Shear Stress Selectively Upregulates Intercellular Adhesion Molecule-1 Expression in Cultured Human Vascular Endothelial Cells

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Abstract

Hemodynamic forces induce various functional changes in vascular endothelium, many of which reflect alterations in gene expression. We have recently identified a *cis*-acting transcriptional regulatory element, the shear stress response element (SSRE), present in the promoters of several genes, that may represent a common pathway by which biomechanical forces influence gene expression. In this study, we have examined the effect of shear stress on endothelial expression of three adhesion molecules: intercellular adhesion molecule-1 (ICAM-1), which contains the SSRE in its promoter, and E-selectin (ELAM-1) and vascular cell adhesion molecule-1 (VCAM-1), both of which lack the SSRE. Cultured human umbilical vein endothelial cells, subjected to a physiologically relevant range of laminar shear stresses (2.5–46 dyn/cm^2) in a cone and plate apparatus for up to 48 h, showed time-dependent but force-independent increases in surface immunoreactive ICAM-1. Upregulated ICAM-1 expression was correlated with increased adhesion of the JY lymphocytic cell line. Northern blot analysis revealed increased ICAM-1 transcript as early as 2 h after the onset of shear stress. In contrast, E-selectin and vascular cell adhesion molecule-1 transcript and cell-surface protein were not upregulated at any time point examined. This selective regulation of adhesion molecule expression in vascular endothelium suggests that biomechanical forces, in addition to humoral stimuli, may contribute to differential endothelial gene expression and thus represent pathophysiologically relevant stimuli in inflammation and atherosclerosis. (*J. Clin. Invest.* 1994. 94:885–891.) Key words: E-selectin • hemodynamic force • leukocyte adhesion • shear stress response element • vascular cell adhesion molecule-1

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Introduction

Endothelial cells, which comprise the lining of blood vessels, are continually subjected in vivo to hemodynamic forces, and in particular fluid shear stresses, by virtue of their direct contact with flowing blood. Exposure of cultured endothelial cells to defined shear stresses in vitro induces morphological changes, as well as alterations in a variety of functionally important products, including coagulation factors, fibrinolytic factors, growth factors, and cytokines (1, 2). A number of these endothelial responses to shear stress have been found to be regulated at the level of gene expression; however, there are significant differences in the temporal pattern of regulation of these genes, some being uniformly increased or decreased, while others exhibit a biphasic pattern of regulation. In addition, a broad range of shear stress levels, as well as temporal and spatial flow patterns, has been reported to influence gene expression in vascular endothelium (1).

Recent work in our laboratory (3) has identified a *cis*-acting shear stress response element (SSRE)[1] within the promoter of the PDGF-B gene which is required for the transcriptional upregulation of this effector molecule in endothelial cells by laminar shear stress in vitro (4). This SSRE is also present in the promoters of several other endothelial genes that have been shown previously to be shear stress responsive (3), thus suggesting a common mechanism linking biomechanical forces to gene expression. Interestingly, intercellular adhesion molecule-1 (ICAM-1), a member of the immunoglobulin superfamily of adhesion molecules, was found to contain the SSRE within its promoter (5), while E-selectin and vascular cell adhesion molecule-1 (VCAM-1), two other endothelial-expressed adhesion molecules, did not (6, 7). These molecules appear to participate in the recruitment of various types of leukocytes into inflamed tissues and atherosclerotic lesions in vivo (8–11) and are coordinately induced in cultured endothelium by humoral stimuli, such as cytokines and bacterial products, although with different expression kinetics (12, 13). To date, however, no comparative study of the regulation of these adhesion molecules by hemodynamic forces has been reported.

In the current report, we provide the first evidence that physiologically relevant levels of laminar shear stress can differentially regulate the expression of endothelial-leukocyte adhe-

1. *Abbreviations used in this paper:* FIA, fluorescence immunobinding assay; HUVEC, human umbilical vein endothelial cells; ICAM-1, intercellular adhesion molecule-1; SSRE, shear stress response element; VCAM-1, vascular cell adhesion molecule-1.

sion molecules. This selective upregulation of ICAM-1, in contrast to the coordinate induction of ICAM-1, VCAM-1, and E-selectin by soluble mediators, suggests that hemodynamic forces, in addition to humoral stimuli, may play a significant role in vivo in pathophysiological conditions such as inflammation and atherosclerosis.

