Adenovirus-Mediated Overexpression of c-Jun and c-Fos Induces Intercellular Adhesion Molecule-1 and Monocyte Chemoattractant Protein-1 in Human Endothelial Cells

Nanping Wang, Lynne Verna, Stephen Hardy, John Forsayeth, Yi Zhu, Michael B. Stemerman

Abstract—As distal targets and mediators of signal transduction pathways, activator protein-1 (AP-1), c-Jun, and c-Fos are among the primary regulators of genes involved in cell function, proliferation, and differentiation. By using adenovirus-mediated gene transfer, we show that overexpression of AP-1 proteins directly causes coinduction of gene expression of an adhesion molecule, intercellular adhesion molecule-1 (ICAM-1), and a chemokine, monocyte chemoattractant protein-1 (MCP-1), in human vascular endothelial cells (ECs). The AP-1–induced gene expression occurs through a mechanism independent of nuclear factor-κB. Because the induced expression of ICAM-1 and MCP-1 in ECs has been implicated in endothelial activation and a number of important vascular disorders, it is suggested that AP-1 activation may play an important role in the pathogeneses of inflammation, angiogenesis, and atherogenesis. (Arterioscler Thromb Vasc Biol. 1999;19:2078-2084.)

Key Words: endothelial activation ■ AP-1 ■ adenovirus ■ adhesion molecules ■ chemokines

The vascular endothelium in its basal state performs a number of critical functions for vascular homeostasis. Endothelial cell (EC) perturbation or activation can be caused by various mechanical, chemical, or biological stimuli. Usually associated with the induction of adhesion molecules and chemokines, EC activation is a major feature of the development of a number of vascular processes, including inflammation, angiogenesis, and atherogenesis.1 Although nuclear factor-κB (NF-κB) has been shown to play a pivotal role in EC activation,2–4 increasing evidence indicates the importance of the transcription factor activator protein-1 (AP-1) in endothelial homeostasis and dysfunction. AP-1 is a regulatory protein complex composed of members of the Jun and Fos families. These trans-acting elements interact with AP-1 binding sites in target genes to mediate cellular responses to environmental stimuli.5 In ECs, AP-1 activity can be induced by a variety of factors, such as cytokines,6 bacterial endotoxin, antioxidants,7 hypoxia,8 fluid shear stress,9–11 and LDL.12 Sequence analysis indicates that AP-1 binding sites are recurrent elements in the promoters of genes encoding adhesion molecules and chemokines. However, the regulatory effects of AP-1 proteins in human ECs remain unclear. It is not known whether AP-1 proteins are sufficient to induce phenotypic changes or whether they must act in concert with other effectors. Using an adenovirus-mediated gene transfer protocol, we found that the AP-1 proteins c-Fos and c-Jun directly cause phenotypic activation and induction of intercellular adhesion molecule-1 (ICAM-1) and monocyte chemoattractant protein-1 (MCP-1) in human ECs. The coinduction of ICAM-1 and MCP-1 may represent coordinated events in endothelial activation processes, such as inflammation, angiogenesis, and atherogenesis.

Methods

Adenoviral Vectors

To construct the recombinant adenoviruses expressing human c-jun (AdJun) and c-fos (AdFos) genes, the cDNA fragments containing full-length coding regions were obtained from parental plasmids pRSV-jun and pRSV-fos (generous gifts from Dr R. Tjian, University of California, Berkeley) and subcloned into a shuttle plasmid pAdlox (Genebank access No. U62024)13 downstream from a tetracycline-responsive promoter operator and minimal cytomegalovirus promoter (from pUHD10-3).14 Shuttle plasmids were cotransfected into CRE8 cells (expressing Cre recombinase) with E1- and E3-deleted adenovirus-5 genome DNA (pAdE3Δ5).15 An intermolecular recombination of the shuttle vector and pAdE3Δ5 creates a new virus that carries the c-jun or c-fos cDNA under the control of a tetracycline-responsive transactivator (tTA).16 The recombinant adenovirus carrying the gene for the inhibitor of NF-κB (rAdIκBα, provided by Dr F.H. Bach, Harvard Medical School, Boston, Mass) was constructed to express the porcine IκBα gene (ECI-6).17 The adenoviruses were plaque-purified, amplified, and titrated in 293 cells.17 The functional titers as determined by plaque assays on 293 cells were 4×1011 plaque-forming units per milliliter (AdtTA), 1.6×1011 pfu/mL (Adβ-gal), 1.6×1011 pfu/mL (AdJun), 1.2×1011 pfu/mL (rAdIκBα), and 1.3×1011 pfu/mL (AdFos), and 1.5×1011 pfu/mL (AdJun, AdFos). For adenoviral infection, confluent human umbilical vein endothelial cells (HUVECs) were exposed to adenoviral vectors at a
multiplicity of infection (MOI) of 100 for 2 hours. Unless specified otherwise, AdTATA was added with AdJun, AdFos, or Adβ-gal to induce transgene expression (with a combined MOI of ~100). The cells were incubated for the indicated time courses after viruses were washed off.

