Matrix metalloproteinases are thought to play an important role in endothelial cell migration and matrix remodeling. We have used an in vitro wound healing migration model and newly generated anti-membrane type 1-matrix metalloproteinase (MT1-MMP) monoclonal antibodies (mAbs) to characterize the role of MT1-MMP during this process. First, the expression and shedding of MT1-MMP are up-regulated upon induction of migration in endothelial cells, as demonstrated by flow cytometry and Western blot analysis. Furthermore, MT1-MMP is concentrated at discrete areas in migrating endothelial cells, in contrast to the diffuse pattern observed in confluent cells. Interestingly, migration of endothelial cells results in the stimulation of MT1-MMP activity, as shown by its ability to process pro-MMP-2 and to degrade fibrinogen assessed by zymography. Moreover, MT1-MMP-mediated gelatin degradation is enriched at migration sites. mAbs generated against the MT1-MMP catalytic domain are shown to inhibit MT1-MMP enzymatic activity and to impair both phorbol 12-myristate 13-acetate-induced endothelial migration and invasion of collagen and fibrin gels. Furthermore, a reduction in the formation of capillary tubes in Matrigel is also observed when endothelial cells are pretreated with the blocking anti-MT1-MMP mAbs. Altogether, these data demonstrate that MT1-MMP plays an important role during endothelial cell migration, and its activity can modulate endothelial migration, invasion, and formation of capillary tubes during the angiogenic response.

The endothelium constitutes a dynamic barrier between the bloodstream and the subendothelial tissue. Endothelial cells are normally quiescent and form a tight monolayer by interacting with the extracellular matrix beneath and with surrounding endothelial cells (1). However, this situation is broken during the angiogenic response, i.e. the formation of new vessels from preexisting capillaries. Angiogenesis is critical for different physiologic and pathologic processes including wound healing, tissue remodeling, chronic inflammatory diseases, and tumorigenesis (2). During angiogenesis, endothelial cells go through several steps including the loosening of matrix and intercellular adhesion, degradation of subendothelial matrix, migration, proliferation, and formation of new tubes (3).

The receptors likely involved in one of the first critical steps, endothelial migration, are not well characterized yet. However, it is known that αvβ3 localizes at the tip of growing vessel sprouts and at the lamellipodia of migrating endothelial cells (4, 5). αvβ3 integrin/tetraspanin complexes also play an important role in regulating endothelial motility, and in angiotensin-induced angiogenesis (6, 7). Moreover, αvβ3 integrin may also be involved in endothelial migration through its interaction with thrombospondin (8). The advancing front of the migrating endothelial cells presumably focuses proteolytic activity to create a defect in the vascular basement membrane, and this degradation is associated with migration of endothelial cells out of the vascular channel toward the angiogenic stimulus (9, 10). During this process, the subendothelial basement membrane, a dense meshwork of collagen, glycoproteins, and proteoglycans, must be proteolytically disrupted to allow formation of new capillaries (11). Migrating endothelial cells elaborate a battery of enzymes that mainly belong to the matrix metalloproteinase (MMP) family to degrade this extracellular matrix (ECM) (10).

MMPs are multidomain zinc-dependent endopeptidases that, with a few exceptions, share a basic structural organization comprising a propeptidic, catalytic, hinge, and hemopexin-like domains and that have been largely involved in tissue remodeling and tumor invasion (12). Although most MMPs are secreted, a subfamily of MMPs associated to the cell membrane has recently been described, with membrane type 1-matrix metalloproteinase (MT1-MMP) the first member characterized (13). Its catalytic activity includes ECM components such as fibronectin, laminin, collagens, gelatin, vitronectin, and others (14, 15). Its localization makes this protein particularly suited to function in pericellular proteolysis (16, 17), and its expression has been correlated with the invasive capacity of different tumors (12). It has also been demonstrated that MT1-MMP serves as activator of pro-MMP-13 (18) and, more interestingly,
as a membrane receptor and activator of pro-MMP-2 (progelatinase A) in coordination with the tissue inhibitor of metalloproteinase-2, forming a highly regulated functional trimolecular complex (19–21). However, recent reports have shown that MT1-MMP might play a role during migration of glioma and epithelial cells independently of MMP-2 activation (22, 23).

