Differential transcriptional activation of matrix metalloproteinase-2 and membrane type-1 matrix metalloproteinase by experimental deep venous thrombosis and thrombin

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Objective: **Resolution of deep venous thrombosis (DVT) is involved in the pathogenesis of postthrombotic syndrome. Matrix metalloproteinases (MMPs) are a family of proteolytic enzymes that are critical in angiogenesis and tissue remodeling. We hypothesized that MMP-2 and its membrane-bound activator membrane type-1 matrix metalloproteinase (MT1-MMP) expression would be expressed and activated during the resolution of DVT.**

Methods: **DVT was generated by caval ligation in wild-type and MMP-2 transgenic reporter mice. Ligated and sham-operated (control) cavae were analyzed for MMP-2 transcription (β–galactosidase activity in MMP-2 reporter mice) and MT1-MMP mRNA by real-time polymerase chain reaction. MMP-2 activity was determined by zymography, and immunohistochemical staining for β–galactosidase and MT1-MMP protein was used to localize expression. Human umbilical vascular endothelial cells (HUVEC) were treated with 10 U/mL thrombin and MMP-2 and MT1-MMP mRNA levels and MMP-2 activity was determined.**

Results: **MMP-2 activity increased 71% (n = 5, $P < .05$) at day 8 in ligated vs control cavae by zymography. β-galactosidase activity showed a 1.2-fold (n = 8, $P < .05$) and 1.7-fold (n = 8, $P < .05$) induction in MMP-2 transcription at day 3 and day 8, respectively. No significant MT1-MMP gene induction was seen at day 3 in ligated vs control cavae, but MT1-MMP mRNA was upregulated 2.5-fold (n = 8, $P < .05$) in ligated cavae at day 8. Immunohistochemical staining localized MMP-2 and MT1-MMP expression to the vein wall and cellular infiltrates of the thrombus. Thrombin-treated HUVEC showed differential responses of MMP-2 and MT1-MMP. Zymography of conditioned media and cell lysates illustrated a 220% (152.6 ± 8.6 vs 69.445 ± 5.46 pixels/unit area, n = 5, $P < .05$) and 150% (74.1 ± 7.3 vs 49.2 ± 5.7 pixels/unit area, n = 5, $P < .05$) increases in MMP-2 activity respectively. MMP-2 mRNA levels were downregulated 30% (0.48 ± 0.023 vs 0.63 ± 0.035 copies of MMP-2 mRNA/copy GAPDH, n = 5, $P < .05$), whereas MT1-MMP message was upregulated 250% (0.147 ± 0.009 vs 0.059 ± 0.005 copies of MT1-MMP mRNA/copy GAPDH, n = 5, $P < .05$).**

Conclusions: **Resolution of DVT is associated with increased MMP-2 transcription and activity as well as MT1-MMP expression. Thrombin may mediate the increase in MT1-MMP noted in DVT. This is the first article studying MMP-2 and MT1-MMP transcription in DVT. These findings add DVT resolution to the class of inflammatory and fibrotic disorders in which transcriptional activation of the MT1-MMP/MMP-2 genes occurs and identify a potential therapeutic target to modulate this clinically relevant process.**

Clinical Relevance: **Postthrombotic syndrome remains a significant clinical problem after deep venous thrombosis (DVT), but the cellular and molecular mechanisms involved in thrombus resolution and vein wall fibrosis remain undefined. Matrix metalloproteinase (MMP) enzymes are critical to cell migration and matrix breakdown. We identify gene transcription and activity of two MMP isoforms, MMP-2 and MMP-14 (membrane type MMP 1, MT1-MMP) in the resolution phase of experimental DVT and in thrombin-treated endothelial cells. These studies define new proteases potentially important to resolution of DVT and development of postthrombotic syndrome. (J Vasc Surg 2005;42: 539-45.)**

Deep venous thrombosis (DVT) is a common clinical problem, with more than 2 million new cases per year in the United States despite heightened public awareness and increased prophylaxis. Treatment with anticoagulation therapy prevents propagation of thrombus and pulmonary embolism. However, anticoagulation does not prevent the occurrence of postthrombotic syndrome, a long-term complication that occurs in 25% to 75% of patients with DVT.[1] Postthrombotic syndrome is secondary to chronic obstruction, valve destruction, and scarring after DVT resolution. It comprises a complex of symptoms and problems that can

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include leg swelling, pain, skin changes, and frank ulceration.

