Interleukin-8
A Mitogen and Chemotactant for Vascular Smooth Muscle Cells

Tian-Li Yue, Xinkang Wang, Cheng-Po Sung, Barbara Olson, Patrick J. McKenna, Juan-Li Gu, Giora Z. Feuerstein

Abstract Interleukin-8 (IL-8) is a chemokine produced by a variety of cell types involved in atherogenesis and is chemo-
tactic for neutrophils and lymphocytes. A recent study has shown that IL-8 is angiogenic and induces proliferation and
chemotaxis of endothelial cells. The present study was under-
taken to find out whether IL-8 is also mitogenic and chemo-
tactic for vascular smooth muscle cells. IL-8 induced a con-
centration-dependent (0.1 to 10 nmol/L) stimulation of DNA
synthesis and cell proliferation in both human and rat aortic
smooth muscle cells. In addition, IL-8 stimulated smooth
muscle cells to produce prostaglandin E2, which can inhibit
IL-8–induced smooth muscle cell proliferation. In the pre-
ence of indomethacin (5 μmol/L), IL-8 (1 nmol/L) stimulated
an increase in human and rat aortic smooth muscle cell
number during a 3-day period of incubation by 61±16% and
59±7% (n=4), respectively. IL-8 also increased DNA synthe-
sis in human and rat aortic smooth muscle cells by 98±10%
and 151±27% (n=5), respectively. Moreover, IL-8 stimulated
rat aortic smooth muscle cell migration by 20-fold over the
control value, with an EC50 value of 0.83 nmol/L; this chemo-
tactic activity of IL-8 was also potentiated by indomethacin.
Exposure of smooth muscle cells to IL-8 caused rapid and
transient expression of the immediate-early genes c-fos and
zif268 mRNA. The maximal levels of c-fos and zif268 mRNA
in human and rat aortic smooth muscle cells were observed 30
minutes and 1 hour after stimulation with IL-8, respectively,
followed by rapid decline. Moreover, IL-8 stimulated mitogen-
activated protein (MAP) kinase in smooth muscle cells with a
peak at 5 to 10 minutes after stimulation. At 1 and 10 nmol/L
IL-8, MAP kinase activity increased by 1.5- and 7-fold above
the basal level, respectively. Since vascular smooth muscle cell
proliferation and migration are crucial steps in neointimal
formation in restenosis and atherosclerosis, these results sug-
gest that IL-8 may be an important naturally occurring mito-
gen and chemotactant for vascular smooth muscle cells and
may play a role in the pathogenesis of arterial intimal thick-
ening and atherosclerosis. (Circ Res. 1994;75:1-7.)

Key Words • interleukin-8 • mitogen • chemotaxis •
smooth muscle cells • gene expression

Vascular smooth muscle is the predominant cell
type in the media of the normal mammalian
artery and as such provides the vasculature
with structural support and vasomotion. In vascular
injury, such as that following balloon angioplasty, the
vascular smooth muscle cells (VSMCs) migrate from the
media to the intima and then proliferate and secrete
excessive matrix proteins. These processes are thought
to play a pivotal role in the development of chronic
atherosclerosis and in restenosis after angioplasty of
human coronary arteries (see Reference 1 for review).
However, the mechanisms responsible for these abnor-
mal changes are still incompletely understood.

An increasing body of evidence has indicated that
cytokines such as interleukin-1 (IL-1) and tumor necro-
sis factor (TNF) may modulate local pathological pro-
cesses including atherosclerosis (see Reference 2 for
review). This hypothesis emerges from the observations
that each of the principal cell types involved in athero-
genesis both produces and responds to a variety of
cytokines. Like the growth factors, cytokines are ex-
pressed at low or undetectable levels in normal animal
or human arterial tissue but are increased after injury or
in atherosclerotic lesions.3,4 There are several examples
of an association between expression of particular cyto-
kines and lesions of atherosclerosis. They alter key
functions of vascular cells5,6 that likely contribute to the
atherogenic process at many junctures. Moreover, it has
been reported that cytokines such as IL-1 also activate
VSMCs to produce other cytokines or growth factors.7,8
Thus, cytokines derived from lesion cells may contribute
to multiple aspects of atheroma initiation, progression,
and complication.

