Shear Stress Attenuates Tumor Necrosis Factor-α-Induced Monocyte Chemotactic Protein-1 Expressions in Endothelial Cells

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Abstract

The interplay between shear stress and cytokines in regulating vascular endothelial function remains largely unexplored. In the present study, the potential role of shear stress in regulating tumor necrosis factor-α (TNF-α)-induced gene expression in endothelial cells (ECs) was investigated. The TNF-α-induced monocyte chemotactic protein-1 (MCP-1) mRNA expressions were significantly attenuated in ECs subjected to a high level of shear stress (20 dynes/cm^2) for 4 or 24 h prior to the addition of TNF-α in the presence of flow. Less inhibition of TNF-α-induced MCP-1 mRNA expression was found in ECs pre-exposed to a low level of shear stress (1.2 dynes/cm^2) for 24 h as compared with the cells presheared (pre-exposed to shear stress) for 4 h. Simultaneous exposure of ECs to TNF-α and a high or low level of shear stress down-regulated TNF-α-induced MCP-1 gene expressions, suggesting that the post-flow condition modulates endothelial responses to cytokine stimulation. Individually or combined, an endothelial nitric oxide synthase (eNOS) inhibitor and a glutathione (GSH) biosynthesis inhibitor had no effect on this shear stress-mediated inhibition. Moreover, in ECs either presheared or remained in a static condition prior to stimulation by TNF-α while under shear flow, the ability of TNF-α to induce AP-1-DNA binding activity in the nucleus was reduced. Our findings suggest that shear stress plays a protective role in vascular homeostasis by inhibiting endothelial responses to cytokine stimulation.

Key Words: endothelial cell, cytokine, monocyte chemotactic protein, shear stress

Introduction

Adhesion of circulating monocytes to vascular endothelial cells (ECs) is an initial step in the sequence of events leading to atherosclerosis (1-3). Early atherosclerotic lesions preferentially form at sites of branching and curvature in the arterial system, where the local flow is often disturbed (e.g., flow separation, recirculation, and flow reattachment) (4, 5), suggesting that hemodynamic forces play a role in the initiation and progression of the disease. There is considerable evidence that hemodynamic forces, including fluid shear stress, exert significant influences on ECs, mainly through changes in gene expression. A number of pathophysiologically relevant genes, such as intercellular adhesion molecule-1 (ICAM-1) (6-10), early growth response-1 (Egr-1) (11-13), and platelet-derived growth factor (PDGF)-B chain (14, 15), have been shown to be regulated by shear stress. Moreover, the expression of various chemokines, including monocyte chemotactic protein-1 (MCP-1) (16, 17), is also activated by shear flow, and these chemokines function as adhesion factors of monocytes on ECs subjected to hemodynamic forces.

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In addition to shear stress, a variety of agents including cytokines are well known to be involved in the formation and progression of atherosclerotic lesions (3). Cytokine induction of a number of atherogenesis-related genes has been amply reported (2, 3). However, the effect of shear stress and cytokine synergy on modulating pathophysiologically relevant gene expression in ECs remains largely unexplored. It has been shown that shear stress modulates endothelial adhesiveness by inhibiting the tumor necrosis factor (TNF)-α-induced vascular cell adhesion molecule-1 (VCAM-1) expression on the EC surface (18, 19). Kawai et al. (20) found that IL-1β or TNF-α stimulation under shear stress augments the tissue plasminogen activator (t-PA) expression, whereas shear stress attenuates plasminogen activator inhibitor (PAI-1) secretion induced by IL-β or TNF-α. They further demonstrated that shear stress inhibits TNF-α-induced endothelial expression of tissue factor (TF) gene (21). Recently, Surapisitchat et al (22) demonstrated that steady laminar flow inhibits cytokine-mediated activation of c-Jun NH_2-terminal kinase (JNK) in ECs *via* the activation of the extracellular signal-regulated kinase1/2 (ERK1/2) signaling pathway. These observations suggest a role for shear stress in modulating cytokine stimulation of gene expression in ECs. Since atherosclerosis is a multifactorial disease involving a complex array of contributing factors including shear stress and cytokines, we believe that the effect of shear stress on endothelial function would be more appropriately investigated in the presence of cytokine stimulation.

