Oncostatin M Induces Angiogenesis In Vitro and In Vivo

Marc Vasse, Jérôme Pourtau, Véronique Trochon, Marc Muraine, Jean-Pierre Vannier, He Lu, Jeannette Soria, Claudine Soria

Abstract—Neovascularization of the atherosclerotic plaque is responsible for its weakening and consequently for the complications of vascular disease. Macrophages are a source of growth factors that can modulate angiogenesis. In this study, we analyzed the effect of oncostatin M (OSM) on angiogenesis, as it could be involved in the development of atherosclerosis. The effect of OSM was compared with those of leukemia inhibitory factor (LIF) and interleukin-6 (IL-6). On human dermal microvasculature endothelial cells (HMEC-1s), OSM (22.5 to 112.5 pmol/L) induced a dose-dependent increase in cell proliferation greater than that induced by the classic angiogenic factors vascular endothelial growth factor (VEGF; 543 pmol/L) and basic fibroblast growth factor (bFGF; 1.1 nmol/L). LIF (19 to 475 pmol/L) induced only a 30% increase in cell proliferation, and IL-6 had no effect. Furthermore, in a modified Boyden-chamber model, OSM, LIF, and IL-6 were chemoattractant for HMEC-1s. In a tridimensional gel of fibrin, OSM increased tube formation and tube length, which were already noticeable by day 3. LIF and IL-6 induced a weaker effect that was only obvious by day 10. The angiogenic effect of OSM was also demonstrated in vivo in a rabbit corneal model: OSM was more potent than LIF, the length of the neovessels being longer with OSM than with LIF, whereas IL-6 was without effect. We tested factors that could be involved in the proliferative effect of OSM on HMEC-1s. OSM induced only a slight increase in the urokinase receptor and a 60% increase in VEGF secretion, whereas it does not modify IL-8 secretion or bFGF levels. The effect of OSM seems to depend on endothelial cell origin and cell species: OSM (up to 112.5 pmol/L) did not induce human umbilical vein endothelial cell proliferation and even had a small inhibitory effect (17%) on calf pulmonary artery endothelial cells. In conclusion, OSM induces an angiogenic effect on capillary endothelial cells, which could be, at least in part, implicated in pathological processes such as atherosclerosis or tumor growth. (Arterioscler Thromb Vasc Biol. 1999;19:1835-1842.)

Key Words: angiogenesis ■ atherosclerosis ■ oncostatin M ■ leukemia inhibitory factor ■ vascular endothelial growth factor

It is now established that the functional role of the macrophage extends far beyond its originally recognized role as a scavenger cell. Monocytes in particular are recognized as angiogenesis effector cells that produce a number of growth stimulators and inhibitors, proteolytic enzymes, and cytokines that can influence 1 or more steps in the angiogenesis cascade. It has been shown that activated monocytes or their culture supernatants induce new capillary growth in vitro and angiogenesis in vivo.1,2 However, recent evidence also suggests that capillary regression may be mediated by monocytes by producing antiangiogenic factors in vitro and in vivo.3–5 Thus, macrophages could be involved in both stimulation and suppression of angiogenesis.

Because macrophages reside at the sites of atherosclerotic plaques in the vessel wall and because the formation of highly vascularized granulations could be responsible for the fragility of the plaque,6 we were prompted to analyze the effects of oncostatin M (OSM) on neovascularization. OSM, secreted by activated monocytes, is a cytokine of the interleukin-6 (IL-6) family that includes IL-6, leukemia inhibitory factor (LIF), and ciliary neurotrophic factor.7 All of these cytokines share a common receptor subunit involved in signal transduction, gp130.8 Our interest in OSM is derived from our previous report that OSM could be involved in the development of atherosclerosis to a greater extent than is IL-6.9 This concept is corroborated by the results of Grove et al,10 who showed that OSM, but not IL-6, induced the proliferation and morphological changes of smooth muscle cells isolated from rabbit aorta. This idea is also supported by a recent report of Modur et al,11 who showed that OSM, present in human aortic aneurysms, is an inflammatory mediator contributing to chronic inflammation, but that the other cytokines of the IL-6 family are not involved. We therefore analyzed the other deleterious effects that can be induced by OSM on the endothelium, because endothelial cells are the richest source of receptors for OSM.12 In the current study, we show the...
effect of OSM on angiogenesis both in vitro (proliferation of endothelial cells and the formation of capillary sprouts in a tridimensional model) and in vivo (in a rabbit corneal model). Moreover, we analyzed the effect of OSM on the production of angiogenic factors (IL-8, vascular endothelial growth factor [VEGF], and basic fibroblast growth factor [bFGF]) by endothelial cells and on the expression of the cell-surface urokinase receptor (uPAR), because it can also play a role in angiogenesis.13

