Anti-Inflammatory Mechanisms in the Vascular Wall

Alain Tedgui, Ziad Mallat

Abstract—The role of vascular cells during inflammation is critical and is of particular importance in inflammatory diseases, including atherosclerosis, ischemia/reperfusion, and septic shock. Research in vascular biology has progressed remarkably in the last decade, resulting in a better understanding of the vascular cell responses to inflammatory stimuli. Most of the vascular inflammatory responses are mediated through the IκB/nuclear factor-κB system. Much recent work shows that vascular inflammation can be limited by anti-inflammatory counteregulatory mechanisms that maintain the integrity and homeostasis of the vascular wall. The anti-inflammatory mechanisms in the vascular wall involve anti-inflammatory external signals and intracellular mediators. The anti-inflammatory external signals include the anti-inflammatory cytokines, transforming growth factor-β, interleukin-10 and interleukin-1 receptor antagonist, HDL, as well as some angiogenic and growth factors. Physiological laminar shear stress is of particular importance in protecting endothelial cells against inflammatory activation. Its effects are partly mediated through NO production. Finally, endogenous cytoprotective genes or nuclear receptors, such as the peroxisome proliferator–activated receptors, can be expressed by vascular cells in response to proinflammatory stimuli to limit the inflammatory process and the injury. (Circ Res. 2001;88:877-887.)

Key Words: endothelial cells ◼ smooth muscle cells ◼ inflammation ◼ anti-inflammatory cytokines ◼ shear stress

Inflammation is a basic pathological mechanism that underlies a variety of diseases. The inflammatory reaction involves the complex interactions between inflammatory cells (neutrophils, lymphocytes, and monocytes/macrophages) and vascular cells (endothelial cells [ECs] and smooth muscle cells [SMCs]). The role of vascular cells during the inflammatory process is critical. Multiple cytokines and growth factors are present at sites of inflammation, and each of these can potentially influence the nature of the inflammatory response. ECs and SMCs must integrate the signals generated by these multiple factors to effectively regulate the immunoinflammatory response through the expression of adhesion molecules, cytokines, chemokines, matrix metalloproteinases, and growth factors. Research in vascular biology has progressed remarkably in the last decade, resulting in a better understanding of the vascular cell responses to inflammatory stimuli and resulting in the identification of major intracellular inflammatory signaling pathways, particularly the IκB/nuclear factor-κB (NF-κB) system. Much recent work shows that vascular inflammation can be limited by anti-inflammatory counteregulatory mechanisms that maintain the integrity and homeostasis of the vascular wall. This might be of particular importance in inflammatory diseases, such as atherosclerosis, septic shock, or ischemia/reperfusion. The purpose of the present review is to describe recent advances in the understanding of the anti-inflammatory mechanisms in vascular cells, focusing on anti-inflammatory external signals and intracellular mediators.
Anti-inflammatory effects of glucocorticoids will not be addressed in this review.

**External Anti-Inflammatory Signals**

**Anti-Inflammatory Cytokines**

Critically situated at the boundary between blood and tissues, the endothelium is a focus for inflammatory processes. ECs receive signals from humoral factors, inflammatory mediators, and physical forces from both the circulation and the tissue. Several potential triggers capable of inducing proinflammatory and prothrombotic cellular responses have been identified; these include modified lipoproteins, proinflammatory cytokines, chemokines, vasoactive peptides, neuropeptides, hyperglycemia and advanced glycosylated end products, smoking, oxidative stress, and others. SMCs also are targets of these triggers. Yet the vascular inflammatory responses and their temporal patterns can be regulated by anti-inflammatory cytokines. Anti-inflammatory cytokines that exert inhibitory effects on vascular cells include transforming growth factor-β (TGF-β), interleukin-10 (IL-10), and IL-1 receptor antagonist (IL-1ra).

**TGF-β**

TGF-β family members are secreted in inactive complexes with a latency-associated peptide (LAP), a protein derived from the N-terminal region of the TGF-β gene product. Extracellular activation of these complexes is a critical step in the regulation of TGF-β function in vivo. Cytokine activation of ECs increases TGF-β synthesis and activation of latent TGF-β by the plasminogen/plasmin system. Active TGF-β is produced by ECs in vitro when they are cocultured with pericytes or SMCs. Production of active TGF-β has also been found in human arterial SMCs in culture.