In the present study, to investigate the interplay between shear stress and cytokines in modulating the monocyte recruitment process, we analyzed the effect of shear stress on TNF-α-induced MCP-1 gene expressions in ECs. We found that TNF-α induction of MCP-1 in ECs was attenuated by shear stress, suggesting that shear force acts as an intrinsic modulator of monocyte recruitment to the vascular wall.

Materials and Methods

Materials

The MCP-1 cDNA probe was a gift from Dr. D. L. Wang (Academia Sinica, Taiwan, ROC). Buthionine sulfoximine (BSO) and N^G-monomethyl-L-arginine (L-NMMA) were purchased from Calbiochem (San Diego, CA, USA). The AP-1 consensus oligonucleotide was obtained from Promega (E3201; Madison, WI, USA). All other chemicals, of reagent grade, were obtained from Sigma (St. Louis, MO, USA).

Endothelial Cell Culture

ECs were isolated from fresh human umbilical cords by means of the collagenase perfusion technique as described previously (23). The cell pellet was resuspended in a culture medium consisting of medium 199 (M199, Gibco, Grand Island, NY, USA) supplemented with 20% fetal bovine serum (FBS, Gibco) and 1% penicillin/streptomycin (Gibco). ECs were grown in Petri dishes for 3 days and then seeded onto glass slides (75 by 38 mm, Corning, NY, USA) precoated with fibronectin (Sigma) to reach confluence. The culture medium was then exchanged with medium that was identical except that it contained only 2% FBS, and the cells were further incubated for 24 h prior to the experiment.

Flow Apparatus

The slide with cultured ECs was mounted in a parallel-plate flow chamber, which has been characterized and described in detail elsewhere (24). The chamber was connected to a perfusion loop system, kept in a constant-temperature controlled enclosure, and maintained at pH 7.4 by continuous gassing with a mixture of 5% CO_2 in air. The osmolality of the perfusate was adjusted to 285 to 295 mOsm/kg H_2O during the perfusion. The flow channel width (w) was 1 cm, and the channel height (h) was 0.025 cm. The wall shear stresses (τ) produced by the flow chamber in the present study were estimated to be 20 and 1.2 dynes/cm^2, using the formula $\tau = 6\mu Q/wh^2$, where τ is the shear stress, μ is the viscosity of the perfusate, and Q is the flow rate.

Experimental Procedure

To test the inhibitory effect of shear stress on TNF-α-induced MCP-1 gene expressions in ECs, EC monolayers were incubated in the static condition or pre-exposed to flow for 4 or 24 h at designated shear stress, and then subjected to the identical flow condition for another 4 h in the presence of TNF-α (100 U/ml). ECs treated with TNF-α for 4 h were used as positive controls. In some experiments, ECs were individually or simultaneously pretreated with L-NMMA (500 or 1000 μmol/l), an endothelial nitric oxide synthase (eNOS) inhibitor, and BSO (10 or 30 μmol/l), a glutathione (GSH) biosynthesis inhibitor, for 1 h, and then stimulated by TNF-α in the presence of the same reagents under flow. In additional experiments, nuclear protein extracts were obtained from the experimental cells for elucidating the effect of shear stress on TNF-α-induced AP-1-DNA binding activities in the nucleus by using the electrophoretic mobility shift assay (EMSA).

RNA Isolation and Northern Blot Analysis

After the treatment, total RNA was isolated from ECs by the guanidium isothiocyanate/phenochloroform method as described previously (10). The RNA (10 μg/lane) was separated by electrophoresis on a 1% agarose formaldehyde gel and transferred onto a nylon membrane (Nytran, Schleicher & Schuell Inc., Germany) by a vacuum blotting system (VacuGene XL, Pharmacia, Piscataway, NJ, USA). After hybridization with the ^{32}P-labeled MCP-1 cDNA probes, the membrane was washed with 1x saline-sodium citrate (SSC) containing 1% sodium dodecylsulfate (SDS) at room temperature for 30 min and then exposed to X-ray film (Kodak X-Omat-AR, Rochester, NY, USA) at -70°C. Autoradiographic results were scanned and analyzed by using a densitometer (Computing Densitometer 300S, Molecular Dynamics, Sunnyvale, CA, USA).

