**Oncostatin M induces basic fibroblast growth factor expression in endothelial cells and promotes endothelial cell proliferation, migration and spindle morphology**

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**SUMMARY**

Oncostatin M (OSM), a pleiotropic cytokine originally isolated from supernatants of the U937 histiocytic lymphoma cell line, has been shown to have regulatory effects on a wide variety of cultured and tumor cells. We investigated the effects of OSM on basic fibroblast growth factor (bFGF) gene expression in bovine arterial endothelial (BAE) cells. Levels of bFGF mRNA transcripts were low in uninduced BAE cells, were maximal at 8 hours of exposure to OSM, and returned to control levels by 24 hours. Induction of bFGF mRNA transcripts by OSM was dose-dependent. Nuclear transcriptional run-on analysis demonstrated that exposure of BAE cells to OSM stimulated bFGF gene transcription. OSM treatment of BAE cells enhanced the synthesis of bFGF protein as determined by ELISA assays. Immunocytochemistry studies demonstrated the presence of low levels of bFGF protein within the cytoplasm in uninduced cells. After stimulation for 8 hours with OSM there was significant staining for bFGF in the cytoplasm. However, 24 hours after exposure to OSM, bFGF antigen was located only within the nuclei. Western blot analysis demonstrated that OSM stimulated predominantly the synthesis of a 22 kDa form of bFGF. In addition, OSM stimulated endothelial cell proliferation and migration as well as acquisition of a spindle shape. Phosphorothioate antisense oligonucleotide directed against bFGF inhibited OSM induced BAE cell proliferation and spindle shape formation but had only a minimal effect on migration. The levels of the 22 kDa form of bFGF were reduced by antisense treatment indicating that OSM induced proliferation and morphology change is likely to be regulated by intracellular bFGF. Our studies suggest that OSM released at sites of vascular injury could stimulate angiogenesis by inducing bFGF synthesis, endothelial cell proliferation and migration.

Key words: Oncostatin-M, Endothelial cell, bFGF, Proliferation, Migration, Spindle morphology

**INTRODUCTION**

Angiogenesis, the growth of new blood vessels, is highly regulated during wound healing, embryogenesis and tumor growth. Persistent unregulated angiogenesis is believed to play a major role in the progression of diseases such as inflammatory arthritis, diabetic retinopathy and cancer (Folkman and Klagsburn, 1987). Macrophages appear to play an important role in modulating the angiogenic response, particularly during wound healing, by secreting factors which may either directly or indirectly influence angiogenesis (Folkman and Shing, 1992; Sunderkotter et al., 1994). Two well-characterized angiogenic factors, basic fibroblast growth factor (bFGF) and vascular endothelial growth factor, are both produced by macrophages (Joseph-Silverstein et al., 1988; Fava et al., 1994) as well as transforming growth factor-β (TGF-β) which inhibits endothelial cell proliferation and promotes matrix synthesis (Heimark et al., 1986; Mardi et al., 1988) both essential components of angiogenesis. Recently a polypeptide growth factor, oncostatin M (OSM) was isolated, characterized and cloned from the U937 human histiocytic lymphoma cell line (Malik et al., 1989) and shown to be produced by activated monocytes/macrophages, T-lymphocytes and AIDS-Kaposi’s sarcoma (KS)-derived cells (Zarling et al., 1986; Brown et al., 1987; Miles et al., 1992). OSM is related to the family of cytokines which include leukemia inhibitory factor (LIF), interleukin (IL)-6, IL-11 and ciliary neurotrophic factor (Rose and Bruce, 1991; Bazan, 1991). These cytokines share a common signal transducer receptor component, gp 130, and have overlapping biological activities (Zhang et al., 1994). OSM has been shown to stimulate growth of rabbit aortic smooth muscle cells and AIDS-KS cells (Grove et al., 1993; Nair et al., 1992; Miles et al., 1992) but inhibits the growth of A375 melanoma cells (Zarling et al., 1986). Studies on bovine and human endothelial cells have demonstrated that treatment with OSM promotes the expression of urokinase plasminogen...
activator, IL-6, granulocyte colony-stimulating factor and granulocyte-macrophage colony-stimulating factor. (Hamilton et al., 1991; Brown et al., 1991, 1993). Since OSM is produced by activated monocytes/macrophages and T-lymphocytes, cell types found frequently at sites of inflammatory wound repair, we examined the potential role of OSM in angiogenesis. In this paper, we demonstrate that OSM may influence angiogenesis by promoting synthesis of bFGF and by stimulating endothelial cell proliferation and chemotaxis.