The role that MT1-MMP plays during endothelial cell motility has not been characterized yet. However, different studies suggest that MT1-MMP might be important in the physiology of the endothelium. Thus, it acts as the most potent fibrinolytic enzyme in endothelial cells (16). Moreover, mice deficient in MT1-MMP display defects in cartilage maturation likely due to both defects in collagen turnover and in vascularization at these sites, and they also exhibit a hampered response to angiogenic factors in the mouse corneal model (24, 25). These reports demonstrate that MT1-MMP participates in the angiogenic response, but the underlying mechanisms remain undefined.

Herein, we have characterized the role that MT1-MMP plays during the migration of human endothelial cells and its putative relevance for the angiogenic process.

### Experimental Procedures

**Generation of Anti-MT1-MMP Monoclonal Antibodies**—BALB/c mice were immunized with the peptides REVPYAYIREGHEK (LEM-1) and confirmed by analysis of COS-1 cells transfected with the MT1-MMP cDNA by electroporation. LEM-2/15, LEM-2/63, and LEM-1/58 mAbs are IgG1, IgG2b, and IgG2a, respectively. mAbs were purified from ascitic fluid by affinity chromatography on a protein-A-Sepharose column (Amersham Pharmacia Biotech, Uppsala, Sweden). Antigens—Proteolytic activity was measured in conditioned media and cell lysates as cleavage of the fluorogenic substrate Z-Glu-Val-Leu-Lys-AMC.

**Flow Cytometry Analysis**—HUVEC were stimulated to migrate by disintegrating the monolayer with 3 by 3 injuries, 20 ng/ml PMA, or both. Lysates and supernatants were prepared as described for Western blot analysis, were then resolved under nonreducing conditions on 9% SDS-PAGE gels embedded with 1 mg/ml gelatin or fibrinogen (Calbiochem-Novabiochem Co., Darmstadt, Germany). Gels were rinsed three times in 2.5% Triton X-100 for 30 min at room temperature and then incubated in 50 mM Tris-HCl, pH 7.5, 10 mM CaCl2, and 200 mM NaCl for 12 h at 37 °C. Gels were stained with Coomassie Blue, and areas of gelatinolytic or fibrinolytic activity were visualized as transparent bands.

**Immunofluorescence Microscopy**—HUVEC were grown to confluence on gelatin-coated 24-well plates. Cells were changed to serum-free medium 24 h prior to the assay. Cells were then incubated with different purified mAbs 30 min before the injury. The monolayer was then stimulated with 20 ng/ml PMA, disrupted with a cell scraper of ~1 mm, and filmed at 0, 4, 8, 12, and 24 h in a phase contrast videomicroscope (Nikon E600) with a 10× objective. Experiments were done in duplicate and four fields of each well were recorded. Migrated area was calculated by subtracting the area in μm² between the wound edges at different time points from the area measured at time 0.

**Cell Transmigration Assay**—HUVEC transmigration assays were performed in 8-μm pore Transwell chambers (Costar Corp.). Cells were resuspended in serum-free medium plus 20 ng/ml PMA and seeded at 10,000 cells/well on gelatin-coated filters in the absence or presence of different purified mAbs on the upper chamber. Transmigrated cells onto the lower surface of the filter were stained with toluidine blue and counted after 8 h of migration. Experiments were done in duplicate, and four fields of each transwell were counted with a 40× objective in an Eclipse E400 microscope (Nikon).