Interleukin-8 (IL-8) is a monocyte/macrophage-de-
derived peptide that belongs to a novel cytokine family of
8- to 10-kD molecular mass. Fourteen distinct members
have currently been assigned to this subfamily.9 The
predominant producing cells for IL-8 are monocytes.
However, a variety of cells, such as endothelial cells and
fibroblasts, have been shown to produce significant
amounts of IL-8 on stimulation with various types of
cytokines or mitogens.10 It has also been reported that
IL-8 was expressed in human VSMCs when the cells
were activated by interleukin-1β (IL-1β) or TNF.7 IL-8
has chemotactic activity for neutrophils and lympho-
cytes at nanomolar and picomolar concentrations, re-
spectively. A recent study has shown that IL-8 induces
proliferation and chemotaxis of human umbilical vein
endothelial cells and is a potent angiogenic agent.11
Moreover, IL-8 shares sequence homology with several
known growth-promoting proteins that are believed to
be involved in cell proliferation. These data suggest a possible function for IL-8 in the pathogenesis of neointimal formation. Since VSMC proliferation and migration are crucial steps in the formation of intimal thickening in atherosclerotic lesions and are thought to contribute to restenosis of the arteries following angioplasty and endarterectomies, we investigated the effects of IL-8 on cultured human aortic smooth muscle cells (HASMCs) and rat aortic smooth muscle cells (RASMCs) to determine whether IL-8 is a mitogen and chemotactant for VSMCs.

Materials and Methods

Materials

The sources of the agents were as follows: human IL-8 (recombinant; PeproTech Inc; recombinant human platelet-derived growth factor (PDGF) A/B, Boehringer Mannheim; fetal bovine serum (FBS), Hyclone; myelin basic protein peptide, Upstate Biotechnology Inc; [3H]thymidine (67 Ci/mmol), New England Nuclear; and [α-32P]ATP, [γ-32P]ATP (3000 Ci/mmol), and prostaglandin (PG) E1, 125I assay kit, Amersham Corp. Dulbecco's modified Eagle's medium (DMEM) was prepared by the Media Preparation Laboratory of SmithKline Beecham with materials from GIBCO Laboratories, and the level of endotoxin in the medium was <12.5 pg/mL. Phenyl methylsulfonyl fluoride, aprotinin, sodium orthovanadate, and Nonidet P-40 were purchased from Sigma Chemical Co.

Cell Culture

RASMCs were isolated and cultured as reported previously. Briefly, RASMCs were isolated from medial explants from the thoracic aorta of male Sprague-Dawley rats 300 to 350 g (Charles River) and cultured in DMEM (GIBCO) supplemented with 10% FBS and gentamicin (50 μg/mL). Smooth muscle cells (SMCs) were allowed to grow out from the tissue, which was consequently removed. After confluence was reached, cells were harvested by brief trypsinization and subcultured in T-150 flasks or 24-well plates. The purity of the VSMCs was estimated to be >90% by cell morphology and the immunoexpression of myosin, as described previously. HASMCs as cryopreserved tertiary culture (MyoPack-AOSMC) were provided by Clonetics Corp and subcultured in smooth muscle growth medium containing human epidermal growth factor (10 ng/mL), human fibroblast growth factor (2 ng/mL), dexamethasone (0.39 μg/mL), 5% FBS, gentamicin (30 μg/mL), and amphotericin-B (50 μg/mL) in T-150 flasks. The growth medium was changed every other day until confluence was reached. Both HASMCs and RASMCs under passage 6 were used in the present study.

