Endogenous VEGF-A is responsible for mitogenic effects of MCP-1 on vascular smooth muscle cells

Astrid Parenti, Lydia Bellik, Laura Brogelli, Sandra Filippi, and Fabrizio Ledda

Laboratory of Vascular Pharmacology, Department of Preclinical and Clinical Pharmacology, University of Florence, 50139 Florence, Italy

Submitted 5 May 2003; accepted in final form 16 December 2003

Vessel wall remodeling is a complex phenomenon in which the loss of differentiation of vascular smooth muscle cells (VSMCs) occurs. We investigated the role of rat macrophage chemoattractant protein (MCP)-1 on rat VSMC proliferation and migration to identify the mechanism(s) involved in this kind of activity. Exposure to very low concentrations (1–100 pg/ml) of rat MCP-1 induced a significant proliferation of cultured rat VSMCs assessed as cell duplication by the counting of total cells after exposure to test substances. MCP-1 stimulated VSMC proliferation and migration in a two-dimensional lateral sheet migration of adherent cells in culture. Endogenous vascular endothelial growth factor-A (VEGF-A) was responsible for the mitogenic activity of MCP-1, because neutralizing anti-VEGF-A antibody inhibited cell proliferation in response to MCP-1. On the contrary, neutralizing anti-fibroblast growth factor-2 and anti-platelet-derived growth factor-bb antibodies did not affect VSMC proliferation induced by MCP-1. RT-PCR and Western blot analyses showed an increased expression of either mRNA or VEGF-A protein after MCP-1 activation (10–100 pg/ml), whereas no fms-like tyrosine kinase (Flt)-1 receptor upregulation was observed. Because we have previously demonstrated that hypoxia (3% O₂) can enhance VSMC proliferation induced by VEGF-A through Flt-1 receptor upregulation, the effects of hypoxia on the response of VSMCs to MCP-1 were investigated. Severe hypoxia (3% O₂) potentiated the growth-promoting effect of MCP-1, which was able to significantly induce cell proliferation even at a concentration as low as 0.1 pg/ml. These findings demonstrate that low concentrations of rat MCP-1 can directly promote rat VSMC proliferation and migration through the autocrine production of VEGF-A.

monocyte chemotactic protein-1; endogenous vascular endothelial growth factor-A; remodeling; autocrine proliferation; chemokine

Address for reprint requests and other correspondence: F. Ledda, Dept. of Preclinical and Clinical Pharmacology, Univ. of Florence, Viale G. Pieraccini, 6, 50139 Florence, Italy (E-mail: ledda@pharm.unifi.it).

Methods

Animals. This investigation conforms with the Guide for the Care and Use of Laboratory Animals [published by National Institutes of Health (NIH Publication No. 85-23, Revised 1996)]. Male Wistar rats (body wt, 220–250 g) were purchased from Harlan (S. Pietro al Natisone, Italy).

Cell culture and proliferation. VSMCs were isolated from the thoracic aorta of male Wistar rats and cultured as previously described (24). Cell proliferation was quantified by the total cell number as previously reported (24). Briefly, 5 × 10⁵ cells in 5% bovine calf serum (BCS) were seeded onto 48-well plates and allowed to adhere overnight. Cells were then kept in starving conditions (0.1% BCS) for 48 h, and media were successively replaced with 1% BCS medium (DMEM) that contained rat MCP-1 (1–100 pg/ml). After 96 h, cells were fixed with methanol and stained with Diff-Quick. Proliferation was evaluated as cell duplication by counting the number of cells in 10 random fields of each well at ×200 magnification with the aid of a 21-mm² ocular grid. In the experiments in which the effect of hypoxia was assessed, cells were exposed to severe hypoxia (3% O₂) during the 48-h
Neuroprobe was used for chemotaxis studies. Polyvinyl-pyrrolidone-cell migration was measured by microscopic evaluation of the number of cells in the absence of any stimulus was taken as basal. Fbb, 5 ng/ml) was chosen as a positive control, and the effect of 1% BCS medium was investigated by using an experimental model that allows chem-Novabiochem (San Diego, CA). DMEM, penicillin-streptomycin-glutamine solution, and trypsin-EDTA solution were purchased from Life Technologies, Inc. (1-105 cells in 50 μl) were seeded into the upper wells of the chamber, which was inserted at 37°C for 4 h. Platelet-derived growth factor-bb (PDGF-BB, 5 ng/ml) was chosen as a positive control, and the effect of 1% BCS medium was investigated by using a silicon-template fencing technique as previously reported (23).