Methods

Cytokines
Recombinant human OSM, LIF, bFGF, VEGF, and a mouse monoclonal antibody against VEGF (clone 26503.11) were supplied by R&D Systems. Recombinant human IL-6 was a generous gift of Sandoz (Basel, Switzerland).

Cell Culture
Calf pulmonary artery endothelial cells (CPAECs) were provided by Dr J. Badet, (Laboratoire de Biotechnologie des Cellules Eucaryotes, Université de Créteil, Créteil, France). These cells were cultured in minimal essential medium supplemented with 20% FCS, 2 mmol/L l-glutamine, 100 IU/mL penicillin, and 100 μg/mL streptomycin (Gibco) and used at passage 12 to 20. The HMEC-1 (human microvascular endothelial cell) line cell was provided by Dr E.W. Ades (Centers for Disease Control and Prevention, Atlanta, Ga), who established this line by transfecting human dermal endothelial cells with SV40A gene product and large T antigen.14 HMEC-1s were established this line by transfecting human dermal endothelial cells and on the expression of the cell-surface urokinase receptor (uPAR), because it can also play a role in angiogenesis.13

Flow Cytometry Analysis of uPAR (CD87) on the Surface of HMEC-1s
The surface expression of CD87 was analyzed by direct immunofluorescence and flow cytometry. In brief, the adherent HMEC-1s were detached by a nonenzymatic cell dissociation solution (Sigma), and the cells were washed twice in cold PBS. Approximately 10^6 cells were incubated for 15 minutes at 4°C with 10 μL of phycoerythrin-conjugated CD87 antibody (1 mg/mL, clone VIM5; Pharmingen). After 2 washes in PBS, the cell suspension was analyzed in a flow cytometer (EPICS XL-MCL, Coulter). Data were expressed as the specific channel fluorescence intensity, which was calculated for each sample by subtracting the background channel fluorescence intensity produced by the negative control antibody from the channel fluorescence intensity value generated by the specific antibody. Photomultiplier voltages remained the same for all measurements for the experiments.

Formation of Capillaries by Using Cytodex-3 Microcarriers in a Fibrin Gel
Microcarrier cell culture was devised according to the method of Nehls et al.17 In brief, HMEC-1s were allowed to attach to the cytodex-3 microcarrier beads (Sigma) by incubating the cells with the beads in complete medium with 20% FCS for 3 hours at 37°C. The microcarrier beads were then suspended in a larger volume of the same medium and agitated for 5 minutes every 30 minutes at 30 rpm during the first 12 hours, followed by culture with continuous stirring for 4 days. When the whole surface of the microcarrier beads was covered with endothelial cells, they were concentrated by centrifugation for 5 minutes at 800g at room temperature and embedded in a fibrin matrix, prepared as follows. Purified fibrinogen at 5 μmol/L/L (Kabi) was dialyzed against minimal essential medium and then mixed with 20% FCS % and 1% l-glutamine in the absence or presence of the cytokine to be tested. Human thrombin (Sigma) at 1 IU/mL was added to the fibrinogen solution. Once the fibrin gel had formed, 500 μL of complete culture medium containing 2 μmol/L aprotinin (Bayer Pharma) was added on the surface of the fibrin matrix and changed every 3 days. Kinetic formation of capillary tubes arising from the periphery of microcarrier beads was observed. These capillaries were photographed with a camera on a reverse microscope, and their lengths were measured on the photographs.

Measurement of VEGF, IL-8, and bFGF
The secretion of VEGF and IL-8 was quantified in the conditioned medium of HMEC-1s cultured for 24 hours in the presence of OSM, IL-6, or LIF by ELISA (kits from R&D Systems). Cell lysates were obtained by treatment with 0.1% (vol/vol) Triton X-100 (Merck) in Dulbecco’s PBS (Eurobio) and sonicated at 60 Hz (Vibracell 72434, BioSpec) for 30 seconds. Then the proteins of the cell lysates were assayed by the method of Bradford.18 Results were expressed as the quantity of cytokines secreted per milligram protein of cell lysates. Because bFGF is mainly intracellular, quantification was performed in cell lysates by using an ELISA kit (R&D Systems).

