Differential Regulation of Matrix Metalloproteinase-9 by Monocytes Adherent to Collagen and Platelets

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Abstract—Circulating monocytes adhere to platelets and matrix proteins at sites of vascular injury, where engagement of specific surface tethering molecules mediates outside-in signaling and synthesis of gene products by the leukocytes. Here we demonstrate that interaction of isolated human monocytes with collagen induces matrix metalloproteinase-9 (MMP-9; gelatinase B) synthesis by monocytes, a process that is greatly enhanced in the presence of platelets. MMP-9 is a potent matrix-degrading enzyme implicated in atherosclerotic plaque rupture, aneurysm formation, and other vascular syndromes. Synthesis of MMP-9 by monocytes is tightly regulated and synergistically increased following adhesion to collagen and platelets. Adhesion to control matrix proteins alone did not result in MMP-9 protein production and, similarly, adhesion of monocytes to platelets activated with thrombin in suspension was not sufficient to induce MMP-9 synthesis in the absence of monocyte adhesion to collagen. Interruption of intercellular contact between platelets and monocytes dramatically inhibited MMP-9 synthesis. These observations demonstrate that discrete adhesion-dependent signaling pathways govern MMP-9 synthesis by monocytes. The synthesis of MMP-9 by monocytes may be critical in vascular syndromes and other pathological processes that are dependent on dysregulated cell-cell and cell-matrix interactions. (Circ Res. 2001;89:509-516.)

Key Words: metalloproteinases ■ leukocytes ■ platelets ■ matrix ■ inflammation

Complex signaling events that lead to cellular phenotypic changes and synthesis of inflammatory and thrombotic mediators occur when human platelets and monocytes interact. One setting for these interactions is damage to the endothelial lining of blood vessels, which exposes the subendothelial matrix and induces circulating platelets to adhere, aggregate, and form hemostatic plugs or pathologic thrombi. During this process, leukocytes are recruited and adhere to the platelets and exposed matrix proteins. Recent evidence indicates that there is a disproportionate number of monocytes among the thrombus-bound leukocytes: their number increased nearly 4-fold compared with that in circulating blood. This suggests that important cell-cell and cell-extracellular matrix (ECM) signaling involving adhesive interactions of monocytes occurs following vascular injury and that the signal transfer influences inflammatory responses, thrombotic and hemostatic processes, and wound healing. Such events may occur, for example, in atherosclerotic plaque rupture and its progression or resolution.

We previously demonstrated that adhesion of platelets to monocytes delivers outside-in signals that induce the expression of key inflammatory gene products. Engagement of P-selectin glycoprotein-1 (PSGL-1) on the monocyte by P-selectin on the activated platelet is critical. Parallel events occur when monocytes adhere to extracellular matrix (ECM). In this setting, numerous transcripts are generated when monocytes adhere to ECM proteins that are recognized by β integrins, but a second signal is required for these messenger RNAs (mRNAs) to be translated into the corresponding gene products. The pattern of expression of mRNAs and their protein products is differentially modulated in monocytes when different adhesion molecules are engaged (Reference 7 and our unpublished observations, 2000). At sites of vascular injury, combinatorial signaling mediated by monocyte adhesion to ECM and cell-cell interactions with platelets may alter expression of monocyte gene products and subsequent inflammatory outcomes. Here, we demonstrate that coordinate interaction of monocytes with activated platelets and collagen induces the expression of matrix metalloproteinase-9 (MMP-9) and show for the first time that adhesion-dependent signals from matrix and from other cells differentially modulate gene expression in monocytes. Our results indicate that ECM proteins and platelets deliver discrete signals to monocytes that lead to the synthesis of MMP-9, a potent mediator of tissue injury, inflammation, and wound remodeling, and...
establish a paradigm for gene expression in complex adhesive interactions of monocytes in vascular injury.

Materials and Methods

Cell Isolation
Washed human platelets were isolated according to protocols previously published by our laboratory. The cells were resuspended in 37°C Medium M199 (M199) for subsequent biological studies. Human peripheral blood monocytes were isolated by countercurrent elutriation from the same donors as platelet isolates using methods that we have previously described in detail. Purified monocytes were resuspended in 37°C M199. For coincubation studies, the cells were incubated at a ratio of 100:1 (1×10⁸ platelets:1×10⁸ monocytes).