Active TGF-β is detectable in the aortic wall of mice and is decreased in transgenic mice expressing apo(a) as a consequence of apo(a) inhibition of the plasminogen/plasmin system. TGF-β was first reported to be a deactivating factor of macrophages capable, for example, of suppressing inducible nitric oxide synthase (iNOS) protein expression in macrophages. TGF-β also has potent anti-inflammatory effects on vascular cells. TGF-β downregulates cytokine-induced expression of E-selectin and vascular cellular adhesion molecule-1 (VCAM-1) in ECs as well as VCAM-1 in SMCs. TGF-β1 significantly decreases monocyte chemotactic protein-1 (MCP-1) expression in human umbilical vein ECs (HUVECs) stimulated with tumor necrosis factor-α (TNF-α) or IL-1β but not with interferon-γ (IFN-γ). The expression of TNF-α receptors seems to be downmodulated by TGF-β1. Furthermore, TGF-β1 inhibits the elaboration of IL-8 by TNF-activated ECs and inhibits the IL-8–dependent migration of neutrophils through the activated endothelial monolayer. TGF-β is able to restore endothelial-dependent vasodilation impaired by TNF-α. In addition, TGF-β suppresses iNOS induction in the vascular wall, leading to the prevention of lipopolysaccharide (LPS) shock in the rat. TGF-β expressed by vascular cells may also operate as a paracrine anti-inflammatory factor: glomerular mesangial cells express TGF-β in an active form that inhibits the production of proinflammatory cytokines by emigrated macrophages. Such cross-communications between vascular cells and infiltrating macrophages may play an important role in the recovery from the inflammatory process.

The pleiotropic effects of TGF-β are mediated from membrane to nucleus through distinct combinations of three types of cell-surface receptors (types I, II, and III), types I and II being serine and threonine kinases and their downstream effectors, known as Smad proteins. Smad-mediated effects result from a competitive interaction between Smad proteins activated by TGF-β and NF-κB proteins activated by proinflammatory stimuli. Smad proteins interact with the limited amount of cAMP response element–binding protein (CREB)-binding protein (CBP) present in ECs, therefore blocking the association of CBP with p65/NF-κB that is required for maximal transcriptional NF-κB activity (Figure 1). This type of signaling mechanism may play an important role in the immunomodulatory actions of this cytokine/growth factor in the cardiovascular system.

Most of the anti-inflammatory effects of TGF-β on vascular cells were documented in vitro. However, the relevance of in vitro findings to in vivo conditions is substantiated by the observation that TGF-β–deficient mice die in utero or in the perinatal period because of widespread uncontrolled inflammation. The TGF-β1 knockout mice have multifocal inflammatory disease in many tissues, but the heart and lungs are most severely affected. Increased adhesion of leukocytes to the endothelium of pulmonary veins and increased expres-
IL-10 exerts its biological effects on cells by interacting with a specific cell-surface receptor. Functionally active IL-10 receptor is composed of two distinct subunits. Both subunits belong to the class II cytokine receptor family. The IL-10 receptor alpha chain (or IL-10R1) plays the dominant role in mediating high-affinity ligand binding and signal transduction. The IL-10 receptor beta subunit (IL-10R2, also known as the orphan receptor CRF2-4) serves as an accessory chain essential for the active IL-10 receptor complex and to initiate IL-10–induced signal transduction events. Studies using macrophages from mice with disrupted genes for Jak1, Stat1, or Stat3 have revealed an obligate role for Jak1 and Stat3 in mediating the anti-inflammatory actions of IL-10. In addition to the Janus kinase/signal transducers and activators of transcription (JAK-STAT) pathway, the presence of a carboxyl-terminal sequence containing at least one functionally critical serine on the intracellular domain of the IL-10 receptor alpha chain is required for expression of the anti-inflammatory actions of IL-10.

IL-10 functions to block NF-κB activity through both the suppression of IkB kinase activity, preventing IkBα degradation, and the suppression of NF-κB DNA-binding activity (Figure 1). IL-10 also affects signaling through extracellular signal–regulated kinase (ERK) 1 and 2 other mitogen-activated protein kinase (MAPK) pathways that are potentially important for chemokine and cytokine induction and destabilizes the mRNA of proinflammatory genes with clustered AU-rich elements motifs. So far, nothing is known concerning the expression of IL-10 receptor alpha and beta chains and the complex IL-10 signaling pathway in vascular cells.

