Chapter 6
Regulation of Endothelial Activation and Vascular Inflammation by Shear Stress

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Abstract Atherosclerosis is a multifactorial disorder caused by genetic and environmental factors such as cholesterol, obesity, hypertension, diabetes, and smoking and is the primary cause of morbidity and mortality worldwide. Blood flow is known to exert shear stress on the vascular endothelium. Atherosclerotic lesions occur predominantly at sites of low shear, whereas regions of the vasculature exposed to high shear are protected. Low shear stress leads to activation of endothelial cells which in turn can initiate inflammation. Shear stress can also modulate several signalling pathways mediated by the activated endothelial cells. The molecules involved in these signalling pathways can be atheroprotective or atherogenic. The aim of this chapter is to discuss the effects of low shear stress on the regulation of endothelial activation and subsequent vascular inflammation.

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Abbreviations

MKP-1	Mitogen activated protein kinase phosphatase 1
KLF2	Kruppel like factor 2
Nrf2	Nuclear factor erythroid 2-related factor 2
VCAM-1	Vascular cell adhesion molecule-1
PECAM-1	Platelet-endothelial cell adhesion molecule-1
ICAM-1	Intracellular cell adhesion molecule-1
MCP-1	Monocyte chemoattractant protein 1
JNK	c-Jun N-terminal kinase
ATF2	Activating transcription factor 2
NF-κB	Nuclear factor κ-light-chain-enhancer of activated B cells
NOS	Nitric oxide

6.1 Introduction

Inflammation of vascular tissues is induced in response to harmful stimuli such as chemicals, pathogens or injury. Vascular inflammation involves the recruitment of leukocytes, also known as white blood cells (monocytes, macrophages, dendritic cells, neutrophils, and lymphocytes) to the site of injury. The vascular system is lined with a monolayer of endothelial cells termed as the endothelium. This monolayer of cells performs various functions such as acting as a mechanical barrier and an anticoagulant, maintaining an anti-inflammatory environment, facilitating physiological control of vasoregulation, and modulating vascular permeability. Endothelial infiltration by circulating leukocytes is comprised of a multistep process that involves rolling of the leukocytes on the endothelium, attachment of the leukocytes to the endothelium, and transmigration of the leukocytes across the endothelial cells lining the blood vessel walls [1]. These processes are mediated by several different receptors called selectins, addressins, and integrins on the leukocytes and their subsequent interaction with adhesion molecules expressed on the surface of the endothelial cells. The adhesion molecules on endothelial cells include E-selectin, intracellular cell adhesion molecule-1 ICAM-1 (CD54), and vascular cell adhesion molecule-1 VCAM-1 [2,4].

The location of endothelial cells within the vascular endothelium is such that they are constantly exposed to mechanical forces such as pressure, circumferential stretch or tension and shear stress. Shear stress is a biomechanical quantity that is determined by factors such as blood flow velocity, vessel geometry and local fluid viscosity. Shear stress may be computationally estimated using fluid dynamics models. The numerical calculation of wall shear stress has been the topic of numerous studies, both in relation to larger scale flow features such as flow detachment and recirculation zones (e.g., [26]) as well as on significantly smaller scales (e.g., [24]). Particularly the study of the role of nitric oxide and the development of atherosclerotic plaque has been well studied in the past (e.g., [23]).

In a biomedical context shear stress is typically expressed in units of dynes/cm^2. Physiological arterial-level shear stress is variable, due to anatomical variation and pulsatility, but is approximately >15 dynes/cm^2 [18]. The magnitude of the shear stress may also be estimated in most of the vasculature by Poiseuille's law which states that shear stress is proportional to blood flow viscosity, and inversely proportional to the third power of the internal radius [13]. A change in the physiological amount of shear stress has been implicated in the pathogenesis of cardiovascular diseases. As pointed out by Cunningham and Gotlieb, three aspects of the way in which shear stress affects the endothelial surface can be distinguished. First, *laminar flow* gives the straightforward steady Poiseuille's flow effect (averaged over the physiological pulsatory cycle). Second, *oscillatory flow,* expresses cycle-to-cycle variations, which are normally zero or very low. Third, there are local regions of disrupted flow comprising separation, recirculation and reattachment [9]. Indeed in a combined numerical and in vitro study it was demonstrated that oscillatory low shear stress present in recirculation zones can lead to a significant activation of endothelial cells by enhancing ICAM-1 expression [34].

