Stat3 Mediates Interleukin-6 Inhibition of Human Endothelial Nitric-oxide Synthase Expression*

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The acute phase response (APR) is associated with atherosclerosis. Elevated levels of interleukin-6, the major inducer of the APR, are associated with an increased risk of cardiovascular events. One of the clinical hallmarks of atherosclerosis is endothelial dysfunction, characterized by a decrease in endothelial production of nitric oxide (NO). We hypothesized that interleukin-6 (IL-6) decreases endothelial NO synthase (eNOS) expression. We now show that IL-6 treatment of human aortic endothelial cells (HAEC) decreases steady-state levels of human eNOS mRNA and protein. This decrease in eNOS expression is caused in part by IL-6 inhibition of transactivation of the human eNOS promoter. To explore the mechanism by which IL-6 affects eNOS expression, we examined activation of signal transducer and transactivator-3 (Stat3). The IL-6 receptor (IL-6R) is expressed in HAEC, and Stat3 is phosphorylated in response to IL-6 stimulation of the IL-6R. We identified four consensus sequences for Stat3 binding (SIE) in the eNOS promoter at positions −1520, −1024, −840, and −540. Transfection of eNOS promoter mutants revealed that the SIE at −1024 mediates Stat3 inhibition of eNOS promoter activity. Gel-shift analysis of nuclear extracts from HAEC treated with IL-6 confirms that Stat3 binds to a complex containing the SIE at −1024. RNA silencing of STAT3 blocks the inhibitory effect of IL-6 on eNOS expression. Our data show that IL-6 has direct effects upon endothelial cells, inhibiting eNOS expression in part through Stat3. Decreased levels of eNOS may be an important component of the pro-atherogenic effect of the APR.

Chronic activation of the acute phase response (APR) is associated with atherosclerosis. Elevated levels of interleukin-6, the major inducer of the APR, are associated with an increased risk of cardiovascular events. One of the clinical hallmarks of atherosclerosis is endothelial dysfunction, characterized by a decrease in endothelial production of nitric oxide (NO). We hypothesized that interleukin-6 (IL-6) decreases endothelial NO synthase (eNOS) expression. We now show that IL-6 treatment of human aortic endothelial cells (HAEC) decreases steady-state levels of human eNOS mRNA and protein. This decrease in eNOS expression is caused in part by IL-6 inhibition of transactivation of the human eNOS promoter. To explore the mechanism by which IL-6 affects eNOS expression, we examined activation of signal transducer and transactivator-3 (Stat3). The IL-6 receptor (IL-6R) is expressed in HAEC, and Stat3 is phosphorylated in response to IL-6 stimulation of the IL-6R. We identified four consensus sequences for Stat3 binding (SIE) in the eNOS promoter at positions −1520, −1024, −840, and −540. Transfection of eNOS promoter mutants revealed that the SIE at −1024 mediates Stat3 inhibition of eNOS promoter activity. Gel-shift analysis of nuclear extracts from HAEC treated with IL-6 confirms that Stat3 binds to a complex containing the SIE at −1024. RNA silencing of STAT3 blocks the inhibitory effect of IL-6 on eNOS expression. Our data show that IL-6 has direct effects upon endothelial cells, inhibiting eNOS expression in part through Stat3. Decreased levels of eNOS may be an important component of the pro-atherogenic effect of the APR.

The acute phase response (APR) is a systemic innate inflammatory response to acute injury (1, 2). A variety of triggers such as infection or trauma lead to the rapid synthesis and circulation of a large number of acute phase effector molecules, including anti-microbial proteins, pro-coagulant factors, and metabolic regulators. Although the APR is a rapid response to acute stress, chronic activation of the APR is associated with various diseases including cancer, autoimmune diseases, and coronary artery disease. For example, levels of acute phase effector proteins such as C-reactive protein (CRP), fibrinogen, and sICAM-1 are higher in patients with atherosclerosis or acute coronary syndromes compared with healthy controls (3–8). The APR may play a role in the pathogenesis of endothelial dysfunction, an early stage in atherogenesis.

