Role of Activating Protein-1 in the Regulation of the Vascular Cell Adhesion Molecule-1 Gene Expression by Tumor Necrosis Factor-α*

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Endothelial cell surface expression of VCAM-1 is one of the initial steps in the pathogenesis of atherosclerosis. The inflammatory response transcription factor nuclear factor (NF)-κB plays an important role in the regulation of VCAM-1 expression by various stimuli including tumor necrosis factor (TNF-α). Other transcription factors may modulate this response through interaction with NF-κB factors. Since c-Fos/c-Jun (activating protein-1 (AP-1)) are expressed in vascular endothelium during proinflammatory conditions, we investigated the role of AP-1 proteins in the expression of VCAM-1 by TNF-α in SV40 immortalized human microvascular endothelial cells (HMEC). TNF-α induced expression of both early protooncogenes, c-fos and c-jun. The ability of TNF-α to activate the κB-motif (κB) dependent VCAM-1 promoter-claramphenicol acetyltransferase (CAT) reporter gene lacking a consensus AP-1 element was markedly inhibited by co-transfection of the expression vector encoding c-fos ribozyme, which decreases the level of c-fos by degrading c-fos mRNA, or c-fos or c-jun oligonucleotides. Conversely, co-transfection of c-Fos and c-Jun encoding expression vectors potentiated the p65/NF-κB-mediated transactivation of the VCAM-1 promoter-CAT reporter gene. Furthermore the c-Fos encoding expression vector potentiated by 2-fold the transactivation activity of a chimeric transcriptional factor Gal/p65 (containing the transactivation domain of p65 and the DNA binding domain of the yeast transcriptional factor Gal-4). Consistent with the promoter studies, curcumin and NDGA, inhibitors of AP-1 activation, markedly inhibited the ability of TNF-α to activate the expression of VCAM-1 mRNA levels at concentrations that did not inhibit the activation of NF-κB. In gel mobility supershift assays, the antibodies to c-Fos or c-Jun inhibited the binding of TNF-α-activated nuclear NF-κB to the κB (κB), suggesting that both c-Fos and c-Jun interacted with NF-κB. These results suggest that AP-1 proteins may mediate the effect of TNF-α in the regulation of VCAM-1 expression through interaction with NF-κB factors in endothelial cells.

" Several proinflammatory agents (e.g. tumor necrosis factor-α)

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1 The abbreviations used are: TNF-α, tumor necrosis factor-α; CAT, chloramphenicol acetyltransferase; VCAM, vascular cell adhesion molecule; DTT, dithiothreitol; HMEC, SV40-transformed human microvascular endothelial cells; AP-1, activating protein-1; NDGA, nordihydroguaiaretic acid; NF, nuclear factor; ICAM, intercellular adhesion molecule.
done by the calcium phosphate co-precipitation technique. Overexpression studies were performed by co-transfection of the expression vectors encoding the p65 subunit of NF-kB and/or c-Fos or c-Jun with the reporter gene p85VCAMCAT (5–10 μg) into HMEC cells as described previously (23). The promoterless plasmid, pUC19 (24), was used for adjusting the amount of transferred DNA. The cells were har vested and cell extracts were prepared by three cycles of rapid freeze-thaw in 0.25 M Tris, pH 8.0. Protein content was determined using the Bradford (25) technique. The same amounts of proteins were assayed for chloramphenicol acetyltransferase (CAT) activity according to standard protocols (26). The CAT activity was expressed as percent of chloramphenicol converted to acetyl chloramphenicol and normalized to the amount of chloramphenicol were separated on thin layer chromatography, and their amounts were quantified by phosphorimaging. Each assay was performed in duplicate or triplicate, and the results reported are representative of at least two separate experiments.

Expression Vectors and CAT Reporter Genes—The eucaryotic expres sion vector, cytomegalovirus-p65, contains the p65 cDNA cloned be tween a cytomegalovirus promoter, β-globin intron, and simian virus 40 poly(A) signal (27). Gal/p65 contains the DNA binding domain of the yeast transcription vector Gal-4 and the transactivation domain of p65. pGal-CAT is a reporter gene containing four tandem elements of yeast transcription vector Gal-4 and the transactivation domain of p65. The reporter gene Gal-4 contains coordinates −85 to −12 of the yeast human VCAM-1 promoter (1). These reporter genes were generous gifts of Dr. D. Dean (Washington University, St. Louis, MO). The expression vectors encoding c-Fos and c-Jun protein were kindly provided by Dr. Tjian, University of California, San Francisco. The expression vector encoding c-fos ribosome (28) was a generous gift of Dr. Scanlon, National Medical Center, City of Hope, California.