**Cell Culture and Reagents**

HUVECs were harvested by collagenase treatment of umbilical cords and cultured on plates coated with collagen. Cells were maintained in medium 199 supplemented with 20% FBS, 20 mmol/L HEPES (pH 7.4), 1 ng/ml recombinant human fibroblast growth factor, 90 μg/ml heparin, and antibiotics.19 Cells within 3 passages were used in these experiments. Phorbol 12-myristate 13-acetate (PMA) was purchased from Sigma Chemical Co, human recombinant tumor necrosis factor-α (TNF-α) from Knoll Pharmaceuticaal, and carbobenzoxyl-leucinyl-leucinyl-leucinyl-H (MG-132) from Biomol.

**RNA Isolation and Northern Blot Analysis**

Total RNA was isolated by using Trizol reagent (GIBCO/BRL), fractionated on formaldehyde/agarose gels, transferred to a nylon membrane, and hybridized to random-primed cDNA probes for human c-jun, c-fos, ICAM-1, MCP-1, and von Willebrand factor.

**Protein Extraction and Western Blot Analysis**

Nuclear proteins were extracted from HUVECs as previously described.20 Protein concentration was measured with the biochino nic acid protein assay kit (Pierce). Twenty micrograms of protein per sample was resolved by SDS–polyacrylamide gel electrophoresis (PAGE), transferred onto an Immobilon-P membrane (Millipore), and analyzed with primary rabbit polyclonal antibodies to c-Jun and c-Fos (1:2000), c-Fos (1:1000), IκBα (1:1000) (all from Santa Cruz), and a horseradish peroxidase–conjugated secondary antibody (sheep anti-rabbit, 1:5000; Sigma), followed by enhanced chemiluminescence detection (Amersham).

**Electrophoretic Mobility Shift Assay (EMSA)**

Six micrograms of nuclear extract was mixed with 1 μg of poly(dI- dC) and DNA binding buffer and incubated at room temperature for 10 minutes. 32P-labeled oligonucleotides were then added to the reaction and incubated for 20 minutes at room temperature. The oligonucleotides were end-labeled by using T4 polynucleotide kinase and [γ-32P]ATP (ICN). Additionally, a 100-fold molar excess of unlabeled competing oligonucleotides was used in some experiments to test the specificity of binding. The sequences of oligonucleotides are as follows: (1) consensus AP-1, 5′-GCT GCT GCC TCA GTT TCC CAG-3′; (2) NF-xB, 5′-AGT TGA GAG GAT TTC CAG AGG C-3′; (3) SP1, 5′-ATT CGA TCG GGG CGG GGC GAG C-3′; (4) GATA, 5′-CAC TTG ATA ACA GAA AGT GAT AAC TCT-3′; (4) ICAM–TRE-responsive element (TRE) (−321), 5′-TAG ACC GTG ATT CAA GCT TAG-3′; (5) ICAM–NF-xB (−223), 5′-TGT AGC TTC CCA GTA CCG GAG CT-3′; (6) ICAM–AP-1/Ets (−940), 5′-GCT GCT GCC TCA GTT TCC CAG-3′; and (7) ICAM–TRE/ AP-1 (−1290), 5′-TGG CCA GTG ACT CGC AGC CCC-3′.