Interleukin-6 (IL-6) is a major activator of the acute phase response (1, 9). IL-6 induces hepatocytes to activate or suppress the synthesis of a variety of acute phase response effector proteins. IL-6 initiates its action by binding to the IL-6 receptor, which is composed of two subunits: an 80-kDa IL-6-binding protein and a 130-kDa transmembrane signal-transducing component (gp130) (9). Activation of IL-6 signal transduction involves gp130 dimerization, ligand-dependent tyrosine phosphorylation of Jak1, Jak2, and Tyk2, followed by tyrosine phosphorylation of signal transducer and activator of transcription 3 (Stat3).

Stat3 is a transcription factor that mediates the cellular response to IL-6 (10–13). Stat3 is expressed in most cell types and can be activated by other cytokines and growth factors including: IFN-α, IL-10, IL-6, IL-11, LIF (leukemia inhibitory factor), CNTF (ciliary neurotrophic factor), G-CSF (granulocyte-colony-stimulating factor), IL-12, IL-2, GH (growth hormone), EGF, PDGF (platelet-derived growth factor), and CSF-1. Jak phosphorylates Stat3 on a tyrosine residue (Tyr705) within a conserved SH2 domain, allowing homodimerization or heterodimerization with other Stat family members, nuclear translocation, and transcription activation. The specific regulatory elements that Stat3 interacts with include the APRE (acute phase response element) and the SIE (c-sis inducible element).

IL-6 and other factors can also activate phosphorylation of Stat3 at a single residue (Ser727), which enhances Stat3 transcriptional activation.

Since effectors of the APR are elevated in apparently healthy subjects who later develop coronary artery disease, the APR may play a role in the early stages of atherogenesis, including endothelial dysfunction (14, 15). Endothelial dysfunction is...
characterized by decreased bioavailability of NO, caused in part by increased oxidant stress and by decreased NO synthesis (16, 17). In the vasculature, NO is normally synthesized by the endothelial isoform of NO synthase (eNOS), where it plays a protective role by inhibiting leukocyte trafficking and by decreasing platelet adhesion and aggregation (18–23). NO production by endothelial cells is regulated by changes in eNOS enzyme activity and gene expression. The expression of eNOS can be regulated by biophysical stimuli (such as shear stress or hypoxia), growth factors (such as TGF-β, FGF, VEGF, or PDGF), hormones (such as estrogens, insulin, angiotensin II, or endothelin 1), or NO itself (23).

We now show that the APR inducer IL-6 decreases eNOS expression in human aortic endothelial cells by activating the binding of Stat3 to a specific site in the human eNOS promoter.

**EXPERIMENTAL PROCEDURES**

**Materials**—Recombinant human IL-6 was purchased from Sigma (St. Louis, MO and Oxon, UK). ECB and growing supplements were purchased from Clonetics (Barcelona, Spain). [32P]ATP was purchased from Amersham Biosciences (3000 Ci/mmol). Antibody to eNOS, iNOS and full-length Stat3 were from BD Biosciences, Madrid, Spain). Antibody against phosphorylated Stat3 on Tyr705 was from Cell Signaling (Beverly, MA) and antibodies against Stat3 (C 20) and Stat1 were from Santa Cruz Biotechnology (Santa Cruz, CA). All other reagents were purchased from Sigma (Oxon, UK).

**Cell Culture**—Human aortic endothelial cells (HAEC) were obtained from Clonetics. HAEC were grown on gelatin-coated plates in endothelial growth media plus growth supplements. Studies were performed on confluent monolayers at passages 2–7. Toxicity was measured by measuring trypan blue dye exclusion and lactate dehydrogenase activity in the incubation media.

**eNOS Promoter Constructs**—The 1.6-kb fragment of the eNOS 5′-flanking region was a generous gift from Dr. Thomas Michel (Brigham and Women’s Hospital, Boston, MA) (24). An eNOS promoter reporter plasmid was constructed by inserting the 1.6-kb fragment of the human eNOS promoter upstream of the firefly luciferase gene in the plasmid pGL3, as described previously (25). This promoter was designated −1624 eNOS-Luc. Deletion mutants of the eNOS promoter were generated by PCR, as described previously (25).

**Immunoblotting**—Immunoblotting was performed as described previously (25). In brief, cellular monolayers were washed with PBS and harvested in protein lysis buffer (1% Triton X-100, 10 mM Tris/HCl, pH = 7.6, 1 mM EDTA, 0.1% sodium deoxycholate, 500 mM sodium orthovanadate, 50 mM NaF, 0.5 mM phenylmethylsulfonyl fluoride, and 1 mg/ml of antipain and leupeptin). 25 μg of total protein were separated in a 10% SDS-polyacrylamide gel electrophoresis and transferred to a polyvinylidene difluoride membrane (Polyscreen, PerkinElmer Life Sciences). For protein detection, blocked membranes were incubated with specific antibodies, washed, and incubated with a secondary antibody. The immunoreactive bands were visualized with the SuperSignal detection system according to the manufacturer’s procedures (Pierce).

