Oscillatory Shear Stress Stimulates Adhesion Molecule Expression in Cultured Human Endothelium

David C. Chappell, Signe E. Varner, Robert M. Nerem, Russell M. Medford, R. Wayne Alexander

Abstract—Low and oscillatory shear stresses are major features of the hemodynamic environment of sites opposite arterial flow dividers that are predisposed to atherosclerosis. Atherosclerosis is a focal inflammatory disease characterized initially by the recruitment of mononuclear cells into the arterial wall. The specific characteristics of the hemodynamic environment that facilitate the generation of arterial inflammatory responses in the presence of, for example, hyperlipidemia are unknown. We show here that prolonged oscillatory shear stress induces expression of endothelial cell leukocyte adhesion molecules, which are centrally important in mediating leukocyte localization into the arterial wall. Vascular cell adhesion molecule-1 was upregulated an average 9-fold relative to endothelial monolayers in static culture. Intercellular adhesion molecule-1 and E-selectin exhibited 11-fold and 7.5-fold increases, respectively. Uprogulation of these adhesion molecules was associated with enhanced monocyte adhesion. Cytokine stimulation of surface vascular cell adhesion molecule-1 was maximally induced after 6 and 8 hours of cytokine incubation. Oscillatory shear stress for these time periods elicited respective vascular cell adhesion molecule-1 levels of 16% and 30% relative to those observed for cytokine stimulation. Surface intercellular adhesion molecule-1 induction by cytokine stimulation for 24 hours was found to be approximately five times the level detected after 24 hours of oscillatory shear stress. Experiments performed in the presence of the antioxidant N-acetylcysteine demonstrated that the expression of vascular cell adhesion molecule-1 could be almost totally abolished, whereas that of intercellular adhesion molecule-1 was typically reduced by ≈70%. These results imply that oscillatory shear stress per se is sufficient to stimulate mononuclear leukocyte adhesion and, presumptively, migration into the arterial wall. These results further indicate that atherosclerotic lesion initiation is likely related, at least in part, to unique signals generated by oscillatory shear stress and that the mechanism of upregulation is, to some extent, redox sensitive. (Circ Res. 1998;82:532-539.)

Key Words: flow ■ endothelium ■ monocyte ■ adherence ■ immunofluorescence

Atherosclerosis is an inflammatory disease of the vasculature and, initially, is highly focal in nature, occurring primarily in regions of branching and at bifurcations. It has been shown1,2 that the occurrence of atherosclerotic lesions in the human carotid bifurcation and the abdominal aorta strongly correlates with low shear regions experiencing an almost purely oscillatory flow. It was found that marked intimal thickening was most extensive along the outer wall of the carotid sinus, where the mean shear stress was low, and the instantaneous shear stress was calculated to oscillate between −7 and +4 dynes/cm².

One of the initial events in atherogenesis is the adherence of mononuclear leukocytes to the endothelial cell surface.3 This binding phenomenon is mediated, at least in part, by adhesion molecules,4 particularly VCAM-1, ICAM-1, and, to a lesser extent, E-selectin. Hemodynamic forces influence the vascular wall and, in particular, endothelial cell functions, including elongation and alignment in the direction of flow,5 increased release of both prostacyclin6 and nitric oxide,7 and a decrease in the rate of endothelial cell proliferation.8 Our concurrent studies show9,10 that exposure of HUVEC monolayers, in vitro, to a steady laminar shear stress of 5 dynes/cm² for 24 hours produces almost complete inhibition of the post–shear stress cytokine-stimulated expression of VCAM-1 relative to that detected on cytokine-stimulated statically maintained monolayers. This decrease in adhesion molecule expression correlated with a reduction in the number of monocytes bound to the previously sheared monolayers. Although these observations may help to explain the relative protective effect of steady shear stress against development of atherosclerosis, there are few data addressing the issue of how oscillatory flow may contribute to lesion formation. In an attempt to examine this aspect of the hemodynamic environment thought to exist at flow dividers in vivo, we have designed a system to determine the possible biological significance of an almost purely oscillatory shear stress. In the present study, the effect of oscillatory shear stress on the level of adhesion molecules expressed by HUVEC monolayers in vitro, both in the presence and absence of the antioxidant NAC, was investigated.

