

## Flow Shear Stress and Atherosclerosis: A Matter of Site Specificity

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### Abstract

It is well accepted that atherosclerosis occurs in a site-specific manner especially at branch points where disturbed blood flow (d-flow) predisposes to the development of plaques. Investigations both *in vivo* and *in vitro* have shown that d-flow is pro-atherogenic by promoting oxidative and inflammatory states in the artery wall. In contrast, steady laminar blood flow (s-flow) is atheroprotective by inhibition of oxidative stress and inflammation in the vessel wall. The mechanism for inflammation in endothelial cells (ECs) exposed to d-flow has been well studied and includes redox-dependent activation of apoptosis signal-regulating kinase 1 (ASK1) and Jun NH2-terminal kinase (JNK) that ultimately lead to the expression of adhesive molecules. In contrast, s-flow leads to the activation of the mitogen extracellular-signal-regulated kinase kinase 5/extracellular signal-regulated kinase-5 (MEK5/ERK5) pathway that prevents pro-inflammatory signaling. Important transcriptional events that reflect the pro-oxidant and pro-inflammatory condition of ECs in d-flow include the activation of activator protein 1 (AP-1) and nuclear factor kappaB (NFκB), whereas in s-flow, activation of Krüppel-like factor 2 (KLF2) and nuclear factor erythroid 2-like 2 (Nrf2) are dominant. Recent studies have shown that protein kinase c zeta (PKCζ) is highly activated under d-flow conditions and may represent a molecular switch for EC signaling and gene expression. The targeted modulation of proteins activated in a site-specific manner holds the promise for a new approach to limit atherosclerosis. *Antioxid. Redox Signal.* 15, 1405–1414.

### Introduction

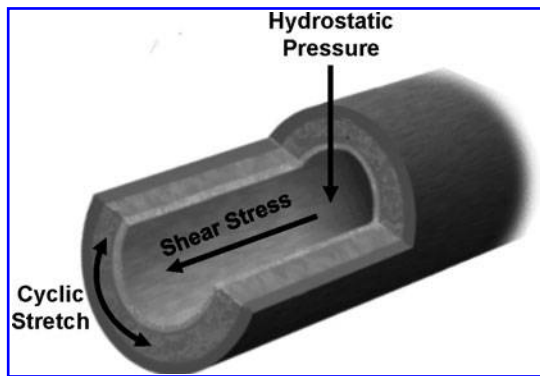
IN THE LATE 15TH CENTURY, Leonardo Da Vinci documented the potential importance of hemodynamics for cardiovascular tissue. Da Vinci wrote that he expected blood flow in the heart to be subjected to similar principles as those he had observed in the flow of water around obstacles in nature. In the 19th century, the great pathologists Rokitsansky (78) and Virchow (90) recognized that the distribution of atherosclerotic lesions was nonuniform and postulated that mechanical forces operating in different regions of the arterial tree may be responsible. Fry (31) and Caro and coworkers (8, 10) investigated the fluid dynamics of lesion-susceptible sites of large arteries and established several important concepts with respect to the role of hemodynamics in atherogenesis. Classic investigations of the hemodynamic role in lesion distribution have been carried out by Glagov, Zarins, Giddens, and coworkers (47, 48, 98). The molecular mechanisms linking endothelial flow responses to the pathogenesis of atherosclerosis are complex and difficult to dissect (38). However, recent publications have presented new steps toward the understanding of how the cell senses

shear stress, and what signaling pathways and genes are regulated. The main objectives of this review are (i) to document the current state of knowledge concerning endothelial response to mechanical forces related to blood flow, (ii) to present specific differences in both signaling mechanisms and gene expression that occur under steady laminar blood flow (s-flow) and/or disturbed blood flow (d-flow) patterns, and (iii) to suggest new candidates and future directions into endothelial mechanotransduction to prevent atherogenesis.

### Flow Confers an Atheroprotective Force

Blood vessels are constantly exposed to various types of hemodynamic forces (including fluid shear stress, cyclic stretch, and hydrostatic pressure) induced by the pulsatile blood flow and pressure (Fig. 1). Shear stress is the force per unit area created when a tangential force (blood flow) acts on a surface (endothelium) (22). The magnitudes of shear stress range from 0.1 N/m<sup>2</sup> (1 dyn/cm<sup>2</sup>) to 0.6 N/m<sup>2</sup> (6 dyn/cm<sup>2</sup>) in the venous system and from 1 N/m<sup>2</sup> (10 dyn/cm<sup>2</sup>) to 7 N/m<sup>2</sup> (70 dyn/cm<sup>2</sup>) in the arterial vessels (56, 63).

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**FIG. 1. Hemodynamic forces on the vessel wall.** A section of the artery wall showing that blood flow generates three major mechanical forces: shear stress (parallel to the vessel wall), hydrostatic pressure (perpendicular to the vessel wall), and cyclic stretch (due to the pressure and consisting in a circumferential stretching of the vessel wall).

