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Induction of the Urokinase Plasminogen Activator System by Oncostatin M Promotes Endothelial Migration

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Abstract Oncostatin M (OSM) is an inflammatory cytokine produced by activated macrophages and T-lymphocytes. We have previously demonstrated that OSM-induced endothelial cell migration, unlike endothelial cell proliferation and spindle formation, is independent of basic fibroblast growth factor expression (Wijelath et al. [1997] J. Cell. Sci. 110:871–879). To better understand the mechanism of OSM-induced endothelial cell migration, this study examined the potential role of the plasminogen activator system in promoting OSM mediated endothelial cell migration. OSM stimulated increased mRNA levels of urokinase-plasminogen activator (uPA) and urokinase-plasminogen activator receptor (uPAR) in a time and dose-dependent manner. Transcriptional run-off and mRNA stability analysis demonstrated that the increase in uPA and uPAR mRNA levels was due to both increased gene transcription and mRNA stability. The increase in mRNA correlated with increased protein levels of both uPA and uPAR. This increase was reflected in elevated levels of membrane-bound plasmin activity. OSM-induced endothelial cell migration was only partially dependent on plasmin activity since incubating endothelial cells without plasminogen or, in the presence of aprotinin, resulted in suppression of endothelial cell migration, indicating that OSM promoted endothelial cell migration through both a plasmin-dependent and -independent mechanism. Our results imply a role for OSM in promoting endothelial cell migration via a plasmin-dependent pathway and a uPAR-mediated pathway. Together, these and other recent studies support a role for OSM in modulating the different phases of angiogenesis. J. Cell. Biochem. 79:239–248, 2000. © 2000 Wiley-Liss, Inc.

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Angiogenesis, the growth of new blood vessels from pre-existing capillaries, is essential for normal growth and differentiation, wound healing, and inflammation. However, unregulated angiogenesis can lead to pathological conditions such as cancer, diabetic retinopathy, and inflammatory diseases [Folkman and Shing, 1992; Risau and Flamme, 1995]. Angiogenesis is a complex process involving three major phases [Pepper et al., 1996; Sage, 1996; Stromblad, 1996]. The first phase involves degradation of the extracellular matrix to allow the sprouting capillaries to invade the surrounding stroma [Ausprunk and Folkman, 1977]. The second phase involves endothelial cell migration and proliferation. Finally, the cells differentiate and mature into capillaries with the deposition of a basement membrane. Matrix dissolution requires the expression of proteolytic enzymes by the invading capillary and surrounding tissue. A major protease involved in this process is plasmin [Saksela and Rifkin, 1988]. The plasminogen activators (PA) urokinase and tissue-type plasminogen activator (uPA/tPA) generate plasmin by converting the zymogen plasminogen, to plasmin. A broad range of extracellular matrix proteins are degraded by plasmin either directly or indirectly

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by activating latent metalloproteinases and collagenases [He et al., 1989; Pepper et al., 1996]. uPA appears to be involved mainly in tissue remodeling and cellular invasion whereas tPA's role is primarily restricted to thrombolysis [Chapman, 1997; Vassalli et al., 1991]. The major inhibitor of uPA and tPA is plasminogen activator inhibitor-1 (PAI-1) [Pepper et al., 1996]. Thus, the regulation of uPA/tPA and its inhibitor PAI-1 is critical in maintaining the angiogenic activity of endothelial cells.

Recent studies have demonstrated a number of endothelial mitogens that can modulate the PA system, such as vascular endothelial growth factor (VEGF) and basic fibroblast growth factor (bFGF) [Mandriota and Pepper, 1997; Pepper et al., 1990]. We recently demonstrated that oncostatin-M (OSM), a macrophage/T-lymphocyte cytokine stimulated endothelial cell proliferation, migration, and spindle morphology [Wijelath et al., 1997]. In that study, we performed antisense experiments to establish that OSM-induced endothelial cell proliferation and spindle formation were mediated through basic fibroblast growth factor (bFGF). However, the migratory effects of OSM were independent of bFGF expression. In an effort to further define the mechanism of migration induced by OSM, we sought to determine whether the PA system is involved in promoting endothelial cell migration. Several studies have demonstrated a coordinated expression of uPA/uPAR at the leading edge of migrating cells suggesting that uPA/uPAR may promote migration by enhancing detachment from matrix proteins [Saksela and Rifkin, 1988]. On the other hand, recent studies have also demonstrated a non-proteolytic role for uPAR in promoting cell migration involving direct binding to matrix [Waltz and Chapman, 1994; Wei et al., 1994] and association with integrins [Wei et al., 1996]. In this study, we demonstrate that OSM promotes endothelial cell migration through a mechanism that is partially dependent on proteolytic activity.

MATERIALS AND METHODS

Cell Culture

Human microvessel endothelial cells (HMVEC) obtained from Clonetics were cultured in MCDB 131 growth medium (Clonetics Corp., San Diego, CA). Endothelial cells used in this study were between passage four through seven. MCDB 131 medium containing insulin, transferrin, and selenium (ITS+3, Sigma Chemical Co., St. Louis, MO) were used when endothelial cells were cultured under serum-free conditions.