Materials and Methods

Cell Culture

Primary HUVECs were obtained from Clonetics Corp, grown in tissue culture dishes, and maintained with medium 199 (GIBCO...
Selected Abbreviations and Acronyms

HUVEC = human umbilical vein endothelial cell  
ICAM-1 = intercellular adhesion molecule-1  
IL-1α, IL-1β = interleukin-1α and -1β  
MPFI = mean peak fluorescence intensity  
NAC = N-acetylcysteine  
VCAM-1 = vascular cell adhesion molecule-1

Laboratories) containing l-glutamine (2 mmol/L) and supplemented with 50 IU/mL penicillin G (GIBCO), 50 μg/mL streptomycin (GIBCO), 16 U/mL heparin (Sigma Chemical Co), 50 μg/mL endothelial cell growth supplement (Biomedical Technologies Inc), and 20% (vol/vol) fetal bovine serum (Intergen Co). This mixture constituted HUVEC culture medium. HUVECs were cultured in a humidified incubator that provided an atmosphere of 95% air/5% CO2 at 37°C. Cells were detached from their substratum using versene (0.2 g/L EDTA • 4Na in PBS, GIBCO) subcultured at a 1:3 ratio, and used up to the fifth passage. In order to assist cell deposition, rectangular plastic slides were coated with 0.1% (wt/vol) sterile aqueous gelatin solution, followed by seeding the cells onto the slides. The medium was changed every 2 days, and flow experiments were initiated 1 day after cells had reached confluence. Cells for use as statically maintained samples were plated similarly. The cells of the human monocytic leukemia cell line THP-1, obtained from American Type Culture Collection, were cultured using RPMI 1640 medium (GIBCO) containing 1-glutamine (2 mmol/L) and 10% (vol/vol) fetal bovine serum as well as the same amounts of penicillin and streptomycin as used for HUVECs. Cells were maintained at a density of 2.5 × 10⁵ cells/mL.

Flow System

The flow system used has been described elsewhere. In brief, the plastic slide containing the monolayer was inserted into a parallel-plate flow chamber that was installed between an upper and lower reservoir connected by tubing. A motor-driven syringe pump was inserted into this system in order to generate the longitudinal oscillatory motion. This oscillatory component allowed pulsatility to be superimposed on the steady flow via a displacement of the medium back and forth above the endothelial cell surface. In order to dampen the motion so as to ensure a sinusoidal waveform, a small reservoir was placed between the syringe and the flow chamber. The frequency and amplitude of the oscillatory flow were controlled by adjusting the speed of the motor and by changing the amplitude of the syringe motion, respectively. The pulsatile flow rate was monitored using an electromagnetic flowmeter probe, calibrated with an in-line flowmeter. The endothelial monolayers were subjected to a very low mean shear stress, with instantaneous oscillatory shear stresses between +5 and −5 dynes/cm², at a periodicity of 1 Hz. This frequency was chosen to approximate the normal resting heart rate. A very small forward flow was allowed (which produced a mean shear stress of ≈0.2 dynes/cm²), in order to provide for the delivery of nutrients and for the removal of potentially toxic substances released by the cells. This regime was imposed for 24 hours. Subsequently, the endothelial cells were rinsed with 0.15 mol/L PBS and isolated, followed by assay for VCAM-1, ICAM-1, and E-selectin expression using immunofluorescent flow cytometry. Some experiments were performed in which endothelial monolayers were subjected to oscillatory shear for 24 hours using medium containing 20 mmol/L NAC (Sigma) after pretreatment under static conditions for 30 minutes. This cell-permeant antioxidant has been reported to increase the intracellular glutathione content of various cell types. The object of these experiments was to determine the effect of the antioxidant, in combination with the oscillatory shear stress stimulus, on the adhesion molecule expression. The statically maintained controls were incubated with the antioxidant for 24 hours before assay. Experiments were also performed in which HUVEC monolayers were subjected to a nonreversing pulsatile flow of 5.5 dyne/cm², also at a frequency of 1 Hz. This flow type is achieved by allowing a steady flow of 5 dynes/cm² and superimposing an oscillatory component using the syringe pump as previously described.