**Collagen and Fibrin Gel Invasion Assays**—Collagen or fibrin gels were prepared by diluting type I collagen (ICN Biomedicals Inc., Costa Mesa, CA) in medium to a final concentration of 500 μg/ml by adding 0.1 units of thrombin to a 3 mg/ml fibrinogen solution in 199 medium, respectively. Then, 500 μl of each solution was dispensed in 24-well plates and allowed to solidify for 1 h at 37 °C. HUVEC were grown to confluence in serum-free medium on top of type I collagen or fibrin gels. Cells were then stimulated with 20 ng/ml PMA (29) and incubated for 24 h in the absence or presence of different purified mAbs. Invasive cells (dendritic shaped cells, whose plane of focus was beneath the surface monolayer) were counted in four 10× randomly selected fields on a phase contrast videomicroscope (Nikon ECLIPSE 80i, 40× objective). Results were expressed as a percentage of the number of invaded cells to the total number of cells present in the monolayer.

**Generation of Anti-MT1-MMP Monoclonal Antibodies**—BALB/c mice were immunized with the peptides REVPYAYIREGHEK (LEM-1) and confirmed by analysis of COS-1 cells transfected with the MT1-MMP cDNA by electroporation. LEM-2/15, LEM-2/63, and LEM-1/58 mAbs are IgG1, IgG2b, and IgG2a, respectively. mAbs were purified from ascitic fluid by affinity chromatography on a protein-A-Sepharose column (Amersham Pharmacia Biotech, Uppsala, Sweden). Antigens—Proteolytic activity was measured in conditioned media and cell lysates as cleavage of the fluorogenic substrate Z-Glu-Val-Leu-Lys-AMC.

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**In Vitro Enzymatic Assays**—MT1-MMP recombinant catalytic domain (0.05 μg) from Calbiochem was incubated with 10 μg of fibrinogen in 20 μl of 50 mM Tris-HCl, pH 7.5, 150 mM NaCl, and 5 mM CaCl2, 12 h at 37 °C in the absence or presence of different mAbs, or 1 mM 1,10-phenanthroline. Samples were analyzed under reducing conditions by SDS-PAGE.

**Wound Healing Assays**—HUVEC were grown to confluence on gelatin-coated 24-well plates. Cells were changed to serum-free medium 24 h prior to the assay. Cells were then incubated with different purified mAbs 30 min before the injury. The monolayer was then stimulated with 20 ng/ml PMA, disrupted with a cell scraper of ~1 mm, and filmed at 0, 4, 8, 12, and 24 h in a phase contrast videomicroscope (Nikon ECLIPSE 80i) with a 10× objective. Experiments were done in duplicate and four fields of each well were recorded. Migrated area was calculated by subtracting the area in μm² between the wound edges at different time points from the area measured at time 0.

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cellular extensions linking cell masses or branch points. Experiments were done in duplicate.

Densitometry—Densitometric analysis was performed on scanned images with the Multi-Analyst software from Bio-Rad.

Statistical Analysis—Tested and control samples in the functional assays were compared for statistical significance by using Student’s t test.

RESULTS

Up-regulation of Expression and Shedding of MT1-MMP during Migration of Human Endothelial Cells—To analyze the role that MT1-MMP might have during endothelial cell migration, a wound healing migration model was used. For this purpose, novel mAbs against the catalytic domain of MT1-MMP were generated and their specificity assessed by both flow cytometry and Western blot analysis of MT1-MMP transiently transfected COS-1 cells (Fig. 1A). MT1-MMP expression was then analyzed on the surface of endothelial cells under different migratory stimuli by flow cytometry. Basal expression of MT1-MMP on resting confluent endothelial cells (thick solid line; MFI = 6) is up-regulated after 6 or 24 h of stimulation (thick solid line) with the injuries (MFI = 10.6 and 10.9), PMA (MFI = 10.7 and 14.2), or both (MFI = 10.8 and 15.2). Pretreatment of endothelial cells with 1 μM cycloheximide during migration largely inhibited the up-regulation of basal MT1-MMP expression (MFI = 6.3) induced by wounds (MFI = 4.3), PMA (MFI = 5.8), or both (MFI = 6.3) for 24 h (compare thick solid versus thin solid line). A representative out of three independent experiments is shown.