Preparation of Immobilized Proteins on Surfaces
Plates (24 mm) were coated with gel-purified human placental collagen type I or laminin (Sigma) using methods previously described. Laminin was suspended in Hanks Balanced Salt Solution (HBSS). Collagen was suspended in 50 mmol/L acetic acid. The plastic surfaces were coated with 50 μg/mL of each protein overnight at 4°C. After the overnight incubation, the unbound matrix proteins were removed and the surfaces were blocked with 1% human serum albumin in HBSS for 4 hours, 25°C. The plates were washed 3 times with HBSS containing 0.1% Tween-20 and then twice with HBSS before use. In selected experiments, transwell coculture dishes (Corning Incorporated) were used to prevent platelets and monocytes from contacting one another. In these studies, both the transwell (0.4 μm) and the bottom well were coated with immobilized protein using the methods described above for collagen type I. For studies in purified systems that did not contain platelets, plastic surfaces were coated with purified P-selectin (2 μg/mL) isolated from platelet membranes as described previously by our laboratory.

Platelet-Monocyte Adherence to Extracellular Matrices
Monocyte adherence to immobilized collagen and laminin was conducted as previously described by our laboratory. In brief, 111indium-labeled monocytes were allowed to adhere to extracellular matrices in the presence or absence of unlabeled platelets. Adherence was expressed as the percentage of the labeled cells that bound after a 2-hour incubation on each surface. In parallel studies, unlabeled platelets, monocytes, or platelets and monocytes were adhered to immobilized collagen or laminin for 2 hours. After this time period, nonadherent cells were washed away and adherent cells were fixed with 4% paraformaldehyde for 20 minutes. After permeabilization with 0.1% Triton X-100, the cells were also left in suspension and activated with phallolidin, which stains polymerized actin in platelets and monocytes, and propidium iodide, which stains nuclei of monocytes. The cells were subsequently viewed by confocal microscopy.

Studies of Platelet-Monocyte Interactions
In experiments in which monocytes interacted with immobilized matrix proteins and with platelets, the platelets were placed on protein-coated surfaces for 5 minutes before the addition of monocytes. In parallel, platelets and monocytes were incubated alone on the immobilized matrix protein. MMP-9 levels were then measured in each sample. Unless otherwise indicated, the time of incubation was 18 hours. After this incubation period, the supernatants were removed, centrifuged, and the cell-free supernatants were stored for subsequent MMP-9 analysis. The pellet cells obtained from this centrifuge were pooled with the adherent cells that were scraped off the tissue culture wells. These cells were lysed in RIPA buffer containing aprotonin (10 μg/mL), leupeptin (10 μg/mL), and 1 mmol/L phenylmethylsulfonyl fluoride for 30 minutes on ice. After this period, the insoluble fraction was pelleted and the soluble lysate was collected for measurement of MMP-9 levels. In parallel studies, platelets and monocytes were also left in suspension and incubated with 0.1 U/mL of thrombin as we previously described to determine whether cellular adherence to matrix is required for MMP-9 synthesis. In selected experiments, the platelet and monocyte suspensions were preincubated for 5 minutes with neutralizing monoclonal antibodies against β₁ integrins (mAb P4/C10) or P-selectin glycoprotein-1 (mAb PL1) before being placed on immobilized protein surfaces. For studies of monocytes in suspension or adherent to P-selectin, the monocytes were left untreated or stimulated with an activating antibody against β₁ integrins (mAb TS2/16) or an isotype-matched control antibody directed against glycoporphin (mAb 10F7).

Zymography
Metalloproteinases generated by platelet-monocyte suspensions were determined using gelatin zymography. Platelet-monocyte conditioned medium was placed in 4× nonreducing SDS sample buffer and electrophoresed in a 10% polyacrylamide gel containing 0.1% gelatin. The gel was then rinsed in 2.5% Triton X-100 for 1 hour on a shaking platform at room temperature followed by rinsing with dH₂O until all the Triton was removed. The gel was subsequently incubated at 37°C for 24 hours in substrate buffer containing 50 mmol/L Tris-base (pH 8.5) and 5 mmol/L CaCl₂, followed by staining with Coomassie Blue. After destaining, the presence of gelatinases was identified as clear bands on a uniform blue background.