In vivo, IL-10 most likely exerts its anti-inflammatory effects on the vascular system through inhibition of leukocyte-EC interactions and inhibition of proinflammatory cytokine and chemokine production by macrophages.
or lymphocytes.41–43 In an acute lung injury model, IL-10 significantly decreased lung injury and ICAM-1 levels through decrease in TNF-α levels.37 LPS-induced expression of ICAM-1 and VCAM-1 in the vasculature of the small intestine and leukocyte adhesion in mesenteric venules are markedly increased in IL-10-deficient mice compared with wild-type animals, underlining the inhibitory role of endogenous IL-10 in the control of intestinal vascular inflammation.38,40 Transfected viral IL-10 decreases leukocyte vein extravasation through a decrease in endothelial expression of P- and E-selectin and ICAM-1.40 IL-10 similarly blunts inflammation secondarily to myocardial ischemia/reperfusion through an ICAM-1-dependent mechanism44 and reduces liver injury and mortality in a mouse septic shock model through decreased neutrophil margination and ICAM-1 and VCAM-1 expression.45 Endogenous IL-10 might also be produced during myocardial ischemia/reperfusion by lymphocytes infiltrating the reperfused myocardium and could limit myocardial macrophage activation.46

The protective effect of IL-10 against the development of diet-induced atherosclerosis could also be attributed to inflammatory cell deactivation. Expression of IL-10 in the atherosclerotic lesion42,47 is associated with low iNOS expression by macrophages and low levels of cell death.42 In addition, atherosclerotic lesions of IL-10+/− mice fed an atherogenic diet are characterized by increased infiltration of inflammatory cells, particularly activated T cells, and by increased production of proinflammatory cytokines, underscoring the anti-inflammatory actions of IL-10 produced within the atherosclerotic plaque.43 In a model of balloon angioplasty or stent implantation in hypercholesterolemic rabbits, treatment with recombinant human IL-10 markedly reduced macrophage infiltration and intimal hyperplasia.48 Additional mechanisms of vascular protection by endogenous IL-10 include decreased superoxide anion production in blood vessels in response to LPS, which prevents the impairment of endothelium-dependent relaxation.49

It is noteworthy that IL-10 most likely exerts its anti-inflammatory effect when produced locally in the vascular wall. Chronic production of high levels of IL-10 in the systemic circulation may instead lead to immunostimulatory effects.

**IL-1 Receptor Antagonist**

IL-1 is one of the most potent proinflammatory cytokines acting on both ECs and SMCs.1 Processed mature IL-1 signals via the type I IL-1 receptor but also binds to a nonsignaling receptor (IL-1 receptor type II). The IL-1 receptor antagonist (IL-1ra) is an endogenous secreted protein that binds to IL-1 type I and II receptors without signaling. An intracellular form of IL-1ra is expressed by human ECs and SMCs,50,51 but its role remains unclear. However, in vivo studies reveal that IL-1ra does have vascular protective effects. Treatment with recombinant IL-1ra inhibits fatty streak formation in apoE−/− mice.52 More importantly, IL-1ra knockout mice develop lethal chronic inflammation of the arterial wall, associated with massive transmural infiltration of neutrophils, macrophages, and CD4+ lymphocytes in branch points and flexures of the aorta and in its primary and secondary branches.53 Additional support for a vascular role of IL-1ra is provided by the recent observation of an association between IL-1ra gene polymorphism and coronary artery disease.54

**Th2 Anti-Inflammatory Cytokines IL-4 and IL-13**

Th2 cytokines IL-4 and IL-13 suppress the production of inflammatory cytokines by macrophages and monocytes and are considered anti-inflammatory cytokines. However, IL-4 and IL-13 selectively induce VCAM-1 and P-selectin expression on ECs with no effect on ICAM-1 or E-selectin.55–57 IL-13 markedly enhances IL-8 and MCP-1 release by cytokine-stimulated human SMCs but inhibits NOS II expression in LPS-activated rat SMCs.58 In vivo studies indicate that IL-4 and IL-13 are capable of promoting angiogenesis57 and that IL-4 plays a role in the progression of early inflammatory atherosclerotic lesions driven by immunization against heat shock protein 65.59 This may be consistent with the recently reported switch from Th1 to Th2 responses during atherosclerosis progression in severely hypercholesterolemic apoE−/− mice.60 However, it remains unknown to what extent this switch might affect lesion progression. In general, it seems that the macrophages deactivating cytokines IL-4 and IL-13 display proinflammatory activities in the vascular system.