In Vivo Angiogenesis Assay
Adult New Zealand white rabbits (Charles River, St Aubin-les-Elbeuf, France) weighing 1.5 to 2 kg were anesthetized with ketamine (Ketalar, 25 mg/kg IP, Parke Davis). The eyes were topically anesthetized with oxybuprocaine (Cibéline, Laboratoire Chauvin), and the globes were propoised with a jeweler’s forceps. With the use of an operating microscope, a central intrastromal linear keratotomy was performed with a surgical blade. A lamellar micro-pocket was then dissected and extended to within 2 mm of the superior limbus. Before the surgical procedure, dehydrated (1×2-mm) pellets made of 70% hydratable hydrogel were imbibed with vehicle only (sterile water or RPMI-1640, Eurobio) or various concentrations of cytokines and placed into the preformed corneal pocket.

The eyes were routinely examined by slit-lamp biomicroscopy on day 8 after implantation. Rabbits were anesthetized with ketamine, and the eyes were propoised. Photographs were made, and the area of neovascularization was evaluated and scored as follows: 0=ab-
**TABLE 1. Effect of OSM on Proliferation of HMEC-1s, HUVECs, and CPAECs**

<table>
<thead>
<tr>
<th>Cytokine</th>
<th>Concentration, pmol/L</th>
<th>HMEC-1s</th>
<th>HUVECs</th>
<th>CPAECs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td></td>
<td>2.67±0.04</td>
<td>2.64±0.04</td>
<td>5.28±0.22</td>
</tr>
<tr>
<td>OSM</td>
<td>22.5</td>
<td>4.45±0.19*</td>
<td>3.01±0.11</td>
<td>5.18±0.22</td>
</tr>
<tr>
<td></td>
<td>45</td>
<td>4.86±0.32†</td>
<td>2.98±0.13</td>
<td>4.43±0.12‡</td>
</tr>
<tr>
<td></td>
<td>112.5</td>
<td>4.85±0.10*</td>
<td>2.76±0.08</td>
<td>4.30±0.34†</td>
</tr>
<tr>
<td>Heat-inactivated OSM</td>
<td>112.5</td>
<td>2.93±0.28</td>
<td></td>
<td></td>
</tr>
<tr>
<td>LIF</td>
<td>19</td>
<td>3.58±0.14*</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>95</td>
<td>4.03±0.43†</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>475</td>
<td>3.60±0.16*</td>
<td>2.68±0.08</td>
<td>5.46±0.21</td>
</tr>
<tr>
<td>IL-6</td>
<td>380</td>
<td>2.90±0.12</td>
<td>2.72±0.16</td>
<td>5.05±0.41</td>
</tr>
<tr>
<td>bFGF</td>
<td>1100</td>
<td>4.00±0.06‡</td>
<td>3.98±0.22*</td>
<td>7.81±0.21*</td>
</tr>
<tr>
<td>VEGF</td>
<td>543</td>
<td>3.68±0.10†</td>
<td>3.37±0.12*</td>
<td>7.02±0.06*</td>
</tr>
</tbody>
</table>

Results of 10 experiments in triplicate are expressed as the ratio of number of cells harvested/number of cells seeded±SEM. †P<0.05, *P<0.01, ‡P<0.001.

**Results**

**Stimulation of Endothelial Cell Proliferation by OSM, LIF, and IL-6**

On HMEC-1s, OSM induced a dose-dependent increase in cell proliferation, with a plateau from 45 pmol/L (1 ng/mL). LIF at concentrations from 19 to 475 pmol/L (1 to 25 ng/mL) also induced a proliferative effect, but less than than that of OSM. IL-6 (up to 380 pmol/L; 10 ng/mL) was devoid of activity (Table 1). OSM at 45 pmol/L induced a greater proliferative effect (P<0.05) than did the 2 well-known angiogenic factors VEGF (1.1 nmol/L; 20 ng/mL) and VEGF (543 pmol/L; 25 ng/mL). The proliferative effects of OSM and LIF were not observed on HUVECs, whereas both VEGF and bFGF induced an increase in cell proliferation similar to that induced on HMEC-1s.