Immunocytochemistry
Immunocytochemical studies were performed as described previously by our laboratory. For immunocytochemical detection of latent and active MMP-9 in monocytes adherent to laminin or collagen, the cells were incubated with mouse monoclonal antibody against human MMP-9 (Clone IIA5, Neomarkers). Red immunoreactive protein for MMP-9 was detected using an ABC kit from Vectorstain (Vector Laboratories) for alkaline phosphatase detection. The nuclei of these cells were stained blue with Gill’s hematoxylin No.3. For immunocytochemical detection of latent and active MMP-9 in monocytes adherent to P-selectin and stimulated with the mouse anti-human β₁ integrin activating antibody, the cells were incubated with a rabbit anti-human antibody directed against MMP-9 (H-129, Santa Cruz). In these studies, MMP-9 was detected with an anti–rabbit secondary antibody fluorescein isothiocyanate tagged with Oregon Green (Molecular Probes). The nuclei were stained with propidium iodide.

Western Analysis
Cell suspensions were collected and pelleted as described above. The cell-free supernatants were precipitated with acetone. The samples were then placed in 100 μL of SDS-PAGE nonreducing buffer and electrophoresed on an 8% SDS-polyacrylamide gel. Western analysis was conducted using affinity-purified, rabbit polyclonal (5980-0907, Biogenes) or mouse monoclonal (Ab-2, Oncogene) antibodies that preferentially recognize latent MMP-9. The Biogenesis antibody also recognizes an additional band of unknown specificity in nonreducing conditions (Manufacturer’s Data Sheet). Immunoreactive protein was detected by affinity-isolated goat anti-rabbit or anti-mouse antibody conjugated to horseradish peroxidase (Bio-source International) followed by an enhanced chemiluminescence detection reagent (Amersham Life Sciences).

Reverse Transcription–Polymerase Chain Reaction (RT-PCR)
Total RNA was isolated from purified human platelets, monocytes, or both cells as described by our laboratory. In brief, RNA was isolated with TRizol using conditions indicated by the vendor (Gibco Incorporated). One microgram of total RNA served as a template for single-stranded cDNA synthesis. RT-PCR was conducted as previously described by our laboratory with specific primers for MMP-9 (5′-GAGTACTCC-3′, 3′-TGTACAGTC-GAGTACTCC-5′).
ELISA
For most studies, total MMP-9 generated by monocytes was measured using a specific ELISA (R&D Systems). In selected experiments, latent MMP-9 (pro–MMP-9) was measured using a specific ELISA (Amersham Pharmacia). The assays did not cross-react with MMP-1, MMP-2, MMP-3, TIMP-1, or TIMP-2 (data not shown).

Activity Assays
Active MMP-9 was measured in selected studies using an activity assay system (Amersham Pharmacia). In these studies, total MMP-9 from the cell lysates or supernatants was captured with an antibody that recognizes both the proform and active form. The captured MMP-9 was then incubated with a pro-detection enzyme that is cleaved into an active detection enzyme in the presence of active MMP-9. The ratio of active to total MMP-9 was determined in parallel samples that were activated by 1 mmol/L p-aminophenylmercuric acetate (APMA). The concentration of active and total MMP-9 was interpolated from pro-MMP standards that were simultaneously activated with APMA. The assay has a detection sensitivity of 0.5 ng/mL and did not cross-react with MMP-1, MMP-2, MMP-3, MMP-8, TIMP-1, or TIMP-2 (data not shown).

Statistics
Statistical significance among multiple groups was determined using analysis of variance (ANOVA). When significance was found, individual differences between each group were tested by Bonferroni post hoc analysis. Statistical significance was set at $P<0.05$.