**High-Density Lipoprotein**

There is abundant evidence from epidemiological studies that HDL plasma concentration is inversely correlated with the occurrence of coronary artery disease. Besides the effects of HDL on the promotion of cholesterol efflux and protection against lipid peroxidation, it exerts potent anti-inflammatory activities on ECs. HDL inhibits cytokine-induced expression of E-selectin, ICAM-1, and VCAM-1 on ECs at the transcriptional level.61–64 The effects of HDL seem to be related to its phospholipid content.65 The anti-inflammatory effects of HDL on ECs could involve the sphingosine kinase (SphK) pathway through the generation of sphingosine 1 phosphate (S1P).65 HDLs inhibit the TNF-induced SphK activity and S1P generation and are expected to subsequently reduce the activation of ERK and NF-κB signal cascades (Figure 1). However, other studies reported that the anti-inflammatory effects of HDL are not mediated by a direct inhibition of the NF-κB pathway, because HDLs do not inhibit IκBα degradation or the nuclear translocation of NF-κB.62 Furthermore, HDLs inhibit E-selectin expression in response to proinflammatory cytokines but have no effect on the expression of NF-κB-dependent genes, such as granulocyte-macrophage colony-stimulating factor (GM-CSF) and COX-2. Interestingly, HDLs also stimulate COX-2 expression in SMCs,66 which suggests that the anti-inflammatory effect of HDL might be restricted to a specific set of genes.

Whatever the molecular mechanisms of the anti-inflammatory effects of HDL, the pathophysiological relevance of the ability of HDL to exert these activities is substantiated by several studies showing that HDLs inhibit endothelial cell adhesion molecule expression in vivo. Elevation of the circulating levels of HDL inhibits E-selectin expression by microvascular ECs in a porcine model of acute
inflammation, and reconstituted HDL containing human apoA-I reduces VCAM-1 expression in common carotid artery after inflammation associated with periadventitial cuff-induced injury in apoE/ mice.

Angiogenic and Growth Factors

VEGF is a potent factor in increasing permeability of endothelial cells that leads to the passage of plasma components and leukocytes from the blood vessel into the tissues and may therefore contribute to the inflammatory response. Yet VEGF might exert anti-inflammatory protective functions by stimulating endothelial NO production, which may inhibit leukocyte recruitment (see below). The receptor mediating VEGF-induced NO production in ECs is likely to be VEGF receptor-2. This effect is mediated by a signaling cascade initiated by flk-1/KDR activation of c-Src, leading to phospholipase Cγ1 activation, inositol 1,4,5-trisphosphate formation, release of intracellular Ca2+, and NOS activation.

Agiopoiitin-1 (Ang-1), the ligand of the endothelium-specific tyrosine kinase receptor Tie-2, has been shown to be an anti-inflammatory agent in vitro. Ang-1 pretreatment of ECs abolished TNF-α–induced transmigration. This effect likely results from enhanced platelet EC adhesion molecule-1 (PECAM-1) localization to the EC junctions. Interestingly, hypoxia and inflammatory cytokines upregulate Tie2 receptor in HUVECs and human microvascular EC-1. In this context, enhancement of PECAM-1 or PECAM-1 engagement mediated by Ang-1 might promote a noninflammatory phenotype of ECs.

Growth factors not only modulate vascular cell survival and growth but also may act as modulators of inflammatory responses. FGF-2 can inhibit endothelial expression of tissue factor and other inflammatory genes, including tissue plasminogen activator, plasminogen activator inhibitor-2, and factor and other inflammatory genes, including tissue plasminogen activator, plasminogen activator inhibitor-2, and IL-8, in response to phorbol myristate acetate. Exposure of minogen activator, plasminogen activator inhibitor-2, and factor and other inflammatory genes, including tissue plasminogen activator, plasminogen activator inhibitor-2, and factor to FGF-2 or FGF-1/heparin also inhibits cytokine-stimulated expression of tissue factor expression in fibroblasts, monocytes, and SMCs. In SMCs, growth factors may have ambiguous effects. Platelet-derived growth factor induces MCP-1 and ICAM-1, whereas it inhibits cytokine-stimulated expression of NOS II and NO release.