Methods

Cell culture. Primary cultures of human umbilical vein endothelial cells (HUVEC) were established from normal term umbilical cords as described previously (14). For experimental use, second passage cells were plated on tissue culture–treated polystyrene (Costar Corp., Cambridge, MA, and Modern Plastics, Peabody, MA) coated with 0.1% gelatin (Difco Laboratories Inc., Detroit, MI) and grown to confluency in Medium 199 (with 25 mM Hepes; GIBCO BRL, Gaithersburg, MD) supplemented with 10% FBS (GIBCO BRL), 2 mM glutamine, 100 U/ml penicillin, 100 μg/ml streptomycin, 25 μg/ml endothelial cell growth supplement (Collaborative Research Inc., Bedford, MA), 50 μg/ml heparin (Sigma Chemical Company, St. Louis, MO), and 250 ng/ml amphotericin B (Fungizone®; GIBCO BRL). Suspension cultures of human JY lymphocytic cells (15), kindly provided by Dr. T. Springer (Center for Blood Research, Boston, MA), were maintained in RPMI 1640 medium (with 25 mM Hepes; BioWhittaker, Inc., Walkersville, MD) supplemented with 10% FBS, 20 mM glutamine, 100 U/ml penicillin, and 100 μg/ml streptomycin. In certain experiments, HUVEC monolayers were activated by treatment with recombinant human IL-1β (Biogen, Cambridge, MA), as indicated.

Shear stress apparatus. The cone and plate flow apparatus used to expose cultured HUVEC monolayers to defined fluid shear stresses has been described in detail previously (16, 17). The essential components consist of a stainless steel cone rotating over a stationary base plate, which supports either 12 12-mm diameter polystyrene coverslips or a single 17.8-cm diameter polystyrene plate. The culture medium present between the cone and base plate (15 ml total volume) is gradually exchanged (0.5 ml/min) without recirculation. The entire apparatus is maintained at 37°C in a humidified 5% CO_2 and 95% air atmosphere. The shear stress on the surface of the plate, τ, can be described by:

$$\tau = \frac{\mu\omega}{\alpha}(1 - 0.4743\tilde{R}^2),$$

where

$$\tilde{R} = \frac{r^2\omega\alpha^2}{12\nu},$$

and where μ is medium viscosity, ω is angular velocity of cone, α is cone angle, r is radial location on cone, and ν is medium kinematic viscosity. The flow is laminar for $\tilde{R} \ll 1$ (17). For the experiments reported here, fluid mechanical parameters were adjusted such that endothelial monolayers were subjected to a laminar shear stress of 2.5–46 dyn/cm^2 for variable time intervals. Dextran (476,000 mol wt, 1% wt/vol; Sigma Chemical Company) was used to alter the medium viscosity, when required, and the viscosity was measured with a coaxial cylinder viscometer at 37°C (Haake, Berlin, Germany). Shear stressed and static monolayers were cultured in the same medium for each experiment. Control monolayers on coverslips were maintained in cell culture dishes (Costar Corp.) under static (no flow) conditions at 37°C in a humidified 5% CO_2 and 95% air atmosphere for equivalent time intervals.

Fluorescence immunobinding assay (FIA). Endothelial monolayers were incubated on ice for 1 h with saturating concentrations of mAbs specific for human endothelial–leukocyte adhesion molecules (Hu 5/3, purified IgG, anti–human ICAM-1; E 1/6, ascites, anti–human VCAM-1; H18/7, purified IgG, anti–human E-selectin), followed by an FITC-labeled F(ab')$_2$ anti–mouse IgG (Caltag Laboratories, South San Francisco, CA) for 1 h, then lysed with a 0.01% NaOH/0.1% SDS solution and fluorescence measured in a Pandex plate reader (Travenol Laboratories, Mundelein, IL).

Table I. Shear Stress Induces Cell-Surface Expression of ICAM-1 in Cultured HUVEC

Experiment duration	Cell-surface immunobinding (fluorescence units)		
	Static	Shear stress	Shear stress/static
h			
4	1974±109	2259±545	1.1
4	886±180	838±88	1.0
8	2819±116	3689±223	1.3*
8	1618±319	1590±160	1.0
24	599±48	1427±327	2.4‡
24	673±98	1333±268	2.0‡
24	2443±371	2753±597	1.1
48	207±144	3413±406	16.5*
48	767±154	2419±111	3.2*
48	395±132	1080±71	2.7‡
48	997±72	2474±283	2.5*
48	944±19	941±637	1.0

Confluent HUVEC monolayers were either exposed to shear stress (10 dyn/cm^2) or maintained under static (no flow) conditions for the times indicated. Cell-surface expression of ICAM-1 was measured using fluorescence immunobinding assay, as described in Methods. Data are expressed as mean±SD; n = 2–4 replicate coverslips in each separate experiment; * $P < 0.01$ and ‡ $P < 0.05$ shear stress versus static control (Student's t test).