It is not clear why some patients with DVT will remain free from postthrombotic syndrome whereas in others with similar initial patterns of thrombosis chronic symptoms of venous obstruction develop. These disparate clinical outcomes suggest that the process of DVT resolution is important in determining whether chronic venous obstruction and reflux will develop. Resolution of DVT occurs clinically over several weeks[2] and often results in recanalization of the vein. This requires thrombus resolution, which is a complex process involving clot retraction, fibrinolysis, and angiogenesis. Studies of experimental venous thrombosis have shown that thrombus resolution involves infiltration of the thrombus by inflammatory cells, capillary endothelial cells, and fibroblasts. The cellular and molecular mediators of thrombus resolution remain largely undefined, but include urokinase-type plasminogen activator (uPA)[3] and the CXCR2 cytokine receptor.[4]

The matrix metalloproteinase (MMP) family is essential for cell migration, matrix remodeling, and angiogenesis. The expression of the principal collagenases (MMP-2 and MMP-9) is increased in experimental venous thrombosis,[4] and these enzymes play a critical role in vascular remodeling induced by altered arterial flow,[5] tissue ischemia,[6] and aortic aneurysms.[7] MMP-2 activity is critical for migration of endothelial cells[8] and monocytes/macrophages.[9] Targeted deletion of either MMP-2[10,11] or its cell surface activator membrane-type 1 MMP (MT1-MMP, MMP-14)[12] abolishes angiogenesis in vivo. Thrombin, a critical proteolytic mediator in venous thrombosis, cannot cleave pro-MMP-2 to its active form,[13] but increases MMP-2 activity in cultured endothelial cells by increasing expression of MT1-MMP.[14] Pro-MMP-2 can also be proteolytically activated in vitro by the coagulation proteins activated protein C (APC)[15] and factor Xa.[16] Despite these suggestive in vitro reports and the potential importance of MMP-2 in thrombus resolution, there are no in vivo studies of MMP-2 and MT1-MMP gene expression and activity in DVT. The purpose of this study was to characterize the expression and activation of the MT1-MMP/MMP-2 proteins in experimental DVT and to identify the potential mechanisms mediating their expression.

MATERIALS AND METHODS

Cell culture. Primary HUVEC cultures were obtained from Cambrex Bio Science (Walkersville, Md) and grown on 1% gelatin-coated 100-mm dishes.[14,17,18] Cells were cultured in M199 media supplemented with 20% fetal bovine serum, 50 μg/mL endothelial cell growth factor (Biomedical Technologies, Stroughton, Mass), and 10 U/mL heparin, in 5% CO_2 at 37°C. All experiments were performed on cells between passages 3 to 6. Cells were washed twice with serum-free M199 media and incubated for 24 hours in serum-free media supplemented with 1% (w/v) Bovine Serum Albumin (BSA) with or without 10 U/mL bovine plasma thrombin (T7513, Sigma, St. Louis, Mo) stimulation. The conditioned media was collected and centrifuged 5000*g* for 10 minutes to remove cellular debris. Cells were lysed in M-Per mammalian protein cell lysis buffer per manufacture instructions (Pierce, Rockford, Ill). Supernatants were stored at −70°C for use in zymography.

Transgenic mouse construction. The starting materials were plasmid p41 containing a 5-kb genomic fragment of the rat MMP-2 gene extending from −1686 (relative to the transcriptional start site) to the middle of the second exon, and p1.2 containing a polylinker 5′ to an SV40 polyadenylation signal and paired NotI sites flanking the entire insert. The MMP-2 coding sequence start codon was first mutated to TAG using mutagenic oligonucleotides. The entire 5-kb fragment was amplified by polymerase chain reaction (PCR) adding 5′ MluI and a 3′ KpnI sites and cloned into p1.2 upstream from the SV40 polyadenylation site. An *Escherichia coli* β-galactosidase gene was isolated by PCR adding a 5′ KpnI site (and Kozak sequence) and a 3′ XhoI site. This was ligated between the MMP-2 promoter and the SV40 polyadenylation sequence to produce F8- βgal. The transcriptional unit was freed from the plasmid backbone using the paired flanking NotI sites. Purified endotoxin-free plasmid DNA was injected into outbred CD-1 mouse embryos. Stable founder lines were created and bred to homozygosity using standard mouse breeding and backcrossing techniques. Tail DNA from each animal was analyzed before experimental use to confirm transgenic genotype.