C. HUVEC were stimulated with injuries, PMA, or both for 6 or 24 h. Lysates or supernatants were analyzed by Western blot using the anti-MT1-MMP mAb LEM-2/15. Densitometric analysis of lysate Western blots (n = 4) was performed, and the arithmetic mean ± S.D. is represented. The MT1-MMP increase induced by the different stimuli is statistically significant (p < 0.05).
the up-regulation of MT1-MMP expression by wound healing started at ~6 h, and it was more consistently observed 24 h after stimulation (Fig. 1B). Moreover, MT1-MMP wound-induced up-regulation depended on de novo protein synthesis, since it was largely prevented in the presence of cycloheximide as it happened with PMA-induced increased expression (Fig. 1B).

The effect of migration in MT1-MMP expression was also analyzed by Western blot of endothelial cell lysates. As shown in Fig. 1C, mature MT1-MMP (60 kDa) was up-regulated ~2-fold in response to the injury at 6 and 24 h. This increase in expression was greater upon stimulation with either PMA or a combination of both stimuli. Interestingly, a major MT1-MMP soluble form of 53 kDa was detected in the supernatant of endothelial cells stimulated to migrate with wound healing, PMA, or both for 6 or 24 h compared with quiescent cells, in which it was barely observed, suggesting an induction of MT1-MMP shedding under these conditions (Fig. 1C).

All these data show that endothelial cell response to injury includes the induction of MT1-MMP synthesis, resulting in an increase of MT1-MMP expression on the cell membrane as well as in the supernatant, and suggest that MT1-MMP might be playing a role in the cell migration associated to wound healing.

**MT1-MMP Relocalizes to Motility-associated Structures in Migrating Endothelial Cells**—We next investigated the localization of MT1-MMP in endothelial cells that are migrating during wound repair. As shown in Fig. 2, cells migrating to repair the wound acquired a migratory phenotype with an expansion of lamellipodia and cellular protrusions compared with the polygonal shape of cells within the confluent monolayer. The net movement direction of the cells is indicated by the arrow. Note that MT1-MMP localization detected by immunofluorescence with the anti-MT1-MMP mAb LEM-2/15 (green) changes upon stimulation of migration from a diffuse pattern in confluent resting cells to discrete enriched-areas of MT1-MMP at the lamellipodia and cytoplasmic projections of migrating cells (arrowheads). Filamentous actin was visualized by staining with Texas Red-phalloidin. Bar, 20 μm.

**FIG. 2.** MT1-MMP relocalizes along the motility-associated structures of actively migrating endothelial cells. HUVEC were seeded on gelatin-coated coverslips and grown until confluence. They were stimulated with lesions or with both wounds and PMA for 6 h. Cells migrating to repair the wound acquire a migratory phenotype with lamellipodia and cellular protrusions compared with the polygonal shape of cells within the confluent monolayer. The net movement direction of the cells is indicated by the arrow. Note that MT1-MMP localization detected by immunofluorescence with the anti-MT1-MMP mAb LEM-2/15 (green) changes upon stimulation of migration from a diffuse pattern in confluent resting cells to discrete enriched-areas of MT1-MMP at the lamellipodia and cytoplasmic projections of migrating cells (arrowheads). Filamentous actin was visualized by staining with Texas Red-phalloidin. Bar, 20 μm.