Northern Blot Analysis

HMVEC were plated on to 60-mm^2 plates and cultured in MCDB 131 medium containing 5% FBS. Prior to experiments, plates were rinsed in PBS and replaced with serum-free MCDB 131 medium containing insulin, transferrin and selenium (ITS+3, Sigma Chemical Co.). HMVEC were then stimulated with OSM (Genzyme, Cambridge, MA) for the indicated times and concentration. RNA was extracted using RNeasy kit (Qiagen, Valencia, CA). Equal amounts of total RNA (10 μg/lane) were separated by electrophoresis on a 1.2% formaldehyde gel and transferred overnight onto Nytran membranes (Schleicher and Schuell, Keene, NH) by downward capillary transfer using 10 × SSC as transfer buffer. The filters were then hybridized in Quickhyb solution (Stratagene, La Jolla, CA) to one of the following random primed (Pharmacia, Alameda, CA) ^{32}P-labeled cDNA probes: (a) 1 kb uPA fragment; (b) 1 kb uPA-R fragment; (c) 1.2 kb tPA fragment; (d) 0.6 kb PAI-1 PCR fragment; and (e) 1 kb G3PDH PCR fragment. Except for PAI-1 and G3PDH, all other cDNA's were obtained from American Type Culture Collection (Rockville, MD). Filters were washed at 68°C in 0.2 × SSC/0.2% SDS and exposed to X-ray film for 48 h at −70°C. For comparison of RNA loading, filters were rehybridized with G3PDH probe.

Nuclear Run-Off Analysis

Nuclei (2 × 10^7) from OSM stimulated (20 ng/ml) endothelial cells were isolated and in vitro transcription was carried out in 100 μl of 10 mM Tris-HCl (pH 8.0) buffer containing 5 mM $MgCl_2$, 300 mM KCl, 0.1 mM EDTA, 1 mM DTT, 0.5 mM CTP, GTP, ATP, and 200 μCi [α-^{32}P] UTP (NEN, Boston, MA) for 30 min at 30°C [Marzluff, 1985]. The reaction was terminated by adding 1 μl proteinase K (20 mg/ml) and 10 μl of 10% SDS followed by incubation at 40°C for 1 h. Radiolabeled RNA was precipitated by LiCl after acid phenol/chloroform extraction. Linearized, denatured

uPA, uPAR, G3PDH, and pGEM-4Z (Promega, Madison, WI) plasmid DNA (5 μg) was vacuum transferred onto nylon membranes (Schleicher and Schuell) using a slot blot apparatus. The nylon membranes were hybridized with radiolabeled RNA (3×10^7 cpm) in Quick-hyb solution for 24 h. The membranes were then washed with $0.5 \times$ SSC/0.2% SDS at 60°C before autoradiography for 72 h at −70°C.

RNA Stability Analysis

HMVEC were stimulated for 4 h with 20 ng/ml OSM. Actinomycin D (ACD, 5 μg/ml; Boehringer Mannheim, Indianapolis, IN) was then added to the cultures and RNA was extracted after every hour for 5 h. Northern analysis was then performed and the membranes were probed with uPA, uPAR, and G3PDH. Autoradiographic signals were analyzed on a Macintosh 9600 computer using the public domain NIH Image analysis program (developed at the U.S. National Institutes of Health and available on the Internet at http://rsb.info.nih.gov/nih-image/). The signal density of each RNA sample hybridized to uPA or uPAR was divided by that hybridized to the G3PDH. The corrected density was then plotted as a percentage of the 0-hour value (log scale) against time.

Plasmin Activity Assay

Endothelial membrane bound plasmin activity was assayed using the chromogenic substrate S-2251 (DiaPharma, Franklin, OH) as previously described [Barnathan et al., 1990]. Briefly, endothelial cells were stimulated with 20 ng/ml OSM for time course studies. For dose-response studies, HMVEC were stimulated for 24 h with different doses of OSM. All experiments were carried out in serum-free MCDB 131 containing ITS+3. At the indicated times, endothelial monolayers were rinsed twice with phosphate buffered saline and incubated at 37°C for 2 h in 100 μl of 50 mM Tris-HCL, pH 7.4 containing 5 U/ml plasminogen (Calbiochem, San Diego, CA), 50 μg/ml CNBr-cleaved fibrinogen (American Diagnostica, Greenwich, CT), S-2251 (275 μg/ml), 0.1% Triton X-100 and 10 mM EDTA. Optical density was read at 402 nm.

