Extracellular Matrix Metalloproteinase Inducer (EMMPRIN) Is Induced Upon Monocyte Differentiation and Is Expressed in Human Atheroma

Terry C. Major, Liang Liang, Xiaokang Lu, Wendy Rosebury, Thomas M.A. Bocan

Objective—Because extracellular matrix metalloproteinase inducer (EMMPRIN), a tumor cell–derived protein, induces matrix metalloproteinases (MMPs) in fibroblasts and because MMPs are important in atheroma formation, we investigated if EMMPRIN was expressed in granulocyte/macrophage-colony stimulating factor (GM-CSF)–differentiated human peripheral blood monocytes (HPBM) and macrophage foam cells. In addition, EMMPRIN was studied for its expression in human atheroma.

Methods and Results—After 10 days of GM-CSF–induced monocyte differentiation, EMMPRIN mRNA increased 5- to 8-fold relative to undifferentiated monocytes. GM-CSF treatment of HPBM revealed that both EMMPRIN mRNA and protein were upregulated by day 2 over undifferentiated monocytes. GM-CSF–differentiated HPBM showed characteristic macrophage phenotype by showing increases in pancake-like morphology and increases in biochemical markers such as apolipoprotein E, MMP-9, and cholesterol ester (CE). While acetylated LDL treatment of the 10-day GM-CSF–differentiated HPBM increased CE mass 13- to 321-fold, EMMPRIN expression was unchanged relative to nonlipid-loaded macrophages. In human coronary atherosclerotic samples, EMMPRIN was observed in CD68(+) macrophage-rich areas as well as areas of MMP-9 expressions.

Conclusions—Based on these data, we conclude that monocyte differentiation induces EMMPRIN expression, CE enrichment of foam cells has no further effect on EMMPRIN expression, and EMMPRIN is present in human atheroma. Therefore, EMMPRIN may play a role in atherosclerosis development. (Arterioscler Thromb Vasc Biol. 2002;22:xxxxxxx.)

Key Words: atherosclerosis ■ EMMPRIN (extracellular matrix metalloproteinase inducer) ■ cholesterol ester ■ human peripheral blood monocyte (HPBM) ■ matrix metalloproteinase-9 (MMP-9) ■ matrix metalloproteinase-1 (MMP-1)

Atherosclerosis is a complex pathological process that has been characterized as the accumulation of modified lipids, monocytes, and cholesterol ester (CE)-containing macrophage foam cells.1-4 Associated with the accumulation of arterial wall macrophages and macrophage foam cells are active forms of matrix metalloproteinases (MMPs) and it is presumed that in these areas extracellular matrix degradation and remodeling may ultimately weaken the atherosclerotic lesion and promote plaque rupture.5-8 Numerous reports have shown that MMP-9 (92-kDa gelatinase), MMP-2 (72-kDa gelatinase),9 and MMP-1 (interstitial collagenase)10 are present in various human atherosclerotic lesions.

Another pathological process that involves the degradation of extracellular matrix to promote cellular invasion is the metastasis of human tumor cells into stromal tissue. A recently discovered membrane protein on tumor cells promotes the invasion and metastasis of tumor cells. This human tumor protein has been termed EMMPRIN or extracellular matrix metalloproteinase inducer. EMMPRIN, also known as basigin, M6 antigen, or CD147, is a 58 kDa cell surface glycoprotein and is a member of the immunoglobulin superfamily that is enriched on the surface of most tumor cells and shown to stimulate underlying stromal cells to produce elevated levels of MMPs, including MMP-1.11-13 These elevated levels of MMPs become concentrated and provide a mechanism for tumor cells to invade and metastasize into the extracellular matrix.14,15 Recently, Guo et al16 have demonstrated that, in addition to inducing MMPs, EMMPRIN forms a complex with MMP-1 at the tumor cell surface, and this additional function may provide an important mechanism for directed modification of extracellular matrix to promote invasion.