Electrophoretic Mobility Shift Assay (EMSA)

To prepare nuclear protein extracts, ECs were washed with cold PBS and then immediately removed by scraping in PBS. After centrifugation of the cell suspension at 2000 rpm, the cell pellets were resuspended in cold buffer A (containing, in mmol/L, KCl 10, ethylenediamine tetraacetate (EDTA) 0.1, dithiothreitol (DTT) 1, and phenyl methylsulfonyl fluoride (PMSF) 1) for 15 min. The cells were lysed by adding 10% Nonidet P40 (NP-40) and then centrifuged at 6000 rpm to obtain pellets of nuclei. The nuclear pellets were resuspended in cold buffer B (containing, in mmol/L, 4-(2-hydroxyethyl)-1-piperazine-ethane-sulfonic acid [HEPES] 20, EDTA 1, DTT 1, and PMSF 1, and NaCl 400), vigorously agitated, and then centrifuged. The supernatant containing the nuclear proteins was used for the EMSA or stored at -70°C until used. Double-stranded oligonucleotides (21 bp; Promega Corp. Madison, WI, USA) containing the consensus DNA binding site for AP-1 (5'-TGAGTCAG-3') were end labeled with [γ-^{32}P]ATP. Extracted nuclear proteins (10 μg) were incubated with 0.1 ng ^{32}P-labeled DNA for 15 min at room temperature in 25 μl binding buffer containing 1 μg poly(dI-dC). The mixtures were electrophoresed on 5% nondenaturing polyacrylamide gels. Gels were dried and imaged by autoradiography.

Statistical Analysis

Results were expressed as mean ± SEM. Significance was determined by using the Student's *t*-test, and the level of statistical significance was defined as $P < 0.05$ from four separate experiments for all comparisons.

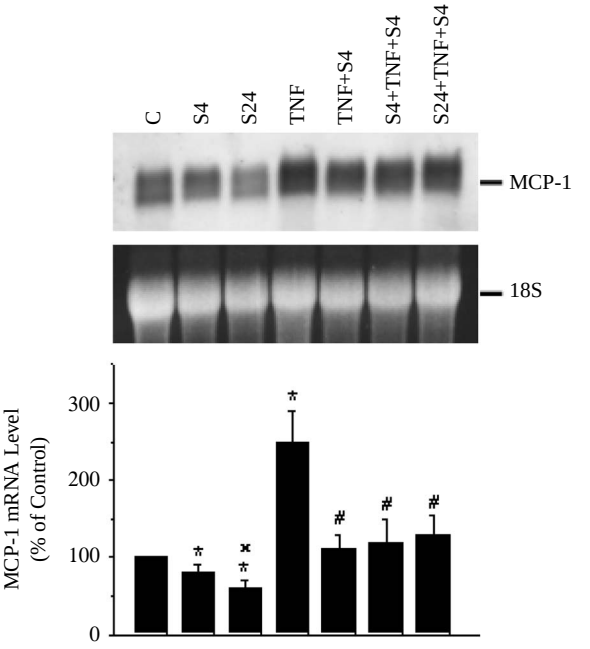

Fig. 1. A high level of shear stress attenuates TNF-α-induced MCP-1 gene expression in ECs. ECs were remained in a static condition (C) or exposed to shear stress of 20 dynes/cm^2 for 4 (S4) and 24 h (S24). In additional experiments, ECs were remained in a static condition (TNF+S4) or exposed to shear stress of 20 dynes/cm^2 for 4 (S4+TNF+S4) and 24 h (S24+TNF+S4) before addition of TNF-α (100 U/ml) under flow for 4 h, and their mRNA expressions were determined using Northern blot analysis, as described in Materials and Methods. ECs treated with TNF-α (100 U/ml) for 4 h were used as positive controls (TNF). Data are presented as a percentage change (relative to static controls) in band density, normalized to 18S RNA levels and are shown as mean ± SEM from four independent experiments. $^{*}P < 0.05$ vs static control ECs. $^{\times}P < 0.05$ *vs* ECs exposed to flow for 4 h. $^{\#}P < 0.05$ *vs* TNF-α-treated ECs.