DNA Synthesis

DNA synthesis in SMCs was assessed by measurement of the incorporation of [3H]thymidine into the cells, as reported previously. Briefly, VSMCs were grown in 24-well plates to 70% to 80% confluence and then made quiescent with DMEM containing insulin (5 μg/mL), transferrin (5 μg/mL), and sodium selenite (5 ng/mL) for 48 hours. Unless otherwise indicated, the cells were incubated with indomethacin (5 μmol/L) for 20 minutes and then challenged with IL-8 for 24 hours. [3H]Thymidine (0.5 μCi per well) was added for a further 4-hour incubation. DNA synthesis was assessed by measuring the radioactivity incorporated into the trichloroacetic acid-insoluble fraction of the cells.

Mitogenic Assay

VSMCs were subcultured into 24-well plates to reach 60% to 70% confluence and then made quiescent, as described above. The cells were stimulated by IL-8 for 3 to 5 days in the presence of indomethacin (5 μmol/L), unless otherwise described. At the end of incubation, medium was removed, and cells were treated with 0.25 mL of 0.05% trypsin–0.53 mmol/L EDTA, harvested, and counted.

Cell Migration Assay

RASMC migration was monitored in a Transwell cell culture chamber by using a polycarbonate membrane with pores of 8 μm (Costar), as reported previously. Briefly, SMCs were suspended in DMEM supplemented with 0.2% bovine serum albumin at a concentration of 2.5 x 106 cells per milliliter. In the standard assay, 0.2 mL of cell suspension was placed in the upper compartment of the chamber. The lower compartment contained 0.6 mL of DMEM supplemented with 0.2% bovine serum albumin and IL-8 or vehicle. Incubation was at 37°C in an atmosphere of 95% air/5% CO2 for 24 hours. After incubation, nonmigrated cells on the upper surface were scraped gently, and the filters were fixed in methanol and stained with 10% Giemsa stain. The number of SMCs that had migrated to the lower surface of the filters was determined microscopically, and four high-power fields (x100) were counted per filter. Experiments were performed in duplicate or triplicate.

PGE2 Assay

HASMCs cultured in 24-well plates were challenged with IL-8 in the presence or absence of indomethacin (5 μmol/L) for 48 hours. PGE2 released from HASMCs into the medium was extracted with ethyl acetate (pH 3 to 4) and determined by use of a PGE2 125I radioimmunoassay kit (Amersham) as per the manufacturer's instructions.

Northern Blot Analysis

Total cellular RNA was prepared from serum-deprived and IL-8-stimulated VSMCs by using an acid guanidinium thiocyanate, phenol, and chloroform extraction procedure. RNA samples (10 μg per lane) were electrophoresed through formamide-agarose slab gels and transferred to GeneScreen Plus membranes (Du Pont–New England Nuclear). Northern hybridization to the cDNA probes of c-fos (a gift of R. Taub, University of Pennsylvania), c-fos17 (a gift of B.A. Christie, University of Texas Health Science Center at San Antonio), and ribosomal protein L32 cDNA (a gift of R.P. Perry, Institute for Cancer Research) was described previously. Briefly, cDNA fragments for probes were isolated by restriction enzyme digestions followed by electrophoresis. DNA probes were uniformly labeled with [α-32P]dATP by using a random-priming DNA labeling kit (Boehringer Mannheim). Hybridization was carried out overnight with 1 x 106 cpm/mL of probe at 42°C in 5 x SSPE buffer (750 mmol/L NaCl, 50 mmol/L NaH2PO4, [pH 7.6], and 5 mmol/L EDTA), 50% formamide, Denhardt’s solution, 2% sodium dodecyl sulfate (SDS), and 200 μg/mL boiled salmon sperm DNA. The membranes were washed in 2 x SSPE and 2% SDS at 65°C for 1 to 2 hours with a change every 30 minutes and then autoradiographed at ~70°C with a Cronex Lightning-Plus intensifying screen for various times, depending on the signal intensity. PhosphorImager was used to quantify the band intensities of the Northern blots, which were analyzed with IMAGEQUANT-TM Software v3.0 (Molecular Dynamics). The probes were stripped from the membranes by boiling in 10 mmol/L Tris (pH 7.5), 1 mmol/L EDTA (pH 8.0), and 1% SDS for 20 minutes before rehybridization with another probe.