Numerous biological mediators such as cytokines, reactive oxygen species,17 and cholesterol18 have been shown to induce expression of MMPs and their endogenous inhibitors, tissue inhibitors of MMPs, or tissue inhibitor of metallo-
preteinases in atherosclerotic plaques. However, it has remained unclear what regulates EMMPRIN expression in tumor cells. Given the similarity of cellular migration, differentiation, and MMP expression between tumor cell metastasis and the transmigration of monocytes during the atherosclerotic inflammatory process, we proceeded to determine if EMMPRIN is expressed in cultured human peripheral blood monocytes (HPBMs), granulocyte/macrophage-colony stimulating factor (GM-CSF)–differentiated macrophages and macrophage foam cells. In this report, we demonstrated that GM-CSF induces EMMPRIN expression in differentiated human macrophages and this expression is not altered by increased cholesterol. For the first time, EMMPRIN has been shown to be expressed in macrophage-rich atheromas from human coronary arteries.

**Methods**

**Materials**

Dulbecco’s modified Eagle’s medium (DMEM), penicillin/streptomycin (pen/strep), 100,000 U/mL each), and Trizol reagent for RNA isolation were purchased from Life Technologies, Inc. Fetal bovine serum (FBS) was obtained from HyClone Laboratories. The monoclonal antibodies to human EMMPRIN (CD147) and CD68 were obtained from Pharmingen. Mouse monoclonal antibodies to human apolipoprotein E (apo E) and α-actin were from Santa Cruz. The monoclonal antibody to human MMP-9 was obtained from Labvims-Neomarkers. The monoclonal antibody to human MMP-1 was obtained from Calbiochem. All other reagents used for these studies were of the highest commercial purity available.

**Cell Culture**

Fresh human peripheral blood monocytes, supplied by Advanced Biotechnology, Inc, were elutriated from the blood of healthy, adult donors as previously described. The first set of cell culture experiments determined the effects of GM-CSF treatment on EMMPRIN expression during a 0- to 8-day monocyte differentiation period. The second set of in vitro experiments evaluated the effects of lipid loading after a 10-day monocyte differentiation induced by GM-CSF. In these experiments, HPBMs were maintained for 10 days in DMEM containing 10% FBS, 1% pen/strep, and 1 ng/mL GM-CSF from R&D Systems to promote monocyte differentiation. On day 10, the cells were washed and incubated with DMEM, 1% Hu-Nutrildoma (Boehringer Mannheim) and 1% pen/strep for an additional 48 hours. On day 12, the cells were incubated with fresh medium; acetylated LDL (acLDL; Intracell Inc) was added for an additional 1, 3, or 7 days (total of 13, 15, or 19 days in culture, respectively). To assess the level of neutral lipid accumulation in macrophages, fluorescent labeling with Nile red stain (Sigma Chemical Co) was used. MMP activity was also assessed from the medium; acLDL (0.5% Nonidet 40 containing 50 mmol/L Tris-HCl, pH 7.5, 1 mmol/L EDTA, 1 mmol/L EGTA, 150 mmol/L NaCl, 10% glycerol, 50 mmol/L sodium fluoride, 10 mmol/L sodium pyrophosphate, 1 mmol/L sodium orthovanadate, 80 μmol/L β-glycerophosphate, 1 mMol/L PMSF, 10 μg/mL aprotinin, 100 μg/mL soybean trypsin inhibitor, and 10 μg/mL leupeptin). Cellular lysates were incubated on a rotary mixer for 1 hour at 4°C. Protein concentrations were measured by using the Bradford reagent (Bio-Rad). Protein extracts (30 μg) were electrophoresed on separate sodium dodecyl sulfate-polyacrylamide gels and transferred onto nitrocellulose for 2 hours at 1 amp. Filters were stained with ponceau red to show protein loading and transfer completeness. Filters were blocked with Tris-buffered saline with 0.05% Tween-20 and 3% nonfat dry milk for 1 hour at room temperature followed by incubation overnight at 4°C with anti-EMMPRIN (1:500 dilution), anti–apo E (1 μg/mL), and anti–α-actin (1 μg/mL) with gentle rocking. Filters were then washed twice with TBS-T for 5 minutes each. The secondary antibodies were incubated with the filters on a rocker for 1 hour at room temperature. After 2 30 minutes TBS-T washes, the filter signals were detected by chemiluminescence (Pierce) and exposed to Kodak Biomax MS film. Densitometric signals were imaged by scanning the film with Adobe Photoshop.