Two-dimensional lateral sheet migration and proliferation of adherent cells in culture. Two-dimensional lateral sheet migration and proliferation of adherent cells in culture were evaluated by using a silicon-template fencing technique as previously reported (24). Briefly, cells in 10% BCS were seeded onto a rectangular silicon gasket that was inserted in the wells of six-well cell culture dishes and allowed to reach confluence. The gasket was then removed, confluent cell monolayers were washed with medium, and four edges of the rectangular cell monolayers were marked with a scalpel on the outside of the tissue culture dish to define the starting line of cell progression. MCP-1 (1% BCS medium) was added, and the experiment was stopped after 3 days. Cell migration and proliferation were quantified by 1) microscopically measuring the distance of migrated cells from the starting lines to the migration front (the farthest cells with an aid of an ocular grid (225 × 225 μm = 1 grid unit), and 2) counting the total number of cells in each grid unit according to a method described elsewhere (23).

Immunoprecipitation and immunoblotting. VSMCs were lysed in lysis buffer before being centrifuged at 14,000 g for 10 min at 4°C as previously reported (24). Aliquots (50 μg) of total proteins were used to immunoprecipitate VEGF-A with a monoclonal mouse IgG antibody (Santa Cruz Biotech). The immunoprecipitates were run on 10% SDS-PAGE gels. Proteins were then transferred to a polyvinylidene difluoride (PVDF) membrane and treated with the VEGF-A antibody. Immunoreactive proteins were detected by enhanced chemiluminescence (ECL).

Differential RT-PCR analysis. A competitor technology for quantitative RT-PCR (QuantumRNA Universal 18S; Ambion; Austin, TX) was used as previously described (25). Briefly, total RNA was extracted using the PureScript RNA isolation kit (Genta; Minneapolis, MN). Reverse transcription of 1 μg of total RNA was carried out, and VEGF-A was amplified with specific primers designed to detect the four distinct VEGF-A isoforms (VEGF121, VEGF145, VEGF165, and VEGF189; Ref. 25) in sequential cycles (35) including 30 s of denaturation at 94°C, 30 s of annealing conditions at 60°C, and 30 s of extension at 72°C. The Flt-1 receptor was amplified with specific primers in sequential cycles (35) including 30 s of denaturation at 94°C, 30 s of annealing conditions at 55°C, and 30 s of extension at 72°C. Primers and competimers to amplify the 18S rRNA were chosen as the internal standard (optimal 18S primer-competimer dilution ratios were 2:8 and 3:7 for VEGF-A and Flt-1, respectively; Ref. 25). Amplified proteins were electrophoresed in 3% agarose gel, and quantitative evaluations were obtained as ratios between the optical density of the target genes and the 18S rRNA amplification products.

Materials. Rat MCP-1 and PDGF-BB were purchased from Calbiochem-Novabiochem (San Diego, CA). DMEM, penicillin-streptomycin-cin-glutamine solution, and trypsin-EDTA solution were purchased from Life Technologies (St. Louis, MO). BCS was purchased from Hyclone (Logan, UT). Rabbit anti-VEGF-A and rabbit anti-PDGF-BB polyclonal antibodies (neutralizing) were from Preprotech (EC; London, UK), mouse anti-fibroblast growth factor (FGF)-2 monoclonal antibody clone bFM-1 (neutralizing) was from Upstate Biotechnology (Lake Placid, NY). Acrylamide, N,N',N'-tetramethylethylenediamine, ammonium persulfate, and Coomassie brilliant blue were from Bio-Rad Laboratories (Richmond, CA). Cells were grown on sterile plastic (Costar Europe).

Statistical evaluation. Data are reported as means ± SE. Each experiment was run in duplicate or triplicate. Statistical analysis was performed using Student’s t-test for unpaired data. P < 0.05 was considered significant.

RESULTS

Effects of MCP-1 on VSMC proliferation. Serum-starved rat VSMCs were stimulated with increasing concentrations of rat MCP-1 (0.1–100 pg/ml) and left in culture for 4 days. Proliferation was assessed as the total number of cells counted in each well. PDGF-BB (5 ng/ml) was chosen as a positive control owing to its known mitogenic effect on VSMCs. MCP-1 induced VSMC proliferation in concentrations as low as 1 pg/ml (27 ± 10% over basal proliferation, n = 5), as shown in Fig. 1. The maximum stimulating effect, which was observed at 100 pg/ml, amounted to 47 ± 6% over basal proliferation and ~80% of the effect obtained with PDGF-BB, which increased proliferation by ~83%. The increase in MCP-1 concentration to 1 ng/ml did not induce any additional stimulation of VSMC proliferation (data not shown).