Surgery. Transgenic and outbred CD-1 mice (Charles River Laboratories, Wilmington, Mass) weighing between 20 and 30 g under ketamine (80 mg/kg I.P) and xylazine (16 mg/kg intra-peritoneal) anesthesia underwent a laparotomy in which the infrarenal inferior vena cava (IVC) was ligated with 6-0 nonabsorbable suture and lumbar branches of the IVC were divided. Control animals underwent a sham ligation in which the IVC was dissected free and branches were divided, but proximal ligation of the IVC was not performed. Mice were euthanized at 3 and 8 days postoperatively. Control vena cavae and ligated vena cavae (with thrombus that is inseparable) were excised and processed for either RNA, protein, or immunohistochemistry. In more than 90% of ligated mice a reproducible large (1 cm) thrombus developed, and cavae that did not show large thrombi were not included in the study. All procedures were performed according to protocols approved by the Institutional Animal Care and Use Committee.

Quantitative real-time PCR. Surgically excised IVC (n = 8 each) were immediately homogenized in 1 mL of Tri-Reagent (Molecular Research Center, Cincinnati, Oh) per manufacturer instructions, and 1 to 3 μg of total RNA was used to generate cDNA with a Super Script III First Strand Synthesis Kit (Invitrogen, Carlsbad, Calif). Using SYBR Green Master Mix and an ABI Prism 7900HT detection system (Applied Biosystems, Foster City, Calif), cDNA was amplified, for 40 cycles, 15 seconds at 95°C and 60 seconds at 60°C. MT1-MMP mRNA levels were normalized to GAPDH mRNA in each sample. Samples were run in triplicate to ensure amplification integrity. The primers used were as follows: MT1-MMP: (forward) 5′-TGGT-

GGCTGTGCATGAGTTG-3′ and (reverse) 5’27-GT-GACCCTGACTTGTTCCATA-3′ GAPDH : (forward) 5′-TGCACCACCAACTGCTTAG- 3′ and (reverse) 5′-GGATGCAGGGATGATGTTC-3′.

Zymography and β-galactosidase assay. Control-operated (n = 8) and ligated IVC (n = 8) were homogenized in the M-Per mammalian protein cell lysis buffer (Pierce) or Promega β-galactosidase reporter lysis buffer (Promega, Madison, Wis). Samples were then centrifuged (14,000 rpm, 15 min) and supernatants were frozen at −70°C. Total protein in all samples was determined by BCA assay (Pierce). Zymography was performed on IVC and HUVEC media as previously described[19,20] using 25 μg of protein loaded on a 10% SDS-polyacrylamide/1% gelatin gel (Invitrogen) run at 125 V for 90 minutes. Molecular weights of protein bands were confirmed by using Mark 12 unstained zymographic molecular weight marker (Invitrogen). Densitometry was done with NIH Image J software (public domain). For β-galactosidase assay, 20 μg lysate was incubated at room temperature with 200 μL chemiluminescent substrate for 1 hour (BD Biosciences, Palo Alto, Calif). β-galactosidase activity was determined with a luminometer (TD 20/20, Turner Designs, Sunnyvale, Calif).

Immunohistochemistry. Control (n = 5) and ligated IVCs (n = 5) were excised, fixed in 4% buffered paraformaldehyde, paraffin embedded, and serially cross-sectioned 5 μm thick. Slides were deparaffinized in xylene, followed by rehydration in graded ethanol washes. Endogenous peroxidase activity was blocked by washing sections in 3% H_2O_2 for 5 minutes. Sections were incubated with avidin-biotin blocking solution (Vector Laboratories, Burlingame, Calif) and stained with a mouse-on-mouse kit (Vector Laboratories). Sections were incubated with either a monoclonal anti-β-galactosidase (catalog No. CRM7001M, Cortex Biochem, San Leandro, Calif) or a monoclonal MT1-MMP (catalog No. RDI-MT1MMPabm-B7, Research Diagnostics, Flanders, NJ) antibody at a 1:250 dilution overnight at 4°C, followed with the anti-mouse immunoglobulin (Ig) G biotinylated secondary antibody at a 1:250 dilution for 30 minutes. Sections were incubated with Vectastatin Elite ABC reagent for 5 minutes and developed with Vector VIP Substrate Kit for Peroxidase (Vector Laboratories). Slides were subsequently mounted with VectaMount (Vector Laboratories). Staining controls included staining with no primary antibody and mouse IgG (Santa Cruz Biotechnology, Santa Cruz, Calif). Staining intensity was quantitated by pixel analysis (NIH Image).