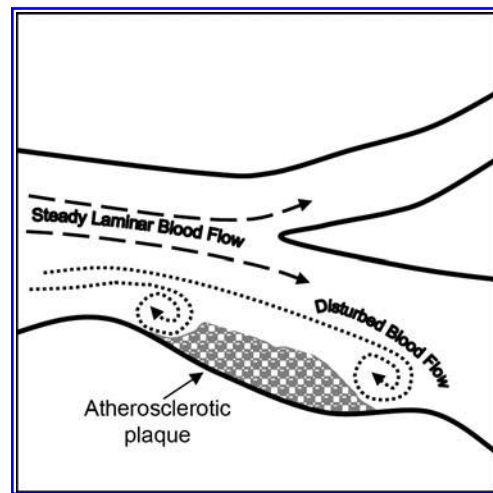
There is a considerable amount of evidence that hemodynamic characteristics determine the location of lesions and contribute to the pathogenesis of atherosclerosis (56, 93). In fact, atherosclerotic lesions are preferentially located at the outer walls of the arterial branches and curvatures, where the local flow is disturbed (*e.g.*, nonuniform, irregular oscillation, and recirculation) (3, 9, 48, 98).

S-flow and sustained high shear stress, as seen in the straight part of the arterial tree, modulate the expression of genes and proteins in endothelial cells (ECs) to protect against atherosclerosis. d-flow and reciprocating shear stress with little forward direction, as seen in vascular branch points and other regions of complex flow, cause the expression of atherogenic genes and proteins that predispose these areas to atherosclerosis.

Direct measurements and fluid mechanical analyses of models of these lesion-prone areas have revealed that shear stress is on the order of  $\pm 0.4 \text{ N/m}^2$  ( $4 \text{ dyn/cm}^2$ ) in d-flow areas and  $>1.2 \text{ N/m}^2$  ( $12 \text{ dyn/cm}^2$ ) in the s-flow areas (56). Thus, s-flow plays protective roles against atherosclerosis, whereas d-flow may act as detrimental mechanical stimuli contributing to atherogenesis (33, 56, 63, 93) (Fig. 2).

### Effect of Flow Shear Stress on EC Phenotype

ECs are in direct contact with the flowing blood and therefore bear most of the wall shear stress. Evidence of direct effects of shear stress on endothelial structure and function has been obtained primarily from *in vitro* studies using cultured ECs exposed to different types of flow shear stress (*e.g.*, laminar, pulsatile, disturbed, or reciprocating flow). These studies have suggested that ECs not only sense and respond to shear stress, but also react specifically to different types of shear stress (5, 37, 74). Importantly, continually sheared ECs exhibit a new phenotype. In particular, sustained s-flow determines a cell cycle arrest in the  $G_0$  or  $G_1$  phase (52). In contrast, EC turnover is accelerated in d-flow (17). This effect may be related to the release of p21 suppression of cyclin-dependent kinase activity *via*  $G_0/G_1$ -S transition (2, 23). Such accelerated cell turnover may explain the enhanced macromolecular permeability and increased lipid uptake in the



**FIG. 2. Vascular bifurcation and flow patterns at an area of atherosclerotic plaque.** Straight regions of arteries are exposed to s-flow and are protected from atherosclerosis. Regions of bifurcations are characterized by d-flow that predisposes to atherosclerosis. Atherosclerotic lesions determine an increased velocity of flow through the narrowed luminal space and create a flow separation in the regions immediately downstream the plaque. d-flow, disturbed blood flow; s-flow, steady laminar blood flow.

d-flow areas of the vascular tree, resulting in a particular propensity to develop atherosclerosis (15). In addition to the new phenotype changes, continually sheared ECs exhibit a different morphology (12, 57, 91, 92). In response to sufficient magnitude and duration of s-flow, ECs become aligned and elongated in the direction of the flow, with a significant alteration in cytoskeletal architecture. In contrast, in response to d-flow, cells appear with a more polygonal appearance without a clear orientation. This observation on EC morphological responses induced by different pattern of flow *in vitro* recapitulates many of the morphological features that have been described *in vivo* (50).

These profound morphological adaptations to shear stress are driven by a novel reorganization of actin in stress fibers (67), redistribution of focal adhesion complexes (34, 67), and partial disassembly and reassembly of cell-cell junctional complexes (66). A recent article shows that Krüppel-like factor 2 (KLF2) is essential for shear stress-induced cell alignment, concomitant shear fiber assembly, and inhibition of Jun NH2-terminal kinase (JNK) and its downstream targets ATF2/c-Jun (7).