Effects of MCP-1 on VSMC migration. The ability of MCP-1 to stimulate either chemotaxis of single cells in suspension or migration of cultured cells was assessed. It was observed that MCP-1, when used at the same concentrations tested on cell growth assays, slightly stimulated VSMC chemotaxis in a statistically insignificant manner (data not shown). Therefore, the effects of MCP-1 on anchorage-dependent cell movement and proliferation were investigated by using an experimental model that allows measurement of the combination of migration and proliferation of adherent cells in culture. Under control conditions (1% BCS), cell migration progressed up to 388 ± 24 μm from the starting line; this distance was almost tripled by the addition of PDGF-BB (912 ± 70 μm), which was taken as a positive control. MCP-1, at concentrations ranging from 1 to 100 pg/ml, stimulated the progression of a significant number of cells from the starting line toward the periphery with maximal effects observed at 10 pg/ml (Fig. 2, A and D). Under these conditions, the distance covered by the cell

![Fig. 1. Effects of rat macrophage chemoattractant protein (MCP-1) (0.1–100 pg/ml) on vascular smooth muscle cell (VSMC) proliferation assessed as cell duplication and expressed as number of cells counted per well. Bars represent means ± SE of 6 experiments in duplicate. **P < 0.001 vs. basal proliferation [5% bovine calf serum (BCS), 5,170 ± 127 cells]. Platelet-derived growth factor-bb (PDGF-BB, 5 ng/ml) is reported as positive control.](http://ajpheart.physiology.org/Downloadedfrom/10220.33.5)
sprouting from the edge increased by 43% over control unstimulated cells; this distance amounted to ~60% of the value recorded with PDGFββ. MCP-1 (10 pg/ml) also significantly increased the total number of cells compared with control unstimulated cells (Fig. 2, B and D), thus showing that proliferation plays a role in this experimental model.

**Effects of anti-VEGF-A antibody on VSMC growth in culture.** Because we have previously shown that rat VSMCs may be responsive to VEGF-A (25), and because it is known that VSMCs produce this growth factor (21, 32), we investigated whether a cooperation between MCP-1 and endogenous VEGF-A exists. The results of this study showed that the growth-promoting effect of MCP-1 on VSMCs was mediated by endogenous VEGF-A, because the addition of a neutralizing VEGF-A antibody (1 μg/ml) significantly inhibited VSMC proliferation induced by all of the MCP-1 concentrations, whereas nonimmune IgG was ineffective. Moreover, the antibody did not affect basal proliferation (Fig. 3A). The possible role of other endogenous mitogens such as FGF-2 and PDGFββ were then assessed, as it is known that these growth factors may stimulate VSMC growth in an autocrine manner (20, 24, 26). The neutralizing anti-FGF-2 antibody did not influence the proliferative effect of MCP-1 on VSMCs as shown in Fig. 3B. In the same manner, the block of endogenous PDGFββ did not influence cell proliferation in response to MCP-1 (data not shown).

**Effects of MCP-1 on VEGF-A and Flt-1 receptor production.** We tested the hypothesis that MCP-1-induced VSMC proliferation was associated with VEGF-A upregulation. Serum-starved cells were stimulated with MCP-1 (10–100 pg/ml), and VEGF-A production was measured as either mRNA or protein. Immunoblotting of unstimulated rat VSMCs showed a double band for VEGF-A (nonreducing conditions) located at 46 kDa, which is higher than the human recombinant one as previously detected by Zheng et al. (36). The addition of MCP-1 (10–100 pg/ml) to starved VSMCs significantly increased VEGF-A production. The increase was maximal after 8 h of exposure and remained high after overnight incubation (Fig. 4).

To assess whether VEGF-A upregulation by MCP-1 reflected an increase in the steady-state levels of VEGF-A mRNA, differential RT-PCR analysis was performed. As shown in Fig. 5A, 6 h of exposure to MCP-1 (10–100 pg/ml) significantly increased all VEGF-A mRNA isoforms. The effect was particularly evident for the VEGF145 and VEGF165 isoforms, which are almost undetectable in unstimulated cells.
Because we previously demonstrated that VEGF-A stimulates VSMCs by acting through Flt-1 receptors (25), the expression of these receptors was also measured in response to MCP-1. MCP-1 did not increase mRNA Flt-1 expression either after a 6-h stimulation (Fig. 5B) or after overnight stimulation (data not shown).