Immunocytochemistry. For microscopic visualization of cell-surface–associated proteins, endothelial monolayers were fixed in 2% paraformaldehyde at 4°C and incubated at room temperature for 1 h with specific mAbs directed to human endothelial–leukocyte adhesion molecules, as described above, followed by biotinylated anti–mouse IgG, a peroxidase-conjugated biotin–avidin complex, and finally an amino-ethyl-carbazole developing reagent (Vector Laboratories, Inc. Burlingame, CA).

Northern blot analysis. Total cellular RNA was extracted from endothelial monolayers by the acid guanidinium thiocyanate-phenol-chloroform method (Cinna/Biotecx Laboratories International, Inc., Friendswood, TX). Samples (15 μg each) were electrophoresed through 1% agarose gels containing formaldehyde, transferred to nitrocellulose membranes (Schleicher & Schuell, Inc., Keene, NH) and hybridized with human ICAM-1, VCAM-1, or E-selectin cDNA probes labeled by [α-^{32}P]dCTP (Amersham Corp., Arlington Heights, IL), using random hexanucleotide primers (Pharmacia LKB Biotechnology, Inc., Piscataway, NJ). The cDNA fragments used were as follows: a 1.8-kb SalI-KpnI fragment of ICAM-1 cDNA from pGEM4, kindly provided by Dr. T. Springer; a 1.0-kb XbaI fragment of E-selectin cDNA from pCDM8; and a 1.0-kb EcoRI/BamHI fragment of VCAM-1 cDNA from pBSM13, kindly provided by Dr. T. Collins (Brigham and Women's Hospital, Boston, MA).

Leukocyte adhesion assay. Endothelial monolayers on 12-mm coverslips were coincubated for 15 min at room temperature under static conditions in 24-well cell culture plates (Costar Corp.) with JY cells (1.5×10^5 cells/ml), a human lymphocytic cell line which expresses an ICAM-1 ligand, LFA-1 (CD11a/CD18) (15), in 75 μl of RPMI 1640 plus 10% FBS. To remove unattached JY cells, each coverslip was washed by immersion three times in RPMI 1640 plus 1% FBS, then once in PBS, and fixed in 2% paraformaldehyde at 4°C. After staining with hematoxylin, adherent JY cells were counted in five randomly selected high power microscopic fields (×100) on each of two coverslips for each controlled variable.