**Results**

**Adenovirus-Mediated Overexpression of c-Jun and c-Fos Induces AP-1 Activation in ECs**

The Jun-Fos complex is the major component of cellular AP-1.19,20 To induce AP-1 activation specifically, a straight-forward approach is to overexpress c-jun and c-fos genes in ECs. However, owing to the difficulties in transfecting primary ECs, there has been no successful report regarding overexpression of AP-1 components in HUVECs. Recently, recombinant adenoviral vectors have been used to transiently express foreign proteins with high efficiency in various types of cells, including human ECs. Therefore, we explored a recently developed method13 to construct replication-deficient recombinant adenoviruses in which the transgene is under the control of a tet (Figure 1A). Expression of the transgene requires coinfection with an additional adenovirus encoding the rTA transactivator. As shown with X-gal staining and the β-gal activity assay (Figure 1B), coinfection of HUVECs with Adβ-gal and AdTATA induced a high level of
β-gal gene expression in ≈100% of HUVECs. The protein expression of transgenes in HUVECs can be detected as early as 16 hours after infection, reaching a peak at 24 hours. At the titers tested (up to 500 MOI), infection with Adβ-gal and AdtTA did not cause either cytopathological changes or the nonspecific induction of endogenous genes, such as c-jun, c-fos, and ICAM-1 in HUVECs. Similarly, the adenoviruses expressing c-jun and c-fos were generated. After infection of HUVECs with AdFos or AdJun, total RNA and nuclear protein were extracted to examine the expression of c-fos and c-jun mRNA and protein. As shown in Figure 1C, the adenoviral vector–encoded c-fos and c-jun transcripts were detectable within 12 hours and reached a maximal level 24 to 48 hours after infection. The adenovirally encoded transcripts can be distinguished from endogenous transcripts because the adenoviral vector–encoded c-fos and c-jun mRNAs (the transcripts of AdJun and AdFos) are 3.6 and 2.2 kb, respectively. Adenovirus-mediated overexpression of c-jun and c-fos markedly potentiated ICAM-1 and MCP-1 induction by PMA (10 ng/mL) (Figure 3B). These findings indicate that adenovirus-mediated coexpression of c-jun and c-fos could effectively and specifically establish a model for AP-1 activation in human ECs.

**Figure 2. AP-1 activation in HUVECs.** A, DNA binding induced by overexpression of AP-1. Nuclear extracts were prepared from HUVECs 48 hours after coinfection with AdtTA and either AP-1 viruses or Adβ-gal or 2 hours after exposure to PMA (100 ng/mL). EMSA was performed with 32P end-labeled oligonucleotides containing consensus AP-1, NF-κB, SP-1, and GATA sequences. For competition EMSAs, unlabeled AP-1 or NF-κB was added (in 100-fold molar excess) to the binding reactions. B, Enhanced AP-1–dependent promoter activity. HUVECs were cotransfected with 5 μg of pGL–AP-1–Luc and 1 μg of pRSV-β-gal plasmids, followed by coinfection with AdtTA and AdJun and/or AdFos, or infection with AdtTA alone as a control. Results shown are representative of 3 individual experiments and are presented as fold induction of basal promoter activity (average luciferase activities of triplicate cultures). *P<0.05.

For the binding induced by overexpression of c-jun and c-fos. In addition, DNA binding activity to other sequences, including NF-κB, SP-1, and GATA, was not affected by overexpression of c-fos and c-jun. For a functional analysis, a promoter transactivation assay was performed with a chimeric promoter-luciferase reporter, pGL–AP-1–Luc, which contains 3 copies of the consensus AP-1 motifs. Expression of this AP-1–dependent gene was enhanced 3-fold in HUVECs coinfected with AdJun and AdFos (Figure 2B). Taken together, these results confirmed that adenovirus-mediated coexpression of c-jun and c-fos could effectively and specifically establish a model for AP-1 activation in human ECs.

**Figure 3. Gene induction by overexpression of AP-1.** A, HUVECs were coincubated with AdtTA, AdJun, and AdFos for the indicated time courses or exposed to PMA (100 ng/mL, 6 hours). RNA blots were hybridized to 32P–labeled cDNA probes for human ICAM-1, MCP-1, and von Willebrand factor, respectively. B, Twenty-four hours after coinfection with AdtTA, AdFos, and AdJun or with Adβ-gal, HUVECs were exposed to PMA (10 ng/mL) for 6 hours. Data are representative of 3 individual experiments.