MATERIALS AND METHODS

Cell cultures
Bovine aortic endothelial (BAE) cells were grown in Dulbecco’s modified minimal essential medium (DMEM, Sigma Chemical Co., St Louis, MO) with 10% fetal calf serum (Hyclone, Logan, UT) and 25 μg/ml gentamycin sulfate (growth medium) at 37°C in 5% CO2. Prior to experiments and incubations, endothelial cells were incubated for 24 hours in serum-free DMEM containing transferrin, insulin, selenium, oleic acid and bovine serum albumin (UBI, Lake Placid, NY). For cell growth studies, endothelial cells were incubated with OSM, LIF or bFGF (10 ng/ml; Genzyme, Boston, MA) for 48 hours. This time point was chosen since preliminary experiments demonstrated that OSM did not have any effect on cell growth at 24 hours but caused near doubling of cell numbers at 48 hours (data not shown). To obtain cell counts, BAE cells were dissociated by exposure to trypsin and counted in a hemocytometer.

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, endothelial cells (1×105) were placed in the upper chamber in DMEM/0.5% bovine serum albumin. OSM was added to the bottom chambers at the indicated concentrations in the same medium. Transwells were incubated for 6 hours at 37°C. Cells remaining on the upper membrane were removed with a cotton bud and cells attached to the lower membrane were stained with 0.1% Crystal Violet in 50 mM borate pH 8.8, 2% ethanol. Membranes were then washed in phosphate buffered saline (PBS) and the Crystal Violet stain eluted with 10% acetic acid and quantified at 600 nm.

RNA isolation and northern analysis
Total cellular RNA was isolated with Trizol reagent (Gibco, Grand Island, NY) and samples (10 μg) were subjected to electrophoresis on a 1.2% agarose-2.2 M formaldehyde gel before transfer to Nytran filters (Schleicher and Schuell, Keene, NH). Filters were prehybridized and hybridized (Quick-Hyb, Stratagene, La Jolla, CA) with cDNA probes according to the manufacturer’s instructions. cDNA probes were labeled by random priming (Pharmacia, Alameda, CA). Filters were washed at a final stringency of 0.5x SSC and 0.1% SDS at 68°C. Probes used were, bFGF 800 bp human cDNA fragment (kindly provided by Dr Michael Reidy, University of Washington, Seattle) and a 1 kb canine glyceraldehyde-3-phosphate dehydrogenase (G3PDH) PCR cDNA fragment obtained from canine smooth muscle cells. TGF-β, acidic fibroblast growth factor (aFGF), platelet-derived growth factor (PDGF)-A and B chain human cDNAs were obtained from the American Type Culture Collection (Rockville, MD).