Intracellular Anti-Inflammatory Mediators

Regulators of NF-κB Signaling Pathway

The expression of inducible genes leading to the synthesis of cytokines, chemokines, adhesion molecules, and autacoids relies on transcription factors. Among the primary transcription factors, NF-κB plays a central role in the regulation of inflammatory mediators. Events leading to the activation of NF-κB rely on the phosphorylation of IκBα followed by its ubiquitination and proteolytic degradation into the proteasome. Phosphorylation of IκBα depends on a IκB kinase (IKK) complex containing two kinases, IKKα and IKKβ, and the regulatory protein NEMO (NF-κB essential modifier, also named IKKγ). Moreover, NF-κB itself induces the synthesis of IκBα to regenerate an inactive form of NF-κB and ensures the transiency of NF-κB activation. The intracellular redox status of the cell is extremely important in the regulation of NF-κB/IκB by preventing the activation of IκB kinase. Antioxidants, aspirin, N-acetyl-L-cysteine (NAC), and flavonoids may inhibit the activation of NF-κB. We have seen that TGF-β and IL-10 negatively regulate the NF-κB pathway. Glucocorticoids enhance the formation of IκB, and several constitutive or inducible cytoprotective genes have been shown to inhibit NF-κB activity in ECs (see below). NO also inhibits NF-κB activity in ECs through the induction and stabilization of IκBα (see below). The activation of NF-κB can also be attenuated by inhibiting the proteolytic degradation of IκB in the proteasome. A naturally occurring antibacterial peptide, PR39, which reversibly binds the 26S proteasome and blocks the degradation of IκBα by the ubiquitin-proteasome pathway, suppresses VCAM-1 and ICAM-1 gene expression in TNF-α–activated human ECs and reduces the size of myocardial infarction in an in vivo mouse model of coronary ligation. Another possibility to interrupt the NF-κB pathway is to block the interaction between NEMO and IKKβ. A cell-permeable NEMO-binding domain (NBD) peptide able to disrupt the IKKβ-NEMO interaction efficiently reduces E-selectin expression in TNF-α–treated HUVECs. These anti-inflammatory agents (PR39 and NBD peptide) that directly interact intracellularly with the NF-κB pathway (Figure 1) might prove to be useful tools to block proinflammatory activation in the vascular wall.

Protective Genes

Endogenous protective genes can be expressed by vascular cells to limit the inflammatory process and injury. Indirect arguments suggest that both ECs and VSMCs may develop an autoprotective phenotype during inflammation.

Cytoprotective Genes

In addition to protecting ECs from apoptosis, several antiapoptotic genes have been shown to possess potent anti-inflammatory properties (Figure 2). As a consequence, they have been named cytoprotective genes. These include members of the Bcl-2 family (Bcl-2, Bcl-xL, and A1), A20,
and heme oxygenase-1 (HO-1). A1 and A20 are induced in response to inflammatory stimuli to protect ECs from unfettered activation and from undergoing apoptosis even when NF-κB is blocked.\textsuperscript{84} Overexpression of Bcl-2, Bcl-x\textsubscript{L}, A1, or A20 inhibits VCAM-1, E-selectin, and IL-8 expression in ECs by inhibiting NF-κB activation.\textsuperscript{85,86} In vivo experiments underscore the importance of these protective genes in organ xenografts. ECs in hamster hearts that accommodate themselves in rats express certain genes, such as A20 and bcl-2, whereas hearts that are rejected do not express these genes.\textsuperscript{87} In addition, vessels of rejected hearts show florid transplant arteriosclerosis, whereas those of accommodated hearts do not. Moreover, studies in mice deficient for A20 confirm the critical role of this protective gene for limiting TNF-α-dependent NF-κB activation and inflammation.\textsuperscript{88} A20 knockout mice develop severe inflammation and cachexia, whereas hypertensive to both LPS and TNF, and die prematurely. Taken together, these data suggest that A1 and A20 offer the mean of achieving an anti-inflammatory effect in the vascular wall.

HOs also belong to the family of cytoprotective genes. HOs catalyze the rate-limiting step in the degradation of heme to yield equimolar amounts of biliverdin, carbon monoxide, and iron. Besides their antipapoptotic effect, there is a growing body of evidence that ascribes an anti-inflammatory role for the products of the inducible form of HO (HO-1).\textsuperscript{89} HO-1 can be upregulated in human ECs by TNF and IL-1.\textsuperscript{89} In particular, exogenous administration of HO-1 by gene transfer protects the rat lung against hyperoxia-induced neutrophil infiltration and tissue injury.\textsuperscript{90} Moreover, HO-1 deficiency in humans is associated with the presence of severe and persistent endothelial damage.\textsuperscript{92}

**Fas and Fas Ligand**

Fas ligand (FasL) is a death factor that induces apoptosis in Fas-bearing cells. FasL is constitutively expressed on ECs but not in SMCs.\textsuperscript{93} Local administration of TNF-α to arteries downregulates endothelial FasL expression and induces mononuclear cell infiltration, whereas FasL overexpression markedly attenuates TNF-α-induced cell infiltration. Moreover, adherent mononuclear cells undergo apoptosis rather than diapedesis under these conditions as a result of Fas-FasL ligation. These data suggest that endothelial FasL plays an active role in inhibiting leukocyte extravasation and vascular inflammation. Recent experiments in FasL-deficient mice additionally support this contention.\textsuperscript{94} In a model of flow restriction in the common carotid artery, vascular T lymphocyte and macrophage infiltration after flow restriction is notably enhanced in FasL knockout mice compared with wild-type mice. Moreover, the flow-restricted common carotid arteries develop greater neointima formation in FasL knockout mice than in wild-type mice.