Shear stress, or rather, lower levels of shear stress have long been associated with the development of atherosclerosis. Atherosclerosis is a multifactorial disorder caused by genetic and environmental factors such as cholesterol, obesity, hypertension, diabetes, and smoking. It is the primary cause of morbidity and mortality worldwide. The pathogenesis of atherosclerosis involves biochemical and biomechanical changes in the arterial walls. Atherosclerosis is a chronic inflammatory disease that involves complex interactions between various modified lipoproteins, monocyte-derived macrophages, T lymphocytes, endothelial cells, and smooth muscle cells [25]. Atherosclerotic lesions occur predominantly in areas such as inner curvatures of the coronary arteries where there is lower shear stress compared to the average physiological shear stress levels and also in areas that demonstrate bifurcations where the shear stress is oscillatory [6,22].

At physiological levels of shear stress, the endothelial cells elongate and orient themselves parallel to the direction of the flow [17]. However, at lower levels of shear stress, the endothelial cells are found to being more rounded in shape [17]. Interestingly, such a rounded endothelial cell morphology has been observed in atherosclerotic lesions, and this is consistent with the finding that atherosclerosis develops at branches and bends that are exposed to lower levels of shear stress [6,22].

6.2 Signalling Pathways Involved in Shear Stress Mediated Endothelial Activation and Inflammation

Dysfunction of endothelial cells has been believed to be one of the main factors in initiating the pathogenesis of atherosclerosis [25]. This dysfunction in turn can lead to changes in gene expression by the endothelial cells. Various signalling pathways have been implicated in being upregulated or downregulated following endothelial

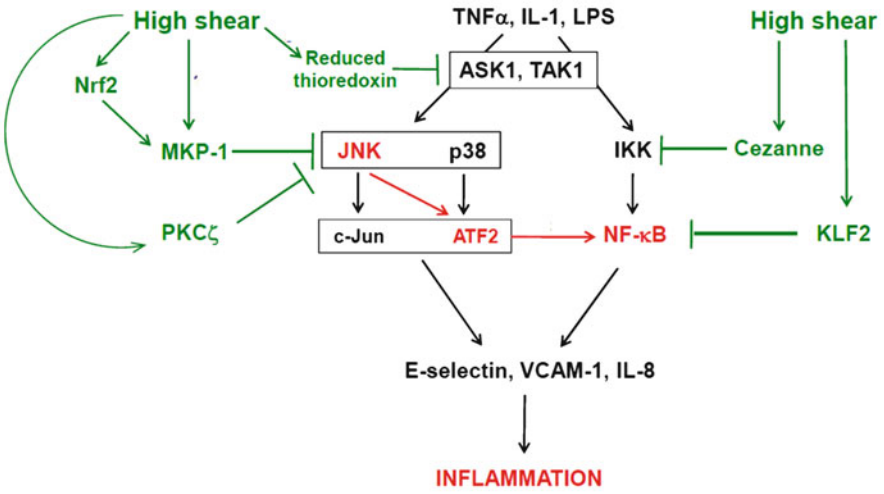

Fig. 6.1 Illustration of various signalling pathways that are involved in the pathogenesis of atherosclerosis due to differential levels of shear stress. High, laminar shear is atheroprotective and induces expression of anti-inflammatory factors such as MKP-1, Nrf2, Cezanne, and KLF2. The expression of these anti-inflammatory factors can down regulate the activation of inflammatory pathways. Low, oscillatory shear stress on the other hand induces the JNK-ATF2 –NF-KB pathway that in turn leads to recruitment of inflammatory leukocytes modulated by the expression of E-selectin and VCAM-1

activation by lower levels of shear stress. Upregulation of pro-inflammatory signalling molecules such as JNK, p38, and NF-κB are implicated in the pathogenesis of atherosclerosis whilst upregulation of Nrf2, KLF2, and MKP-1 and activation of eNOS is more atheroprotective. The inflammatory mechanisms that are involved in atherosclerosis and modulated by shear stress are hence explained in detail (Fig. 6.1).