Nuclear run-on transcription analysis
Endothelial cell monolayers were washed with cold PBS, harvested and lysed in 0.1% Triton X-100 buffer and nuclei were harvested by pelleting through a 2.2 M sucrose gradient as previously described (Marzull and Huang, 1985). Nascent RNA was elongated as described by Merscher et al. (1994). Briefly, nuclei were resuspended in 50 μl glycerol buffer (50 mM Tris-HCl, pH 8.3, 5 mM MgCl2, 0.1 mM EDTA, 40% glycerol) and incubated in an equal volume of reaction buffer containing 10 mM Tris-HCl, pH 8, 5 mM MgCl2, 0.3 M KCl, 0.2 mM EDTA, 4 mM ATP, CTP, GTP, and 3 ul DIG-UTP (250 mM, Boehringer Mannheim, Indianapolis, IN) at 30°C for 30 minutes. The reaction was stopped by adding 10 μl 10% SDS to 1 μl proteinase K (20 mg/ml). The samples were incubated for 1 hour at 37°C and RNA precipitated after extraction with phenol and chloroform. Samples of denatured plasmid DNA (5 μg) were bound to nylon membranes (Boehringer Mannheim, Indianapolis, IN) using a slot blot apparatus. DIG-UTP labeled RNA was hybridized to the membrane bound plasmid DNAs in 0.5 M sodium phosphate, pH 7, containing 7% SDS for 16 hours at 65°C. The filters were washed once in 0.5 M sodium phosphate buffer, 1% SDS and twice in 0.1 M sodium phosphate buffer, 1% SDS for 15 minutes each at 65°C. Detection of DIG-UTP labeled RNA (Boehringer Mannheim, Indianapolis, IN) was performed according to the manufacturer’s instructions.

Extraction and western blot analysis of bFGF
BAE cell monolayers were rinsed with PBS and harvested with a cell scraper. Cells were lysed in 10 mM sodium phosphate buffer, pH 7.2, containing 1% Nonidet P-40, 0.5% sodium deoxycholate, 0.5 mM EDTA, 150 mM NaCl, 1 mM PMSF and 10 μg/ml aprotinin. Lysates were clarified by centrifugation at 30,000 g for 30 minutes at 4°C. Supernatants adjusted to yield the same amount of protein (5 mg) were incubated with heparin Sepharose for 4 hours at 4°C. After washing three times with 10 mM Tris-HCl, pH 7.4, containing 1 M NaCl, the bound proteins were eluted with SDS sample buffer and loaded onto a 12% SDS-polyacrylamide gel for electrophoresis. After electrophoresis proteins were transferred to nitrocellulose membranes and bFGF detected by immunoblot (ECL Amersham, Arlington, IL) using a monoclonal antibody to bFGF (UBI, Lake Placid, NY).

Immunocytochemistry
BAE cells were stimulated with OSM (25 ng/ml) for the appropriate times and fixed in 3.7% paraformaldehyde in PBS for 20 minutes then treated with 0.1 M glycine in PBS for 10 minutes. Fixed monolayers were then permeabilized with 0.5% Triton X-100 for 10 minutes. Following a PBS wash, monolayers were blocked with 2% BSA in PBS for 30 minutes before sequential incubation at room temperature with 1 μg/ml monoclonal anti-bovine bFGF (UBI, Lake Placid, NY) or control mouse IgG for 1 hour, biotinylated horse anti-mouse IgG (1:200; Vector Laboratories, Burlingame, CA) for 30 minutes, and avidin-peroxidase (ABC kit, Vector Laboratories, Burlingame, CA) for an additional 30 minutes. Each incubation step was followed by a PBS wash. Staining was developed with 3-amino-9-ethylcarbazole kit (Vector Laboratories, Burlingame, CA).

bFGF immunoassay
After treatment of BAE cells with indicated agents, cell monolayers were harvested, washed in PBS and resuspended in 10 mM Tris-HCl, pH 7.4, containing 1 M NaCl, 1 mM PMSF and 10 μg/ml aprotinin. The cells were disrupted by three cycles of freeze-thawing and sonication for 1 minute. The homogenate was centrifuged at 30,000 g for 30 minutes and the supernatant dialyzed overnight in PBS at 4°C. After correcting for protein content, bFGF levels were quantified using a standard ELISA procedure (R&D systems, Minneapolis, MN).

Oligodeoxynucleotides
Synthesized phosphorothioate oligodeoxynucleotides were purified by HPLC, ethanol precipitated, lyophilized to dryness and dissolved in culture medium. The 24 mer phosphorothioate oligonucleotide used in this study was targeted against the first splice donor-acceptor site at codon 60 of the human bFGF gene (Enscoli et al., 1994). The sequence of the oligonucleotide used is as follows: antisense bFGF 5’-TTGATGGATGTGAGGGTGCATAUC-3’ (AS-bFGF). Sense

bFGF probes
Oligodeoxynucleotides were obtained from the American Type Culture Collection (Rockville, MD).