Results

Monocytes Synthesize MMP-9 When They Bind to Collagen Type I and Platelets
In vivo adhesive interactions between platelets and monocytes occur in areas of vascular damage where subendothelial ECM is exposed. Because proteases expressed by leukocytes are critical for vascular injury, repair, and thrombus remodeling we asked whether synthesis of MMP-9 is induced by adhesive interactions of monocytes. Platelets, monocytes, or the two cell types together were allowed to adhere to collagen type I, one of the most abundant extracellular matrix proteins in the fibrous cap of the atherosclerotic plaque. Using an ELISA specific for pro–MMP-9, we detected no secreted MMP-9 in conditioned medium collected from platelets adherent to collagen type I. Monocytes adherent to collagen type I released low levels of MMP-9, as previously reported for this cell type. This response was greatly increased when platelets were also adherent (Figure 1A). Incubation of platelets and monocytes on immobilized collagen and maximal levels found between 18 and 24 hours (data not shown). We found that the majority of MMP-9 is secreted from adherent monocytes, although significant amounts remain associated with the cells (Figure 1B; also see Figures 4 and 5C). Western analysis and zymography of secreted proteins confirmed the ELISA data and demonstrated that monocytes release pro–MMP-9 (Figures 1C and 1D). Additional analysis revealed that approximately 30% of pro–MMP-9 is converted to its active form under the conditions of these incubations (Figure 1E). MMP-9 expression and secretion were observed in every experiment (n=11), although the magnitude varied among donors (range, 7 to 46 ng/mL pro–MMP-9 measured by ELISA). The release of MMP-9 was time-dependent, with increases in MMP-9 levels detected as early as 4 hours after incubation with platelets and collagen and maximal levels found between 18 and 24 hours (data not shown).

Synthesis of MMP-9 Requires Adhesion to Type I Collagen
We next determined whether MMP-9 expression by monocytes interacting with platelets is differentially regulated by adhesion to specific ECM components. In these studies, the cells were allowed to adhere to collagen type I or laminin. Monocyte adherence to laminin and collagen was comparable in the presence of platelets (Table). Microscopy also revealed that platelets and monocytes adhered to both matrices (Figure 2A). However, cell spreading and polarization were more marked when platelets and monocytes adhered to collagen compared with laminin (Figure 2A). We next detected MMP-9 mRNA by RT-PCR. Freshly isolated platelets (data not shown) or platelets adherent to the ECM proteins did not contain mRNA for MMP-9 although they did contain low levels of GAPDH as previously reported (see Figure 2B and
Reference 8). Freshly isolated monocytes also did not contain mRNA for MMP-9 (data not shown), but transcripts were present following adherence to collagen or laminin (Figure 2B). Platelets further enhanced MMP-9 mRNA levels in monocytes adherent to each ECM surface. Despite this increase in mRNA, monocytes adherent to laminin secreted only a trivial amount of pro–MMP-9 compared with those adherent to collagen when incubated with platelets, as analyzed by Western immunoblotting and zymography (Figure 2C).

Since previous reports from our laboratory demonstrated that adhesion of activated platelets to monocytes induces new expression of genes when the two cells interact in suspension,1 we questioned whether MMP-9 is induced under these conditions (ie, in the absence of concomitant adhesion to collagen). We found that incubation of monocytes in suspension with platelets activated by thrombin resulted in formation of heterotypic cell clusters, and the synthesis and secretion of MCP-1 and IL-8 (Figure 3A and data not shown), as previously reported.1 Monocytes adherent to collagen alone secreted MCP-1 (Figure 3A) and IL-8 (not shown) in concentrations similar to those found in platelet-monocyte suspensions. However, we were unable to detect intracellular MMP-9 (data not shown) and levels of secreted MMP-9 in the same cell suspensions were greatly reduced compared with the levels released by monocytes adherent to collagen and coincubated with platelets (Figure 3B). These results demonstrate that adhesion-dependent synthesis of individual gene products by monocytes is differentially regulated and that discrete outside-in signals control the magnitude of synthesis of MMP-9.