**Nitrite Production**—Nitrite release from endothelial cells was evaluated by a fluorometric assay using 2,3-diaminonaphthalene as described. Fluorescence was determined in a PerkinElmer Life Sciences LS 50B fluorometer using excitation and emission wavelengths of 365 and 450 nm, respectively.

**RNA Isolation and Northern Blot Analysis**—Total cellular RNA from HAEC was isolated with the guanidinium thiocyanate-phenol-chloroform method. Total RNA 10 μg was fractionated by electrophoresis through denaturing 1% agarose, 0.66 mM formaldehyde gels, transferred to Hybond-N (Amer sham Biosciences, Buckinghamshire, UK), and UV cross-linked before hybridization. For Northern analysis, a 1700-bp fragment of human eNOS cDNA were labeled with [32P]dCTP and random hexamers using a kit (Redi Prime, Amersham Biosciences). Hybridization was performed at 42 °C for 12–16 h; membranes were then washed and exposed with XAR Kodak film, using an intensifying screen. Blots were re-hybridized with a human glyceraldehyde-3-phosphate dehydrogenase cDNA.

**eNOS Promoter Activity**—Cell transfection was performed using methods previously reported. HAECs grown to 50–60% confluence in 6-well plates were preincubated in 0.5% endothelial growth media for 30 min at 37 °C. The eNOS promoter constructs (1 μg) and a plasmid containing SV40-driven Renilla luciferase gene (Promega) were mixed with Lipofectamine 2000 (Invitrogen). HAEC were incubated with the plasmids for 6 h and then fed with media. Luciferase activity was determined using the dual Luciferase reporter kit (Promega) according to manufacturer’s directions. Firefly luciferase activity was determined using a luminometer (Monolight 2010, Analytical Luminescence Laboratory, Ann Arbor, MI) and normalized against Renilla activity. The results were normalized as relative luciferase light units/μg of protein. Transfection efficiency was between 30 and 40%.

**Site-Directed Mutagenesis**—Mutagenesis of the eNOS 5′-flanking region was performed with the QuikChange site-directed mutagenesis kit (Stratagene, La Jolla, CA). The SIE consensus sites present in the eNOS promoter were mutated by substituting CC for the TT nucleotides within the SIE consensus binding site TTN(4–5)AA.

**Electrophoretic Mobility Shift Assays**—Proteins were prepared from nuclear extracts as described previously (26). Nuclear extracts (10 μg) from HAECs were incubated with a [γ-32P]ATP-labeled oligonucleotide containing the −1024 eNOS promoter SIE binding element at 22 °C for 30 min. DNA-protein complexes were then electrophoresed through a 5% non-denaturing polyacrylamide gel. The gel was dried and autoradiographed. For competition experiments, a 200-fold molar excess of competitor DNA was incubated in the mixture prior to the addition of the nuclear extracts. For supershift experiments 2 μg of specific antibody against Stat3 or Stat1 (Santa Cruz Biotechnology) were included in the reaction.

**RNA Interference**—STAT-3 expression was knocked down in BAEC with the Silencer siRNA Construction kit from Ambion (Madrid, Spain), according to the manufacturer’s guidelines (26). Predesigned and annealed STAT-3 dsRNA, as well as STAT-3-negative control dsRNA, were also from Ambion (catalog numbers 16704 and 4611, respectively).
Statistical Analysis—Experiments were performed in duplicate and each experiment was repeated at least three times. The variability of the data were described by analysis of variance followed by Dunnett’s modification of the t test whenever comparisons were made with a common control. The unpaired two-tailed Student’s test was used for other comparisons. Results are expressed as mean ± S.E. with p < 0.05 considered statistically significant.