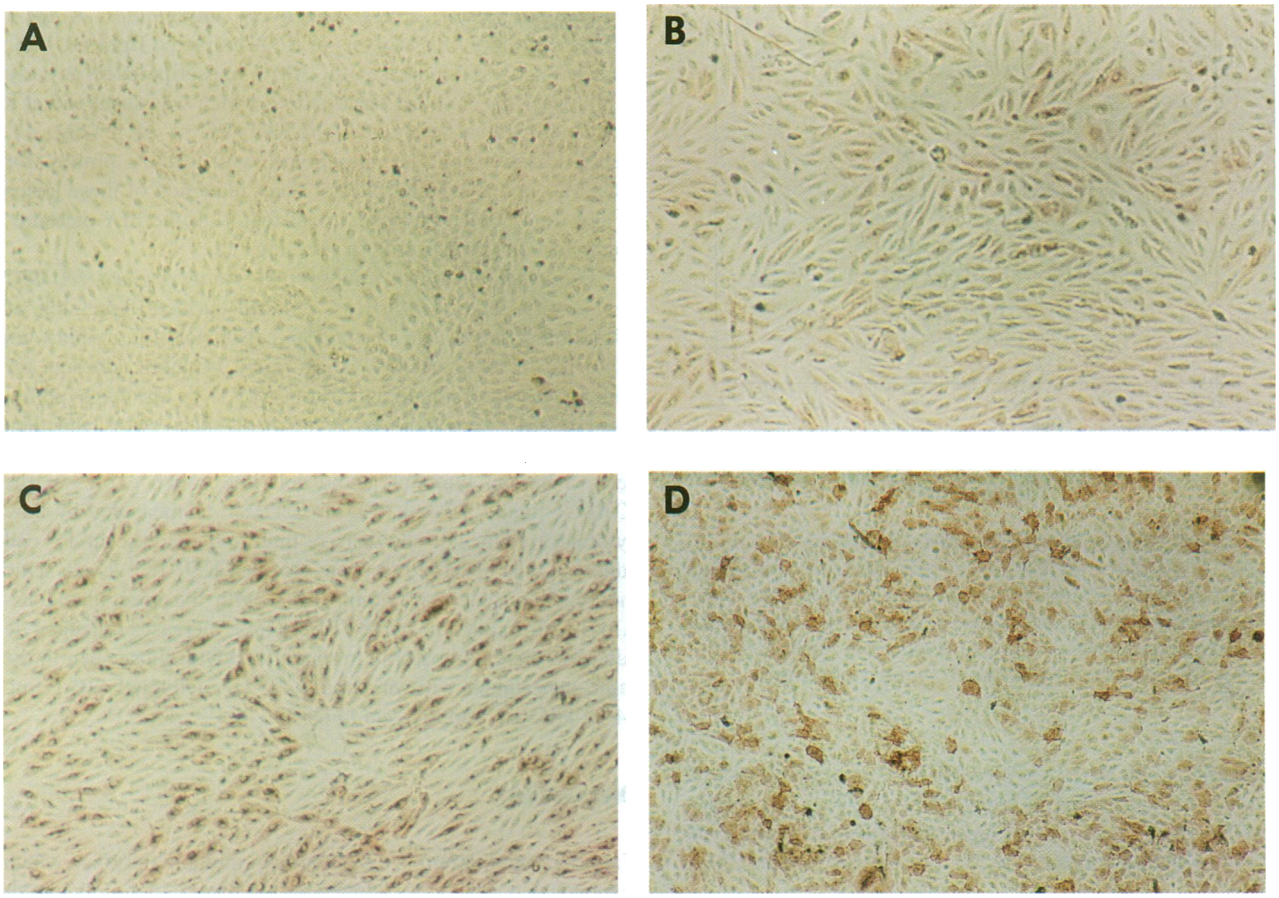

Figure 1. Immunocytochemistry of ICAM-1 surface expression on HUVEC exposed to shear stress. HUVEC monolayers were either maintained under no flow conditions (static) or subjected to high (10 dyn/cm^2) or low (3 dyn/cm^2) laminar shear stress for variable time periods: (*A*) static, (*B*) high shear stress, 24 h, (*C*) high shear stress, 48 h, or (*D*) low shear stress, 24 h. The monolayers were then fixed with 2% paraformaldehyde and immunocytochemically stained using an ICAM-1–specific mAb, as described in Methods. ×45.

Results

Shear stress upregulates ICAM-1 surface expression in a time-dependent and force-independent manner. ICAM-1 expression on the surface of HUVEC monolayers which had been either incubated under static (no flow) conditions or subjected to laminar shear stress of 10 dyn/cm^2 for 4, 8, 24, and 48 h was measured quantitatively by an FIA. Table I summarizes the results obtained from a total of 12 separate experiments in which confluent endothelial monolayers on multiple coverslips were simultaneously exposed to the same fluid shear stress or static conditions. Significant elevations in cell-surface immunoreactive ICAM-1 were observed as early as 8 h after the onset of shear stress and increased progressively up to 48 h. The maximum level of ICAM-1 expression induced by shear stress was comparable with that observed in HUVEC activated with a maximally effective concentration of recombinant human IL-1β (10 U/ml) (see Fig. 3 below). Conditioned effluent medium collected from the shear stress apparatus, at intervals ranging from 15 min to 24 h, failed to induce ICAM-1 upregulation when incubated with static HUVEC monolayers for 3 and 24 h (data not shown).

As illustrated in Fig. 1, immunocytochemical staining of paraformaldehyde-fixed HUVEC monolayers subjected to laminar shear stress of 10 dyn/cm^2 also demonstrated the progressive upregulation of cell-surface ICAM-1 at 24 and 48 h. Morphological responses of cells, namely elongation and alignment in the flow direction, were clearly evident in these shear stressed monolayers at both time points. However, HUVEC monolayers exposed to a lower level of shear stress (3 dyn/cm^2) for 24 h displayed a similar increase in surface ICAM-1 expression, without any detectable elongation or alignment of the cells. In both of these relatively high (10 dyn/cm^2) and low (3 dyn/cm^2) shear stress conditions, ICAM-1 induction was not uniform across the monolayer.

To further investigate the potential force dependence of ICAM-1 induction, confluent HUVEC monolayers were exposed for 24 h to a broad range of laminar shear stresses (2.5–46 dyn/cm^2), comparable with those encountered in vivo in large vessels (18). In a series of 12 experiments, as illustrated in Fig. 2, significant increases in immunoreactive surface ICAM-1 expression, measured by FIA, were observed at each level of shear stress tested; however, the amount of induction appeared to be independent of the magnitude of the applied force.