Imposition of shear stress on cultured endothelium also induces planar cell polarity (PCP) in the downstream direction (89). PCP occurs when cell organelles, cytoskeleton, and/or adhesion complexes exhibit unidirectional organization along an axis that lies in the plane of a cell monolayer. McCue *et al.* showed a novel mode of mechanosensitive PCP that is tightly regulated by glycogen synthase kinase 3 beta (GSK-3 $\beta$ ), proving that manipulation of GSK-3 $\beta$  could induce polarity of these cells to reverse direction (58). This is a capacity not displayed by any other cell type studied to date. Remarkably, ECs display PCP *in vitro* and in intact blood vessels (77). However, the origins of this polarity are controversial because microtubule polarity *in vivo* varies among blood

vessels (76, 77). Specifically, PCP is often directed upstream in arteries and downstream in veins, a finding that has led to the inference that it is independent of shear stress.

Polarity of many cell types is controlled by the partitioning-defective (PAR) complex consisting of PAR-3, PAR-6, and protein kinase c zeta (PKC $\zeta$ ). This ternary complex is part of an evolutionary conserved molecular cassette with fundamental roles in cell polarity in a variety of biological context (6, 45, 46, 80).

We have recently reported that PKC $\zeta$  is highly modulated by shear stress patterns and is able to decrease the shear stress-induced KLF2 promoter activity (65).

### Difference in Signaling Mechanism in s-Flow Compared to d-Flow Patterns

As shear stress acts at the surface of luminal cells, local membrane structure can participate in mechanotransduction. Several mechanosensing candidates have been proposed although it is not clear how they orchestrate responses to shear stress. Examples include activation of ion channels, G proteins, glycocalyx, and primary cilia, and changes in phospholipid metabolism and membrane fluidity (22). *In vitro* studies using parallel-plate flow chamber or cone-and-plate viscometer (75) have shown that the activation of mechanosensors by shear stress leads to the activation of specific signaling pathways, with Ras serving as an upstream molecule and with three key downstream mitogen-activated protein kinases (MAPKs) molecules: extracellular signal-regulated kinase (ERK), JNK, and p38.

A crucial s-flow-mediated atheroprotective mechanism is the mitogen extracellular-signal-regulated kinase kinase 5 (MEK5)-ERK5-KLF2 pathway. ERK5 is critical to EC functions as shown by the fact that ERK5 knockout mice have impaired vascular development (73) and the inhibition of ERK5 promotes EC apoptosis (71). We have shown that s-flow potently activates ERK5 (86) and inhibits leukocyte binding as well as adhesion molecule expression (18). We have also reported that s-flow-induced peroxisome proliferator-activated receptor  $\gamma$ 1 (PPAR $\gamma$ 1) activation *via* ERK5 contributes to the anti-inflammatory and atheroprotective effects of s-flow. In addition, we and others have demonstrated that s-flow-mediated ERK5 activation induces KLF2, a recently identified transcriptional activator of endothelial nitric oxide synthase (eNOS) and inhibitor of EC inflammation (1, 69).

Moreover, using both *in vitro* cultured ECs (54, 86) and *in vivo* intact vessels (96), we have demonstrated that inhibition of tumor necrosis factor- $\alpha$  (TNF $\alpha$ )-mediated activation of the Apoptosis signal-regulating kinase 1 (ASK1) pathway is one mechanism by which s-flow is atheroprotective. ASK1 has been shown to play a key role in vascular dysfunction. Yamashita *et al.* (94) reported that ASK1 contributes to endothelial dysfunction by reducing eNOS activity and activating reduced nicotinamide adenine dinucleotide phosphate (NADPH) oxidase. ASK1 is a 170-kDa protein that functionally is composed of an inhibitory N-terminal domain, an internal kinase domain, and a C-terminal regulatory domain. The C-terminal domain of ASK1 binds to TNF receptor-associated factor 2 (TRAF2) and this association is required for ASK1 activation by cytokines. The C-terminal domain contains Ser967, which when phosphorylated creates a motif for 14-3-3 binding that inhibits ASK1. We recently showed that

14-3-3 is an important regulator in s-flow-mediated inhibition of TNF $\alpha$ -stimulated ASK1-mediated JNK activation (54). It has also been shown that thioredoxin-1 (Trx1), in a reduced form, binds to the N-terminal part of ASK1 and blocks activation of ASK1 by TNF $\alpha$  (35, 53, 79). Apoptotic stimuli (TNF $\alpha$  or reactive oxygen species [ROS]) activate ASK1 in part by oxidizing Trx1 to release Trx1 from ASK1 (35, 53, 79). Our recently published data (95, 96) suggest that s-flow increases Trx1 activity, thereby inhibiting ASK1-JNK activation. These data suggest that s-flow modulates the EC redox state, subsequently regulating Trx1 binding to ASK1. This may be an important mechanism to explain the observations that s-flow prevented EC apoptosis induced by TNF $\alpha$  (27).