Statistical analysis. All data are presented as mean ± standard error. All experiments were repeated at least three times with similar findings to confirm reproducibility. Power calculations were done to ensure adequate sample sizes. Unpaired Student’s *t*-test was used to compare control and ligated samples. $P < .05$ was defined as statistical significance.

RESULTS

Deep venous thrombus induces MMP-2 activity. Caval ligation reproducibly produced a thrombus at 3 and 8 days after infrarenal ligation of the IVC (Fig 1A). Hematoxylin and eosin staining of both control and ligated IVC at day 8 showed cellular infiltrates within the thrombus as well as vein wall inflammation (Fig 1B). We studied MMP-2 and MT1-MMP in thrombus at day 8 because prior studies have shown that thrombus resolution occurs at this time point.[4]

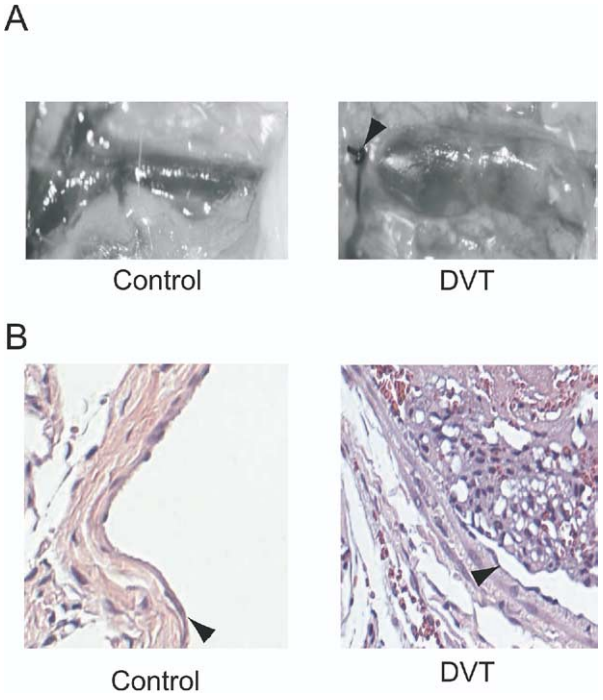

Fig 1. Mouse model of deep venous thrombosis. **A,** Ligation of the infrarenal inferior vena cava (IVC) causes thrombus to develop. *Arrowhead* represents the location of the infrarenal ligature of the IVC. **B,** Representative sectioning of control and deep venous thrombosis (DVT) with hematoxylin and eosin counterstaining 8 days after IVC ligation (400× magnification). The vein wall is labeled by a *single arrowhead*.

In ligated IVC, we noted induction of MMP-2 activity (Fig 2A) by zymography at day 8. Densitometry of zymogram gels confirmed a 70% increase in active MMP-2 in ligated vs control cavae (140.2 ± 14.3 vs 83.5 ± 7.85 pixels/unit area, n = 5, $P < .05$). Similarly there was an increased ratio of active MMP-2 to pro-MMP-2 in the ligated cavae vs the control (1.31 ± 0.089 vs 1.01 ± 0.046, n = 5, $P < .05$). At day 3, when thrombus size is increasing,[4,21] MMP-2 activity (Fig 2A) was not statistically different between ligated and control IVC (82.8 ± 2.73 vs 79.5 ± 3.84 pixels/unit area, n = 5, NS). As expected, the ratio of active MMP-2 to pro-MMP-2 was statistically the same (0.623 ± 0.019 vs 0.601 ± 0.023, n = 5, P = NS) at day 3.