**Synthesis of MMP-9 by Monocytes Signaled by Platelets Requires Intercellular Contact**

The observations in Figures 1 through 3 demonstrated that both adhesion to type I collagen and signaling by platelets are essential for MMP-9 synthesis by monocytes, but it was not clear that cell-cell contact is required. To examine this issue,
we first assayed MMP-9 protein by immunocytochemistry and found that in mixed cell incubations on collagen type I, platelets readily adhered to monocytes forming numerous intercellular aggregates, consistent with our findings in Figure 2A. MMP-9 was robustly expressed by monocytes in these intercellular clusters (Figure 4B). In contrast, there was little or no staining for MMP-9 in monocytes adherent to laminin and platelet-monocyte aggregates were less frequent (Figure 4A), consistent with the results in Figure 3B. These data suggested that MMP-9 synthesis requires contact between platelets and monocytes in addition to adhesion of monocytes to collagen.

To directly test whether intercellular contact is required for MMP-9 synthesis by monocytes, we separated the two cell types from one another using transwell culture chambers. This system allows both cells to adhere to collagen and the semipermeable membrane permits free exchange of soluble products, but cellular contact is prevented. In incubations under these conditions, pro–MMP-9 secretion was not augmented (Figure 5A), indicating that intercellular contact is essential for enhanced synthesis of this matrixin when monocytes adhere to collagen as signaled by platelets. Consistent with this interpretation, conditioned medium obtained from platelets adherent to collagen type I did not induce MMP-9 synthesis when transferred to isolated adherent monocytes (data not shown).

Since MMP-9 expression requires adherence to collagen and platelets, we asked whether engagement of \( \beta_1 \) integrins and/or P-selectin glycoprotein-1 (PSGL-1) on monocytes, a critical ligand for P-selectin displayed by activated platelets, is required for this response. Neutralizing antibodies against \( \beta_1 \) (Figure 5B) and \( \alpha_5 \) (data not shown) integrin subunits reduced the total number of adherent platelets and monocytes to collagen and attenuated MMP-9 synthesis (Figure 5B), whereas an antibody against \( \alpha_5\beta_1 \) integrin did not inhibit either response (see online Figure 1 available in the data supplement at http://www.circresaha.org and data not shown). However, neutralization of \( \beta_1 \) did not block intercellular adherence between platelets and monocytes (Figure 5B). A neutralizing antibody against PSGL-1 prevented platelets and monocytes from binding to one another and attenuated MMP-9 synthesis despite the fact that platelets and monocytes still adhered to collagen (Figure 5B). Although incubation of the cells with both neutralizing antibodies greatly reduced adherence of platelets and monocytes to collagen and formation of platelet-monocyte aggregates, it did not further reduce MMP-9 synthesis.

We next asked whether coengagement of \( \beta_1 \) integrins and PSGL-1 on monocytes elicits MMP-9 generation. Using P-selectin purified from platelet membranes and an activating \( \beta_1 \) antibody, we found that PSGL-1 and \( \beta_1 \) integrins synergistically induced MMP-9 synthesis (Figure 5C). Engagement of \( \beta_1 \) integrins alone did not induce MMP-9 synthesis (Figure 5C). Similarly, adherence to immobilized P-selectin or incubation with soluble P-selectin alone did not directly induce MMP-9 synthesis by monocytes (Figure 5C and data not shown).

**Discussion**

In this report, we examined cellular interactions that are relevant to plaque fissuring and further destabilization of atherosclerotic lesions and found that MMP-9 is synthesized by human monocytes when they are coordinately adherent to collagen and platelets. Adhesion of monocytes to collagen or platelets alone is not sufficient to induce MMP-9 expression indicating that, under these circumstances, convergent signaling pathways are required for the leukocytes to synthesize this enzyme. The adhesion dependency of expression of MMP-9 indicates that its synthesis by monocytes may be spatially restricted to areas of vascular injury and inflammation.