OSM, LIF or bFGF (10 ng/ml; Genzyme, Boston, MA) for 48 hours. This time point was chosen since preliminary experiments demonstrated that OSM did not have any effect on cell growth at 24 hours but caused near doubling of cell numbers at 48 hours (data not shown). To obtain cell counts, BAE cells were dissociated by exposure to trypsin and counted in a hemocytometer.
bFGF (S-bFGF) with reverse complementary sequence was used as control.

**Transfection of BAE cells**

BAE cells were cultured in growth medium until approximately 80% confluent. Cells were trypsinized, washed in PBS and resuspended in growth medium. Transfection of cells was carried out by electroporation (BTX ECM 600, San Diego, CA). Briefly, 400 µl of BAE cells (3x10⁶ cells/ml) in 4 mm gap cuvettes were electroporated under the following settings: 1.000 µF, and 260 V. For cell growth and morphology, 3x10⁶ and 5x10⁴ cells, respectively, were plated into 24-well plates and incubated overnight in growth medium. Cultures were then replaced with fresh serum-free DMEM containing supplements and incubated for 48 hours with the appropriate stimulants. Cell counts were determined as described above. For migration studies, BAE cells were incubated for 8 hours in growth medium prior to performing chemotaxis studies.

**RESULTS**

**Induction of bFGF transcripts by OSM**

We initiated studies to investigate the expression of several angiogenic factors at the mRNA level in endothelial cells stimulated with OSM. BAE cells were incubated in the presence of OSM at 10 ng/ml for different time periods before RNA was extracted and analyzed by northern blotting for angiogenic factors. OSM appear to specifically up-regulate the 3.7 kb and 7 kb bFGF mRNA transcript (Fig. 1A). mRNA levels of other angiogenic factors such as aFGF, PDGF-A, PDGF-B and TGF-β were not altered by OSM treatment (data not shown). Uninduced endothelial cells expressed bFGF transcripts at near undetectable levels. The level of bFGF transcripts increased after 6 hours of exposure to OSM treatment, reached a peak by 8 hours, then returned to that of control cells by 24 hours (Fig. 1A). Dose-response experiments showed that OSM caused enhancement of bFGF mRNA steady state levels over a wide range of concentrations reaching near maximal levels at 25 ng/ml (Fig. 1B). Since OSM binds to the high affinity LIF receptor (OSM type 1 receptor), we investigated the effects of LIF on bFGF mRNA induction. LIF did not alter the steady state mRNA levels of bFGF (data not shown).

**bFGF-expression is partially controlled at the transcriptional level**

An increase in the level of bFGF transcripts could be due to an increase in the rate of transcription, stabilization of previously transcribed mRNAs or a combination of both mechanisms. Since bFGF mRNA in uninduced BAE cells was barely measurable, mRNA stability studies were not performed. To determine whether a change in transcription rate of bFGF might contribute to the increased expression after OSM stimulation, nuclear run-on experiments were performed. In unstimulated cells, the rate of bFGF transcription was very low (Fig. 2). In contrast, exposure to OSM for 6 hours significantly increased bFGF gene expression, whereas the transcription rate of the G3PDH gene was unaffected (Fig. 2). Thus, results from our northern analysis studies, together with the nuclear run-on experiments suggest that the increase in bFGF mRNA induced by OSM reflects specific activation of the bFGF promoter and enhanced bFGF gene transcription. However, these studies do not rule out the possibility that some of the observed increase in bFGF mRNA induced by OSM may reflect posttranscriptional regulation such as an increase in mRNA stability.