6.2.1 Mitogen Activated Protein Kinase Phosphatase 1

Mitogen activated protein kinase phosphatase 1 (MKP-1) belongs to the family of dual specificity protein phosphatases that are ubiquitously located within the body and is upregulated by various extracellular stimuli [29]. This early gene is upregulated in vascular and nonvascular cells by an array of factors that include heat shock [14], oxidative stress [14], pro-inflammatory cytokines such as tumor necrosis factor-α (TNF-α) [31], lipopolysaccharides (LPS) from bacteria [28], peptide ligands such as thrombin [5], and growth factors such as vascular endothelial growth factors [15]. A primary function of MKP-1 is to inactivate the mitogen activated protein kinase (MAPK) by dephosphorylation of p38 and JNK at specific tyrosine and threonine residues. Shear stress controls the expression of MKP-1 by

endothelial cells. For instance, MKP-1 is over expressed in regions of vascular endothelium subject to laminar unidirectional high shear stress. These regions are termed atheroprotective regions as they do not exhibit any signs of developing atherosclerosis. The consequence of MKP-1 expression by these endothelial cells is that it negatively regulates the JNK and p38 pathways and thus hampers inflammation [36]. On the other hand, it also suppresses VCAM-1 expression [36] which promotes the recruitment of leukocytes to the vessel walls. Thus, high shear stress can prove to be atheroprotective in that it protects arteries from induction of persistent endothelial expression of MKP-1, which in turn, suppresses the activities of p38 and JNK [36]. Prior studies revealed that MKP-1 exerts anti-inflammatory effects in EC by inhibiting the expression of adhesion molecules and chemokines [5,31,36]. MKP-1 also suppresses the activation of macrophages and their capacity to produce the pro-atherogenic cytokine TNFα [7]. In addition, MKP-1 can be induced by low density lipoprotein (LDL) [19] and cyclic strain in smooth muscle cells [16] and reduces their proliferation. Thus, MKP-1 may exert anti-atherogenic effects in vascular endothelium by suppressing EC activation and also influence lesion development by regulating macrophage physiology and smooth muscle cell accumulation. Hence more research has to be carried out with respect to MKP-1 expression in regions that are susceptible to low, oscillatory shear stress and exploitation of this expression to yield therapeutic benefits.

6.2.2 Nuclear Factor Erythroid 2-Related Factor 2

Nuclear factor erythroid 2-related factor 2 (Nrf2) is a member of the "cap 'n' collar" family of basic leucine zipper transcription factors that is important in protecting cells against oxidative damage by reactive oxygen species (ROS) [3]. Nrf2 plays an important role in the protection of endothelial cells via antioxidant response element (ARE)-mediated gene expression of phase II detoxification antioxidant proteins. Both types of shear stress, laminar and oscillatory, have shown to increase the expression of Nrf2. However, stabilization of Nrf2 and expression of genes modulated by Nrf2 is only induced by laminar shear stress. In studies on human umbilical vein endothelial cells (HUVEC), induction of Nrf-2 regulated genes such as heme oxygenase 1, NAD(P)H quinone oxidoreductase1, glutamate-cysteine ligase modifier subunit, and ferritin heavy chain is carried out by laminar shear stress [32,33]. The regulation of these genes is inhibited when treated with Nrf2 siRNA. Laminar shear stress has been observed to induce gene expression of cytoprotective enzymes for glutathione biosynthesis and detoxification which are regulated by Nrf2. Laminar shear stress might activate Nrf2 via a phosphoinositol 3-kinase/Akt-dependent signalling pathway [10].

Following activation by laminar shear stress, Nrf2 acts in an anti-inflammatory manner similar to that of MKP-1. Nrf2 is constitutively active in endothelial cells in atheroprotected regions and has been observed to reduce the proinflammatory activities of p38 MAP kinase by inactivating it [35]. Nrf2 also suppresses the

VCAM-1 expression. The other manner in which Nrf2 controls endothelial activation is by reducing MKK3/6 signaling to p38 and by enhancing the activity of MKP-1. The mechanism behind the MKK3/6 suppression by Nrf2 is likely to involve redox regulation too because ASK1, a MAP kinase that acts upstream from MKK3/6, is known to be inhibited by reduced forms of glutathione and thioredoxin [35]. Further evidence suggests that Nrf2 can enhance the catalytic activity of MKP-1 by promoting a reducing environment via the induction of multiple antioxidants. Thus, it is proposed that laminar shear stress suppresses endothelial cell activation at atheroprotected sites by inducing MKP-1 and by simultaneously enhancing MKP-1 activity via activation of Nrf2 [35].

