Pergamon

0021-9290(95)00099-2

J. Biomechanics, Vol. 28, No. 12, pp. 1515–1528, 1995
Copyright © 1995 Elsevier Science Ltd
Printed in Great Britain. All rights reserved
0021-9290/95 $9.50 + .00

CONTROL OF ENDOTHELIAL CELL GENE EXPRESSION BY FLOW

Adel M. Malek* and Seigo Izumo†

* Department of Neurosurgery, Brigham & Women's Hospital, Children's Hospital and Harvard Medical School, Boston, MA 02215, U.S.A.; and †‡Cardiovascular Research Center, University of Michigan, Ann Arbor, MI 48109, U.S.A.

Abstract—The vessel wall is constantly subjected to, and affected by, the stresses resulting from the hemodynamic stimuli of transmural pressure and flow. At the interface between blood and the vessel wall, the endothelial cell plays a crucial role in controlling vessel structure and function in response to changes in hemodynamic conditions. Using bovine aortic endothelium monolayers, we show that fluid shear stress causes simultaneous differential regulation of endothelial-derived products. We also report that the downregulation of endothelin-1 mRNA by flow is a reversible process, and through the use of uncharged dextran supplementation demonstrate it to be shear stress- rather than shear rate-dependent. Recent work on the effect of fluid shear stress on endothelial cell gene expression of a number of potent endothelial products is reviewed, including vasoactive substances, autocrine and paracrine growth factors, thrombosis/fibrinolysis modulators, chemotactic factors, surface receptors and immediate-early genes. The encountered patterns of gene expression responses are classified into three categories: a transient increase with return to baseline (type I), a sustained increase (type II) and a biphasic response consisting of an early transient increase of varying extent followed by a pronounced and sustained decrease (type III). The importance of the dynamic character of the flow stimulus and the magnitude dependence of the response are presented. Potential molecular mechanisms of shear-induced gene regulation, including putative shear stress response elements (SSRE), are discussed. These results suggest exquisite modulation of endothelial cell phenotype by local fluid shear stress and may offer insight into the mechanism of flow-dependent vascular remodeling and the observed propensity of atherosclerosis formation around bifurcations and areas of low shear stress.

Keywords: Atherosclerosis; Shear stress; Endothelin-1; Growth factors; Mechanical stress.

NOTATION

Ac-LDL	acetylated low-density lipoprotein
BAE	bovine aortic endothlium
bFGF	basic fibroblast growth factor
$[Ca^{2+}]_i$	intracellular calcium
cAMP	cyclic adenosine monophosphate
cGMP	cyclic guanosine monophosphate
CHX	cycloheximide
EC	endothelial cell
ET-1	endothelin-1
GAPDH	glyceraldehyde phosphate dehydrogenase
HUVE	human umbilical venous endothelium
ICAM	intercellular adhesion molecule
IE	immediate-early
MCP	monocyte chemotactic protein
NO	nitric oxide
NOS	nitric oxide synthase
PAI	plasminogen activator inhibitor
PDGF-A/B	platelet-derived growth factor A/B
PGI_2	prostacyclin
PKC	protein kinase C
SMC	smooth muscle cell
SSRE	shear stress responsive element
TGF-β	transforming growth factor-β
TM	thrombomodulin
t-PA	tissue-type plasminogen activator
u-PA	urokinase-type plasminogen activator
VCAM	vascular cell adhesion molecule

Address for correspondence: Adel M. Malek M.D., Ph.D. Department of Neurosurgery, Brigham & Women's Hospital, 75 Francis Street, Boston, MA 02215, U.S.A. Tel: (617) 732-6660; Fax: (617) 732-4144; E-mail: ammalek@bics.bwh.harvard.edu.

INTRODUCTION

The mechanical environment to which a blood vessel is exposed is known to regulate its structure during development (Langille, 1993) and in response to iatrogenic changes such as those induced by arterio-venous shunts and vessel constriction (Kamiya and Togawa, 1980; LaBarbera, 1990; Zarins *et al.*, 1987). Recent work in cellular and molecular biology has uncovered a set of complex interactions between the various components of the blood vessel including endothelium, smooth muscle, matrix and blood-borne elements such as platelets and leukocytes (monocytes, lymphocytes, and polymorphonuclear leukocytes) (Ross, 1993). The blood vessel (Fig. 1) is no longer considered to be simply a non-thrombogenic passive conduit for blood flow. Rather it is increasingly viewed as a continually-adapting, physically and chemically interdependent network of elements with the common goal of maintaining optimal function in response to constantly changing hemodynamic and metabolic conditions (Davies and Tripathi, 1993; Langille, 1993; Ross, 1993). Mechanically, the vessel wall is grossly subjected to two types of force, flow-induced shear stress acting on the endothelial cell (EC) at the luminal side, and tangential stress acting on smooth muscle/fibroblast cell and extracellular matrix constituents in the media of the wall (Fung, 1984; Goldsmith and Turitto, 1986). Endothelium secretes a large repertoire of substances with both paracrine and autocrine action on a number of target cells (Ross, 1993). The activity of these

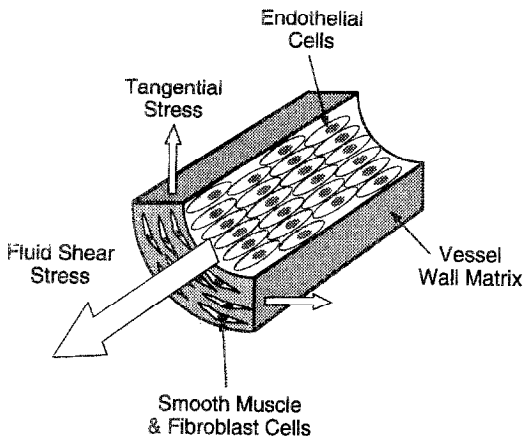


Fig. 1. Vessel wall constituents and hemodynamic environment. Schematic cut-away view of an arterial blood vessel. Smooth muscle cells and fibroblast cells constitute the bulk of the vessel wall and generate tension to counteract vessel expansion in response to blood pressure. The endothelial layer is constantly subjected to fluid shear stress on its surface while the smooth muscle cells and vessel wall constituents are exposed to tangential stress resulting from transmural pressure. Endothelial and smooth muscle cells produce a number of potent substances with autocrine and paracrine properties many of which are regulated by biomechanical stress (Table 1).

substances includes growth regulation (bFGF, PDGF-A and B, TGF-β) (D'Amore and Smith, 1993), vasoactive and mitogenic control (ET-1, NO) (Rubanyi *et al.*, 1986; Yanagisawa *et al.*, 1988), fibrinolysis/thrombolysis (Esmon, 1987) and matrix remodeling (t-PA, TM, PAI-1) (Mignatti and Rifkin, 1993) and cell adhesion/activation (MCP-1, VCAM-1, ICAM-1) (Hynes, 1992) (Table 1). The EC is known to play a crucial role in mediating both short-term vasomotor (Koller and Kalley, 1991) and long-term structural changes in vessel structure in response to blood flow changes (Langille, 1993). Given the optimal position of the endothelium as a transducer of fluid shear stress, the goal of the present paper is to study the effect of shear on the in vitro regulation of EC-derived products with vasoactive, growth regulatory and hemostatic properties in an effort to understand the mechanism of EC-mediated flow vessel remodeling. Using the mRNA level of the vasoconstrictor endothelin-1 (ET-1) as a flow-regulated signal, experiments were conducted to better define the time course and magnitude dependence of gene regulation by flow. Additional experiments were carried out to determine whether shear rate or shear stress magnitude is the critical regulator of ET-1 mRNA and ascertain the reversibility of the regulatory process upon cessation of flow. A review of the reported effects of flow and shear on endothelial gene expression is presented along the lines of functional relevance and a classification system is proposed to better characterize types of gene control based on time profile, dependence on shear stress magnitude, and sensitivity to the dynamic character of the flow stimulus.