### Difference in Gene Expression in s-Flow Compared to d-Flow Patterns

The activation of the pathways described above leads to the regulation of pathophysiologically relevant genes, whose expression is modulated by different types of shear stress.

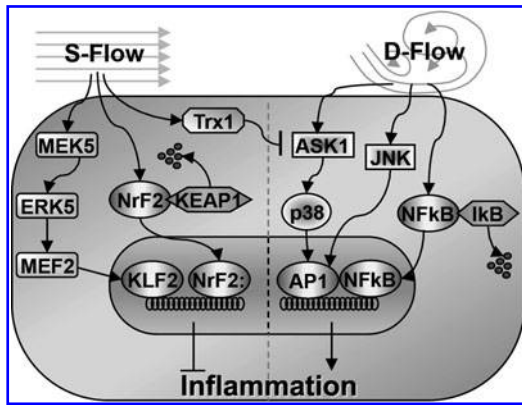
Several studies have been performed to identify the differences, on the transcriptomic level, of ECs exposed to shear stress compared to control conditions. One of the first studies described that ECs in s-flow (atheroprotected) regions of pig aortas have lower expressions of both pro- and anti-inflammatory genes (70). Using a similar full-genome approach, Dekker *et al.* (25) identified KLF2 as a transcription factor specifically induced by s-flow in ECs. Further, KLF2 was found not to be induced by other stimuli that govern the endothelial transcriptome. Nuclear factor erythroid 2-like 2 (Nrf2) was also identified as a shear-induced transcription factor that is responsible for antioxidant gene expression (14). It has also been shown that both of these transcription factors are induced in s-flow regions of the vasculature *in vivo* as well (20, 26).

In addition, it was shown that KLF2 improves the nuclear localization of Nrf2 and the combined actions of these two factors constitute about 70% of the s-flow-induced endothelial gene expression (29). Nrf2 potently induces anti-inflammatory/antioxidant enzymes, whereas KLF2 induces anti-inflammatory and anticoagulant proteins, most specifically eNOS and thrombomodulin. KLF2 also inhibits proinflammatory and antifibrinolytic genes through inhibition of the proinflammatory transcription factors nuclear factor kappaB (NF $\kappa$ B) and activator protein (AP-1).

On the other hand, d-flow activates NF $\kappa$ B and AP-1 in ECs. Nagel *et al.* (62) demonstrated that ECs subjected to d-flow exhibit increased levels of localized NF $\kappa$ B, c-Jun, and c-fos in their nuclei, as compared with the cells exposed to s-flow or maintained under static conditions. The activation of these transcription factors leads to the upregulation of pro-inflammatory and pro-atherogenic genes such as intercellular adhesion molecule 1 (ICAM-1), E-selectin, platelet-derived growth factor (PDGF)-BB, interleukin (IL)-1 $\alpha$  (21, 97), bone morphogenic protein-4 (BMP-4) (84), monocyte chemoattractant protein-1 (MCP-1) (39), and the vasoconstrictor endothelin-1 (ET-1) (99). Based on these data, s-flow activates KLF2 and Nrf2, which inhibit inflammation, whereas d-flow activates NF $\kappa$ B and AP-1, which promote inflammation (Fig. 3).

More recently Jo's group reported flow-sensitive gene changes using the mouse model of d-flow (64), discovering numerous novel mechanosensitive molecules and providing





**FIG. 3. Signaling pathways activated by s-flow and d-flow.** s-flow activates the MEK5/ERK5/KLF2 cascade and also determines the translocation of Nrf2 to the nucleus after Keap1 is dissociated and degraded. KLF2 and Nrf2 mediate the anti-inflammatory effects of s-flow. s-flow also increases Trx1 activity, which inhibits ASK1 activation. On the other hand, d-flow, through the activation of ASK1 and JNK, as well as induction of IκB degradation, determines the activation of the NFκB and AP-1, which are responsible for the pro-inflammatory effects of d-flow. Keap1 and IκB degradation is represented in the figure as circle grouping. AP-1, activator protein-1; ASK1, apoptosis signal-regulating kinase 1; ERK5, extracellular signal-regulated kinase-5; JNK, jun NH2-terminal kinase; KLF2, Krüppel-like factor 2; MEK5, mitogen extracellular-signal-regulated kinase kinase 5; NFκB, nuclear factor kappaB; Nrf2, nuclear factor erythroid 2-like 2; Trx1, thioredoxin-1.

additional support for the flow-dependent regulation of KLF2.

Conklin *et al.* (19) also demonstrated that d-flow increases vascular endothelial growth factor (VEGF) expression. These changes in VEGF expression may suggest a possible molecular mechanism for increased endothelial permeability in regions of d-flow. Other findings demonstrate that d-flow increases the interaction between the white blood cells in the flowing blood and ECs, contributing to the regional propensity for inflammatory cell recruitment in areas prone to atherosclerosis (13, 16).