Mitogen-Activated Protein Kinase Assay

RASMCs were made quiescent for 48 hours in DMEM. Fresh DMEM was added before the addition of IL-8 or vehicle, and cells were further incubated for 5 minutes or at the indicated time with IL-8. Incubation medium was decanted, and cell monolayers were washed once with cold Dulbecco’s phosphate-buffered saline containing 1 mmol/L
TABLE 1. Interleukin-8 Increases Prostaglandin E₂ Release From Cultured Human Aortic Smooth Muscle Cells

<table>
<thead>
<tr>
<th>Treatment</th>
<th>PGE₂, pg per Well</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle</td>
<td>23±2</td>
</tr>
<tr>
<td>IL-8</td>
<td></td>
</tr>
<tr>
<td>0.1 nmol/L</td>
<td>600±140</td>
</tr>
<tr>
<td>1 nmol/L</td>
<td>1325±213</td>
</tr>
<tr>
<td>1 nmol/L+indomethacin</td>
<td>20±3</td>
</tr>
<tr>
<td>1 nmol/L IL-1β</td>
<td>5400±436</td>
</tr>
</tbody>
</table>

PGE₂ indicates prostaglandin E₂; IL, interleukin. Values are mean±SEM of four samples (each sample was pooled from three wells).

Human aortic smooth muscle cells cultured in 24-well plates were pretreated with indomethacin (5 μmol/L) or vehicle for 30 minutes and then challenged with IL-8 or IL-1β for 48 hours. PGE₂ was measured as described in “Materials and Methods.”

sodium orthovanadate. Cells were lysed (20 minutes at 4°C) with lysing solution containing 20 mmol/L Tris-Cl (pH 8.0), 137 mmol/L NaCl, 10% glycerol, 1 mmol/L phenylmethylsulfonyl fluoride, 0.15 U/mL aprotinin, 1 mmol/L sodium orthovanadate, and 1% Nonidet P-40. Lysed cells were transferred to microcentrifuge tubes and centrifuged at 16,000g for 10 minutes at 4°C. Mitogen-activated protein (MAP) kinase activity was measured according to the method of Koide et al. with modification. Briefly, assay (total volume, 30 μL) containing 10 μL of the sample to be assayed plus 10 μL of the assay cocktail consisted of 50 mmol/L β-glycerophosphate (pH 7.5), 10 mmol/L magnesium acetate, 1 mmol/L dithiothreitol, 1.5 mmol/L EGTA, 60 μmol/L ATP, and 1 μg (γ-32P)ATP. The assay was initiated by the addition of substrate, myelin basic protein peptide (sequence ARTPGGR, which includes amino acids 95-98 of bovine myelin basic protein; final concentration, 1 mmol/L), and terminated after 20 minutes in room temperature by spotting 25 μL onto P-81 phosphocellulose paper inside the scintillation vial. The paper was washed five times with 4 mL of 180 mmol/L phosphoric acid and radioactivity-counted.

Data Analysis

Statistical analysis was performed by Dunnett’s test21 with a program provided by MicroComputer Specialists. A value of P<.05 was considered to be significant.

Results

IL-8 Stimulates DNA Synthesis and Cell Proliferation in VSMCs

Since it has been reported that PGs produced by stimulated VSMCs can inhibit the proliferative response of these cells to PDGF22 or IL-1β3, we first examined whether IL-8 stimulated VSMCs to produce PGE₂. The results shown in Table 1 indicate that IL-8 stimulated HASMCS to produce PGE₂. Therefore, we performed the experiments by preincubating cells with 5 μmol/L indomethacin for 20 minutes, which completely blocks the synthesis of PGE₂, before the addition of IL-8. Indomethacin itself did not affect cell proliferation or DNA synthesis (data not shown) in VSMCs.