RESULTS

IL-6 Decreases eNOS Expression—To test our hypothesis that IL-6 decreases eNOS expression, HAECs were incubated for 24 h with increasing concentrations of IL-6, and eNOS protein expression was analyzed by immunoblotting. IL-6 decreases steady-state eNOS protein levels in a dose dependent manner (Fig. 1A). As little as 10 ng/ml of IL-6 can decrease eNOS expression. IL-6 affects eNOS expression within 8–16 h of treatment (Fig. 1B). We next determined the effect of IL-6 upon eNOS mRNA expression. IL-6 decreases steady-state levels of eNOS within 6 h of treatment (Fig. 1C). We also measure the nitrite levels produced by the cells in response to IL-6 stimulation. IL-6 decreases the basal nitrite release from HAECs in a dose-response manner (Fig. 1D). Taken together, these data show that IL-6 decreases eNOS expression and NO synthesis in endothelial cells in vitro.

IL-6 Does Not Affect eNOS mRNA Stability—To determine whether IL-6 decreases eNOS mRNA by destabilizing eNOS mRNA, we treated HAEC with IL-6 or vehicle for 8 h, then stopped transcription by adding actinomycin D (5 μM), and then harvested total RNA at increasing times for use in Northern analysis of eNOS mRNA. IL-6 does not affect the stability of the eNOS mRNA transcript (Fig. 2). These results indicate that IL-6 induced changes in eNOS expression involved a transcriptional effect rather than a decrease in mRNA stability.

IL-6 Decreases eNOS Promoter Activity—We first studied the effects of IL-6 upon the human eNOS 5′-flanking region extending 1600 bp upstream from the transcriptional start site. We transfected HAEC with an eNOS promoter-luciferase reporter construct extending from 0 to −1592 upstream of the transcriptional start site, stimulated with increasing amounts of IL-6, and the luciferase activity measured by a luminometer (n = 5 ± S.D.; * p < 0.05 versus 0 ng/ml). B, structure of the eNOS promoter, with the Stat3 binding elements (SIE) in gray boxes.

statistical analysis—Experiments were performed in duplicate and each experiment was repeated at least three times. The variability of the data were described by analysis of variance followed by Dunnett’s modification of the t test whenever comparisons were made with a common control. The unpaired two-tailed Student’s test was used for other comparisons. Results are expressed as mean ± S.E. with p < 0.05 considered statistically significant.
**IL-6 Inhibition of eNOS Expression**

**A.** Fold increase in eNOS promoter activity (RLU/μg prot) for different deletion mutants of the eNOS promoter. The promoter activity is normalized against the activity of the full-length eNOS promoter. IL-6 treatment decreases the promoter activity in a dose-dependent manner.

**B.** Renilla luciferase activity (RLU/μg protein) for different eNOS promoter mutants after IL-6 treatment. The mutants include WT, −1540, −1024, and −840 bp upstream of the eNOS transcription start site. IL-6 treatment significantly decreases luciferase activity.

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eNOS promoter activity in endothelial cells in a dose-dependent manner (Fig. 3A). Since IL-6 activates Stat3, we examined the eNOS promoter region for potential Stat3 binding elements (SIE). The eNOS promoter contains four consensus SIE sites TTN(4–5)AA (at −1501, −1021, −840, and −640) (Fig. 3B).

**Stat3 Binding Element at −1024 Mediates IL-6 Inhibition of eNOS Expression**—To identify which of the SIE mediate IL-6 inhibition of eNOS expression, we first constructed plasmids containing deletions of the human eNOS promoter driving luciferase expression. We co-transfected HAEC with these human eNOS promoter-luciferase plasmids (along with plasmids constitutively expressing Renilla luciferase as internal controls). We then added IL-6 to transfected HAEC and after 24 h measured luciferase activity in cell lysates. IL-6 decreases the activity of the −1500-bp eNOS promoter (Fig. 4A, top row). IL-6 also decreases transactivation of the −1300-bp truncated eNOS promoter (Fig. 4A, second row). However, IL-6 does not affect transactivation of the eNOS promoter deletion constructs lacking bp −1600 to −1000 upstream of the eNOS regulatory region (Fig. 4A, lower three rows). These data suggest that elements within −1600 to −1000 bp upstream of the eNOS transcription start site mediate IL-6 suppression of the eNOS promoter.