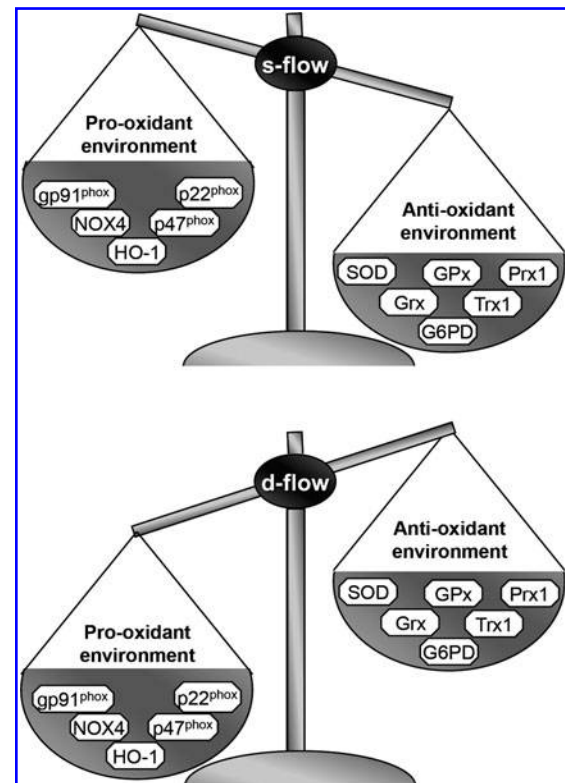
All together, these results suggest that different patterns of shear stress selectively regulate gene expression tipping the balance toward anti-inflammatory and antiatherogenic effect in s-flow conditions *versus* pro-inflammatory and pro-atherogenic status in d-flow settings.

#### Different Patterns of Flow Shear Stress and ROS

ROS acts as mediators in EC responses to shear stress. The basic product of enzymatic ROS production is the superoxide anion ( $O_2^{\bullet-}$ ), which is converted quickly into hydrogen peroxide ( $H_2O_2$ ) by superoxide dismutase (SOD). The  $H_2O_2$  is transformed in its turn by two enzymes, catalase and glutathione peroxidase (GPx). The most relevant sources of ROS, with respect to vascular disease, appear to be NADPH oxidase, xanthine oxidase (XO), uncoupled eNOS, cytochrome P450, and the mitochondrial respiratory chain (51, 85). Conversely, ROS production is kept in check by antioxidant defenses such as SOD, catalase, and GPx, but also Trx1 and heme oxygenase (HO-1).

Laurindo *et al.* (49) observed ROS generation after flow changes *in vitro* and *in vivo* using electron paramagnetic resonance spectroscopy. Since then, it has been well established that shear stress causes the generation of not only NO, but also ROS in isolated ECs as well as in intact vessels and tissue. In particular, continuous oscillatory shear in human umbilical vein endothelial cells (HUVECs) results in oxidative stress by increasing of intracellular  $O_2^{\bullet-}$ . The increased  $O_2^{\bullet-}$  production is dependent on reduced nicotinamide adenine dinucleotide (NADH) oxidase activity, and HO-1 mRNA (24). Further,  $O_2^{\bullet-}$  production is increased by the upregulation of subunits of the NADPH oxidase complex, such as  $p47^{phox}$  (42),  $p22^{phox}$  (81),  $gp91^{phox}$ , and NOX4 expression (41) (Fig. 4).  $O_2^{\bullet-}$  generation by oscillating shear is also reliant upon the balance of XO and dehydrogenase in ECs (59, 60). Under this condition of oxidative stress, the activity of both  $O_2^{\bullet-}$  and NO-generating enzymes results in protein nitrosylation through ONOO<sup>-</sup> formation, as found in areas at risk of atherosclerosis (40). This modulation of gene expression and increased ROS production correlates well with low-density lipoprotein oxidation, MCP-1 expression, and monocyte-EC binding (42).

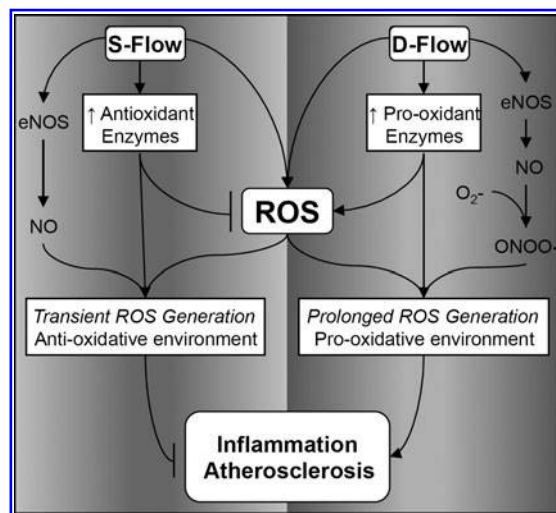
Moreover, abnormally high flow was recently shown to upregulate NADPH oxidase-dependent ROS production chronically in carotid arteries (11), suggesting that supra-physiological levels of shear stress have a protracted influence on the oxidative status.