**Effects of hypoxia on VSMC proliferation in response to MCP-1.** The data reported above show that MCP-1 proliferation is partially due to VEGF-A upregulation in VSMCs. Because we recently demonstrated that VEGF-A is able to increase VSMC mitogenesis after severe hypoxia through an upregulation of Flt-1 receptor (25), the effects of hypoxia on MCP-1-induced mitogenesis of VSMCs were investigated. When cells were exposed for 48 h to severe hypoxia (3% O2) and then stimulated with increasing concentrations of MCP-1, potentiation of VSMC proliferation was observed. In fact, the chemokine induced statistically significant cell proliferation under these experimental conditions at concentrations as low as 0.1 pg/ml (31% over basal proliferation; n = 5; Fig. 6).

**DISCUSSION**

This study focuses on the still-debated problem of mitogenic activity of MCP-1 on VSMCs and demonstrates that this chemokine promotes in vitro proliferation of rat VSMCs at very low concentrations (picograms per milliliter) on the condition that species specificity is respected. In addition, it shows that direct activity of MCP-1 on VSMCs promotes VEGF production and that inactivation of this endogenous factor inhibits MCP-1-induced effects.

MCP-1 was previously shown by different authors to either stimulate or inhibit VSMC growth; these findings were obtained by using human MCP-1 on rat (12, 27) or rabbit (34)
VSMCs. More recently, Selzman et al. (31) have stressed the importance of species specificity for MCP-1 activity on VSMC proliferation in experiments where human recombinant MCP-1 was used to stimulate human VSMC growth. The discrepancy between the results obtained in previous studies and those observed in the present work are probably due to differences in experimental planning because species homology was respected in our study in which rat VSMCs were stimulated with rat MCP-1. A growth-promoting effect on rat VSMCs was previously observed by Porreca et al. (27) only in the presence of very high human-MCP-1 concentrations (>100 ng/ml). This observation suggests that differences may exist between rat and human CC chemokine receptor-2 (CCR2). Although human VSMCs were recently demonstrated to possess CCR2 receptors (11), no information has yet been reported regarding CCR2 receptors and rat VSMCs. Moreover, it has been suggested (29) that the receptor of human VSMCs may be different from that of monocytes. The low concentrations of MCP-1 found to be effective in our study seem to be physiologically relevant, because it was recently reported that the MCP-1 concentration increases to nearly 100 pg/ml in injured vessels (8).