**RNA Isolation and Northern Blot Analysis**

Total RNA was extracted by using the guanidine isothiocyanate method with Trizol (Life Tech.) according to the manufacturer’s protocol. Total RNA (10 μg) was electrophoresed in a 1% formaldehyde/agarose gel and blotted onto a nylon membrane (Ambion) in 20X SSC by capillary transfer. The Northern blot was baked at 80°C for 20 minutes, UV cross-linked, and prehybridized with prehybridization/hybridization buffer. Blots were hybridized at 42°C overnight with radiolabeled [α-32P]dCTP cDNA probes for human EMMPRIN (a 0.591-kb fragment inactivating an EcoRI-ligated pCRII plasmid) and, as an internal control, human S9 ribosomal cDNA (0.9-kb fragment; Clontech). Blots were washed at 55°C twice in low-stringency wash for 10 minutes to remove unbound probe and then twice in high-stringency wash for 15 minutes per wash to remove nonspecific hybridization to the blot. The blots were exposed to either X-ray film or directly on a Storm 860 PhosphorImager for 24 hours, and then resulting signals were quantified by densitometry using Quantscan software (BioSoft) or ImageQuant software (Molecular Dynamics), respectively.

**High-Pressure Liquid Chromatography (HPLC)**

Cell culture experiments designed to measure the free cholesterol (FC), CE, and triglyceride (TG) contents of the HMDM were performed concomitant with those experiments designed for RNA isolation. Cellular lipids were quantified by using a HPLC method. Briefly, HMDM cells were plated into 6-well plates, differentiated for 10 days with 1 ng/mL GM-CSF, incubated for 2 days in 1% apolipoprotein E (apo E) and 50 mmol/L Tris-HCl, pH 7.5, 1 mmol/L EDTA, 1 mmol/L EGTA, 150 mmol/L NaCl, 10% glycerol, 50 mmol/L sodium fluoride, 10 mmol/L sodium pyrophosphate, 1 mmol/L sodium orthovanadate, 80 μmol/L β-glycerophosphate, 1 mMol/L PMSF, 10 μg/mL aprotinin, 100 μg/mL soybean trypsin inhibitor, and 10 μg/mL leupeptin). Cellular lysates were incubated on a rotary mixer for 1 hour at 4°C. Protein concentrations were measured by using the Bradford reagent (BioRad). Protein extracts (30 μg) were electrophoresed on separate sodium dodecyl sulfate-polyacrylamide gels and transferred onto nitrocellulose for 2 hours at 1 amp. Filters were stained with ponceau red to show protein loading and transfer completeness. Filters were blocked with Tris-buffered saline with 0.05% Tween-20 and 3% nonfat dry milk for 1 hour at room temperature followed by incubation overnight at 4°C with anti-EMMPRIN (1:500 dilution), anti–apo E (1 μg/mL), and anti–α-actin (1 μg/mL) with gentle rocking. Filters were then washed twice with TBS-T for 5 minutes each. The secondary antibodies were incubated with the filters on a rocker for 1 hour at room temperature. After 2 30 minutes TBS-T washes, the filter signals were detected by chemiluminescence (Pierce) and exposed to Kodak Biomax MS film. Densitometric signals were imaged by scanning the film with Adobe Photoshop.