Nuclear Extract Preparation—Confluent HMEC cells were exposed to TNF-α (1000/ml) for 1–2 h. Nuclear extracts were prepared by a modification of the method of Dignam et al. (29). Briefly, after washing with phosphate buffered saline, cells were centrifuged and the cell pellet suspended in 500 μl buffer A (10 mM HEPES, pH 7.9, 1.5 mM MgCl2, 10 mM KCl, and 1 mM EDTA). After centrifugation, the nuclei were resuspended in nuclei extract buffer with a volume of buffer A containing 0.1% Triton X-100. After incubating for 10 min at 4 °C, the homogenate was centrifuged, and the nuclear pellet was washed once with buffer A and resuspended in 70 μl buffer C (20 mM HEPES, pH 7.9, 25% (v/v) glycerol, 0.42 mM NaCl, 1.5 mM MgCl2, 0.2 mM EDTA, 1 mM DTT). After centrifugation at 20,000 × g for 10 min. The resulting supernatant (nuclear extract) was stored at −70 °C. Protein concentrations were determined by the Bradford (25) method. To minimize prot olysis, all manipulations were done by the calcium phosphate co-precipitation technique. Northern Blot Analysis—Total cellular RNA was isolated by a single extraction using an acid guanidinium thiocyanate-phenol-chloroform (32). The total cellular RNA (10 μg) was size fractionated using 1% agarose formaldehyde gels in the presence of 1 μg/ml ethidium bromide. The RNA was transferred to a nitrocellulose filter and covalently linked by ultraviolet irradiation using a Stratalinker UV cross-linker (Stratagene, La Jolla, CA). Hybridizations were performed at 68 °C for 1 h in 5× SSC (1 × 150 mM NaCl, 15 mM sodium citrate), 1% sodium dodecyl sulfate, 5 × Denhardt’s solution, 50% formamide, 10% dextran sulfate, and 100 μg/ml sheared denatured salmon sperm DNA. Approximately 1–2 × 106 cpm/ml of labeled probe (specific activity, >108 cpm/μg of DNA) was used per hybridization. Following hybridization, filters were washed with a final stringency of 0.2 × SSC at 65 °C. The nitrocellulose membrane was soaked for stripping the probe with boiled water prior to rehybridization. mRNA bands were quantified using PhosphorImager.

RESULTS

TNF-α Induces c-fos and c-jun in HMEC Cells—Because TNF-α is known to induce c-fos and c-jun in various cell types (7, 35, 36), we tested whether similar responses occur in HMEC. Confluent HMEC cells were treated with TNF-α (100 units/ml) for 15–120 min and RNA was prepared for analysis of c-fos and c-jun expression. As shown in Fig. 1, TNF-α treatment produced a severalfold induction of c-fos and c-jun mRNA. c-fos mRNA was elevated 15 min after TNF-α treatment, peaked at nearly 30 min, and return to basal level at 2 h, kinetics similar to c-fos induction by serum and growth factors (37–39). The kinetics of induction of c-jun was similar to that of observed for c-fos.