Adhesion Molecule Analysis

The flow cytometric procedure has been described elsewhere. Briefly, after detachment of the cells from their substratum, the cells were incubated with 10 μg/mL of the respective monoclonal antibody in HUVEC culture medium for 30 minutes at 4°C (mouse anti-human VCAM-1, mouse anti-human ICAM-1, or mouse anti-human E-selectin, R & D Systems). Cells were then washed and spun (500g for 5 minutes), and the pellet was incubated with 25 μg/mL fluorescein-conjugated goat anti-mouse IgG (F(ab')₂ fragment (Chemicon). Cells were then washed and fixed with paraformaldehyde in PBS at a final concentration of 1% (wt/vol). Replica cells maintained in static culture were similarly processed for use as control specimens. Sets of these cells were processed using a nonbinding primary antibody (mouse anti-neurofilament monoclonal antibody, Sigma) to serve as a control for nonspecific binding. The relative levels of cell surface fluorescently labeled protein were quantified by immunofluorescent flow cytometry with log amplification using a fluorescence-activated cell sorter (FACScan IV, Becton Dickinson). The nonspecific binding control was used as a reference sample.

Northern Blot Analysis

Extraction of total cellular RNA was achieved using TriPure reagent (Boehringer-Mannheim), and 20 μg aliquots were size-fractionated using 1% agarose-formaldehyde gels containing 1 μg/mL ethidium bromide. The RNA was then transferred to a nitrocellulose filter and covalently linked by ultraviolet irradiation using a Stratalinker UV cross-linker (Stratagene Inc). Approximately 10⁵ cpm/mL of 32P-labeled probes were used per hybridization, which was performed at 68°C for 1 hour in QuickHyb solution (Stratagene). Subsequent to hybridization, filters were washed with a final stringency of 0.2 × SSC at 60°C for 30 minutes.

Monocyte Adhesion Assay

The human monocyte leukemia cell line THP-1 was used in the adhesion assay as a model of the human monocyte. The assay protocol was adapted from a documented procedure. In brief, 50 μg of BCECF-AM (Molecular Probes Inc) was dissolved in 50 μL dimethyl sulfoxide. THP-1 cells were resuspended in protein-free HUVEC culture medium at a concentration of 5 × 10⁵ cells/mL. Probe solution was added (~5 μL/mL of medium), and the mixture was incubated at 37°C for 45 minutes with frequent agitation. Cells were centrifuged, aspirated, and resuspended in fresh protein-free medium. Culture medium was removed from the HUVEC monolayers, and the latter were rinsed with 0.15 mol/L PBS. The fluorescently labeled THP-1 cell suspension was added to the monolayers, and the mixture was incubated at 37°C for 45 minutes. The monolayers were then rinsed three times with 0.15 mol/L PBS, followed by fixation (2% [wt/vol] paraformaldehyde/PBS) and mounting with a glass coverslip using 50% (vol/vol) glycerol/PBS. The plates were visualized via fluorescence microscopy, and adherent THP-1 cells were counted using 25 high-power representative fields on each plate and expressed as the number of cells bound per mm² endothelium. Percentages were expressed as mean ± SD.

Statistical Analyses

Statistical analyses were performed by using an unpaired t test.

Results

Adhesion Molecule Expression

Fig 1A, 2A, and 3A represent the flow cytometric histograms for assay of VCAM-1, ICAM-1, and E-selectin, respectively. The cells, which were preconditioned by oscillatory flow, exhibit an increased MPFI relative to statically maintained monolayers. This increase is indicative of enhanced expression of the respective adhesion mole-
cules. In the case of VCAM-1, it was observed that the percentage of cells that exhibited enhanced expression was 91±6%. For ICAM-1 and E-selectin, the percentages were 94±3% and 74±6%, respectively.