Western Blotting

HMVEC were stimulated with the indicated dose of OSM for 24 h. Endothelial cells were then lysed in ice-cold 10 mM Tris-HCl, pH 7.5 containing 1% Triton-X 100, 150 mM NaCl, and a protease inhibitor cocktail (Boehringer Mannheim). Protein concentrations were determined and equal amounts of protein were separated on a 10% Bis-Tris polyacrylamide gel (Novex, San Diego, CA). The separated proteins were transferred to nitrocellulose filters for 2 h at 1.5 mA/cm^2 (Bio Rad, Hercules, CA) by semi-dry electrotransfer and blocked with 20 mM Tris-HCl (pH 7.5) containing 0.15 M NaCl, 3% gelatin and 0.5% Tween-20 for 2 h at room temperature and then probed with a monoclonal antibody to uPAR (dilution 1:2000; American Diagnostica) in 20 mM Tris-HCl (pH 7.5) containing 1% gelatin, 0.15 M NaCl, and 0.05% Tween-20 for 2 h at room temperature. Blots were then washed with 20 mM Tris-HCl (pH 7.5) containing 0.15 M NaCl and incubated with a peroxidase-linked goat anti-mouse antibody (dilution 1:30,000; Bio Rad). After washing, bands were developed using Super Signal chemiluminescent reagent (Pierce, Rockford, IL).

ELISA

HMVEC in MCDB-131 medium containing ITS+3 were stimulated with 20 ng/ml OSM for the indicated times. Supernatants were assayed for uPA, tPA, and PAI-1 by ELISA as described by the manufacturer (American Diagnostica).

Chemotaxis Assay

Effects of OSM on endothelial cell migration were studied using 6.5 mm Transwells, (Costar, Cambridge, MA) as described previously [Leavesley et al., 1993]. Briefly, the underside of transwells were pre-incubated with 10 μg/ml vitronectin (Calbiochem, San Diego, CA) overnight at room temperature. Endothelial cells (1×10^5) were placed in the upper chamber in MCDB 131 containing 0.5% bovine serum albumin and 100 μg/ml plasminogen (Calbiochem). OSM was added to the bottom chambers at the indicated concentrations in the same medium. Transwells were incubated for 6 h at 37°C. Cells remaining on the upper membrane were removed with a cotton bud. Cells attached to the lower membrane were stained with 0.1% crystal violet in 50 mM borate pH 9, 2% ethanol. Membranes were subsequently washed in phosphate buffered saline (PBS) and the crystal violet stain was eluted

with 10% acetic acid and then quantified at 600 nm. Monoclonal antibodies to uPA and uPAR (American Diagnostica) at concentrations of 30 μg/ml and aprotinin at 100 μM were used for studies on the effects of inhibiting uPA, uPAR, and protease activity on endothelial cell migration.

RESULTS

OSM Promotes uPA and uPAR mRNA Expression

We reported previously that OSM promotes endothelial cell proliferation, spindle formation, and migration [Wijelath et al., 1997]. Since other studies have demonstrated an important role for plasmin activity in endothelial cell proliferation and migration, the present study was designed to determine whether OSM-induced endothelial cell migration was regulated by the PA system. First, we determined whether OSM induced PA genes. Endothelial cells were incubated with OSM (10 ng/ml) and at varying time points, uPA, tPA, PAI-1, and uPAR mRNA levels were measured by Northern blot analysis (Fig. 1A). OSM stimulated uPA and uPAR mRNA levels without having any effect on tPA or PAI-1 levels. Both uPA and uPAR mRNA levels were elevated within the first hour, by more than two-fold for uPA and three-fold for uPAR (Fig. 1B,C). Both mRNA levels remained elevated for up to 8 h. When the response of these transcripts to varying concentrations of OSM dose was investigated after 2 h of incubation, both uPA and uPAR transcripts responded in a dose-dependent manner (Fig. 2). The increase in uPA and uPAR mRNA transcripts could be a consequence of gene activation, mRNA stability, or a combination of both mechanisms. We performed nuclear run-on analysis and mRNA stability studies to address this question. Nuclear extracts were made from 4 h OSM-stimulated endothelial cell cultures. As shown in Figure 3, uPA mRNA levels were increased in OSM-stimulated nuclear extracts whereas uPAR mRNA levels remained unchanged. Studies of mRNA half-life indicate both uPA and uPAR mRNA half-lifes were increased by 90 and 240 min, respectively (Fig. 4A,B). Although nuclear run-off studies indicated that uPAR mRNA levels remained unchanged, Northern blotting (Fig. 1A,C) demonstrated a four-fold increase in uPAR mRNA levels whereas mRNA stability studies demonstrated only a 20% increase in uPAR mRNA stability following 1 h stimulation with OSM. Therefore, the four-fold increase in uPAR mRNA following 1 h stimulation with OSM cannot be accounted for by mRNA stability alone. Together, these data indicate that the observed increase in uPA and uPAR