Damage to blood vessels exposes the subendothelial matrix and results in the adhesion of platelets and monocytes at the site of vascular injury. Type I collagen is abundant in the fibrous cap of atherosclerotic plaques and in restenotic coronary lesions, in addition to abdominal aortic aneurysms. Platelets and monocytes avidly adhere to type I collagen and monocytes from binding to one another and attenuated MMP-9 synthesis (Figure 5B). Adhesion to type I collagen induces selective patterns of gene expression and enhances differentiation of monocytes into macrophages. In vitro, monocyte adhesion to isolated immobilized extracellular matrix components, including collagen, induces expression of multiple transcripts but is not sufficient for translation and secretion of the corresponding proteins without a second signal. Consistent with this observation, we found that transcription of MMP-9 mRNA occurs when monocytes adhere to collagen. Synthesis of this enzyme also occurs as previously reported, but the addition of platelets greatly increases protein production indicating that platelets provide a second signal that is required for translation of the MMP-9 message. When platelets adhere to collagen, they spread and express P-selectin on their surfaces, making them capable of binding and stimulating adjacent leukocytes. Consistent with these observations, we found that platelets spread on adherence to collagen and cluster around monocytes forming platelet-monocyte aggregates. In contrast, we
observed little platelet spreading on laminin, consistent with a previous report. The ability of collagen to induce platelet activation and platelet-monocyte interaction may be a required step that then allows mRNA for MMP-9, which is expressed as a result of adhesion of the monocytes to collagen, to be translated into protein.

Previous reports have demonstrated that spreading and activation of platelets following adhesion to collagen type I is primarily regulated through αβ1 integrins, although other adhesion molecules are also involved in flowing blood. Integrin αβ1 also regulates binding of monocytes to type I collagen. We found that neutralizing antibodies directed against either α or β1 integrin subunits attenuated MMP-9 synthesis by monocytes costimulated with platelets and collagen. In contrast, blocking integrin αvβ3, which recognizes collagen determinants under some conditions, had no effect.

Whereas ligation of β1 integrins was necessary for enhanced MMP-9 synthesis (Figure 5B), it was not sufficient for the maximal response (Figure 5C).

The finding that platelets deliver a second signal for MMP-9 synthesis by adherent monocytes extends earlier observations demonstrating that platelets induce gene expression in monocytes and also shows for the first time that engagement of monocytes by platelets and immobilized ECM proteins delivers coordinate signals that differentially regulate expression of specific gene products. Our previous experiments demonstrated that specific genes, including IL-8 and MCP-1, are induced when monocytes bind to activated platelets in suspension. Induction of these inflammatory mediators is regulated by intercellular contact between the two cells and parallel stimulation by platelet-derived signaling molecules, with binding of P-selectin on the platelet to PSGL-1 on the monocyte being a critical component. We found, however, that thrombin-stimulated platelets did not induce MMP-9 synthesis by monocytes when the two cell types were incubated together in suspension, indicating that P-selectin and platelet-derived signaling molecules are insufficient for maximal synthesis of this matrixin. This contrasts with expression of other gene products by monocytes (Figure 4 and Reference 1). Although insufficient to trigger the full response, outside-in signaling through PSGL-1 regulates the ability of monocytes to synthesize MMP-9 when they are adherent to collagen. This was

![Figure 5](http://circres.ahajournals.org/)

**Figure 5.** Intercellular contact between platelets and monocytes is required for MMP-9 synthesis. A, Platelets and monocytes were coincubated on immobilized collagen type I, which allowed them to contact one another, or intercellular contact was prevented using transwell culture chambers as described in the Materials and Methods and Results sections. Secreted MMP-9 was measured by ELISA. The bars represent the mean±SEM of 5 experiments. P<0.05 compared with all other experimental groups. B, Platelets and monocytes were pretreated for 5 minutes with a neutralizing antibody against PSGL-1 (mAb PL1, 10 μg/mL), β1 integrins (mAb P4C10, 10 μg/mL), or mouse IgG (IgG) before incubation on immobilized collagen. After 18 hours, MMP-9 was assayed by ELISA. The data are expressed as a percentage of secreted MMP-9 generated from cell suspensions treated with IgG (100%). P<0.05 compared with IgG-treated cells. C, Monocytes were left in suspension or were allowed to adhere to purified immobilized P-selectin in the presence of a β1 activating antibody (10 μg/mL of mAb TS2/16) or a control antibody (CoAb; 10 μg/mL of mAb 10F7). After 4 hours, the cells were prepared for immunocytochemical detection of MMP-9. Green immunofluorescence represents MMP-9 and red immunofluorescence is staining of nuclei with propidium iodide. This figure is representative of 3 independent experiments.
shown by blockade of PSGL-1 with a neutralizing antibody, which attenuated formation of platelet-monocyte aggregates and synthesis of MMP-9 protein by monocytes bound to the collagen matrix. Thus, although the identity of additional platelet-derived signaling molecule(s) is not yet defined, cell-cell contact and signaling through PSGL-1 are clearly required.

