor in plaque progression: (1) Expression of EMMPRN and MT1-MMP on circulating monocytes is enhanced in acute MI; and (2) EMMPRN, which shows very low constitutive expression on resting cardiovascular cells (monocytes, endothelial cells, smooth muscle cells), becomes upregulated on various inflammatory stimuli and seems to induce MMP synthesis and secretion during cellular interactions. Indeed, monocytes induced to produce EMMPRN stimulate MMP-2 activity of adjacent smooth muscle cells, and reduction of EMMPRN expression inhibits inflammation-stimulated MMP-9 secretion in monocytes. These data imply that EMMPRN inhibition in vivo might reduce the propensity of plaques to rupture, flagging this protein as a potential new therapeutic target in patients with atherosclerosis.
Extracellular Matrix Metalloproteinase Inducer Regulates Matrix Metalloproteinase Activity in Cardiovascular Cells: Implications in Acute Myocardial Infarction
Roland Schmidt, Andreas Bültmann, Martin Ungerer, Nader Joghetaei, Özgür Bülbü, Sven Thieme, Triantafyllos Chavakis, Bryan P. Toole, Meinrad Gawaz, Albert Schömig and Andreas E. May

_Circulation._ 2006;113:834-841; originally published online February 6, 2006; doi: 10.1161/CIRCULATIONAHA.105.568162
_Circulation_ is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2006 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7322. Online ISSN: 1524-4539

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circ.ahajournals.org/content/113/6/834

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in _Circulation_ can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to _Circulation_ is online at:
http://circ.ahajournals.org//subscriptions/