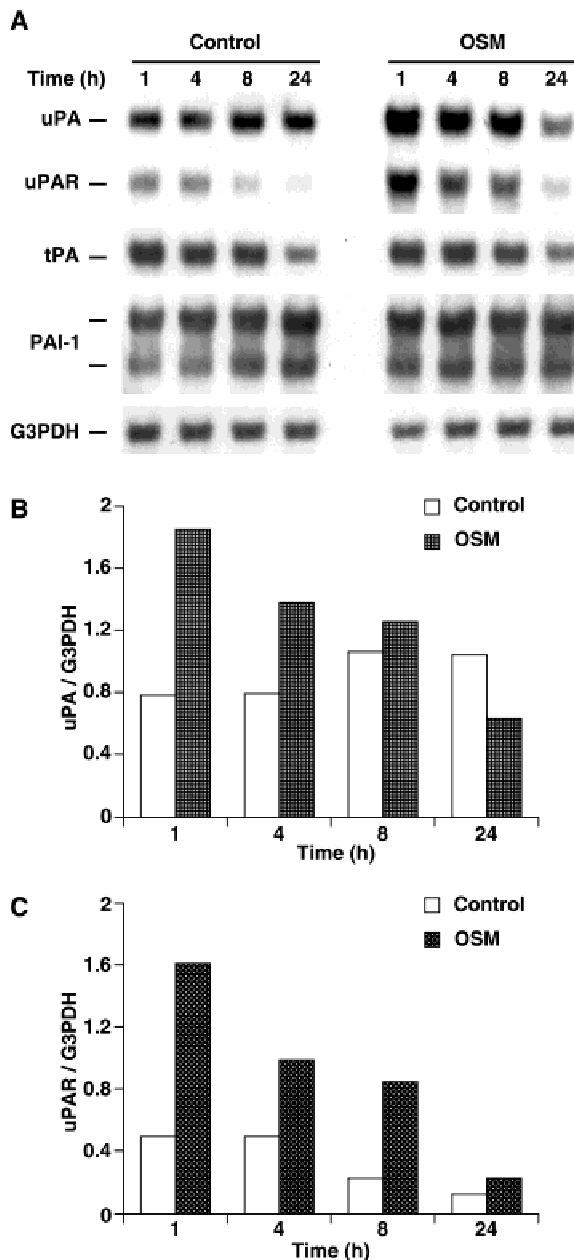


Fig. 1. OSM induces uPA and uPAR mRNA. **A**: Kinetics of uPA, uPAR, tPA, PAI-1 mRNA induction by OSM in HMVEC. Total RNA was harvested from HMVEC exposed to OSM (10 ng/ml) at the indicated times and mRNA expression was assessed by Northern blot. The blot was rehybridized with G3PDH to demonstrate equal RNA loading. **B,C**: Densitometry analysis of uPA and uPAR mRNA expression. Data is expressed as ratio of uPA or uPAR to G3PDH expression.

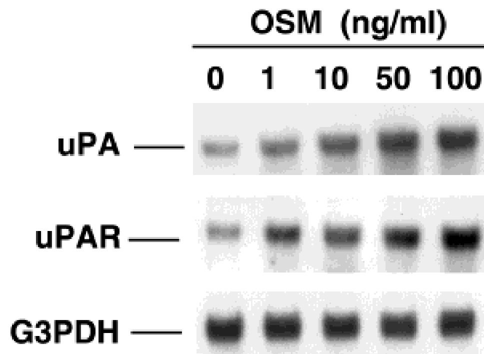


Fig. 2. Dose-response effect of OSM on uPA and uPAR mRNA expression. HMVEC were incubated for 2 h with the indicated concentrations of OSM and total RNA was extracted from the cells at the end of each incubation time and analyzed as described in Materials and Methods.

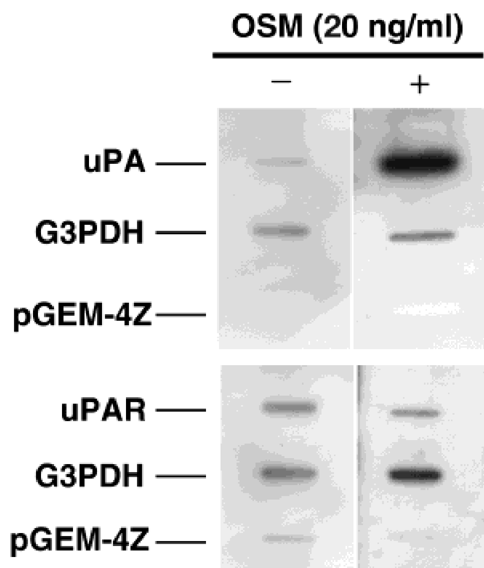
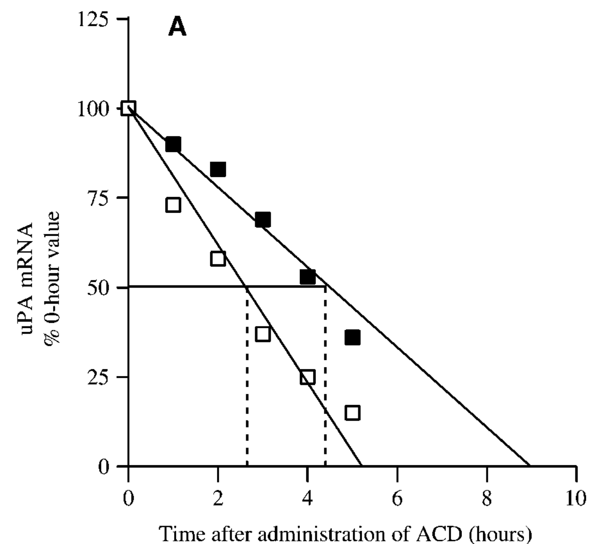