MT1-MMP Activity Is Induced by Migration on Human Endothelial Cells—One of the most relevant catalytic activities of MT1-MMP is the processing of pro-MMP-2 (pro-gelatinase A) to MMP-2 (13). In our model, activation of MMP-2 during endothelial migration was analyzed by gelatin zymography. The 62-kDa active form of MMP-2 was induced in the cell lysate and usually in the culture supernatant of endothelial cells stimulated to migrate by wound healing for 6 or 24 h (Fig. 3, A and C, and data not shown). The intrinsic enzymatic activity of MT1-MMP in migrating cells was also analyzed by zymography of fibrinogen-embedded gels. Endothelial cells stimulated to migrate during 6 or 24 h degraded fibrinogen more efficiently than resting endothelial cells similarly to the effect of PMA (Fig. 3, A and B, and not shown). In both cases, the induction was more clearly observed in the cell lysates according to the anchorage of MT1-MMP to the membrane and an additive effect in MT1-MMP activation was usually observed when both the wound and PMA were used in combination (Fig. 3, A and B). The molecular masses of the proteolytic activities detected in fibrinogen-embedded gels were 60 kDa for the lysate and 53 kDa for the supernatant, which correlated with the MT1-MMP species revealed on endothelial cells by Western blot (see Fig. 1C). The activation of pro-MMP-2 as well as the fibrinogen degradation induced by injuring were partially dependent on the increase in MT1-MMP expression since they were largely inhibited in the presence of cycloheximide (Fig. 3, C and D). However, a faint proteolytic activity remained, suggesting that other factors such as relocalization of MT1-MMP might also be playing a role in MT1-MMP activation.

To investigate whether relocalized MT1-MMP to the motility-associated structures of migrating endothelial cells might be related to MMP-2 (gelatinase A) activation and to migration of endothelial cells, we analyzed the in situ gelatinolytic activity in the wound healing model. Endothelial cells were grown on fluorescein-labeled gelatin, and degradation of this matrix was tested upon stimulation with different migratory stimuli. In confluent quiescent endothelial cells, degradation areas were barely observed (Fig. 4A). However, an increase in gelatin degradation was detected in areas nearby the wound where...
endothelial cells were actively migrating to repair the injury (Fig. 4A). This effect was more noticeable when endothelial cells were stimulated with wounds, PMA, or both for 24 h (Fig. 4A, and data not shown). Altogether, these data demonstrate that migration of endothelial cells induced the activity of MT1-MMP and subsequently of MMP-2 in areas nearby the wound, suggesting that this proteolytic activity might be directly involved in the migration process.

**MT1-MMP-mediated Pro-MMP-2 Processing and Fibrinogen Degradation Are Inhibited by Anti-MT1-MMP mAbs**—The enrichment of gelatinolytic areas around migratory sites suggested a relation between proteolysis and endothelial migration. We therefore tested the ability of the anti-MT1-MMP mAbs generated against the catalytic domain to modulate MT1-MMP activity. As shown in Fig. 5A, anti-MT1-MMP mAbs LEM-2/15, LEM-2/63, and LEM-1/58 inhibited by an average of 34, 41, and 85% pro-MMP-2 processing and by an average of 20, 40, and 75% fibrinogen degradation induced by wounding and PMA on endothelial cells, compared with no effect of the control anti-VE-cadherin TEA1/31 mAb. Furthermore, the anti-MT1-MMP LEM-1/58 mAb largely prevented the induction of gelatinolytic areas around the wound compared with no effect of the same control mAb (Fig. 4B, and data not shown).

To determine whether the inhibitory effect was directly exerted on the MT1-MMP catalytic activity, the anti-MT1-MMP mAbs were tested in enzymatic assays using the recombinant catalytic domain of MT1-MMP. As shown in Fig. 5B, the anti-MT1-MMP mAbs LEM-2/15, LEM-2/63, and LEM-1/58 significantly decreased the degradation of the Aα and Bβ chains of fibrinogen (by 50 and 87% for LEM-2/15, 30 and 90% for LEM-2/63, and 75 and 99% for LEM-1/58, respectively) compared with no effect of the control mAb anti-β1 integrins TS2/16. These data demonstrate that the anti-MT1-MMP mAbs directly interfered with the enzymatic activity of the metalloproteinase, constituting very useful tools to investigate the role of MT1-MMP proteolytic activity during distinct cellular processes.