Results

Shear Stress Attenuates TNF-α-Induced MCP-1 Gene Expression in ECs

To investigate the synergistic effect of shear stress and cytokines on modulating endothelial gene expression, ECs were pre-exposed to shear stress of 20 or 1.2 dynes/cm^2 for 4 or 24 h, and then subjected to TNF-α stimulation under the identical flow condition for 4 h. In addition, ECs were simultaneously subjected to the designated shear stress and TNF-α stimulation for 4 h to elucidate the post-flow effect on TNF-α-induced gene expression in ECs. The MCP-1 mRNA expression in the cells was examined by Northern blot analysis, as described in Materials and Methods. As shown in Figure 1, ECs exposed to shear stress of 20 dynes/cm^2 for 4 h had significantly attenuated MCP-1 mRNA levels as compared with cells in the static control ($P < 0.05$). The MCP-1

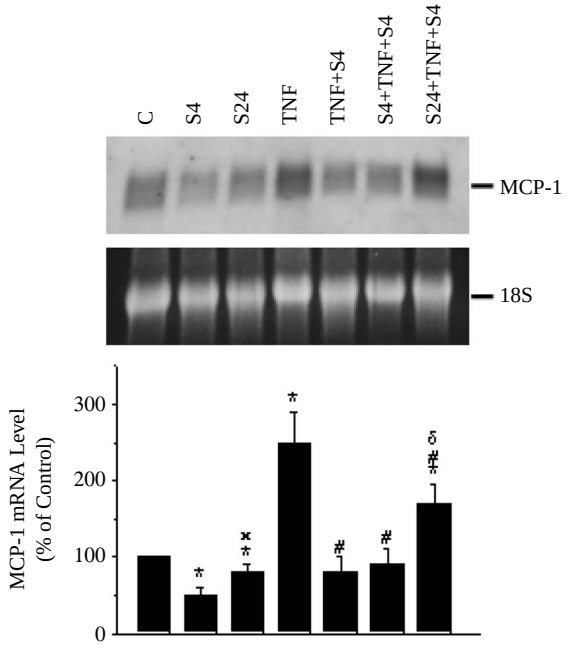

Fig. 2. Prolonged pre-exposure of ECs to a low level of shear stress results in less inhibition of TNF-α-induced MCP-1 gene expression in ECs. ECs were remained in a static condition (C) or exposed to shear stress of 1.2 dynes/cm^2 for 4 (S4) and 24 h (S24). In additional experiments, ECs were remained in a static condition (TNF+S4) or exposed to shear stress of 1.2 dynes/cm^2 for 4 (S4+TNF+S4) and 24 h (S24+TNF+S4) before addition of TNF-α (100 U/mL) under flow for 4 h, and their mRNA expressions were determined using Northern blot analysis, as described in Materials and Methods. ECs treated with TNF-α (100 U/ml) for 4 h were used as positive controls (TNF). Data are presented as a percentage change (relative to static controls) in band density, normalized to 18S RNA levels and are shown as mean ± SEM from four independent experiments. $^*P < 0.05$ *vs* static control ECs. $^{\times}P < 0.05$ *vs* ECs exposed to flow for 4 h. $^{\#}P < 0.05$ *vs* TNF-α-treated ECs. $^{\delta}P < 0.05$ *vs* ECs pre-exposed to flow for 4 h.

mRNA expression of ECs exposed to 24 h of flow (20 dynes/cm^2) was more inhibited than that found in the ECs exposed to flow for only 4 h ($P < 0.05$). These results are consistent with those of Shyy et al. [16]. Simultaneous exposure of ECs to shear stress of 20 dynes/cm^2 and TNF-α for 4 h significantly reduced TNF-α-induced MCP-1 mRNA expression ($P < 0.01$). Moreover, ECs presheared for 4 and 24 h and then treated with TNF-α under flow also reduced TNF-α-induced MCP-1 gene expression ($P < 0.01$, respectively). The inhibitory effect of low shear stress (1.2 dynes/cm^2) on TNF-α-induced MCP-1 gene expression in ECs is shown in Figure 2. In contrast to high shear stress, low shear stress resulted in higher MCP-1 mRNA levels in ECs exposed for 24 h than in cells exposed for 4 h ($P < 0.05$). Less inhibition of TNF-α-induced MCP-1 mRNA expression was found in ECs pre-exposed to shear stress of 1.2 dynes/cm^2 for 24 h as compared with the cells presheared for 4 h ($P < 0.05$). This result implies that prolonged exposure of ECs to low shear stress may render the cells more vulnerable to cytokine-stimulated MCP-1 gene expression and thereby to monocyte adhesion.