We next sought to identify the precise sites within the eNOS promoter that mediate IL-6 suppression of eNOS expression. Based on our promoter deletion data (Fig. 4), we selected the SIE elements between −1000 and −1600 bp for further analysis. To determine which of the SIE elements at −1501 or −1024 mediate IL-6 suppression of the human eNOS promoter, we used site-directed mutagenesis to construct a set of eNOS promoter mutants, each construct containing a 2-bp mutation disrupting one of the three distal SIE elements. HAEC were transfected with these mutants and stimulated with IL-6 for 24 h, and cell lysates were analyzed for luciferase activity. IL-6 decreases the eNOS promoter activity of the wild-type eNOS promoter, and IL-6 also decreases eNOS promoter activity of the eNOS promoter lacking the functional SIE at −1540 bp (Fig. 4B). However, IL-6 does not affect promoter activity of the eNOS promoter lacking the SIE at −1024. Taken together, our data suggest that the SIE at −1024 upstream of the eNOS transcriptional start site mediates IL-6 suppression of the eNOS promoter.

**IL-6 Stimulates Stat3 Phosphorylation in Human Endothelial Cells**—IL-6 activates the APR in part by interacting with the IL-6 receptor which in turn triggers Jak phosphorylation of Stat3. The IL-6 receptor is composed of two subunits, IL-Rα and gp-130. To further investigate the molecular mechanism by which IL-6 inhibits eNOS expression, we analyzed Stat3 phosphorylation in response to IL-6 in endothelial cells. We treated HAEC with IL-6 for 0–240 min and then measured phosphorylation of Stat3. Phosphorylated Stat3 levels increase within 15 min of IL-6 treatment, decrease within 60 min, but remain above basal levels for up to 240 min (Fig. 6A, top). The IL-6 receptor subunit gp-130 is expressed in HAECs, and IL-6 does not change its expression (Fig. 6A, bottom). The treatment of HAECs with a Jak inhibitor (tyrphostin A) was able to completely prevent IL-6 induced Stat3 phosphorylation (Fig. 6B). Thus IL-6 is capable of initiating the JAK/stat3 pathway in HAEC.

To confirm that IL-6 activates the ability of Stat3 to interact with its DNA response element, we measured binding activity in endothelial nuclear extracts. We treated HAEC with IL-6,
harvested nuclear extracts, and incubated nuclear extracts with a radiolabeled eNOS fragment spanning −1031 to −1011 that contained the Stat3 binding site SIE at −1024. Nuclear protein-DNA complexes were fractionated by non-denaturing polyacrylamide gel and autoradiographed. IL-6 increases SIE binding activity in endothelial cells 15 min after treatment and peaks 30 min after treatment (Fig. 5C). Excess non-labeled SIE oligonucleotide competes for this binding activity. Addition of an antibody to Stat3 produces a supershift in the binding to the SIE element, indicating that Stat3 is present in the protein-DNA complex. In contrast, an antibody to Stat1 has no effect on the protein-DNA complex. Normal mouse IgG does not affect the protein-DNA complex. Thus IL-6 activates Stat3 binding to a complex containing the eNOS SIE −1024 site oligonucleotide. These data suggest that IL-6 activates Stat3 interaction with the eNOS promoter at an SIE site at position −1024.

**Stat3 Pathway Mediates eNOS Inhibition by IL-6**—To confirm our results that Stat3 mediates IL-6 suppression of eNOS, we tested the effects of other Stat3 activating cytokines upon eNOS protein expression. Onconstatin M (OSM), tumor necrosis factor α (TNF-α), and EGF are each capable of stimulating Stat3. HAEC were stimulated with OSM, TNF-α, EGF, or IL-6 at increasing concentrations for 24 h. OSM and TNF-α reduced eNOS expression in parallel to IL-6 in a dose dependent manner (Fig. 6A). However, EGF treatment had little effect on eNOS expression. Furthermore, IL-6, OSM, and TNF-α also reduced nitrite production whereas EGF did not (Fig. 6B). Thus, cytokines that activate the Stat3 pathway also reduced eNOS expression and activity.

We next used RNA interference to test the role of Stat3 in IL-6 suppression of eNOS. HAEC were transfected with Stat3 siRNA or a nonspecific siRNA as control. Stat3 siRNA decreases Stat3 levels (Fig. 7A, middle panel). We then treated HAEC with IL-6 for 24 h. As before, IL-6 decreases eNOS expression in control transfected cells (Fig. 7A, right top lanes). In contrast, IL-6 has no effect upon eNOS expression in HAEC transfected with the Stat3 siRNA (Fig. 7A, left top lanes).