Fig. 3. Effects of OSM on the transcriptional rate of uPA and uPAR. Nuclear extracts were harvested from HMVEC treated with OSM (20 ng/ml) for 4 hours compared to unstimulated cultures. The transcription rates of uPA and uPAR genes were assessed by nuclear run-off assay. Equal amounts of ^{32}P-labeled in vitro transcribed RNA probes were hybridized to 5 μg of denatured uPA, uPAR, G3PDH, and pGEM-4Z cDNA.



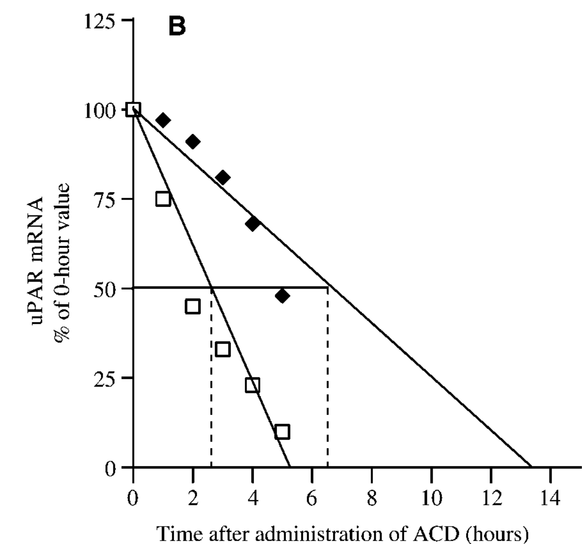


Fig. 4. Effect of OSM on uPA and uPAR mRNA stability. **A,B**: HMVEC were exposed to vehicle (□) or 20 ng/ml OSM (■) for 4 h before addition of actinomycin D (ACD). Total RNA was extracted at the indicated times following the addition of ACD (5 μg/ml). Northern blots were performed and probed with uPA, uPAR, and G3PDH.

mRNA following OSM stimulation is through gene activation and increased mRNA stability.

Effect of OSM uPA and uPAR Protein Expression

To determine whether the mRNA levels correlated with protein expression, supernatants from OSM-stimulated endothelial cells were analyzed by ELISA for uPA, tPA, and PAI-1 antigen levels. While there was no significant difference in the antigen levels for tPA and PAI-1 between control and OSM-stimulated supernatant (data not shown), uPA supernatant and cell-associated levels increased in a time-dependent fashion by approximately 10-fold and five-fold, respectively at 24 h (Fig. 5). Western-blot analysis was performed to determine the effect of OSM on uPAR protein ex-

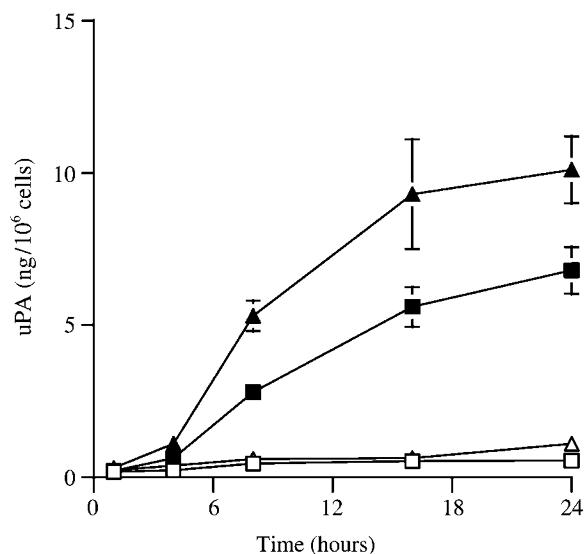


Fig. 5. OSM promotes uPA-cell bound and supernatant levels. HMVEC were treated with OSM (10 ng/ml). At the indicated times, supernatant collected and cell-lysates obtained by lysing in 0.1% Triton-X 100. Supernatant and diluted lysate were then incubated in microtitre plates containing immobilized anti-uPA (Immunobind uPA kit, American Diagnostica) analyzed for uPA according to manufactures instructions. Cell lysate (■) and supernatant (▲) from OSM stimulate cultures. Open symbols for control cultures. Results are expressed as mean ng/10^6 cells ± s.e.m. of triplicate cultures.