Inhibition of c-fos or c-jun blocks TNF-α Activation of the VCAM-1 Promoter—TNF-α induces p85VCAMCAT (a minimal VCAM-1 promoter–coordinates −85 to +12, containing two NF-xB motifs, κB-xR, but lacking a consensus AP-1 binding site, attached to the reporter gene CAT) through activating NF-xB proteins (1–3, 23). We investigated whether endogenous c-Fos contributes to the TNF-α-mediated transactivation of p85VCAMCAT. The endogenous level of c-fos was inhibited by co-transfection of an expression vector encoding anti c-fos ribozyme that specifically degrades c-fos mRNA (28). As expected, TNF-α (100 units/ml) activated p85VCAMCAT severalfold above the basal levels (Fig. 2, lanes 3 and 4) in transient transfection assays in HMEC cells compared with untreated cells (lanes 1 and 2). In cells co-transfected with the expression vector encoding c-fos ribozyme, TNF-α was unable to transactivate p85VCAMCAT (lanes 5 and 6). Under similar conditions the expression vector expressing c-fos ribozyme had no effect on the constitutive activity of the SV40 promoter-driven reporter gene CAT, pSV2CAT (compare lanes 7 and 8 with lanes 9 and 10). Under similar conditions antisense oligonucleotides c-fos or c-jun (30, 31) inhibited the activity of TNF-α to activate p85VCAMCAT nearly 80% (data not shown). These results suggest that endogenous c-Fos and c-Jun contribute to TNF-α-
mediated transactivation of p85VCAMCAT.

Co-expression of the Expression Vectors Encoding c-Fos or c-Jun Potentiates the p65-mediated Transactivation of p85VCAMCAT—p65 is a member of the rel family and a component of the NF-κB heterodimers. p65 potently transactivates p85VCAMCAT (23, 40). We investigated whether overexpression of c-Fos and c-Jun potentiates the p65-mediated transactivation of p85VCAMCAT. Eucaryotic expression vectors encoding c-Fos or c-Jun and/or p65 were co-transfected with p85VCAMCAT into HMEC cells and the promoter activity assessed. Consistent with previous observations, co-expressed p65 transactivated p85VCAMCAT (Fig. 3) (23, 40). Co-expression of the expression vectors encoding c-Fos and c-Jun dose-dependently potentiated the p65-mediated transactivation of p85VCAMCAT with maximum potentiation above 30-fold at 5 μg of the each expression vector (Fig. 3A). When co-transfected individually, both c-fos and c-jun expression vectors also potentiated the p65-mediated transactivation of p85VCAMCAT (Fig. 3B). These results suggest that both c-Fos and c-Jun functionally interact with the p65 subunit of NF-κB to potentiate p65-mediated transactivation of p85VCAMCAT.

Co-expression of the Expression Vector Encoding c-Fos Potentiates the Gal/p65-dependent Transactivation of pGal-CAT—p65 has a DNA binding domain and two transactivation domains (41). The transactivation domains of p65 require co-factor proteins for transactivation activity (42). We investigated whether c-Fos could interact with the transactivation domains of p65. We utilized an expression vector encoding chimeric transcriptional factor Gal/p65 and a Gal/p65 driven reporter gene pGal-CAT. Gal/p65 contains a DNA binding domain of the yeast transcriptional factor Gal-4 and transactivation domains of p65 subunit of NF-κB. pGal-CAT contains four multimerized DNA elements of the transcriptional factor Gal-4 attached to a reporter gene, CAT. Co-transfected Gal/p65 potently transactivated pGal-CAT in transient transfection assays (42) (Fig. 4). Co-transfection of the expression vector encoding c-Fos potentiated the Gal/p65-mediated transactivation 2-fold. Co-transfection of the expression vector encoding the p65 subunit of NF-κB completely inhibited the Gal/p65-mediated transactivation of pGal-CAT. This was likely due to the lack of one or both of the NF-κB-like transcription factors acting as transactivators of the Gal4 DNA binding motif. These results suggest that c-Fos interacts with transactivation domains of p65.

Antibodies to c-Fos and c-Jun Inhibit the Binding of TNF-α-Activated NF-κB-like Proteins to the NF-κB Motifs (κL-κR) of the VCAM-1 Promoter—To determine whether c-Fos and/or c-Jun were part of the NF-κB-like complex, electrophoretic mobility shift assays were performed. Consistent with the previous observations in human umbilical vein endothelial cells (23), TNF-α induces a major NF-κB-like complex A1 in the nucleus of HMEC cells (Fig. 5, lanes 2 and 6) compared with the control (lanes 1 and 5). Preincubation of nuclear extracts with antibodies to either c-Fos or c-Jun inhibited the binding of NF-κB-like proteins to the κL-κR motif (lane 4). Similarly, in the presence of c-Jun antibody, the antibody c-Jun was unable to inhibit the binding of the κL-κR motif (lane 7). To test the specificity of this interaction, the nuclear extracts were incubated with antibodies to either c-Fos or c-Jun in the presence of preincubated with antibodies to either c-Fos or c-Jun inhibited the binding of NF-κB-like proteins to the κL-κR motif (lane 3 and 7). These results suggest that both c-Fos and c-Jun are part of the NF-κB proteins in the nuclear extract of HMEC cells activated by TNF-α.