The Inhibitory Effect of Shear Stress on TNF-α-Induced MCP-1 Expressions is not via the eNOS and GSH Redox Cycle Pathways

ECs subjected to shear stress constantly release NO (18, 25-27) and adjust their intracellular redox status (28, 29). To investigate whether NO and intracellular redox system are involved in shear stress regulation of TNF-α-induced gene expression, ECs were pretreated with L-NMMA, an eNOS inhibitor, and/or BSO, a GSH biosynthesis inhibitor, at varied concentrations (L-NMMA: 500 or 1000 μmol/l; BSO: 10 or 30 μmol/l) for 1 h. Then the cells were subjected to TNF-α stimulation under flow (20 dynes/cm^2) in the presence of the same reagents. As shown in Figure 3A, ECs pretreated with either L-NMMA (500 μmol/l) or BSO (10 μmol/l) or the combination of these reagents did not alter the inhibitory effect of shear stress on TNF-α-induced MCP-1 mRNA expressions. Moreover, pretreatments of ECs with a higher concentration of L-NMMA (1000 μmol/l) and/or BSO (30 μmol/l) also had no effect on this shear stress-mediated inhibition of TNF-α-induced gene expression (Figure 3B). The same treatments with these inhibitors had no significant effect on the mRNA expression of MCP-1 gene in the control cells (data not shown). These results indicate that the eNOS and intracellular GSH cycle system may not be involved in the regulatory effect of shear stress on TNF-α-stimulated MCP-1 expressions in ECs.

Shear Stress Attenuates AP-1-DNA Binding Activity in the Nucleus Induced by TNF-α

The transcriptional pathway mediated by AP-1 is activated by various stimuli including cytokines and shear stress (17, 30). Since the promoter region of the MCP-1 gene contains the AP-1 binding domain which has been shown to be responsive to shear stress (17), we postulated that shear stress regulates TNF-α-induced gene expression in ECs by changing AP-1-DNA binding activity. To test this hypothesis, nuclear proteins of ECs that were either remained in the static condition or exposed to 20 dynes/cm^2 for 4 or 24 h before being subjected to flow in the presence of TNF-α were compared. As shown in Figure 4, when nuclear proteins extracted from TNF-α-treated ECs were incubated with oligonucleotides corresponding to the AP-1 binding sequences, increased binding activity occurred. Simultaneous exposure of ECs to