VSMC proliferation and migration are key events during pathological vessel-wall remodeling. Expansive remodeling, with lumen enlargement, also occurs during arteriogenesis, which is a process that compensates for a persistent insufficiency by increasing collateral flow (5). MCP-1 is well known to be implicated in all of these processes, and in vivo studies (15) have shown that vessel walls express MCP-1 within 4 h of endothelium removal. MCP-1 is also secreted by VSMCs in atherosclerotic plaques as well as by endothelial cells and macrophages; this suggests that MCP-1 may contribute to the pathogenesis of atherosclerosis (30). Moreover, MCP-1 has been colocalized in both resident and inflammatory cells in plaque (17). In several vascular experimental models, antagonism of MCP-1 or its receptor CCR2 appears to inhibit lesion development (3, 8). Aside from its putative role in atherosclerosis, MCP-1 represents a potent stimulus for arteriogenesis: it has been demonstrated (13) that MCP-1 is able to increase collateral vessel development after arterial occlusion in experimental models in vivo. However, although MCP-1 has arteriogenic properties (28), its beneficial potential in the course of ischemia may be hampered by its inflammatory and profibrotic effects. MCP-1 activity is attributed to its effect on mononuclear cells, which are recruited at the site of injury where they become macrophages that are able to secrete numerous cytokines and growth factors (28). Nevertheless, on the basis of recent experimental findings including the present one, a direct, monocyte-independent effect of MCP-1 on VSMC growth can be suggested. In fact, present data demonstrate that MCP-1 is able to promote migration and/or proliferation of cultured VSMCs in a bidimensional lateral-sheet migration model (1, 23). Chemotaxis of VSMCs was previously found to be unaffected by MCP-1 (8). Our present data obtained with a modified Boyden chamber are in agreement with those of Furukawa et al. (8), in that no significant VSMC chemotaxis was detectable even in the presence of MCP-1 concentrations that are able to stimulate VSMC growth. However, it is noteworthy that we were able to demonstrate activity of MCP-1 on VSMCs by using an experimental model that allows measurement of the combination of migration and proliferation of adherent cells in culture. In fact, growth-arrested cells cultured in silicon gaskets responded to MCP-1 by forming a significant sprouting when the gasket was removed. This was a potent effect, because the maximum activity was produced by 10 pg/ml MCP-1. On the basis of these findings, we suggest that MCP-1 can directly activate VSMCs and thus induce both proliferation and migration; it can be speculated that this mechanism, in association with the well-known monocyte recruitment effect, is involved in vascular wall remodeling. However, the major finding of the present study consists of the original observation that the growth-promoting effect of MCP-1 is mediated by the production of endogenous VEGF-A. During vascular remodeling, VEGF-A is secreted by many cell types and is involved in the modulation of angiogenesis and re-endothelialization of injured vessels (7, 16). The strong and specific activity on endothelial cells displayed by VEGF-A has led to a proposal (19) that this growth factor can be considered a therapeutic agent for inducing angiogenesis in hypoxic tissues of patients suffering from coronary artery and peripheral vascular diseases. We recently demonstrated (25) that experimental conditions mimicking vascular injury increase the responsiveness of VSMCs to VEGF-A. Results from the present study clearly show that the proliferative effect of MCP-1 is partially dependent on endogenous VEGF A production, because a VEGF-A-neutralizing antibody was able to significantly inhibit VSMC proliferation. In agreement with this observation, an upregulation of this growth factor, measured either as mRNA or protein, was observed in VSMCs exposed to MCP-1 at picogram-per-milliliter concentrations. On the contrary, an upregulation of Flt-1 was not observed, which thus excludes the possibility that MCP-1 effects may be linked to upregulation of VEGF receptors in VSMCs. Also the demonstration that significant cell stimulation was detected only in experiments on adherent cells in culture is in line with our hypothesis that the effects of MCP-1 are mediated by endogenous VEGF-A production. In fact, it can be expected that the 4-h incubation period of cell chemotaxis experiments may be inadequate to induce a significant VEGF-A upregulation. Conversely, a significant production of endogenous factor can be expected in the three-day
observation period used in the experiments on two-dimen-
sional lateral-sheet migration of adherent cells in culture.

In the present study, the hypothesis that the proliferative effects of MCP-1 may be mediated by endogenous FGF-2 and PDGFbb was also tested, because it is well known that these growth factors have a prominent role in VSMC prolif-
eration (20, 24, 26). It was found that unlike endogenous VEGF-A, neither FGF-2 nor PDGFbb were involved in the growth-promoting effects of MCP-1, in that the neutralizing anti-FGF-2 and anti-PDGFbb antibodies did not modify the effects of the chemokine. Finally, potentiation of the cell growth-promoting effects induced by MCP-1 was observed in cells previously exposed to severe hypoxia. This finding reinforces the conclusion reached in this study; we have recently shown (25) that exposure to hypoxia induces up-
regulation of the VEGF receptor Flt-1 in VSMCs. Thus it is conceivable that a condition that increases the cell respon-
siveness to VEGF-A could enhance the proliferative re-
sponse to an agent such as MCP-1, which acts through
VEGF-A secretion.

In conclusion, all of these observations support the hypoth-
thesis that MCP-1 exerts direct monocyte-activity-independent
stimulating effects on VSMCs, and that these effects are
mediated by the induction of VEGF-A, which in turn is
responsible for an autocrine-paracrine stimulation of VSMC
function by interaction with VEGF-A receptors. Moreover, the
demonstration that MCP-1 potently activates VSMC prolif-
eration and reorganization via endogenous VEGF-A production
adds to our knowledge on the role of the chemokine in vascular
remodeling and reinforces the hypothesis that autocrine regu-
lation of VSMC functions may be involved in pathological
processes characterized by vascular remodeling.

GRANTS

This work is supported by a grant from the Italian Ministry of University and Scientific and Technological Research (to F. Ledda).

REFERENCES

1. Augustin-Voss HG and Pauli BU. Quantitative analysis of autocrine-
regulated, matrix-induced, and tumor cell-stimulated endothelial cell mi-
gration using a silicon template compartmentalization technique. Exp Cell

Expression of intercellular adhesion molecule-1 on rat cardiac myocytes
1994.