We studied MMP-2 activity in the superior vena cava after IVC ligation to ensure that MMP-2 activity in the IVC was not a result of a systematic inflammatory response. There was approximately a 3.0-fold difference (28.5 ± 5.26

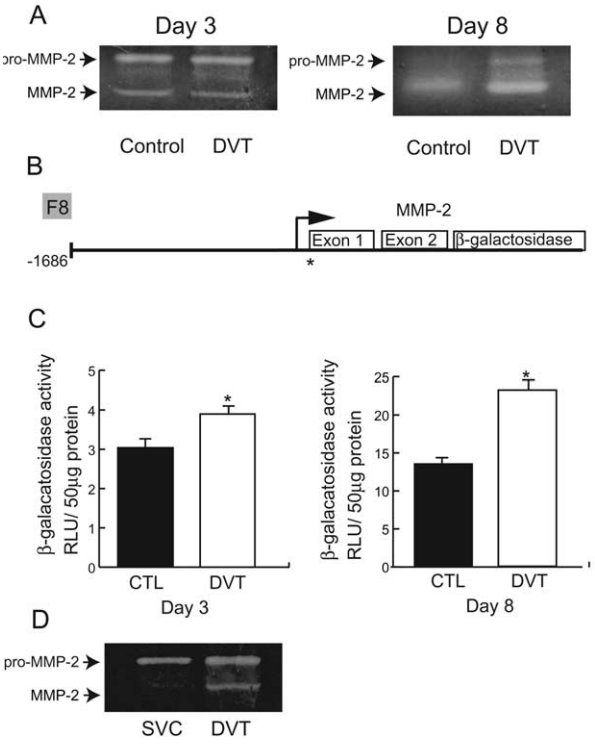

Fig 2. Deep venous thrombosis (DVT) and matrix metalloproteinase (MMP)-2 activity and gene transcription in F8 transgenic mouse. **A,** Representative gelatin zymography 3 and 8 days after inferior vena cava (IVC) ligation. MMP-2 activity showed no significant difference at 3 days, but a noticeable induction in MMP-2 activity is observed at day 8. **B,** A schematic diagram of the F8 MMP-2 reporter mouse: 5086 bp of rat MMP-2 genomic DNA (including first two exons) with β-galactosidase reporter cassette. *Arrow* indicates MMP-2 transcription start site. *Asterisk* indicates mutated translational start site. **C,** β–galactosidase assay of control and DVT 3 and 8 days after ligation shows increased activity representing transcriptional activation of the MMP-2 gene; n = 8, *$P < .05$. **D,** Representative gelatin zymography of the IVC and superior vena cava of a mouse in which DVT was induced to illustrate that elevated levels of MMP-2 in DVT are not a result of a systematic inflammatory response.

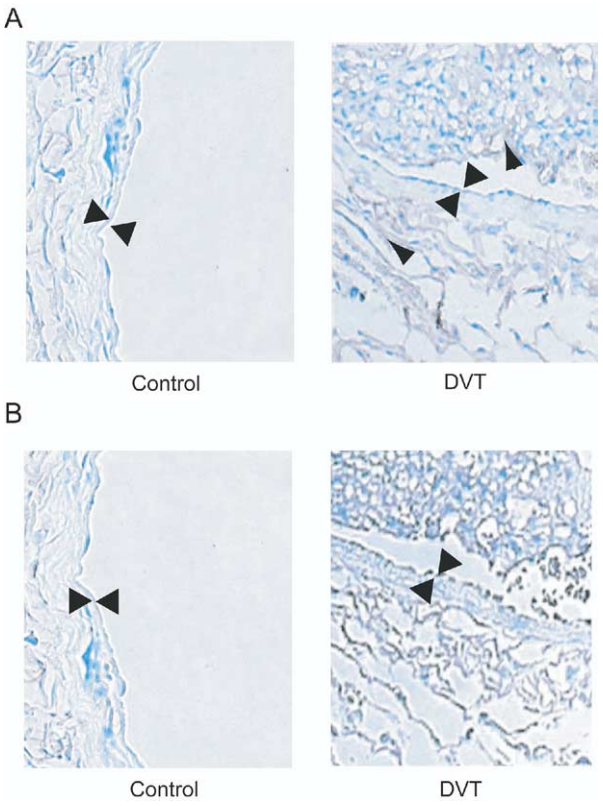

Fig 3. Localization of deep venous thrombosis (DVT)-induced matrix metalloproteinase (MMP)-2 expression. **A,** Immunohistochemical staining for β-galactosidase (*brown color*) in control and DVT vein. Staining (*single arrowheads*) is evident in both the vein wall and thrombus of the DVT (400× magnification). **B,** Mouse immunoglobulin G was used in place of the primary antibody as control (400× magnification). The vein wall is labeled by *double arrowheads*, and staining is indicated by a *single arrowhead*.

vs 78.4 ± 4.49 pixels/unit area, n = 5, $P < .05$) in active levels of MMP-2 between ligated IVC and the superior vena cava (SVC) (Fig 2D). In addition, there was an increased ratio of active MMP-2 to pro-MMP-2 in the IVC as compared with the SVC (0.703 ± 0.013 vs 0.336 ± 0.0765, n = 5, $P < .05$).