METHODS

BAE cells were harvested from fresh descending thoracic aortas, verified for Ac-LDL uptake, and cultured in Dulbecco's modified Eagle medium (Gibco) with 10% calf serum and penicillin/streptomycin in a humidified incubator (37°C, 5% CO2, pH 7.4). A cone-plate viscometer was used to subject confluent BAE monolayers to well-defined laminar fluid shear stress. Shear stress magnitude and Reynolds values were computed using numerical integration (Malek and Izumo, S., 1992; Malek *et al.*, 1995). Confluent BAE monolayers plated in 10 cm tissue culture plates (Falcon #3003, Oxnard, CA) last fed 24–48 h with either serum-free or 10% calf serum in DMEM were exposed to shear stress of 15 or 36 dyn/cm^{-2} for the indicated time period by using a rotating 0.5 or 1° cone (Malek *et al.*, in press). In some experiments, viscosity of the medium was increased by 2.94-fold by addition of 5% uncharged dextran (average M.W. 76,000). Following exposure to shear, cells were

Table 1. Endothelial-derived products, their actions and target sites of activity

Endothelical products	Actions and properties	Target
ET-1 (Yanagisawa *et al.*, 1988)	Vasoconstrictor, paracrine mitogen	Smooth muscle, fibroblast
NO (Rubanyi *et al.*, 1986)	Vasodilator, growth/protein-synthesis inhibitor	Smooth muscle, fibroblast, platelet, endothelium
bFGF (Rifkin and Moscatelli, 1989)	Matrix-bound autocrine/paracrine mitogen	Endothelium, smooth muscle, fibroblast
PDGF-A & PDGF-B (D'Amore and Smith, 1993)	Soluble paracrine mitogen	Smooth muscle, fibroblast
TGF-β (Spron and Robert, 1992)	Soluble autocrine/paracrine growth inhibitor	Endothelium, smooth muscle, fibroblast
t-PA (Mignatti and Rifkin, 1993)	Soluble fibrinolytic substance	Fibrin clot, matrix
TM (Esmon, 1987)	Surface-bound antithrombotic/antifibrinolytic	Thrombin, protein C cascade
PAI-1 (Mignatti and Rifkin, 1993)	Inhibits plasminogen activators t-PA, u-PA	Plasmin action
MCP-1 (Springer, 1990)	Monocyte chemotactic protein	Monocyte
VCAM-1 (Springer, 1990)	Monocyte/lymphocyte surface-bound receptor	Monocyte, lymphocyte, leukocyte
ICAM-1 (Springer, 1990)	Monocyte/lymphocyte surface-bound receptor	Monocyte, lympocyte, leukocyte

lysed for total cellular RNA isolation and purification (Chomczynski P. and Sacchi, 1987; Malek and Izumo, 1992). Quantitative Northern blot hybridization was performed with a random-primer ^{32}P-labeled fragment of either preproendothelin-1 (ET-1), c-sis (for PDGF-B) (Devare *et al.*, 1983), bFGF (Schweigerer *et al.*, 1987), t-PA (ATCC #67585), TM (Jackman *et al.*, 1986), or GAPDH cDNA. Expression of GAPDH mRNA was found not to be affected by fluid shear stress and was thus used to normalize other mRNA species for variations in loading and transfer (Malek and Izumo, 1992). Part of the data in Figs 3A and B has been presented in a previous report (Malek and Izumo, 1992).

RESULTS

Differential effect of fluid shear stress on endothelial gene expression

Physiological steady laminar fluid shear stress (6 h duration) regulates the expression of a number of endothelial products in a specific fashion (Fig. 2). ET-1 and PDGF-B, factors which cause both vasoconstriction and increase smooth muscle and fibroblast growth (Yanagisawa *et al.*, 1988; D'Amore and Smith, 1993), show decreased expression under steady shear stress in a magnitude-dependent fashion (15 and 36 dyn cm^{-2}). In contrast, elevated shear (36 dyn cm^{-2}) increases production of bFGF, a heparin-binding factor usually stored in the matrix underlying endothelial cells which is thought to induce growth of both endothelial cells and smooth muscle cells (Rifkin and Moscatelli, 1989). A similar pattern of concerted differential regulation is seen in response to shear with thrombomodulin and t-PA (Esmon 1987, 1989; Mignatti and Rifkin, 1993), with the former anticoagulant receptor undergoing decreased and the latter fibrinolytic substance sustaining increased expression under shear. The magnitude of the shear stress also plays a crucial role: although shear decreases ET-1, PDGF-B and TM mRNA at both 15 and 36 dyn cm^{-2} (to a greater extent at the higher magnitude), it increases bFGF and t-PA mRNA levels to a significant extent only at magnitudes greater than 15 dyn cm^{-2}.

Endothelin-1 regulation by fluid shear stress

The downregulation of ET-1 by shear is a time- and magnitude-dependent phenomenon, showing saturation behavior at magnitudes greater than 15–20 dyn cm^{-2} (up to 36 dyn cm^{-2}) and remaining decreased at up to 24 h (Figs 3A and B; Noris *et al.*, 1995). In addition, the ET-1 mRNA decrease is dependent on shear stress magnitude rather than shear rate or fluid velocity (Fig. 3C) since endothelium exposed to shear stress of 17 dyn cm^{-2} at a rotational velocity (proportional to fluid velocity and shear rate) of 2.5 rev s^{-1} reveals greater downregulation than at the lower shear stress magnitude but higher rotational velocity of 14 dyn cm^{-2} and 4 rev s^{-1}, respectively. The process of ET-1 downregulation by shear is reversible (Fig. 3D) since halting flow at 6 h, following ET-1 mRNA downregulation, results in a gradual recovery of ET-1 expression over the next 24 h. This phenomenon is similar to that described for thrombomodulin mRNA, albeit with a longer recovery time constant (Malek *et al.*, 1994).

INFLUENCE OF SHEAR ON ENDOTHELIAL GENE EXPRESSION

Vasoactive products

Endothelial-produced vasoactive substances have particular significance because of the well recognized process of flow-induced vasodilation both in the short-term (Koller *et al.*, 1993) and in long-term structural remodeling (LaBarbera, 1990; Langille, 1993). Prostacyclin, PGI_2, release was first shown to be increased in HUVE cells exposed to flow, with greater extent in response to step changes (Frangos *et al.*, 1984; Grabowski *et al.*, 1985). Here we consider the regulation of the endothelin-1 (Malek *et al.*, 1993b) and nitric oxide synthase (Nishida *et al.*, 1992) genes by flow. ET-1 was the first gene product reported to be regulated by fluid flow (Yanagisawa *et al.*, 1988). The initial report describing this 21-amino acid vasoconstrictor and smooth muscle/fibroblast mitogen described downregulation in response to shear stress (Yanagisawa *et al.*, 1988). Later transient upregulation by shear stress of 5 dyn cm^{-2} was found in porcine aortic endothelium (Yoshizumi *et al.*, 1989). Other groups have since described decreased ET-1 mRNA in both HUVE and BAE cells at shear stress levels above 15 dyn cm^{-2} (Malek and Izumo 1992; Noris *et al.*, 1995; Sharefkin *et al.*, 1991). Our studies have revealed a mild (20–40%) early transient increase in ET-1 mRNA levels between 0.5 and 1 h following onset of shear (Fig. 3A; Malek and Izumo, 1992). Measuring ET-1 peptide levels rather than mRNA expression, Kuchan and Frangos suggested the existence of two mechanisms of ET-1 regulation by flow, an early upregulation prominent at low shear stress, largely independent of shear magnitude, and mediated by PKC activation and a late NO-dependent downregulation (Kuchan and Frangos, 1993). We have failed to prevent shear-induced ET-1 mRNA downregulation with the NO-synthase inhibitor L-NMMA (Malek, 1994). A recent report by Noris *et al.* (1995) also suggests that ET-1 mRNA downregulation occurs independently of NO since it was observed under turbulent shear where measured NO production was not changed (Noris *et al.*, 1995). Morita *et al.* have suggested that the early transient upregulation of ET-1 mRNA is mediated by G-actin levels which increase in response to shear by a process that is partially dependent on $[Ca^{2+}]_i$ and protein kinase C (Morita *et al.*, 1994). Our group found the downregulation of ET-1 mRNA to be independent of protein kinase C and transcriptionally mediated, not involving significant changes in messenger stability, and requiring the region between -2.5 and -2.9 kb at the 5′ end of the ET-1 transcription initiation site (distinct from AP-1 and GATA-2 DNA binding sites) (Malek *et al.*, 1993b). The process leading to decreased ET-1 mRNA requires ongoing protein synthesis, while the upregulation component

does not. The downregulation was found to be dependent on the mean magnitude of shear rather than on small shear variations.

Flow has been known to increase NO production in endothelial cells (Cooke *et al.*, 1990; Rubanyi *et al.*, 1986). Recent work reveals that shear stress induces increased mRNA expression and immunoreactivity of NO synthase in response to shear of 15 dyn cm^{-2} (Nishida *et al.*, 1992). The mechanism of upregulation is thought to require on-going protein synthesis and to be independent of protein kinase C (same as for ET-1 downregulation) (Uematsu *et al.*, 1993). Recently, Noris *et al.* have shown that although NO production and NOS mRNA levels were elevated under steady laminar shear stress (8 dyn cm^{-2}), neither showed a significant increase when turbulent shear of similar magnitude was used (Noris *et al.*, 1995). Taking advantage of the breakdown of [^{3}H]L-arginine into NO and [^{3}H]L-citrulline, NO production was measured and found to be similarly increased under steady and pulsatile flow, but maximally increased (up to 25-fold) in response to periodic square wave time functions (15 min cycle) of shear. It is not clear whether the higher increased levels of [^{3}H]L-citrulline fully reflect higher production of NO rather than increased rate of pinocytosis in response to the step changes in shear, a previously documented phenomenon (Davies *et al.*, 1983) which would have increased the effective intracellular concentration of [^{3}H]L-arginine. Distinct from the upregulation of NOS at the mRNA level, the mechanism of short-term increase in NO in response to flow does not involve gene upregulation and appears to be independent of shear stress magnitude (range 6–25 dyn cm^{-2}) (Davies, in press). The intricate control in opposite directions of the vasoconstrictor ET-1 and vasodilators NO and PGI_2 suggests a balanced interplay between mechanical stimuli and vasoactive substance production which may lie at different steady states depending on the type of vascular bed.