**OSM promotes synthesis and nuclear localization of bFGF**

To determine whether the induction of the bFGF gene reflects increased expression of bFGF protein, we analyzed by ELISA the abundance of bFGF protein content over a 48 hour period following OSM stimulation. Fig. 3 shows that lysates from unstimulated BAE cells contained low levels of bFGF. OSM stimulation caused an approximate 4-fold increase in bFGF antigen by 8 hours reaching maximal levels by 24 hours. To
examine the subcellular localization of bFGF following OSM stimulation, indirect immunocytochemistry experiments were carried out using a monoclonal antibody to bovine bFGF. Unstimulated endothelial cells showed diffuse staining throughout the cytoplasm without any staining in the nucleus (Fig. 4B) a finding which is consistent with our observation that unstimulated endothelial cells contain low levels of bFGF mRNA and protein (Figs 1 and 3). Increased staining of bFGF protein in the cytoplasm was observed after 8 hours (Fig. 4C). By 24 hours bFGF was detected only within the nucleus (Fig. 4D). Furthermore, these cells had a spindle-shaped appearance compared to either the unstimulated (Fig. 4B) or 8-hour stimulated cells (Fig. 4C). There was no significant difference in membrane bound bFGF protein between the control and OSM stimulated cells as determined by immunocytochemistry (data not shown). bFGF protein was also not detected in supernatants from OSM-stimulated BAE cultures (data not shown).

### OSM promotes endothelial cell proliferation, migration and modulation of morphology

We next investigated the effects of OSM on endothelial cell proliferation and compared its effect with LIF. Incubation of endothelial cells with OSM or bFGF (10 ng/ml) for 48 hours resulted in a 2-fold and 2.5-fold increase in cell number, respectively, when compared to control cells (Fig. 5). LIF (10 ng/ml) did not stimulate proliferation (Fig. 5) and in high concentrations could not displace the effects of OSM on endothelial cell proliferation (data not shown) suggesting that the effects of OSM are not mediated through the LIF high affinity receptor, but more likely through an OSM specific receptor. In order to determine whether released bFGF may contribute to some of the proliferative effect of OSM, monoclonal antibodies to bFGF were added to OSM-stimulated cultures. The antibodies failed to prevent endothelial cell proliferation in this assay, suggesting that extracellular bFGF did not contribute to OSM induced endothelial cell proliferation (data not shown). In the presence of OSM, a striking change in the morphology of endothelial cells was observed within 24 hours, changing from a ‘cobblestone’ shape typical of endothelial cells in static culture (Fig. 6A) to a spindle morphology typical of smooth muscle cells (Fig. 6B). Previous studies have demonstrated that nuclear bFGF is critical in inducing spindle shape formation in endothelial cells. In this study we have also demonstrated the presence of nuclear bFGF after 24 hours of exposure with OSM (Fig. 4D). To determine the potential role of OSM in endothelial cell migration, cells were exposed to a chemotactic

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**Fig. 3.** Time course analysis of OSM-induced bFGF protein expression. BAE cells were stimulated with OSM (25 ng/ml). At the indicated times cell extracts were prepared and bFGF content analysed as described in Materials and Methods. Results are shown as means ± s.d. (n=3).

**Fig. 4.** Subcellular localization of bFGF protein. BAE cells were stimulated for: (B) 0, (C) 8, (D) 24 hours with OSM (25 ng/ml). Cells were then fixed, permeabilized and probed with monoclonal bFGF antibody or control mouse IgG (A) and signal developed by Vectastain ABC kits. The arrows in B, C and D indicate positive staining for bFGF.
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gradient of OSM. Endothelial cells migrated in a dose dependent manner becoming maximal at 10 ng/ml (Fig. 7). Addition of OSM to both chambers abolished migration demonstrating that this was not merely a chemokinetic effect of OSM (data not shown). In order to rule out the possibility that some released bFGF may contribute to the migratory effects of OSM, experiments were also carried out in the presence of monoclonal antibodies to bFGF (data not shown). We did not observe any inhibition of migration of endothelial cells in response to OSM in the presence of anti-bFGF.