E-selectin and VCAM-1 are not upregulated in HUVEC by shear stress. In contrast to the induction of ICAM-1 by shear stress, no significant changes were observed in either E-selectin or VCAM-1 expression, as measured by cell-surface FIA, at any time point (4–48 h) or shear stress level (2.5–46 dyn/cm^2) studied (data not shown). In particular, as seen in Fig. 3,

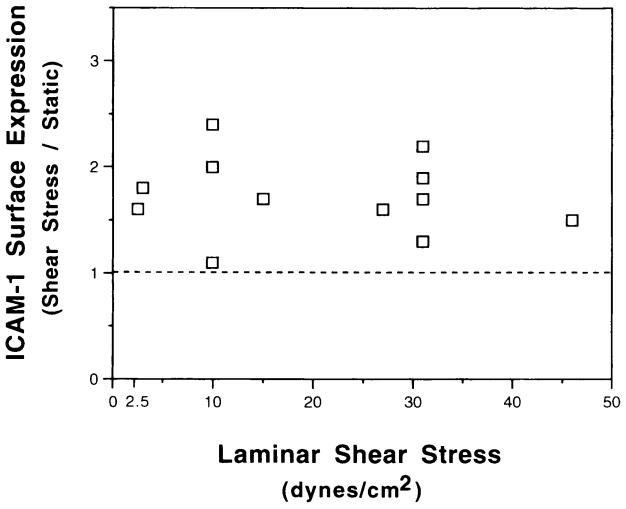

Figure 2. Upregulation of ICAM-1 by a range of laminar shear stress levels (2.5–46 dyn/cm^2). HUVEC monolayers were either maintained under static conditions or subjected to various levels of laminar shear stress, as indicated, for 24 h. Cell-surface protein was determined using a fluorescence immunobinding assay, as described in Methods, and displayed as the ratio (shear stress/static) for each experiment.

at time points when IL-1β–treated HUVEC cultures displayed peak levels of E-selectin (4 h) and VCAM-1 (24 h), no changes in the cell-surface expression of these molecules were observed in HUVEC cultures subjected to 10 dyn/cm^2 of laminar shear stress.

To test whether shear stressed endothelial cells were still responsive to other activating stimuli, such as cytokines, we subjected HUVEC monolayers to 24 h of laminar shear stress (10 dyn/cm^2) followed by a 4- or 24-h treatment with IL-1β (10 U/ml) under static conditions. Immunocytochemical staining revealed marked upregulation of E-selectin (after 4 h) and VCAM-1 (after 24 h) comparable with that observed in HUVEC that were not exposed to shear stress (data not shown).

Laminar shear stress selectively upregulates ICAM-1 mRNA. Northern blot analysis of RNA extracted from HUVEC revealed a marked increase in ICAM-1 transcript levels as early as 2 h after the onset of shear stress, which was sustained at 8 h and still elevated above static HUVEC at 24 h (Fig. 4). Rehybridization of the membrane with cDNA probes for VCAM-1 and E-selectin did not show induction of these genes at any time point examined, consistent with the FIA measurements of cell-surface protein (see Fig. 3).

Enhanced leukocyte adhesion to shear stressed HUVEC monolayers. The shear stress–induced surface expression of immunoreactive ICAM-1 suggested that HUVEC monolayers subjected to laminar shear stress might be more adhesive for leukocytes expressing ligands for ICAM-1. To test this hypothesis, HUVEC were exposed to 48 h of shear stress and then coincubated in a standard monolayer adhesion assay with JY cells, a human lymphoblastoid cell line which expresses the ICAM-1 ligand, LFA-1 (CD11a/CD18), as measured by flow cytometry (data not shown). A threefold increase in JY cell adhesion was observed to shear stressed HUVEC versus static monolayers, which was comparable with the upregulated ICAM-1 expression detected by FIA. These increases in leukocyte adhesion were comparable in magnitude with those seen in the same experiment with maximally IL-1β–activated (10 U/ml, 24 h) HUVEC monolayers (Fig. 5).

Discussion

Vascular endothelium is a dynamically mutable interface which responds to a variety of endogenous mediators, including blood-borne and locally generated cytokines, hormones, growth factors, as well as exogenous products, such as bacterial endotoxins (19, 20). In addition to these biochemical stimuli, there is ample evidence that biomechanical forces, generated by the pulsatile flow of blood through the branched tubular geometry of the circulatory system, can act on the endothelium to modify its structure and function (1, 2, 21, 22). Several of these alterations, which involve increases (or decreases) in the production