Curcumin Inhibits the Ability of TNF-α to Activate the Expression of VCAM-1 without Affecting the Activation of NF-κB—To explore the role of c-Fos/c-Jun in the TNF-activation of

FIG. 2. Effect of transiently transfected expression vector encoding ribozyme c-fos on the TNF-α-mediated activation of p85VCAMCAT. A, HMEC cells were transiently transfected with p85VCAMCAT (10 μg) and the plasmid encoding ribozyme c-fos (200 ng). After overnight transfection, the cells were washed, the medium was replaced and the cells were allowed to recover 5–15 h. Then the cells were stimulated with TNF-α (100 units/ml) for 16–24 h, and the CAT activity was determined. B, as a control, HMEC cells were transiently transfected with p85VCAMCAT (10 μg) and the plasmid encoding ribozyme c-fos (200 ng) as described earlier. These experiments are representative of two to three experiments performed in duplicate.

FIG. 3. Effect of the co-transfected plasmids encoding c-Fos or c-Jun on the p65-mediated transactivation of p85VCAMCAT. A, HMEC cells were co-transfected with the plasmid encoding c-Fos and c-Jun, and/or p65 and 10 μg of p85VCAMCAT by calcium phosphate precipitation. After overnight transfection, the cells were washed and the medium was replaced. The CAT activity was determined 24 h later. B, the transfections were performed as described above. The experiments presented are representative of two to three independent experiments performed in duplicate.

FIG. 4. Effect of the eucaryotic expression vectors encoding c-Fos or p65 subunit of NF-κB on the Gal/p65-dependent transactivation of pGal-CAT reporter gene. HMEC cells were transiently transfected with the expression vectors encoding c-Fos or p65 subunit of NF-κB and/or Gal/p65 (1 μg) and the reporter gene pGal-CAT (5 μg) as described under “Materials and Methods.” After overnight transfection, the cells were washed, the medium was replaced, and the cells were harvested after 24 h for CAT activity measurements. The experiments presented are representative of two to three independent experiments performed in duplicate.
endogenous VCAM-1 expression, we investigated the effect of curcumin, an inhibitor of c-jun/c-fos expression (43). We investigated the dose-dependent effect of curcumin on the expression of the VCAM-1 gene by TNF-α. Pretreatment of HMEC cells with 10 μM curcumin inhibited TNF-α-activation of VCAM-1 but not ICAM-1 expression at the mRNA levels (Fig. 6A). At the same concentration, curcumin did not inhibit the TNF-α-activation of NF-κB (Fig. 6B). These results suggest that curcumin at 10 μM inhibits the TNF-α activation of VCAM-1 through a mechanism independent of NF-κB activation.

NDGA Inhibits the Expression of VCAM-1 by TNF-α without Inhibiting the Activation of NF-κB—NDGA inhibits several of the effects of TNF-α by inhibiting c-fos expression (7). We investigated the effect of NDGA on the TNF-α-activation of VCAM-1 expression in HMEC cells. Pretreatment of the cells with NDGA at all concentrations tested (1–10 μM) inhibited the TNF-α-activation of VCAM-1 expression (Fig. 7A). Pretreatment of the cells with NDGA at the same concentrations did not inhibit the TNF-α activation of NF-κB (Fig. 7B). These results suggest that activation of the lipoxygenase pathway and c-Fos activation may mediate, at least in part, the effect of TNF-α in regulating the expression of VCAM-1 in HMEC cells.

**DISCUSSION**

We demonstrate that AP-1 proteins c-Fos/c-Jun are involved in TNF-α-activation of VCAM-1 expression. At least a part of the effect of c-Fos/c-Jun is mediated through interaction with NF-κB, and is independent of the AP-1 element present on the VCAM-1 promoter. Furthermore, we demonstrate that anti-inflammatory agents and inhibitors of c-Fos/c-Jun expression inhibit the TNF-α-activation of VCAM-1 in HMEC cells through a pathway that is independent of NF-κB activation (Fig. 8). These studies suggest that in addition to NF-κB, c-Fos/c-Jun also mediate the effect of TNF-α in the regulation of VCAM-1 expression.