Growth factors

Heparin-binding growth factors have potent growth-regulating activity which may play an important role in long-term control of vascular structure (D'Amore and Smith, 1993; Ross, 1993). PDGF-A and PDGF-B are paracrine growth factors secreted by the endothelium in a vectorial pattern into the substratum (Zerwes and Risau, 1987) which induce smooth muscle and mesenchymal cell growth. Using HUVE cells, Hsieh *et al.* have demonstrated a transient increase in both PDGF-A (10-fold) and PDGF-B (2–3-fold) peaking at 1.5–2 h following the onset of shear and returning to control levels by 4 h. The shear stress magnitude dependence showed a brisk jump with increasing shear magnitude up to 6 dyn cm^{-2} then reached a plateau (Hsieh *et al.*, 1991). Using BAE cells, other groups have also reported increased PDGF-B chain mRNA expression, which was in one instance sustained at 24 h (Mitsumata *et al.*, 1993) and in the other not studied beyond 4–6 h (Resnick *et al.*, 1993). The mechanism of this upregulation was suggested to be PKC-mediated (Hsieh *et al.*, 1992; Mitsumata *et al.*, 1993). Our work using BAE cells revealed, following a mild early increased expression at 1–2 h, a significant 5–10-fold decrease in PDGF-B chain mRNA without any detectable PDGF-A expression (Fig. 2; Malek *et al.*, 1993a). The downregulation, which persisted at long periods of time and was shear stress-dependent, showed greater decrease at 36 than at 15 dyn cm^{-2} (Fig. 2). The shear-induced decreased PDGF-B mRNA was of similar extent under both laminar, pulsatile and tubulent shear stress (Malek *et al.*, 1993a). Like ET-1, the downregulation of PDGF-B was inhibited by CHX while the early transient upregulation component was not (Malek and Izumo, 1994).

Basic FGF is an endothelial-produced autocrine growth factor (Rifkin and Moscatelli, 1989; Sato and Rifkin, 1988) with high binding affinity to the extracellular matrix which may play a role in vessel remodeling via its autocrine and paracrine action as well as its ability to induce t-PA and collagenase (Mignatti and Rifkin, 1993). Although initial experiments on HUVE cells using quantitative PCR failed to detect shear-induced differences at 24 h in response to shear stress of 21 dyn cm^{-2} (earlier time points were not reported), our group has uncovered a clear shear-induced increase which is magnitude-dependent, reaching a peak at 6 h and remaining elevated at 9 h up to 5-fold (Fig. 2; Malek *et al.*, 1993a). This finding may be of importance *in vivo* given the autocrine effect of bFGF. Shear-induced bFGF increased production may account for the increased EC density seen in response to step increases in flow *in vivo* (cervical AV-shunt model) (Masuda *et al.*, 1989) and conversely decreased bFGF at lower shear may help explain the decreased EC density observed in response to decreased flow (external carotid ligation model) (Langille *et al.*, 1989). The mechanism underlying bFGF upregulation is not at present well understood. Furthermore, since bFGF can induce increased t-PA mRNA expression, it remains to be seen whether bFGF upregulation is a necessary step for t-PA upregulation or whether the two processes are mechanistically independent. Transforming growth factor β is of particular interest because it appears to be a context-sensitive factor (D'Amore and Smith, 1993), acting as a smooth muscle growth inhibitor at low concentrations and as a growth inducer at higher levels through the induction of PDGF (Moses *et al.*, 1990). TGF-β is secreted by EC in a latent form which requires activation (Yasafumi *et al.*, 1993) and acts as an inhibitor of growth and motility of a wide variety of cells (Sporn and Robert, 1992). Recent work has shown that fluid shear stress induces a magnitude-dependent increase in TGF-β mRNA, transcription, and active product (Ohno *et al.*, 1995). These levels remain elevated at 12 h following onset of flow, and are thought to be mediated by a protein synthesis-independent mechanism. It is unclear what effect shear-induced TGF-β would have *in vivo*.

Mediators of thrombosis and fibrinolysis

Given the importance of the anti-thrombogenic properties of the endothelial cell (Esmon 1987; Vassalli *et al.*, 1991), there is clear interest in determining flow-induced

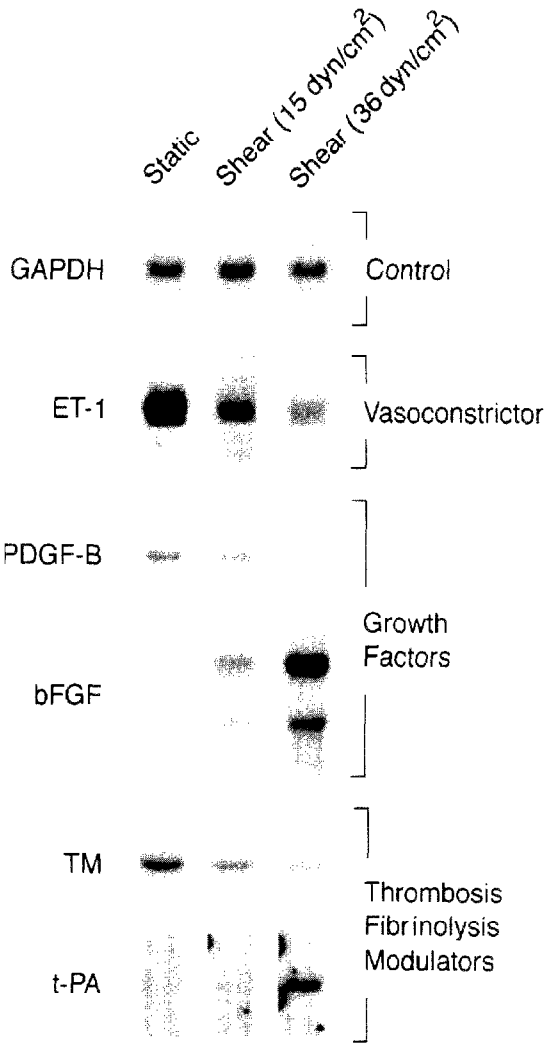


Fig. 2. Fluid shear stress affects endothelial cell gene expression in a time- and magnitude-dependent manner. Northern blot analysis revealing the pattern of endothelial messenger RNA (mRNA) expression changes of a number of functional products induced by physiological levels of steady laminar fluid shear stress in BAE monolayers at 6 h following onset of flow.