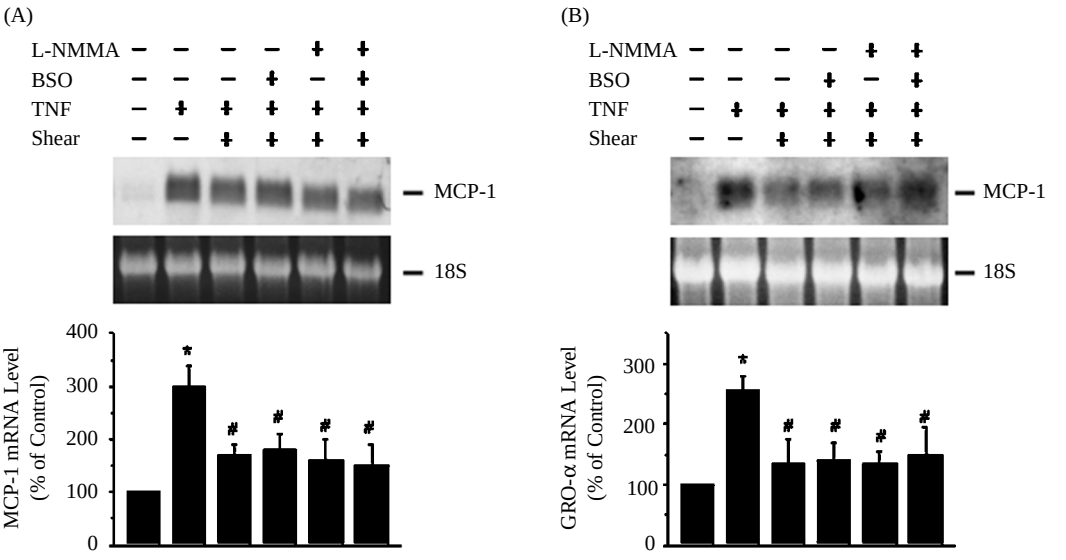

Fig. 3. The inhibitory effect of shear stress on TNF-α-induced MCP-1 gene expression is not *via* eNOS and GSH redox cycle pathways. ECs were untreated or treated with BSO or L-NMMA at a (A) lower concentration (10 or 500 μmol/l, respectively) or (B) higher concentration (30 or 1000 μmol/l, respectively), or the combination of these reagents for 1 h before being subjected to TNF-α (100 U/ml) stimulation under flow for 4 h in the absence or presence of these reagents. Autoradiographic results were analyzed by a densitometer. Data are presented as a percentage change (relative to static controls) in band density, normalized to 18S RNA levels and are shown as mean ± SEM from four independent experiments. $^{*}P < 0.05$ *vs* static control ECs. $^{\#}P < 0.05$ *vs* TNF-α-treated ECs.

shear stress and TNF-α caused an attenuation of this binding activity. Pre-exposure of ECs to flow for 4 and 24 h further attenuated this TNF-α-induced AP-1-DNA binding activity. This binding was obviously specific for AP-1, because it was abolished by co-incubation with 20-fold excess unlabeled oligonucleotides. These results indicate that shear stress inhibits the transcriptional pathway stimulated by TNF-α, which may consequently contribute to its inhibitory effect on TNF-α-induced MCP-1 gene expression in ECs.

Discussion

Atherosclerosis is a multifactorial disease that develops from the interaction of various cytokines, growth factors, and circulating blood cells with vascular wall, and involves the effects of hemodynamic forces on mass transfer. Within days of exposure to a high-cholesterol diet, monocytes begin to adhere to the vascular endothelium (31). This phenomenon is thought to be mediated by alterations in the endothelial adhesiveness due to the induced expression of adhesion molecules and chemotactic proteins, such as MCP-1. Cytokine and hemodynamic forces including shear stress are well known to activate a number of pathophysiologically relevant gene expressions in ECs (2, 3). However, the interplay between these factors in regulating endothelial responses to various stimuli (e.g., chemical and mechanical) remains largely unclear. It is conceivable that the synergistic effect of cytokines and hemodynamic forces may influence endothelial function in response to environmental stimuli that ultimately affect vascular biology.

The present study provides several lines of evidence to demonstrate that the fluid shear stress plays a pivotal role in mediating endothelial responses to cytokine stimulation. First, the TNF-α-induced MCP-1 mRNA expressions of ECs were significantly attenuated by subjecting these cells to shear stress of 20 or 1.2 dynes/cm^2 for 4 or 24 h prior to the addition of TNF-α under flow conditions. Second, the simultaneous exposure of ECs to shear stress and TNF-α down-regulated TNF-α-induced MCP-1 gene expressions in ECs. This suggests that the post-flow condition exerts a modulating influence on endothelial responses to cytokine stimulation. Third, individual or simultaneous addition of L-NMMA, an eNOS inhibitor, and BSO, a GSH biosynthesis inhibitor, had no effect on this shear stress-mediated inhibition of TNF-α-induced gene expression1. This implies that both eNOS and GSH redox cycle system may not be involved in the regulatory effect of shear stress on the TNF-α-induced MCP-1 expressions in ECs. Finally, the AP-1-DNA binding activity was reduced in the nucleus of ECs that were either exposed to shear stress or remained in a static condition prior to TNF-α stimulation under flow. These findings suggest that shear stress plays a role in modulating the cytokine-