**Gel Zymography**

For Western blot analysis, cells (30×10⁶ cells) were washed with PBS, pH 7.4, and then solubilized with 100 to 300 μL of lysis buffer (0.5% Nonidet 40 containing 50 mmol/L Tris-HCl, pH 7.5, 1 mmol/L EDTA, 1 mmol/L EGTA, 150 mmol/L NaCl, 10% glycerol, 50 mmol/L sodium fluoride, 10 mmol/L sodium pyrophosphate, 1 mmol/L sodium orthovanadate, 80 μmol/L β-glycerophosphate, 1 mMol/L PMSF, 10 μg/mL aprotinin, 100 μg/mL soybean trypsin inhibitor, and 10 μg/mL leupeptin). Cellular lysates were incubated on a rotary mixer for 1 hour at 4°C. Protein concentrations were measured by using the Bradford reagent (BioRad). Protein extracts (30 μg) were electrophoresed on separate sodium dodecyl sulfate-polyacrylamide gels and transferred onto nitrocellulose for 2 hours at 1 amp. Filters were stained with ponceau red to show protein loading and transfer completeness. Filters were blocked with Tris-buffered saline with 0.05% Tween-20 and 3% nonfat dry milk for 1 hour at room temperature followed by incubation overnight at 4°C with anti-EMMPRIN (1:500 dilution), anti–apo E (1 μg/mL), and anti–α-actin (1 μg/mL) with gentle rocking. Filters were then washed twice with TBS-T for 5 minutes each. The secondary antibodies were incubated with the filters on a rocker for 1 hour at room temperature. After 2 30 minutes TBS-T washes, the filter signals were detected by chemiluminescence (Pierce) and exposed to Kodak Biomax MS film. Densitometric signals were imaged by scanning the film with Adobe Photoshop.
Coomassie blue and destained with buffer consisting of 10% acetic acid, 50% methanol, and 40% distilled water for 30 minutes to visualize the zymogen bands. An image of each gel (after drying) was scanned into a computer with a Hewlett-Packard scanner, the zymogen bands were quantified by using densitometry, and results are expressed in arbitrary densitometric units. To validate that the zymogen bands were MMPs, 20 mmoL/L EDTA was added to the developing buffer to abolish catalytic activity and 0.1 mmol/L P-aminophenylmercuric acetate, which is known to convert latent zymogens to their active forms, was also added to the developing buffer.

**Immunohistochemistry**

For immunohistochemistry, formalin-fixed, paraffin-embedded sections were used for each human coronary artery atherectomy specimen. For comparison, the internal mammary arteries were used as normal arterial specimens. Nonspecific binding was blocked by preincubation with PBS containing 5% normal horse serum for 30 minutes at room temperature. Serial sections were incubated with mouse antihuman CD147 (EMMPRIN) primary antibody (1:100 dilution), mouse antihuman CD68 (macrophage-specific) primary antibody (1:100 dilution), mouse antihuman α-actin primary antibody (1:50 dilution), mouse antihuman MMP-9 primary antibody (1:10 dilution) or mouse antihuman MMP-1 primary antibody (1:300 dilution) for 1 hour at room temperature. The sections were then incubated with biotinylated conjugated horse antimouse IgG for 30 minutes at room temperature. Antigen-antibody complexes were localized by incubation with avidin/biotin/horseradish peroxidase or alkaline phosphate complex for 30 minutes at room temperature and by subsequently using 3,3′-diaminobenzidine/0.01% hydrogen peroxide or Vector Red chromagen (Research Genetics). Nonimmune controls were performed by substituting mouse serum for specific primary antibodies during the incubation of arterial sections.

**Statistical Analysis**

Data are expressed as mean±SEM. Comparison between undifferentiated monocytes and various times of macrophage differentiation were performed by using nonparametric analysis for unpaired data (Wilcoxon/Kruskal Wallis test).

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**Results**

**Effects of GM-CSF on EMMPRIN Expression and HPBM Differentiation**

To ascertain if EMMPRIN was expressed in human monocytes, western analysis was performed on human peripheral blood monocytes that were differentiated into macrophages in culture by GM-CSF. As shown in Figure 1, EMMPRIN expression increased on day 2 of GM-CSF treatment over the untreated HPBMs. EMMPRIN continued to increase in expression through day 8. Proteins that mark differentiation of monocytes into macrophages such as apo E and MMP-9 activity (by gel zymography) were found to increase in their expression as GM-CSF treatment continued through day 8.

Because GM-CSF-treated HPBMs become macrophages by day 8, EMMPRIN expression appears to be directly correlated to the differentiation process.