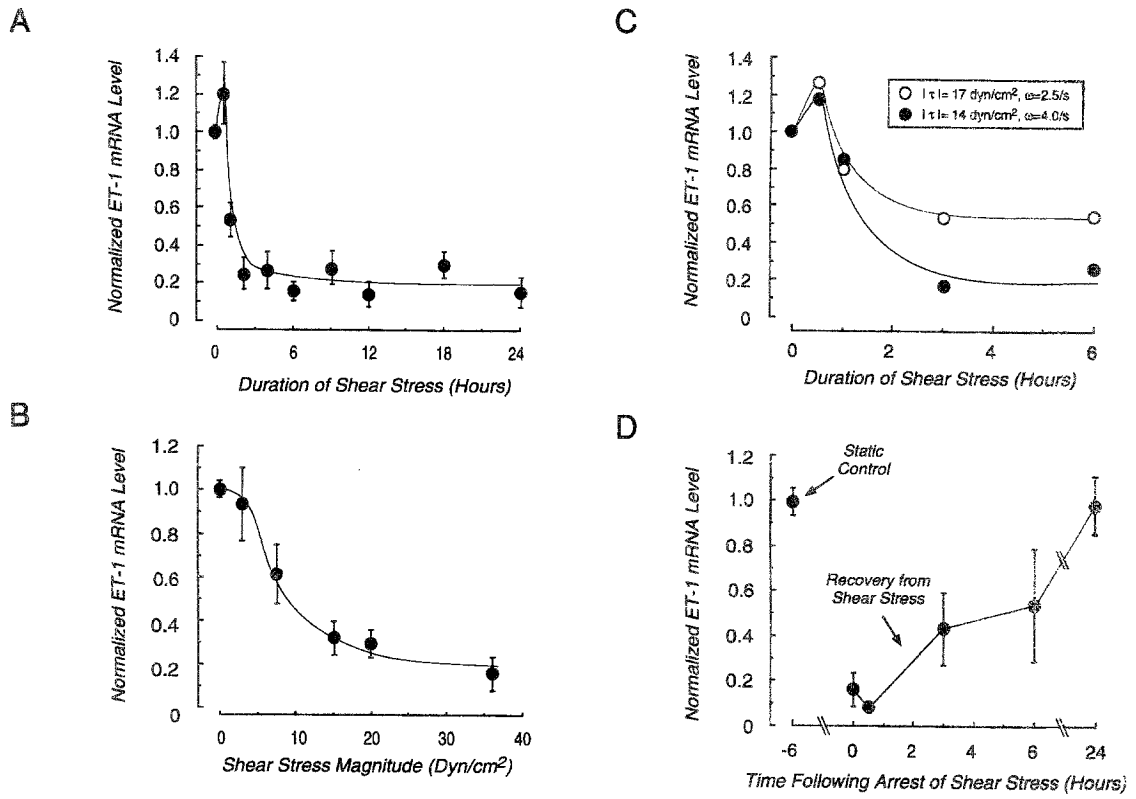


Fig. 3. Endothelin-1 mRNA regulation by fluid shear stress is time-dependent, magnitude-dependent, shear stress- rather than shear rate-dependent, and is reversible upon cessation of the flow stimulus. (A) Effect of steady laminar shear of 15 dyn cm^{-2} on ET-1 mRNA content reveals a small 20–40% increase at 0.5–1 h followed by a marked 5–10-fold decrease at times greater than 3 h. (B) ET-1 mRNA is decreased by steady laminar fluid shear in a magnitude-dependent manner at 4 h. (C) Downregulation of ET-1 mRNA is dependent on shear stress magnitude rather that shear rate or flow velocity. (D) The downregulation of ET-1 mRNA is a reversible process.

changes in expression of EC-derived thrombomodulators. Diamond *et al.* showed initially that HUVE cells exposed to elevated (arterial level, 21 dyn cm^{-2}) but not low (venous level, 4 dyn cm^{-2}) shear stress secreted significantly higher levels of t-PA (Diamond *et al.*, 1989) with unchanged production of PAI-1 by a process that relied on shear-induced increased t-PA mRNA (Diamond *et al.*, 1990). Similar findings were also made in BAE cells exposed to elevated shear (36 dyn cm^{-2}) and to a lesser extent moderate shear (15 dyn cm^{-2}) magnitudes when compared to static control cells (Malek *et al.*, 1994). In addition to their well-known fibrinolytic activity, plasminogen activators are thought to be crucial in matrix dissolution (Mignatti and Rifkin 1993) and vascular wall remodeling (Langille, 1993). This feature may be contributing to structural vascular response to chronic increases in flow (Kamiya and Togawa, 1980).

TM is an endothelial cell surface receptor that plays a crucial role in the activation of the protein C anticoagulant pathway which serves to counteract thrombin-induced clot formation (Esmon, 1989). In addition to anticoagulant properties, TM helps in the internalization of thrombin (Esmon, 1989), has been proposed to decrease EC activation by agonists (Parkinson *et al.*, 1993) and to contribute to the inactivation of plasminogen activators such as u-PA and t-PA (Majerus *et al.*, 1991). TM mRNA and protein levels are downregulated by shear in a time- and magnitude-dependent (greater than 6-fold at 9 h and 36 dyn cm^{-2}) fashion (Malek *et al.*, 1994). The process is reversible, since halting flow returns TM mRNA to baseline levels, and time invariant because the process can then be repeated with similar results (Malek *et al.*, 1994).

Inflammatory mediators and adhesion molecules

These receptors found on the endothelial cells have an important role in the adhesion of blood-borne inflammatory cells such as monocytes, lymphocytes, and polymorphonuclear leukocytes (Hynes, 1992; Springer, 1990). The process of inflammation is increasingly being considered to be crucial in atherosclerosis (Ross, 1993). Monocyte chemotactic protein-1 is a glycoprotein which increases the chemotaxis of monocyte cells. It is expressed in various types of cells including endothelium, smooth muscle, fibroblast and monocyte cells themselves. Fluid shear stress induces a transient transcriptional activation of MCP-1 in HUVE cells which peaks at 2.5-fold then returns to baseline by 4 h (Shyy *et al.*,

Table 2. Flow-responsive gene regulation of endothelial products and its classification according to dependence to shear stress magnitude

Magnitude-dependent process		Magnitude-independent process	
Downregulation	Upregulation	Downregulation	Upregulation
ET-1	bFGF	—	PDGF-A
TM	t-PA		PDGF-B
PDGF-B	TGF-β		MCP-1
VCAM-1			ICAM-1

1994a). The MCP-1 induction is not shear stress magnitude-dependent, showing similar increase at 6, 16, and 32 dyn cm^{-2}. Of equal and perhaps more significance is the downregulation of MCP-1 at times greater than 4 hours (Shyy *et al.*, 1994b). The downregulation is reversible following cessation of flow, a similar phenomenon to that described for TM and ET-1 in this report. However, unlike TM which is specific to endothelial (not seen in smooth muscle cells), MCP-1 is similarly controlled in cells not usually exposed to flow, namely HeLa, skin fibroblast, and glioma cells (Shyy *et al.*, 1994), raising a question about the physiological importance of such regulation.

Vascular adhesion molecule-1 (VCAM-1) is an endothelial surface receptor which mediates lymphocyte adherence to the vessel wall and plays an important part in the inflammatory response. Increased activity of lymphocytes and monocytes has been proposed to participate in the focal process of atherosclerosis (Jonasson *et al.*, 1986; Ross, 1993). Ando *et al.* (1994) have recently used mouse lymphatic endothelium to show that fluid shear stress induces a time- and magnitude-dependent downregulation in VCAM-1 expression which persists at 24 h. This process was ascertained both immunochemically and functionally and shown to be sensitive to shear stress rather than shear rate by using high-viscosity medium and independent of prostacyclin and nitric oxide (Ando *et al.*, 1994). In contrast, Nagel *et al.* (1994) failed to detect any changes in VCAM-1 in BAE cells, probably owing to the low level of expression compared to the lymph node EC used by Ando *et al.* (1994).

Intercellular adhesion molecule-1 is a surface adhesion molecule belonging to the immunoglobulin superfamily with affinitiy to β_2-type integrin receptors. ICAM-1 is a participant in the recruitment of lymphocytes, monocytes and polymorphonuclear leukocytes (Springer, 1990). It is proposed to play a role in local areas of inflammation and atherosclerosis (Cybulsky and Gimbrone, 1991). Nagel *et al.* have reported that fluid shear stress of 10 dyn cm^{-2} increases the expression of ICAM-1 surface expression (assessed both immunocytochemically and functionally) (Nagel *et al.*, 1994). ICAM-1 mRNA levels are elevated at 2 and 8 h and remain higher than static control at 24 h. Unlike the downregulation of VCAM-1 or ET-1 (Table 2), ICAM-1 increase is observed at shear levels as low as 3 dyn cm^{-2} by a process which appears to be independent of shear stress magnitude. This behavior is in essence similar to that of MCP-1

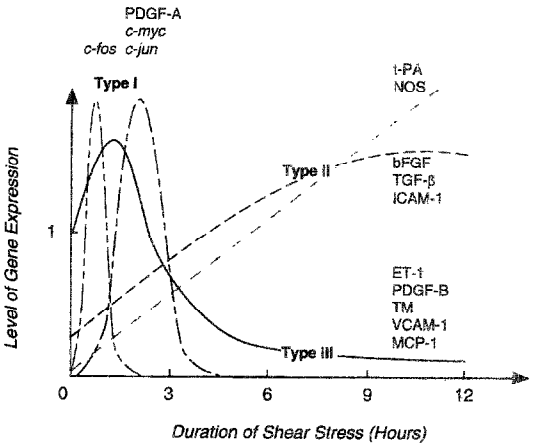


Fig. 4. Classification of observed endothelial gene expression times profiles in response to flow. The response of endothelial-derived products to flow can be categorized into three types: type I is an early transient increase (*c-fos*, *c-jun*, *c-myc*, PDGF-A), type II is a steady and continued increase above baseline (t-PA, NOS, bFGF, TGF-b, ICAM-1) and type III reveals biphasic regulation, increasing transiently to a varying extent followed by a significant and sustained decrease below baseline (ET-1, PDGF-B, TM, VCAM-1, MCP-1).

(Shyy *et al.*, 1994) and the transient increase of PDGF-A and B reported by Hsieh *et al.* (1993b). The physiological significance and specificity of such type of regulation is not clear at present, especially given the lack of magnitude dependence. Further experiments with increased medium viscosity (Ando *et al.*, 1994; Malek and Izumo, 1992; Ohno *et al.*, 1995) will need to be carried out to address this point.