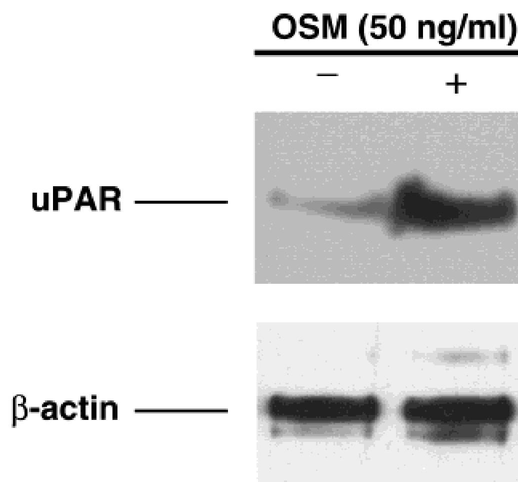


Fig. 6. Stimulation of uPAR protein synthesis by OSM. HMVEC were treated with the indicated dose of OSM for 16 h. Total cellular protein (30 μg) was separated on a 10% SDS-polyacrylamide gel and transferred to a nylon filter . uPAR was visualized by incubating the filter with a monoclonal antibody as described in Methods. Unstimulated cells served as controls. Blots were striped and reprobed with anti-β actin to demonstrate equal loading.

pression. As shown in Figure 6, an increase in uPAR antigen levels was observed in OSM-stimulated endothelial cell cultures.

OSM-Induced Endothelial Cell Migration is Dependent on Endothelial Proteolytic Activity

To test whether the activation of the uPA/uPAR system resulted in increased proteolytic activity on the endothelial cell surface, we incubated HMVEC in serum-free medium containing plasminogen (100 μg/ml). After stimulation with OSM (10 ng/ml) for the indicated times, the endothelial monolayers were incubated in plasmin substrate buffer. As shown in Figure 7A, increased total cell proteolytic activity was observed with increasing time. We also observed a dose-dependent effect of OSM on endothelial cell proteolytic activity (Fig. 7B). Supernatant from stimulated cultures also showed increased proteolytic activity (data not shown). This increase in proteolytic activity was partly responsible for the OSM-induced migration, since endothelial cells incubated in the absence of plasminogen or in the presence of aprotinin, a serine protease inhibitor, suppressed OSM-induced migration by approximately 55% and 45% (Fig. 8A). To demonstrate that the induction of uPA and uPAR was responsible for the migratory effects of OSM, we incubated endothelial cells with antibodies to uPA and uPAR. Anti-uPAR and anti-uPA inhibited OSM-induced endothelial cell migration by approximately 75% and 45%, respectively. A combination of both antibodies totally inhibited endothelial migration cell compared to control (Fig. 8B). The antibodies had no effect on cell adhesion to vitronectin, suggesting that inhibition of cell migration by these antibodies were not due to diminished cell adhesion (data not shown). When both uPA and uPAR antibodies were present, plasmin activity was completely inhibited whereas anti-uPAR on its own inhibited plasmin activity by only 20% (data not shown). Since unbound uPA, which will not be neutralized by uPAR antibodies, can still contribute to plasmin generation, we therefore cannot rule out a role for proteolytic activity in mediating uPAR induced endothelial cell migration. Altogether, these studies indicate that both a proteolytic-dependent and-independent pathway mediate OSM-induced endothelial cell migration.