Interestingly, in regions of low shear stress that are more susceptible to atherosclerosis, Nrf2 seems to be expressed in a nonactive form and is incapable of suppressing the pro-inflammatory milieu that follows the formation of atherogenic lesions [35]. In addition to these observations, HUVEC challenged with laminar shear stress and simultaneously treated with Nrf2 siRNA (that will interefere with the expression of biologically active Nrf2) showed an upregulation of expression of adhesion molecules and chemokines (Takabe et al. 2011). In the same study, arterial endothelial cells isolated from Nrf2 deficient mice also demonstrated a similar result. The collective observation from the above studies suggest that Nrf2 might be an important therapeutic candidate to suppress inflammation in the vascular endothelium, as it can inhibit expression of adhesion molecules and recruitment of chemokines.

6.2.3 *Kruppel-like Factor*

Kruppel-like factor (KLF2) is an endothelial transcription factor, the expression of which is specifically induced by laminar shear stress [21]. It is an anti-inflammatory transcription factor that aids in maintaining the atheroprotective phenotype of vascular endothelial cells. KLF2 induces the expression of atheroprotective endothelial nitric oxide (eNOS) and thrombomodulin whilst downregulating the expression of pro-atherogenic MCP-1 and endothelin [11]. KLF2 also inhibits the expression of VCAM-1 and E-selectin, and thus, it suppresses the initiation of the inflammatory cascade that precedes the rolling and adhesion of inflammatory cells [27].

6.2.4 *JNK-NF-κB Pathway*

The nuclear factor κ-light-chain-enhancer of activated B cells (NF-κB) family of transcription factors regulates vascular inflammation by inducing adhesion proteins and other proinflammatory molecules in vascular endothelial cells [30]. There are five different subunit forms of NF-κB and the RelA/p50 heterodimer is the most

abundant form of NF-κB found in vascular endothelial cells. RelA expression is preferentially found in athero-susceptible regions of the arteries [8]. The low, oscillatory shear stress that is exerted in these regions initiate the signalling pathway that induces the expression of RelA [8]. Murine studies that involved the exposure of vascular endothelial cells to low levels of shear stress exhibited an enhanced activation of the mitogen activated protein kinase JNK1 and a downstream transcription factor ATF2 [8]. Simultaneously, the expression of RelA was also observed to be increased in the vascular endothelium. However, when JNK1 was genetically abrogated, activation of ATF2 was hindered and RelA expression was also reduced in the murine vascular endothelium. These observations indicated that low, oscillatory shear stress influences ATF2 and RelA activity through a JNK1-dependent mechanism [8]. The important roles JNK isoforms play in cardiovascular injury and disease have been demonstrated previously. This is supported by animal studies that have revealed that JNK can be activated in arteries in response to injury, during the development of aneurysms or in atherosclerotic lesions [37]. Other studies also report that the gene transfer of a dominant negative form of JNK1 reduced neointimal formation in injured arteries [12] and genetic deletion of JNK2 reduced foam cell formation and EC dysfunction in hypercholesterolemia [20].

Thus, the JNK-ATF2-NK-κB signalling pathway might be a key role in promoting inflammation in the vascular endothelium during atherosclerosis influenced by low, oscillatory shear stress.

6.3 Conclusion

Laminar shear stress is vital in maintaining vascular homeostasis and preventing atherosclerosis. Low, oscillatory shear stress can activate vascular endothelial cells through different inflammatory mechanisms. This activation can lead to recruitment of leukocytes that can lead to the development of atherogenic lesions. By comparing and contrasting the gene expression of various molecules in regions of laminar shear stress and oscillatory shear stress, the relationship between blood flow and development of atherogenesis can be understood more clearly. The conclusion from these studies are that high, laminar shear stress is atheroprotective and does not activate endothelial cells whereas low, oscillatory shear stress is pro-atherogenic and can initiate the activation of vascular endothelial cells, thus leading to inflammation seen in the pathogenesis of atherosclerosis. Therefore, the control of shear stress can be of potential therapeutic interest in the treatment of atherosclerosis and other diseases where fluctuations of shear stress can play a role in the pathogenesis.

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