Inhibition of endogenous c-Fos/c-Jun by the expression vector encoding ribozyme c-fos (28) or by phosphorothioate antisense oligonucleotides c-fos or c-jun (30, 31) inhibited the TNF-activation of 85VCAMCAT, a minimal NF-κB-driven VCAM-1 promoter-reporter gene lacking the AP-1 element. These studies suggest that the effect of c-fos and c-jun is mediated, at least in part, through interaction with NF-κB factors. While it is tempting to speculate that antisense c-fos and c-jun, and ri-

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**Fig. 5.** The effect of the antibodies to c-Fos or c-Jun on the binding of TNF-α-activated nuclear NF-κB protein to the κB motif of VCAM-1 promoter. HMEC cells were treated with TNF-α (100 units/ml) for 1–2 h, and nuclear extracts were prepared as described under "Materials and Methods." Nuclear proteins (2 μg) were incubated with 32P-labeled double-stranded oligonucleotide (5′-CTGCTGAGTTTCCTGAAGGGATTTCCTGCGCCTGCACAAGAGCTGAGATCCTATG-3′) encompassing two double underlined NF-κB motifs (κB) of the VCAM-1 promoter. The κB binding proteins were separated from free labeled probe on 4% polyacrylamide gel. A, the basal and TNF-α-activated binding proteins are shown in lanes 1 and 2, respectively. The TNF-α-activated nuclear extracts (2 μg) were incubated with the polyclonal antibodies to c-Fos (1 μg, Santa Cruz catalog no. SC-52) for 15 min before incubating with the labeled oligonucleotide (lane 3). As a control, the TNF-α-activated nuclear extract of HMEC cells was incubated with the polyclonal antibodies to c-Fos (1 μg, Santa Cruz catalog no. SC-52) for 15 min in the presence of the peptide to which the antibody was raised, and then the nuclear extracts were incubated with the labeled oligonucleotide for 15 min at 30 °C (lane 4). B, the basal and TNF-α-activated binding proteins are shown in lanes 1 and 2, respectively. The TNF-α-activated nuclear extracts (2 μg) were incubated with the polyclonal antibodies to c-Jun (1 μg, Santa Cruz catalog no. sc-45) for 15 min before incubating with the labeled oligonucleotide (lane 3). As a control, the TNF-α-activated nuclear extract of HMEC cells was incubated with the polyclonal antibodies to c-Jun (1 μg) for 15 min in the presence of 1 μl of the peptide to which the antibody was raised, and then the nuclear extracts were incubated with the labeled oligonucleotide for 15 min at 30 °C (lane 4). These experiments are representative of two to three experiments performed in duplicate.

**Fig. 6.** Effect of curcumin on the TNF-α-mediated expression of VCAM-1 and NF-κB. A, confluent HMEC cells were pretreated with curcumin 2–50 μM for 1 h before stimulation with TNF-α (100 units/ml) for 4 h. Then the cells were harvested for mRNA preparation and the Northern blot analysis was performed as described under "Materials and Methods." VCAM-1 mRNA was probed using human VCAM-1 cDNA. The ethidium bromide staining of 18 and 28 S RNA is shown for equal loading. B, HMEC cells were pretreated with curcumin 2–10 μM for 1 h before stimulating with TNF-α (100 units/ml) for 1 h. Then the nuclear extracts (2 μg) prepared from these cells were incubated with 32P-labeled double-stranded oligonucleotide encompassing κB motif for 15 min at 30 °C. The DNA-bound proteins were separated from the free oligonucleotide on 4% polyacrylamide gel electrophoresis.