**FIG. 4. Balance between antioxidant and pro-oxidant enzymes under s-flow and d-flow conditions.** S-flow induces the expression of antioxidant enzymes that tip the balance toward a reducing environment, whereas d-flow upregulates pro-oxidant enzymes that contribute to increased oxidative stress.

Interestingly, onset of s-flow also appears to induce ROS (4, 24). However, in the course of several hours, the effects of flow onset are compensated by antioxidant defenses, such as increased Cu/Zn SOD expression (24, 43) and Mn/SOD (88), upregulation and activation of eNOS, and the subsequent downregulation, by NO, of NADPH oxidase subunits (28), as well as the increased expression of GPx (87), Prx1 (61), and Trx1 (36) (Fig. 4). We have recently shown that s-flow increases G6PD activity (68). G6PD is the rate-limiting enzyme for NADPH formation in ECs and also appears to mediate increases in reduced glutathione (GSH). As a consequence of increased GSH, both Trx1 and glutaredoxin (Grx) are maintained in a reduced form. Reduced Grx and Trx1 bind and inactivate ASK1 decreasing inflammation (vascular cell adhesion molecule 1 [VCAM-1] expression) and atherosclerosis. These data explain how s-flow inhibits inflammation by creating a reducing environment.

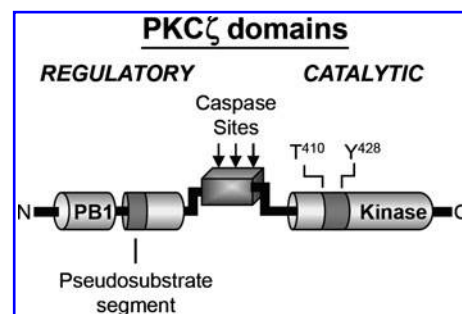
Thus, with continuous s-flow applied to cultured cells, ROS are produced, but their bioavailability is reduced over time by ROS-neutralizing enzymes and are overwhelmed by NO availability (Fig. 5). These combined studies explain how s-flow and d-flow produce divergent effects on ROS-producing and ROS-neutralizing enzymes, tipping the balance toward an antioxidative environment in the former condition and oxidative state in the latter (Fig. 4). Hence, ROS generation in ECs exposed to s-flow is a transient response to the change in shear rate, whereas ROS production persists in ECs exposed to d-flow, generating a pro-oxidant and athero-prone condition (Fig. 5).



**FIG. 5. ROS generation in s-flow versus d-flow settings.** ROS are generated under s-flow as well as under d-flow conditions. However, s-flow induces only a transient ROS generation because it also increases the expression of antioxidant enzymes that neutralize ROS and contribute to an antioxidative environment. d-flow causes a sustained increase in ROS production, derived in part from pro-oxidant enzyme and uncoupled eNOS. Further,  $O_2^{\bullet-}$  produced under d-flow conditions reacts with NO to form  $ONOO^-$ , which contributes to further oxidative damage. eNOS, endothelial nitric oxide synthase; ROS, reactive oxygen species.

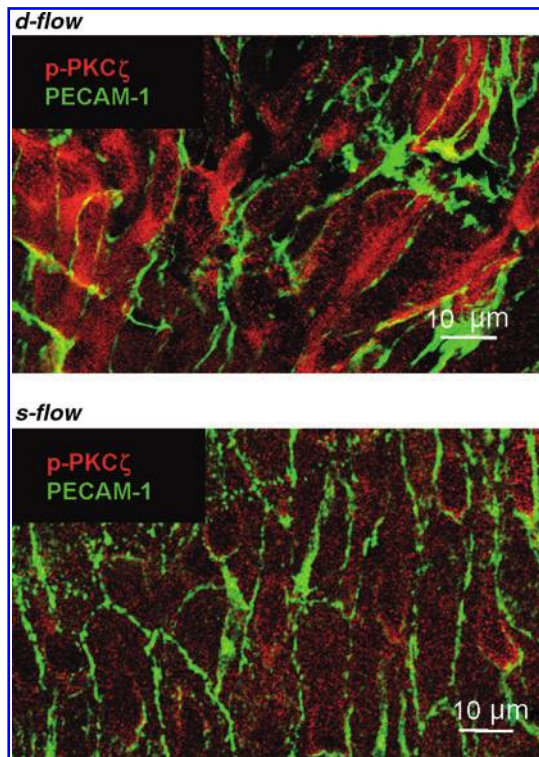
### Site Specificity Mechanisms in d-Flow Areas: Focus on PKC $\zeta$