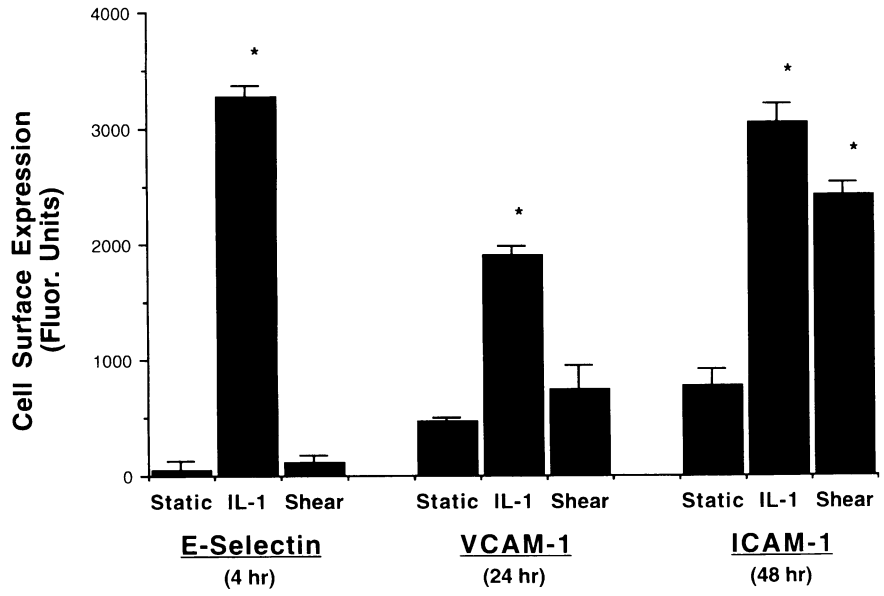

Figure 3. Peak cell-surface expression of endothelial–leukocyte adhesion molecules in HUVEC subjected to IL-1β or shear stress. HUVEC monolayers were maintained under static conditions, exposed to laminar shear stress (10 dyn/cm^2), or treated with a maximally effective concentration of IL-1β (10 U/ml) for time periods corresponding to the peak cytokine-induced surface expression of E-selectin (4 h), VCAM-1 (24 h), or ICAM-1 (48 h), respectively. Cell-surface protein was measured using a fluorescence immunobinding assay, as described in Methods. n = 3–4 replicate coverslips for each controlled variable; data expressed as mean±SD. *$P < 0.01$ stimulus versus static (Student's t test).

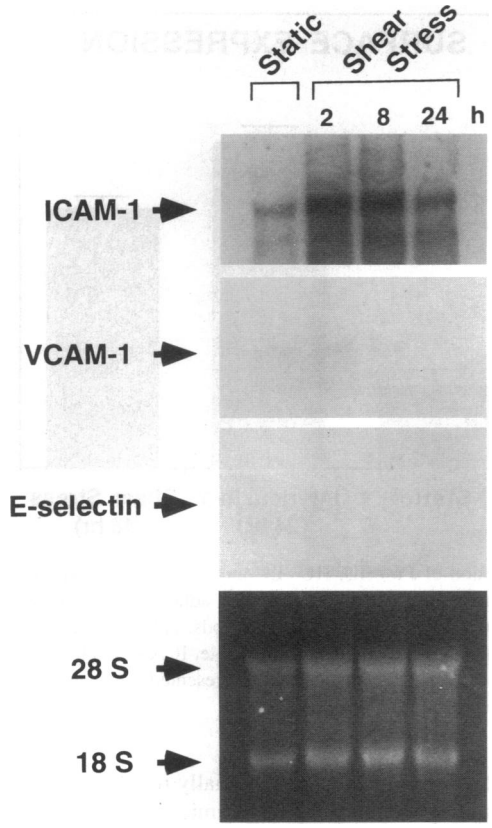

Figure 4. Northern blot analysis of adhesion molecule induction by shear stress in HUVEC. HUVEC monolayers were either maintained under static conditions or exposed to laminar shear stress (10 dyn/cm^2) for 2, 8, or 24 h. Total cellular RNA was isolated for Northern blot analysis as described in Methods, and each lane was loaded with 15-μg aliquots (*bottom panel,* ethidium bromide staining of 18S and 28S ribosomal RNA). The membrane was sequentially hybridized with radiolabeled cDNA probes for ICAM-1, VCAM-1, and E-selectin.

of key biological effector molecules, are dependent directly on gene expression. The recent discovery of a shear stress response element in the promoter of the PDGF-B chain gene, which is shared by other shear stress–inducible endothelial genes (3), provides a potential genetic regulatory mechanism to explain certain of these biomechanically induced changes.