Exposure of the endothelial monolayers to the oscillatory flow regime for 24 hours, produced an average 9-fold upregulation of the expression of VCAM-1 relative to that present on statically maintained unstimulated monolayers. The mean level detected on the sheared samples was found to be 56% of that present on the statically cultured cytokine (IL-1β, 10 U/mL, 6 hours)–stimulated monolayers (Fig 1B). HUVECs were found to exhibit a moderate constitutive expression of ICAM-1, and this level was increased by an average 11-fold when subjected
to the oscillatory flow regime. The amount detected on the oscillatory shear–preconditioned specimens was observed to be 52% of that found on statically maintained, cytokine-stimulated monolayers (Fig 2B). For E-selectin, the level detected on the flow preconditioned endothelial monolayers was found to be increased 7.5-fold relative to cells maintained in static culture. This level was observed to be 53% of that detected on the cytokine-stimulated monolayers (Fig 3B). Monolayers preconditioned with oscillatory shear for 24 hours followed by stimulation with IL-1β (10 U/mL) for 6 hours produced no significant change in either VCAM-1 or ICAM-1 surface expression relative to that observed for stimulated statically maintained control cells (Tables 1 and 2).

In order to determine whether oscillatory shear stress promoted the autocrine activation of the HUVEC monolayers by release of stimulatory agents, conditioned effluent medium was extracted from oscillatory flow experimental systems (after 6 and 24 hours of oscillatory flow) and was incubated with sets of statically maintained HUVEC monolayers for periods of 3, 6, and 24 hours. The cells were then processed and analyzed for adhesion molecule expression as described. For the assay of VCAM-1, the MPFI values for cells incubated statically for periods of 3, 6, and 24 hours with conditioned medium extracted after 24 hours of oscillatory flow were observed to be 5±1 (n=3), 4±2 (n=3), and 3±2 (n=4), respectively (compare with Fig 1B). In the case of ICAM–1, the MPFI values

TABLE 1. Effect of IL-1β and NAC on Endothelial Expression of VCAM-1 for Both Static and Oscillatory Presheared Monolayers

<table>
<thead>
<tr>
<th>Condition</th>
<th>MPFI</th>
<th>Mean, % Change</th>
</tr>
</thead>
<tbody>
<tr>
<td>Static control</td>
<td>4±1 (n=3)</td>
<td></td>
</tr>
<tr>
<td>Static+NAC</td>
<td>2±1 (n=3)</td>
<td>-50*</td>
</tr>
<tr>
<td>Static+IL-1β</td>
<td>64±5 (n=3)</td>
<td></td>
</tr>
<tr>
<td>Static+IL-1β+NAC</td>
<td>8±3 (n=3)</td>
<td>-88†</td>
</tr>
<tr>
<td>24-h Osc Unstim</td>
<td>36±2 (n=3)</td>
<td></td>
</tr>
<tr>
<td>24-h Osc+NAC</td>
<td>2±1 (n=3)</td>
<td>-94†</td>
</tr>
<tr>
<td>24-h Osc+IL-1β</td>
<td>70±11 (n=3)</td>
<td></td>
</tr>
<tr>
<td>24-h Osc+IL-1β+NAC</td>
<td>3±1 (n=3)</td>
<td>-96†</td>
</tr>
</tbody>
</table>

*Osc indicates oscillatory shear stress; Unstim, unstimulated. Values are mean±SD.

TABLE 2. Influence of IL-1β and NAC Endothelial Expression of ICAM-1 for Both Static and Oscillatory Presheared Monolayers

<table>
<thead>
<tr>
<th>Condition</th>
<th>MPFI</th>
<th>Mean, % Change</th>
</tr>
</thead>
<tbody>
<tr>
<td>Static control</td>
<td>15±4 (n=3)</td>
<td></td>
</tr>
<tr>
<td>Static+NAC</td>
<td>6±3 (n=3)</td>
<td>-60*</td>
</tr>
<tr>
<td>Static+IL-1β</td>
<td>317±31 (n=3)</td>
<td></td>
</tr>
<tr>
<td>Static+IL-1β+NAC</td>
<td>50±15 (n=3)</td>
<td>-84†</td>
</tr>
<tr>
<td>24-h Osc Unstim</td>
<td>165±19 (n=3)</td>
<td></td>
</tr>
<tr>
<td>24-h Osc+NAC</td>
<td>55±11 (n=3)</td>
<td>-67†</td>
</tr>
<tr>
<td>24-h Osc+IL-1β</td>
<td>238±27 (n=3)</td>
<td></td>
</tr>
<tr>
<td>24-h Osc+IL-1β+NAC</td>
<td>53±12 (n=3)</td>
<td>-78†</td>
</tr>
</tbody>
</table>