In addition to the EMMPRIN protein expression observed in Figure 1, EMMPRIN mRNA also increased through day 4 of GM-CSF treatment (Figure 2A). As with the protein expression, little or no EMMPRIN mRNA was observed in untreated HPBMs. MMP-9 mRNA also increased after 1 day of GM-CSF treatment and marks, as did MMP-9 protein activity, the differentiation of HPBMs into macrophages (Figure 2B).

**EMMPRIN Expression in 10-Day GM-CSF-Differentiated HPBMs and Effects of Lipid Loading**

To establish a protocol that would be able to assess EMMPRIN expression in cultured macrophages and foam cells, the experimental design of HPBM differentiation and lipid loading, as shown in Figure 3A, was used. Human monocytes were differentiated into adherent macrophages for 10 days under the influence of GM-CSF and then lipid-loaded...
by incubating for 1, 3, or 7 days with acLDL under serum and GM-CSF–free conditions. Phase contrast images of each stage of monocyte differentiation are shown at each point of cellular harvest (Figure 3A). A pancake-like cellular morphology of the differentiated macrophages and the “soapy-like” lipid vesicles of the cytoplasm due to lipid accumulation in the macrophage foam cells.

To assess EMMPRIN mRNA expression in differentiated macrophages, a Northern analysis was done. As shown in Figure 3B, EMMPRIN increased 5- to 8-fold in differentiated macrophages, 13 to 19 days after GM-CSF treatment, relative to the monocytes. In all 3 to 4 experiments with individual HPBM populations studied, EMMPRIN expression relative to the housekeeping gene ribosomal S9 RNA was consistently upregulated. The 19-day EMMPRIN expression appears to be comparable to that level observed after 8 days of GM-CSF treatment.

To explore the relationship between lipid loading and EMMPRIN induction, concentration-response and time course experiments were performed with acLDL. Neutral lipid accumulation in differentiated macrophages after acLDL treatment is tabulated in the Table. The maximum accumulation of cellular CE increased 7-, 39-, and 321-fold at 1, 3, and 7 days after acLDL treatment. In addition, acLDL increased TG levels in a concentration- and time-dependent manner while FC remained virtually unchanged (Table). After 3 days of acLDL treatment, the amount of neutral lipids was increased in the macrophages as measured by the bright yellow staining of nile red as compared with the reddish staining due to nile red background (Figure 4A). Similar results were observed at 1 and 7 days of acLDL treatments (data not shown).

To assess if this marked increase in neutral lipids would effect EMMPRIN mRNA expression in macrophage foam cells, a Northern analysis was done. As shown in the representative blot in Figure 4B, EMMPRIN expression was unchanged in macrophage foam cells after 6 to 100 μg/mL acLDL treatment for 3 days relative to the unloaded macrophages. EMMPRIN protein expression followed the same changes as the mRNA expression (data not shown). To assess the effects of foam cell formation on MMP-9 expression, gelatin zymography was done. Shown in Figure 4C, 3 days of lipid loading did not change MMP-9 activity relative to unloaded cultured macrophages.

EMMPRIN Expression in Human Atherosclerotic Specimens

Because nothing is known about the expression of EMMPRIN in human atheromatous plaques, experiments with immunohistochemistry were carried out. EMMPRIN was found to be expressed in all 5 human coronary atheroma samples and not in normal internal mammary artery specimens examined (Figure 5). Serial sections were stained for EMMPRIN, CD68 macrophage-specific stain, and α-actin smooth muscle cell (SMC)-specific stain using monoclonal antibodies. EMMPRIN was observed to be localized to lesion areas of the human atheromas. The area of strong EMMPRIN protein expression (Figure 5) coincided with areas of CD68-positive macrophage staining and not to areas of α-actin–positive SMC staining. In addition, MMP-9 expression coincided with CD68-positive macrophage staining, but MMP-1
expression appeared to be more associated with the SMCc of the media and intima.