Temporal response of shear-induced gene regulation

The gene regulatory responses to flow that have been documented so far in the literature suggest a classification into three types (Fig. 4). Type I response is a transient and early response which subsides back to baseline levels following a period of stimulation ranging from 1 to 4 or more hours. This would include the early responses of genes such as *c-fos*, and PDGF-A, *c-myc*, and *c-jun* described above. Type II consists of an upregulation which is steadily increasing or remains at elevated levels in response to shear stress and would include genes such as t-PA, NOS, bFGF, TGF-β and ICAM-1. Finally, type III regulation is characterized by an early upregulation which is transient, subsiding back to basal levels, and is

Table 3. Characterization of response to dynamic shear stress stimuli

Gene product	Response to dynamic stimulus
c-fos (Hsieh *et al.*, 1993a)	Higher increase with pulsatile vs steady flow
c-myc (Hsieh *et al.*, 1993a)	No difference between plusatile and steady flow
c-jun (Hsieh *et al.*, 1993a)	No difference between plusatile and steady flow
ET-1 (Malek *et al.*, 1992)	Downregulation similar under pulsatile, steady and turbulent*
NOS (Noris *et al.*, 1995)	Upregulation more pronounced in periodic on–off–on flow
PDGF-A (Hsieh *et al.*, 1991)	Upregulation higher with pulsatile vs steady flow
PDGF-B (Hsieh *et al.*, 1991)	Upregulation greater with steady flow, downregulation same as ET-1*
bFGF (Malek *et al.*, 1993a)	Upregulation sensitive to mean shear magnitude not dynamic character
TGF-β (Ohno *et al.*, 1995)	NA
t-PA (Malek *et al.*, 1994b)	Upregulation similar in response to both pulsatile and steady flow
TM (Malek *et al.*, 1994b)	Downregulation similar under pulsatile, steady and turbulent*
MCP-1 (Shyy *et al.*, 1994)	NA
VCAM-1 (Ando *et al.*, 1994)	NA
ICAM-1 (Nagel *et al.*, 1990)	NA

*Shown to be mostly dependent on the mean shear magnitude rather than to small dynamic variations.
NA = data not available.

followed by a sustained decrease in gene expression at longer time points. This type of regulation would characterize ET-1, PDGF-B, TM, VCAM-1 and MCP-1. In the case of ET-1 (Malek *et al.*, 1993b) and PDGF-B (Malek and Izumo, 1994), we were able to uncouple the two components of the type III response by using the protein synthesis inhibitor cycloheximide: the early transient increase was found to be independent of, while the sustained decrease component was found to be dependent on protein synthesis.

The response of EC gene expression to flow can also be usefully divided into two categories: genes whose expression is regulated by a process which is shear stress magnitude-dependent in a graded fashion, and those whose level of expression appears be sensitive to the presence or absence of flow, in a binary fashion, as evidenced by a response at low shear levels which is largely independent of shear magnitude (Table 2). The mechanism of the latter type of shear stress magnitude-independent processes may be similar in some respects to the flow-induced ATP-dependent $[Ca^{2+}]_i$ increase which is known to be the result of flow-induced boundary layer changes (Dull and Davies, 1991; Mo *et al.*, 1991).

Importance of dynamic character of flow stimulus

Given the pulsatile nature of blood flow and pressure *in vivo* (Fung, 1984; Goldsmith and Turitto, 1986), it is of paramount importance to determine the role of the dynamic character of the flow stimulus on endothelial gene expression. Other aspects of EC function, including PGI_2 production (Grabowski *et al.*, 1985), pinocytosis (Davies *et al.*, 1983) and entry into the cell cycle (Davies *et al.*, 1986), have previously been shown to be dependent on the time characteristics of the applied shear stress. In our studies, we felt it important to maintain the same root mean square magnitude of shear stress for any comparison and arbitrarily chose 15 dyn cm^{-2}. We used a steady laminar and turbulent regimes at 15 dyn cm^{-2}, a pulsatile regimen varying between 12 and 18 dyn cm^{-2} at 2.5 Hz and a reversing $\pm$21 dyn cm^{-2} wave. We found no significant difference in gene regulation between steady, turbulent or pulsatile for ET-1 (Malek and Izumo, 1992), PDGF-B, bFGF (Malek *et al.*, 1993a), or TM (Malek *et al.*, 1994). Of note, reversing shear (0 mean magnitude) did not induce significant change in gene expression of ET-1, suggesting that the EC response for these genes at the time point examined was dependent on the net shear vector, rather that on changes in or peak values of the stimulus wave. In contrast, Hsieh *et al.* (1993a) found *c-fos* upregulation to be exquisitely sensitive to the pulsatile nature of the shear stress whereas *c-myc* and *c-jun* were not. Noris *et al.* (1995) have also shown that NO production and NOS mRNA induction were also sensitive to the dynamic content of the stimulus. The reports from the literature are summarized in Table 3. Further studies are required to characterize fully the importance of time-varying stimuli, especially since the final EC response may not only be dependent on cytoskeletal and structural considerations at the cellular level (Adams, 1992; Fung and Liu, 1993) but also be affected by the myriad temporal characteristics of the signaling systems involved in the final outcome.

DISCUSSION

The results presented here clearly illustrate the concept of endothelial functional modulation by flow and fluid shear stress. Such difference in phenotype has been described between endothelium freshly isolated *ex vivo* compared to *in vitro* (Liaw and Schwartz, 1993). PDGF-B was found expressed in much higher quantities in static cultures (zero shear) compared to freshly isolated EC samples (physiological shear) (Barrett and Benditt, 1988) raising the possibility that, consistent with our findings

in vitro (Malek *et al.*, 1993a) PDGF-B may have been downregulated as a result of the constant fluid shear stress stimulus *in vivo*. The results presented above suggest a switch in endothelial phenotype at low shear when compared to moderate and high shear. For instance, shear increases expression of a vasodilator (NO) while it decreases that of vasoconstrictors such as ET-1 and PDGF-B. Similarly, it induces expression of substances that may promote expansile vascular remodeling such as t-PA and bFGF while decreasing substances that may have a contractile growth response such as ET-1 and PDGF-B. Finally, shear decreases expression of an anticoagulant with anti-fibrinolytic properties such as TM while it increases expression of the fibrinolytic substance t-PA. These push-pull patterns of control, regulating the mRNA levels of agonists and antagonists in opposite directions, may provide a certain level of redundancy and safety in shear-regulated endothelial gene expression control.

We have expanded here our previous findings on the time course and magnitude dependence of ET-1 downregulation. The phenomenon is, unlike that reported upregulation of PDGF-A and B or *c-fos*, *c-myc* and *c-jun*, not transient but rather sustained at 24 h. The magnitude dependence shows saturation behavior above 15–20 dyn cm^{-2}, the average optimal level of shear stress in mammalian ciculatory networks. This may represent an important phenomenon which may support the involvement of ET-1 in the chronic vascular structure optimization. The reversibility of ET-1 downregulation, similar in principle to that of TM, suggests that the regulatory response is not the result of irreversible injury but may instead be physiologically important. Further it is of interest that the time course of downregulation (2–4 h) is significantly shorter than that of recovery (12–24 h). The complex regulatory events induced by flow and shear described above raise multiple questions about the potential molecular mechanism; these are addressed next.

Immediate early genes

Immediate early genes are activated in the short-term following agonist stimulation and may participate in the propagation of the response by acting as modulators of transcriptional activity. These include the proto-oncogenes *c-fos*, *c-jun*, and *c-myc* whose levels have been shown to increase in cardiac myocytes subjected to mechanical strain (Komuro *et al.*, 1990; Sadoshima *et al.*, 1992). The only available data on shear regulation of IE genes were obtained in HUVE cells exposed to either steady or pulsatile (1 Hz) shear stress by Hsieh *et al.* (1993a). Both steady and pulsatile shear induced dramatic transient increased *c-fos* mRNA at 0.5 h, with the latter being nearly 2.5 times greater than the former (62 vs 24-fold, respectively). In contrast, both steady and pulsatile shear increased *c-jun* and *c-myc* mRNA expression transiently to a similar extent by approximately 2-3-fold relative to stationary controls over a period of 4 h. The increase in *c-fos* mRNA was shown to be dependent on shear stress magnitude under pulsatile flow (Hsieh *et al.*, 1993a). This result is difficult to interpret since increasing shear stress magnitude under pulsatile flow also increases the value of the shear stress derivative with respect to time. The finding presents the attractive possibility that *c-fos* expression may be related more to temporal changes in shear stress (time derivative) than in shear stress absolute magnitude. This phenomenon may have important pathophysiological ramifications.