*P=NS and †P<.05 vs corresponding value without NAC.
were found to be 17±2 (n = 3), 14±4 (n = 3), and 12±3 (n = 3) respectively (compare with Fig 2B). These data show that in HUVECs treated with conditioned medium from cells exposed to oscillatory shear, there were no increases in the level of the adhesion molecules compared with control samples. Moreover, there is no evidence for bacterial or endotoxin contamination in this model system. Similar results were obtained when it was further investigated whether the effect of the shear regime could be to induce the surface expression of IL-1α, which may, in turn, upregulate adhesion molecule expression. Oscillatory presheared endothelial monolayers as well as isolated cells were treated with goat anti-human IL-1α (R & D Systems), followed by the fluorescent secondary antibody. Examination of the monolayer en face as well as assay of the isolated cells by flow cytometry showed no significant increases in fluorescence output relative to control levels (data not shown).

The upregulation of VCAM-1 expression by oscillatory flow could be almost totally abolished when the cells were sheared in the presence of 20 mmol/L NAC. This phenomenon was also observed for statically maintained monolayers incubated with NAC for 24 hours, followed by IL-1β (10 U/mL) for 6 hours as well as monolayers presheared in the presence of NAC followed by IL-1β stimulation (Table 1). Northern blot analysis of VCAM-1 mRNA derived from HUVEC monolayers previously subjected to oscillatory flow for periods of 4, 6, 8, 16, and 24 hours in the presence of NAC showed no induction of VCAM-1 mRNA (data not shown). This indicates that the inhibitory action of NAC is probably occurring at the transcriptional level. This finding is similar to that seen for cytokine-treated human endothelial cells. The ICAM-1 level present on the endothelial cell surface after oscillatory flow conditioning in the presence of NAC was decreased by ≈70% relative to that observed for oscillatory flow in the absence of NAC. This reduction is similar to that detected for statically maintained monolayers incubated with NAC for 24 hours followed by IL-1β stimulation for 6 hours, as well as that observed for monolayers presheared in the presence of NAC followed by IL-1β (10 U/mL) stimulation for 6 hours (Table 2).

In order to determine whether the very small forward flow component, present in the oscillatory shear regimen, could produce the observed effects, HUVEC monolayers were exposed to steady laminar flow for 24 hours at a shear stress of 0.2 dynes/cm². The cells were then analyzed for adhesion molecule expression as previously described. For VCAM-1, the MPFI was 9±3 (n = 4) compared with 4±1 for static cells. In the case of ICAM-1, the MPFI was 27±5 (n = 3) compared with 15±4 for static cells. Further experiments in which monolayers were exposed to laminar shear stress at 2 dynes/cm² for 24 hours elicited no increases in the levels of either VCAM-1 or ICAM-1 relative to static control cells (VCAM-1 MPFI was 7±2 [n = 3]; ICAM-1 was 19±5 [n = 3]).

Northern blot analysis of homogenates obtained from HUVEC monolayers subjected to oscillatory shear stress for a range of time periods is shown in Fig 4. The level of mRNA detected after 8 hours of shear is not significantly altered up to 24 hours of shear stress. One possible interpretation of this result is that the flow regime could decrease turnover of VCAM-1 protein. HUVEC monolayers exposed to pulsatile shear stress of 5±5 dynes/cm², ie, flow containing an oscillatory component but with no flow reversal, did not induce either VCAM-1 mRNA or surface VCAM-1 expression for all time periods up to 24 hours.