**Fig. 7.** Effect of NDGA on the TNF-α-mediated expression of VCAM-1 and NF-κB. A, confluent HMEC cells were pretreated with NDGA 1 and 10 μM for 1 h before stimulation with TNF-α (100 units/ml) for 4 h. Then the cells were harvested for total RNA preparation and the Northern blot analysis was performed as described under "Materials and Methods." VCAM-1 mRNA was probed using human VCAM-1 cDNA. B, HMEC cells were pretreated with NDGA (10 μM) for 1 h before stimulating with TNF-α (100 units/ml) for 1–2 h. Then the nuclear extracts (2 μg) prepared from these cells were incubated with 32P-labeled double-stranded oligonucleotide encompassing κB motif for 15 min at 30 °C. The DNA-bound proteins were separated from the free oligonucleotide on 4% polyacrylamide gel electrophoresis.
Regulation of VCAM-1 by AP-1 and NF-κB-like Proteins

bozyme c-fos functions by decreasing the endogenous concentration of c-Fos or c-Jun in the κB-bound NF-κB complex, we have not directly assessed this in these studies and thus cannot rule out an indirect effect of the antisense oligonucleotides and ribozyme c-fos on the expression of other transcriptional factors.

Co-transfection of the expression vectors encoding c-Fos and c-Jun dose-dependently potentiated the expression of the VCAM-1 promoter by co-expressed p65. Since low levels of basal c-Fos/c-Jun are present in various cell types and TNF-α activates both c-Fos/c-Jun and NF-κB, it is likely that the level of proinflammatory response is determined by the interaction of these two class of transcriptional factors.

Co-transfection of the expression vector encoding the p65 subunit of NF-κB completely inhibited the Gal/p65-driven activity of pGal-CAT in HMEC cells as observed earlier in COS cells (42). These studies suggested that certain cofactors such as bridging proteins, mediators, or intermediary proteins that associate or interact with the transactivation domain of p65 are necessary for transactivation by p65. We investigated whether c-Fos or c-Jun could act as a co-factor in the transactivation by p65. Consistent with the 85VCAMCAT studies, co-transfection of the expression vector encoding c-fos potentiated the Gal-p65 driven transactivation of pGal-CAT. Under similar conditions, co-transfection of the expression vector encoding c-Jun did not potentiate the transactivation activity of Gal/p65 suggesting that the interaction of c-Jun with p65 may require its DNA binding domain. These studies at least suggest that c-Fos could act as a cofactor through interaction with the transactivation domain of p65 in HMEC cells.

AP-1 proteins can regulate the expression of several genes in various ways. 1) Through binding to the AP-1 element, AP-1 proteins can activate the transcription of various genes containing the AP-1 element; 2) after binding to their DNA element, AP-1 proteins can synergize with other transcriptional factors and modulate the transactivation mediated by them; and 3) independent of their DNA binding element, AP-1 proteins can interact with other transcriptional factors and modulate the transactivation mediated by them (44–46). Since deletion or mutation of the AP-1 element present in the VCAM-1 promoter had no effect on activation of the VCAM-1 promoter by TNF-α (1, 2), the AP-1 element does not appear to play a role in TNF-α-activation of the VCAM-1 promoter. This would suggest that the regulation by AP-1 proteins of VCAM-1 is mainly through interaction with NF-κB factors and is independent of the AP-1 element.

Curcumin inhibits transcriptional activation of the c-jun gene by PMA and TNF-α and blocks the increase in TRE binding activity of c-Jun/AP-1 protein (43, 47). Curcumin decreases TPA-induced nuclear abundance of c-Fos protein through the induction of a hyperphosphorylated unstable form of c-Fos (48). Curcumin has also been shown to inhibit the activation of NF-κB (49). In our experimental conditions, curcumin (10 μM) blocked the TNF-α-activation of VCAM-1 expression in HMEC cells without inhibiting NF-κB activation. These studies suggest that transcriptional factors other than NF-κB, such as c-Fos/c-Jun/AP-1, may also play an important role in the regulation of VCAM-1 gene expression by TNF-α.

NDGA, an inhibitor of fos expression (7), inhibits the TNF-α activation of VCAM-1 expression without blocking NF-κB activation. These results suggest that NDGA inhibited the transactivation activity of NF-κB either by directly changing the phosphorylation of nuclear NF-κB or by inhibiting the activity of other factors that interact with NF-κB. In recent studies, the phosphorylation of nuclear NF-κB was analyzed under conditions that inhibited TNF-α-activation of VCAM-1 expression but did not inhibit nuclear translocation of NF-κB (50). These studies suggest that factors other than phosphorylation of NF-κB are involved in inhibiting the transactivation activity of NF-κB. Consistent with these observation, our studies suggest that AP-1 proteins may play an important role in regulating the activity of NF-κB.