Intracellular bFGF promotes OSM-induced BAE cell proliferation and spindle morphology

To examine the significance of bFGF expression in OSM induced BAE cell proliferation, migration and spindle morphology, we specifically inhibited the synthesis of intracellular bFGF protein using antisense phosphorothioate oligodeoxynucleotide targeted against bFGF mRNA. As shown in Fig. 8, AS-bFGF inhibited the proliferation of BAE cells stimulated with OSM in a dose dependent fashion. BAE cells treated with 0.5 μM, 1 μM and 5 μM AS-bFGF oligomers inhibited OSM induced cell growth over a 48 hour period by 45%, 55% and 83%, respectively, relative to untreated OSM stimulated cells. In contrast, S-bFGF oligomers at the same concentrations had no significant effect. To assess the effect of AS-bFGF oligonucleotide on bFGF protein synthesis, BAE cell cultures were electroporated with concentrations of AS or S-bFGF oligomers as indicated. Following stimulation with OSM (10 ng/ml) for 48 hours, BAE cell lysates were prepared and bFGF protein content assessed by ELISA and western blot. Fig. 9 shows that AS-bFGF oligonucleotide inhibited OSM induced bFGF protein synthesis in a dose-dependent manner. In contrast, S-bFGF oligonucleotides had no significant effect. The 5 μM AS and S-treated BAE cell lysates from the same experiments were purified with heparin Sepharose and subjected to western blot analysis. Fig. 10 shows that unstimulated BAE cell extract contained mainly the 18 kDa bFGF isoform. However, stimulation with OSM increased predominantly the content of 22

**Fig. 5.** OSM stimulates endothelial cell proliferation. BCAE cells \((7 \times 10^4)\) were incubated in medium containing 10% FBS overnight after which fresh medium containing 0.1% FBS (control), 0.1% FBS plus 10 ng/ml OSM, bFGF or LIF were added to wells. Cells were trypsinized and counted on a hemocytometer at the start of the experiment and after 48 hours. The results are presented as the mean ± s.e.m. of triplicate determination. This experiment was repeated twice with similar results.

**Fig. 6.** OSM promotes endothelial cells to acquire a spindle cell shape. (A) Untreated BAE cells or (B) OSM (10 ng/ml) treated BAE cells were incubated for 24 hours at 37°C and 5% CO₂. BAE cells stimulated with OSM acquired a spindle morphology compared to normal cobble-stone shaped endothelial cells.

**Fig. 7.** Chemotactic response of endothelial cells to OSM. BAE cells \((1 \times 10^5)\) were placed in the upper compartment of the Transwell cell culture chamber. OSM or medium alone was added to the lower compartment and incubated for 6 hours. Migration rate is expressed as a percentage relative to non-OSM treated cells with 100% equal to the absorbance in vehicle-treated group. Values are shown as mean ± s.e.m. of triplicate determination. Similar results were obtained in three separate experiments.
When BAEC cells were treated with AS-bFGF oligonucleotide, OSM induced 22 kDa bFGF synthesis was inhibited. Control S-bFGF oligonucleotide had no significant effect. To test whether induction of intracellular bFGF by OSM affects BAEC cell morphology and migration, we added OSM to BAEC cells in the presence of AS or S-bFGF oligonucleotides. Significant inhibition in OSM induced spindle morphology was observed in cells receiving AS-bFGF oligonucleotide compared to untreated cultures (Fig. 11B,D). In contrast, cultures treated with S-bFGF oligonucleotide were not affected (Fig. 11C). To determine whether intracellular bFGF contributed to OSM induced migration, BAEC cells were treated with various concentrations of AS or S-bFGF oligomers. Migration assays were then performed using 10 ng/ml OSM. As shown in Fig. 12, concentrations of 1 µM and 5 µM AS-bFGF oligomer inhibited OSM induced migration by 23% and 27%, respectively, while the S-bFGF oligomer had no significant effect.