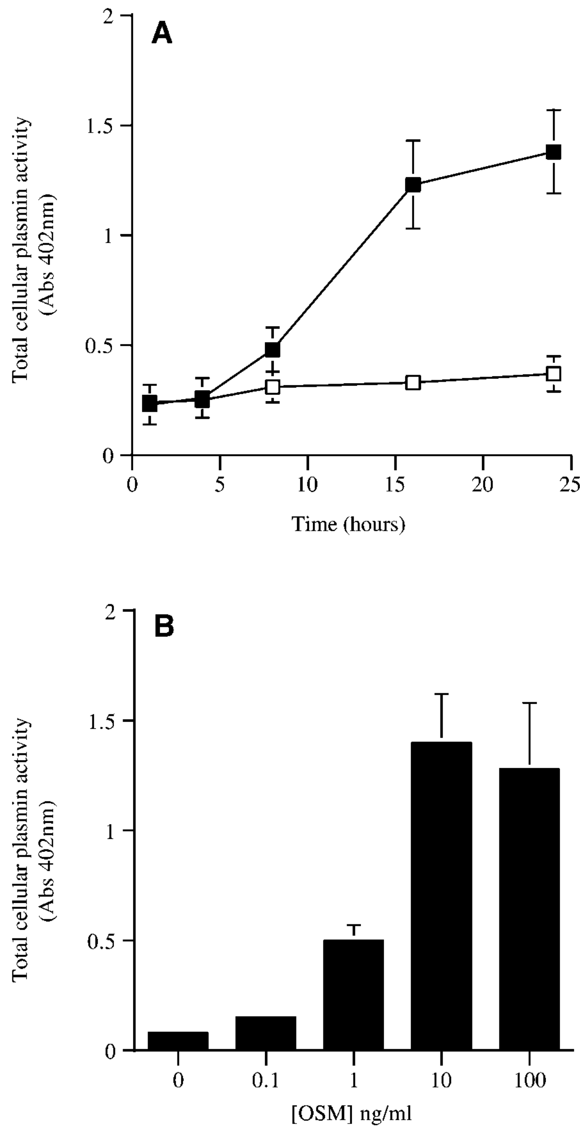


Fig. 7. OSM stimulates endothelial cell associated proteolytic activity. **A**: Kinetics and (**B**) dose-response effect of OSM on HMVEC of proteolytic activity. HMVEC were stimulated with OSM (20 ng/ml) for kinetic studies (control, □; OSM, ■) and for 24 h for the dose-response studies. Cultures were lysed and plasmin activity was assayed using the chromogenic substrate S-2251 as described in Materials and Methods. Results are expressed as mean ± s.e.m. of triplicate cultures.

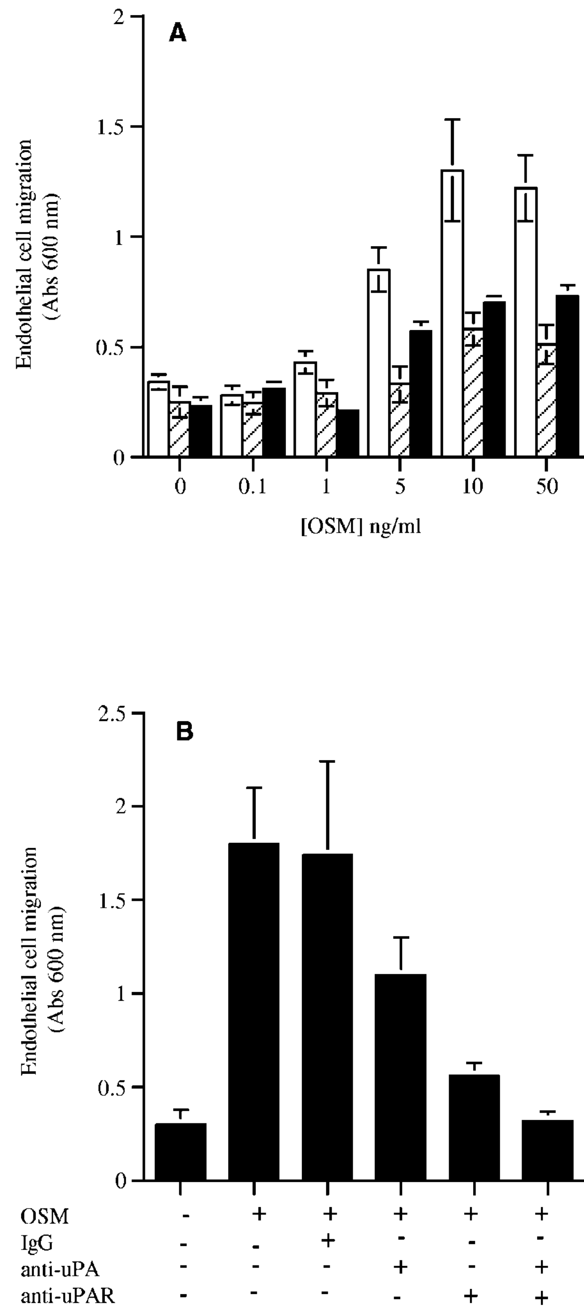


Fig. 8. OSM promotes migration dependent on proteolytic effect. **A**: Effect of aprotinin on OSM induced HMVEC migration. HMVEC were incubated with the indicated concentrations of OSM for 6 h in 100 μg/ml plasminogen (open bar), absence of plasminogen (shaded bar) or in the presence of 100 μM aprotinin (closed bar). **B**: Effects of monoclonal antibodies to uPA (30 μg/ml) and uPAR (30 μg/ml) on OSM induced HMVEC migration. Results are expressed as mean ± s.e.m. of triplicate cultures.

DISCUSSION

It is now well established that macrophages are key effector cells during angiogenesis [Clark et al., 1976]. These cells secrete numerous cytokines that are growth stimulators or inhibitors that can influence one or more phases in the angiogenic processes. One such cytokine is OSM, a macrophage/T-lymphocyte cytokine [Zarling et al., 1986]. OSM is related to the family of cytokines which include leukemia inhibitory factor (LIF), interleukin (IL)-6, IL-11, and ciliary neurotrophic factor [Bazan,

1991; Rose and Bruce, 1991]. We recently demonstrated that OSM promoted endothelial cell proliferation, spindle formation and migration suggesting a role for this cytokine in promoting angiogenesis [Wijelath et al., 1997]. In that study, OSM stimulated bFGF expression which was localized within the cytoplasm and nucleus. The proliferative and spindle formation effects of OSM were attributed to the induction of bFGF since antisense bFGF could reverse these effects. However, the migratory effects of OSM were independent of bFGF expression suggesting an alternate pathway. In the present study, we provide evidence that OSM promotes endothelial cell migration via the urokinase PA system. This conclusion is based on the observation that OSM promoted the expression of uPA and its receptor, and that antibodies to both uPA and uPAR inhibited endothelial cell migration. Similar observations were noted in OSM stimulated smooth muscle cell migration (data not shown).