DVT induces MMP-2 transcription in the F8 reporter mouse. We examined DVT-induced MMP-2 transcription by using the F8 transgenic mouse, which contains 1686 to +3400 bp of the rat MMP-2 gene (including the first two exons), coupled to a β-galactosidase reporter cassette (Fig 2B). β-galactosidase assays of the ligated IVC relative to control showed a 1.2-fold (n = 8, $P < .05$) and 1.7-fold (n = 8, $P < .05$) induction in MMP-2 transcription at days 3 and 8 respectively (Fig 2C). Immunohistochemical staining for the β-galactosidase protein was done to identify cells in which MMP-2 transcription occurred. Expression was seen in both the vein wall and thrombus (Fig 3A), and quantitation of immunostaining showed similar intensity of β-galactosidase immunoreactivity between vein wall and thrombus (10,207 ± 349 vs 10,090 ± 151, n = 5, P = NS). β-galactosidase staining outside the vein wall suggests the possible importance of MMP-2 in perivenous inflammation. Staining with mouse IgG in place of a primary antibody showed minimal staining in all specimens (Fig 3B).

MT1-MMP gene expression and protein are increased in DVT. We examined MT1-MMP mRNA and protein in DVT by real-time PCR and immunohistochemistry, respectively. Although no significant induction of MT1-MMP mRNA was seen at day 3 (Fig 4A, 0.07 ± 0.014 vs 0.06 ± 0.005 copies of MT1-MMP mRNA/copy of GAPDH, n = 8, NS), MT1-MMP mRNA was upregulated more than twofold (0.39 ± 0.068 vs 0.14 ± 0.043 copies of MT1-MMP mRNA/copy of GAPDH, n = 8, $P <$

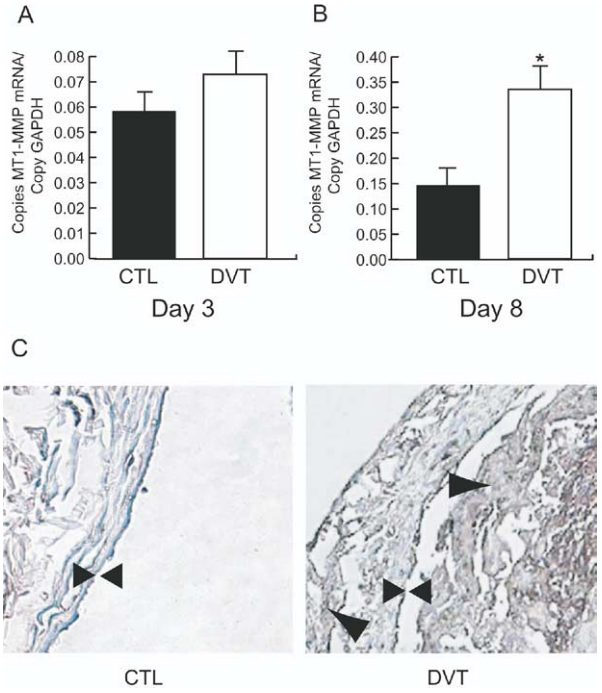

Fig 4. Membrane-bound activator membrane type-1 matrix metalloproteinase (MT1-MMP) gene and protein expression is elevated in deep venous thrombosis (DVT). **A,** Real-time polymerase chain reaction analysis of MT1-MMP mRNA levels in control and ligated inferior vena cavae at 3 and 8 days after ligation. n = 8 each, *$P < .05$. **B,** Histologic staining with a MT1-MMP antibody shows increased expression relative to control of MT1-MMP in both the vein wall and thrombus (400× magnification). The vein wall is labeled by *double arrowheads*, and staining is indicated by a *single arrowhead*.