3. Boring L, Gosling J, Cleary M, and Charo IF. Decreased lesion
formation in CCR2/−/− mice reveals a role for chemokines in the

Gorlach A. Thrombin-induced MCP-1 expression involves activation of
the p21-goh-containing NADPH oxidase in human vascular smooth muscle

5. Buschmann I and Schaper W. The physiopathology of the collateral

Kreuzer J. Expression of monocyte chemotactrant protein-1 cDNA in
vascular smooth muscle cells: induction of the synthetic phenotype: a
possible clue to SMC differentiation in the process of arteriogenesis.

7. Ferrara N and Davis-Smyth T. The biology of vascular endothelial

8. Furukawa Y, Matsumori A, Ohashi N, Shiot T, Ono K, Harada A,
Matsushima K, and Sasayama S. Anti-monocyte chemotactrant pro-
tein-1/monocyte chemotactic and activating factor antibody inhibits neo-
imal hyperplasia in injured rat carotid arteries. *Circ Res* 84: 306–314,
1999.

9. Galindo M, Santiago B, Alcami J, Rivero M, Martin-Serrano J, and
Pablos JL. Hypoxia induces expression of the chemokines monocyte
chemoattractant protein-1 and IL-8 in human dermal fibroblasts. *Clin Exp


Roach AG, Westwick J, and Williams RJ. Human vascular smooth muscle
cells express receptors for CC chemokines. *Arterioscler Thromb Vasc

Monocyte chemotactrant protein-1 inhibits growth of rat vascular

Monocyte chemotactic protein-1 increases collateral and peripheral
conductance after femoral artery occlusion. *Circ Res* 80: 829–837,
1997.

autocrine growth factors modulate vascular smooth muscle cell growth

15. Landry DB, Cooper LL, Bryant SR, and Lindner V. Activation of the
NF-kappaB and IkappaB system in smooth muscle cells after rat arterial
injury. Induction of vascular cell adhesion molecule-1 and monocyte

16. Lee SH, Wolf PL, Escudero R, Deutsch R, Jamieson SW, and This-
thelhoit AE. Early expression of angiogenesis factors in acute myocard-

17. Martin-Ventura JL, Ortego M, Esprit P, Hernandez-Presa MA, Or-
tega I, and Egido J. Possible role of parathyroid hormone-related protein
as a proinflammatory cytokine in cytosclerosis. *Stroke* 34: 1783–1789,
2003.

18. Marumo T, Schini-Kerth VB, and Busse R. Vascular endothelial growth
factor activates nuclear factor-kappaB and induces monocyte chemotact-
rant protein-1 in bovine retinal endothelial cells. *Diabetes* 48: 1131–
1137, 1999.

19. Melillo G, Scociatti M, Kovesdi I, Safi J Jr, Riccioni T, and Cape-
grossi MC. Gene therapy for collateral vessel development. *Cardiovasc

20. Mikhail N, Fukuda N, Tremblay J, and Hamet P. Effect of high glucose
on vascular endothelial growth factor expression in vascular smooth

chemoattractant protein-1 in human atheromatous plaques. *J Clin Invest*
1127, 2002.

tachykinin NK-1 receptor mediates the migration-promoting effect of
substance P on human skin fibroblasts in culture. *Naunyn Schmiedebergs

23. Parenti A, Brogelli L, Donnini S, Ziche M, and Ledda F. Angiotensin
II potentiates the mitogenic effect of noradrenaline in vascular smooth

24. Parenti A, Brogelli L, Filippini S, Donnini S, and Ledda L. Effect of
hypoxia and endothelial loss on vascular smooth muscle cell responsi-
212, 2002.

25. Peißen KA and Winkles JA. Angiotensin II and endothelin-1 increase
fibroblast growth factor-2 mRNA expression in vascular smooth muscle

26. Perez E, Di Felbo C, Reale M, Castellani ML, Baccanti G, Barbac-
ance R, Conti P, Cucurullo F, and Poggi A. Monocyte chemotactic
protein-1 (MCP-1) is a mitogen for cultured rat vascular smooth muscle

27. Schaper W and Buschmann I. Arteriogenesis, the good and bad of it.

28. Schecker AD, Rollins BJ, Zhang YJ, Charo IF, Fallon JT, Rossikhina
M, Giesen PL, Nemerson Y, and Taubman MB. Tissue factor is induced
by MCP-1, ENDOGENOUS VEGF-A, AND VSMC GROWTH
H1983

Downloaded from http://ajpheart.physiology.org/ by 10.220.33.5 on April 9, 2017

AJP-Heart Circ Physiol • VOL 286 • MAY 2004 • www.ajpheart.org