Molecular mechanisms of shear-induced gene regulation

An altered level of mRNA can be due either to a change in the rate of transcription of the gene or in the stability of its mRNA molecule (Fig. 5). ET-1 mRNA contains multiple AUUUA repeats in its 3′ region (Inoue *et al.*, 1989) which are thought to confer instability and short half-life to the associated mRNA (Brawerman, 1989). Kinetic assays using actinomycin D to inhibit transcription have revealed no effect of shear stress on ET-1 mRNA stability (Malek *et al.*, 1993b). Rather, in the case of ET-1 the downregulation appeared to be transcriptional and to be mediated by (or at least dependent on) a yet-undefined 5′ sequence present between −2.5 and 2.9 kb upstream of the transcription initiation site. Recently, Davies' group showed that steady laminar shear of 12 dyn cm^{-2} stimulated two transcription factors AP-1 and NFκB (Lan *et al.*, 1994). NFκb/DNA complex formation was stimulated as soon as 20′, increasing up to 3-fold at 60′ and remaining elevated, while AP1/DNA complex formation revealed a biphasic response, increasing 3-fold by 20′ decaying back at 30′ then increasing again up 30-fold at 120′. AP1/DNA complex formation is thought to be mediated by Fos/Jun heterodimers following the phosphorylation of *c-jun*. Table 4 shows the various shear stress-activated response elements (SSRE) and the genes that contain them. Resnick *et al.* (1993) have implicated the involvement of the core sequence GAGACC in the upregulation of PDGF-B transcription in BAE cells by measuring the shear-stress-induced activity of multiple deletion mutants of the PDGF-B gene promoter linked to reporter CAT construct. Furthermore, BAE cells exposed to shear stress revealed increased specific retardation in gel shift assays using the sequence GAGACC, in a cell-specific manner since nuclear extracts from smooth muscle did not show binding. The activation is quite rapid and is seen as soon as 10′ following exposure to shear. The factor that binds to the sequence has not yet been determined. The GAGACC SSRE is found in a number of shear-responsive genes including t-PA, NOS, ICAM-1, *c-fos*, *c-jun*, TGF-β and MCP-1, although the activation of MCP-1 has recently been reported to be mediated via the TRE element (Shyy *et al.*, 1994) independently of GAGACC. Furthermore, even though the GAGACC sequence is found at −1219 in the TGF-β promoter, shear-induced activation was found to occur in constructs containing the sequence between −453 and +11 of the TGF-β promoter region which neither contains GAGACC nor its complementary sequence (Ohno *et al.*, 1995).

Recently, Gimbrone's group has shown that the PDGF-A induction by shear in HUVE is mediated inde-

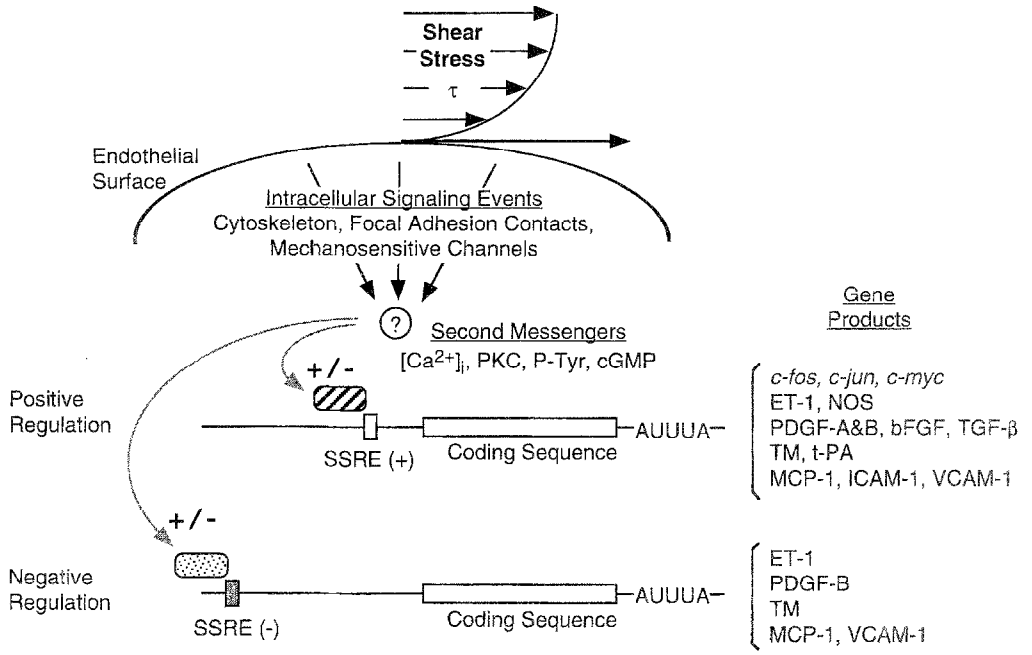


Fig. 5. Molecular biological model of the earlier steps in shear stress regulation of gene expression. Shear activates mechanosensitive structures including cytoskeletal subsets, focal adhesion contacts, channels, or alters boundary layer thickness with resultant altered receptor activity. This initial step is then translated into altered second messenger levels, such as intracellular calcium, $[Ca^{2+}]_i$, protein tyrosine phosphorylation, protein kinase C, or cGMP among others, with eventual change in the activation state of DNA binding factors. This final step alters the transcriptional activity of various genes via either positive shear stress response elements SSRE(+) or negative SSRE(−) or a combination of the two acting at different times. The steps preceding the change in activity of SSREs, the identity of the SSREs and their associated DNA binding factors are still being defined.

Table 4. Nuclear transcription factors, their binding sequences and their potential contribution to gene regulation

DNA binding factor & sequence	Gene containing sequence
SSRE-BP *GAGACC* (Resnick *et al.*, 1993)	NOS PDGF-B, TGF-β t-PA, MCP-1, ICAM-1, *c-fos, c-jun,*
AP-1 *TGACTCC* *TCACTCA* (Shyy *et al.*, 1994)	ET-1, PDGF-A & PDGF-B, TGF-β t-PA, MCP-1, ICAM-1
NFκB *GGAAGATCCCT* (Lan *et al.*, 1994)	VCAM-1

pendently of the GAGACC SSRE (Halnon *et al.*, 1994). Given that the response of VCAM-1 mRNA to shear appears to parallel that of ET-1, it will be interesting to determine whether the transcriptional control in response to flow is the same, especially since neither of their promoter regions contains the PDGF-B SSRE sequence. These results, taken together, suggest that a number of different potential SSREs may be present in the EC and may be activated under different conditions by various mechanisms (Fig. 5). The involvement of the various binding factors, their interactions at various levels and regimes of shear as well as their eventual contribution to the final transcriptional balance are currently being defined.

Significant evidence has accumulated which links the focal nature of atherosclerosis and other vascular pathological situations such as post-stenotic dilatations and vascular graft failure to local wall shear stress and blood pressure. Multiple studies have by now shown a higher propensity for focal atherosclerotic plaque development in areas of low and time-varying shear stress magnitude (Asakura and Karino, 1990; Gibson *et al.*, 1993; Glagov *et al.*, 1988). The *in vitro* results presented above would suggest that endothelium in such areas would be producing higher levels of ET-1, PDGF-B and VCAM-1 and lower levels of NO and TGF-β, conditions that would encourage growth of underlying smooth muscle cells and fibroblasts and migration into the wall of various inflammatory cells. These processes that have been postulated to play a role in the evolution of atherosclerosis (Ross, 1991, 1993). A more complete characterization of the endothelial response to flow and its mechanism should improve understanding of vascular development and pathophysiology and lead to therapeutic interventions to

interrupt the progression of vascular disorders such as atherosclerosis.

Acknowledgements—This work was supported by a Whitaker Foundation Research Grant, a Johnson & Johnson Foundation Research Grant (through the Harvard-MIT Health Sciences and Technology Division), and by the National Institutes of Health Medical Scientist Training Program.

REFERENCES

Adams, D. S. (1992) Mechanisms of cell shape change: the cytomechanics of cellular response to chemical environment and mechanical loading. *J. Cell Biol.* **117,** 83–93.

Ando, J., Tsuboi, H., Korenaga, R., Takada, Y., Toyama-Sorimachi, N., Miyasaka, M. and Kamiya A. (1994). Shear stress inhibits adhesion of cultured mouse endothelial cells to lymphocytes by downregulating VCAM-1 expression. *Am. J. Physiol.* **267,** C679–687.

Asakura, T. and Karino, T. (1990) Flow patterns and spatial distribution of atherosclerotic lesions in human coronary arteries. *Circ. Res.* **66,** 1045–1066.

Barrett, T. B. and Benditt, E. P. (1988) Platelet-derived growth factor gene expression in human atherosclerotic plaques and normal artery wall. *Proc. Natl. Acad. Sci. U.S.A.* **85,** 2810-2814.

Brawerman, G. (1989) mRNA decay: finding the right targets. *Cell* **57,** 9–10.

Chomczynski, P. and Sacchi, N. (1987) Single-step method of RNA isolation by acid guanidium thiocyanate–phenol–chloroform extraction. *Analyt. Biochem.* **162,** 156–159.

Cooke, J. P., Stamler, J. S., Andon, N., Davies, P. R. and Loscalzo, J. (1990) Flow stimulates endothelial cells to release a nitrovasodilator that is potentiated by reduced thiol. *Am. J. Physiol.* **28,** H804–812.