**Anti-MT1-MMP mAbs Inhibit Migration of Human Endothelial Cells**—The role of MT1-MMP activity in endothelial cell migration was assessed by the use of anti-MT1-MMP mAbs. As shown in Fig. 4A, migration of endothelial cells nearby the wound was prevented by the anti-MT1-MMP mAbs LEM-2/15, LEM-2/63, and LEM-1/58 compared with no effect of the control mAb. These data demonstrate that MT1-MMP plays a critical role in the regulation of endothelial cell migration.
Motility was directly assessed using the inhibitory anti-MT1-MMP mAbs in different migration assays. As shown in Fig. 6A, anti-MT1-MMP mAbs LEM-2/15, LEM-2/63, and LEM-1/58 consistently retarded the kinetics of migration and subsequent repair of the wound in PMA-treated endothelial cells by an average of 22, 24, and 35% at 8 h, respectively. The role of MT1-MMP in endothelial cell migration was also determined by using transwell chamber assays. Again, PMA-induced transmigration of human endothelial cells through gelatin-coated filters was consistently inhibited by anti-MT1-MMP.
mAbs LEM-2/15, LEM-2/63, and LEM-1/58 by an average of 30, 27, and 47%, respectively (Fig. 6B). No additive inhibitory effect on cell migration was achieved when two different anti-MT1-MMP mAbs were used in combination, and interestingly no major effects of these mAbs on PMA-induced endothelial cell adhesion to gelatin could be observed (data not shown). Control anti-VE-cadherin TEA1/31 mAb had no effect, whereas the activatory anti-β1 integrin TS2/16 prevented cell migration, as described previously (6). These data demonstrate that MT1-MMP plays an important role during the migration of human endothelial cells and its activity can regulate endothelial cell motility.

Anti-MT1-MMP mAbs Inhibit Human Endothelial Cell Invasion of Collagen and Fibrin Gels as Well as Capillary Tube Formation—During angiogenesis, migration of endothelial cells requires a focused degradation of the matrix to allow endothelial cells to advance. Thus, we next addressed whether MT1-MMP might also be modulating matrix remodeling and subsequent cell invasion together with endothelial migration. Anti-MT1-MMP mAbs LEM-2/15, LEM-2/63, and LEM-1/58 consistently inhibited PMA-induced invasion of type I collagen gels by endothelial cells by an average of 20, 40, and 53%, respectively, without significantly interfering with PMA-induced cell adhesion to this matrix (Fig. 7A and data not shown). Likewise, anti-MT1-MMP mAbs LEM-2/15, LEM-2/63, and LEM-1/58 inhibited PMA-stimulated endothelial cell invasion and formation of tubule-like structures on top of fibrin gels by an average of 36, 27, and 55%, respectively (Fig. 7A). Control mAbs anti-VE-cadherin TEA1/31 and anti-β1 integrin TS2/16 had no effect or an inhibitory effect, respectively (Fig. 7A).

Interestingly, since migration and invasion are processes required for the formation of new capillaries, anti-MT1-MMP mAbs were also tested in Matrigel assays in which endothelial cells undergo the complex processes that lead to the formation of capillary tubes in vitro. Anti-MT1-MMP mAbs LEM-2/15, LEM-2/63, and LEM-1/58 consistently inhibited the spontaneous formation of capillary tubes from endothelial cells in the Matrigel model by an average of 46, 50, and 20%, respectively (Fig. 7B). Control mAbs anti-β1 integrins LIA1/2 and TS2/16 inhibited and increased tube formation, respectively, as described previously (7). These data demonstrate that MT1-MMP is directly involved in matrix remodeling by endothelial cells and the resulting formation of capillary tubes, which might explain its important role in angiogenesis.