**Induction of Coordinated Expression of ICAM-1 and MCP-1.**

To elucidate the consequences of AP-1 activation, we further examined its effect on the transcriptional induction of genes involved in phenotypic modulation of ECs. ICAM-1 and MCP-1 genes were induced in HUVECs earlier than 24 hours after infection with AdFos and AdJun (Figure 3A). Peak levels of steady-state mRNA for ICAM-1 and MCP-1 were found 24 to 48 hours after coinfection. Infection with Adβ-gal and AdtTA did not induce ICAM-1 or MCP-1 expression. Therefore, coinduction of ICAM-1 and MCP-1 genes appeared to be the specific result of increased levels of c-Jun and c-Fos expression. Coexpression of c-Fos and c-Jun did not regulate gene expression of von Willebrand factor, which is considered an EC-specific molecular marker. None of these genes was found to be induced by infection with Adβ-gal and AdtTA as the controls for adenoviral vectors. In addition, overexpression of c-jun and c-fos markedly potentiated ICAM-1 and MCP-1 induction by PMA (10 ng/mL) (Figure 3B). These findings indicate that AP-1 is an important signaling pathway for PMA induction of ICAM-1.

Many vascular adhesion molecule genes (including those for ICAM-1) contain functional NF-κB binding sites in their promoter regions, which have been suggested as the critical cis-elements for cytokine-induced transcriptional induction. In addition, interaction between AP-1 and NF-κB has recently been reported to play a role in the activation of another vascular adhesion molecule, VCAM-1. Therefore, to determine whether the ICAM-1 induction and the EC activation induced by the AP-1 activation process were dependent on an NF-κB pathway, we examined the induction of ICAM-1 in...
the absence of NF-κB activation. MG-132, an aldehyde peptide, is an inhibitor of proteasomes and an effective antagonist of NF-κB activation. Pretreatment of HUVECs with MG-132 can block NF-κB activation and largely abolish TNF-α-induced ICAM-1 activation (Figure 4A). As a more specific NF-κB antagonist, rAdIkBα was used to overexpress IkBα in HUVECs (Figure 4B), which was previously demonstrated to specifically inhibit NF-κB activation in ECs. As a result, TNF-α induction of ICAM-1 was similarly inhibited in the rAdIkBα-infected HUVECs. However, ICAM-1 induction by AP-1 overexpression was not blocked by either MG-132 or IkBα (Figure 4C). These data strongly suggest that AP-1–induced ICAM-1 activation can occur independently of the NF-κB pathway.

Enhancement of Promoter Activity and Binding to AP-1 Sites in the ICAM-1 Promoter

Activation of ICAM-1 gene expression is known to occur at the level of transcription. To further elucidate the mechanism by which overexpression of AP-1 proteins activates ECs, a transactivation assay with the use of a 1.3-kb ICAM-1 promoter–driven luciferase reporter construct (ICAM-Luc) was first used to test whether adenovirally mediated AP-1 (c-Jun/c-Fos) overexpression could enhance ICAM-1 promoter activity. As shown in Figure 5B, expression of the ICAM-Luc reporter (pGL1.3) was enhanced ∼4-fold by coinfec- tion with AdJun and AdFos but not with control virus AdTA. Because this 1.3-kb region of the ICAM-1 promoter contains multiple putative AP-1–like sequences and NF-κB motifs (Figure 5A), EMSA with the oligonucleotides corresponding to these motifs was performed to precisely localize the sequences that are functionally involved in AP-1 induction of ICAM-1 gene expression. As shown in Figure 5C, individual overexpression of c-Jun or c-Fos induced little binding activity to any of the sequences, whereas coexpression of c-Jun and c-Fos resulted in strong binding activity to the proximal AP-1/TRE site, moderate binding to the distal AP-1, but no binding to the AP-1/Ets sequence. This result suggests that the proximal AP-1/TRE site may function as the cis-element for AP-1 (likely, c-Jun–c-Fos heterodimers) to activate ICAM-1 transcription. DNA binding activity at the ICAM-1 NF-κB sequence, on which cytokine induction critically depends, was not found to be induced by AdFos and/or AdJun. This result, consistent with the antagonist experiment, indicates that NF-κB, either as the trans-activator or the cis-element, is not indispensable for ICAM-1 gene activation when the AP-1 pathway is activated. AP-1 proteins may act through an independent regulatory mechanism to cause EC phenotypic activation by using specific recognition sites in the target genes.