OSM was recently shown to promote angiogenesis in vivo and that cyclogenase-2 activity was required for OSM-induced microvessel endothelial cell proliferation [Pourtau et al., 1999; Vasse et al., 1999]. Endothelial cell migration and the role of proteases in regulating migration are a critical aspect of angiogenesis [Pepper et al., 1996]. Two studies have reported the induction of uPA by OSM in synovial fibroblasts [Hamilton et al., 1991] and in the liver cell line, HepG2 [Okada et al., 1996]. Our work confirms these observations in human microvessel endothelial cells and provides the first evidence for the induction of uPAR by OSM and a possible mechanism of OSM-induced endothelial migration. The induction of uPA by OSM was due both to gene activation and to mRNA stability, consistent with studies on the induction of uPA by bFGF [Gualandris and Presta, 1995]. The increase in uPAR mRNA observed in this study was also due to both gene activation and mRNA stability. Interestingly, unlike bFGF and VEGF, which can stimulate both uPA and tPA [Mandriota and Pepper, 1997; Pepper et al., 1991], OSM had no effect on the expression of tPA in endothelial cells and smooth muscle cells (unpublished observation). Our study suggests a partial requirement of plasmin generation for OSM to promote endothelial cell migration, since endothelial cells incubated without plasminogen or in the presence of aprotinin (a serine protease inhibitor) inhibited endothelial migration by 55% and 45%, respectively. Similar observations on the role of proteolytic activity have been made for endothelial migration [Barnathan et al., 1990; Fibbi et al., 1988; Pepper et al., 1993]. Initiation of angiogenesis requires dissolution of the basement membrane and the surrounding matrix by plasmin and matrix metalloproteases [Pepper et al., 1996]. However, plasminogen has to be activated to plasmin. The binding of uPA to uPAR serves to stabilize uPA and increase the rate of plasminogen activation [Ellis et al., 1989; Hajjar et al., 1986]. Another important function of uPA binding to uPAR is to localize uPA activity to the cell surface and therefore limit the proteolytic activity (plasmin generation) to the leading front of migrating cells [Estreicher et al., 1990; Pyke et al., 1991]. Recent studies in mice deficient in the PA genes suggest that the role of the PA/plasmin system may be limited at least in normal mouse development and physiology to situations where newly developing capillaries encounter fibrin [Carmeliet et al., 1994].

The issue of whether the PA/plasmin system contributes to cell migration in wound healing and tumor growth is still unclear. A role for uPA and uPAR in cellular migration was recently demonstrated using a gene disruption approach. Neointimal cell accumulation was reduced in $uPA^{-/-}$ mice but not $tPA^{-/-}$ mice [Carmeliet et al., 1997]. The migratory response of smooth muscle cells isolated from $uPA^{-/-}$ and $uPAR^{-/-}$ mice to bFGF was reduced suggesting a role for uPA and uPAR in smooth muscle cell migration [Herbert et al., 1997]. However, in that study, the binding of uPA to uPAR promoted migration in a plasmin-independent manner. Several other studies have demonstrated that uPA catalytic activity is not always required for cell migration [Odekon et al., 1992]. Our studies suggest that OSM promoted endothelial cell migration via a proteolytic-dependent and-independent mechanism. The observation that uPAR is critical for OSM-induced migration is consistent with previous results associating uPAR expression with cell migration [Gyetko et al., 1994; Pepper et al., 1993]. While uPA binding to uPAR can contribute to OSM-induced migration via proteolysis, it is conceivable that in this study, ligation of uPA to uPAR may promote migration via uPAR mediated signal

transduction [Dumler et al., 1998]. Recently, it was demonstrated that uPAR signal transduction following uPA ligation induced cell migration [Busso et al., 1994; Del Rosso et al., 1993]. Another possibility is that the uPA/uPAR complex can bind vitronectin [Kanse et al., 1996; Waltz and Chapman, 1994; Wei et al., 1994] and promote motility that is dependent on the $\alpha_v\beta_5$ integrin [Yebra et al., 1996]. Interestingly, we did not observe any induction of PAI-1 mRNA (Fig.1) or protein synthesis (data not shown), unlike other studies demonstrating that angiogenic factors like bFGF and VEGF co-induce uPA/uPAR and PAI-1. Based on the increased expression of uPA and uPAR relative to the PAI-1 level in endothelial cells stimulated by OSM, it is likely that the net effect of OSM on endothelial cells is a shift towards increased proteolysis.

In summary, based on data from our previous study and our studies described here, it is clear that OSM can influence at least two phases of angiogenesis, that is matrix dissolution by promoting proteolytic activity and endothelial cell proliferation and migration. Given that macrophages are a major source of OSM and that these cells are frequently found at inflammatory sites, it is likely that OSM released at these sites plays a major role in promoting angiogenesis.

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