The time course of oscillatory flow and IL-1β induction of VCAM-1 (Fig 5) indicates that cytokine induced surface VCAM-1 expression peaks at ≈6 to 8 hours. The levels of VCAM-1 elicited by oscillatory shear stress at these times are ≈16% and 30% of those peak values for IL-1β. VCAM-1 protein levels decrease after 16 hours of stimulation and are further reduced at 24 hours. Oscillatory flow–induced VCAM-1 surface expression increases 3-fold after 8 hours of shear stress relative to that observed at 6 hours of flow. This is consistent with the Northern blot data indicating that mRNA VCAM-1 levels peak after 4 to 6 hours of oscillatory shear stress (compare with Fig 4). The time course of ICAM-1 induction (Fig 6) shows increasing levels of ICAM-1 with increasing duration of stimulation either by oscillatory shear stress or by IL-1β. The level of surface ICAM-1 after IL-1β stimulation for 24 hours was found to be approximately five times that detected for 24 hours of oscillatory shear stress. Since it is likely that ICAM-1 induction does not peak at 24 hours of stimulation, as suggested by the Northern blot analysis, it is difficult to ascertain whether there is an altered rate of protein degradation in this case.

**Monocyte Adhesion**

The results from the THP-1 cell adhesion assay (Fig 7) indicate that the increases in adhesion molecule expression after oscillatory shear preconditioning are associated with an increase in THP-1 cell adherence. It was found that relative to the statically cultured endothelial cells, the preconditioned monolayers exhibited a 10-fold mean enhancement in binding. This level of adherence was observed to be ≈30% of that detected.
for the cytokine-stimulated monolayers. Antibody blocking studies of the monolayers presheared for 24 hours, using a combination of anti-human VCAM-1 (40 μg/mL) and antihuman ICAM-1 (40 μg/mL), showed a decrease in the level of monocyte adherence of 71 ± 6% relative to that for untreated presheared monolayers.

**Discussion**

Previous studies in which endothelial cell monolayers were subjected to shear containing a reversed flow component have focused on alterations in the morphology and c-fos expression of the cells, as well as modulation of cell-matrix attachment proteins. The present study indicates that oscillatory flow, imposed in an in vitro environment, has the capacity to induce adhesion molecule expression on HUVECs. The results suggest that areas of endothelium experiencing oscillatory flow in vivo may express enhanced levels of surface VCAM-1, ICAM-1, and E-selectin. The observations are consistent with the evidence that areas of presumed oscillatory flow in vivo exhibit recruitment of circulating monocytes that may contribute to plaque formation. Thus, the increased particle residence time, which was due to the extremely low positive flow, may not be the sole factor accounting for the occurrence of enhanced monocyte binding at susceptible areas of the vasculature. An active signal generated by the oscillatory flow pattern appears likely to be involved.

The data from the study to determine whether autocrine activation of the cells was occurring provide additional evidence that the imposition of oscillatory flow directly modifies intracellular signaling mechanisms, leading to enhanced adhesion molecule expression. These data mitigate against the possibility that the in vitro oscillatory flow system stimulates production of cytokine that could enhance adhesion molecule expression through an autocrine or paracrine mechanism. Since it was found that very low steady shear stresses did not have significant effects on the upregulation of the adhesion molecules studied, it may be concluded, therefore, that the observed effects are a result of the oscillatory shear regime. Furthermore, the induction of adhesion molecule expression appears to be functionally significant, since THP-1 cell adhesion is markedly enhanced, and the extent of binding can be dramatically reduced by prior treatment with antibodies to both VCAM-1 and ICAM-1. E-Selectin is known to contribute to the rolling phenomenon exhibited by circulating polymorphonuclear neutrophils when in contact with the endothelium. In the present study, E-selectin is the least upregulated and exhibited the lowest percentage of immunoreactive cells relative to both VCAM-1 and ICAM-1. This
suggests that oscillatory flow primarily increases the adhesivity of the endothelial cells.

It has been demonstrated\textsuperscript{\textasciitilde} that protracted exposure of HUVEC monolayers to a laminar shear stress of 10 dynes/cm\textsuperscript{2} did not significantly increase VCAM-1 expression, whereas ICAM-1 levels were selectively enhanced. The present study shows that VCAM-1 (as well as ICAM-1) is markedly upregulated by oscillatory shear stress. Whether there are common, but undefined, elements in the gene promoters responding to this stimulus or whether oscillatory shear stress stimulates more proximal common signaling pathways remains to be determined.