Furthermore, OSM induced a small but significant inhibition of cell proliferation on CPAECs, whereas bFGF and VEGF induced a proliferative effect similar to that observed with HMEC-1s and HUVECs. LIF and IL-6 were devoid of effect (Table 1). The inhibiting effect on CPAEC proliferation by OSM was due to blockage of the cell cycle in the G2/M phase, because there was an increase in propidium iodide incorporation, whereas the binding of annexin V was not modified (data not shown), indicating that the inhibitory effect was not related to apoptosis.

**OSM, LIF, and IL-6 Act as Chemotactic Factors In Vitro**

In a second part of this study, we evaluated whether OSM was chemotactic for HMEC-1s. As shown in Table 2, OSM, LIF, and IL-6 (respectively, 112.5, 475, and 380 pmol/L) added to the lower chamber of the Transwell-Col inserts significantly (P<0.05, n=3 in triplicate) increased the migration of HMEC-1s from the upper to the lower chamber. bFGF (1.1 nmol/L) and VEGF (543 pmol/L) were used as positive controls. Preincubation of HMEC-1s with these cytokines did not significantly modify the migration rate. These results indicate that OSM, LIF, and IL-6 act as chemoattractant factors for endothelial cells.

**Variation of uPAR Expression on the HMEC-1 Surface by OSM**

Because uPAR is involved in angiogenesis and cell migration,13 we studied the modulation of uPAR expression at the surface of endothelial cells by OSM, LIF, and IL-6. After a 24-hour incubation of HMEC-1s with OSM (9 to 112.5 pmol/L), a weak increase in the expression of uPAR was observed (1.3-fold increase for 112.5 pmol/L of OSM, P<0.01, n=3). LIF induced a weaker effect (1.18-fold for 475 pmol/L, P<0.05, n=3), but IL-6 did not induce any change in uPAR expression (Table 3).

**TABLE 2. Cytokine-Dependent Chemotactic Migration of Microvascular Endothelial Cells**

<table>
<thead>
<tr>
<th>Cytokines and Concentration, pmol/mL</th>
<th>VEGF (453)</th>
<th>bFGF (1100)</th>
<th>OSM (112.5)</th>
<th>LIF (475)</th>
<th>IL-6 (380)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Untreated</td>
<td>1.85±0.05*</td>
<td>1.43±0.20†</td>
<td>2.19±0.32†</td>
<td>1.70±0.21†</td>
<td>2.23±0.17†</td>
</tr>
<tr>
<td>Pretreatment</td>
<td>NT</td>
<td>1.66±0.20†</td>
<td>2.22±0.24†</td>
<td>1.66±0.21†</td>
<td>2.16±0.21†</td>
</tr>
</tbody>
</table>

NT indicates not tested. Cells (2×10⁶) were seeded in the upper chamber of a 3-μm-pore diameter Transwell-Col, and cytokines at indicated concentration were added in the lower compartment to test their chemoattractant activity. After a 24-hour incubation, cells on the lower face of the membrane were scraped, added to the cells in the lower compartment, and then counted in a hemocytometer. Migration assay was performed either on untreated cells or on cells pretreated with cytokines for 24 hours. Results of 3 experiments in triplicate are expressed as the ratio of the number of migrated cells in the presence of cytokines/number of migrated cells in the absence of cytokines±SEM. †P<0.05, ‡P<0.01 versus control.
Effect of OSM, LIF, and IL-6 on Capillary Tube Formation in a Fibrin Matrix

Because we previously observed that OSM induced HMEC-1 proliferation, we used a tridimensional matrix to determine whether OSM could stimulate endothelial cell invasion. Microcarrier beads seeded with HMEC-1s were embedded in a fibrin gel. After 3 days of culture, the formation of small, endothelial cell–linked, hollow tubelike structures was observed under phase-contrast microscopy. As shown in Figure 1, OSM (112.5 pmol/L) induced a 2-fold increase of the mean tube length (data from 15 microcarrier beads for each condition) and an increase in the number of tubes around the microcarrier beads. The difference was more obvious after 10 days of incubation. The addition of LIF (190 pmol/L) or IL-6 (380 pmol/L) was also able to increase the lengths of the capillaries that invaded the fibrin gel, but the effect was small and began at day 6, although it was more evident at day 10. Therefore, the effect of IL-6 and LIF on capillary tube formation was shown to be weaker than that of OSM.