**DISCUSSION**

The interaction of cytokines released by macrophages and T-lymphocytes with endothelial cells is critical in modulating the angiogenic response, particularly during wound healing or in inflammatory diseases such as rheumatoid arthritis. In vitro studies have shown that bFGF is a potent mitogen for endothelial cells (Schweigerer et al., 1987), as well as stimulating their migration (Sato and Rifkin, 1988). In this study, we demonstrate that OSM, a macrophage/T-lymphocyte derived cytokine stimulates bFGF gene and protein expression as well as promoting cell replication, migration and morphological changes in BAEC cells. Northern analysis of cultured BAEC cells stimulated with OSM demonstrated a time and dose dependent expression of bFGF mRNA. The cDNA probe hybridized to 3.7 and 7 kb mRNA species consistent with previous reports demonstrating alternate spliced forms of bFGF (Abraham et al., 1986). Although we did not perform bFGF mRNA stability studies due to almost undetectable levels in uninduced cultures, nuclear run-on experiments clearly demonstrated that OSM induced bFGF mRNA accumulation could be due at least in part to transcriptional activation. In this study we showed that the increased rate of bFGF mRNA expression following OSM stimulation of BAEC cells correlated with an increased accumulation of predominantly the 22 kDa isoform of bFGF. The presence of multiple forms of the bFGF protein ranging from 18 kDa to 26 kDa in endothelial and smooth muscle cells have been recently demonstrated (Yu et al., 1993; Alberts et al., 1994; Rifkin et al., 1994). At least some of these multiple forms of bFGF (18, 21.5 and 22.0 and 24 kDa) are synthesized via the use of alternate initiation codons. The 18 kDa form initiates at an AUG codon while the higher molecular mass forms initiate at CUG codons (Florkiewicz and Sommer, 1989; Powell and Klagsburn, 1991; Rifkin et al., 1994). The localization of bFGF protein in endothelial cells may be dependent...
Oncostatin M induces bFGF expression on its molecular size. The 18 kDa protein is thought to be predominantly localized within the cytoplasm, in contrast to the 21.5-26 kDa forms which are found predominantly in the nucleus (Renko et al., 1990; Yu et al., 1993; Rifkin et al., 1994). Consistency with this view, our western blot studies suggest that OSM preferentially stimulates the synthesis of a high molecular mass bFGF isoform (22 kDa) over the 18 kDa cytoplasmic bFGF. Furthermore, immunocytochemical studies demonstrate that the induced 22 kDa bFGF isoform is present in the nucleus. Consistent with the studies of Sherman et al. (1993) and Stachowiak et al. (1994), we did not detect bFGF protein in culture medium of OSM stimulated BAE cells. Taken together, our results suggest that the bFGF protein synthesized in response to OSM is initially localized within the cytoplasm, but is subsequently found in the nucleus.