From the present study, it is clear that oscillatory flow represents a stimulus different from that of steady flow, with respect to adhesion molecule expression. It has previously been shown\textsuperscript{5} that prior treatment of endothelial monolayers with the antioxidant pyrrolidine dithiocarbamate can suppress the level of cytokine-induced VCAM-1 relative to untreated controls. These findings parallel our concurrent studies\textsuperscript{9,10} in which chronic laminar shear stress is also able to suppress inducible VCAM-1 expression by \textasciitilde\textasciitilde\textasciitilde80% compared with static IL-1\beta-stimulated controls. This leads to the conclusion that prolonged laminar shear stress may act as an antioxidant stimulus on the endothelial monolayer. Conversely, prolonged oscillatory shear stress does not suppress the subsequent induction of VCAM-1 by IL-1\beta on HUVEC monolayers (Table 1). This finding indicates that the action of oscillatory flow seems not to hypersensitize the cells to the action of IL-1\beta but to upregulate the inducible levels of the adhesion molecules studied. Furthermore, the data derived from the use of NAC suggest that oscillatory shear stress may upregulate VCAM-1 (Table 1) and, to a lesser extent, ICAM-1 (Table 2) via a redox-sensitive mechanism. The finding that pulsatile shear stress of 5\textpm{}5 dynes/cm\textsuperscript{2} does not increase VCAM-1 mRNA for all time periods suggests that the flow-reversal component of the oscillatory shear regimen is an important factor in the upregulation. These data indicate that HUVECs are capable of discriminating between various flow types. The differential responses to the flow environment are also reflected in morphological responses. In contrast to endothelial cell monolayers exposed to high laminar steady shear, which assume an elongated morphology,\textsuperscript{19} and, in vivo, possess overlapping borders,\textsuperscript{20} oscillatory shear–exposed HUVEC monolayers tend to maintain their polygonal morphology, as displayed by statically cultured monolayers. This lack of effect of oscillatory shear to cause elongation and alignment has also been found for confluent bovine aortic endothelial cells.\textsuperscript{21} It has been suggested\textsuperscript{15} that in the human carotid sinus at areas distant to the flow divider, increased ingress of plasma constituents across the endothelium occurs as a result of the destabilizing influence of oscillatory shear stress on the intercellular junctions of the endothelial cells at these regions.

We have attempted to mimic, in vitro, the effect of the hemodynamic forces thought to be exerted on the endothelium at the human carotid sinus as far as adhesion molecule expression is concerned. In future studies, it will be of interest to determine whether alterations in frequency and amplitude of the oscillations produce similar, enhanced, or diminished effects from those demonstrated here. As shown in Figs 5 and 6, the pattern of adhesion molecule induction observed under oscillatory flow is similar to that seen after cytokine stimulation, although the relative magnitudes differ and the kinetics of the former are slower than those of the latter. IL-1\beta-stimulated HUVEC monolayers may be typically induced to express maximal levels to VCAM-1 within 8 hours of the commencement of stimulation. ICAM-1 levels do not peak up to 24 hours of induction.

These data provide the initial evidence that oscillatory shear stress in vitro provides a proinflammatory stimulus to human endothelial cells, leading to enhanced expression of VCAM-1, ICAM-1, and E-selectin. This upregulation correlates with an increased level of monocyte adherence relative to the statically maintained monolayers. It is likely that the observed augmentation proceeds through a mechanism involving an oxidative pathway(s) and that the flow-reversal component of the oscillatory flow stimulus is a critical factor. These observations are in contradistinction to the inhibitory effect on endothelial cells of steady laminar shear stress on cytokine-induced VCAM-1 expression,\textsuperscript{22,23} which may correlate with the apparent protective effects of steady shear stress to inhibit atherosclerosis development in vivo.\textsuperscript{1} These results with oscillatory shear may explain, at least in part, the striking propensity to
develop atherosclerotic lesions opposite arterial flow dividers, ie, areas that are thought to exhibit oscillatory flow. Further defining the molecular mechanisms involved in the stimulation of adhesion molecule expression by oscillatory shear stress may enhance our understanding of the pathogenesis of atherosclerosis.

Acknowledgments
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