PKC $\zeta$  is a key mediator of shear stress responses, ROS generation, and inflammation. PKC $\zeta$  has a conserved carboxyl-terminal catalytic domain and an amino-terminal regulatory domain. The regulatory segment contains a PB1 domain (Phox and Bem1p), which constitutes a recently recognized protein-protein interaction domain found in the atypical protein kinase C (aPKC), and also an autoinhibitory sequence (pseudosubstrate) (Fig. 6). Previous work has shown that TNF $\alpha$  and cycloheximide treatment induces PKC $\zeta$  (72 kDa) processing into a shorter form, named catalytic domain of PKC $\zeta$  (CAT $\zeta$ , 50 kDa) (82). This caspase-dependent processing promotes relief of the autoinhibitory state by separating the kinase domain (aa268–335) from the pseudo-substrate autoinhibitory sequence (aa116–122) (83). Caspase 3-dependent processing was also shown to increase PKC $\zeta$  kinase activity, likely due to loss of endogenous negative regulation (82). We have recently reported that CAT $\zeta$ , generated by caspase-3 cleavage of PKC $\zeta$ , potentiates a feedback loop that activates JNK to amplify caspase-3 activation and the cleavage of more PKC $\zeta$  (32). The same pathway was shown both in HUVEC and EC fractions from rabbit aorta (32). Notably, we have found that exposure of ECs to s-flow inhibited JNK-caspase-3-CAT $\zeta$  generation, reducing apoptosis and pro-inflammatory endothelial adhesion protein expression. In addition, PKC $\zeta$  promotes the adhesive phenotype of ECs when activated by TNF $\alpha$  (72), *via* stimulation of NF $\kappa$ B-dependent ICAM-1 expression (44, 72). Also, Frey *et al.* demonstrate a novel function of PKC $\zeta$  in signaling oxidant generation in ECs by the activation of NADPH oxidase, which may be important in mediating endothelial activation responses (30). Interestingly, Davies group reported that PKC $\zeta$  is highly modulated by shear stress patterns with increased activity in ECs located in d-flow compared to s-flow regions in pig aortas (55). Because



**FIG. 6. PKC $\zeta$  domains.** PKC $\zeta$  contains a regulatory and a catalytic domain. The regulatory domain is comprised of PB1 and the pseudosubstrate segment. The PB1 domain allows PKC $\zeta$  to interact with other PB1 domains of scaffolding and anchoring proteins. The pseudosubstrate segment represents an inhibitory substrate domain. PKC $\zeta$  contains three caspase sites between the regulatory and catalytic domain. Caspase processing releases a functional catalytic domain of the enzyme, CAT $\zeta$ . Two phosphorylation sites, T-410 and T-428, involved in PKC $\zeta$  activation, are highlighted. CAT $\zeta$ , catalytic domain of PKC $\zeta$ ; PKC $\zeta$ , protein kinase C zeta.





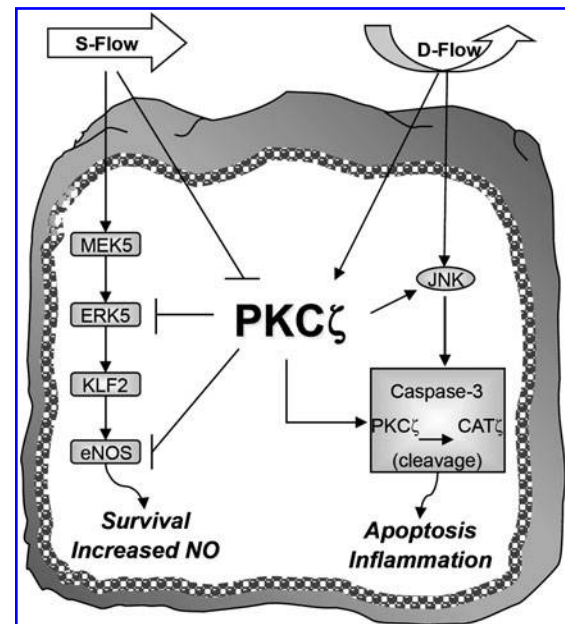
**FIG. 7. PKC $\zeta$  activation in s-flow and d-flow area of *Apoe*<sup>-/-</sup> mice.** PKC $\zeta$  is highly activated/phosphorylated (p-PKC $\zeta$ , red) in atherosclerotic lesions present in athero-prone regions (lesser curvature, d-flow) compared to athero-protected regions (greater curvature, s-flow) of mouse aortas. ECs were identified by PECAM-1 staining (green). 60 $\times$  lens; scale bar = 10  $\mu$ m. ECs, endothelial cells.