**Discussion**

EMMPRIN, which has been found in human tumor cells, has been revealed to exist in human macrophages after GM-CSF-induced differentiation and, for the first time, in human atherosclerotic lesions. In addition, the level of EMMPRIN expression in cultured GM-CSF–differentiated macrophages was maintained during lipid loading.

EMMPRIN expression on monocyte-macrophage differentiation suggests that EMMPRIN may be important in the early phases of directed cell migration and both autocrine and paracrine stimulation of MMP expression, but further work is

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**Figure 3.** EMMPRIN expression in differentiated macrophages after 10 days of GM-CSF treatment. A, Schematic of the cell culture protocol used to differentiate HPBM into macrophages and then lipid load with acLDL for 1, 3, and 7 days. Photomicrographs show the state of differentiation of the HPBMs from monocytes to macrophage foam cells (arrows point to cytoplasmic lipid vesicles). B, Cultured differentiated human macrophages were harvested 1, 3, and 7 days after 10 days of GM-CSF treatment and then after 2 days of GM-CSF removal. EMMPRIN mRNA expression was analyzed by Northern blot analysis as described in the Methods. Graphical representation of EMMPRIN expression after normalization with S9 ribosomal mRNA. Values represent means ±SEM (n=3 to 4). Significance was P<0.07 at 3 days compared with monocyte levels.
needed to test this hypothesis. From our data, EMMPRIN mRNA and protein expression were markedly increased 5- to 8-fold on GM-CSF–induced monocyte to macrophage differentiation. Such increases in a protein may provide the differentiated macrophage with a mechanistic framework to perform specific functional activities at this stage of cellular maturation. In particular, EMMPRIN expression in invading tumor cells is increased significantly and leads to specific upregulation of metastatic tissue MMP levels. These elevated levels of MMPs become concentrated and provide the mechanism for tumor cells to invade and metastasize the extracellular matrix.\(^{22,23}\) Recently, Guo et al.\(^{24}\) have demonstrated an additional mechanism for EMMPRIN in that it forms a complex with MMP-1 at the tumor cell surface, and this additional function may provide an important mechanism for directed modification of extracellular matrix to promote invasion. In 7-day cultured dendritic cells generated by GM-CSF and interleukin-4 (IL-4), CD147, or EMMPRIN was found to be expressed.\(^{25}\) CD147 monoclonal antibodies have recently been shown to induce homotypic cell aggregation of monocytic cell line U937.\(^{26,27}\) Another example of protein upregulation that occurs during the differentiation of monocyte to macrophage is the increase in the cell surface scavenger receptor. The type I scavenger receptor is upregulated during monocyte maturation in order for the macrophage to begin accumulating modified LDL particles that reside in the subendothelial space of the vascular wall. Geng et al.\(^{28}\) demonstrated that a selective increase in type I scavenger receptor mRNA during differentiation from monocyte to macrophage leads to an increased uptake of modified LDL, and such processes precede foam cell formation. The co-expression of EMMPRIN and MMPs on monocyte-macrophage differentiation suggests that EMMPRIN may help establish the differentiated macrophage in the sub endothelial space of the atherosclerotic vessel.

Given that vascular macrophages become lipid loaded, the effect of CE enrichment on EMMPRIN expression was studied in vitro. The present study has shown that CE accumulation and foam cell formation do not appear to have a regulatory role with respect to EMMPRIN expression, because lipid loading did not further increase the levels of EMMPRIN mRNA or protein expression in differentiated macrophages. In contrast to our results, Wang et al.\(^{29}\) have shown that cholesterol loading of the human monocytic cell line THP-1 as well as macrophage-rich areas of human coronary atheromas does influence expression of cellular proteins. They found an increase in the expression of a cytokine, IL-8, after cholesterol treatment. In addition, it has been shown that cholesterol also increased expression of the MMP elastase in human macrophages.\(^{30,31}\) EMMPRIN gene expression has recently been shown in human THP-1 and mouse RAW 246.7 monocyte/macrophage cell lines and under the regulation of non–cholesterol-related transcriptional factors Sp1 and Sp3.\(^{32}\) Based on these observations, one can conclude that GM-CSF stimulates EMMPRIN expression on monocyte differentiation and that this EMMPRIN expression is not altered by cholesterol loading.