**Serpine Proteinase Inhibitor 9**

SMCs and ECs express the 33-kDa precursors of both IL-1α and IL-1β as cell-associated proteins, but SMCs neither contain mature IL-1β nor are able to process recombinant IL-1β precursor into its mature 17-kDa form. Despite this failure, SMCs express IL-1–converting enzyme but possess in their cell membrane compartment an inhibitory factor of IL-1β processing, recently identified as the serpine proteinase inhibitor 9 (PI-9).\textsuperscript{95} PI-9 is homogenously expressed in the normal arterial wall, and its expression inversely correlates with immunoreactive IL-1β in the atherosclerotic plaque, suggesting a potential role for PI-9 in this inflammatory disease.

**Nitric Oxide**

Besides its action on vasomotor tone regulation, endothelium-derived NO has been recognized to be an anti-inflammatory molecule. Endogenous NO synthesis inhibits leukocyte rolling and adhesion as well as cytokine-induced expression of ICAM-1 and VCAM-1.\textsuperscript{96,97} NO inhibits M-CSF synthesis in ECs.\textsuperscript{98} Furthermore, inhibition of basal NO production by N\textsuperscript{G}-nitro-L-arginine in human ECs upregulates and exogenous addition of NO decreases MCP-1 expression.\textsuperscript{99} NO donors inhibit the expression of MCP-1 in SMCs exposed to LPS or oxidized LDL,\textsuperscript{100} and diminish VCAM-1 expression induced by IFN-γ.\textsuperscript{101} The anti-inflammatory effects of NO are attributable, at least in part, to inhibition of NF-κB activation through increased expression and nuclear translocation of IκBα.\textsuperscript{102} (Figure 3). The crucial role of NO as an endogenous anti-inflammatory mediator was later substantiated by in vivo experiments of chronic inhibition of NO synthesis. Administration of the NO synthesis inhibitor \textsuperscript{N=}-nitro-L-arginine methyl ester (L-NAME) induces vascular monocyte infiltration, MCP-1, IL-6, M-CSF, ICAM-1, and VCAM-1 expression as well as NF-κB activation.\textsuperscript{103–106} In vivo transfecion of cis element decy ligoidoxynucleotides against NF-κB prevents the L-NAME–induced early inflammation, suggesting that the NF-κB pathway is essential in this process.\textsuperscript{104} Along with inflammatory changes, vascular superoxide anion production is also increased after chronic NO blockade, and the antioxidant NAC prevents all of these
Therefore, inhibition of NO synthesis increases vascular oxidative stress leading to inflammatory responses. Interestingly, in L-NAME–induced vascular inflammation, treatment with an angiotensin II type 1 receptor antagonist also prevents NF-κB activity and the consequent inflammatory changes.\textsuperscript{105,107} Taken together, these data suggest that endogenous endothelial NO decreases proinflammatory oxidative stress-sensitive signals by suppressing localized activity of angiotensin II in blood vessels.

**Peroxisome Proliferator–Activated Receptors**

Peroxisome proliferator–activated receptors (PPARs) are transcription factors belonging to the nuclear receptor superfamily, of which three different PPAR subtypes have been identified, PPAR\textsubscript{α}, PPAR\textsubscript{β/δ}, and PPAR\textsubscript{γ}. PPARs regulate gene expression by binding with the retinoid receptor RXR as a heterodimeric partner to specific DNA sequence elements termed PPAR-responsive elements. Fatty acid derivatives and eicosanoids have been identified as natural ligands for PPARs.\textsuperscript{108} Furthermore, fibrates are synthetic ligands for PPARs that mediate the lipid-lowering activity of these drugs, and the antidiabetic thiazolidinediones are synthetic ligands for PPAR\textsubscript{γ}. PPAR\textsubscript{α} and PPAR\textsubscript{γ} have been found to be expressed in both ECs\textsuperscript{109,110} and SMCs\textsuperscript{111–113} in vitro and in vivo in the human atherosclerotic plaque.\textsuperscript{114–116} Anti-inflammatory actions of PPARs were first reported in monocytes and macrophages for PPAR\textsubscript{γ}.\textsuperscript{117,118} Thereafter, PPAR\textsubscript{α} activation, but not PPAR\textsubscript{γ}, was shown to repress cytokine-induced activation of COX-2 and IL-6 in human SMCs\textsuperscript{119} and VCAM-1 in human ECs, resulting in reduced functional adhesion of monocytes.\textsuperscript{119} These in vitro findings are in good agreement with an earlier study showing that PPAR\textsubscript{α} knockout mice have increased acute inflammatory responses.\textsuperscript{120} Moreover, recent findings clearly indicate that PPAR\textsubscript{α} has anti-inflammatory properties in the vascular wall; aortic explants isolated from PPAR\textsubscript{α} knockout mice display an exacerbated response to inflammatory stimuli, resulting in increased IL-6 production compared with wild-type mice.\textsuperscript{113} Furthermore, fibrate treatment represses IL-6 mRNA levels in LPS-stimulated aortas of wild-type mice but not of PPAR\textsubscript{α} knockout mice.