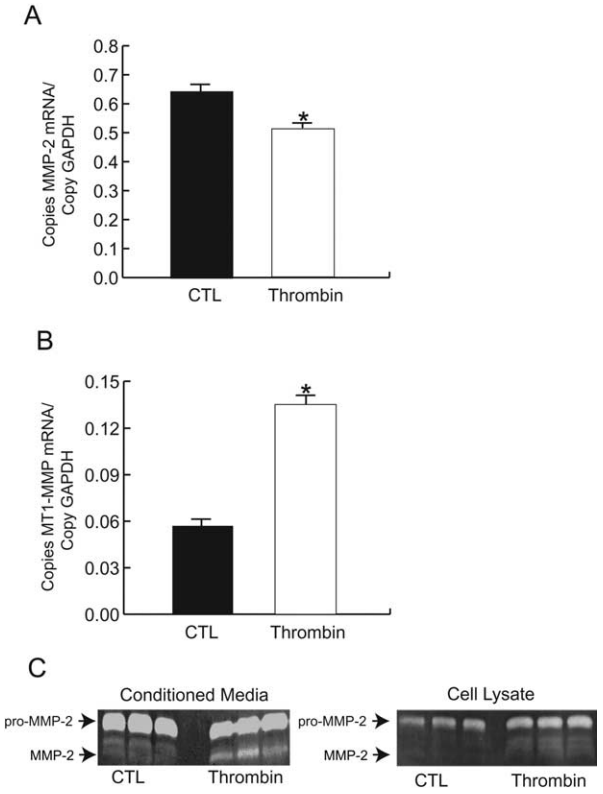

Fig 5. Thrombin induces differential transcriptional expression and activity of matrix metalloproteinases (MMP)-2 and membrane-bound activator membrane type-1 matrix metalloproteinase (MT1-MMP) in cultured endothelial cells. **A,** MMP-2 mRNA levels determined by real-time polymerase chain reaction after a 24-hour 10-U/mL thrombin treatment in human umbilical vascular endothelial cells (HUVEC), n = 5, *$P < .05$. **B,** MT1-MMP mRNA levels in thrombin-treated and control HUVEC, n = 5, *$P < .05$. **C,** Gelatin zymography of conditioned media and cell lysates of thrombin-treated and control HUVEC.

.05) in ligated IVC relative to controls at day 8 (Fig 4B). Immunohistochemical staining for MT1-MMP at day 8 showed a distribution similar to the expression pattern noted in the F8 transgenic mouse. Staining was present in both the vein wall and cellular components of the thrombus (Fig 4C).

Thrombin induces MMP-2 activity and MT1-MMP mRNA in HUVEC. We treated HUVEC with activated thrombin and measured MMP-2 activity, MMP-2 mRNA, and MT1-MMP mRNA. Zymography of conditioned media from HUVEC cells 24 hours after thrombin treatment illustrated a 2.2-fold increase in secreted active MMP-2 (Fig 5C) relative to untreated cells (152.6 ± 8.6 vs 69.445 ± 5.46 pixels/unit area, n = 5, $P < .05$). The ratio of active to pro-MMP-2 was two-fold greater in the treated cells vs control (0.855 ± 0.046 vs 0.396 ± 0.056, n = 5, $P < .05$). Zymography of cellular lysates (Fig 5C), paralleling conditioned media, showed a 1.5-fold increase in active MMP-2 (74.1 ± 7.3 vs 49.2 ± 5.7 pixels/unit area, n = 5, $P < .05$). However, the ratio of active to pro-MMP-2 in thrombin treated cells vs control was not significant (0.461 ± 0.052 vs 0.424 ± 0.052, n = 5, n = NS). Although MMP-2 activity increased, MMP-2 mRNA levels were downregulated 30% by real-time PCR in thrombin-treated cells (Fig 5A, 0.48 ± 0.023 vs 0.63 ± 0.035 copies of MMP-2 mRNA/copy of GAPDH, n = 5, $P < .05$). This thrombin treatment caused a 2.5-fold upregulation in MT1-MMP gene levels after 24 hours (Fig 5B, 0.147 ± 0.009 vs 0.059 ± 0.005 copies of MT1-MMP mRNA/copy of GAPDH, n = 5, $P < .05$).