The targeting of high molecular mass forms of bFGF to the nucleus is determined by RG amino acid repeats located at multiple sites within the amino-terminal extension (Rifkin et al., 1994). The specific function of these nuclear isoforms of bFGF is not presently clear. However, studies by Bouche et al. (1987) suggest a direct effect of bFGF on ribosomal gene expression. Several other studies have demonstrated that the selective expression of high molecular mass bFGF to the nucleus in 3T3 cells induced a transformed phenotype (Quarto et al., 1991; Rifkin et al., 1994). Further, Tsuboi et al. (1990) demonstrated that clones of bovine capillary endothelial cells that expressed high levels of bFGF had a spindle morphology. Cells transfected with high, but not low, molecular mass bFGF cDNA could proliferate when cultured in low serum conditions, indicating a role for intracellular nuclear bFGF in cell replication (Bikfalvi et al., 1995). Viewed in the context of these observations, a possible explanation for the OSM-induced BAE cell proliferation and spindle formation is the induction and localization of the 22 kDa bFGF isoform to the nucleus. In this regard, blocking the synthesis of bFGF by treating BAE cells with AS-bFGF oligomers inhibited OSM-induced BAE cell replication and spindle formation while the corresponding S-bFGF oligomers had no significant effects. Direct biologic effects of nuclear bFGF on cell proliferation are not yet precisely characterized. It is conceivable that nuclear bFGF may act as a mitogenic factor by initiating activation of genes or enzymes responsible for cell cycling. For example, Imamura et al. (1990) and Wiedlocha et al. (1994) demonstrated a requirement for nuclear localization for acidic proteins.
FGF to stimulate DNA synthesis. The observation that AS-bFGF can inhibit OSM-induced spindle formation is intriguing. Miles et al. (1992) and Nair et al. (1992) demonstrated that OSM could promote primary cultures of AIDS-KS cells to change from a cobblestone shape typical of endothelial cells to a spindle cell shape and that these cells can produce OSM and proliferate in response to OSM. Furthermore, Enscoli et al. (1994) demonstrated that antisense oligonucleotide to bFGF inhibited proliferation and migration of AIDS-KS cells suggesting a key role for this growth factor in the development of KS lesions. Interestingly, Fiorelli et al. (1995) recently demonstrated that cytokines from activated T-lymphocytes could induce normal endothelial cells to acquire phenotypic and functional features of AIDS-KS spindle cells. Thus, it is conceivable that the shape change of endothelial cells observed in their study may be due to the effects of OSM released by activated T-lymphocytes. Furthermore, our finding that OSM promotes bFGF synthesis may be relevant to the mechanism of OSM-induced AIDS-KS cell proliferation. Unlike the proliferative and spindle shape change induced by OSM, the migratory effects of OSM on BAEC cells was only partially inhibited by AS-bFGF oligomers. Although a direct role for OSM as a chemotactic factor cannot be ruled out in this study, a more plausible explanation for the lack of inhibition of BAEC cell migration by AS-bFGF oligomers is the induction of urokinase plasminogen activator (uPA) and its receptor (uPAR; data not shown). Previous studies have demonstrated an important role for uPA and uPAR in endothelial cell migration (Pepper et al., 1993) therefore suggesting that OSM-induced BAEC cell migration in our study may be due to the expression of uPA and its receptor. Taken together, data from the bFGF antisense experiments indicate that intracellular bFGF play a role in OSM-induced BAEC cell proliferation and spindle formation but is not sufficient for migration.

An interesting finding in this study is the inability of LIF to mimic the effects of OSM since OSM has been shown to bind to the LIF high affinity receptor. Both of the cytokines are members of a family of structurally and functionally related cytokines that include ciliary neurotrophic factor, IL-6 and IL-11. All of these cytokines share a common receptor subunit, gp 130 (Zhang et al., 1994). Two types of OSM receptor complexes have been identified to date. Type I is identical to the high affinity LIF receptor and is composed of the gp130 signal transducer and the LIF binding subunit (Gearing et al., 1992). The ability of OSM to bind to the high affinity LIF receptor probably accounts for the broad overlap of the bioactivities of the two cytokines. However, the OSM type II receptor is specific for OSM (Toma et al., 1994). The presence of OSM specific type II receptor is consistent with the observation that some of the biological activities of OSM are unique to OSM and are not observed with LIF such as the growth inhibition of A375 melanoma cell line. The inability of LIF in this study to induce bFGF mRNA, stimulate endothelial cell proliferation and morphological changes as well as its inability to displace OSM in its stimulatory effects on endothelial cell proliferation suggests that the effects of OSM in our study are mediated through the OSM type II receptor. Consistent with our finding is the recent demonstration that AIDS-KS cells proliferate in response to OSM but not LIF due to the presence of OSM specific receptors (Murakami-Mori et al., 1995). In contrast to our study, Ferrara et al. (1992) and Pepper et al. (1995) demonstrated inhibition of BAEC cell proliferation by LIF suggesting the presence of LIF receptors in their cell line.

In summary, this study has demonstrated that OSM stimulates bFGF expression at the RNA and protein levels in endothelial cells. We have also shown that OSM stimulates endothelial cell proliferation, migration and conversion to a spindle shape morphology. Our data further suggest that the OSM effect in this study is most likely mediated via the type II OSM receptor.

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