In preliminary studies, we have found that mononuclear cells embedded in platelet-rich areas of ruptured atherosclerotic plaque express abundant MMP-9 (see online Figure 2). When platelets and monocytes adhere to areas of exposed matrix after disruption or fissuring of atherosclerotic plaques, they are likely to encounter collagen type I because it is the predominant matrix component in human atherosclerotic plaques. Induction of the gene for MMP-9 in monocytes and release of active enzyme may then locally destablize the region over time, contributing to plaque rupture and additional rounds of platelet and leukocyte accumulation. Induction of MMP-9 by monocytes adherent to collagen may explain overexpression of this matrixin in vulnerable regions of human atherosclerotic plaques. Expression of MMP-9 by monocytes also has the potential to alter the vascular wall in other ways. By locally degrading elastin and other extracellular matrix components, MMP-9 may enhance leukocyte emigration from the vascular compartment into atherosclerotic tissues or generate chemotactic peptides. Thus, the production of MMP-9 by monocytes as a result of adhesive interactions with collagen and platelets can contribute to local accumulation of additional leukocytes, including T lymphocytes and newly recruited monocytes, in the region. Increased production of MMP-9 by mononuclear cells is thought to contribute to the progressive deterioration of the elastic lamellae characteristic of aneurysm formation, and targeted gene disruption of this matrixin suppresses the development of abdominal aortic aneurysms. Thus our observations, in addition to providing new insights into the mechanisms of MMP-9 expression by monocytes, suggest that matrixin inhibitors may prove beneficial in the treatment of atherosclerosis and its complications if these agents prevent injurious actions when the enzyme is inappropriately synthesized in the vessel wall.

Acknowledgments
This work was supported by grants from the National Institutes of Health (HL 56713 to A.S.W., HL 44525 to G.A.Z., and KO8 HL03799 to E.S.H.), the NHLBI/Lifeline Foundation (to L.W.K.), and Department of Veteran Affairs Medical Research Funds (to S.W.G.). We thank the technical staff of the Vascular Biology group for their help with cell isolation, our colleagues in the Program in Human Molecular Biology and Genetics (HMGB) and elsewhere at the University of Utah for their helpful discussions and critical reading of the manuscript, and Diana Lim for preparation of the figures.

References


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Circ Res. 2001;89:509-516; originally published online August 30, 2001;
doi: 10.1161/hh1801.096339

Circulation Research is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0009-7330. Online ISSN: 1524-4571

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Supplemental Figure 1. Mononuclear cells embedded in platelet-rich ulcerated plaques express MMP-9. Serial tissue sections from the shoulder region of a ruptured carotid atherosclerotic plaque were obtained by endarterectomy and prepared for immunohistochemistry using an antibody that recognizes the pro- and active form of MMP-9 (Clone IIA5, Neomarkers). Tissue sections are counterstained with Gill’s hematoxylin #3 which stains nuclei blue. (a) The MMP-9 antibody was omitted to provide a negative control. (b) A section adjacent to that shown in panel 2A, taken at the same magnification, demonstrates a mononuclear cell positive for MMP-9 (brown reaction product). (c) A section taken from a different area of the ruptured plaque shown in panels a and b also stained positive for MMP-9. (d) Mononuclear cells, taken from vasa vasorum of the same tissue section as figures a-c, that are negative for MMP-9. The arrows point to mononuclear cells in all panels.

Supplemental Figure 2. Neutralization of α,β3 integrins does not block intercellular aggregates or adhesion of monocytes and platelets to collagen. The left panel depicts platelets and monocytes adherent to collagen following pretreatment with 10 μg/ml of mouse IgG (Control). The right panel shows platelets and monocytes adherent to collagen following pretreatment with α,β3 antibody (10 μg/ml of anti-α,β3; LM609, Chemicon). This figure is representative of 3 independent experiments.