Effects of OSM, LIF, and IL-6 on VEGF, IL-8, and bFGF Production by HMEC-1s

To more accurately analyze the effect of OSM on HMEC-1 proliferation, we tested the ability of OSM to modulate the secretion of 3 well-known angiogenic factors, VEGF, IL-8, and bFGF. HMEC-1s spontaneously secreted VEGF and IL-8 (Table 4). OSM (from 22.5 to 450 pmol/L) induced an increase in VEGF secretion, which was significant from 112.5 pmol/L (P<0.001 versus control, n=5). Concentrations up to 450 pmol/L did not induce any further increase (data not shown). Surprisingly, LIF (190 pmol/L) induced a slight (33%) but significant (P<0.05 versus control, n=5) decrease in VEGF secretion but had no effect on IL-8 secretion. IL-6 (190 pmol/L) did not modify either VEGF or IL-8 secretion. OSM, LIF, or IL-6 did not modify intracellular levels of bFGF.

Therefore, because LIF, which also induces a proliferative effect on HMEC-1s, did not induce secretion of VEGF, we can assume that the angiogenic effect of OSM was not only due to the increased secretion of VEGF. This hypothesis is supported by the fact that a neutralizing VEGF antibody inhibited only 21±12% (P<0.05, versus control, n=6) of the proliferative effect of OSM.

In Vivo Angiogenesis Assays

The former results suggest that OSM and LIF, 2 cytokines of the IL-6 family, are angiogenic factors in vitro. Thus, we used a rabbit corneal model to evaluate the properties of these...
cytokines in vivo. In all experiments, the angiogenic activity of OSM, LIF, and IL-6 was compared with that of bFGF, a known angiogenic factor. OSM at a dose of 2.25 pmol per implant promoted new vessel growth, progressing over half the way to the lens by day 7 (Figure 2). The maximum score was obtained for the 9 pmol per implant dose of OSM (Table 5); 2.25 pmol per implant of OSM or 3.8 pmol per implant of LIF induced angiogenesis in the rabbit cornea with a score equal to that produced by bFGF (2.75 pmol per implant), whereas IL-6 at 7.6 pmol per implant had no effect over the same period. The corneal sections did not show any inflammatory infiltrate (Figure 3).

**Discussion**

It has been demonstrated that in the aortic wall, the number of capillary-like structures is correlated with the extent of inflammatory cell infiltrate that can act by cytokine secretion. Neovascularization may play a role in the progression of the atherosclerotic plaque as well in the development of complications such as intraplaque hemorrhage or aneurysms. However, the mechanism and stimuli for neovascularization in atherosclerotic plaque remain unknown. Some observations indicate the role of smooth muscle cells through the secretion of VEGF; of mast cells, because they accompany

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**TABLE 4. Effects of OSM, LIF, and IL-6 on the Secretion of VEGF or IL-8 in the Supernatant and Intracellular bFGF Content of HMEC-1s**

<table>
<thead>
<tr>
<th>Cytokines in pmol/L</th>
<th>Control</th>
<th>OSM (112.5)</th>
<th>LIF (190)</th>
<th>IL-6 (190)</th>
</tr>
</thead>
<tbody>
<tr>
<td>VEGF</td>
<td>4.06±0.66</td>
<td>6.38±0.81*</td>
<td>2.70±0.20</td>
<td>3.08±0.54</td>
</tr>
<tr>
<td>IL-8</td>
<td>5.42±1.47</td>
<td>4.45±0.98</td>
<td>4.38±1.76</td>
<td>5.42±1.31</td>
</tr>
<tr>
<td>bFGF</td>
<td>3.18±1.03</td>
<td>2.30±0.47</td>
<td>2.85±1.05</td>
<td>2.94±0.25</td>
</tr>
</tbody>
</table>

Confluent monolayers of HMEC-1s were incubated for 24 hours with the various cytokines at indicated concentrations, supernatants were collected for VEGF and IL-8 measurements, and the cells were lysed for intracellular bFGF determination. Results are mean±SEM of 5 independent experiments and are expressed in picograms of VEGF per milligram of protein in cell lysates and in nanograms of IL-8 and bFGF per milligram of protein.

†P<0.05, *P<0.001 versus control.