In addition to regulating gene transcription via PPAR responsive elements, PPARs have recently been shown to modulate gene transcription by interfering with other transcription factor pathways in a DNA binding–independent manner.\textsuperscript{111,113,117,119} PPARs have been shown to downregulate inflammatory response genes by negatively interfering with the STAT, AP-1, and NF-κB transcriptional pathways.\textsuperscript{109,111,113,117,119} For example, direct protein-protein interactions between PPAR\textsubscript{α} and AP-1 and NF-κB proteins have been invoked as mechanisms of transrepression.\textsuperscript{113} In addition, by regulating antioxidant enzyme activities, such as catalase,\textsuperscript{121} PPAR\textsubscript{α} activators reduce the oxidative stress and, as a result, may inhibit NF-κB activation. Finally, PPAR\textsubscript{α} activators may antagonize NF-κB activation through the expression of the inhibitory protein IκB\textsubscript{α}, as shown in IL-1β–stimulated human aortic SMCs in the presence of fibrates\textsuperscript{122} (Figure 1).

Several years ago, the n-3 fatty acid docosahexaenoic acid docosahexaenoic acid was reported to limit cytokine-induced expression of VCAM-1 and other proinflammatory mediators in human ECs.\textsuperscript{123} We now know that this is likely attributable to the anti-inflammatory activities of PPAR\textsubscript{α}, which is a target for various long-chain fatty acids, including n-3 fatty acids.\textsuperscript{124}

The anti-inflammatory activities of PPAR\textsubscript{α} take on particular significance in view of the findings that fibrate treatment decreases plasma concentrations of inflammatory cytokines in patients with angiographically established atherosclerosis.\textsuperscript{111} Furthermore, the recent Veteran’s Administration HDL Intervention Trial showed a beneficial effect of the fibrate gemfibrozil on atherosclerotic events that could not be accounted for by reductions in LDL concentrations.\textsuperscript{125}

**Anti-Inflammatory Effects of Shear Stress**

Apart from humoral stimuli, ECs are constantly exposed to a spectrum of hemodynamic forces generated by pulsatile blood flow, hydrostatic pressure, cyclic strains, and wall shear stresses.\textsuperscript{126} Biomechanical forces induce endothelial structural changes and modulate gene expression.\textsuperscript{127} Most in vitro experiments using ECs suggest that physiological levels of shear stress are essentially anti-inflammatory and anti-adhesive. Prolonged exposure of ECs to laminar flow, as occurs in vivo, results in downregulation of ICAM-1 and VCAM-1,\textsuperscript{128} whereas prolonged low or oscillatory shear-stress conditions enhance monocyte adhesion and VCAM-1, ICAM-1, and E-selectin expression.\textsuperscript{129,130} Anti-inflammatory effects of laminar shear stress may also prevail in conditions of activated ECs. Chronic laminar shear stress suppresses VCAM-1 expression by IL-1β–stimulated ECs, whereas oscillatory shear stress has no effect.\textsuperscript{130} Similarly, monocyte adhesion to ECs in the presence of oxidized lipids is markedly reduced by physiological pulsatile unidirectional flow, whereas oscillatory flow promotes monocyte adhesion.\textsuperscript{131} These in vitro studies are in agreement with in vivo experiments showing that low or oscillatory shear stress provides a proinflammatory stimulus to ECs.\textsuperscript{30,132,133} Constitutive NF-κB activation and VCAM-1 expression are seen in ECs located in aortic regions of high probability for atherosclerotic lesion development, which are known to be regions of altered hemodynamics forces.\textsuperscript{30,133} Moreover, chronically decreased blood flow in rabbits stimulates VCAM-1 expression and enhances monocyte adhesion.\textsuperscript{132}