In our initial database search; the SSRE core sequence (GAGACC), defined to be functionally important for human PDGF-B chain responsiveness to shear stresses in vitro, was also found in the 5′ flanking region of the human ICAM-1 gene (5), which previously had not been reported to be shear stress inducible. Given the current interest in regulation of adhesion molecule expression in endothelial cells and their broad pathophysiological implications, we undertook the studies reported here to examine the shear stress inducibility of ICAM-1, as well as E-selectin and VCAM-1, in cultured HUVEC. Several findings are of note. First, the differential pattern of adhesion molecule expression elicited by laminar shear stress is in contrast with the coordinate activation profile typically observed in cultured HUVEC with cytokines such as TNFα, IL-1β, or bacterial endotoxins (9, 12, 13), thus suggesting that distinct transduction pathways and/or transcriptional and posttranscriptional regulatory mechanisms are involved. Second, the lack of E-selectin expression, at the level of both message and cell-surface protein, after exposure to laminar shear stress at all times examined, argues strongly against the induction of an endogenous cytokine (e.g., IL-1β or IL-1α) as an autocrine mechanism of activation in this system. Finally, the correlation of the shear stress inducibility of ICAM-1 with the presence of the SSRE in its promoter region, and, conversely, the noninducibility of E-selectin and VCAM-1, two endothelial-expressed genes that lack the SSRE, further suggests that this putative genetic regulatory element may be pathophysiologically relevant.

Recent studies in several laboratories have implicated various transcription factors, including NFκB and related Rel family members, as well as AP-1 and GATA binding proteins, in the regulation of ICAM-1, E-selectin, and VCAM-1 expression by cytokines (23, 24). Certain of these *trans*-acting factors also have been found to be modulated in endothelial cells by shear stress (3, 25), but their direct involvement in the transcriptional regulation of shear stress–responsive genes in endothelium has not yet been demonstrated. Gel shift experiments using a 30-bp probe encompassing the SSRE in the ICAM-1 promoter, in parallel with a comparable SSRE probe from the PDGF-B promoter, have revealed the formation of similar DNA–protein complexes with nuclear extracts derived from shear stressed cells (Resnick, N., unpublished observations). This result implies that the SSRE, interacting with yet-to-be-defined DNA-binding proteins, may play a role in the induction of the ICAM-1 gene by laminar shear stress. However, dependence on transcriptional activation and the functionality of the SSRE in this process will require further experimental analyses, as reported previously for the PDGF-B gene (3). Recent reports also have demonstrated biphasic patterns of regulation of several endothelial genes (e.g., PDGF-B, bFGF, endothelin-1, and thrombomodulin) by shear stress (26–28), consisting of a rapid induction followed by downregulation below control levels. For endothelin-1, the decrease in steady state message appears to involve downregulation of transcription mediated by a region within the promoter that does not contain the SSRE (29). Thus, it is becoming apparent that the transcriptional activity of different shear stress–responsive endothelial genes may be regulated by a complex interplay of synergistic and antagonistic factors, many of which remain to be characterized.

In this report, we have demonstrated a selective upregulation of ICAM-1 in cultured HUVEC exposed to a broad range of laminar shear stresses (2.5–46 dyn/cm^2). In the same HUVEC monolayers, E-selectin (which is normally a silent gene) (12) and VCAM-1 (which shows a relatively low level of constitutive expression) (13) remained unchanged in response to shear stress. Recently, however, VCAM-1 has been reported to be downregulated in a cultured line of murine endothelial cells, derived from lymph nodes, in response to increasing levels of shear stress in the physiologic range (0–7.2 dyn/cm^2) (30). Of interest, in contrast to the HUVEC cultures used in our study, this murine endothelial cell line shows a high level of constitutive VCAM-1 expression. These intrinsic differences in VCAM-1 expression or, conceivably, other properties related to the distinct tissue sources of the endothelial cells, may account for the different effects of shear stress observed.

In this study we have used a single shear stress regime, namely laminar flow, as a model system in which to study the response of endothelial cells to a defined biomechanical force. These experiments actually represent step functions in which endothelial cells are changed from static to shear stress condi-