**TABLE 5. Effect of OSM, LIF, and IL-6 on In Vivo Angiogenesis in the Rabbit Corneal Model Compared With bFGF**

<table>
<thead>
<tr>
<th>Cytokine (pmol/Implant)</th>
<th>Positive Implants/Total Implants</th>
<th>Mean Score</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0/8</td>
<td>0</td>
</tr>
<tr>
<td>OSM (2.25)</td>
<td>6/6</td>
<td>2</td>
</tr>
<tr>
<td>OSM (9)</td>
<td>7/7</td>
<td>3</td>
</tr>
<tr>
<td>IL-6 (7.6)</td>
<td>0/4</td>
<td>0</td>
</tr>
<tr>
<td>LIF (3.8)</td>
<td>4/6</td>
<td>2</td>
</tr>
<tr>
<td>bFGF (2.75)</td>
<td>6/6</td>
<td>2</td>
</tr>
</tbody>
</table>

New Zealand white rabbits were anesthetized, and hydrogel pellets were implanted in the corneal micropocket as described in Methods. Results are expressed as the number of positive (ie, with observable neovascularization) implants over the total number of implants for each condition and also as the mean score of the positive implants.

**Figure 2.** Rabbit cornea with an implant containing vehicle alone (Control), OSM at 2.25 pmol/implant, LIF at 3.8 pmol/implant, and bFGF at 2.75 pmol/implant 7 days after implantation. P indicates implant; arrow indicates neovessels progressing from the limbus to the implant.

**Figure 3.** Histological perpendicular sections of rabbit cornea stained with hematoxylin and eosin. a, Implant with vehicle alone; b, implant with 9 pmol/implant OSM. P indicates implant; arrows indicate neovessel. Lacunae in the corneal tissue represents the sections of vessels. In controls, only a few vessels are noted, whereas in OSM, marked angiogenesis is obvious, without inflammatory cell infiltration.
microvessels in atheroma; or of activated T cells, by production of heparin-binding epidermal growth factor and bFGF, but the role of monocytes remains controversial. Because macrophages, which secrete cytokines, reside at the site of angiogenesis, it is possible that these cells play an important role in the neovascularization of atherosclerotic plaque, as in tumors. In previous work, we have suggested that the macrophage-derivied OSM may play a major role in the development of atherosclerosis and its complications. This concept is supported by the presence of a large amount of OSM in the infiltrate of the human aortic aneurysm. Because endothelial cells have a greater number of OSM receptors than do other cell types (10- to 20-fold greater), we analyzed the effect of OSM on neoangiogenesis both in vitro and in vivo.

First, we analyzed the proliferative effect of OSM and related cytokines (LIF and IL-6) on endothelial cells of different origin and from different species. HMEC-1s are representative of the microvasculature and have properties similar to those of the original primary culture, whereas HUVECs are representative of the macrovasculature. OSM induced a strong proliferative effect on HMEC-1s and was more potent than the 2 angiogenic factors used as a reference, bFGF and VEGF. In contrast, OSM did not modify the proliferation of HUVECs. Furthermore, OSM induced an inhibitory effect on CPAEC proliferation. These results are similar to those of Takashima and Klagsbrun, who presented OSM as an endothelial cell growth inhibitor in vitro by using bovine aortic cells. This inhibitory effect on CPAEC proliferation was due to blockade of the cell cycle in G1/M, as indicated by the increase in propidium iodide and [3H]thymidine incorporation without apoptosis. This was accompanied by an 4-fold increase in the cell surface of CPAECs, as detected by a computerized morphometry analysis (results not shown).

OSM also induced a proliferative effect on HMEC-1s, whereas it had no effect on HUVEC or CPAEC proliferation. Using bovine aortic endothelial cells, Ferrara et al described an inhibitory effect of LIF on cell proliferation. These discrepancies can be attributed to the remarkable biological versatility of LIF, which depends on cell origin, because the same authors also reported that LIF induces a stimulatory effect on the growth of capillary endothelial cells of the adrenal cortex.

Therefore, the action of OSM and related cytokines of the IL-6 family on endothelial cell proliferation depends on endothelial cell origin and the species. These differences of action of these cytokines could be due to different signal transduction pathways of cytokines of the IL-6 family or to differential expression of cytokine receptors on endothelial cells of different origins and from different species. Cytokines OSM, LIF, and IL-6, when added to the lower compartment of a modified Boyden chamber (Transwell), have a chemotactic effect, as shown by an increase in cell-migration through a collagen-coated membrane.