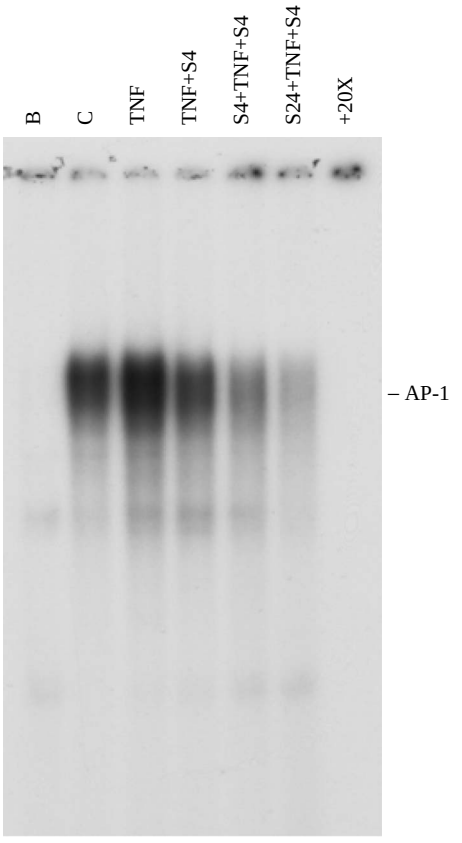

Fig. 4. Shear stress attenuates AP-1-DNA binding activity in the nucleus induced by TNF-α. ECs were in static condition (C) or treated with TNF-α (100 U/ml) for 4 h (TNF). Before treatment of TNF-α under flow conditions, ECs were kept in static condition (TNF+S4) or pre-exposed to shear stress of 20 dynes/cm^2 for 4 (S4+TNF+S4) and 24 h (S24+TNF+S4). Total nuclear extracts were prepared and analyzed by EMSA using a ^{32}P-labeled oligonucleotide probe containing the AP-1 binding sites. The specificity of the retarded complexes (AP-1) was assessed by preincubating the nuclear extracts with excess unlabeled oligonucleotides containing AP-1 binding sequences as a competitor (+20X). ^{32}P-labeled oligonucleotide probe incubated with reaction buffer only was used as a negative control (B). Results are representative of duplicate experiments with similar results.

induced signal transduction pathway, especially at the transcriptional level.

In human carotid and coronary arteries, atherosclerotic plaques are found in the vicinity of arterial bifurcations and bends, where laminar flow is disturbed, and where recirculation establishes areas of low shear stress (4). Experimental reduction of flow in the rabbit carotid artery induces monocyte adherence and transmigration *in vivo* (32). In contrast, regions of carotid bifurcation that experience relatively higher shear stress as the result of ordered laminar blood flow patterns, however, were protected from atherosclerosis. These observations suggest a role for the nature and magnitude of shear stress in long-term maintenance of the structure and function of the blood vessel. In the current study, the TNF-α-induced MCP-1 gene expressions were significantly reduced in ECs pre-exposed to both high (20 dynes/cm^2) and low (1.2 dynes/cm^2) shear stresses for 4 or 24 h and then treated with TNF-α under flow. However, prolonged (24 h) in contrast to short (4 h) exposure to low shear stress caused less inhibition of TNF-α-induced MCP-1 gene expression. This result implies that low shear stress affords ECs less protection from cytokine-activated atherosclerosis-related gene expression. Since 1) vascular ECs are constantly subjected to the shear stress and 2) atherosclerotic lesions usually develop in regions with flow disturbance and low shear stress distribution, it is mostly likely that the less inhibitory effect of low shear stress may render the ECs more vulnerable to environmental stimuli (e.g., chemical, toxic, and viral) that induce MCP-1 expression and thereby monocyte adhesion in these disease-prone sites.