Discussion

The major findings of this study are as follows: (1) Adenovirus-mediated gene transfer of c-jun and c-fos can effectively and specifically establish an experimental model for AP-1 activation in human ECs, (2) AP-1 activation can directly induce gene expression of an adhesion molecule, ICAM-1, and a chemokine, MCP-1, which are considered to be the molecular markers of EC activation and are implicated in various EC pathological processes, from inflammation to atherogenesis, (3) The AP-1–mediated induction of ICAM-1 can occur independently of activation of the NF-κB pathway. AP-1 transcription factors are formed through dimerization between the members of the Fos and Jun families. Recent studies have suggested AP-1 to be an important regulator in endothelial function and pathological processes. First, the AP-1 binding motif has been identified as a recurrent sequence in the promoters of many genes biologically significant in the conversion of ECs into a proinflammatory or procoagulant status, such as ICAM-1, VCAM-1, E-selectin, MCP-1, and tissue factor. Second, AP-1 proteins and activity in ECs can be increased by a variety of
LDL. Because these stimuli have pleiotropic effects on the expression of human primary ECs to many transfection methods, transient transfection systems are used to establish the regulatory effect of AP-1 on endogenous genes and to allow biochemical analysis in transformed cell lines or nonhuman cells and is therefore limited in its physiological relevance to human disease. To assay the regulatory effect of AP-1 proteins on the expression of endogenous genes and to allow biochemical analysis in human ECs, it is necessary to include a highly efficient transfection system. Considering the advantages of a recombinant adenovirus as an effective gene delivery vector into human ECs, we asked whether AP-1 activation could cause EC phenotypic modulation. A pronounced induction of ICAM-1 and MCP-1 gene expression was observed after overexpression of c-jun and c-fos. This is the first report of a direct link between AP-1 activation and the induction of both a chemokine and an adhesion molecule in ECs, which is particularly significant in endothelial biology.

Figure 5. Activation of ICAM-1 promoter by AP-1 overexpression. A, Putative AP-1 and NF-κB elements in the ICAM-1 gene 5′-flanking region. Positions were numbered in relation to ATG. B, ICAM-1 promoter-reporter transactivation assay. HUVECs were cotransfected with 5 μg of pGL1.3 and 1 μg of pRSV-β-gal plasmids, followed by coinfection with AdTATA, AdJun, and AdFos or infection with AdTATA alone as a control. Results are presented as fold induction of basal promoter activity (average luciferase activities of triplicate cultures). *P<0.01. C, EMSA was performed with the nuclear extracts prepared from HUVECs 48 hours after coinfection with AdTATA, AdFos, and/or AdJun: Adβ-gal; or 2 hours after exposure to PMA (100 ng/mL). Oligonucleotides contain the sequences indicated in A. Data are representative of 3 individual experiments.

With successful adenovirus-mediated gene transfer of c-jun and c-fos in HUVECs, we asked whether AP-1 activation could cause EC phenotypic modulation. A pronounced induction of ICAM-1 and MCP-1 gene expression was observed after overexpression of c-jun and c-fos. This is the first report of a direct link between AP-1 activation and the induction of both a chemokine and an adhesion molecule in ECs, which is particularly significant in endothelial biology.

ICAM-1 and MCP-1 are important cell adhesion and chemokine mediators of leukocyte and EC interactions. MCP-1 is primarily chemotactic for monocytes, whereas ICAM-1 is important in mediating the adhesion and extravasation of circulating leukocytes. Therefore, coinduction of ICAM-1 and MCP-1 may represent coordinated events in endothelial activation processes. In particular, both ICAM-1 and MCP-1 have been identified as being involved in mononuclear cell infiltration in experimental models of atherosclerosis and in lesions from human subjects. Atherogenic factors such as LDL have been reported to induce both AP-1 and ICAM-1. This observation will further our understanding of AP-1 as an important regulator in the pathobiological activation of the vascular endothelium. On the basis of the observation that c-jun and c-fos coexpression appeared to be more efficient for induction of ICAM-1/MCP-1 expression (Figure 3), which is consistent with the ICAM-1–AP-1/TRE binding assay and ICAM-1 promoter transactivation assay (Figure 4), we hypothesize that Jun:Fos heterodimers might be a functional form of the AP-1 complex in inducing ICAM-1 and MCP-1 expression. In the future, studies using c-fos and c-jun ribozymes or adenoviruses expressing antisense c-fos and c-jun will be helpful to detail the role of c-Fos and c-Jun in mediating EC activation.