The antibodies to c-Fos and c-Jun inhibited the binding of NF-κB to the κB motif, as observed earlier with Ig/HIV κB motif (44). Assuming that c-Fos and c-Jun are part of the κB-bound NF-κB complex, the antibodies to c-Fos or c-Jun could inhibit the binding of NF-κB to κB by either changing the conformation of the DNA-binding domain of NF-κB or directly interfering with the binding of NF-κB to κB. The other possibility is that c-Fos and c-Jun are not part of the NF-κB complex but that their presence in the nucleus facilitates the binding of NF-κB to the κB motif. These results suggest that the TNF-α-activated signal transduction pathways leading to activation of NF-κB and AP-1 may interact in the nucleus to regulate VCAM-1 gene expression.

In conclusion, our studies have demonstrated that TNF-α induces 85VCAMCAT (a minimal VCAM-1 promoter-CAT reporter gene) and expression of VCAM-1 gene through interaction of c-Fos and c-Jun with NF-κB proteins, suggesting that the AP-1/NF-κB protein complex may be one of the transactivating complexes induced by TNF-α in HMEC cells. Furthermore, c-Fos/c-Jun may play a role in the regulation of VCAM-1 gene expression under conditions such as growth state of the cell and shear stress that affect the levels of c-Fos/c-Jun in endothelial cells.

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REFERENCES
1. Iademarco, M. F., McQuillan, J. J., Rosen, G. D., and Dean, D. C. (1992) J. Biol. Chem. 267, 16223–16228
2. Neish, A. S., Williams, A. J., Palmer, H. J., Whitley, M. Z., and Collins, T. (1992) J. Exp. Med. 176, 1583–1593
3. Marui, N., Offermann, M. K., Sverlick, R., Kunsch, C., Rosen, C. A., Ahmad, M., Alexander, R. W., and Medford, R. M. (1993) J. Clin. Invest. 92, 1866–1874
4. Brand, K., Page, S., Rogler, G., Bartsch, A., Brandl, R., Knuechel, R., Page, M., Kalschmidt, C., Baerence, P. A., and Neumeier, D. (1996) J. Clin. Invest.
Regulation of VCAM-1 by AP-1 and NF-κB-like Proteins

4621

97, 1715–1722
5. Collins, T. (1993) Lab. Invest. 68, 499–508
6. Lo, Y. Y. C., and Cruz, T. F. (1995) J. Biol. Chem. 270, 11727–11730
7. Haliday, R. M., Ramesha, C. S., and Ringold, G. (1991) EMBO J. 10, 109–115
8. Angel, P., and Karin, M. (1991) Biochim. Biophys. Acta 1072, 129–157
9. Cohen, D. R., Ferreira, P. C., Gentz, R., Franza, B. R., Jr., and Curran, T. (1990) Genes Dev. 4, 444–454
10. Curran, T., and Franza, B. R., Jr. (1988) Science 237, 173–184
11. Lee, W., Mitchell, P., and Tjian, R. (1989) Genes Dev. 3, 395–400
12. Schonthal, A., Herrlich, P., Rahmsdorf, H. J., and Ponta, H. (1988) Cell 52, 471–480
13. Ahmad, M., Marui, N., Alexander, R. W., and Medford, R. M. (1994) J. Biol. Chem. 269, 661–663
14. Bradner, J. E., Tate, J. E., and Habener, J. F. (1989) BioTechniques 7, 1111–1116
15. Bradford, M. M. (1976) Anal. Biochem. 72, 248–254
16. Sambrook, J., Fritsch, E. F., and Maniatis, T. (1989) Molecular Cloning: A Laboratory Manual, 2nd Ed., Cold Spring Harbor Laboratory, Cold Spring Harbor, NY
17. Ruben, S., Narayanan, H., Klement, J., Chen, C.-H., and Rosen, C. (1992) Mol. Cell. Biol. 12, 444–454
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