DISCUSSION

We studied the expression of MMP-2 and MT1-MMP (MMP-14) in experimental DVT and cultured endothelial cells. The significant findings in this study are: first, venous thrombosis induces MMP-2 transcription and activity; second, MT1-MMP transcription and protein expression are increased by venous thrombosis; third, thrombin treatment of endothelial cells induces MMP-2 activity, but not MMP-2 gene transcription; and fourth, thrombin treatment of endothelial cells induces MT1-MMP gene transcription.

We constructed and used a unique MMP-2 transgenic reporter mouse to identify areas within the resolving thrombus and the vein wall where MMP-2 transcription occurs.

We studied MMP gene activation at day 3 and day 8 because thrombus size is increasing on day 3 and thrombus resolution is ongoing at day 8. Time points beyond day 8 are not associated with significant further decreases in thrombus size,[4] and the lack of activation of MMP-2 and MMP-14 at day 3 specifically links these enzymes to the period of thrombus resolution. Comparison with the distribution of MT1-MMP expression within the vein wall and thrombus reveals co-localization of MMP-2 and MT1-MMP immunoreactivity, a finding that is consistent with the requirement for activation of pro-MMP-2 by cell surface MT1-MMP. Comparison of the pro and active MMP-2 isoforms indicates that DVT in vivo and thrombin treatment of HUVEC increased pro-MMP-2 levels and concurrently the ratio of active to pro-MMP-2. The finding of MMP-2 transcription and MT1-MMP protein within the thrombus shows that these proteases are being produced by cells infiltrating the thrombus, rather than by deposition of circulating MMP-2. These cells within the thrombus may be both inflammatory cells and capillary endothelial cells, supporting the study of MMP gene activation in cultured endothelial cells. Both MMP-14 and MMP-2 may have diverse functions in thrombus resolution, because MMP-14 can cleave fibrinogen and thus contribute to plasmin-independent fibrinolysis.[22]

Our finding that thrombin induces MT1-MMP expression in endothelial cells provides a potential mechanism to explain the induction of MT1-MMP identified in vivo within the thrombus. We used HUVEC because these cells have been extensively studied with regard to thrombin-induced MMP-14 expression and MMP-2 activation.[13,14] This finding is in agreement with previous studies that have suggested that the activation of MMP-2 protein by thrombin is not caused by increased transcriptional activation of MMP-2, but rather by increased pro-MMP-2 processing by MT1-MMP.[14] The effect of thrombin on MMP-14 expression requires proteolytic activity, and thus anticoagulation may inhibit thrombin-induced MMP14 expression and MMP-2 activation.[14] The decrease in MMP-2 mRNA in response to thrombin (Fig 5A) may reflect feedback inhibition of MMP-2 transcription by the net increase in active MMP-2 induced by thrombin.

Our finding that MMP-2 transcription is induced by DVT in vivo but not by thrombin in vitro suggests that there are thrombin-independent factors that drive MMP-2 transcription in the resolving thrombus and vein wall. The cellular and molecular mechanisms that drive MMP-2 transcription after thrombosis will thus require study in vivo, and transgenic reporter mice carrying specific deletions in the MMP-2 promoter are under development in our laboratory to aid in these investigations.

This is the first study of expression of the MMP-2/MT1-MMP genes in thrombosis in vivo, and our finding that MMP-2 gene transcription is induced by thrombus formation is significant given the lack of induction of MMP-2 gene expression by thrombin and other stimuli in prior in vitro studies.[14] Cell culture studies of MMP-2 transcription are hampered by the fact that cultured cells continuously produce pro-MMP-2 and do not increase transcription with cytokine treatment, phorbol esters, or other stimuli, leading investigators to consider MMP-2 expression as constitutive.[17] By contrast, in vivo studies of limb ischemia,[23] neointimal hyperplasia,[24] and aneurysm formation[7] show induction of MMP-2 transcription by a variety of inflammatory and tissue-remodeling stimuli. The importance of MMP-2 as a mediator of disease is supported by the elevated circulating MMP-2 levels in patients with cerebral aneurysms,[25] liver fibrosis,[26] advanced breast cancer,[27] and hypertrophic cardiomyopathy.[28] Our finding of thrombus-induced MMP-2 transcription adds thrombus resolution to the class of disorders in which transcriptional activation of the MT1-MMP/MMP-2 genes occurs. As further studies delineate whether MMP-2 activity accelerates or impairs thrombus resolution, augmentation or inhibition of these genes may be a potential therapeutic target to modulate this clinically relevant process.

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