IL-8 induced a concentration-dependent stimulation of cell proliferation after a 3- to 5-day period of incubation as shown in Fig 1. In the presence of 5 μmol/L indomethacin, the maximal increases in HASMC and RASMC number induced by 1 nmol/L IL-8 were 61±16% (n=4) and 59±7% (n=4) above the control value, respectively. Under the same conditions, IL-1β (10 nmol/L)–induced increases in HASMC and RASMC proliferation were 94±30% (n=3) and 96±24% (n=5) over the control value, respectively. In the absence of indomethacin, the responses of the VSMCs to IL-8 were significantly reduced and quite variable (Fig 1).

IL-8 also stimulated [3H]thymidine incorporation into both HASMCs and RASMCs in a concentration-dependent manner, as shown in Fig 2. The maximal increase in DNA synthesis over the control value was...
98±10% (n=5) at 10 nmol/L IL-8 in HASMCs and 151±27% (n=5) at 1 nmol/L IL-8 in RASMCs. Under the same conditions, the maximal increases in DNA synthesis induced by IL-1β (10 nmol/L) in HASMCs and RASMCs were 219±30% (n=3) and 193±29% (n=6), respectively.

**IL-8 Stimulates RASMC Migration**

As shown in Fig 3, IL-8 stimulated RASMC migration in a time- and concentration-dependent manner, with an EC₅₀ value of 0.83 nmol/L. The maximal stimulatory effect was observed at ~1 to 10 nmol/L IL-8, and the number of migrated SMCs was 20-fold higher than the basal level. In the presence of 5 μmol/L indomethacin, the stimulatory effect of IL-8 on SMC migration was potentiated, whereas indomethacin itself did not affect SMC migration, as shown in Fig 3. IL-8–induced VSMC migration was also inhibited by the direct addition of PGE₂ to the cells. At 0.1 and 1 nmol/L PGE₂, 1 nmol/L IL-8–induced cell migration was reduced by 67±3.2% and 92±5.2%, respectively (data not shown). Table 2 compares the maximal migration of RASMCs in response to a variety of VSMC mitogens. The value of maximal cell migration for each mitogen was obtained from a concentration-response curve over a 24-hour incubation period (data not shown). When IL-8 (1 nmol/L) was added to the upper chamber or both chambers (upper and lower chamber), the number of the migrated RASMCs was only 3±2 and 4±2 (n=3), respectively, indicating that the response of SMCs to IL-8 was chemotactic in nature.

**IL-8–Induced mRNA Expression of Immediate-Early Genes c-fos and zif268**

Two critical immediate-early genes, c-fos and zif268, were selected to monitor the IL-8–induced cellular proliferation of SMCs. The mRNA levels of these two genes in unstimulated RASMCs were almost undetectable and rapidly induced by IL-8, as shown in Fig 4. The maximal levels of c-fos and zif268 mRNAs were obtained.

**TABLE 2. Comparison of Maximal Rat Aortic Smooth Muscle Cell Migration Induced by Interleukin-8 and Other Mitogens**

<table>
<thead>
<tr>
<th>Agent</th>
<th>Maximal RASMC Migration, Cells per 4 HPF</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle</td>
<td>3±2</td>
</tr>
<tr>
<td>IL-8</td>
<td>59±7</td>
</tr>
<tr>
<td>Ang II</td>
<td>12±3</td>
</tr>
<tr>
<td>α-Thrombin</td>
<td>30±4</td>
</tr>
<tr>
<td>ET-1</td>
<td>15±2</td>
</tr>
<tr>
<td>ET-3</td>
<td>9±1</td>
</tr>
<tr>
<td>PDGF A/B</td>
<td>243±10</td>
</tr>
</tbody>
</table>