Molecular mechanisms of anti-inflammatory actions of shear stress involve protection against oxidative stress and inhibition of NF-κB and jun-N-terminal kinase (JNK)–AP-1 pathways (Figure 3). Laminar shear stress induces Cu/Zn superoxide dismutase,\textsuperscript{134,135} suggesting that absence or decrease of shear stress results in increased production of superoxide radicals. In contrast, low or oscillatory flow patterns induce a sustained activation of pro-oxidant processes, resulting in redox-sensitive gene expression.\textsuperscript{129,136} The antioxidant pyrrolidine dithiocarbamate, but not NAC, strongly inhibits low shear–induced NF-κB activation, expression of VCAM-1, and monocyte adhesion.\textsuperscript{129} Because NAC seems to have no effect on superoxide radical,\textsuperscript{137} it is tempting to hypothesize that low shear stress allows O$_{2}^{-}$-dependent activation of NF-κB and subsequently VCAM-1. However, both pyrrolidine dithiocarbamate and NAC inhibit NF-κB activation and VCAM-1 expression induced by oscillatory shear stress or cytokines, suggesting that other pro-
oxidant pathways may be involved (H_2O_2/OH^- sensitive mechanisms).^{129,130} Taken together, these data also indicate that ECs discriminate between various types of flow and between flow and cytokine stimulation. Other mechanisms may account for the anti-inflammatory actions of shear stress. The MAPK JNK is activated by exposure of cells to cytokines or environmental stress and contributes to inflammatory responses.^{138} Laminar shear stress specifically inhibits cytokine-induced JNK activity but has no effect on the other MAPKs.^{139} Shear stress also abrogates the complement-induced IL-8 and MCP-1 expression in ECs through upregulation of the complement-inhibitory protein clustering.^{140} In addition, shear stress upregulates the expression of the inhibitory adapter protein tumor necrosis receptor-associated factor (TRAF)-3, and transfection of a dominant-negative TRAF3 mutant reverses the inhibitory effect of shear stress on CD40-induced MCP-1 expression.^{141} Finally, shear stress is known to be the physiological activator/inducer of NOS III, and NO has other MAPKs.^{139} Shear stress also abrogates the complement-inhibits cytokine-induced JNK activity but has no effect on the other MAPKs.^{139} Shear stress also abrogates the complement-induced IL-8 and MCP-1 expression in ECs through upregulation of the complement-inhibitory protein clustering.^{140} In addition, shear stress upregulates the expression of the inhibitory adapter protein tumor necrosis receptor-associated factor (TRAF)-3, and transfection of a dominant-negative TRAF3 mutant reverses the inhibitory effect of shear stress on CD40-induced MCP-1 expression.^{141} Finally, shear stress is known to be the physiological activator/inducer of NOS III, and NO has other MAPKs.^{139}

Conclusions
The involvement of vascular inflammation in several pathological conditions, including atherosclerosis, ischemia/reperfusion, hypertension, restenosis, angiogenesis, septic shock, and cerebral malaria, has only recently been fully revealed. In the context of atherosclerosis, the inflammatory response seems to determine the development of the disease from even the site and extent of endothelial activation to lesion growth and lesion complications. This is consistent with clinical studies in humans showing a major prognostic and independent value for markers of inflammation in predicting the occurrence of ischemic vascular events.^{142} Great progress has been made during the last decade in the understanding of the molecular mechanisms that mediate the inflammatory responses in vascular cells. The identification of protective anti-inflammatory mechanisms is much more recent. Interestingly, several effects of some well-known antiatherogenic and vasculoprotective agents, including HDL, statins, fibrates, NO, shear stress, antioxidants, and n3-fatty acids, might in fact be attributable, at least in part, to their anti-inflammatory properties. More importantly, recent data suggest that vascular cells may develop genetically determined intrinsic anti-inflammatory mechanisms.^{143} Delineating these anti-inflammatory pathways will be one of the challenging future objectives in vascular biology, which could yield promising novel therapeutic possibilities. Yet such anti-inflammatory strategies should be specifically targeted at the diseased tissue, particularly when long-term therapy is necessary, to limit potential side effects.

Acknowledgments
This work was supported by INSERM, Fondation de France, and Fondation pour la Recherche Médicale.

References
cytokine production by mononuclear phagocytes and endothelial cells.


Anti-Inflammatory Mechanisms in the Vascular Wall
Alain Tedgui and Ziad Mallat

_Circ Res._ 2001;88:877-887
doi: 10.1161/hh0901.090440

_Circulation Research_ is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2001 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7330. Online ISSN: 1524-4571

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circres.ahajournals.org/content/88/9/877

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in _Circulation Research_ can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to _Circulation Research_ is online at:
http://circres.ahajournals.org//subscriptions/