Cybulsky, M. I. and Gimbrone, M. A. Jr (1991) Endothelial expression of a mononuclear leukocyte adhesion molecule during atherogenesis. *Science* **251,** 788–791.

D'Amore, P. A. and Smith, S. R. (1993) Growth factor effects on cells of the vascular wall. *Growth Factors* **8,** 61–75.

Davies, P. F. Flow-mediated endothelial mechanotransduction. *Physiol. Rev.* (in press).

Davies, P. F., Dewey, F. C. Jr, Bussolari, S. R., Gordon, E. J. and Gimbrone, M. A. Jr (1983) Influence of hemodynamic forces on vascular endothelial function, *in vitro* studies of shear stress and pinocytosis in bovine aortic cells. *J. Clin. Invest.* **73,** 1121–1129.

Davies, P. F., Remuzzi, A., Gordon, E., Dewey, F. Jr and Gimbrone, M. A. Jr (1986) Turbulent fluid shear stress induces vascular endothelial cell turnover *in vitro*. *Proc. Natl. Acad. Sci. U.S.A.* **83,** 2114–2117.

Davies, P. F. and Tripathi, S. C. (1993) Mechanical stress mechanisms and the cell, an endothelial paradigm. *Circ. Res.* **72,** 239–245.

Devare, S. G., Reddy, E. P., Law, J. D., Robbins, K. C. and Aaronson, S. A. (1983) Nucleotide sequence of the simian virus genome: demonstration that its acquired cellular sequences encode the transforming gene product $p23^{sis}$. *Proc. Natl. Acad. Sci. U.S.A.* **80,** 731–735.

Diamond, S., Eskin, S. and McIntire L. (1989) Fluid flow stimulates tissue plasminogen activator secretion by cultured human endothelial cells. *Science* **243,** 1483–1485.

Diamond, S. L., Sharefkin, J. B., Dieffenbach, C., Frasier-Scott, K., McIntire, L. V. and Eskin, S. G. (1990) Tissue plasminogen activator messenger RNA levels increase in cultured human endothelial cells exposed to laminar shear stress. *J. Cell. Physiol.* **143,** 364–371.

Dull, R. O. and Davies, P. F. (1991) Flow modulation of agonist (ATP)-response (Ca^{2+}) coupling in vascular endothelial cells. *Am. J. Physiol.* **261,** H149-154.

Esmon, C. T. (1987). The regulation of natural anticoagulant pathways. *Science* **235,** 1348-1352.

Esmon, C. T. (1989) The roles of protein C and thrombomodulin in the regulation of blood coagulation. *J. Biol. Chem.* **264,** 4743–4746.

Frangos, J. A., Eskin, S. G., McIntire, L. V. and Ives, C. L. (1984) Flow effects on prostacyclin production by cultured human endothelial cells. *Science* **227,** 1477–1479.

Fung, Y. C. (1984) *Biodynamics: Circulation.* Springer, New York.

Fung, Y. C. and Liu, S. Q. (1993) Elementary mechanics of blood vessels. *J. Biomech.* **115,** 1–12.

Gibson, C. M., Diaz, L., Kandarpa, K., Sacks, F. M., Pasternak, R. C., Sandor, T., Feldman, C. and Stone, P. H. (1993). Relation of vessel wall shear stress to atherosclerosis progression in human coronary arteries. *Arterioscl. Thromb.* **13,** 310–315.

Glagov, S., Zarins, C., Giddens, D. P. G. and Ku, D. N. (1988) Hemodynamics and atherosclerosis, insights and perspectives gained from studies of human arteries. *Arch. Pathol. Lab. Med.* **112,** 1018-1031.

Goldsmith, H. L. and Turitto, V. T. (1986) Rheological aspects of thrombosis and haemostasis: basic principles and applications. *Thromb. Haem.* **55,** 415–435.

Grabowski, E. F., Jaffe E. A. and Weksler, B. B. (1985) Prostacyclin production by cultured endothelial cell monolayers exposed to step increases in shear stress. *J. Lab. Clin. Med.* **105,** 36–43.

Halnon, N. J., Collins, T., Gimbrone, M. A. Jr and Resnick, N. (1994) Regulation of the endothelial PDGF-A gene by shear stress. *Circulation* **90,** I88 (Abst. 0468).

Hsieh, H.-J., Li, N.-Q. and Frangos, J. A. (1991) Shear stress increases platelet-derived growth factor mRNA levels. *Am. J. Physiol.* **260,** H642–646.

Hsieh, H.-J., Li, N.-Q. and Frangos, J. A. (1992) Shear-induced platelet derived growth factor gene expression in human endothelial cells is mediated by protein kinase C. *J. Cell Physiol.* **150,** 552–558.

Hsieh, H.-J., Li, N.-Q. and Frangos, J. A. (1993a) Pulsatile and steady flow induces *c-fos* expression in human endothelial cells. *J. Cell Physiol.* **154,** 143–151.

Hsieh, H.-J., Shyy, Y.-J., Hu, Y.-L., Rafii, K. and Chien, S. (1993b) MCP-1 gene expression in endothelium is modulated by shear stress (Abstract). *FASEB* **7,** (A54), 308.

Hynes, R. O. (1992) Integrins: versatility, modulation, signalling and cell adhesion. *Cell* **69,** 11–25.

Inoue, A., Yanagisawa, M., Takuwa, Y., Mitsui, Y., Kobayashi M. and T. Masaki, (1989) The human preproendothelin-1 gene: complete nucleotide sequence and regulation of expression. *J. Biol. Chem.* **264,** 14, 954–14,959.

Jackman, R. W., Beeler, D. L., VanDeWater, L. and Rosenberg, R. (1986) Characterization of a thrombomodulin cDNA reveals structural similarity to the low density lipoprotein receptor. *Proc. Natl. Acad. Sci. U.S.A.* **83,** 8834–8838.

Jonasson, L., Holm, J., Skalli, O., Bonders, G. and Hansson, G. L. (1986) Regional accumulations of T cells, macrophages, and smooth muscle cells in the human atehrosclerotic plaque. *Arteriosclerosis* **6,** 131–138.

Kamiya, A. and Togawa, T. (1980) Adaptive regulation of wall shear stress to flow change in the canine carotid artery. *Am. J. Physiol.* **239,** H14-21.

Koller, A. and Kalley, G. (1991) Endothelial regulation of wall shear stress and blood flow in skeletal muscle microcirculation. *Am. J. Physiol.* **260,** H862-868.

Koller, A. Sun, D. and Kaley, G. (1993) Role of shear stress and endothelial prostaglandins in flow- and viscosity-induced dilation of arterioles *in vivo*. *Circ. Res.* **72,** 1276–1284.

Komuro, I., Kaida, T., Shibazaki, Y., Kurabayashi, M., Katoh, Y., Hoh, E., Takaku, F. and Yazaki, Y. (1990) Stretching cardiac myocytes stimulates protooncogene expression. *J. Biol. Chem.* **265,** 3595–3598.

Kuchan, M. J. and Frangos, J. A. (1993) Shear stress regulates endothelin-1 release via protein kinase C and cGMP in cultured endothelial cells. *Am. J. Physiol.* **264,** H150–156.

LaBarbera, M. (1990) Principles of design of fluid transport systems in zoology. *Science* **249,** 992–1000.

Lan, Q., Mercurius, K. O. and Davies, P. F. (1994) Stimulation of transcription factors NFkB and AP1 in endothelial cells subjected to shear stress. *Biochem. Biophys. Res. Comm.* **201,** 950–956.

Langille, B. L. (1993) Remodeling of developing and mature arteries: endothelium, smooth muscle and matrix. *J. Cardiovasc. Pharm.* **21,** S11–17.

Langille, B. L., Pendeck, M. P. and Keeley, F. W. (1989) Adaptations of carotid arteries of young and mature rabbits to reduced carotid blood flow. *Am. J. Physiol.* **256,** H931–H939.

Liaw, L. and Schwartz, S. (1993) Comparison of gene expression in bovine aortic endothelium *in vivo* versus *in vitro*: differences in growth regulatory molecules. *Circ. Res.*, **13,** 985–993.

Majerus, P. W., De Munk, G. A. W., Groeneveld, E. and Rijken, D. C. (1991) Acceleration of the thrombin inactivation of single chain urokinase-type plasminogen activator (prourokinase) by thrombomodulin. *J. Clin. Invest.* **88,** 1680–1684.

Malek, A. M. (1994) Molecular and functional characterization of the vascular endothelial response to fluid shear stress. Ph.D. thesis, MIT.

Malek, A. M., Gibbons, G. H., Dzau, V. J. and Izumo, S. (1993a) Fluid shear stress differentially modulates expression of genes encoding basic fibroblast growth factor and platelet-derived growth factor B-chain in vascular endothelium. *J. Clin. Invest.* **92,** 2013–2021.