As transcription factors, AP-1 proteins may activate gene expression through their binding to specific cis-elements within the promoters. Alternatively, AP-1 may exert its role in modifying the activity of other transcription factors.
regulatory effects through complex interaction with other regulatory proteins such as NF-κB.\textsuperscript{45} In fact, many vascular adhesion molecule genes have both AP-1 and NF-κB elements in their promoters. Recently, NF-κB has been shown to be a pivotal player in transcriptional regulation of EC activation.\textsuperscript{21} However, among the various stimuli, some, such as proinflammatory cytokines, are activators of both AP-1 and NF-κB, while others, such as LDL,\textsuperscript{12} are AP-1 but not NF-κB inducers. Furthermore, some excitants, such as the antioxidant pyrrolidine dithiocarbamate,\textsuperscript{46} are activators of AP-1 but inhibitors of NF-κB. Therefore, it would be intriguing to block NF-κB to test whether this AP-1–mediated effect occurred independently or was dependent on an interaction with NF-κB. Typically, NF-κB (a p50/p65 heterodimer) is retained in the cytoplasm by association with IκB protein in quiescent ECs. After stimulation, IκB is phosphorylated by IκB kinase,\textsuperscript{47} which action causes IκB degradation and release of NF-κB to the nucleus.\textsuperscript{2} In the current study, 2 strategies were used to block NF-κB activation in ECs. The proteasome inhibitor MG-132 has been widely used because it can reduce nuclear translocation of p50/p65 and block TNF-α–induced activation of NF-κB–dependent promoters.\textsuperscript{24} A further specific blockade, the adenovirus rAdIκBα, can effectively block TNF-α–induced (Figure 4) and even basal levels of NF-κB activation in ECs.\textsuperscript{16} As a result, TNF-α–stimulated ICAM-1 can be largely blocked by treatment with NF-κB antagonists. However, neither of the NF-κB blockers could reduce AP-1–mediated ICAM-1 induction, suggesting that this induction is indeed an NF-κB–independent event. This evidence supports our hypothesis that AP-1 activation can circumvent the NF-κB pathway and independently induce certain types of gene expression in ECs. In light of the fact that certain chronic vascular stimulating agents such as LDL (antioxidants as well) selectively induce AP-1 activation, this AP-1–specific event may reflect a gene activation pathway distinct from that used by many acute vascular stimuli, such as proinflammatory cytokines and bacterial endotoxin.

The 5′-flanking region of the human ICAM-1 gene contains a variety of putative regulatory enhancer elements, including multiple AP-1–like sites and NF-κB sites.\textsuperscript{15} Our EMSA data have revealed that infection with AdJun and AdFos induced the most pronounced binding to the proximal AP-1/TRE site and, to a lesser extent, to the distal AP-1 site. This finding is in accordance with a previous report that transcriptional induction of ICAM-1 by tissue plasminogen activator in human neuroblastoma cells involves Jun- and Fos-containing complexes binding to this AP-1/TRE site.\textsuperscript{48} It was also described that in human ECs, the antioxidant pyrrolidine dithiocarbamate can induce binding to the AP-1 site of the ICAM-1 promoter by complexes composed of Fos and Jun proteins.\textsuperscript{46} Moreover, promoter deletion analysis has indicated that the inducibility of ICAM-1 by LDL largely depends on the presence of this proximal AP-1/TRE element (Y.Z. et al, unpublished data, 1999). Although the responsiveness of the MCP-1 promoter and the functionality of the putative AP-1 site have not been examined in the present study, an AP-1/TRE site was similarly found to be responsible for the inducibility of a 550-bp MCP-1 promoter by fluid shear stress.\textsuperscript{49} Taken together, our data indicate that AP-1 regulatory proteins may mediate EC activation through the cognate sequence in the promoter regions of the target genes. Future studies with site-directed mutagenesis experiments will be helpful to explore whether ICAM-1 induction depends on the proximal AP-1/TRE site and the potential roles of its flanking sequences and interaction with other sites.

In conclusion, we have presented a unique model system in which AP-1 complexes are overexpressed by adenoviral vectors in primary human ECs. Our study demonstrates that AP-1 overexpression is itself sufficient to induce adhesion molecule and chemokine genes in ECs through an NF-κB–independent pathway. These results provide the important insight that AP-1 plays a key regulatory role, whereby a variety of stimuli activate ECs in a specific pattern of gene expression and subsequently contribute to the development of vascular pathological processes.

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