In addition to the proliferative and chemotactant effect observed on capillary endothelial cells, OSM induces a strong effect on capillary tube formation in a tridimensional system (a fibrin gel). As early as 3 days of culture, OSM incorporated into the fibrin gel increased the length and number of tubes around the microcarrier beads. The increase was also observed after 10 days of culture. Addition of OSM induced an increase in mean tube length (data from 15 microcarrier beads for each condition). With LIF (up to 190 pmol/L) and IL-6 (380 pmol/L), the effect was not observed within 3 days, but the increase became evident only after 6 and 10 days of incubation with the cytokines. Furthermore, OSM specifically induced an angiogenic process on microcapillary endothelial cells, an effect that is more potent than that of bFGF. LIF also induced a proliferative and morphogenic effect, but it was weaker than that of OSM. However, for IL-6, there is a discrepancy between the response of microvessel endothelial cells in 2-dimensional versus 3-dimensional culture: whereas IL-6 had no effect on cell proliferation in 2-dimensional culture, it induced an increase in capillary tube formation in a fibrin matrix (3-dimensional). This type of difference between 2- and 3-dimensional models has already been described for transforming growth factor-B.

Among the mechanisms involved in OSM-induced endothelial cell proliferation, we observed that OSM stimulated the secretion of VEGF on HMEC-1s, a factor known to induce proliferation of endothelial cells. However, because LIF, which induces a proliferative effect, did not induce secretion of VEGF, we can assume that the proliferative effect of OSM is not totally due to the secretion of VEGF. This hypothesis is also supported by the fact that a neutralizing VEGF antibody only partially inhibited its proliferative effect. In contrast to the results of Wijelath et al using bovine aortic endothelial cells, we have not found any induction of bFGF by OSM in HMEC-1s. Mechanisms by which OSM stimulates cell proliferation could be related to the induction of phosphorylation of mitogen-activated protein kinases (MAPKs), since OSM has recently been found to activate the MAPK/ERK kinase on AIDS-derived Kaposi sarcoma cells, or by a specific tyrosine phosphorylation. Binding studies of endothelial cells suggest the presence of a high-affinity receptor for OSM, with an apparent Kd of 15 pmol/L, and a low-affinity receptor, with an apparent Kd of 1 nmol/L. The angiogenic effect of OSM observed at low concentrations (45 to 90 pmol/L) suggests that the high-affinity receptor, which also induces P-selectin, IL-6, and growth-related cytokine secretion, is involved in the angio-
Our results demonstrate that OSM also promotes angiogenesis in vivo in the rabbit cornea, because a single inoculation of OSM resulted in the rapid formation of vessels around the lens where the OSM was adsorbed. LIF also induces an in vivo angiogenic effect, but as expected from the proliferation experiment, its effect was less than that of OSM. The growth of corneal neovessels induced by OSM and LIF was not associated with an inflammatory cell infiltrate, and therefore we can conclude that OSM and LIF have a direct angiogenic effect. In contrast, IL-6 had no effect on corneal angiogenesis, despite its chemoattractant effect that is comparable to that of LIF and OSM. However, in some murine models, IL-6 was suggested to be angiogenic. Giraud et al. described an autocrine IL-6 secretion that induced murine endothelial cell proliferation. In contrast, in our model, neither exogenous recombinant human IL-6 nor endogenous IL-6 supported proliferation. In contrast, in our model, neither exogenous recombinant human IL-6 nor endogenous IL-6 supported macrovascular and microvascular endothelial cell proliferation (since OSM is a potent inducer of IL-6 on HUVECs).

The physiological significance of the angiogenic property of IL-6 on vascular cells is strengthened by the fact that OSM is 1 of the most potent stimulators of Kaposi sarcoma cell growth, since some authors have demonstrated that Kaposi sarcoma spindle cells can be derived from endothelial cells activated by proinflammatory cytokines. The physiological significance of the angiogenic property of LIF can be considered in embryonic genesis, since Gendron et al have demonstrated that LIF is a potent mitogen for rabbit vascular smooth muscle cells. Our results demonstrate that OSM also promotes angiogenesis in vivo and in vitro after blastocyte injection. This work was supported by grants from the Fondation de France and the Ministe`re de l’Education Nationale, de la Recherche et de la Technologie of France. The authors thank Richard Meioiros for his editorial assistance and Eliane Abdelouad for her excellent technical assistance.

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