DISCUSSION

In this report, MT1-MMP is shown to be regulated in human endothelial cells during migration. Regulation took place at several levels including expression, subcellular localization, and activation. The functional relevance of the regulation of MT1-MMP is underscored by the fact that the blockade of MT1-MMP activity with specific mAbs prevented migration and invasion of endothelial cells as well as the formation of capillary tubes, thus highlighting the critical role that MT1-MMP plays during the angiogenic response.

In our in vitro model of migration, a consistent increase of ∼2-fold in the expression of MT1-MMP in endothelial cells was observed, pointing to the importance of the tme regulation of these proteases. This is in agreement with the increments induced by angiogenic factors, inflammatory cytokines, phorbol esters, lectins, as well as three-dimensional collagen lattices and mechanical release (30–34). Moreover, the migration-dependent increase of MT1-MMP expression reported herein was largely dependent on de novo protein synthesis. Since the transcription factor Egr-1 has been implicated in the ECM-induced up-regulation of MT1-MMP expression (35), Egr-1-mediated transcription might also be involved in the MT1-MMP increase induced by migration. In this regard, Egr-1 levels are up-regulated in endothelial cells upon wounding or increased shear stress (36, 37). Additionally, we have shown that migra-
cell migration during angiogenesis, a process that requires the generation and degradation of extracellular matrix (ECM). MMP-2, a member of the MMP family, is involved in the breakdown of the extracellular matrix during angiogenesis. In the study, MT1-MMP relocalized to structures associated with motility in migrating endothelial cells and this relocalization of MT1-MMP activity with mAbs prevents PMA-induced migration of endothelial cells. It is known that MMP-2 can activate MT1-MMP in primary human endothelial cells and its dynamic relocalization to motility-associated structures during migration as suggested previously for osteoclasts (41). Moreover, filamentous actin was shown to be rearranged at areas where MT1-MMP was particularly enriched, suggesting that cytoskeletal interactions might be involved in the mobilization of preexistent or de novo synthesized MT1-MMP to certain areas of the cell membrane as proposed for melanoma cells (40). Other mechanisms that might play a role in the recruitment of MT1-MMP to specific endothelial cell sites include the association to membrane microdomains or to other membrane receptors. In this regard, MT1-MMP colocalizes with caveolin-1 in microvascular cells upon lectin stimulation (42), and biochemical evidence for this association and for the regulation of MT1-MMP activity within the caveolae has recently been demonstrated in tumor cells (43). A putative interaction or close vicinity of MT1-MMP with the integrin receptor αvβ3 has also been proposed in endothelial and carcinoma cells (42, 44). Moreover, membrane localization rather than MT1-MMP activity seems to be critical for cell migration and invasion since deletion of the MT1-MMP transmembrane or cytoplasmic domains resulted in an altered localization of the protein together with a defect in MT1-MMP-mediated functions (45, 39, 40, 16, 17). Herein, it is shown that MT1-MMP relocalized to structures associated with motility in migrating endothelial cells and this correlates with a migration-induced increase of pro-MMP-2 processing and of gelatin degradation in areas around the wound, indicating that membrane localization of MT1-MMP might also be important for its function in endothelial cells. In fact, our data demonstrate that probably both the increase in MT1-MMP expression and its relocalization at specific membrane sites allow migrating endothelial cells to concentrate their catalytic activity at certain areas and are responsible for migration-induced MT1-MMP activation.