RASMC indicates rat aortic smooth muscle cell; HPF, high-power field; IL, interleukin; ET, endothelin; and PDGF, platelet-derived growth factor. Values are mean±SEM of three to five experiments performed in duplicate. The maximal effect was obtained from the dose-response curve of each mitogen (four doses, at least, were used for each agent). The concentrations for inducing maximal RASMC migration were as follows: PDGF, 1 nmol/L; Ang II, 10 nmol/L; α-thrombin, ET-1, and ET-3, 100 nmol/L; and IL-8, 10 nmol/L.
Fig 4. Northern analysis (A) and graph (B) showing quiescent and interleukin-8 (IL-8)–stimulated rat aortic smooth muscle cells (RASMCs). A, RASMCs were made quiescent for 48 hours and stimulated with 1 nmol/L IL-8 for vehicle for the indicated times. Total cellular RNA was extracted, resolved (10 µg per lane) by electrophoresis, transferred to a nylon membrane, and hybridized sequentially to c-fos, Zif268, and ribosomal protein L32 (rpl 32) cDNA probes as described in "Materials and Methods." The mRNA size was determined by comparison with the migration of the RNA ladder (GIBCO BRL) and marked on the right. B, The relative mRNA levels of the Northern blot were quantified by PhosphorImager analysis, and the mRNA signals were displayed graphically. The amount of RNA loaded for each lane was normalized by rpl 32 mRNA. Each probe has a sum value of 100%.

erved 30 minutes and 1 hour, respectively, after addition of the cytokine and then declined rapidly. Results very similar to those in RASMCs were observed in HASMCs (data not shown). Fig 5 shows a comparison of IL-8–induced, IL-1β–induced, and PDGF-induced c-fos and Zif268 mRNA expression in RASMCs under the same conditions.

Effect of IL-8 on the Activity of MAP Kinases in RASMCs

On stimulation with IL-8, the MAP kinase activity was markedly increased in the extracts of RASMCs. As shown in Fig 6A, IL-8–stimulated MAP kinase was detectable as early as 2 minutes, peaked at 5 to 10 minutes, and then declined. The dose-response relation for MAP kinase activation elicited by IL-8 is shown in Fig 6B. At 1 and 10 nmol/L IL-8, MAP kinase activity increased by ≈1.5- and 7-fold, respectively.

Discussion

Our results show that IL-8 is mitogenic for both HASMCs and RASMCs. The responses of the cells to IL-8 were quite variable in the absence of indomethacin, suggesting that IL-8–stimulated SMCs might release factors that modulate the proliferative effect of IL-8 on SMCs. It was previously reported that PGE₂ inhibited PDGF-induced VSMC proliferation23 and that IL-1β stimulated VSMCs to produce PGs, which masked the mitogenic effect of IL-1β. We have also observed in the present study that IL-8 stimulated VSMCs to produce PGE₂, as shown in Table 1. On the basis of the above information, we studied the effects of IL-8 on VSMCs in the presence of indomethacin. Under this condition, IL-8 showed a clear stimulatory effect on both HASMCs and RASMCs and increased DNA synthesis and cell proliferation. The effective concentration of IL-8 was >0.1 nmol/L, and the maximal stimulation was observed at 1 to 10 nmol/L. The IL-8–induced maximal stimulation of cell proliferation in both HASMCs and RASMCs approximated 60% of
the maximal increase stimulated by IL-1β. IL-8-induced maximal increase in DNA synthesis in VSMCs was also ≈40% to 60% of IL-1β-induced maximal increase. These results suggest that IL-8 is a somewhat less potent mitogen compared with IL-1β.

VSMC migration from the media to the intima is a key step in the development of intimal lesions, but the factors that control migration or of their biochemical basis are poorly understood. The present data indicate that IL-8 is a chemoattractant for RASMCs, which is consistent with observations in HASMCs and more effective than all tested mitogens in the present study except PDGF. It is interesting to find that IL-8–induced VSMC migration was also potentiated by indomethacin. Moreover, a direct inhibition of IL-8–stimulated VSMC migration by PGE2 was observed. We have also observed that PDGF (1 nmol/L)–induced SMC migration was inhibited by PGE2 and PGF2α, with IC50 values at nanomolar levels (authors’ unpublished data).

Since the lipoxxygenase-mediated eicosanoid metabolites, such as 12-hydroxyeicosatetraenoic acid and leukotriene B4, were found to stimulate SMC migration mildly, it is possible that indomethacin also augmented IL-8–induced VSMC migration via increasing the production of lipoxxygenase metabolites by shifting eicosanoid metabolism from the cyclooxygenase pathway to the lipoxxygenase pathway. In view of the inhibitory effects of PGs on both VSMC proliferation and migration, our data suggest that the production of PGs could be an important regulatory mechanism in vivo to limit uncontrolled cell proliferation and migration in response to a variety of mitogens and chemoattractants of VSMCs. Moreover, this could be the reason, at least in part, for the failure of cyclooxygenase inhibitors such as aspirin to prevent restenosis in animal models as well as in humans.