Taken together, these results suggest that Stat3 mediates IL-6 suppression of eNOS expression by interacting with an SIE element at −1024 within the human eNOS regulatory region.

**DISCUSSION**

The main finding of our study is that IL-6 decreases eNOS gene expression in part by activating Stat3 binding to the eNOS 5'-flanking region.

**Inflammatory Signals Decrease eNOS Expression**—Our results support the findings of others that inflammatory mediators decrease eNOS expression (27–29). TNF-α can decrease eNOS expression by inhibiting eNOS promoter transactivation and also by destabilizing eNOS mRNA (30–32). CRP, an effector of the APR, decreases eNOS expression by destabilizing its mRNA (33, 34). Our current study shows that IL-6, a major trigger of the APR, also decreases eNOS expression. Our work

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**FIGURE 5. IL-6 activates Stat3 in endothelial cells.** A, HAEC were treated with 10 ng/ml IL-6 for 0–4 h, and cell lysates were analyzed by immunoblotting. B, HAEC were treated with 10 ng/ml IL-6 for 30 min in the presence or the absence of tyrphostin A (1 µM). C, nuclear extracts from HAEC stimulated with IL-6 were incubated with radiolabeled SIE probe, non-labeled probe (C), mutant non-labeled probe (M), or antibody to Stat1 or Stat3 or IgG. These experiments were repeated three times with similar results.

**FIGURE 6. Cytokines that activate the Stat3 pathway also decrease eNOS expression.** A, immunoblot analysis of HAEC treated with IL-6, OSM, TNF-α, or EGF for 24 h. B, nitrite production of HAEC treated with control (CT), 10 ng/ml IL-6, 10 ng/ml TNF-α, 10 ng/ml EGF, or 1 µM OSM for 24 h (n = 3 ± S.D.; *, p < 0.05 versus control).

**FIGURE 7. Silencing of Stat3 reverses IL-6 inhibition of eNOS expression.** Immunoblot analysis of HAEC transfected with Stat3 RNAi or control RNAi and then stimulated with 0–10 ng/ml IL-6 for 24 h.
add to the findings of others who have identified transcription factors regulating eNOS promoter transactivation, including Sp1, GATA, AP-1, NF-1, and Smad2 (26, 27, 35–37).

Stat3 Isoforms and eNOS Regulation—The Stat3 gene is alternatively spliced into two distinct mRNA species encoding separate isoforms, Stat3α and Stat3β (38, 39). Stat3α contains a DNA binding domain and a transcriptional activation domain, so Stat3 can interact with SIE elements and drive transcription of genes. However, Stat3β only contains a DNA binding domain and lacks a transcriptional activation domain, so Stat3β can interact with SIE elements but does not drive transcription. Traditionally, Stat3β has been considered a dominant negative isoform of Stat3. The ratio of Stat3α and Stat3β varies in cells, ranging from 1:3 to 10:1 at the protein level. This variation may have important biologic consequences. Specific ablation of the Stat3β isoform dramatically affects the pattern of gene expression in endotoxic shock, indicating a central role of this isoform in modulating systemic inflammation (40, 41). Recently, an important study showed that Stat3α and Stat3β have distinct functions and that Stat3β is not merely a dominant-negative factor, as has been generally thought. Expression of Stat3β can rescue the embryonic lethality of the Stat3α null mutation, and it can, by itself, induce the expression of specific Stat3 target genes. Nevertheless, Stat3α is the isoform that mediates the cell response to cytokines such as IL-6 or IL-10 (41). Although our studies show that IL-6 treatment leads to Stat3 phosphorylation and binding to the eNOS promoter, our studies do not distinguish which Stat3 isoform represses eNOS transcription.

IL-6 May Decrease eNOS Expression in Atherosclerosis—Recent studies point to IL-6 as a marker of cardiovascular disease as well as systemic inflammation (3–8). IL-6 plasma levels are elevated in myocardial infarction, unstable angina, and atherosclerosis. Chronic inflammation may trigger synthesis of IL-6, which activates Stat3; Stat3 in turn drives transcription of acute phase response reactants such as CRP which contribute to the development and progression of atherosclerosis (42, 43). Risk factors for atherosclerosis are associated with endothelial dysfunction, characterized in part by a deficient production or response to nitric oxide. Our finding that IL-6 decreases eNOS expression provides a mechanism by which the acute phase response contributes to the progression of cardiovascular disease (14, 15).

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