New mAbs generated against the catalytic domain of MT1-MMP were shown to inhibit the enzymatic activity of the native metalloproteinase in endothelial cells as well as of its recombinant catalytic domain. These mAbs have allowed dissecting the roles of MT1-MMP during the angiogenic response. Thus, our data reveal that MT1-MMP participated in endothelial cell migration, an essential step during angiogenesis, since inhibition of MT1-MMP activity with mAbs prevents PMA-induced migration of endothelial cells. It is known that MMP-2 can influence cell migration (46). Recently, MT1-MMP has also been directly implicated in migration of glioma and epithelial cells on specific substrates (22, 23). In these cases, MT1-MMP regulates cell motility by degradation of migration inhibitory proteins or by exposure of cryptic adhesion sites on the ECM.
(47). In our model, it is possible that other mechanisms such as direct interactions of MT1-MMP with cell adhesion receptors or ECM components might be involved, and this issue deserves further investigation. On the other hand, both MT1-MMP and MMP-2 might be acting coordinately in gelatin degradation during migration of endothelial cells in response to wounding and PMA, as proposed for laminin and collagen in other cell systems (23, 48). Nevertheless, MT1-MMP could probably be the main player during wound-induced endothelial migration since not only pro-MMP-2 processing but also MT1-MMP fibrinolytic activity is up-regulated by the migratory stimulus. Supporting this idea is the fact that similar inhibition of migration with anti-MT1-MMP mAbs is achieved in a human microvascular endothelial cell line that does not activate MMP-2 under these conditions.2 Thus, our data provide a link between MT1-MMP-mediated proteolysis and endothelial migration likely involving an amplification feedback mechanism since MT1-MMP activity is up-regulated by migration.

The role of MT1-MMP in matrix remodeling has mainly been investigated in tumor cell invasion, although it is also thought to be important for endothelial invasion. We have shown that MT1-MMP activity is essential for efficient collagen matrix remodeling by endothelial cells, thus allowing their subsequent matrix invasion. In this regard, it was known that type I collagen is a substrate for MT1-MMP (14, 15), that type I collagen gels can regulate the expression and activity of MT1-MMP in rat endothelial cells (33), and that MT1-MMP can act as the main pericellular collagenase in transdifferentiated epithelial cells (17). On the other hand, when an injury affects the vascular bed, a provisional matrix of fibrin is deposited. The role of MT1-MMP as a pericellular fibrinolysin in endothelial cells has been reported previously (16). Our data demonstrate not only the important role played by MT1-MMP in fibrin remodeling by endothelial cells but also that this fibrinolytic activity is susceptible of regulation by a physiologic stimulus such as migration. Interestingly, the inhibition of MT1-MMP enzymatic activity by the different mAbs correlates with their ability to prevent endothelial migration and invasion pointing to a direct involvement of MT1-MMP proteolytic activity in these processes.

Previous reports had suggested a functional role for MT1-MMP during angiogenesis (24, 25, 30). Herein, MT1-MMP activity is shown to be required for the spontaneous formation of capillary tubes from primary endothelial cells in the Matrigel system, in accordance with the first description of MT1-MMP in which a role in Matrigel invasion by tumor cells was observed (13). In our system, anti-MT1-MMP mAbs are probably affecting several steps that take place during this complex process including migration and matrix remodeling as previously discussed. These findings demonstrate that MT1-MMP is important for angiogenesis in vitro as has been previously suggested by the analysis of MT1-MMP-deficient mice in vivo. However, no role of MT1-MMP in Matrigel remodeling has been reported in a different cell system using epithelial cells stably transfected with MT1-MMP (17). This apparent discrepancy might arise from the fact that other components of the proteolytic machinery such as MMP-2 and tissue inhibitor of metalloproteinase-2 can also be involved in the Matrigel remodeling.

Finally, angiogenesis is being tried as a novel therapeutic target in different pathologies including tumoral and chronic inflammatory diseases (49–51). Thus, it could be interesting to confirm the inhibitory effects of the novel generated anti-MT1-MMP mAbs during in vivo angiogenic processes, since previous anti-metalloproteinase reagents were affecting more than a proteolytic pathway and it is known how important is the tune regulation of MMP balance. Moreover, recent reports have suggested roles for MT1-MMP in pathologies including cardiovascular disease (52, 53), liver fibrosis (54), platelet aggregation (55), and others encouraging the trial of in vivo applications of these specific tools.

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