Of more interest in our current study is that the post-flow conditions attenuated TNF-α induction of MCP-1 in ECs. Exposure of ECs to both shear stress and TNF-α simultaneously or exposure of ECs to shear stress for 4 h prior to TNF-α resulted in similar reductions of TNF-α-induced gene expressions. This result indicates that the inhibitory effects of pre- and post-flow conditions were not additive. Whether the post-flow effect controls or dominates the preshear effect on the inhibition of TNF-α-induced gene expression remains unclear. However, our recent study demonstrated that the MCP-1 mRNA levels in ECs presheared (20 dynes/cm^2) for 4 h and then treated 1 h with TNF-α was lower than that in cells not presheared (data not shown). These results imply that preshearing may exert a stronger inhibitory effect at an earlier stage of stimulation.

The mechanisms by which shear stress regulates TNF-α-induced gene expression in ECs remain unclear. Shear stress applied to ECs increases their eNOS mRNA level and NO production (18, 25-27). Studies by Tsao et al. (18, 19) indicated that ECs pre-exposed to shear stress had less adhesiveness for monocytes. They concluded that this phenomenon was due to flow-induced NO rather than alterations in the expression of adhesion molecules. De Caterina et al. (33) reported that NO could inhibit cytokine-induced expression of adhesion molecules in ECs such as VCAM-1 and E-selectin. Khan et al. (34) further demonstrated that this inhibitory effect was NO concentration dependent and regulated at the transcriptional level by a redox-sensitive mechanism in ECs. We have recently demonstrated that NO regulates shear stress-induced Egr-1 expression *via* the extracellular signal-regulated kinase (ERK) pathway in ECs (13). These results suggest a role of NO in modulating the responses of ECs to mechanical

and chemical stimuli. On the other hand, shear stress has been shown to alter the intracellular redox status in the cells (28, 29). Our recent study [10] demonstrated that shear flow to ECs induces intracellular reactive oxygen species (ROS) generation that may result in an increase of ICAM-1 mRNA levels *via* transcriptional events. Whether the shear stress inhibition of TNF-α-induced MCP-1 expressions in ECs resulted from the alteration of intracellular redox system and NO production in sheared ECs remains unclear. In the present study, addition of an eNOS inhibitor (L-NMMA) or a GSH biosynthesis inhibitor (BSO) to the sheared ECs did not restore the TNF-α-induced MCP-1 mRNA level to that of cells without shearing. Moreover, combined administration of L-NMMA and BSO also had no effect on this shear stress modulation of TNF-α-induced gene expression. These results rule out the involvement of the intracellular redox system and NO production in the shear stress modulation of TNF-α-induced gene expression in ECs. The precise molecular mechanism by which shear stress regulates TNF-α-induced gene expression in ECs remains a complicated issue that warrants further investigation.

Cytokines and mechanical forces are well known mediators of gene expression in cells via activation of transcriptional factors, including nuclear factor-κB (NF-κB) and activator protein-1 (AP-1) (2, 15, 17, 30). Molecular cloning of MCP-1 has provided evidence for NF-κB- and AP-1-binding domains in the promoter region of this gene. Shear stress inhibition of TNF-α-induced NF-κB-DNA binding activity in ECs has been reported (19). However, the regulatory effect of shear stress on cytokine-stimulated AP-1-DNA binding activity remains unclear. The recent study by Shyy et al. (17) has demonstrated that the AP-1 binding element in the promoter region of MCP-1 gene is responsible for the shear stress inducibility of this gene. Thus, the inhibitory effect of shear stress on TNF-α-induced MCP-1 gene expression could be due to the inhibition of AP-1-DNA binding activity induced by TNF-α. In the present study, the TNF-α-induced AP-1-DNA binding activity was significantly reduced in ECs that were exposed to shear stress or remained in a static condition before TNF-α treatment under flow conditions. This result suggests that shear stress exerts significant influences on the signal transduction pathway, especially at the transcriptional level. Thus, although the precise mechanism by which shear stress inhibits cytokine-induced endothelial gene expression is not well understood, our data clearly indicates that this inhibition could be due to the modulation of signal events at the transcriptional level.

In summary, our present study clearly demonstrates that shear stress inhibits TNF-α-induced MCP-1 gene expressions in ECs. The regulatory effect of shear stress on this cytokine-induced gene expression could be attributed to effects on the signal transduction pathway at the transcriptional level. Our findings suggest that shear stress acts as a protective regulator of cytokine-stimulated gene expression in ECs.

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