Consistent with the in vitro observations, EMMPRIN is localized to macrophage-rich regions of human atherosclerotic plaques. Several lines of evidence exist showing an experimental basis for the potential mechanisms for stabilization of atherosclerotic plaques. Bocan et al.\(^{33}\) showed that

### Table 1

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Data are mean ± SEM. N = 4.
inhibition of vascular ACAT by the ACAT inhibitor, avasimibe, can directly limit macrophage accumulation and can potentially stabilize pre-established atherosclerotic lesions by reducing MMP expression within the lesion. Shah et al. showed that the accumulation of human monocyte-derived macrophages in the fibrous cap region of atherosclerotic plaques induce collagen degradation due to the overexpression of MMPs and that an MMP inhibitor partially blocked this process. In a more recent study, lipid lowering by dietary manipulation has been documented to show a decrease in MMP activity and increase collagen content of established atheroma in rabbits. This lipid lowering was found to decrease the number of macrophages present in the lesions. Lipid lowering in the vascular wall either by diet or ACAT inhibition may decrease matrix-degrading protease expression by limiting the stimuli for gene expression and/or by reducing activation or secretion of these enzymes. Past work has concluded that proinflammatory cytokines, such as tumor necrosis factor-α or IL-1β, potently stimulate MMP expression in macrophages and vascular SMCs. AIkawa et al. speculated that lipid lowering may decrease the stimulus for cytokine gene expression in turn decreasing the expression of vascular wall MMPs. However, cytokine regulation of MMPs may contribute to the overall MMP expression and activation in the atherosclerotic plaque. Novel, previously unknown proteins that can induce MMP expression in the vessel wall may provide other potential mechanisms for the transcriptional regulation of extracellular matrix-degrading proteases and, in part, contribute to overall plaque destabilization. As revealed in this study, a tumor-derived protein, EMMPRIN, may be one of these MMP-inducing proteins in atherosclerosis but further investigation is needed.

In summary, this study reveals for the first time that differentiation of human monocytes into macrophages by the cytokine GM-CSF results in increased expression of the tumor-derived MMP inducing protein EMMPRIN as early as 1 day after the cytokine treatment. Further, our data show that EMMPRIN expression appears to be unaltered by cholesterol treatment in cultured macrophage foam cells. EMMPRIN has also been shown to be expressed in human coronary atherectomy tissue by immunohistochemistry. Because this study has found EMMPRIN present in human atheroma, we hypothesize that the role of EMMPRIN in the atherogenic process is possibly through the induction of MMPs by invading both monocytes and vascular SMCs through EMMPRIN binding with its yet unidentified receptor. EMMPRIN expression, as in tumor growth, may provide a mechanism for atherosclerotic plaque growth and potentially for lesion destabilization.
at sites of active monocyte invasion and differentiation, but this hypothesis still remains untested.

We are grateful to Dr Mark Rehkter (Pfizer Global Research and Development) for providing us with sections of human coronary and internal mammary artery specimens.

References

Figure 5. EMMPRIN expression in human coronary artery atheroma as depicted by photomicrographs. EMMPRIN expression was determined by immunohistochemistry. Immunostaining for both formalin-fixed, paraffin-embedded human coronary atheroma (A through E) and control (normal internal mammary artery) tissues (F through J). Arrows indicate specific EMMPRIN staining (dark areas, A), specific CD68 staining (B), α-actin staining (C), specific MMP-9 staining (dark red areas, D), and MMP-1 staining (E). Arrow indicates lack of specific MMP-1 staining. EMMPRIN, macrophage-specific CD68, SMC-specific α-actin, MMP-9 and MMP-1 stains were labeled by anti-human EMMPRIN, CD68, α-actin, MMP-9, or MMP-1 antibodies, respectively, and visualized by diaminobenzidine or Texas Red chromogens; magnification, ×100. The normal arterial tissues used a nonimmune mouse serum as a control for all immunostaining.


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