Malek, A. M., Gibbons, G. H., Dzau, V. J. and Izumo, S. A cone-plate apparatus for the *in vitro* biochemical and molecular *analysis* of the effect of shear stress on adherent cells. *Meth. Cell Sci.* **17,** (in press).

Malek, A. M., Greene, A. L. and Izumo, S. (1993b) Regulation of endothelin-1 gene by fluid shear stress is transcriptionally mediated and independent of protein kinase C and cAMP. *Proc. Natl. Acad. Sci. U.S.A.* **90,** 5999–6003.

Malek, A. and Izumo, S. (1992) Physiological fluid shear stress causes down-regulation of endothelin-1 mRNA in bovine aortic endothelium. *Am. J. Physiol.* **32,** C389–396.

Malek, A. M. and Izumo, S. (1994) Molecular aspects of signal transduction of shear stress in the endothelial cell. *J. Hypertension* **12,** 989–999.

Malek, A. M., Jackman, R. W., Rosenberg, R. D. and Izumo, S. (1994) Endothelial expression of thrombomodulin is reversibly regulated by fluid shear stress. *Circ. Res.* **74,** 852–860.

Masuda, H., Kawamura, K., Tohda, K., Shozawa, T., Sageshima, M. and Kamiya, A. (1989) Increase in endothelial cell density before enlargement in flow-loaded canine carotid artery. *Arteriosclerosis* **9,** 812–823.

Mignatti, P. and Rifkin, D. B. (1993) Biology and biochemistry of proteinases in tumor invasion. *Physiol. Rev.* **73,** 161–195.

Mitsumata, M., Fishel, R. S., Nerem, R. M., Alexander, R. W. and Berk B. C. (1993) Fluid shear stress stimulates platelet-derived growth factor expression in endothelial cells. *Am. J. Physiol.* **262,** H3–8.

Mo, M., Eskin, S. and Schilling, W. P. (1991) Flow-induced changes in Ca^{2+} signalling in vascular endothelial cells: effect of shear stress and ATP. *Am. J. Physiol.* **260,** H1698–1707.

Morita, T., Kurihara, H., Maemura, K., Yoshizumi, M., Nagai, R. and Yazaki, Y. (1994) Role of Ca^{2+} and protein kinase C in shear stress-induced actin depolymerization and endothelin 1 gene expression. *Circ. Res.* **75,** 630–636.

Moses, H. L., Yang, E. Y. and Pietenpol, J. A. (1990) TGF-β stimulation and inhibition of cell proliferation: new mechanistic insights. *Cell* **63,** 245–247.

Nagel, T., Resnick, N., Atkinson, W. J., Dewey C. F. Jr and Gimbrone, M. A. Jr (1994) Shear stress selectively upregulates intercellular adhesion molecule-1 expression in cultured human vascular endothelial cells. *J. Clin. Invest.* **94,** 885–891.

Nishida, K., Harrison, D. G., Navas, J. P., Fisher, A. A., Docjery, S. P., Uematsu, M., Nerem, R. M., Alexander, R. W. and Murphy, T. J. (1992) Molecular cloning and characterization of constitutive bovine aortic endothelial cell nitric oxide synthase. *J. Clin. Invest.* **90,** 2092–2096.

Noris, M., Morigi, M., Donadelli, R., Aiello, S., Foppolo, M., Todeschini, M., Orisio, S., Remuzzi, G. and Remuzzi, A. (1995) Nitric oxide synthesis by cultured endothelial cells is modulated by flow conditions. *Circ. Res.* **76,** 536–543.

Ohno, M., Cooke, J. P., Dzau, V. J. and Gibbons, G. H. (1995) Fluid shear stress induces transforming growth factor beta-1 transcription and production. *J. Clin. Invest.* **95,** 1363–1369.

Parkinson, J. F., Bang, N. U and Garcia, J. G. N. (1993) Recombinant human thrombomodulin attenuates human endothelial cell activation by human thrombin. *Arterioscl. Thromb.* **13,** 1119–1123.

Resnick, N., Collins, T., Atkinson, W., Bonthron, D. T., Dewey, C. F. J. and Gimbrone, M. A. (1993) Platelet-derived growth factor B chain promoter contains a cis-acting fluid shear stress-responsive element. *Proc. Natl. Acad. Sci. U.S.A.* **90,** 4591–4595.

Rifkin, D. B. and Moscatelli, D. (1989) Recent developments in the cell biology of basic fibroblast growth factor. *J. Cell Biol.* **109,** 1–6.

Ross, R. (1991) Polypeptide growth factors and atherosclerosis. *Trends Cardiovasc. Med.* 277–282.

Ross, R. (1993) The pathogenesis of atherosclerosis: perspective for the 1990s. *Science* **362,** 801–809.

Rubanyi, G. M., Romero, J. C. and Vanhoutte, P. M. (1986) Flow-induced release of endothelium derived relaxing factor. *Am. J. Physiol.* **250,** H1145–1149.

Sadoshima, J., Jahn, L., Takahashi, T., Kulik, T. J. and Izumo, S. (1992) Molecular characterization of the stretch-induced adaptation of cultured cardiac cells. An *in vitro* model of load-induced cardiac hypertrophy. *J. Biol. Chem.* **267,** 10, 551–10,560.

Sato, Y. and Rifkin, D. B. (1988) Autocrine activities of basic fibroblast growth factor: regulation of endothelial cell movement, plasminogen activator synthesis, and DNA synthesis. *J. Cell Biol.* **107,** 1199–1205.

Schweigerer, L., Neufeld, G., Friedman, J., Abraham, J. A., Fiddes, J. C. and Gospodarowicz D. (1987) Capillary endothelial cells express basic fibroblast growth factor, a mitogen that promotes their own growth. *Nature* **325,** 257–259.

Sharefkin, J. B., Diamond, S. L., Eskin, S. G., McIntire L. V., and Dieffenbach, C. W. (1991) Fluid flow decreases preproendothelin mRNA release and suppresses endothelin-1 peptide release in cultured human endothelial cells. *J. Vasc. Surg.* **14,** 1–9.

Shyy, Y.-J., Hsieh, H.-J., Usami, S. and Chien, S. (1994a) Fluid shear stress induces a biphasic response of human monocyte chemotactic protein 1 gene expression in vascular endothelium. *Proc. Natl. Acad. Sci. U.S.A.* **91,** 4678–4682.

Shyy, J. Y.-J., Lu, Y.-J., Chen, W., Lin, M.-C. and Chien, S. (1994b) Shear stress activation of monocyte chemotactic protein-1 is mediated through the phorbol ester TPA-responsive element. *Circulation* **90,** I572 (Abst. 3079).

Sporn, M. B. and Robert, A. B. (1992) Transforming growth factor-β: recent progress and new challenges. *J. Cell Biol.* **119,** 1017–1021.

Springer, T. A. (1990) Adhesion receptors of the immune system. *Nature* **346,** 425–434.

Uematsu, M., Navas, J. P., Nishida, K., Ohara, Y., Murphy, T. J., Alexander, W., Nerem, R. M. and Harrison, D. G. (1993) Mechanisms of endothelial cell NO synthase induction by shear stress. (Abstract). *Circulation* **88,** I184, 978.

Vassalli, J. D., Sappino, A. P. and Belin, D. (1991) The plasminogen activator/plasmin system. *J. Clin. Invest.* **88,** 1067–1072.

Yanagisawa, M., Kurihara, H., Kimura, S., Tomobe, Y., Kobayashi, M., Mitsui, T., Yazako, Y., Goto K. and Masaki, T. (1988). A novel potent vasoconstrictor peptide produced by vascular endothelial cell. *Nature* **332,** 411–415.

Yasafumi, S., Fumio, O., Mayumi, A., Tadashi, S., Michihiko, K., Seiji, S., Akiko, F., Nobuo, H. and Tatsuya, T. (1993) The mechanism for the activation of latent TGF-β during coculture of endothelial cells and smooth muscle cells: cell-type specific targeting of latent TGF-β to smooth muscle cells. *J. Cell Biol.* **123,** 1249–1254.

Yoshizumi, M., Kurihara, H., Sugiyama, T., Takaku, F., Yanagisawa, M., Masaki, T. and Yazaki, Y. (1989) Hemodynamic shear stress stimulates endothelin production by cultured endothelial cells. Biochem. *Biophys. Res. Comm.* **161,** 859–864.

Zarins, C. K., Zatina, M. A., Giddens, D. P., Ku, D. N. and Glagov, S. (1987) Shear stress regulation of artery lumen diameter in experimental atherogenesis. *J. Vasc. Surg.* **5,** 413–420.

Zerwes, H.-G. and Risau, W. (1987) Polarized secretion of a platelet-derived growth factor-like chemotactic factor by endothelial cells *in vitro*. *J. Cell Biol.* **105,** 2037–2041.