Immediate-early gene induction is a common occurrence after mitogen stimulation of quiescent VSMCs in culture and has also been demonstrated in vivo models of vascular injury. c-fos has been widely used as a representative of immediate-early proto-oncogene, and zif268 is the first example of a growth-regulated gene encoding a zinc finger protein. Both c-fos and zif268 proteins are likely to be localized in the nucleus and serve as transcriptional regulators in cellular proliferation. The maximal levels of c-fos and zif268 mRNA were observed 30 minutes and 1 hour, respectively, after the addition of IL-8, and the kinetics of both genes induced by IL-8 coincide with those stimulated with IL-1β and oncostatin M in VSMCs. The ability of IL-8 to rapidly increase c-fos and zif268 levels in both HASMCs and RASMCs provides further evidence that IL-8 may trigger early events in the commitment process for cell growth. As shown in Fig 5, the potency of IL-8 in modulating both c-fos and zif268 message levels was lower than that of PDGF or IL-1β; this finding also coincides with the results observed in promoting cell proliferation by IL-8.

Protein-tyrosine phosphorylation has been considered to be involved in cell proliferation, because several oncogene products and a number of growth factor receptors possess a tyrosine kinase activity. MAP kinase, a serine/threonine-specific protein kinase, has been found to be activated in many proliferative cells in response to mitogenic stimulation by various growth factors or oncogenes and is one of the most widely studied tyrosine-phosphorylated proteins. Recently, MAP kinase has been shown to phosphorylate in vitro the products of the proto-oncogenes c-fos, c-jun, and c-myc. Since IL-8 has been shown in the present study to stimulate VSMC proliferation and the expression of the proto-oncogenes, we were interested in exploring whether MAP kinase is involved in IL-8–stimulated VSMCs. As shown in Fig 6, IL-8 at 1 and 10 nmol/L stimulated MAP kinase activity. The activation was detectable as early as 2 minutes, peaked at 5 to 10 minutes, and declined to nearly the basal level 30 minutes after stimulation. MAP kinase was activated by IL-8 in a concentration-dependent manner (Fig 6); however, the maximal effect was shown at an IL-8 concentration of 10 nmol/L, which was higher than that required for stimulating cell proliferation and migration. The exact reason for this difference is not known at present. The possibility is that even the modest extent of MAP kinase activation by IL-8 at low concentrations is sufficient to induce the maximal increase in cell proliferation and migration. The peptide APRTPGGRR, which constitutes a MAP kinase phosphorylation site in myelin basic protein, was used as a substrate for MAP kinase assay in the present study. This peptide is now used more frequently by investigators in the field because of its apparent specificity over myelin basic protein. However, there is no published data so far suggesting it to be absolutely specific for MAP kinase. Whether other serine/threonine kinases can be activated by IL-8 and also phosphorylate the myelin basic protein peptide is not known. This possibility cannot be completely eliminated at the present time. MAP kinase is considered to play an important role as an intermediate in the signaling pathways from receptor to the ribosomes and nucleus. However, the precise role of IL-8–induced MAP kinase activation in signal transduction in VSMCs remains to be clarified until the IL-8 receptor in VSMCs is characterized and specific inhibitors of MAP kinase become available.

In summary, IL-8 is a mitogen and chemoattractant for VSMCs. IL-8 stimulates VSMCs to release PGs that modulate both VSMC proliferation and migration. IL-8 rapidly and transiently induces proto-oncogene c-fos and zif268 expression and activates MAP kinase in VSMCs. These results suggest that IL-8 may be an important naturally occurring mitogen and chemoattractant for VSMCs and that it plays a role in arterial intimal thickening.

References


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