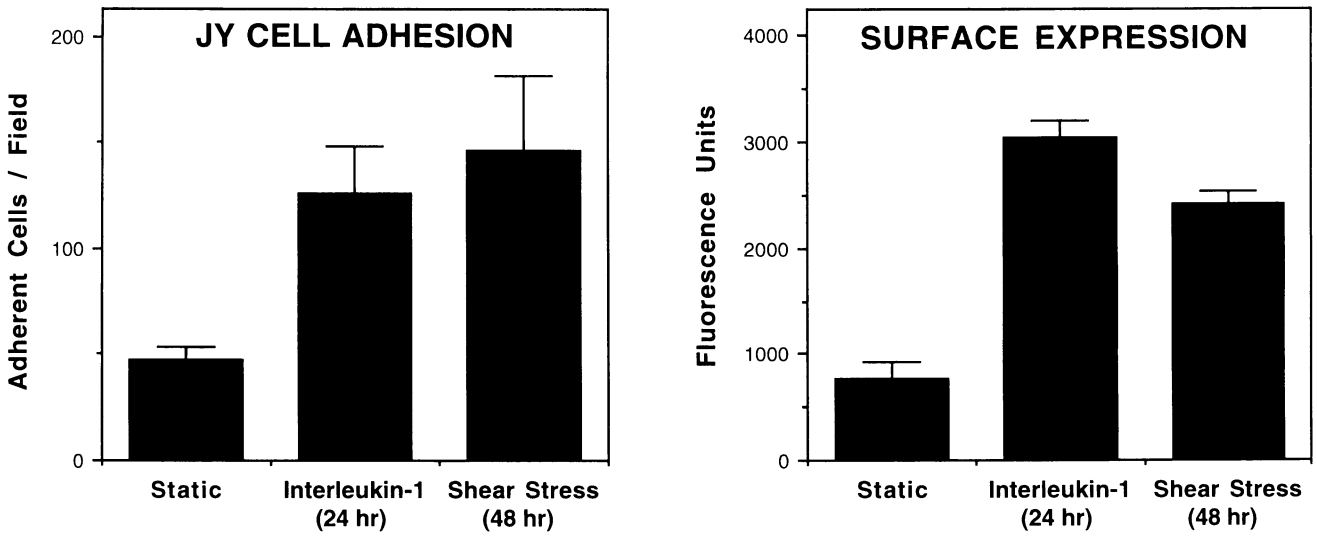

Figure 5. ICAM-1 induction by shear stress in HUVEC is correlated with an increased adhesion of JY cells. HUVEC monolayers were exposed to laminar shear stress (10 dyn/cm^2, 48 h) or treated with IL-1β (10 U/ml, 24 h), and then were incubated in a standard adhesion assay with human JY cells, a lymphocytic cell line that expresses the ICAM-1 ligand, LFA-1 (CD11a/CD18), as described in Methods. Adherent JY cells (*left*) were counted in five randomly selected high power (×100) fields on each of two coverslips for each controlled variable. ICAM-1 cell-surface expression (*right*) was measured in parallel by a fluorescence immunobinding assay, as discussed in Methods. Data are presented as mean±SD.

tions. The effective signal, responsible for initiation of gene regulation, may be a discrete alteration in shear stress magnitude above a minimum threshold. In vitro studies with shear stress regimes other than laminar flow have indicated that endothelial cells can respond to different shear stress parameters. For example, pulsatile shear stress, with its inherent temporal variations, has been shown to be more effective than steady flow in altering gene expression, in the case of c-*fos,* and less effective than steady flow, in the case of PDGF-A and -B (31). When compared with laminar shear stress of comparable magnitude, turbulent flow, which has fluctuations in both its temporal and spatial components, causes entry into cell cycle (32), but is essentially indistinguishable from laminar shear stress in regulating the expression of various genes, including PDGF-B, bFGF, endothelin-1, and thrombomodulin (26–28). In vivo, the endothelium is exposed to complex shear stress patterns, due to the pulsatile flow of blood through a branched tubular network (33). In an in vitro disturbed laminar flow field, created within the cone and plate apparatus and designed to mimic in vivo wall shear stress patterns at arterial bifurcations, the localized responses of the endothelial monolayer were found to vary with the shear stress gradient, rather than the absolute force (34). Thus, it will be instructive to compare the modulation of ICAM-1 expression in endothelial cells exposed to various flow regimes, including pulsatile, turbulent, and especially disturbed laminar flow. Such studies hopefully will provide insights into the potential relevance of shear stress–induced endothelial changes in different pathophysiologic settings (e.g., atherosclerotic lesions that typically develop in arterial geometries associated with flow disturbances, or inflamed microvessels in which flow is acutely increased). In addition, characterization of the nature of effective shear stress stimuli may lead to a better understanding of the cellular transduction mechanisms that link these externally applied forces to genetic regulatory events (35).

In conclusion, we have demonstrated that leukocyte adhesion molecule expression can be differentially regulated in vascular endothelium by a defined hemodynamic force, in contrast to the coordinate pattern of induction typically elicited by humoral stimuli, such as cytokines. These observations have important implications for the basic mechanisms of gene regulation in vascular endothelial cells. Furthermore, the selective upregulation of ICAM-1 by physiological levels of laminar shear stress suggests that the biomechanical regulation of this molecule may have significance, in vivo, in settings such as inflammation and atherosclerosis.

Acknowledgments

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