atherosclerotic susceptibility correlates with sites of d-flow we measured PKC $\zeta$  activation in atherosclerotic regions of mouse aortas. We have found that endothelial PKC $\zeta$  activation/phosphorylation was elevated in atherosclerotic lesions (d-flow) of the aortic arch compared with nonatherosclerotic (s-flow) regions (Fig. 7) (65). Further, we have demonstrated a critical crosstalk between the pro-inflammatory PKC $\zeta$  pathway and the anti-inflammatory MEK5/ERK5/KLF2 pathway (Fig. 8) (65). Mechanistically, we have proven that PKC $\zeta$  binds and phosphorylates ERK5, thereby decreasing eNOS protein stability and contributing to early events of atherosclerosis (65). All these observations suggest that PKC $\zeta$  activity could be an important determinant of atherogenesis susceptibility *via* regulation of inflammatory pathways. We will continue to pursue research in our lab with the expectation that it will lead to further evidence in substantiating the role PKC $\zeta$  has toward the progression of atherosclerosis.

## Conclusions

Studies described in this review highlight current evidence supporting the influence of hemodynamics in the pathogenesis of atherosclerosis.

It is widely recognized that s-flow is atheroprotective, whereas d-flow near arterial bifurcations, branch ostia, and



**FIG. 8. PKC $\zeta$  crosstalk between anti-inflammatory and pro-inflammatory pathways.** The pro-inflammatory effects of PKC $\zeta$  are mediated both by inhibiting the MEK5/ERK5/eNOS pathway and by promoting JNK-caspase-3-CAT $\zeta$  generation. These events are modulated by flow shear stress. In fact, s-flow inhibits the activation of JNK, which is required for caspase-3 cleavage, whereas d-flow is able to activate PKC $\zeta$ .

curvatures promotes atherosclerosis. Additionally, vascular endothelium has been shown to sense different flow patterns and to have different, flow-specific behavioral responses both at the molecular and at the cellular levels. Modulation of vascular wall behavior in regions of d-flow in conjunction with the impact of systemic vascular factors such as smoking, hyperlipidemia, hyperglycemia, and hypertension would be an appropriate approach to prevent atherosclerosis and to provide a useful therapeutic intervention. The most common approach to inhibit atherosclerosis has been to activate atheroprotective mechanisms, such as increasing nitric oxide bioavailability. However, we believe that a more elegant approach is to inhibit the atheropromoting mechanisms that occur uniquely in areas of d-flow. Our lab and others have shown that PKC $\zeta$ -dependent signaling is a unique atheropromoting mechanism that occurs solely in areas of d-flow. Therefore, PKC $\zeta$  is a prominent example of a regulatory signaling pathway that links extracellular events to intracellular responses, and has been directly implicated in atherogenesis.

Although inconclusive at present, we believe that PKC $\zeta$  could be an innovative therapeutic target to improve endothelial dysfunction that has been directly implicated in atherogenesis.

## Acknowledgment

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#### Abbreviations Used

AP-1 = activator protein 1  
aPKC = atypical protein kinase C  
ASK1 = apoptosis signal-regulating kinase 1  
BMP-4 = bone morphogenic protein-4  
CAT $\zeta$  = catalytic domain of PKC $\zeta$   
d-flow = disturbed blood flow  
ECs = endothelial cells

eNOS = endothelial nitric oxide synthase  
ERK5 = extracellular signal-regulated kinase-5  
ET-1 = endothelin-1  
GPx = glutathione peroxidase  
Grx = glutaredoxin  
GSH = glutathione  
GSK-3 $\beta$  = glycogen synthase kinase 3 beta  
HO-1 = heme oxygenase  
HUVECs = human umbilical vein endothelial cells  
ICAM-1 = intercellular adhesion molecule 1  
I $\kappa$ B = inhibitor of kappa B  
IL-1 = interleukin 1  
JNK = Jun NH<sub>2</sub>-terminal kinase  
Keap1 = Kelch-like ECH-associated protein 1  
KLF2 = Krüppel-like factor 2  
MAPKs = mitogen-activated protein kinases  
MCP-1 = monocyte chemotactic protein-1  
MEK5 = mitogen extracellular-signal-regulated kinase kinase 5  
NADH = nicotinamide adenine dinucleotide  
NADPH = nicotinamide adenine dinucleotide phosphate  
NF $\kappa$ B = nuclear factor kappaB  
Nrf2 = nuclear factor erythroid 2-like 2  
PAR = partitioning-defective  
PCP = planar cell polarity  
PDGF = platelet-derived growth factor  
PKC $\zeta$  = protein kinase C zeta  
PPAR $\gamma$ 1 = peroxisome proliferator-activated receptor gamma 1  
ROS = reactive oxygen species  
s-flow = steady laminar blood flow  
SOD = superoxide dismutase  
TNF $\alpha$  = tumor necrosis factor-alpha  
TRAF2 = TNF receptor-associated factor 2  
Trx1 = thioredoxin-1  
VCAM-1 = vascular cell adhesion molecule 1  
VEGF = vascular endothelial growth factor  
XO = xanthine oxidase

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